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Contents

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MINIREVIEWS

Contents

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MINIREVIEWS

Role of radiotherapy in the pre-operative management of carcinoma of the esophagus

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Abstract

The use of radiotherapy in the management of carcinoma of the esophagus and gastro-esophageal junction has undergone much evolution over the past 2 decades. Advances to define its role have been slow with meta-analyses often providing the most useful data. In spite of this many institutions around the world are divided about the role of radiotherapy in this disease and attribute different roles to radiotherapy based on clinical stage, tumor site and histology. The purpose of this review is to try to define the role of radiotherapy given our current knowledge base and to review which current and future trials may fill the gaps of knowledge that we currently have. It will also highlight the difficulties in making firm recommendations about the use of radiotherapy especially in a time when technology and treatments are rapidly evolving.

Key words: Esophageal cancer; Preoperative therapy; Neoadjuvant therapy; Chemoradiotherapy; Surgery

Core tip: This review describes the history and development of radiotherapy in the pre-operative setting for resectable esophageal cancer. In particular it focuses on data from multicenter phase Ⅱ and phase Ⅲ trials as well as meta-analyses from across the world. The review concludes with a discussion about the role of new radiation technologies in the management of esophageal cancer.

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INTRODUCTION

The issue of improving loco-regional control and possibly survival for resectable esophageal cancer has been a subject of interest for about the last 3 decades. Prior to 1980 radiotherapy was used mostly as sole modality for therapy when patients were not suitable for surgery. Response rates and survival however were low and it was only when chemotherapy was added to radiotherapy that response rates improved and long term remissions

from the disease were noted. At the time, squamous cell carcinoma (SCC) was the predominant cell type and being a relatively responsive disease, most early clinical trials involving pre-operative therapy were confined to that histology. It was only in the late 1990s that some investigators began to include adenocarcinoma (AC) in neoadjuvant clinical trials on the basis that if surgery was to be the major treatment modality, histological subtype might not play a major role in outcomes. In this review I trace the progress of the early trials involving SCC only, the combined histology trials and finally some of the meta-analyses, most of which have included both histological subtypes. The dilemma however is far from resolved with the issue of whether radiotherapy adds real benefit to systemic therapy in terms of survival and loco-regional control yet to be determined in a randomized trial.

PRE-OPERATIVE RADIATION THERAPY

Following the suggestion that radiotherapy may lead to a complete pathological response (pCR) in some patients prior to surgery in esophageal cancer, some investigators did report non-randomized retrospective comparisons of surgery alone *vs* pre-operative radiotherapy followed by surgery. Radiation doses ranged from 20-60 Gy and there were some reports of a survival improvement in those treated with both radiotherapy and surgery, although these studies were non-randomized and clearly a possibility of better performance status patients having combined modality therapy existed. It was not long however before randomized trials were devised to assess outcomes following pre-operative radiotherapy. Between 1981 and 1992 five randomized trials were reported[1-5]. All of these involved SCC and radiation doses varied from 20-40 Gy. Survival outcomes were inconsistent with 3 trials reporting improved survival with pre-operative radiotherapy[2,3,5] and 2 had better survival in the surgery alone arm[1,4]. None of these results however reached statistical significance. Nevertheless an average of 15% of patients did achieve a pCR and local failure rates ranged from 20%-58%. Since that time radiotherapy as a single modality is seldom used as a sole modality in the pre-operative setting.

PRE-OPERATIVE CHEMORADIOTHERAPY

The use of pre-operative chemoradiotherapy in esophageal cancer was first used in 2 phase Ⅱ studies based in the United States[6,7]. The Southwestern Oncology Group trial accrued 113 patients treated with pre-operative chemoradiotherapy using concurrent cisplatin, fluorouracil and 30 Gy. Median survival was 12 mo and 3 year survival 16%[7]. The RTOG study had even poorer survival[6]. Several other phase Ⅱ trials incorporating tri-modality therapy have been done, including some incorporating newer chemotherapy agents including paclitaxel, carboplatin and oxaliplatin. Most show similar median survival times of 20%-58% although the selection criteria for these studies vary and may well affect the different outcomes.

There have been numerous randomized trials comparing surgery alone with pre-operative chemoradiotherapy followed by surgery[8-14]. The earlier studies only involved patients with SCC but more recent ones included both histologies. The first to report a positive outcome in favor of tri-modality therapy was that by Walsh *et al*[12] who only reported the outcomes of a subset of AC patients despite the fact that both subtypes were included in the trial[12]. Although the outcome was significantly beneficial in terms of survival for those receiving chemoradiotherapy, the trial has been criticized as the workup imaging did not include computed tomography (CT) scanning of the chest and abdomen and the outcomes of the surgery alone arm were exceptionally poor. More recently the trials by Tepper *et al*[15] and the CROSS study by van Hagen *et al*[16] have clearly shown benefits in survival and these have both been based on high tumor response rates including pCR rates in the experimental arm. Some of the other chemoradiotherapy trials have shown benefits for subgroups. The Australian trial by Burmeister *et al*[14] showed a benefit for patients with SCC but not AC and then because AC constituted the majority of patients recruited to the trial, there was no overall survival benefit. In the trial by Urba, the benefit was seen in overall survival at 3 years but the difference did not reach statistical significance[13]. Other trials in this area have been negative in terms of survival but have also been criticized because of having low doses of radiotherapy and split courses of treatment.

OUTCOMES OF META-ANALYSES

Meta-analyses (MAs) are frequently used to reach conclusions about absolute trial endpoint such as survival when individual trials don't have enough numbers to provide statistically significant outcomes. The problem is that when trials are included in MAs the sample populations may be different with different eligibility criteria, requirements for clinical staging and in esophageal cancer, different histological subtypes. Requirements for clinical staging may be evolutionary in that earlier trial investigators may not have had access to more contemporary forms of imaging such as CT or positron emission tomography (PET). This means that some of the earlier trials may have included patients with more advanced disease that would be excluded by more modern forms of imaging. Another issue with MAs is that they often fail to address secondary endpoints such as loco-regional control, pCR rates, resectability rates, toxicity and quality of life, unless all the trials included these endpoints and the same methodology in assessing them was used.

Radiotherapy plus surgery vs surgery alone

For patients having pre-operative radiotherapy alone, there is only one MA which has been published on multiple occasions as a Cochrane review[17]. This review involved all 5 randomized trials comparing surgery alone with pre-operative radiotherapy followed by surgery. The dominant histology was SCC although some trials

did include AC. The outcomes was an improvement in survival in those patients receiving combined therapy although this did not reach statistical significance (P = 0.06).

Chemoradiotherapy plus surgery vs surgery alone

There are multiple MAs involving a comparison of surgery alone with pre-operative chemoradiotherapy[18-23]. These have evolved with the completed trials. The first of these to be published was by Urschel *et al*[18] in 2003 and involved more than 1000 patients from 9 randomized trials[18]. They concluded there was a benefit for pre-operative chemoradiotherapy in terms of survival at 3 years. The most widely quoted meta-analysis was published by Gebski *et al*[22] in 2007 combining results of 10 trials and more than 1200 patients. They found a significant benefit in terms of all-cause mortality for those patients receiving pre-operative chemoradiotherapy with a hazard ratio of 0.81 (95%CI: 0.70-0.93; P = 0.002). Three years later the same group updated their results with outcomes from 12 trials and 1854 patients[23]. The hazard ratio for all-cause mortality with patients receiving pre-operative chemoradiotherapy was 0.78 (95%CI: 0.70-0.88, P < 0.0001), indicating a more definite result in favor of neoadjuvant therapy.

Chemoradiotherapy plus surgery vs chemotherapy plus surgery

The meta-analysis by Sjoquist *et al*[23] also included a subgroup review of comparisons of pre-operative chemotherapy *vs* pre-operative chemoradiotherapy. Only 2 trials were able to be included. Both included only patients with AC and both were underpowered. The first one published by Stahl *et al*[24] included junctional tumours and a long extended course of neoadjuvant chemotherapy. The second by Burmeister *et al*[25] had a much shorter chemotherapy regimen and included only esophageal cancer patients. It did suggest that trimodality therapy did improve resectability rates. Neither trial showed a survival benefit for trimodality over bimodality therapy, both being concluded prematurely due to poor accrual. The hazard ratio for these 2 trials combined was 0.77 (95%CI: 0.53-1.12; P = 0.17) in favor of neoadjuvant chemoradiotherapy. This however is clearly not significant and more trials comparing these 2 approaches are required and are currently being conducted.

Individual patient data meta-analysis

In 2013 Ronellenfitsch *et al*[26] published a more detailed MA involving individual patient data (IPD). This is a more sophisticated form of MA in that it enables one to look at subgroups and secondary outcomes in more detail. Unfortunately this MA also did not include the CROSS trial, and some trial chairs refused to provide IPD. Not only did this MA confirm the survival benefit seen in patients receiving neoadjuvant chemotherapy and chemoradiotherapy, but it also detected an improved benefit for junctional tumours. Improved benefits were also noted for other subgroups, such as Eastern Co-operative Oncology Group performance status 0, male gender and age < 65 years. They were also able to look at disease-free survival in some trials which mirrored outcomes in overall survival, although not reaching statistical significance. They also found no difference in post-operative morbidity or mortality. They were however unable to further define the role of radiotherapy in the pre-operative management of esophageal cancer using the IPD that they managed to procure.

NEW RADIOTHERAPY TECHNOLOGIES

Radiotherapy technology has been rapidly evolving over the past decade with most tumors and sites now being able to be treated with highly conformal therapies including intensity modulated radiotherapy and volumetric modulated arc therapy. These new technologies have enabled the oncologist to deliver high doses of radiation with more precision to the tumor and at the same time spare surrounding tissues and organs which has dramatically reduced morbidity. In esophageal cancer the uptake of these modalities has been slow but in currently imbedded in most new protocols involving definitive treatment. It is only a matter of time until they are routinely used in the neoadjuvant setting where the big gain will be in offsetting radiation related surgical morbidity with high doses. It also may enable one to delay surgery which is currently conducted 4-8 wk post radiotherapy to 10-12 wk post radiotherapy. This in turn may make it possible to avoid surgery in some patients where a complete endoscopic and PET related response has been achieved. This concept of "surgery as needed" is increasingly being adopted at other sites where neoadjuvant chemoradiotherapy is used and has the potential to not only reduce health costs but improve quality of life for patients during their cancer journey.

DISCUSSION

One of the big issues around assessing the value of the local treatment modality is deciding on the magnitude of its benefit if there is indeed one. Local treatment modalities such as surgery and radiotherapy aim primarily to control tumors at the primary site and/or regional lymph nodes in order to reduce or eliminate the problems associated with uncontrolled tumor and the effects it may have function, cosmesis, and control of local symptoms. In the esophagus elimination of dysphagia is a principle aim of local treatment with surgery being able to do this effectively with all small tumors and radiotherapy with some small tumors especially SCC. Better control rates are theoretically obtained with these 2 modalities combined in more advanced tumors. Radiotherapy given in the pre-operative setting has the advantage of being able to downstage tumours and make resections easier with less chance of having involved margins.

However in order to have an impact on survival, comparisons of different local treatment modalities require large numbers of patients because improvements in local

control will only result in a survival benefit in a small proportion of those who experience a local control benefit. This means that most published randomized clinical trials comparing local modalities have not shown a survival benefit but rather one in terms of local control and improved resectability where surgery has been involved.

On the other hand trials that have added a systemic therapy such as chemotherapy to one arm of the trial could be expected to possibly confer a survival benefit. This is on the basis of many patients already having subclinical metastatic disease that a systemic agent may be able to control or delay converting to overt metastases. In addition the chemotherapy agents used in esophageal cancer have useful radio-sensitizing properties which may enhance the effects of radiotherapy and further improve local outcomes. It is however unknown whether an exceptionally good local outcome such as pCR at time of surgery is always associated with prolonged survival although many studies suggest that it does.

Many of the randomized trials involving chemotherapy have shown a survival benefit in their own right. Some such as the Walsh trial[12] have been interpreting as having a suboptimal result in the control arm whilst other more recent trials such as the CROSS trial clearly show a survival benefit for combined modality therapy and an improvement in local outcomes as well[16]. So in summary we still need to decide which patients require radiotherapy in addition to surgery plus platinum-based chemotherapy. The MAs and some trials suggest the all SCC patients may benefit. However the case in AC is far less clear, with possible more advanced tumors, those with ill defined resection margins on CT and those with nodal disease more likely to benefit in terms of local control. This hypothesis however is far from confirmed.

So what is clear right now is that although the most recent complete MA by Sjoquist *et al*[23] clearly demonstrates an improved survival advantage for neoadjuvant chemoradiotherapy when compared to neoadjuvant chemotherapy, this approach may not be required in all patients. More trials comparing these 2 modalities are being done but will take several years to analyze and may not have enough patients to give answer to all the endpoints apart from survival. Issues such as local control, respectability, toxicity and cost all are relevant in the current era. By doing more MAs involving IPD one has the potential to look at subgroups and secondary endpoints more rigorously and hopeful identify optimum therapies for patients.

CONCLUSION

At the time of writing this review there remains controversy on the role of radiotherapy as part of a combined package with chemotherapy as a neoadjuvant therapy for operable esophageal cancer. Completion of the ongoing clinical trials investigating this role and sharing of the data as part of further MAs will add to our knowledge base in the management of esophageal cancer.

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers with a grim overall 5-year survival rate of 5%. Advances in surgical techniques, critical care, molecular diagnosis, diagnostic imaging, endosonology and adjuvant therapy have improved outcomes; but still more needs to be achieved. There is an urgent need to discover new avenues that may impact survival. Radiofrequency ablation (RFA) has attracted attention as an adjunctive treatment in PDAC. A review of English literature in PubMed was done using the MESH terms for PDAC and RFA. All the articles were reviewed and core information was tabulated for reference. After a comprehensive review of all articles the data was evaluated to discover the role of RFA in PDAC management. Indications, contraindications, feasibility, success rate, safety, complications and impact on survival were reviewed and are discussed further. RFA appears to be an attractive option for non-metastatic locally advanced PDAC. RFA is feasible but has a significant morbidity. At the present time the integration of RFA into the management of pancreatic ductal adenocarcinoma is evolving. It should be considered as having a complimentary role to current standard therapy in the multimodal management care model. It is likely that indications and patient selection for pancreatic RFA will expand.

Key words: Pancreatic cancer; Radiofrequency ablation

Core tip: Radiofrequency ablation of pancreatic cancer is rapidly emerging as an attractive adjunct in locally advanced inoperable disease and is a part of modern multimodal hepatobiliary teams. Due to technological advances, refinements in thermokinetic principles and ongoing advances in medicinal oncology; it is likely that the role of radiofrequency in management of pancreatic cancer is going to increase in future. In this article we summarize the current evidence of application of radiofrequency ablation in pancreatic cancer.

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INTRODUCTION

Pancreatic ductal adenocarcinoma is the commonest form of pancreatic cancer and is characterized by delayed diagnosis, aggressive tumour biology and dismal survival. At presentation, only 10% of the tumours are potentially curable[1]. Currently, surgery is the only curative treatment which provides long-term survival benefit for patients with pancreatic cancer[2,3]. The median survival of untreated patients is 3-4 mo and less than 5% of patients are alive one year after diagnosis[4]. The 5 years survival rate after a combination of resection and adjuvant therapy does not exceed 30%. Patients with locally advanced and inoperable disease have limited options[5]. Stagnation in surgical and oncological advances has challenged the medical community to explore alternative avenues. While molecular and genetic advances may have a future impact, thermal ablative techniques are increasingly being explored since last decade.

RADIOFREQUENCY ABLATION OF PANCREAS

Principles

Radiofrequency ablation (RFA) is the commonest thermal ablative technique used to treat solid abdominal organ tumours. Apart from the thermal destructive effect of RFA, secondary anticancer immunity due to activation of tumour-specific T lymphocytes appears to play a role[6]. Increasing evidence suggests that RFA might stimulate anti-tumour immunity through an alternative pathway by inducing expression of heat shock protein 70[7].

The past

First animal application of pancreas RFA was done in 1999[8]. However, due to retroperitoneal location, distal bile duct traversing head of pancreas, proximity to major vascular structures and close relation to duodenum and stomach were the major hurdles which curtailed the widespread acceptance of RFA. The increased risk of thermal injury during RFA of pancreatic ductal adenocarcinoma also relates to its diffuse nature and vessel encasement[9]. Earlier reports of RFA of pancreatic adenocarcinoma quoted severe complications with unacceptable mortality[10]. Some serious complications of RFA of pancreas include gastro-intestinal haemorrhage, pancreatic fistula, biliary leak, portal vein thrombosis, pancreatic pseudocyst and sepsis[11,12].

The present

Thermokinetic principles: It was the systematic efforts of Manchester group that helped define and validate the thermokinetic principles[13]. Although the ideal temperature for optimal thermal ablation of the pancreatic adenocarcinoma has been validated in experimental model there is still lack of consensus on the optimal RFA parameters and standardization of operative technique[13]. In a porcine experiment, Fegrachi *et al*[14] has recommended a probe distance of 10 mm from duodenum and portomesenteric vessels along with continuous duodenal cooling with 100 mL/min saline at 5 ℃[14]. Using these settings in six animals, they did not encounter major morbidity and there was no mortality at two weeks. The same group has also demonstrated that duodenal cooling does not affect the ablation efficacy[15]. Performing concomitant biliary and gastric bypass procedures can reduce some complications[9]. RFA of the distal pancreas cancer may be performed without duodenal cooling as the bile duct and duodenum are some distance away. Figure 1 shows general principles underlying the application of RFA in pancreatic lesions.

Technical approaches: The pancreas can be accessed directly by an open laparotomy, endoscopically *via* transgastric or transduodenal approach and percutaneously by a posterior retroperitoneal approach. Endoscopic ultrasound guided RFA (EUS-RFA) appears attractive as it avoids surgery. In a study involving ten adult mini pigs, Kim *et al*[16] has demonstrated safety, feasibility and efficacy for pancreatic body and tail EUS-RFA. In a study involving five Yucatan pigs, Gaidhane *et al*[17] have demonstrated that EUS-RFA of pancreatic head was well tolerated with minimal pancreatitis. Pai *et al*[18] has reported EUS-RFA on eight patients with pancreatic cystic or neuroendocrine tumours with good results and acceptable safety profile. At the 2010 annual conference of International Hepatopancreaticobiliary Association, we presented a report of percutaneous RFA in a patient with local recurrence following a Whipple's operation for a lower bile duct cholangiocarcinoma[19]. We performed duodenal cooling *via* a nasogastric tube and splenomesenteric occlusion to reduce heat sink effect. This patient survived for nine months after RFA.

Multimodal cancer care: RFA is increasingly recognized as an attractive adjunct treatment modality in reducing tumour burden and compliments other adjuvant therapies with potential for improved palliation. Although the effectiveness of RFA have been estimated by reductions in carbohydrate antigen 19-9, improvement of abdominal/back pain and/or non-progression of tumour on repeat interval imaging, such end points are surrogate measurements only. The desired endpoint is ultimately improvement in survival. RFA has shown to improve survival in patients with locally advanced inoperable pancreatic cancer[20,21]. Concomitant octreotide, antiproteases and chemotherapy (systemic or transarterial liver directed) or local

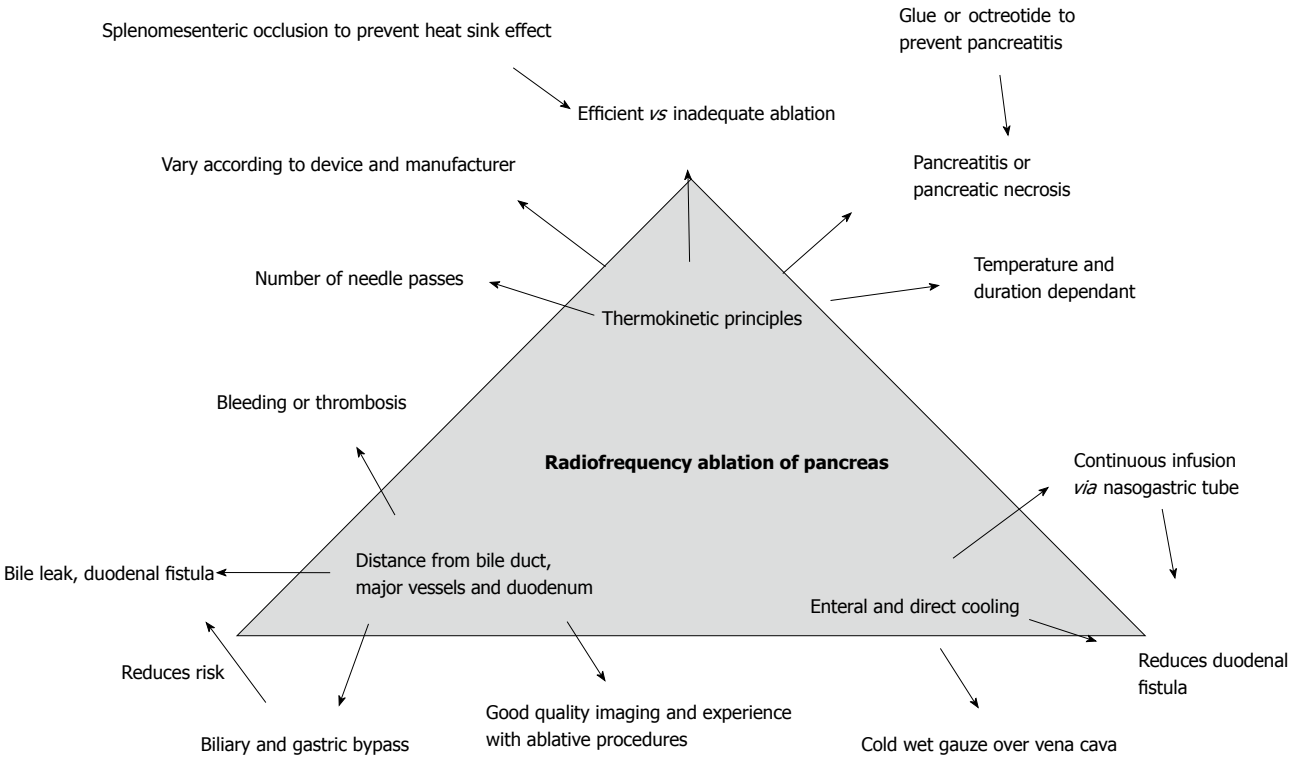

Figure 1 Principles of pancreatic radiofrequency ablation.

Table 1 Case series on radiofrequency ablation of pancreatic ductal adenocarcinoma-themokinetic principles

Ref.	*n*	Age (yr)	Tumour size (cm)	Thermokinetics
Matsui *et al*[24]	20	59	5.3	15 min at 50 ℃ in 2 × 2 × 2 cc field
Date *et al*[25]	1	58	3	RITA probe, 90 ℃ for 10 min each
Hadjicostas *et al*[26]	4	70	8.5 (3-12)	Cooltip© RFA for shorter duration of 2-8 min with 17-gauge electrode
Varshney *et al*[27]	3	58	6.5	4200 W of energy was delivered using a saline perfused needle with the aim of producing a 3 cm diameter necrosis
Wu *et al*[28]	16	67	5[1]	Cooltip© RFA probe with up to 200 W energy, 12 min and tip temperature < 30 ℃. A 5 mm safe distance between probe and major vessel
Spiliotis *et al*[20]	12	67	3.5	Cooltip© 17-gauge RFA electrode which achieved 80-90 ℃. Cooltip© at < 10 min each
Casadei *et al*[29]	3	66	4.7	Cooltip© ablation at 90 ℃ for 5 min each
Girelli *et al*[11]	50	65	4	RITA system was used. Initial temperature of 105 ℃ (first 25 patients) was reduced to 90 ℃ after interim review
Zou[3] *et al*[30]	32	68	4-12[2]	17 gauge electrode at 100-150 W energy with tip temperature of 90-100 ℃ for 12 min each After RFA, ^{125}Iodine seed was implanted
Ikuta *et al*[31]	1	60	4	Cooltip© 17-gauge RFA electrode for 3-4 min each and a temperature of 99 ℃

[1]82% tumors were > 5 cm; [2]Mean/median size not mentioned; [3]Simultaneous ^{125}I seed implantation. RFA: Radiofrequency ablation.

application of radioactive seeds could also modify the clinical response. It is evident from the current reports that RFA should not be done in an obviously resectable pancreatic cancer or a metastatic disease. While RFA of pancreas cancer may not be worthwhile in this clinical context, RFA of liver metastases from pancreatic cancer have been attempted in the setting of multimodal approach. Park *et al*[22] have reported a retrospective review of RFA ablation for liver metastases from pancreatic ductal adenocarcinoma. They performed RFA on 34 patients over a period of seven years including patients with less than six liver lesions and size ⩽ 3 cm and excluding patients with extrahepatic metastatic disease. Median survival time was 14 mo. Patients with oligometastatic disease showed improved survival after RFA compared to patients without liver metastases and no treatment. Huang *et al*[23] reported a median survival of 11 mo with transarterial chemoembolization plus RFA and/or ^{125}I radioactive seed implantation on unresectable pancreatic cancer in a series of 71 patients. In this study the one-year survival was 32.4% for all patients and 25.5% for patients with liver metastases. Multiple case series of RFA application have been published and they generally testify its safety and feasibility. Table 1 provides details of

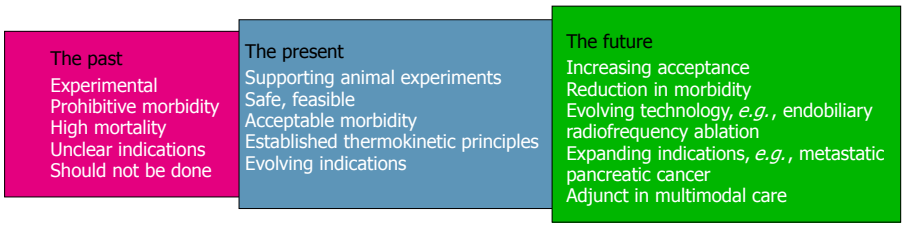

Figure 2 The past, the present and the future of pancreatic radiofrequency ablation.

Table 2 Case series on radiofrequency ablation of pancreatic ductal adenocarcinoma-outcomes and comments

Ref.	Survival	Morbidity and mortality	Comments
Matsui *et al*[24]	3 mo (median)	Morbidity (10%)-septic shock and gastrointestinal bleeding Mortality (5%)-patient with septic shock	All patients had a laparotomy
Date *et al*[25]	3 mo (overall)	Patient developed polyuria. No major complication	Single patient
Hadjicostas *et al*[26]	7 mo (median)	No major complications occurred	Sandostatin was administered prophylactically. Palliative bypass procedures were performed. One patient had significant pain relief
Varshney *et al*[27]	7 mo (mean)	Self-limiting complications occurred in two patients	One patient had percutaneous CT guided RFA. All patients had endobiliary stenting All patients received 7 d of antibiotics
Later this group has updated their results in 10 patients with 10% morbidity and no mortality. Eight patients received post RFA chemotherapy. One patient developed a 2 cm pseudocyst. Overall survival range was 9-36 mo[32]			
Wu *et al*[28]	Not reported	Pancreatic fistula 18.8% (3/16). Overall morbidity 43%. Mortality 25% Massive and mortal gastrointestinal bleeding occurred in 3 patients	Initially performed only for body and tail lesions. Later expanded for head of pancreas lesions, but had 50% mortality in this group 50% patients had relief of back pain 5 patients had liver metastases 5 mm distance to portal vein may not be safe
Spiliotis *et al*[20]	33 mo (mean)	Overall morbidity 25% and nil mortality	Mean survival without RFA was 13 mo RFA in parallel to palliative therapy provided survival benefit for patients with unresectable pancreatic cancer
Casadei *et al*[29]	4 mo (mean)	3 patients developed ascites 1 patient developed biliary fistula	Prospective study. Included 3 patients Complete necrosis achieved in all patients All patients had a laparotomy and double bypass. Study was stopped at interim analysis
Girelli *et al*[11]	Not reported	Abdominal complications occurred in 24%. 30 d mortality 2%. Three patients with surgery related complicated required reoperation	Prospective study RFA was the only treatment in 19 patients All patients received antibiotics, octreotide and gabexate mesilate. Reduction of RFA temperature from 105 ℃ to 900 ℃ resulted in significant reduction in complications
Later this group has updated their experience of 107 patients (Cantore *et al*[21]). They performed a group wise comparison between upfront RFA *vs* RFA following primary therapy and concluded that RFA following primary treatment improves survival (14.7 mo *vs* 25.6 mo)			
Zou[1] *et al*[30]	17.6 mo (mean)	Three patients experienced complications, but no mortality	Somatostatin analogues were used post-operatively The overall 12 mo survival was 65.6%
Ikuta *et al*[31]	Alive at 18 mo	No complications	Laparotomy with bypass procedure followed by chemoradiotherapy to induce pancreatic fibrosis. This was followed by second laparotomy and RFA

[1]Simultaneous ^{125}I seed implantation. CT: Computerized tomography; RFA: Radiofrequency ablation.

thermokinetic principles applied by various authors and Table 2 summarizes outcomes with reference to survival and morbidity/mortality. RFA appears to have a role in treating locally advanced disease; however heterogeneity in the current reports makes it difficult to draw any robust recommendation about RFA applicability. RFA is being explored for improved palliation in malignant obstructive jaundice. Endobiliary RFA along with self-expanding metal stents is reported to be safe, feasible and associated with improved stent patency rates in patients with malignant biliary obstruction[33]. In the first *in vivo* study involving 22 patients with locally advanced pancreatic cancer, Arcidiacono *et al*[34] demonstrated feasibility and safety of endoscopic ultrasound guided cryothermal ablation with technical success in 16 patients (72.8%) and median post-ablation survival of 6 mo. They described late complications of jaundice, duodenal stricture and cystic fluid collection in four patients. Keane *et al*[35]

conducted a systematic review on novel ablative methods in locally advanced pancreatic cancer and concluded that despite proven safety, feasibility and reproducibility; the benefit of ablative techniques on long term survival remains to be confirmed in large prospective randomized studies. Figure 2 shows the past, the present and the future of RFA application in pancreatic cancer.

The future

At the present time the integration of RFA into the management of pancreatic ductal adenocarcinoma is evolving. It should be considered as having a complimentary role to current standard therapy in the multimodal management care model. It is likely that indications and patient selection for pancreatic RFA will expand.

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Contents

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CASE REPORT

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Contents

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CASE REPORT

Gastric metastasis from primary lung adenocarcinoma mimicking primary gastric cancer

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Abstract

Gastric metastases from lung adenocarcinoma are rare. Because gastric metastasis grossly resembles advanced gastric cancer, it is difficult to diagnose gastric metastasis especially when the histology of the primary lung cancer is adenocarcinoma. We describe a case of gastric metastasis from primary lung adenocarcinoma mimicking Borrmann type Ⅳ primary gastric cancer. A 68-year-old man with known lung adenocarcinoma with multiple bone metastases had been experiencing progressive epigastric pain and dyspepsia over one year. Esophagogastroduodenoscopy revealed linitis plastica-like lesions in the fundus of the stomach. Pathologic examination revealed a moderately differentiated adenocarcinoma with submucosal infiltration. Positive immunohistochemical staining for thyroid transcription factor-1 (TTF-1) and napsin A (Nap-A) confirmed that the metastasis was pulmonary in origin. The patient had been treated with palliative chemotherapy for the lung cancer and had lived for over fifteen months after the diagnosis of gastric metastasis. Clinicians should be aware of the possibility of gastric metastasis in patients with primary lung adenocarcinoma, and additional immunohistochemical staining for Nap-A as well as TTF-1 may help in differentiating its origin.

Key words: Adenocarcinoma of lung; Napsin-A; Thyroid transcription factor-1; Gastric metastasis

Core tip: This report describes the rare case of a 68-year-old patient with gastric metastasis from primary lung adenocarcinoma mimicking Borrmann type Ⅳ primary gastric cancer. When gastric carcinoma is suspected in patients with primary lung adenocarcinoma, a differential diagnosis of primary gastric cancer and gastric metastasis can be done through special immunohistochemical staining with napsin-A and thyroid transcription factor-1,

especially when the biopsy results are ambiguous by histology alone.

Kim MJ, Hong JH, Park ES, Byun JH. Gastric metastasis from primary lung adenocarcinoma mimicking primary gastric cancer. *World J Gastrointest Oncol* 2015; 7(3): 12-16 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i3/12.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i3.12

INTRODUCTION

Gastric metastasis from primary lung cancer is rare. Although most gastric metastases are asymptomatic and not detected during the lifetime of the patients, when overlooked or misdiagnosed, fatal complications such as acute bleeding and perforation can occur. Here, we report a case of gastric metastasis from primary lung adenocarcinoma mimicking Borrmann type IV primary gastric cancer grossly and diagnosed through special immunohistochemical staining of tissue for thyroid transcription factor-1 (TTF-1) and napsin A (Nap-A). This report was approved by our institutional review board, and the approval number is OC14RISI0071.

CASE REPORT

A 68-year-old man with known lung cancer was referred to our hospital in March 2013 with progressive epigastric pain and dyspepsia for one year. He was a lifetime non-smoker, had been diagnosed with adenocarcinoma *in situ* (AIS), and had undergone left lower lobe lobectomy in 1996 at a different hospital. In June 2004, he developed a first recurrence in the lung and underwent posterior segmentectomy of the right upper lobe. In March 2007, a second recurrence was found in the left upper lobe, pleura, and diaphragm and he underwent left upper lobe wedge resection and four cycles of paclitaxel/carboplatin chemotherapy. After eighteen months, surveillance chest computed tomography (CT) showed multiple lung-to-lung metastases, and left third and fourth rib metastases. Because mutations of the epidermal growth factor receptor (*EGFR*) gene were not found in the tumor tissue, he received fifty-five cycles of palliative pemetrexed chemotherapy between March 2009 and February 2013, and had stable disease for approximately four years.

The patient had progressive epigastric pain and dyspepsia since 2012. On March 18, 2013, esophago-gastroduo-denoscopy (EGD) revealed a 4 cm × 5 cm submucosal tumor-like lesion containing central ulceration with fusion and thickened mucosal folds in the stomach fundus (Figure 1). The stomach had insufficient expansion with aeration, compatible with Borrmann type Ⅳ gastric cancer. Laboratory findings revealed mild anemia (hemoglobin level 106 g/L and hematocrit 32.8%), normal lactate dehydrogenase levels, and elevated carcinoembryonic antigen (9.67 μg/L). Abdominal CT showed irregular gastric wall thickening in the fundus associated with perigastric infiltration and diffuse nodular infiltration in the omentum and several enlarged lymph nodes in the perigastric space, suspicious of primary gastric cancer and peritoneal dissemination. Pathologic examination revealed a moderately differentiated adenocarcinoma with submucosal infiltration and presence of endolymphatic emboli (Figure 2A). Especially, the surface epithelium had no precancerous or cancerous lesions, suggesting that this lesion was metastatic or primary gastric cancer of Borrmann type Ⅳ. Positive immunohistochemical staining for TTF-1 and Nap-A confirmed that this lesion had metastasized from the lung (Figure 2B). Finally, the patient was diagnosed with known primary lung adenocarcinoma with gastric and intraperitoneal metastases.

Irinotecan/cisplatin combination regimen as second-line palliative chemotherapy for lung adenocarcinoma was initiated on March 26, 2013 and maintained for eight months. The tumor lesion remained stable for eleven cycles of chemotherapy but was discontinued on November 29, 2013 after the patient had uncontrolled diarrhea and decreasing performance status. Five months later, a surveillance chest CT showed stable lesion, but abdominal CT revealed a new hepatic nodule in segment 8. Third-line gemcitabine monotherapy was started on May 15, 2014 and to date, has been well tolerated by the patient.

DISCUSSION

Gastric metastasis from primary lung cancer is rare. A review of autopsies of 1010 cancer patients found only seventeen patients with gastric metastasis (1.7%)[1]. Breast cancer, lung cancer, esophageal cancer, and skin melanoma are the most frequent primary sites[2]. Lung cancer metastasis to the gastrointestinal (GI) tract is rare (0.5%-10%)[3], and most commonly occurs in the small bowel[4]. In one study, among eighteen patients with lung cancer and GI metastasis, nine had small bowel metastasis, four had gastric metastasis, two had colon metastasis, and one had duodenal metastasis[3]. An analysis of 473 autopsies of patients with primary lung cancer showed 3.4% with gastric metastases[5]. The prevalence of histologic types of lung cancer that metastasize to the stomach is not well known. Hasegawa *et al*[5] reported in 1993 that large-cell lung cancer was the most common histology, accounting for 15.6% of primary lung cancer with gastric metastases. However, recent reports have shown that pulmonary adenocarcinoma was the most frequently reported histologic type of metastasis to the gastric wall[4,6-10].

Because hematogenous metastases usually implant in the gastric submucosa[1], diagnosis can only be made after considerable growth. Therefore, most gastric

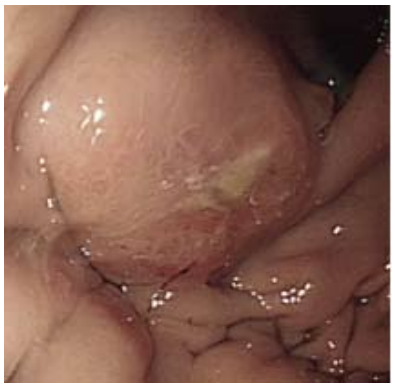

Figure 1 Esophagogastroduodenoscopy shows a Bormann type Ⅳ gastric mucosal lesion and loss of distension of the gastric wall.

metastases from primary lung cancer are asymptomatic and often discovered during autopsy[9]. Among 473 autopsies of primary lung cancer patients, only two of sixteen gastric metastasis cases had been detected clinically in living patients[5]. The most common clinical manifestations of symptomatic patients are nonspecific epigastric pain and chronic bleeding resulting in melena and anemia[1,11]. Perforation and acute bleeding have been reported in some fatal cases[4,10,12-14].

Endoscopic evaluation of 54 patients with gastric metastasis from solid malignant tumors has revealed that gastric metastases mimic submucosal tumors in 28 patients (52%) and primary gastric cancers in 21 patients (39%)[2]. When gastric metastasis mimics primary gastric cancer, it resembles advanced primary gastric cancer rather than early gastric cancer, presenting as bull's eye signs, volcano-like ulcers, or surface umbilication[6-9,11,15]. An infiltrating "linitis plastica" pattern has been seen in only 2% of cases in lung cancer[2,8], while it has been seen in about 50% of gastric metastases from breast carcinoma[1,15].

In the present case, the patient had perigastric lymph node involvement, omental seeding, and progressive liver metastasis, in addition to Bormann type Ⅳ-like advanced gastric cancer lesion in the gastric wall. Kim *et al*[13] reported patients with squamous cell lung cancer with gastric wall metastasis, perigastric lymph node metastasis, and splenic invasion who received total gastrectomy and splenectomy for control of bleeding[13]. However, most gastric metastasis from primary lung cancer manifests as a solitary gastric metastasis[6-9,11]. With the exception of gastric wall metastases, these accompanying intra-abdominal metastases have only been reported in rare and unusual gastric metastasis cases.

TTF-1 regulates gene expression in the thyroid, lung, and diencephalon during embryogenesis[16]. TTF-1 has appeared to be helpful in distinguishing tissues of pulmonary origin from those of others in circumstances for which there is currently no lung-specific tumor marker[17,18]. However, there are some prior reports indicating that 13% to 45% of

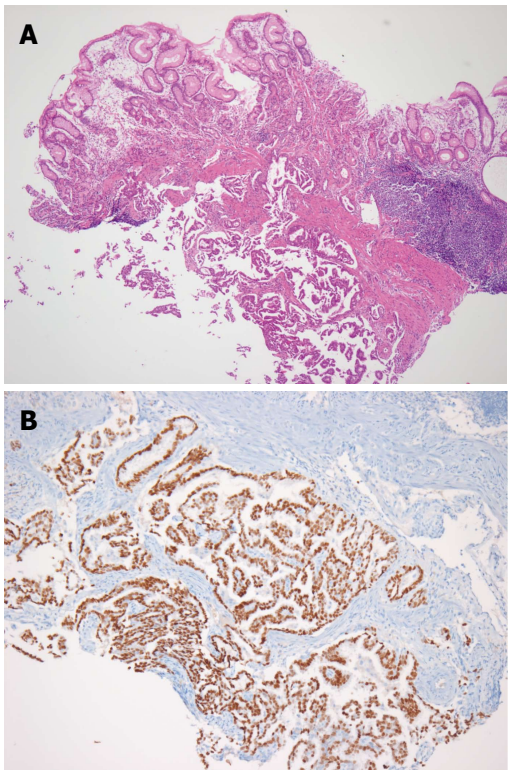

Figure 2 Hematoxylin-and-eosin (× 40) staining of the gastric lesion shows adenocarcinoma cells infiltrating the gastric submucosa (A) and thyroid transcription factor-1 positive staining in the cancerous gastric lesion (× 100) (B).

metastatic adenocarcinomas of pulmonary origin are TTF-1 negative, thereby limiting the sensitivity of this marker. Another marker, Nap-A, is a functional aspartic proteinase expressed in the cytoplasm of healthy lung parenchyma[16] that consists of a 38 kDa protein expressed in type II pneumocytes, alveolar macrophages, renal tubules, and exocrine glands and ducts in the pancreas. Data from tissue microarrays constructed from primary lung cancers indicate that the sensitivity of Nap-A for primary lung adenocarcinoma is similar to that of TTF-1[17]. According to recent reports, Nap-A is more sensitive than TTF-1 in distinguishing primary lung carcinoma from other adenocarcinomas[16,18-20], making it a useful additional immunohistochemical staining to TTF-1 for determining the origin of metastatic adenocarcinomas. In our present case, in addition to the EGD findings, clinical manifestation such as liver metastasis mimicked primary gastric cancer, causing us to question whether it was real gastric metastasis or not. However, immunohistochemical positivity for TTF-1 and Nap-A confirmed the diagnosis of gastric metastasis from primary lung adenocarcinoma.

Optimal management of symptomatic gastric metastasis from primary lung cancer remains controversial because gastrointestinal involvement is considered to represent an advanced stage. Lee *et al*[21] reported longer survival in patients with gastric and/or duodenal metastases that were managed by supportive treatment without surgery. However, surgery is still necessary to

prevent life-threatening complication such as massive hemorrhage, obstruction and perforation thus providing effective palliation and reasonable survival in patients with only a solitary gastric metastasis[21,22]. In the present case, surgical intervention was not performed since the patient had other extrathoracic metastases outside the stomach and the symptoms were not very severe and well controlled by medical treatment.

Because gastric metastasis is a late-stage disease, in many cases, the patient's performance status is poor owing to the high burden of the primary malignancy itself and related complications. Therefore, the prognosis of gastric metastasis from primary lung cancer is very poor. However, our patient has had stable disease with third-line chemotherapy and has lived for over five years after the initial diagnosis of lung cancer and for fifteen months after the diagnosis of gastric metastasis. A possible explanation for this extraordinarily good clinical outcome in our patient, even though his mutation status of EGFR was wild-type, could be the initial pathologic diagnosis of AIS, formerly known as bronchioloalveolar carcinoma. This is a non-mucinous or mucinous type adenocarcinoma composed of tumor cells replacing the alveolar wall without stromal invasion[23], and it is associated with better survival than other invasive adenocarcinomas[24]. Adenocarcinoma in situ and minimally invasive adenocarcinoma are known to have near 100% 5-year survival rates when completely resected[25].

In conclusion, clinicians should be aware of this rare situation of gastric metastasis from primary lung cancer. When gastric carcinoma is suspected in patients with primary lung adenocarcinoma, the differential diagnosis of primary gastric cancer from gastric metastasis should be done through special immunohistochemical staining with Nap-A and TTF-1, especially when the biopsy results are ambiguous by histology alone.

COMMENTS

Case characteristics

A 68-year-old man with known lung adenocarcinoma presented with epigastric pain and dyspepsia.

Differential diagnosis

Malignant tumors (primary or metastatic).

Laboratory diagnosis

Mild anemia (hemoglobin 10.6 gm/dL and hematocrit 32.8%), normal lactate dehydrogenase levels, and elevated carcinoembryonic antigen (9.67 μg/L).

Imaging diagnosis

Esophagogastroduodenoscopy revealed linitis plastica-like lesions in the fundus of the stomach and abdominal computed tomography scan showed diffuse nodular infiltration in the omentum, suspicious of primary gastric cancer and peritoneal dissemination.

Pathological diagnosis

Endoscopy and biopsy revealed an adenocarcinoma with submucosal infiltration and thyroid transcription factor-1 (TTF-1)/napsin A (Nap-A) positive confirmed that the gastric metastasis from pulmonary origin.

Treatment

The patient was treated with palliative chemotherapy for the lung cancer (Irinotecan/cisplatin combination regimen).

Related reports

Gastric metastases from lung adenocarcinoma are rare and difficult to diagnose especially when the histology of the primary lung cancer is adenocarcinoma because it grossly resembles advanced gastric cancer.

Term explanation

Nap-A is a functional aspartic proteinase expressed in the cytoplasm of healthy lung parenchyma, more sensitive than TTF-1 in distinguishing primary lung carcinoma from other adenocarcinomas.

Experiences and lessons

Clinicians should be aware of the possibility of gastric metastasis in patients with primary lung adenocarcinoma, and additional immunohistochemical staining for Nap-A as well as TTF-1 may help in differentiating its origin.

Peer-review

This is a nicely written and interesting case-report.

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REVIEW

Cancer cachexia, mechanism and treatment

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Abstract

It is estimated that half of all patients with cancer eventually develop a syndrome of cachexia, with anorexia and a progressive loss of adipose tissue and skeletal muscle mass. Cancer cachexia is characterized by systemic inflammation, negative protein and energy balance, and an involuntary loss of lean body mass. It is an insidious syndrome that not only has a dramatic impact on patient quality of life, but also is associated with poor responses to chemotherapy and decreased survival. Cachexia is still largely an underestimated and untreated condition, despite the fact that multiple mechanisms are reported to be involved in its development, with a number of cytokines postulated to play a role in the etiology of the persistent catabolic state. Existing therapies for cachexia, including orexigenic appetite stimulants, focus on palliation of symptoms and reduction of the distress of patients and families rather than prolongation of life. Recent therapies for the cachectic syndrome involve a multidisciplinary approach. Combination therapy with diet modification and/or exercise has been added to novel pharmaceutical agents, such as Megestrol acetate, medroxyprogesterone, ghrelin, omega-3-fatty acid among others. These agents are reported to have improved survival rates as well as quality of life. In this review, we will discuss the emerging understanding of the mechanisms of cancer cachexia, the current treatment options including multidisciplinary combination therapies, as well an update on new and ongoing clinical trials.

Key words: Physical exercise; Pharmacological treatment; Cancer cachexia

Core tip: This review aims to present the clinical presentation, the mechanisms, and current treatment options, such as pharmacological treatment and physical exercise for cancer cachexia.

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INTRODUCTION

Although there is no single universally agreed upon definition of cachexia, a recent consensus statement

states that cachexia is a complex metabolic syndrome associated with underlying illness, and is characterized by the loss of muscle with or without loss of fat mass. Cachexia is seen in many medical conditions, including cancer, acquired immunodeficiency syndrome (AIDS), chronic obstructive pulmonary disease, multiple sclerosis, chronic heart failure, tuberculosis, familial amyloid polyneuropathy, mercury poisoning (acrodynia) and hormonal deficiency[1,2]. Cancer cachexia is characterized by systemic inflammation, negative protein and energy balance, and an involuntary loss of lean body mass, with or without wasting of adipose tissue[3]. Clinically, cachexia is represented by significant weight loss in adults and failure to thrive in children[4], accompanied by alterations in body composition and a disturbed balance of biological systems[5-7]. Whilst the loss of skeletal muscle mass is the most obvious symptom of cancer cachexia, cardiac muscle is also depleted, though muscle of other visceral organs tend to be preserved. Though cachexia is seen in several disease states, the loss of muscle mass has been shown to occur most rapidly in cancer patients[8].

Cancer cachexia is an insidious syndrome that not only has a dramatic impact on patient quality of life, but is also associated with poor responses to chemotherapy and survival[9-11]. Indeed, cachexia occurs in the majority of terminal cancer patients and, according to Warren, is responsible for the death of 22% of cancer patients[12,13].

Current therapies focus on palliation of symptoms and the reduction of distress of patients and families rather than cure[14]. In many cases, cachexia remains a largely underestimated and untreated condition[4,15]. Approximately half of all patients with cancer experience cachexia[16,17], with the prevalence rising as high as 86% in the last 1-2 wk of life[18,19], and with 45% of patients losing more than 10% of their original body weight over the course of their disease progression[19]. Death usually occurs when there is 30% weight loss[5]. The best management strategy of cancer cachexia is to treat the underlying cancer as this will completely reverse the cachexia syndrome. Unfortunately, this remains an infrequent achievement with advanced cancers. A second option could be to counteract weight loss by increasing nutritional intake, but since in the majority of cachectic patients anorexia is only a part of the problem, nutrition as a unimodal therapy has not been able to completely reverse the wasting associated with cachexia.

In this review, we discuss the presentation, mechanisms, and current treatment options for cancer cachexia, including diet and exercise therapy to improve quality of life as well as prognosis for affected patients.

CANCER CACHEXIA AND MALIGNANT INFLAMMATION

Multiple mechanisms are involved in the development of cachexia, including anorexia, decreased physical activity, decreased secretion of host anabolic hormones, and an altered host metabolic response with abnormalities in protein, lipid, and carbohydrate metabolism[20]. Due to the complex clinical findings, guidelines for the diagnosis of cachexia have just recently started to appear[3]. Even so, there is great variation in definitions, which presents problems when comparing studies and informing clinical diagnoses[21,22] (Table 1).

One proposed mechanism of cancer cachexia is that it is an integrated physiological response of substrate mobilization driven by inflammation[23]. There is an increase in pro-inflammatory cytokine activity during cancer progression[24,25], and systemic inflammation is a hallmark of cancer cachexia, indicated by the production of acute-phase response (APR) proteins such as C-reactive protein (CRP) and fibrinogen[26,27]. CRP is considered to be an accurate measure of the pro-inflammatory cytokine activity[28] that has been implicated in muscle wasting[29]. The APR is related to the inflammation and weight loss seen in cachexia[30,31] and the reduced quality of life and shortened survival of cachexia patients[10,32-35]. These phenomena increase muscle catabolism and transfer amino acids from muscle anabolism toward the amino acid pool required for APR protein anabolism[36,37]. It has been suggested that eicosanoids also mediate inflammation in cancer cachexia[38-40].

There is considerable evidence that signaling through cytokines and myostatin/activin pathways has a role in cancer cachexia and anorexia[41-43] (Figure 1). Numerous cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), IL-6, and interferon-gamma (IFN-γ), have been postulated to play a role in the etiology of cancer cachexia[44-52]. The cytokines are transported across the blood-brain barrier where they interact with the luminal surface of brain endothelial cells causing release of substances that affect appetite[53]. Receptors of TNF-α and IL-1 are found in the hypothalamic areas of the brain, which regulate food intake. Anorexia induced by both TNF-α and IL-6 can be blocked by inhibitors of cyclooxygenase, suggesting that a prostaglandin, such as PGE2, may be the direct mediator of appetite suppression[54].

The role of TNF-α in mediating cancer cachexia is supported by evidence that intraperitoneal injection of a soluble recombinant human TNF-receptor antagonist improved food intake and weight gain in tumor-bearing rats[55]. TNF-α increases gluconeogenesis, lipolysis and proteolysis, decreases the synthesis of proteins, lipids and glycogen, induces the formation of IL-1[17], and stimulates the expression of Uncoupling proteins (UCP) 2 and UCP3 in cachectic skeletal muscle[8]. Despite the fact that TNF-α induces the symptoms of cachexia, its inhibition has not been shown to stop or to reverse cancer cachexia[49]. This indicates that though TNF-α may be involved in the development of cachexia, it is not solely responsible for the effects seen in cachectic

Table 1 Cancer cachexia

Treatment	Description	Physiologic benefit	Possible mechanism	Ref.
Megestrol acetate	Active progesterone derivative	Improves appetite, caloric intake, nutritional status, quality of life	Unknown; possible neuropeptide Y release	[80-92]
Medroxyprogesterone	Active progesterone derivative	Improves appetite, food intake Weight stabilization	Decreases serotonin, IL-1, IL-6, TNF-α	[93-96]
Ghrelin	Gastric peptide hormone	Improves lean + total body mass, hand grip, cardiac function (CHF cachexia only)	Growth hormone receptor secretagogue	[105]
Delta-9-tetrahydrocannabinol	Cannabinoid	MIXED May improve food intake, weight gain	Possible endorphin receptor activation, Inhibition of prostaglandin, IL-1	[85,106-110]
Melanocortin antagonists	Adrenocorticotropic hormone antagonist	UNTESTED; prevention of anorexia, loss of lean body mass or basal energy (animal only)	Neuropeptide Y alteration or melanocortin-4 receptor antagonism	[112,113]
Thalidomide	Immunomodulatory	Limits weight and lean body mass loss	Decreases TNF-α, pro-inflammatory cytokines, nuclear factor kappa B, cyclooxygenase 2, angiogenesis	[124-126]
Etanercept	Immunomodulatory	Limits fatigue; improves adjuvant therapy adherence	Decreases TNF effect	[127]
Eicosapentaenoic acid/ omega-3-fatty acids	Lipid	MIXED; may improve weight, appetite, quality of life	Decreases pro-inflammatory cytokines, proteolysis inducing factor	[129,130,133,137,140-142,146-152]
Rikkun-shito	Herbal Japanese medicine	Improves median survival with gemcitabine (pancreatic cancer); improves anorexia, GI dysmotility, muscle wasting, anxiety	Unknown	[154,155]
Corticosteroids	Immunomodulatory	Improves appetite and quality of life	Various mechanisms	[156,157]
Formoterol	β2-adrenergic agonist	UNTESTED	Protein and muscle degradation antagonism	[170]
Erythropoetin	Glycoprotein hormone	Improves patient's metabolic and exercise capacity	Decreases production of IL-6	[171-173]
ACE inhibitors	Heart medications	Reduce wasting of muscle mass	Inhibit TNF-α production	[174]
β-blockers	Heart medications	Preserved body weight, and lean and fat mass, and improved the quality of life	Normalized Akt phosphorylation	[175]

IL: Interleukin; TNF: Tumor necrosis factor.

patients.

IL-1 concentrations increase in the cachectic state and have been known to cause similar effects to TNF-α[56]. IL-1 induces anorexia in cachectic patients as it causes an increase in plasma concentrations of tryptophan, which in turn increases serotonin levels, causing early satiety and suppressing hunger[57]. Increased tryptophan leading to associated increased serotonin production from the hypothalamus has been linked to anorexia[57,58]. A conflicting study showed that IL-1 did not affect food intake or weight loss, suggesting that IL-1 has a local effect on a particular tissue or the exogenous doses of IL-1 must be larger in order to see characteristics of cachectic state[59].

IL-6 is an important mediator in the defense mechanism of humans through its regulation of immune responses[60]. Concentration levels of IL-6 increase transferrin in cancer patients[28]. Levels of IL-6 were observed to be higher in patients with cachexia than weight-stable patients. Although IL-6 may have an important role in the development of cachexia, it is not considered to be solely responsible, working through indirect action, indicated by the failure of IL-6 administration to reproduce cachexia in animal model[17]. As such, it is likely that a complex interplay of these factors is responsible for cachexia, rather than each working in isolation[61]. However, since there is limited variation in levels of circulating cytokines[62], and circulating cytokines are produced by isolated peripheral mononuclear cells, it is speculated that local production in affected tissues is more important and relevant to cachexia than systemic circulation of these factors[63].

Signal transducers and activators of transcription 3 (STAT3) is a member of the STAT family of proteins. STAT3 function as essential signal transducing effector proteins of cytokine-induced pathways that control the development, proliferation, differentiation, homeostasis of many cell types[64]. STAT3 activation is a common feature of muscle wasting. STAT3 is activated in muscle by IL-6 and by different types of cancer and sterile sepsis[65]. It is not certain whether the cytokine production is primarily from tumor or host inflammatory cells. It has been hypothesized that either tumor cell production of pro-inflammatory cytokines or the host inflammatory cell response to tumor cells is the source of the APR proteins seen in many malignancies and in cachexia[66].

CATABOLISM

A number of factors in cancer patients are known to

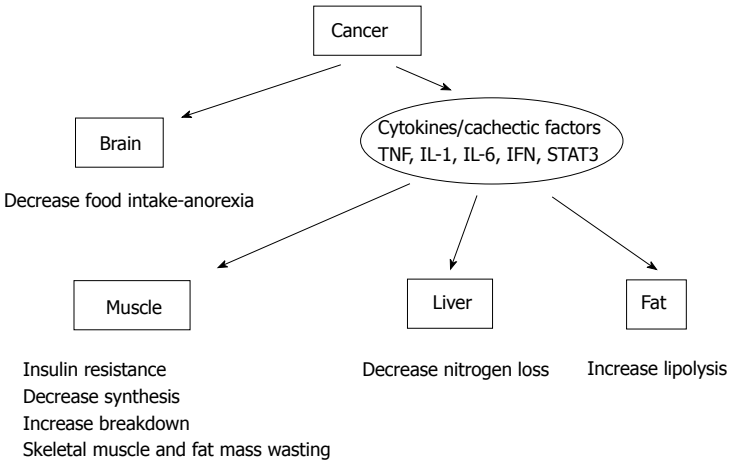

Figure 1 Role of tumor-induced systemic inflammation with metabolic pathways in organs affected by cancer cachexia. IL: Interleukin; TNF: Tumor necrosis factor; IFN: Interferon; STAT3: Signal transducers and activators of transcription 3.

increase the catabolic response, leading to unsustainable levels of fat and muscle mobilization and levels of muscle depletion that cause significant morbidity and mortality.

The metabolic changes found in cachexia resemble those of infection rather than starvation and are multifactorial and complex[67]. Although the weight loss brought on by starvation is mainly from adipose tissue stores, the weight loss of cancer cachexia is caused by loss of both skeletal muscle and adipose tissue mass[68]. In patients with cachexia there is an increase in muscle protein catabolism leading to a net loss of muscle mass. This imbalance of protein synthesis and degradation is one of the most obvious aspects of metabolism disruption in cancer cachexia. It has been widely observed that the rate of muscle protein catabolism increases in cachexia, whilst anabolism of new proteins decreases, resulting in net protein breakdown[8,69-71].

Increased energy expenditure may also contribute to the wasting process. Resting energy expenditure (REE) is increased in the cachectic state, with futile metabolic cycling accounting for much of this increase[72]. About 70% of the total energy expenditure in sedentary people arises from the REE[1]. The REE in cancer patients is strongly determined by the type of tumor. For example, patients with pancreatic and lung cancer had increased REE compared with healthy subjects[73,74]. Patients with gastric and colorectal cancer were reported to have no elevation of REE[73], though it seems that these results reflect how close the patients were to death at the time of measurement. In malnourished patients near death there is an increase in REE and in protein catabolism which could relate to the utilization of the last skeletal muscle mass[75].

Although skeletal muscle is the most important site for thermogenesis in the adult human, brown adipose tissue (BAT) is also known to have an important role in cachexia. Non-shivering thermogenesis takes place in BAT, and in a single study using autopsy samples of peri-adrenal tissue examined by light microscopy, BAT was observed in 20 of the cachectic cancer patients (80%) compared to 2 of the age-matched subjects (13%)[76].

UCPs, related to the regulation of mitochondrial proton gradients and the production of reactive oxygen species in skeletal muscle and adipose tissue, may also play a role in the increased REE observed in cachexia[8]. There are three UCPs: UCP1 found only in BAT, UCP2 found in most tissues, and UCP3 found only in BAT and skeletal muscle[77]. In particular, the expression of UCP2 and UCP3, associated with energy expenditure and metabolism in skeletal muscle, is upregulated in the cachectic state, indicating involvement of these mechanisms[8]. Expression levels of mRNA of UCP1 in BAT were significantly elevated over controls in mice bearing cachexia inducing tumors, while expression levels of UCP2 and -3 did not change in BAT, but were significantly increased in skeletal muscle[78]. This may also be applicable to cancer patients, since UCP-3 mRNA levels are increased in muscle only when weight loss is associated with cancer. UCP-2 mRNA levels in muscle seem unaffected by cancer either with or without weight loss[79]. The increase in UCP3 mRNA might enhance energy expenditure and contribute to tissue catabolism.

PHARMACOLOGICAL TREATMENT

Megace

Megestrol acetate (MEGACE) and medroxyprogesterone (MPA) are synthetic, orally active derivatives of the naturally occurring hormone, progesterone.

MEGACE was first synthesized in England in 1963. Developed as an oral contraceptive, the agent was first tested in the treatment of breast cancer in 1967 and, was later tested for the treatment of endometrial cancer. MEGACE is currently used to improve appetite and to increase weight in cancer-associated anorexia. From September 1993, MEGACE was approved by the Food and Drug Administration in the United States for the treatment of anorexia, cachexia or unexplained weight loss in patients with AIDS. MEGACE has been found to improve appetite, caloric intake and nutritional status in several clinical trials[80-90]. Recently a meta-analysis of 35 trials, comprising 3963 patients, for the effectiveness of MEGACE was conducted[91], demonstrating a benefit of MEGACE compared with placebo, particularly with regard

to appetite improvement and weight gain in cancer. Higher doses were more related to weight improvement than lower doses. Quality of life improvement in patients was seen only when comparing MEGACE *vs* placebo[91]. The mechanism for the associated weight gain is mostly unknown, although MEGACE may stimulate the synthesis, transport, and release of neuropeptide γ, known to produce appetite-stimulating effects in rats[92].

MPA has similarly been shown to increase appetite and food intake with a stabilization of body weight[93]. There is evidence that high-dose synthetic progestins have effects on both appetite and body weight, the two clinical hallmarks most widely identified in patients with cancer anorexia and cachexia[94]. MPA has been shown to reduce the *in vitro* production of serotonin and cytokines (IL-1, IL-6 and TNF-α) by peripheral blood mononuclear cells of cancer patients[92,93,95,96]. These findings have also been replicated in the clinical setting, with IL-1, IL-6, and TNF-α levels in serum reported to be decreased in cancer patients after MEGACE or MPA treatment[93].

Ghrelin

Ghrelin, a 28-amino-acid gastric peptide hormone, was first identified in the rat stomach in 1999 as an endogenous ligand for the growth hormone secretagogue receptor[97]. The functions of ghrelin include food intake regulation, gastrointestinal (GI) motility, and acid secretion in the GI tract. Many GI disorders involving infection, inflammation, and malignancy are correlated with altered ghrelin production and secretion[98]. Circulating levels of ghrelin are noted to be increased when human melanoma cells are implanted in nude mice[99]. In a similar manner, circulating levels of both acyl and des-acyl ghrelin are elevated in cachectic cancer patients with gastric cancer[100,101] and lung cancer[102,103]. The levels of acyl-ghrelin are reported to be 50% higher in cancer patients with cachexia[104]. These elevated levels of ghrelin could represent a counter regulatory mechanism to fight anorexia associated with tumor growth, representing an endocrine response to the so-called "ghrelin resistance" found in cancer patients. This is the rationale behind the clinical studies of high dose ghrelin as a treatment to counteract anorexia in cancer.

An experimental study showed that repeated administration of ghrelin improves cardiac structure and function and attenuates the development of cardiac cachexia in chronic heart failure, with ghrelin thought to regulate energy metabolism through growth hormone dependent and growth hormone independent mechanisms[105]. For cancer cachexia, a phase Ⅱ randomized, placebo-controlled, double-blind study, using an oral ghrelin mimetic was conducted[105]. This study demonstrated an improvement in lean body mass, total body mass and hand grip strength in cachectic cancer patients[105].

Cannabinoids

Cannabinoids, which are present in marijuana, are a class of diverse chemical compounds that activate cannabinoid receptors on cells that repress neurotransmitter release in the brain. Cannabinoids have a definite effect on weight gain and, bearing this in mind, have been used to increase food intake in cancer patients. The main effective constituent of cannabis is delta-9-tetrahydrocannabinol[106,107], but the mechanism by which cannabinoids exert their effects has yet to be clarified. It has been postulated that they may act *via* endorphin receptors, through inhibition of prostaglandin synthesis[108], or by inhibiting IL-1 secretion[85]. Despite high expectations for cannabinoids to be effective against cancer-related anorexia/cachexia syndrome, both of the two separate randomized clinical trials carried out by Jatoi *et al*[109] and Strasser *et al*[110] have failed to show benefit as compared to MEGACE or placebo, respectively.

Melanocortin antagonists

The melanocortin-4 (MC4) receptor subtype plays a pivotal role in body weight regulation[111]. Acute and chronic stimulation of MC4 receptors produces anorexia, weight loss, and an increase in metabolic rate, the cardinal features of disease-associated cachexia. Knock-out or antagonism of MC4 receptors in animal models of cachexia protects from anorexia and the loss of both lean and fat body mass, and it is suggested that an MC4 antagonist may be beneficial in wasting diseases, which are poorly treated by available therapies[112]. The MC4 receptor is involved in the anorexigenic cascade leading to a decrease in neuropeptide γ and, therefore, a decrease in food intake. The use of MC4 antagonists has been proven to be effective in preventing anorexia associated with cachexia, loss of lean body mass and basal energy in animal models[112,113]; however, there is no clinical data at this time. Future clinical trials are needed to prove the efficacy of this antagonist in the treatment of human cachexia.

Thalidomide and etanercept

TNF-α, IL-6, and IFN-c have all been implicated in the pathogenesis of cachexia, and in cachectic tumor bearing murine models treatment with anti-TNF-α, anti-IL-6, and anti-IFN-c antibodies can attenuate the disease process, although it cannot stop or reverse cancer cachexia[49,114-120]. There is also some evidence that cytokines play a role in the pathogenesis of cachexia[121]. It has been suggested that by mimicking the hypothalamic effect of excessive negative feedback signaling from leptin by persistent stimulation of anorexigenic peptides, or by inhibition of the neuropeptide Y pathway, cytokines could induce anorexia[122]. Thus modulating cytokine expression in cancer patients may also affect cancer associated anorexia. Therapeutic strategies have been based on either blocking cytokine synthesis or their action[123].

Thalidomide (a-N-phthalimidoglutaramide) has complex immune-modulatory and anti-inflammatory properties. It has been shown to down-regulate the production of TNF-α and other pro-inflammatory

cytokines in monocytes, to inhibit the transcription factor nuclear factor kappa B (NFκB), down-regulate cyclooxygenase 2, and to inhibit angiogenesis[124,125]. One randomized placebo-controlled trial in patients with cancer cachexia showed that the drug was well-tolerated and effective at attenuating loss of weight and lean body mass in patients with advanced pancreatic cancer[126].

Etanercept, a soluble p75 tumor necrosis factor receptor: FC (TNFR: FC) fusion protein for plasma cytokines, has been used over the last decade for the treatment of immune-mediated rheumatic diseases. In a clinical pilot study, patients with several advanced malignancies treated with etanercept combined with docetaxel had less fatigue and improved tolerability to anti-tumor treatment, although etanercept alone did not show effects[127].

Omega-3-Fatty acides (N-3-FA), eicosapentaenoic acid

Eicosapentaenoic acid (EPA) is one of several omega-3 polyunsaturated fatty acids found abundantly in fish oil. Polyunsaturated fatty acids have been proposed to reduce cachexia-associated tissue wasting[128] as well as tumor growth[129,130]. EPA down-regulates the production of pro-inflammatory cytokines in both healthy individuals and patients with cancer. Furthermore, the effects of proteolysis inducing factor, a cachectic factor produced by cancer, are also inhibited by EPA.

Three systematic reviews have been published regarding n-3-FA. Only one of these formulated a weak recommendation of n-3-FA for patients with advanced cancer and weight loss[131], stating that there was fair evidence to recommend its use (recommendation grade B). The other two reviews found no clear advantages from treatment with n-3-FA. A meta-analysis by Colomer *et al*[131] contained 17 trials[61,132-146], and attempted to evaluate the effectiveness and safety of n-3-FA in relieving symptoms associated with the cancer cachexia syndrome. They reported that EPA improved various clinical, biochemical, and quality of life parameters after 8 wk of treatment. Dewey *et al*[147] showed that data were insufficient to determine whether oral EPA is better than placebo in their analysis of 5 trials[130,137,140,148,149]. Comparison of EPA *vs* MEGACE as an appetite stimulant provided no evidence that EPA improved cachexia-related symptoms[147]. Mazzotta *et al*[150] systematically reviewed several databases including publications until 2006 in order to identify the clinical efficacy of EPA and Docosahexaenoic Acid (DHA), another Omega-3-fatty acid, for the management of cachexia in cancer patients. They analyzed 10 studies and 7 RCTs[133,137,140-142,151,152] and found no clear advantage of either EPA or DHA on weight, lean muscle mass, symptoms, quality of life, or survival. Studies that reported statistically significant differences were found to have only a small clinical difference, not enough to justify the use of EPA or DHA alone as a treatment option. However, it does seem clear that multidimensional treatments represent the most useful approach for cachexia in advanced cancer[150].

Altogether, there is not enough evidence to support a net benefit from n-3-FA in treating cachexia from advanced cancer. On the other hand, adverse effects were infrequent and not severe. More research is needed not only on drugs such as eicosapentaenoic acid or other n-3-FA, but also on multimodal approaches combining drugs and non-drug interventions.

Herbal medicine (kampo)

Kampo is the Japanese herbal medical practice, which is an adaptation of traditional Chinese medicine that came to Japan between the 7th and 9th centuries. Kampo has been shown to have significant clinical benefits for cachexia[153]. Fujitsuka *et al*[154] reported that Rikkun-shito, a Kampo formula, improved anorexia, gastrointestinal dysmotility, muscle wasting, and anxiety-related behavior. Rikkun-shito improved anorexia-cachexia and prolonged survival of tumor-bearing rats in this study. Moreover, Rikkun-shito significantly prolonged median survival of pancreatic cancer patients with ascites who were treated with gemcitabine. These studies suggest that Rikkun-shito may be useful in clinical practice for cachectic cancer patients. Although the mechanisms of how the herbs demonstrate these effects are unclear and remain to be elucidated, they deserve further studies as new potential therapy agents for cancer treatment[155].

Corticosteroids

Corticosteroids are one of the most widely used appetite stimulants. In randomized controlled studies, they have been shown to improve appetite and quality of life compared with placebo[156]. MEGACE and corticosteroids seem equally effective, although for long-term use, corticosteroids result in more serious adverse effects such as protein breakdown, insulin resistance, water retention, and adrenal suppression[157]. Therefore, corticosteroids are not suitable for long-term use and should be used in a limited fashion, such as during the pre-terminal phase of cachexia.

Non-steroidal anti-inflammatory drugs

There are four studies investigating the relationship between non-steroidal anti-inflammatory drugs (NSAIDs) and cancer cachexia[158-161]. These studies demonstrated improved quality of life, performance status, inflammatory markers, weight gain and survival. Notably these reviews show that side effects of NSAIDs use were not remarkable in these reports that were evaluated.

However, two reports concluded data were insufficient for recommending the widespread use of NSAIDs in practice[162,163]. This reflection arises from the large heterogeneity observed in terms of study design, number of patients, type of cancer, clinical parameters, definition of effect criteria, and the weakness of the many individual studies.

β2-adrenergic agonists

β2-adrenergic agonists are potent muscle growth promoters in many animal species resulting in skeletal muscle hypertrophy[164-167], and reduction of the body fat

content[168,169]. The wide variety of physiologic functions controlled by β-adrenergic receptors suggest that the mechanisms underlying effects on carcass composition may be extremely complex.

Formoterol is a long-acting β2 agonist approved for the management of asthma and chronic obstructive pulmonary disease. Formoterol exerts a selective, powerful protective action on heart and skeletal muscle by antagonizing the enhanced protein degradation, which is a characteristic of cancer cachexia. β2-agonists are also proposed to have a protective action against the apoptosis of skeletal muscle. Formoterol may be potential therapeutic tool in pathologic states[170].

Others

Other drugs that are investigated to be used for cancer cachexia include Erythropoetin[171-173], ACE inhibitors[174], and β-blockers[175].

Chemotherapy

At the present time, cancer cachexia cannot be cured. However, several recent randomized trials using combinations of newer chemotherapy agents have shown promising results. Combination chemotherapy was initially assessed with low-efficacy regimens designed for symptomatic management in the palliative setting until effective regimens were discovered that were found to improve survival in the adjuvant setting[176]. Regimens combining multiple drugs are expected to be successful. In a phase Ⅱ study, the combined administration of anti-oxidants, pharmaco-nutritional support, progestagen and anti-cyclooxygenase-2 drugs, was shown to be safe and effective for cancer cachexia[177]. Based on those results, an ongoing randomized phase Ⅲ study began recruiting patients in 2005, with the aim of including more than 300 cachectic cancer patients. Findings to date reinforce the use of multi-modal therapies in the treatment of the cachexia-anorexia syndrome in cancer. Usually the response to therapy is better with early intervention during active adjuvant or palliative cancer therapy, compared to treatment when the patient has progressed to become refractory to anti-cachexia treatment. One of the challenges to undertaking "upfront" randomized trials for cachexia is that the systemic chemotherapy for cancer treatment itself can aggravate weight loss, and for anti-cachexia therapy to show benefit it has to "compete" with chemotherapy.

NON-PHARMACOLOGICALTREATMENT

Dietary treatment

Since cancer cachexia differs from starvation, at the present time no single modality therapies using traditionally applied nutritional regimens has succeeded in demonstrate any efficacy in improving weight gain, including gain in lean body mass, in patients diagnosed with cancer cachexia[178]. The average calorie deficit in a weight-losing patient is reported to be approximately 200 kcal per day in the setting of advanced cancer[132] and 250-400 kcals/d in those patients with cancer cachexia[178]. An average supplementation of 1 calorie/mL has not been shown to improve the nutritional status of patients receiving chemotherapy[140,179].

The average protein intake in patients with cancer cachexia is about 0.7-1.0 g/kg per day[140]. Food energy intake needs to increase by 300-400 kcal per day and protein intake to increase by up to 50% to have an effect on anabolic resistance (recommended intake 1.0-1.5 g/kg per day). The analysis of a randomized trial found that in addition to oral nutritional support, the use of parenteral nutrition resulted in a short (6-8 wk) but significant ($P < 0.001$), prolongation of survival when nutritional goals were achieved[180]. A meta-analysis of oral nutritional interventions in malnourished patients with cancer suggests that oral nutritional interventions have no effect on survival and that the effect on body weight and energy intake is inconsistent, though statistically significant improvements in some aspects of QOL may be achieved. In this study, nutritional intervention was associated with a significant increase in energy intake (430 kcal per day) and a weight gain of 1.9 kg. There was a beneficial effect on appetite and global quality of life[181].

Physical exercise

Physical exercise has been suggested as a promising countermeasure for preventing cachexia[182]. Unfortunately, only a few studies, in both clinical and experimental settings, have been performed to define the effectiveness of exercise against cachexia.

The rationale for the use of exercise relies on the known dramatic reduction of muscle strength and endurance during cachexia[183-186]. Since it is also reported that exercise increases insulin sensitivity, protein synthesis rate, and anti-oxidative enzyme activity[187] it may lead to a suppression of the inflammatory response and enhancement of immune function[188]. There is significant evidence that endurance exercise (*e.g.,* a high number of repetitions performed over extended time periods against relatively low resistance) ameliorates cancer-related fatigue[189]. A randomized trial has also reported that, in patients with advanced-stage cancer, exercise is feasible and that although fatigue is not reduced, physical performance is improved significantly[190]. Combination of resistance and aerobic muscle training has been suggested to be incorporated into cachexia treatment programs[191]. Exercise training is able to increase both strength and endurance in healthy conditions, depending on the type of exercise, and moreover, it has been proven to act as an excellent anabolic drive for skeletal muscle in combination with anabolic steroids or other muscle anabolic drugs[192].

FUTURE DIRECTIONS

Additional directions for study in the field of cancer

cachexia may come from the results of Bossola *et al*[193] who showed hyper-expression of mRNA for ubiquitin and increased proteolytic activity of proteasomes prior to weight loss in cancer patients. This finding could open a new research area in the field of early intervention and of prevention of cancer induced weight loss. Further research is also needed into cancer anorexia, due to the frequent finding of reduced food intake in cancer patients, and the lack of any current powerful therapies to improve appetite and daily caloric intake.

CONCLUSION

Cancer cachexia has been regarded as a non-curable disease, and has been estimated to be responsible for the death of over 20% of cancer patients. The management of cancer cachexia has improved dramatically in the past decade, as the mechanisms involved in the development and progression of the condition continue to be elucidated. Currently all treatments for cancer cachexia are considered palliative, but new agents have improved patient survival as well as their quality of life. Regular anti-neoplastic agents have the ability to treat cancer, but in many cases worsen cachexia. Future progress in the field will be realized through development of treatment agents with the ability to affect cancer progression as well as improve patient quality of life.

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EDITORIAL

Epigenetic reduction of DNA repair in progression to gastrointestinal cancer

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Abstract

Deficiencies in DNA repair due to inherited germ-line mutations in DNA repair genes cause increased risk of gastrointestinal (GI) cancer. In sporadic GI cancers, mutations in DNA repair genes are relatively rare. However, epigenetic alterations that reduce expression of DNA repair genes are frequent in sporadic GI cancers. These epigenetic reductions are also found in field defects that give rise to cancers. Reduced DNA repair likely allows excessive DNA damages to accumulate in somatic cells. Then either inaccurate translesion synthesis past the un-repaired DNA damages or error-prone DNA repair can cause mutations. Erroneous DNA repair can also cause epigenetic alterations (*i.e.*, epimutations, transmitted through multiple replication cycles). Some of these mutations and epimutations may cause progression to cancer. Thus, deficient or absent DNA repair is likely an important underlying cause of cancer. Whole genome sequencing of GI cancers show that between thousands to hundreds of thousands of mutations occur in these cancers. Epimutations that reduce DNA repair gene expression and occur early in progression to GI cancers are a likely source of this high genomic instability. Cancer cells deficient in DNA repair are more vulnerable than normal cells to inactivation by DNA damaging agents. Thus, some of the most clinically effective chemotherapeutic agents in cancer treatment are DNA damaging agents, and their effectiveness often depends on deficient DNA repair in cancer cells. Recently, at least 18 DNA repair proteins, each active in one of six DNA repair pathways, were found to be subject to epigenetic reduction of expression in GI cancers. Different DNA repair pathways repair different types of DNA damage. Evaluation of which DNA repair pathway(s) are deficient in particular types of GI cancer and/or particular patients may prove useful in guiding choice of therapeutic agents in cancer therapy.

Key words: Epigenetic; DNA damage; DNA repair; DNA repair deficiency disorders; Epimutation; Genomic instability; Germ-line mutation; MicroRNAs; Precancerous conditions; Gastrointestinal cancer

Core tip: The primary cause of cancer is DNA damage. DNA damage leads to replication errors and erroneous repair, and can result in driver mutations and epimutations. While germ-line mutations in DNA repair genes cause cancer-prone syndromes, mutations in DNA repair genes are infrequent in sporadic gastrointestinal cancers. However, reduction of DNA repair proteins due to epigenetic repression of DNA repair genes is

very frequent and can cause early steps in sporadic cancers. Evaluation of which DNA repair pathway(s) are deficient in particular types of GI cancer and/or particular patients may prove useful in guiding choice of therapeutic agents.

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REDUCED DNA REPAIR INCREASES CANCER RISK

Germ-line mutations in DNA repair genes cause increased risk of GI cancers. Examples are given in Table 1.

About 5% to 10% of all types of cancers are due to hereditary cancer syndromes[12]. Two reviews on hereditary cancer syndromes list 48 and 55 such syndromes[12,13]. Mutation in any of 37 DNA repair genes, including those listed in Table 1, can cause an hereditary cancer syndrome[14]. That hereditary cancer syndromes are frequently caused by mutations in DNA repair genes indicates that reduction in DNA repair gene expression can be a crucial early event in progression to cancer. If DNA repair gene expression is reduced in a somatic tissue by epigenetic repression, this is also likely to be a crucial early event in progression to cancer in that tissue.

Epimutations in DNA repair genes are frequent during progression to cancer

Vogelstein *et al*[15], reviewing evidence from sequencing 3284 tumors and the 294881 mutations found in those cancers, noted that germ-line mutations that give rise to hereditary cancer syndromes are infrequent in sporadic tumors.

More in depth studies of defects in DNA repair genes O-6-methylguanine-DNA methyltransferase (*MGMT*) and *PMS2*, important in progression to GI cancer, are consistent with the observations of Vogelstein *et al*[15]. In the case of *MGMT*, 113 sequential colorectal cancers were evaluated and only four had a missense mutation in the DNA repair gene *MGMT*, while most had reduced *MGMT* expression due to methylation of the *MGMT* promoter region[16]. Other laboratories, quantifying their results, reported that 40% to 90% of colorectal cancers have reduced *MGMT* expression due to methylation of the *MGMT* promoter region[17-21].

In the case of *PMS2*, when 119 colorectal cancers deficient in DNA mismatch repair gene *PMS2* expression were examined, mutation in *PMS2* was present in 6 cases while in 103 cases the pairing partner of *PMS2*, *MLH1* was repressed due to promoter methylation (PMS2 protein is unstable in the absence of MLH1)[22]. In the remaining 10 cases it was likely that epigenetic over-expression of the miRNA, miR-155, which down-regulates MLH1 messenger RNA (mRNA), caused the loss of *PMS2* expression[23].

These findings suggest that, if an early step in progression to sporadic GI cancer is reduction in function of a DNA repair gene, that reduction is likely due to an epigenetic alteration rather than to a mutation in that gene.

DNA DAMAGES ARE VERY FREQUENT AND AN IMPORTANT CAUSE OF CANCER

An average of more than 60000 endogenous DNA damages occur per cell per day in humans (Table 2). These are largely caused by hydrolytic reactions, interactions with reactive metabolites such as lipid peroxidation products, endogenous alkylating agents and reactive carbonyl species, and exposure to reactive oxygen molecules[28].

However, more important still in causing cancer, are DNA damages caused by exogenous agents. Doll *et al*[29] compared cancer rates for 37 specific cancers in the United States to rates for these cancers in countries where there is low incidence for these cancers. The populations for comparison included Norwegians, Nigerians, Japanese, British and Israeli Jews. They concluded that 75%-80% of the cases of cancer in the United States were likely avoidable. They indicated that the avoidable sources of cancer included tobacco, alcohol, diet (especially meat and fat), food additives, occupational exposures (including aromatic amines, benzene, heavy metals, vinyl chloride), pollution, industrial products, medicines and medical procedures, UV light from the sun, exposure to medical X-rays, and infection. Many of these sources of cancer are DNA damaging agents.

One example of diet-related DNA damaging agents likely important in human GI cancer are bile acids. Bernstein *et al*[30] summarized 14 published reports showing that the secondary bile acids deoxycholic acid and lithocholic acid, formed by bacterial action in the colon, cause DNA damage. Bile acids are increased in the colon after the gall bladder releases bile acids into the digestive tract in response to consumption of fatty foods to aid in their digestion. Bile acids in the colon were doubled in the colonic contents of humans in the United States who were on typical diets and then were experimentally fed a high fat diet[31]. Cancer rate comparisons can be made between two similar populations, one with low levels and one with high levels of colonic bile acids. For instance, deoxycholic acid (DOC) in the feces of Native Africans in South Africa is present at 7.30 nmol/g wet weight stool while for African Americans DOC is present at 37.51 nmol/g wet weight stool, a 5.14 fold higher concentration[32]. Native Africans in South Africa have a colon cancer rate

Table 1 Inherited mutations in DNA repair genes that increase the risk of gastrointestinal cancer

DNA repair gene(s)	Repair pathway(s) affected	Cancers with increased risk
BLM	HRR[1]	Leukemia, lymphoma, colon, breast, skin, lung, auditory canal, tongue, esophagus, stomach, tonsil, larynx, uterus[2]
WRN	HRR, NHEJ, long patch BER[3]	Soft tissue sarcoma, colorectal, skin, thyroid, pancreatic[4]
Fanconi's anemia genes *FANC A, B, C, D1, D2, E, F, G, I, J, L, M, N*	HRR and TLS[5]	Leukemia, liver tumors, solid tumors in many areas including esophagus, stomach and colon[6]
MSH2, MSH6, MLH1, PMS2	MMR[7]	Colorectal, endometrial[7]
MUTYH	BER of A mispaired with 8-OHdG[8]	Colon[8]
P53	HRR, BER, NER, NHEJ, MMR[9]	Sarcoma, breast, osteo-sarcoma, brain, adreno-cortical carcinomas[10] and colon and pancreas[11]

HRR: Homologous recombinational repair; NHEJ: Non-homologous end joining; BER: Base excision repair; TLS: Translesion synthesis; MMR: Mismatch repair; DDR: DNA damage response.

Table 2 Endogenous DNA damages/cell/day for humans

DNA damages	Reported rate of occurrence
Oxidative damages	10000[24]
Depurinations	9000[25]
Depyrimidations	696[26]
Single-strand breaks	55000[26]
Double-strand breaks	Approximately 50/cell cycle[27]
O^6-methylguanine	3120[26]
Cytosine deamination	192[26]

of < 1:100000[33] compared to the incidence rate for male African Americans of 72:100000[34], a more than 72-fold difference in rates of colon cancer.

The likely role of bile acids as causative agents in colon cancer is further illustrated by experiments with mice. When mice were fed a diet supplemented with the bile acid deoxycholate (DOC) for 10 mo, raising their colonic level of DOC to that of humans on a high fat diet, 45% to 56% of these mice developed colon cancers, while mice fed the standard diet alone, with 1/10 the level of colonic DOC, developed no colon cancers[35,36].

Another indication that diet is important in colon cancer is observed in populations migrating from low-incidence to high-incidence countries. Cancer rates change rapidly, and within one generation reach the rate in the high-incidence country. This has been observed, for instance, in the colon cancer incidence of migrants from Japan to Hawaii[37].

MANY GENES INVOLVED IN DNA REPAIR

At least 169 enzymes are either directly employed in DNA repair or influence DNA repair processes[38]. Of these, 139 are directly employed in DNA repair processes including base excision repair (BER), nucleotide excision repair (NER), homologous recombinational repair (HRR), non-homologous end joining (NHEJ), mismatch repair (MMR) and direct reversal of lesions (DR). The other 30 enzymes are employed in the DNA damage response (DDR) needed to initiate DNA repair; chromatin structure modification required for repair; reactions needed for the reversible, covalent attachment of ubiquitin and small ubiquitin-like modifier proteins to DDR factors that facilitate DNA repair; or modulation of nucleotide pools.

When the incidence of endogenous and exogenous DNA damages is high, decreases in expression of DNA repair genes or DDR genes lead to a build-up of DNA damage within a cell. These excessive damages provide more opportunities for replication errors and erroneous repair to occur (see mechanisms below) and cause higher rates of mutation and epimutation. Higher numbers of mutations and epimutations increase the chance of including selectively advantageous driver mutations and epimutations that, in turn, promote progression to cancer.

DNA DAMAGES GIVE RISE TO MUTATIONS AND EPIGENETIC ALTERATIONS

Translesion synthesis (TLS) past a single-stranded DNA damage introduces mutations.

Single-strand DNA damages are the most frequent endogenous DNA damages (Table 2). TLS is a DNA damage tolerance process that allows the DNA replication machinery to replicate past single-strand DNA lesions in the template strand. This permits replication to be completed, rather than blocked (which may kill the cell or cause a translocation or other chromosomal aberration)[39].

Humans have four translesion polymerases in the Y family of polymerases [REV1, Pol κ (kappa), Pol η (eta), and Pol ι (iota)] and one in the B family of polymerases [Pol ζ (zeta)][39]. The temporary tolerance of DNA damage during chromosome replication may allow DNA repair processes to remove the damage later[40], and avoid immediate genome instability[41]. However, translesion synthesis is less accurate than the replicative polymerases δ (delta) and ε (epsilon) and tends to introduce mutations[39].

Deficiency in expression of a DNA repair gene can allow excessive DNA damages to accumulate. Some of the excess damages will likely be processed by translesion synthesis, causing increased mutation.

Kunz *et al*[42] summarized numerous experiments

in yeast, in which forward mutations were measured (by sequence analyses of a few selected genes) in cells carrying either wild-type alleles or one of 11 inactivated DNA repair genes. Their results indicated that DNA repair deficient cells accumulate excess DNA damages that then give rise to mutations after error-prone translesion synthesis. The 11 inactivated DNA repair genes were distributed among MMR, *NER*, *BER* and *HRR* genes. Deficiencies in DNA repair increased mutation frequencies by factors between 2- and 130-fold, but most often by double digit-fold increases. Overall, the authors concluded that 60% or more of spontaneous single base pair substitutions and deletions are likely caused by translesion synthesis.

Stuart *et al*[43] examined spontaneous mutation frequencies in a *lacI* transgene (in a Big Blue mutation assay[44]) in either replicating tissues or in largely non-replicating tissues of mice. If most mutations occur during translesion synthesis, then non-replicating brain tissue, which has little or no synthesis once maturity is reached, would have little or no further mutation accumulation. In mouse brain, after 6 mo of age, there was no increase in mutation frequency, even at 25 mo of age. In bladders of mice, with replicating tissues, mutation frequency increased with age, almost tripling between ages of 1.5 mo and 12 mo of age. The authors concluded that the age related increases in spontaneous mutation frequencies reflect endogenous DNA damages that subsequently gave rise to mutations following DNA replication. This indicates that translesion synthesis is a major source of mutation in mouse replicating tissues.

Mutations are frequently caused by error-prone repair of double-strand breaks

While only a minority of endogenous DNA damages in the average cell are double-strand breaks (Table 2), this type of lesion appears to contribute substantially to the mutation rate as well. As indicated by Vilenchik and Knudson[27], the doubling dose for ionizing radiation (IR) induced double-strand breaks is similar to the doubling dose for mutation and induction of carcinomas by IR. Thus, double-strand breaks likely lead frequently to mutations.

As described by Bindra *et al*[45], non-homologous end-joining (NHEJ) and HRR comprise the two major pathways by which double-strand breaks (DSBs) are repaired in cells. NHEJ processes and re-ligates the exposed DNA termini of DSBs without the use of significant homology, whereas HRR uses homologous DNA sequences as a template for repair. HRR predominates in S-phase cells, when a sister chromatid is available as a template for repair, and is a high-fidelity process. NHEJ is thought to be active throughout the cell cycle, and it is more error-prone than HRR. NHEJ repair comprises both canonical NHEJ and non-canonical pathways. The former pathway results in minimal processing of the DSB during repair, whereas the latter pathway, with or without the use of sequence microhomology for re-ligation, typically results in larger insertions or deletions. Mutagenic NHEJ repair is a robust process, yielding percentages of mutated sites at the position of a DSB ranging from 20% to 60%.

As pointed out by Vilenchik *et al*[27], about 1% of single-strand DNA damages escape repair and are not bypassed, and some of these become converted to double-strand breaks. This may contribute to the impact of double-strand breaks in causing mutations and carcinogenesis.

Epigenetic alterations occur due to DNA damage

Epigenetic alterations can arise due to incomplete repair of DNA double-strand breaks. As an example, O'Hagan *et al*[46] used a cell line stably transfected with a plasmid containing a consensus *I-SceI* cut site inserted into a copy of the *E-cad* promoter. This promoter contained a CpG island. O'Hagan *et al*[46] induced a defined double-strand break in the E-cadherin CpG island, and the CpG island was not currently hypermethylated. As the repair of the break began, they observed that key proteins involved in establishing and maintaining transcriptional repression were recruited to the site of damage, to allow repair of the break. Most cells examined after the DNA break was repaired showed that DNA repair occurred faithfully, with the promoter not hypermethylated and the silencing factors removed. However, a small percentage of the cells retained heritable silencing. In these cells the chromatin around the break site was enriched for most of the silencing chromatin proteins and histone marks, and the region had increased DNA methylation in the CpG island of the promoter. Thus, repair of a DNA break can occasionally cause heritable silencing of a CpG island-containing promoter. Such CpG island methylation is frequently associated with tight gene silencing in cancer.

Morano *et al*[47] also showed that epigenetic alterations can arise as a consequence of DNA damage. They studied a system in which recombination between partial duplications of a chromosomal green fluorescent protein (*GFP*) gene is initiated by a DSB in one copy. Two cell types were generated after recombination: clones expressing high levels of GFP and clones expressing low levels of GFP, referred to as H and L clones, respectively. Relative to the parental gene, the repaired *GFP* gene was hypomethylated in H clones and hypermethylated in L clones. The altered methylation pattern was largely restricted to a segment 3' to the DSB. Although it is 2000 base pairs distant from the strong cytomegalovirus promoter that drives GFP expression, hypermethylation of this tract significantly reduced transcription. The ratio of L (hypermethylated) to H (hypomethylated) clones was 1:2 or 1:4, depending on the insertion site of the GFP reporter. These experiments were performed in mouse embryonic (ES) or human cancer (Hela) cells. HRR-induced methylation depended on DNA

methyltransferase I. These data, taken together, argue for a cause-effect relationship between double-strand DNA damage-repair and altered DNA methylation.

The main function of the proteins in the BER pathway is to repair DNA single-strand breaks and deamination, oxidation, and alkylation-induced DNA base damage. In addition, Li *et al*[48] reviewed studies indicating that one or more BER proteins also participate(s) in epigenetic alterations involving DNA methylation, demethylation or reactions coupled to histone modification. Franchini *et al*[49] also showed that DNA demethylation can be mediated by BER and other DNA repair pathways requiring processive DNA polymerases. Another form of epigenetic silencing also appears to occur during DNA repair. PARP1 [poly(ADP)-ribose polymerase 1] and its product poly(ADP)-ribose (PAR) accumulate at sites of DNA damage as intermediates of a DNA repair process[50]. This directs recruitment and activation of the chromatin remodeling protein ALC1, which can cause nucleosome remodeling[51]. Nucleosome remodeling, in one case, has been found to cause epigenetic silencing of DNA repair gene *MLH1*[52]. These reports, overall, indicate that DNA damages needing repair can cause epigenetic alterations by a number of different mechanisms.

Other causes of epigenetic alterations

Heavy metals and other environmental chemicals cause many epigenetic alterations, including DNA methylation, histone modifications and miRNA alterations[53]. DNA damage itself causes programmed changes in non-coding RNAs, and a large number of miRNAs are transcriptionally induced upon DNA damage[54]. However, it is not clear what proportion of these alterations are reversed or are retained as epimutations after the external sources of damage are removed upon repair of the DNA damages[55].

Mutations in isocitrate dehydrogenase 1 (IDH1) and *2* (IDH2) are frequent in several types of cancer and they can cause epigenetic alterations. As reviewed by Wang *et al*[56], *IDH1* and *IDH2* mutations represent the most frequently mutated metabolic genes in human cancer. These mutations occur in more than 75% of low grade gliomas and secondary glioblastoma multiforme, 20% of acute myeloid leukemias, 56% of chondrosarcomas, over 80% of Ollier disease and Maffucci syndrome, and 10% of melanomas. *IDH1* is also mutated in 13% of inflammatory bowel disease-associated intestinal adenocarcinoma with low-grade tubuloglandular histology but not in sporadic intestinal adenocarcinoma[57]. The *IDH1* and *IDH2* mutations that give rise to epimutations usually occur in the hotspot codons Arg132 of *IDH1*, or the analogous codon Arg172 of *IDH2*. These mutations allow accumulation of the metabolic intermediate 2-hydroxyglutarate (2-HG), and 2-HG inhibits the activity of alpha ketoglutarate (α-KG) dependent dioxygenases, including α-KG-dependent histone demethylases and the TET family of 5-methylcytosine hydroxylases.

Wang *et al*[56] found that histone H3K79 dimethylation levels were significantly elevated in cholangiocarcinoma samples that harbored *IDH1* or *IDH2* mutations (80.8%) compared to tumors with wild-type *IDH1* and *IDH2* (45.0%).

In addition, they surveyed over 462000 CpG sites in CpG islands, CpG shores and intragenic regions, and found that 2309 genes had significantly increased methylation in the presence of *IDH1* or *IDH2* mutations. In particular, Sanson *et al*[58] found that methylation of the DNA repair gene *MGMT* was associated with *IDH1* mutation, since 81.3% of *IDH1*-mutated gliomas were *MGMT* methylated compared with 58.3% methylated in *IDH1* non-mutated tumors.

DNA REPAIR GENES WITH EPIGENETICALLY REDUCED EXPRESSION ARE LIKELY PASSENGERS IN A SPREADING FIELD DEFECT

A DNA repair gene that is epigenetically silenced or whose expression is reduced would not likely confer any selective advantage upon a stem cell. However, reduced or absent expression of a DNA repair gene would cause increased rates of mutation, and one or more of the mutated genes may cause the cell to have a strong selective advantage. The expression-deficient DNA repair gene could then be carried along as a selectively neutral or only slightly deleterious passenger (hitch-hiker) gene when there is selective expansion of the mutated stem cell. The continued presence of a DNA repair gene that is epigenetically silenced or has reduced expression would continue to generate further mutations and epigenetic alterations.

The spread of a clone of cells with a selective advantage, but carrying along a gene with epigenetically reduced expression of a DNA repair protein would be expected to generate a field defect, from which smaller clones with still further selective advantages would arise. This is consistent with the finding of field defects in colonic resections, that have both a cancer and multiple small polyps, such as the one shown in Figure 1.

For any particular type of GI cancer, an epigenetic reduction in expression of a specific DNA repair gene may be common. In cases where a specific epigenetic reduction of expression of a DNA repair gene occurs in a cancer, it is also likely to be evident in the field defect surrounding the cancer (Table 3). The lower frequency in the surrounding field defect that is usually found (Table 3) likely reflects the process whereby the expanding clone laterally displaces the more normal epithelium. This displacement may be only partial. Thus, areas with the DNA repair deficiency would be present at a lower frequency in the field defect than in the cancer. In the cancer, the cells carrying the DNA repair deficiency are members of a founding clone. Thus, in the cancer, the DNA repair defect, along

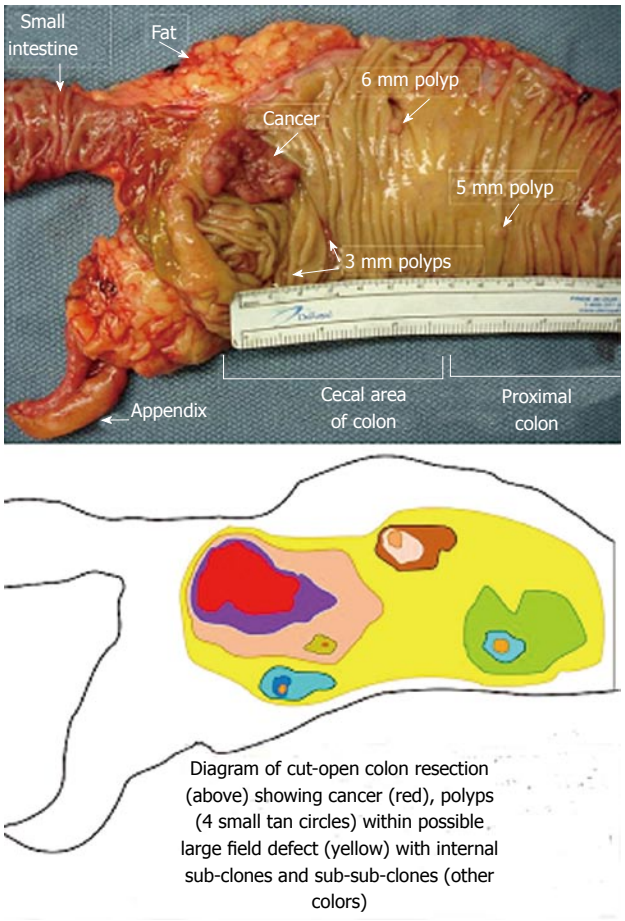

Figure 1 Cut open gross specimen of proximal human colon showing multiple tumors[59].

with other accumulated mutations and epigenetic alterations, would be seen at a relatively higher frequency than in the surrounding field defect.

DECREASED EXPRESSION OF MULTIPLE DNA REPAIR GENES IN GI CANCERS

The protein expressions of three DNA repair genes within a 20 cm colon resection were evaluated at six different locations within the resection (Figure 2)[62]. One of the DNA repair proteins, KU86, was only deficient infrequently, with the deficiencies occurring in small patches (up to three crypts). These KU86 defects are not likely important in progression to colon cancer. However, two of the evaluated DNA repair proteins, ERCC1 and PMS2, were often deficient in patches of tens to hundreds of adjacent crypts at each of the locations evaluated (see Nguyen *et al*[68] at minutes 18 to 24 of a 28 min video of crypts immunostained for ERCC1 or PMS2).

Overall, ERCC1 (NER) was deficient in 100% of 49 colon cancers evaluated, and in an average of 40% of the crypts within 10 cm on either side of the cancer. PMS2 (MMR) was deficient in 88% of the 49 cancers and in an average of 50% of the crypts within 10 cm of the cancer. As reported by Facista *et al*[62], the pattern

Table 3 Epigenetic deficiency of DNA repair genes in gastrointestinal cancers and field defects

Cancer	Gene	Frequency in cancer	Frequency in adjacent field defect
Colorectal[17]	*MGMT*	46%	34%
Colorectal[19]	*MGMT*	47%	11%
Colorectal[60]	*MGMT* with *MSI*	70%	60%
Colorectal[19]	*MSH2*	13%	5%
Colorectal[61]	*MBD4*	Frequent	Frequent
Colorectal[62]	*ERCC1*	100%	40%
Colorectal[62]	*PMS2*	88%	50%
Colorectal[62]	*XPF*	55%	40%
Colorectal[63]	*WRN*	29%	13%
Stomach[64]	*MGMT*	88%	78%
Stomach[65]	*MLH1*	73%	20%
Esophagus[66]	MLH1	77%-100%	23%-79%

MSI: Microsatellite instability.

of expression of ERCC1 in the crypts within 10 cm of a colon cancer indicated that when the ERCC1 protein was deficient, this deficiency was due to an epigenetic reduction in expression of the *ERCC1* gene. When the PMS2 protein is deficient, it is usually due to the epigenetic repression of its pairing partner, MLH1, and the instabilty of PMS2 in the absence of MLH1[22]. In the study of Facista *et al*[62], ERCC1 and PMS2 were also deficient in all 10 tubulovillous adenomas evaluated (precursors to colonic adenocarcinomas). Thus ERCC1 and PMS2 are deficient at early times (in the field defect), at intermediate times (in tubulovillus polyps), and at late times (within the cancer) during progression to colon cancer. Another DNA repair protein, XPF, was deficient in 55% of the cancers, as well[62]. The majority of cancers were simultaneously deficient for ERCC1, PMS2 and XPF.

Deficiencies in multiple DNA repair genes were also observed in gastric cancers. Kitajima *et al*[69] evaluated MGMT (direct reversal repair), MLH1 (MMR) and MSH2 (MMR) and found that synchronous losses of MGMT and MLH1 increase during progression and stage of differentiated-type cancers. In un-differentiated-type gastric cancers, the frequency of MGMT deficiency increased from early to late stages of the cancer, while frequencies of MLH1 and MSH2 deficiencies were between 48% and 74% at both early and late stages. Thus, in un-differentiated-type gastric cancers, MLH1 or MSH2 deficiency, if it is present, is an early step, while MGMT deficiency is often a later step in progression of this cancer.

Farkas *et al*[70] evaluated 160 genes in 12 paired colorectal tumors and adjacent histologically normal mucosal tissues for differential promoter methylation. They found aberrant methylation in 23 genes, including six DNA repair genes. These DNA repair genes (with DNA repair pathways indicated) were *NEIL1* (BER), *NEIL3* (BER), *DCLRE1C* (NHEJ), *NHEJ1* (NHEJ), *GTF2H5* (NER), and *CCNH* (NER).

Lynam-Lennon *et al*[71] found that miR-31 is over-

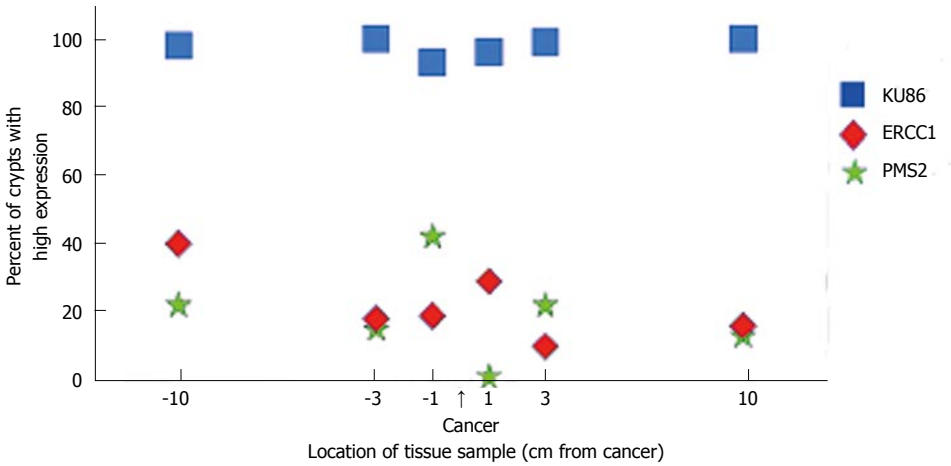

Figure 2 Expression of three DNA repair proteins, KU86, ERCC1 and PMS2, at locations sampled along the 20 cm length of a colon resection that had a cancer at the indicated location[67].

expressed in 47% of esophageal cancers and examined the consequences of over-expression of miR-31 in these cancers. Using a cell line, they first tested the effect of over-expression of miR-31 on the expression of 84 DNA repair genes. They found that 11 DNA repair genes were repressed by over-expression of miR-31. They then evaluated the expression of the five most altered DNA repair genes in 10 esophageal cancers that had high expression of miR-31 and low resistance to radiation treatment (likely low levels of DNA repair). These 10 cancers showed significantly reduced mRNA levels of DNA repair genes *PARP1, SMUG1, MLH1* and *MMS19*. Asangani *et al*[72] showed that miR-31 is an epigenetically regulated microRNA. This microRNA is encoded in an intron of *MIR31HG* (*miR-31* host gene). The transcriptional regulatory region of *MIR31HG* is enriched for histone 3 that could be acetylated on lysine (K) 27 (this is designated H3K27Ac), and H3K27Ac causes an epigentic "mark" that is associated with transcriptionally active genes. If, instead, this histone 3 has triple methylation on lysine 27 (H3K27me3), this causes gene silencing. The regulatory region of *MIR31HG* also has 77 CpG islands surrounding the transcription start site. These observations indicate that miR-31 transcription could be up-regulated by H3K27Ac or silenced by CpG island methylation or by histone H3K27me3. It appears that DNA repair genes *PARP1* (BER and HRR), *SMUG1* (BER), *MLH1* (MMR) and *MMS19* (NER) are epigenetically repressed by over-expressed miR-31 in esophageal cancers.

Based on the examples above, decreased expression of multiple DNA repair genes likely occurs often in GI neoplasia.

EFFECTS LIKELY DUE TO DNA REPAIR DEFECTS

Regression of early lesions

If DNA repair defects are present early in progression to cancer, this should result in increased mutation frequency in those neoplastic lesions. Most new mutations are expected to be deleterious to the cells in which they arise, and thus would cause negative selection of those cells. This expectation is consistent with the observations of Hofstad *et al*[73] who showed that when colonic polyps were identified during a colonoscopy and followed but not removed, between 11% and 46% of polyps smaller than 5 mm diameter were not detectable in the succeeding one to three years. For polyps between 5 and 9 mm in diameter, between 4% and 24% became undetectable in the succeeding one to three years. Of the remaining 68 polyps that were followed for three years, 35% decreased in diameter, 25% remained the same size and 40% increased in diameter. Similarly, Stryker *et al*[74] followed 226 patients with colonic polyps that were $\geqslant$ 1 cm in size for an average of 5.7 years (though some patients were followed for as long as 19 years). Stryker *et al*[74] found that 37% of polyps $\geqslant$ 1 cm enlarged (at least doubled in volume) during the study while 4% of the polyps that had been observed at least twice, previously, were later not found. The risk of these polyps $\geqslant$ 1 cm producing an invasive carcinoma within 20 years was 24%. The data of Hofstad *et al*[73] and Stryker *et al*[74] are also consistent with statistics showing more frequent occurrence of adenomas during colonoscopy and autopsy compared to the frequency of colon cancer, indicating there must be a significant regression rate for adenomas[75].

Subclones in cancers

When infrequent positively selected mutations arise in a cell, this can provide the cell with a competitive advantage that promotes its preferential clonal proliferation, leading to cancer. The continued presence of epigenetically repressed DNA repair genes, carried along as passengers in the development of cancers, also predicts that cancers will contain heterogeneous genotypes (multiple subclones). For instance, as a test for the presence of subclones, in one primary

renal carcinoma with multiple metastases, 101 non-synonymous point mutations and 32 indels (insertions and deletions) were identified[76]. Five mutations were not validated and excluded from the study. Of the remaining 128 mutations, 40 were "ubiquitous" and present in each region of the tumor sampled. There were 59 "shared" mutations, present in several but not all regions, and 29 "private" mutations, unique to a specific region evaluated. The authors constructed a phylogenetic tree and concluded that the evolution in the tumor and its metastases was branching, and not linear.

A deficiency of DNA repair would likely produce genetic clonal diversity, through generation and selection for new mutational variants. In a study by Maley *et al*[77], 268 patients with Barrett's esophagus were followed for an average of 4.4 years during which 37 esophageal adenocarcinomas (EACs) developed. Genetic clonal diversity within Barrett's esophagus proved to be a better predictor of EAC than the presence of specific mutations in genes associated with EAC, such as mutation in *P53*. This finding suggests that DNA repair deficiency is of primary importance in progression to cancer.

EPIGENETIC REPRESSION OF DNA REPAIR GENES, DUE TO ALTERATIONS IN CPG ISLAND METHYLATION IN GI CANCERS

Table 4 gives examples of reports of DNA repair genes repressed by CpG island hypermethylation (or with increased expression due to CpG hypomethylation, which may cause unbalanced repair processes) in GI cancers (this is only a partial list). Nine different DNA repair genes (all listed among the 169 DNA repair and *DDR* genes previously identified[38]) were often hyper- (or sometimes hypo-) methylated in one or more GI cancer. Such alterations in methylation of promoter regions of DNA repair genes can cause deficient repair of DNA damages. Thus, hyper- (or hypo-) methylations of DNA repair genes are frequently important factors responsible for lack of appropriate repair of DNA damages. Faulty DNA repair leads to increased mutation and epigenetic alteration, central to progression to cancer.

DNA REPAIR GENE EXPRESSION MAY BE REPRESSED BY MULTIPLE PROCESSES

A number of the DNA repair genes with reduced expression due to CpG island hypermethylation are also epigenetically repressed by other means. Many protein coding genes are repressed by microRNAs. MicroRNAs (miRNAs) are small noncoding endogenously produced RNAs that play key roles in controlling the expression

Table 4 CpG island hyper- (and hypo-) methylation of DNA repair genes in cancers

Cancer	Gene	Frequency of promoter hyper- (or hypo-) methylation in cancer
Colorectal	*LIG4*	82%[78]
	MGMT	40%-90%[17-21]
	ERCC1	38%[79]
	WRN	29%-38%[63,80]
	MLH1	9%-10%[22,81]
	FEN1	Frequent (hypo-)[82]
	MBD4	Frequent (hyper-)[61]
Esophageal	*MGMT*	23%-79%[65,83,84]
	MLH1	43%[82], 64%[85]
	MSH2	29%[83], 75%[84]
Stomach	*MGMT*	88%[60]
	MLH1	73%[64]
	WRN	24%-25%[80,86]
	FEN1	Frequent (hypo-)[82]
Gastric lymphoma	*ATM*	11%[87]

of many cellular proteins. Once they are recruited and incorporated into a ribonucleoprotein complex, they can target specific messenger RNAs (mRNAs) in a miRNA sequence-dependent process and interfere with the translation into proteins of the targeted mRNAs *via* several mechanisms (see detailed review by Lages *et al*[88]).

As discussed above, when mismatch DNA repair protein PMS2 is deficient in colorectal cancer, this may be due to hypermethylation of its pairing partner MLH1, or due to over-expression of the miRNA miR-155 which targets the *MLH1* gene for repression.

While only 38% of cancers have CpG island methylation of the *ERCC1* promoter (Table 4), Facista *et al*[62] found that 100% of colon cancers have significantly reduced levels of ERRC1 protein expression. In the 49 cancers examined, ERCC1 protein expression varied from 0% to 45% (with a median value of 28%) of the level of ERCC1 expression of neoplasm-free individuals. It is likely that *ERCC1* can be repressed by more than one mechanism. A second mechanism of repression of *ERCC1* may be due to the combined effects of epigenetically deficient miRNA let-7a and resulting over-expression of HMGA2 protein, which then represses *ERCC1,* as discussed below.

As indicated by Motoyama *et al*[89], the let-7a miRNA normally represses the *HMGA2* gene, and in normal adult tissues, almost no HMGA2 protein is present. In breast cancers, for instance, the promoter region controlling let-7a-3/let-7b miRNA is frequently repressed by hypermethylation[90]. Reduction or absence of let-7a miRNA allows high expression of the HMGA2 protein. Regulation of gene expression by HMGA2 is achieved by binding to AT-rich regions in the DNA and/or direct interaction with several transcription factors[91].

HMGA2 targets and modifies the chromatin architecture at the *ERCC1* gene, reducing its expression[92]. As shown by Mayr *et al*[93], using an artificial construct,

the lack of let-7a miRNA repression of HMGA2 could occur through translocation of *HMGA2*, disrupting the 3' UTR of *HMGA2* which is the target of let-7a miRNA, and this can lead to an oncogenic transformation. However, the promoter controlling let-7a miRNA also can be strongly regulated by hypermethylation in intact cells. When human lung cells are exposed to cigarette smoke condensation, the promoter region controlling let-7a becomes highly hypermethylated[94]. It is likely that hypermethylation of the promoter for let-7a miRNA reduces its expression. This allows hyperexpression of *HMGA2*. Hyperexpression of *HMGA2* can then reduce expression of *ERCC1.* The combined effects of reduced let-7a miRNA and hyperexpressed *HMGA2* or other possible epigenetic mechanism(s) may cause the reduced protein expression of ERCC1 in colorectal cancers in addition to the 38% of colorectal cancers in which the *ERCC1* gene is directly hypermethylated.

DNA REPAIR PROTEINS AND MIRNAS

A review by Wouters *et al*[95] lists 74 DNA repair genes that are potentially targeted by miRNAs, and two additional reviews[96,97] list, combined, 30 miRNAs known to target DNA repair genes. The review by Wouters *et al*[95] used "in silico" computer programs (Targetscan and Mirbase) to identify likely miRNAs that could target their 74 DNA repair genes of interest, and, for each of these genes, indicated between 1 and 19 "conserved" miRNAs that were predicted to repress those genes. They define "conserved" miRNAs as miRNAs found in at least five mammalian species. However, about half of the miRNAs they found "in silico" were inducible by UV irradiation, and may have been controlled by transcriptional regulation and not by an epigenetic mechanism. Tessitore *et al*[96] and Vincent *et al*[97] each list about 20 miRNAs that are altered in cancers and which control expression of DNA repair genes. However, they did not indicate how these miRNAs are deregulated.

Deregulation of miRNA expression in cancers has been found to occur by epigenetic as well as non-epigenetic mechanisms[88,98]. One non-epigenetic mechanism includes alterations in genomic miRNA copy numbers and location. Some of these are deletions that include the miRNA clusters *15a/16-1* or *let-7g/mir-135-1,* or else amplification or translocation of the *mir-17-92* cluster. In some cancers miRNAs were deregulated because of defects in the biogenesis mechanism (the process of creating miRNAs, which has a number of steps). Some cancers have deregulated miRNAs due to single nucleotide polymorphisms (SNPs) in the genes coding for the miRNAs, or SNPs in the target gene area to which the miRNA is targeted. Some miRNAs, that target DNA repair genes, are regulated by oncogenes. For instance ATM is down-regulated by miR-421, but miR-421 is regulated by N-Myc[99]. Thus, not all instances of deregulation of DNA repair genes or DDR genes by miRNAs are due to epigenetic alterations affecting expression of the miRNAs.

EPIGENETIC REPRESSION OF DNA REPAIR GENES DUE TO ALTERATIONS OF METHYLATION OF PROMOTERS OF MIRNAS IN VARIOUS CANCERS

Table 5 lists nine miRNAs that have three characteristics: (1) their expression is epigenetically controlled by the methylation level of the promoter regions coding for the miRNAs; (2) they control expression of DNA repair genes; and (3) their level of expression was frequently epigenetically altered in one or more types of GI cancer. This list is not exhaustive. Many of the 30 miRNAs listed by Tessitore *et al*[96] or Vincent *et al*[97] might also meet these criteria upon further examination. Four of the miRNAs on this list are not noted by Tessitore *et al*[96] or Vincent *et al*[97]. Most of the studies of these epigenetically controlled miRNAs have not noted the frequencies with which their alterations occur in cancers. Thus, these studies are somewhat less systematic than those detailing methylation of DNA repair genes in Table 4. However, the nine epigenetically controlled miRNAs listed in Table 5 can repress the 16 DNA repair genes listed in Table 5 and these genes are repressed in various GI cancers.

WHOLE GENOME SEQUENCING INDICATES A HIGH LEVEL OF MUTAGENESIS IN GI CANCERS

Almost 3000 pairs of tumor/normal tissues were analyzed for mutations by whole exome sequencing (sequencing the protein coding parts of whole genomes) and more than a hundred pairs of tumor/normal tissues were analyzed for mutations by whole genome sequencing by Lawrence *et al*[120]. Median mutation frequencies for 27 different types of cancer were found to vary by 1000-fold. When there was a particular median mutation frequency for a type of cancer, the scatter of values (in individual cancers) for that type of cancer, above and below that median value, sometimes also varied by as much as 1000-fold. Some mutation frequencies in GI cancers, given as numerical values of median numbers of mutations per megabase in a review of the literature by Tuna *et al*[121], and recent values for esophageal cancers by Weaver *et al*[122], are shown in Table 6. The values were also converted to mutation frequency per whole diploid genome.

The mutation frequency in the whole genome [not just the exome (protein coding regions)] between generations for humans (parent to child) is about 30-70 new mutations per generation[123-125]. For protein

Table 5 Epigenetic ↑ or ↓ miRNAs, altered in cancers, targeting DNA repair genes

Specific miRNA	DNA repair gene targets	Cancers affected (frequency if measured)	References indicating epigenetic control of miRNA	References indicating target gene(s) of miRNAs	References indicating cancer type(s) affected
miR-103 miR-107	*RAD51,* *RAD51D*	Osteosarcoma, lung, endometrial, stomach	[100]	[101]	[101]
miR-34c	*UNG*	Gastric (70%) field defect gastric (27%) colon (98%) field defect colon (60%) chronic lymphocytic leukemia (18%) small-cell lung cancer (67%) NSCLC (26%)	[102,104]	[103]	[102,105,106]
miR-31	*PARP1* *MLH1* *SMUG1* *MMS19*	Esophagus (47%) colon	[72]	[21]	[71,107,108]
miR-124	*KU70*	Colon	[109]	[110]	[109]
miR-155	*RAD51* *MLH1* *MSH2* *MSH6*	Breast Colon	[90,111]	[23,112]	[23,90]
let-7a repression increases HMGA2; HMGA2 alters chromatin architecture of and represses ERCC1)	*ERCC1*	(Colon) Anaplastic astrocytoma	[90]	[92,113]	[113]
Let-7b repression increases HMGA1; HMGA1 targets P53	*P53*	Prostate Colon	[90]	[114,115]	[114,115]
miR-182	*BRCA1* *NBN* *RAD17*	Breast Colon	[116]	[117,118]	[107,117,119]

coding regions of the genome in individuals without cancer, Keightley[126] estimated there would be 0.35 mutations per parent to child generation. Whole genome sequencing was also performed in blood cells for a pair of monozygotic (identical twin) 100 years old centenarians[127]. Only 8 somatic differences were found between the twins, though somatic variation occurring in less than 20% of blood cells would be undetected. These findings, as well as the data summarized in Table 6, indicate that cancer cell lineages experience substantially higher mutation rates than non-cancer cell lineages.

EPIGENETICALLY REDUCED EXPRESSION OF DNA REPAIR GENES IN GI CANCERS OCCUR IN DIFFERENT REPAIR PATHWAYS

Figure 3[128] indicates some types of DNA damaging agents that may be encountered by cells in the GI tract, some of the DNA lesions they cause and the pathways used to repair these lesions. Many of the genes active in these pathways are included in Figure 3 and are indicated by their acronyms. The acronyms listed in red represent genes whose expression is frequently reduced due to epigenetic alterations in various types of GI cancers, as discussed above. Such reduced expression could be a substantial source of the genomic instability that is characteristic of these cancers.

THE CENTRAL ROLE OF DNA DAMAGE AND EPIGENETIC DEFECTS IN DNA REPAIR DURING PROGRESSION TO GI CANCER

The central role of DNA damage and epigenetic defects in DNA repair are illustrated in Figure 4[129]. When DNA damage results in epigenetic reduction in expression of one or more DNA repair genes, the resulting DNA repair deficiency can allow DNA damage to accumulate at a much increased rate. As indicated in Figure 3, at least 18 DNA repair genes that are frequently epigenetically deficient in one or more GI cancers have been identified. These epigenetic defects in DNA repair are often found to be present in field defects from which the cancers arose, so that such epigenetic reductions in DNA repair are likely early events in progression to cancer. A large increase in unrepaired DNA damage, due to an epigenetic reduction in DNA repair, can then lead to the large increase in mutation frequencies found in GI cancers (Table 6).

An epigenetic reduction of DNA repair may be the key early event that accelerates progression to cancer.

SELECTIVE TUMOR KILLING

DNA-damaging agents have a long history of use in cancer chemotherapy. As pointed out by Cheung-Ong *et al*[130], and indicated in the text earlier in this article,

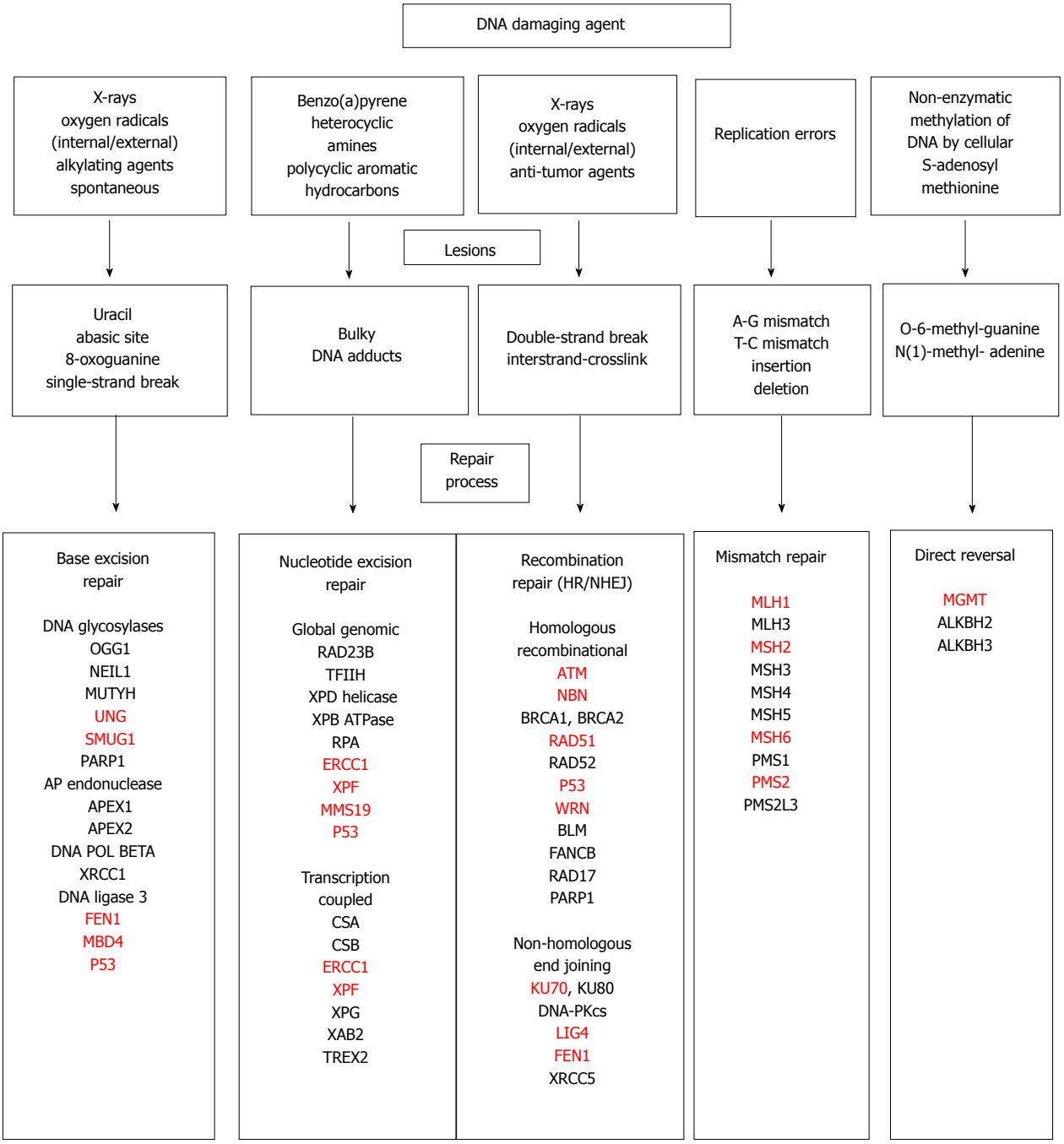

Figure 3 DNA damaging agents, the lesions they produce and the repair pathways that deal with the DNA damages, including acronyms for many of the genes in each of the pathways. Acronyms in red represent genes indicated in the text that have altered (usually reduced) expression due to an epigenetic alteration in one or more types of gastrointestinal cancer[128].

cancer cells are typically deficient in DNA damage-sensing/repair capabilities. That makes them more susceptible to DNA damage than normal cells. As Cheung-Ong *et al*[130] describe, both the earliest as well as the most frequent current cancer chemotherapeutic agents are DNA damaging agents.

A recently developing strategy for more effective and selective treatment of cancer is to inhibit one of the tumor's remaining DDR or DNA repair pathways. This can hyper-sensitize a tumor to radiation or chemotherapeutic agents, compared to the sensitivity of a tumor treated with a DNA damaging agent alone. This strategy is called synthetic lethality.

An early effort to implement synthetic lethality was the successful trial of Fong *et al*[131], in which a PARP inhibitor was given to germ-line mutated *BRCA* carriers. In this case, 12 of 19 (63%) of these patients in a Phase I trial had a clinical benefit from treatment with the PARP inhibitor olaparib alone, with no other chemotherapy. The patients in this Phase I trial had tumors that had been refractory to the 1 - ⩾ 4 therapies that had been tried previously. As noted by O'Sullivan *et al*[132], the BRCA proteins are active in the HRR pathway, and PARP is largely active in BER, though

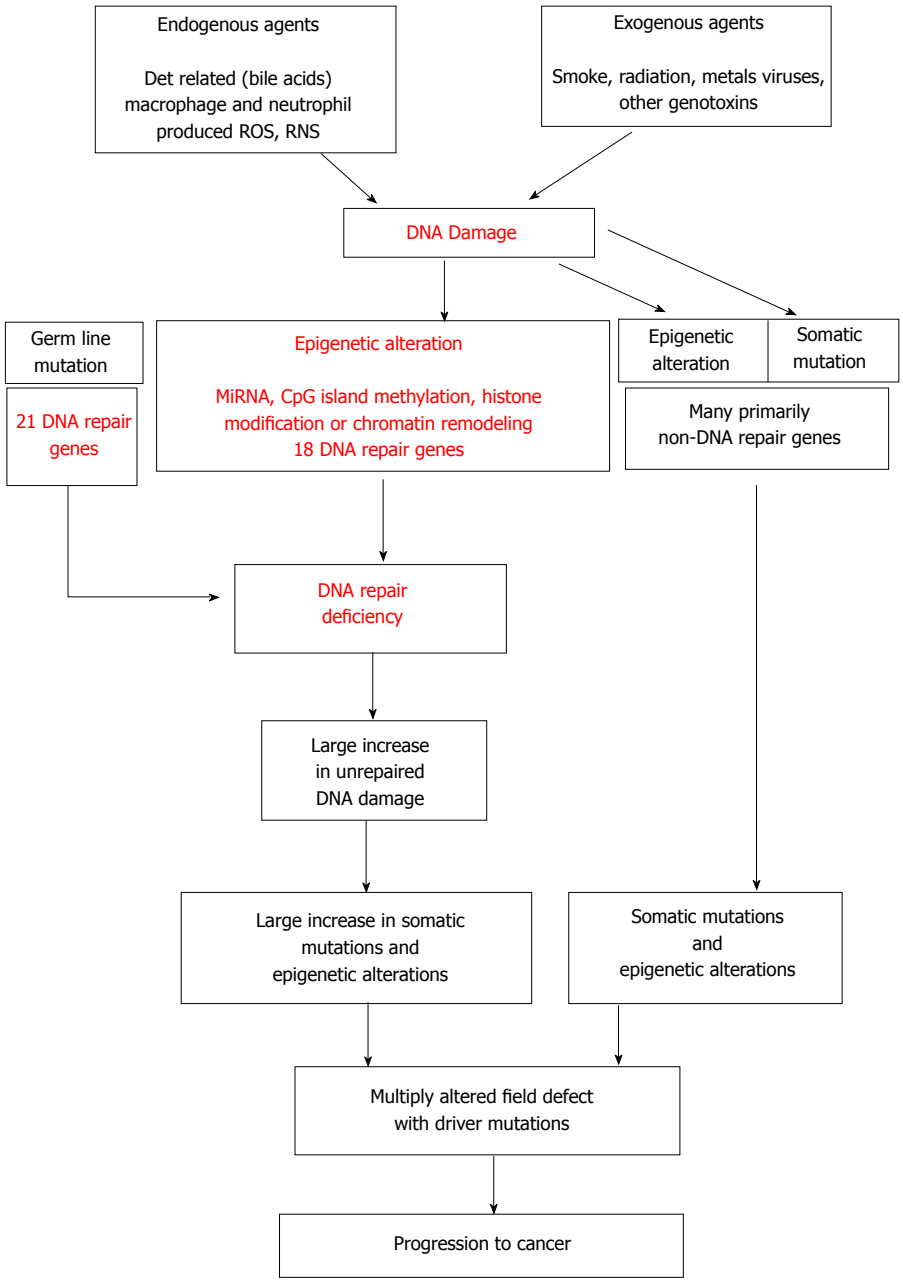

Figure 4 The central role of DNA damage and epigenetic alteration in DNA repair genes in gastrointestinal carcinogenesis[129].

it is also important in HRR. O'Sullivan *et al*[132] indicated that PARP inhibition appears to have synthetic lethality for both BRCA mutation-associated and "BRCA-like" solid tumors. As reviewed by O'Sullivan *et al*[132], PARP inhibitors are currently being evaluated in Phase Ⅰ and Phase Ⅱ trials of many different cancers, including GI cancers in pancreas, liver, colorectum, stomach and esophagus. They summarize some early quantitative results (in the range of 14% to 23% tumor regression or delayed progression) in pancreatic and colorectal cancers. McLornan *et al*[133] summarize positive results (tumor regression or delayed progression), often in the range of about 40% to 50%, with PARP inhibitors used in treatment of advanced solid tumors in other Phase Ⅰ and Ⅱ trials, including one on recurrent or metastatic gastric cancer.

Hosoya *et al*[134] listed a large number of synthetic lethality Phase Ⅰ and Phase Ⅱ trials that included not only PARP inhibitors but also inhibitors of DDR elements CHK1 and CHK2 and inhibitors of DNA repair elements DNA-PK and APE1. In addition they discuss interesting pre-clinical, potentially useful, synthetic lethal experiments with inhibitors of ATM/ATR and the MRN complex, DNA ligases, RAD51, RAD52 and histone deactylases.

Clinical applications of synthetic lethality are just beginning, as Phase Ⅰ and Ⅱ trials, but appear to be a new and potentially effective avenue for cancer therapy. How synthetic lethality may relate to epigenetically repressed DNA repair genes is currently unclear. The

Table 6 Median mutation frequencies and ranges

Parent/child per generation or cancer type	Mutation frequency per million bases	Mutation frequency per diploid genome
Parent/child per generation	0.00000023	30-70
Colorectal carcinoma	Approximately 5	Approximately 30000
MSS colon cancer	2.8	16800
MSI colon cancer (mismatch DNA repair deficient)	47	282000
Hepatocellular carcinoma	4.2	25200
Esophageal carcinoma (single nucleotide variants)	2.8 Range 0.7-9.3	16994 Range 4516-56528
Esophageal carcinoma (small insertions and deletions)		994 Range 262-3573

MSS: Microsatellite stable; MSI: Microsatellite instable.

epigenetic repression of DNA repair genes appears to be important for progression for many types of cancer, for cancer susceptibility to DNA damaging agents, and for increased cancer susceptibility to synthetic lethality. When Phase Ⅲ trials indicate which efforts at synthetic lethality are beneficial therapeutically, synthetically lethal down regulation of DNA repair pathways should be incorporated into standard medical treatments of cancers.

Evaluation of which DNA repair pathway(s) are epigenetically deficient in particular types of GI cancer and/or particular patients may prove useful in guiding choice of radiation, chemotherapeutic and/or synthetic lethality agent.

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Contents

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ORIGINAL ARTICLE

Observational Study

Locoregional therapy and systemic cetuximab to treat colorectal liver metastases

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Abstract

AIM: To investigate efficacy and safety of second-line treatment with irinotecan-loaded drug-eluting beads (DEBIRI) and cetuximab (DEBIRITUX) of unresectable colorectal liver metastases.

METHODS: Patients with the following characteristics were included in the study: unresectable hepatic metastases from colorectal carcinoma (CRC-LM), progression after first line chemotherapy (any type of chemotherapeutic drug and combination was allowed), second line treatment (mandatory), which included for each patient (unregarding the KRas status) two cycles of DEBIRI (using 100-300 μm beads loaded with irinotecan at a total dose 200 mg) followed by 12 cycles of cetuximab that was administered weekly at a first dose of 400 mg/m^2 and then 250 mg/m^2; good

performance status (0-2) and liver functionality (alanine aminotransferase and gamma-glutamyl transferase not exceeding three times the upper limit of normal, total bilirubin not exceeding 2.5 mg/mL). Data were collected retrospectively and included: tumor response (evaluated monthly for 6 mo then every 3 mo), overall response rate (ORR), KRas status, type and intensity of adverse events (G according to the Common Terminology Criteria for Adverse Events v3.0, CTCAE), overall survival (OS) and progression free survival (PFS).

RESULTS: Forty consecutive cases of CRC hepatic metastases were included in the study. Median duration of DEBIRITUX was 4.4 mo (range, 4.0-6.5). Sixteen patients (40%) received the planned 2 cycles of DEBIRI and an average of 10 cetuximab cycles. ORR of the whole sample was 50%, in particular 4 patients were complete responders (10%) and 16 (40%) partial responders. The most observed side effects (G2) were: post-embolization syndrome (30%), diarrhea (25%), skin rushes (38%) and asthenia (35%). The retrospective evaluation of KRas status (24 wild type, 16 mutated) showed that the group of patients with wild type KRas had ORR significantly higher than mutant KRas. Median follow-up was 29 mo (8-48 range); median PFS was 9.8 mo and OS was 20.4 mo. Future randomized trials are required in this setting to establish a role for DEBIRITUX compared with systemic chemotherapy.

CONCLUSION: DEBIRITUX seems to be efficacious after first line chemotherapy for the treatment of unresectable CRC-LM.

Key words: Cetuximab; Irinotecan-loaded drug-eluting beads; Hepatic metastases; Chemoembolization; Colon rectal tumor; Irinotecan

Core tip: Irinotecan-loaded drug-eluting beads (DEBIRI) has shown manageable toxicities and favorable response rates for unresectable colorectal liver metastasis (CRC-LM). This study is the first in the world investigating effectiveness and toxicity of the association DEBIRI and cetuximab (DEBIRITUX) as second line therapy of CRC-LM. Forty cases were enrolled. The overall response rate (ORR) was 50%. Most frequent side effects were: post-embolization syndrome, diarrhea, skin rushes and asthenia. The group of patients with wild type KRas had ORR significantly higher than mutant Kras. The median progression free survival was 9.8 mo and overall survival was 20.4 mo. DEBIRITUX regimen seems effective and safe after first line chemotherapy for CRC-LM.

Fiorentini G, Aliberti C, Sarti D, Coschiera P, Tilli M, Mulazzani L, Giordani P, Graziano F, Marqués Gonzalez A, García Marcos R, Gómez Mugnoz F, Cantore M, Ricci S, Catalano V, Mambrini A. Locoregional therapy and systemic cetuximab to treat colorectal liver metastases. *World J Gastrointest Oncol* 2015; 7(6): 47-54 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i6/47.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i6.47

INTRODUCTION

Colorectal cancer (CRC) is the second most commonly diagnosed cancer in Europe, with an annual incidence of nearly 450000 cases and an annual mortality of more than 200000 patients[1]. Nearly 25% of patients with CRC have synchronous liver metastases, whereas recurrence occurs in almost 70% of patients after resection of the primary tumor. Although surgery remains the only option for potential cure in patients with liver metastases from CRC, many patients have unresectable disease at diagnosis. The long-term survival rate of these patients is very low[2].

In the setting of unresectable metastatic CRC, the best outcome is achieved in patients receiving fluoropyrimidines, oxaliplatin, and irinotecan in their treatment plan[3,4]. Over the last decade, several combination of these three drugs significantly increased the response rate (RR) and overall survival (OS) time, with a RR of 40%-50% and a median OS duration up to 20 mo[5-9].

In a sequential strategy, however, one third of patients are not able to receive second-line chemotherapy due to side effects or liver progression.

The majority of patients with unresectable liver metastasis from CRC receive systemic chemotherapy with one or more agents. Combination chemotherapy regimens consist of fluoropyrimidines with oxaliplatin and/or irinotecan[3,6,10]. The administration of molecular targeted agents (bevacizumab, cetuximab, panitumumab) improve response rates and significantly prolongs median survival times in patients with metastatic CRC[11-13].

Recent advances focus on targeting the pathway of epidermal growth factor receptor (EGFR). The monoclonal antibody cetuximab is an EGFR antagonist that is capable of activating internalization of the receptor and its degradation, leading to increased tumor cell apoptosis[14]. Cetuximab, initially approved for clinical use in patients with detectable EGFR who failed on irinotecan chemotherapy[15], is nowadays widely used in combination with chemotherapy, because of its different toxicity profile in respect to the classical side effects of chemotherapy.

The role of intra-arterial chemotherapy of liver metastases is constantly evolving as the technique and its practical application improve[16-20]. Recent reports show that the application of DEBIRI to the intra-arterial therapy of liver metastasis from CRC (CRC-LM) is effective, feasible and has limited side effects[21-25]. Drug-eluting bead are small particles able to carry the chemotherapeutic agents directly to arterial vessels. In this way toxic agent concentration in the liver

tumors is increased, whereas the systemic exposure to drugs is decreased. The amount of cell death, moreover, is higher with DEBIRI than trans-arterial chemoembolization[26]. Since liver arteries are the main circulation of CRC-LM (90%), DEBIRI method can deliver elevate concentration of toxic agents inside the tumor, resulting in systemic low adverse events. Hence DEBIRI can be particularly useful in patients previously treated with other lines of chemotherapy.

We recently reported the data of FOLFIRI *vs* DEBIRI for the therapy of heavily previously treated patients with CRC-LM. The data analysis showed a statistically significant advantage of DEBIRI compared with FOLFIRI, in terms of OS, progression free survival (PFS), months to extra-hepatic progression, life quality[27]. The association of cetuximab and DEBIRI should be a further clinical research step for CRC-LM therapy, because irinotecan and cetuximab are efficacious and have acceptable, different and not cumulative toxicities. The purpose of our analysis is to assess effectiveness and toxicity of DEBIRI in association with intravenously cetuximab as second-line chemotherapy in unresectable CRC-LM.

MATERIALS AND METHODS

Patients selection

All patients within this study had histological confirmed non-resectable colorectal adenocarcinoma that was metastatic to the liver and were of age > 18 years. Other eligibility criteria were: good clinical conditions; tumor size evaluation with RECIST version 1.1; normal liver and renal functions; normal hematological values; one previous line of chemotherapy for metastatic disease at least 1 mo before DEBIRITUX; estimated life expectancy ⩾ 3 mo. Exclusion criteria were: contraindication to angiographic and selective visceral catheterization; presence of extra hepatic disease; brain or leptomeningeal metastases; bad absorption; inflammatory intestinal disease; psychiatric severe impairment; active infection; peripheral neuropathy ⩾ grade 2; pregnancy or breast feeding; previous cetuximab therapy; other severe clinical impairment.

This was a cohort study, data were collected from 40 consecutive eligible patients that had received the same second-line treatment: DEBIRITUX, notwithstanding the type of first line treatment and the KRas status.

Treatment evaluation

Data collected included: blood-cells levels, biochemistry, anamnesis, objective examination, tumor size (evaluated with abdomen and pelvis computed tomography scan), carcinoembryonic antigen (CEA) levels, life quality and performance status. Positron emission tomography scan was used upon researchers' decision to clarify disease extension. The above data were monitored before each DEBIRI, and every 4 cycles of cetuximab.

Treatment plan

Every patient in the study received the same second line treatment (DEBIRITUX), notwithstanding the type of first line treatment and the KRas status. At the beginning of DEBIRI, an interventional radiologist performed a diagnostic angiography to assess the level of CRC-LM arterial diffusion. DEBIRI treatment consisted in the infusion of 1 mL of DC beads microspheres (100 to 300 microns diameter) charged with irinotecan (100 mg), a second DEBIRI administration was repeated after 30 d, as reported in our previous experience[28-30].

The loaded DC beads were mixed with non-ionic contrast solution and distilled water, to perform a correct infusion.

Systemic Cetuximab (maximum 12 cycles) administration was done at 400 mg/m^2 and was planned one week after the first DEBIRI administration (day 1), on day 8 from study start, and continued on day 15, 22, 29. DEBIRI was repeated on day 36 and, then, cetuximab continued on day 43, 50, 57, 64, 71, 78, 85, 92 at a dose of 250 mg/m^2. Cetuximab was suspended according to physician opinion or in case of progression or unbearable side effects. Cetuximab dosage could be reduced to 200 or 150 mg/m^2 in cases severe side effects.

Safety and effectiveness assessment

National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 was used for side effects assessment, whereas the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1 was used for disease evaluation. CT scan was performed within 1, 3, 6 and 9 mo from the treatment completion according to RECIST indications[31-33].

Analysis of KRas mutation

The detections of *KRas* activating mutations (most frequently at codon 12 and 13) were done as previously reported[34]. Since some centers did not perform the KRas status analysis before treatment beginning, it was made retrospectively in the laboratory of the coordinating center of the study.

Statistical analysis

Kaplan-Meier analysis (MedCalc) was used for survival assessment; in particular, PFS was measured from beginning of DEBIRI to progression or death, whereas OS was computed beginning of DEBIRI to death or last follow-up date. OS analysis was performed for the whole sample, KRas wild type and KRas mutant group, to investigate differences related to KRas status.

χ^2 and Student's *t* test, were used to assess significativity of continous variables ($P < 0.05$).

RESULTS

Sample and tumor description

From April 2011 to December 2014, 40 patients were

Table 1 Patient characteristics

	DEBIRITUX
Number of patients	40
Sex (M-F)	24-16
Age	61 (range 47-74)
Liver involvement (≤ 25%-≤ 50%)	18-22
Synchronous/metachronous disease	0/40
Number of metastases	3.5 (range 3–9)
Largest diameter (cm)	5 (range 2.5-6)
Performance status (0-1 and 2)	25 and 15
Extrahepatic metastases	0
Previous CHT (1 line/2 lines for ≤ 12 mo)	23-17
Types of previous CHT	23 FOLFOX 11 IFL 3 FOLFOX + bevacizumab 3 FU + cetuximab
Weight loss in last 3 mo	16 (40%)
CEA (ng/mL)	90 (range 7.5-1250)
KRas (WT-M)	24-16
LDH (normal-high)	32-8
Albumin, g/dL (median)	4

DEBIRITUX: Irinotecan-loaded drug-eluting beads and cetuximab; CEA: Carcinoembryonic antigen; LDH: Lactate dehydrogenase; CHT: Chemotherapy.

enrolled from three centers, 24 (60%) were males and 16 (40%) females, with a median age of 61 years (range 47-74), 80% of sample had ECOG = 0. All patients had primary disease resection, 23 of them were treated with first-line chemotherapy for an average of 5 mo (range 3.5-6 mo); whereas 17 had one year of previous chemotherapy. The types of previous chemotherapy were reported in Table 1. Four patients had previous radiotherapy on the pelvis. Ninety percent of patients had increased CEA levels and 60% had values more than 10 times the upper limit range.

Treatment compliance

Every patient in the study received the same second line treatment (DEBIRITUX), notwithstanding the type of first line therapy. Median duration of DEBIRITUX was 4.4 mo (range, 4.0-6.5). Sixteen patients (40%) received the planned 2 cycles of DEBIRI and an average of 10 cetuximab cycles (9-12 range). Twenty-five patients (62.5%) had dose reduction of cetuximab because of toxicity, with an overall relative dosage of 85% of the planned dosage.

The most cases had several metastases (5 median; 1-8 range) and 28 (70%) had metastases in both liver lobes. Median diameter of largest lesion was 4.0 cm (range 2.0-6.5 cm), and total disease size was 8.8 cm (4-14 cm range). CRC-LM involved < 25% of liver in 28 (70%) cases, and 26%-50% in 12 (30%) patients.

Intra-arterial treatment

Most affected lobe was the right (24 patients, 60%), whereas the left lobe was treated in 6 patients (15), and 10 patients (25%) received a bilobar treatment. The planned dosage of irinotecan was 200 mg for all patients, however 8 patients (20%) required dose reduction, due to early stasis during the arterial administration of DEBIRI. The median irinotecan dose was of 80% of the planned dosage (range, 50%-90%). Partial occlusion of blood circulation was attained in 48 DEBIRI, and was almost total in 32.

Table 2 Advers events

Adverse events	*n* (%)
Acne-like skin rash	5 (50%)
Skin fissuring	3 (30%)
Skin dryness	3 (30%)
Hypersensitivity	3 (30%)

Safety

The majority of patients (90%) were hospitalized for treatment. Median hospitalization for DEBIRI was 48 h (24-72 range). Main significant effect on laboratory chemistry values was the increase in with blood-cells count from 6400 to 8700/mmc (26%). Median hemoglobin value decreased of 1%, from 12.5 to 12.0 g/dL after the treatment; median platelet count decreased of 2%, from 148 to 135 × 1000/mmc, median bilirubin value increased of 18%, from 0.8 to 1.2 mg/dL; median creatinine value decreased of 11%, from 0.9 to 0.8 mg/dL; and median albumin value decreased of 5%, from 4.1 to 4.0 g/dL. No changes were observed in INR.

Ten patients (25%) showed adverse reactions related to cetuximab. Four patients reported grade 3 adverse reactions, whereas 6 had grade 2 adverse reactions (Table 2).

Post-embolic syndrome was the main side effects, and was observed as a consequence of 30% of DEBIRI. Other treatment-related events included gastritis in 6 (15%) patients, dehydration (G2) in 2 (5%) patients, cholecystitis (G3) in 1 (2.5%) case, and hypertension (G2) for 7 (17.5 %) patients. These side effects, however, were resolved without complications. These symptoms were probably related to the post embolization syndrome (PES). Elevation of liver enzymes occurred almost in every patient, probably due to the more extensive type of embolization performed.

Efficacy, follow up and tumor response

Median follow-up was 29 mo (8-48 range). Overall response rate (ORR) was 50% after three months of therapy. Each patient, moreover, showed a > 50% reduction of CEA levels after 3 mo of treatment. This reduction was observed up to 6-mo of evaluation.

Twenty-five patients (60%) died because of disease progression. None died because of DEBIRITUX toxicity. Median PFS was 9.8 mo and OS was 20.4 mo, with 75.0% and 39.1% of patients alive at 1 and 2 years, respectively (Figures 1 and 2).

KRas analysis

Twenty-four patients (60%) were KRas wild type (WT),

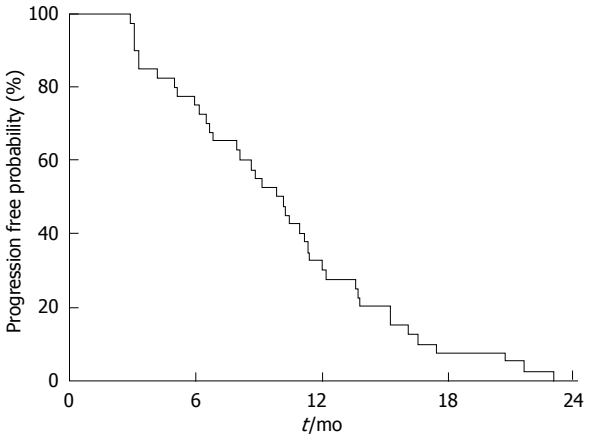

Figure 1 Kaplan-Meier survival curve for progression free survival.

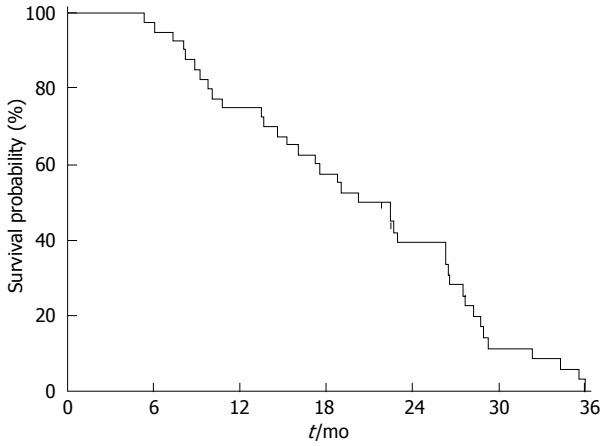

Figure 2 Kaplan-Meier survival curve for overall survival.

whereas 16 (60%) had a mutation in KRas (MUT). Patients of the KRas wild-type group achieved a better ORR than the KRas mutated group 70.8% (95%CI: 52.6-89.0) compared to 37.5% (95%CI: 13.8-61.2) respectively. The toxicity was the same for both groups. PFS was slightly increased in KRas-WT than KRas-MUT [11.3 and 9.9 mo, respectively; HR = 1.55 (95%CI: 0.790-3.054; *P* = 0.148)]. OS was 14.2 mo for KRas-MUT and 22.8 mo for KRas-WT (HR = 1.97; 95%CI: 0.965-4.050; *P* = 0.029) (Figure 3).

DISCUSSION

Surgical resection of CRC-LM is the preferred intervention, but it can be done only 20%-35% of cases[35]. Effective chemotherapy for CRC consists in fluoropyrimidines with oxaliplatin and/or irinotecan[36]. The use of biomolecular agents, such as bevacizumab, cetuximab and panitumumab, increments RR of 50%-80% and increases survival time up to 20-24 mo in CRC-LM[10-16,36].

Many CRC-LM are not indicated for surgery after a first-line chemotherapy or progress after multiple treatment. The second line treatment in these cases is still under discussion, and their prognosis is poor[7,37,38].

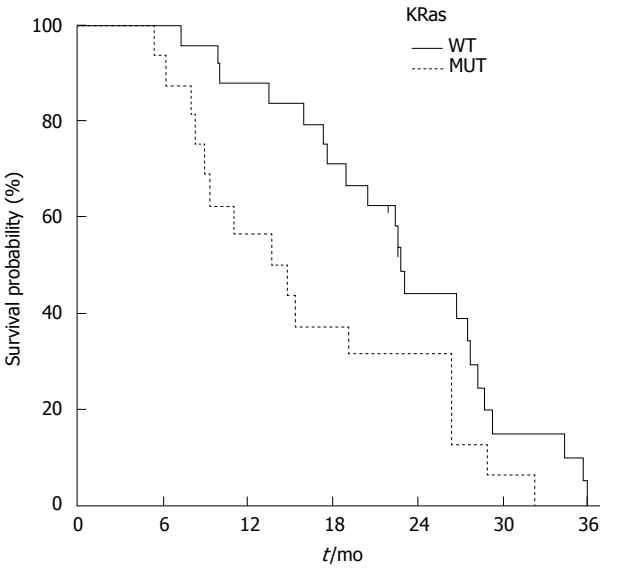

Figure 3 Kaplan-Meier survival curve for overall survival of KRas wild type and mutant. WT: Wild type; MUT: Mutation.

New cytotoxic and biomolecular drugs have been recently introduced for CRC-LM therapy, however, the use of locoregional therapy is increasing for the improvement of RR and OS[39]. The association of systemic and locoregional treatment ia an example of the modern concept of multidisciplinary CRC metastases management, requiring more collaboration with interventional radiologists[40].

The expected advantage of trans-arterial chemoembolization (TACE) is the increase of drug concentrations inside the tumor and the decrease of systemic leakage[39]. Promising results are obtained with TACE in CRC-LM[41].

The palliative role of TACE is assessed in large case series by Vogl *et al*[42]. They show that 463 CRC-LM patients are treated with TACE that is repeated each 4-wk (2441 total TACE; on average 5.3 TACE/patient)[42]. They use chemotherapy with mitomycin C alone, mitomycin C/gemcitabine or mitomycin C/irinotecan, and they perform the embolization with lipiodol and microspheres for vessel occlusion[42]. They show, in particular, partial response in 12% of patients, stable disease in 51% and progressive disease in 37%, with 1-year and 2-year OS in 62% and 38% of cases respectively[42].

Many efforts have made recently to improve TACE, expecially applying new toxic agents to the liver for a longer period. New polyvinyl alcohol beads are capable of being loaded with doxorubicin and irinotecan. They continuously release the drug in the liver after injection in the arterial vessels of the liver[40]. Embolization associated with the delivery of these particles allows to decreasing the flow inside tumor-feeding arteries and, then, the washout of drugs. This procedure can increase the dwell time of anticancer drugs in proximity of tumor cells. In 2007, our group reported the first experience with DEBIRI applied to twenty CRC-LM

patients, which had been previously treated with chemotherapy[23]. Most of the sample (80%) showed a response according to RECIST. As concerning toxicity, they all had G2 fever; 15 patients had G2/3 right upper quadrant pain[23]. Median hospitalization for the procedure was 3 d (1-10 range)[23].

Martin *et al*[43] published data from a trial with CRC-LM 55 patients, which showed a RR of 66% at 6 mo and 75% at 12 mo; OS of 19 mo and and PFS of 11 mo. Eleven (20%) patients had a significant tumor response and reduced disease intensity or stable response without progression that allowed resection, ablation or both[44].

Recently, we reported the results of a randomized trial on DEBIRI *vs* FOLFIRI after second or third lines of systemic chemotherapy in CRC-LM[45]. Patients treated with DEBIRI achieved a 43% OS improvement (22 *vs* 15 mo of FOLFIRI; $P = 0.031$), higher PFS (7 *vs* 4 mo of FOLFIRI; $P = 0.006$), an improved RR (68% *vs* 20% of FOLFIRI)[45]. DEBIRI resulted in increased toxicity (G3 pain, nausea, fever), because of post embolization syndrome[45]. Diarrhea, asthenia, leucopenia, anemia and alopecia were the most observed side effects of FOLFIRI[45]. We reported for the first time that DEBIRI improved OS, reducing cost treatments of systemic therapy. Noteworthy, the median life quality improvement was observed for 8 mo after DEBIRI and 3 after FOLFIRI ($P = 0.00002$).

In this paper we investigated for the first time the tumor response and toxicity of DEBIRI in association to cetuximab as second-line treatment of unresectable CRC-LM.

Our results showed that the association systemic/hepatic intra-arterial therapy was practicable and efficacious. An ORR of 50% was encouraging and comparable with previous reports[46]. The analysis of PFS and OS were also promising and further supported DEBIRITUX efficacy.

DEBIRITUX had low toxicity, showing only the known drug-related (irinotecan and cetuximab) toxicities. Diarrhea was the most observed adverse event, this was probably due to both irinotecan and cetuximab. The management of patients affected by CRC liver metastases with TACE and targeted agents was challenging, and resulted in interesting PFS and OS.

Cetuximab is a non-chemotherapeutic agent targeting the EGFR, is effective and is applied worldwide for the treatment of CRC with KRas wild type[47,48].

The aim and efficacy of loco-regional therapy of the liver is well known, however its several side effect (biliar stenosis, catheter displacement and consequent systemic leakage) may undermine its application[31-34]. DEBIRI may overcome the above disadvantages, because it is a more precise and direct method, resulting in mild intensity general side effects[21-33]. DEBIRI can also be combined with therapies involving monoclonal antibodies, such as cetuximab.

Our results do not show any severe cetuximab or irinotecan general side effects. Most common adverse events are related to the PES, including pain, nausea, and hypertension.

DEBIRI can be performed also after several previous line of therapy, provided that an adequate supportive therapy is guaranteed. This point is crucial as the duration of hospitalization may be limited and tolerability of treatment (less abdominal pain, discomfort, serum transaminases level elevation) may be improved.

KRas analysis, unfortunately, was not performed at the beginning of the treatment, but it was done retrospectively in the central laboratory of the coordinating center. This may have affected the RR, since cetuximab is not efficacious in KRas mutated cases. We show a possible correlation of KRas status and response to DEBIRITUX, this is supported also in other reports[35-39].

In conclusion, DEBIRITUX is efficacious and induces low toxicity for CRC-LM therapy, as it appears from the few side effects that was observed and the high RR, and the prolonged decrease of CEA. More clinical trials are required to address this issue, and to assess when DEBIRITUX should be applied in the therapeutic sequence of CRC-LM. This study is the first to our knowledge that confirms the efficacy of systemic plus intra-arterial therapy association in the management of CRC-LM.

COMMENTS

Background

Colorectal cancer (CRC) is nowadays one of the principal health care concerns and leading tumor in Europe and United States. Surgical resection is feasible only for 20% of patients with liver metastases (LM). The recently introduced loco-regional therapy with drug-eluting beads plus irinotecan (DEBIRI) shows good tumor reduction in unresectable CRC-LM that were previously treated with 2 or more chemotherapy regimens.

Research frontiers

Cetuximab is a monoclonal antibody that antagonize the epidermal growth factor receptor and is widely used in combination with chemotherapy because of its different toxicity profile in respect to the classical side effects of chemotherapy. Cetuximab associated to irinotecan has been recently approved for CRC-LM treatment, as second line therapy. This study investigated the efficacy and safety of the association cetuximab/DEBIRI (DEBIRITUX) as second-line therapy of CRC-LM.

Innovations and breakthroughs

The aim and efficacy of loco-regional therapy of the liver is well known, however its several side effect. DEBIRI has the potential of overcome this disadvantages, since it can be a safer and more direct method, resulting in mild intensity general side effects, as reported in the literature. DEBIRI can also be combined with therapies involving monoclonal antibodies, such as cetuximab. This study is the first to the knowledge that confirms the activity of the combination of systemic plus intra-arterial therapy of CRC-LM.

Applications

DEBIRITUX is efficacious and induces low toxicity for CRC-LM therapy, as it appears from the few side effects that was observed and the high response rate, and the prolonged decrease of carcinoembryonic antigen. More clinical trials are required to address this issue, and to assess when DEBIRITUX should be applied in the therapeutic sequence of CRC-LM.

Terminology

DEBIRI is an intra-arterial therapy adopting drug-eluting beads preloaded with irinotecan. Drug-eluting bead are small particles able to carry the

chemotherapeutic agents directly to arterial vessels of the metastases. In this way the toxic agent concentration in the liver tumors is increased, whereas the systemic exposure to drugs is decreased. The volume of tissue necrosis, moreover, is significantly greater when using drug-eluting beads compared to a conventional trans-arterial chemoembolization. Since liver arteries are the main sustainance of CRC-LM (90%), DEBIRI method can deliver elevate concentration of toxic agents inside the tumor, resulting in systemic low adverse events.

Peer-review

The authors investigated the efficacy and safety of the addition of cetuximab to DEBIRI (DEBIRITUX) as second-line treatment in patients with unresectable liver metastases from CRC. It interesting that the regimen combined with DEBIRI and cetuximab appear to be effective and feasible in second line treatment.

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EDITORIAL

Low rectal cancer: Sphincter preserving techniques-selection of patients, techniques and outcomes

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Abstract

Low rectal cancer is traditionally treated by abdominoperineal resection. In recent years, several new techniques for the treatment of very low rectal cancer patients aiming to preserve the gastrointestinal continuity and to improve both the oncological as well as the functional outcomes, have been emerged. Literature suggest that when the intersphincteric resection is applied in T1-3 tumors located within 30-35 mm from the anal verge, is technically feasible, safe, with equal oncological outcomes compared to conventional surgery and acceptable quality of life. The Anterior Perineal PlanE for Ultra-low Anterior Resection technique, is not disrupting the sphincters, but carries a high complication rate, while the reports on the oncological and functional outcomes are limited. Transanal Endoscopic MicroSurgery (TEM) and TransAnal Minimally Invasive Surgery (TAMIS) should represent the treatment of choice for T1 rectal tumors, with specific criteria according to the NCCN guidelines and favorable pathologic features. Alternatively to the standard conventional surgery, neoadjuvant chemo-radiotherapy followed by TEM or TAMIS seems promising for tumors of a local stage T1sm2-3 or T2. Transanal Total Mesorectal Excision should be performed only when a board approved protocol is available by colorectal surgeons with extensive experience in minimally invasive and transanal endoscopic surgery.

Key words: Low rectal cancer; Sphincter preserving surgery; Intersphincteric resection; Anterior Perineal PlanE for Ultra-low Anterior Resection of the Rectum; Total mesorectal excision; TransAnal Minimally Invasive Surgery; Transanal Total Mesorectal Excision; Quality of life; Oncological outcome; Functional outcome

Core tip: The present review presents the most recent advances in the field of sphincter preserving surgery for the treatment of low rectal cancer patients, providing indications, patients' selection, surgical techniques, multimodality approaches, postoperative course and oncological and functional outcomes. In particular, the review focuses on data deriving from prospective

studies, systematic reviews and meta-analyses. The conclusion makes clear that a customized approach based on current guidelines, as well as specific pathological prognostic factors, is mandatory for obtaining the maximum favorable outcome in each patient.

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INTRODUCTION

Low rectal cancer is defined as any tumor lying in < 5 cm from the anal verge[1]. For more than twenty years, the most fundamental advance in rectal cancer surgery was the advent of total mesorectal excision (TME), proposed by Heald *et al*[2] in 1982. Although TME never compared to the traditional surgical approaches in a prospective randomized fashion, TME demonstrates clear superiority in terms of local recurrence and survival as compared to historical controls[3]. Properly conducted TME reduces the recurrence rate to < 10% and increases overall 5-year survival to over 80%[4]. The Dutch TME trial[5] confirmed the above results, clearly stating an increased risk of local tumor recurrence for patients who had undergone a potentially curative procedure with incomplete mesorectal excision, as compared to patients in whom the specimen showed a completely resected mesorectum[6]. Laparoscopy offers better visualization of the pelvic cavity and therefore facilitates mobilization of the rectum[7]. Although laparoscopic TME is a standardized and reproducible procedure[8], it can be proved a technically difficult operation[9]. In the UK MRC CLASICC trial[10], a high incidence of positive circumferential radial margin (CRM) after laparoscopic anterior resection was observed. Tumor location in the mid and distal rectum may be considered *per se* as an important risk factor for compromised CRM[11,12].

A positive CRM (< 1 mm), places the patient at great risk for local failure and distant metastases, thus reducing the overall survival[13]. Adoption of TME resulted in decreased CRM positivity[3].

Another change in the rectal cancer surgical management was the re-evaluation of the length of distal resection margin (DRM). A 2-cm margin is quite adequate because distal intramural spread and/or retrograde lymphatic extension are rare[14]. Even more, a recent systematic review of 17 studies[15] found no negative impact of DRM < 1 cm or even < 5 mm in terms of local recurrence or overall survival in patients with good risk tumors.

Adoption of TME, tolerance of shorter DRM, and availability of circular stapling devices, have dramatically decreased the abdominoperineal resection (APR) rates.

However, pooled analysis of 14 European rectal cancer studies[16] disclosed 10% positive CRMs, 20% local recurrence rates and 59% 5-year survival for patients who had undergone APR, compared to 5% positive CRMs, 11% local recurrence rates and 70% 5-year survival for patients who had undergone LAR, concluding that the oncological outcome following APR is not superior or at least equal to the LAR, proposing that the inferior outcomes following APR could be due to deficiencies in the surgical technique and/or tumor characteristics.

In recent years, several new techniques for the treatment of very low rectal cancer patients aiming to preserve the GI continuity and to improve both the oncological as well as the functional outcomes, have been emerged. In the present article we present these new techniques providing evidence based data for the oncological and functional outcomes of each of them.

INTERSPHINCTERIC RESECTION

Selection of patients

The selection of patients who may benefit from the intersphincteric resection (ISR) should be based on the results of magnetic resonance imaging (MRI), computed tomography, endoanal ultrasonography, rigid proctoscopy and digital examination[17]. Particularly, digital examination under anesthesia is important for evaluating tumor mobility, tumor relation to the anal sphincter and final decision making[18-20].

A recent systematic review[21] addressed that the method should be ideally applied in T1-3 tumors located within 30-35 mm from the anal verge, with or without internal anal sphincter (IAS) invasion[22].

Absolute contraindications for the method are T4 tumors, invasion of external anal sphincter (EAS), fixed tumors in digital examination (indication that the tumor has broken through the intersphincteric plane), poorly differentiated tumor, poor preoperative sphincter function, distant metastases and presence of mental disease[23].

Surgical technique

ISR was firstly described by Schiessel *et al*[24] in 1994 and the principle of the technique is based on the dissection of the anatomical plane between the IAS, which is the prolonged muscular layer of the rectum, and the EAS. The technique is aiming to increase the preservation of sphincter and to avoid the need for a permanent stoma for low rectal cancer tumors.

The operation consists of an abdominal and a perineal phase. The abdominal phase starts with high ligation of the inferior mesenteric vein and the inferior mesenteric artery immediately after the emergence of the left colic artery[25]. In order to accomplish that, the peritoneum above the inferior mesenteric

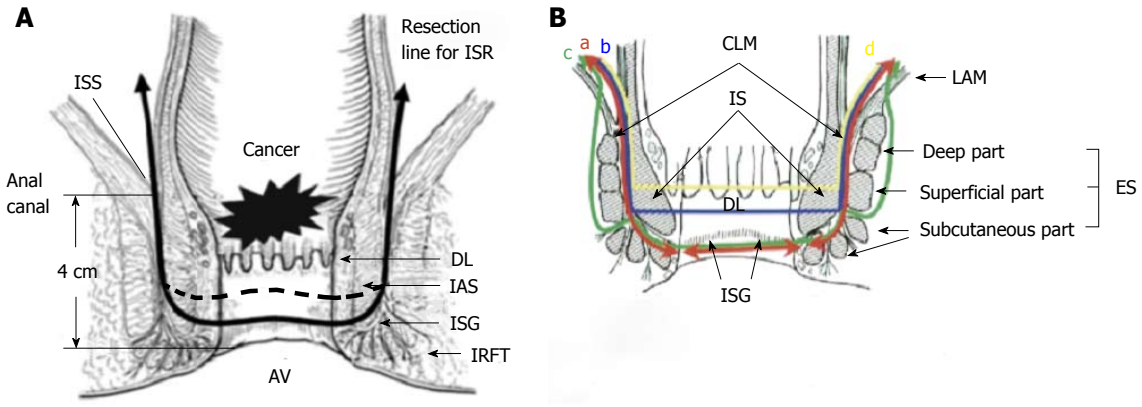

Figure 1 Schematic presentation of the perineal phase of intersphincteric resection. A: Akagi *et al*[37]; B: Saito *et al*[49]. a: Total ISR; b: Subtotal ISR; c: ISR + PESR; d: Partial ISR. CLM: Conjoined longitudinal muscle; ISG: Intersphincteric groove; LAM: Levator ani muscle; IS: Internal sphincter; ES: External sphincter; DL: Dentate line; AV: Anal verge.

vessels is divided, and left mesocolon is mobilized *via* mesofascial separation[26]. After the ligation of the vessels, the parasigmoid and pararectal peritoneal folds are divided and the mesosigmoid is mobilized *via* mesofascial separation. Mesosigmoid is continuous with left mesocolon above and mesorectum below[27]. The dissection continues in the mesorectal plane, with the separation of the mesorectum from adjacent mesorectal fascia[2]. Although not always necessary, mobilization of the splenic flexure might be required[28]. Splenic flexure mobilization, requires freeing of both mesocolic and gastrointestinal components[29]. The dissection of left mesocolon, mesosigmoid and mesorectum *via* mesofascial separation, allows the removal of the specimen with intact fascial layers whilst simultaneously maximizing lymph node yield[27].

Laparoscopic, open[30] and robotic[31] approaches have been used for the abdominal phase.

For the perineal phase, the patient is placed in the high lithotomy position, a self-retaining retractor is applied for perineal exposure[28] and 1 mg epinephrine diluted in 20 mL of saline solution is injected at several points beneath the anal mucosa, for minimization of bleeding and facilitation of intersphincteric dissection[32]. A circumferential incision in the anal mucosa, at a distance of at least 1 cm from the macroscopic distal edge of the tumor for T1 lesions and 2 cm for T2-3 lesions is made in such a way, to include in the specimen the whole rectal wall as well as a part or the whole of the IAS[32] (Figure 1). The anal orifice is then closed transanally with purse string sutures to prevent tumor cell dissemination during the perineal approach[22]. Under direct vision, the dissection is continued cephalad through the intersphincteric space to be connected with the TME plane developed transabdominally[3]. The specimen is usually delivered per anus. The continuity of GI tract is then restored by a hand-sewn coloanal anastomosis. Many types of hand sewn anastomosis have been used such as, colonic J-pouch, transverse coloplasty, or straight coloanal hand-sewn anastomosis, according to surgeons preference[22]. Finally, diverting ileostomy or colostomy is performed[21].

There are three types of ISR (partial, subtotal or total) depending on the extent of the IAS resection. Therefore, partial ISR is defined as a one-third resection of the upper part of the IAS, subtotal ISR as a two-third resection of the IAS, and total ISR as a complete resection of the IAS[21]. Combined resection of the EAS (external sphincter resection, ESR) is sometimes performed for tumors with suspected invasion into the intersphincteric space and/or external sphincter muscles[33,34].

ISR differs from conventional coloanal anastomosis performed after ultra low anterior resection, because it's characterized by resection of internal sphincter by dissection in the intersphincteric plane[35].

Early postoperative outcome

Operative mortality varies between 0% and 1.7%, while postoperative morbidity rate ranges between 8% and 64%[22]. Main causes of morbidity are anastomotic leakage, anastomotic stricture, fistula formation, pelvic sepsis development, wound complications, bleeding and ileus[22]. Particularly anastomotic leakage, has been reported as related to postoperative anastomotic stricture formation, cancer recurrence, poor postoperative function and increased operative mortality[36]. In a meta-analysis by Martin *et al*[17], a 25.8% cumulative morbidity rate is reported, with an anastomotic leak rate of 9.1% and a pelvic sepsis rate of 2.4%. Akagi *et al*[37], reported Dindo Grade Ⅱ complication rate of 12% and anastomotic leak rate of 5.6%, while Saito *et al*[38], reported a 10% leak rate.

Oncological outcome

Tilney and Tekkis[36] performed a literature search to identify studies reporting outcomes following ISR. Twenty-one studies, accumulating a total of 612 patients were identified. The pooled rate of local

recurrence was 9.5%, the average 5-year survival was 81.5%, while distant metastases occurred in 9.3%.

In Martin *et al*[17]'s systematic review, the mean distal free resection margin was 17.1 mm (range: 12-29 mm), a CRM negative margin was achieved in 96% (range: 89%-100%) of the patients, an R0 resection was performed in 97% of the patients, the overall local recurrence rate was 6.7% (range: 0%-23%), the 5-year disease-free survival rate was 78.6% (range: 69%-87%) and the 5-year overall survival was 86.3% (range: 62%-97%), at a median follow-up of 56 mo.

A large prospective study published in 2013[37], with 124 patients with low rectal T1-3 tumors without preoperative chemo-radiotherapy (CRT), showed total (local and distant) postoperative recurrence rate of 16.1%, including 4.7% at stage Ⅰ, 19.5% at stage Ⅱ and 25% at stage Ⅲ disease. Local recurrence (including recurrence in anastomotic site, lateral lymph nodes and pelvic floor recurrence) occurred in 4.8% of patients, including 4.7% at stage Ⅰ, 4.9% at stage Ⅱ and 5.0% at stage Ⅲ disease. There was no anastomotic site recurrence. Lateral lymph node metastasis was observed in 2.4% of patients (2% at stage Ⅰ, 2.4% at stage Ⅱ and 2.5% at stage Ⅲ). Pelvic floor recurrence was also found in 2.4% patients (2% at stage I, 2.4% at stage Ⅱ, and 2.5% at stage Ⅲ). The rate of distant metastasis was 10.5% in total. The cumulative recurrence-free 5-year survival rate at each stage was 92.2%, 81.9%, and 69.6% respectively and the cumulative cancer-specific 5-year survival rate at each stage was 90.5%, 91.0% and 83.6%, respectively. The overall 5-year survival rate at each stage was 84.2%, 85.2%, and 78.6% respectively. Moreover, the authors compared the oncologic outcomes after ISR with those after APR performed during the same period. No significant differences were noticed in the pathological stage distribution between the two operative methods. The overall recurrence-free survival and local recurrence rates after ISR were similar to those after APR.

In Saito *et al*[38]'s prospective study, 199 patients underwent ISR. Among them 25% had undergone neoadjuvant CRT and 20.6% underwent concomitant partial EAS resection. After a median follow-up of 6.5 years (range: 12-164 mo), pulmonary metastases were occurred in 14.1%, local recurrence with or without distant metastasis in 13.6%, liver metastasis in 7.5% and combined recurrence in 4.5% of the patients. Positive CRM was reported as high as 19.6%, but T4 tumors were also included in the study (19 T4 tumors out of 199). The estimated 7-year overall survival, disease free survival and local relapse free survival rates were 78%, 67%, and 80%, respectively.

Most of the studies comparing LAR, APR and ISR[19,37,39], concluded in non-statistically significant differences regarding their oncological outcome, although Saito *et al*[40] disclosed that the 5-year overall survival was worse in APR than in ISR (61.5% *vs* 80%).

In a recent study[41], 77 patients who underwent ISR, compared to 33 patients who underwent APR and 68 patients who underwent LAR. There were no significant differences in the disease stage status between the ISR and the LAR group of patients, although in the APR group more advance TNM stage was noticed. Overall recurrence (both local and distant), was noticed in 7.8% of the patients after ISR compared to the 11.7% in the LAR group and to the 12.1% in the APR group (P = 0.67). The local recurrence rate was 2.6% for the ISR group of patients compared to the 5.9% in the LAR group and to the 6.1% in the APR group (P = 0.57). With a median follow-up of 69 mo (range: 56-87), the 5-year local recurrence-free survival was 93.5% for the ISR group, 88.2% for the LAR group and 87.9% for the APR group; although these differences were not statistically significant. The 5-year overall survival rate after ISR was 76.4%, better than the APR (51.2%) and similar to the LAR (80.7%), probably reflecting the higher frequency of advanced cancers in APR group of patients. The 5-year overall survival rate according to the TNM stage in patients who underwent ISR was 90.0% for stage Ⅰ, 79.8% for stage Ⅱ, and 65.6% for stage Ⅲ. In stage Ⅲ patients, the 5-year overall survival rate for the ISR, LAR, and APR groups was estimated at 65.6%, 56.3%, and 33.3%, respectively (P = 0.02). These long-term results suggest that ISR is a suitable technique based on the oncologic outcome. However, T3 tumor and patients with positive microscopic resection margins were more likely to have local recurrence after ISR[42,43].

Finally, the CRM is a powerful indicator for local recurrence[44] and the CRM around the anal canal has been proposed as a risk factor for local recurrence when ISR is performed[21]. The group of patients with positive CRM displayed significantly worse overall survival, disease free survival and local relapse free survival rates than the negative CRM group of patients (P < 0.001)[38].

Other risk factors for local recurrence include, de-differentiation of the tumor, and preoperative CA19-9 levels above 37 U/mL[45], while pathological N1 and N2 tumor and poorly differentiation of the tumor, have been reported as risk factors for distant recurrence[45].

Functional outcome: Quality of life

Although postoperative anal function represents a particularly important clinical outcome measure after sphincter-preserving surgery for lower rectal cancer, only few studies have reported short-term postoperative results[46-51]. After ISR, resting pressure is not greatly restored, but gradually recovers overtime[18,47]. In contrast, the maximum squeeze pressure is not affected. Anal function seems to improve over time[49,52].

Köhler *et al*[53] reported a 29% reduction in resting anal pressure following ISR, while the squeeze pressure recovered to preoperative levels after 12 mo.

In Martin's *et al*[17] systematic review, the mean number of bowel movements per day was 2.7. Nearly half (51.2%) of the patients reported "perfect continence", about a third (29.1%) experienced fecal soiling, 23.8% had flatus incontinence and 18.6% had urgency.

In a large study assessing functional outcomes after ISR, Denost *et al*[54] reported that half of the patients had a "good functional result", 39% had minor fecal incontinence and 11% had major incontinence.

Saito *et al*[38] reported the long term functional results of 199 patients. In this study mean stool frequency per 24 h was 4.0 ± 3.7 and the median Wexner score was 8.5 (range: 0-20) in all patients at 5 years after stoma closure. Approximately 50% of patients had stool fragmentation and incontinence to gas, 30% still experienced soiling during the day and at the nighttime, while a quarter suffered from difficulties in evacuation. Multivariate analysis disclosed male gender and preoperative CRT as independent factors predisposing to a worse continence score, although the type of surgery (partial or total ISR) did not affect the long-term functional outcomes. Similarly, Ito *et al*[51] reported that preoperative CRT was the risk factor with the greatest negative impact on anal function after ISR.

In contrast, multivariate analysis in Yamada *et al*[32]'s study disclosed patient's age at surgery as the only risk factor for postoperative fecal incontinence.

Bretagnol *et al*[48] reported that frequency, urgency, the Wexner score and the Fecal Incontinence Severity Index were significantly improved following colonic J-pouch reconstruction, compared to the straight coloanal anastomosis.

Denost *et al*[54] reported that the risk of fecal incontinence after ISR was directly related to the tumor level and the height of the anastomosis, stating that for a "good" continence result, distance of tumor greater than 1 cm from the anorectal ring and anastomosis higher than 2 cm from the anal verge, are required.

In a recent study[41], comparing the functional outcomes following ISR and LAR, the authors disclosed that the postoperative defecation functions in terms of the frequency of defecation, urgency, ability to distinguish gas emission and perianal skin irritation, were equal between the two techniques, the Wexner score was lower in the LAR group, but no significant difference was observed in the fecal incontinence quality of life (FIQL) score between the ISR and LAR.

Bretagnol *et al*[48] using both SF-36 and FIQL questionnaires to compare quality of life (QoL) between patients undergoing ISR and conventional coloanal anastomosis, found no differences in the QoL between the two groups throughout the physical and mental subscales of the SF-36.

Saito *et al*[38] reported that patients after ISR with or without partial EAS excision have QoL at 5 years equal to or better than those preoperatively, but patients with preoperative CRT showed significantly worse FIQL scores after long-term follow-up.

Conclusion

ISR was developed as an alternative to the classical surgical approaches for the treatment of low rectal cancer patients who might be benefited from a sphincter preserving technique and who otherwise, should have undergone an APR. Literature results suggest that when the method is applied in T1-3 tumors located within 30-35 mm from the anal verge with or without IAS invasion, is technically feasible, safe (in terms of early postoperative outcome), with equal oncological outcomes compared to LAP and APR and acceptable QoL. APR should be reserved for locally advanced tumors.

ANTERIOR PERINEAL PLANE FOR ULTRA-LOW ANTERIOR RESECTION OF THE RECTUM

Selection of patients

The Anterior Perineal PlanE for Ultra-low Anterior Resection of the Rectum (APPEAR) procedure was developed to allow sphincter-preserving rectal resection for both benign and malignant pathology, which would traditionally required APR or completion proctectomy, if treated by conventional means[55]. In recent case reports, APPEAR is indicated for patients with low rectal cancer, 2-5 cm from the anal verge[56,57].

Surgical technique

The APPEAR technique, was firstly described by Williams *et al*[55] in 2008. The technique consists of abdominal and perineal approach, allowing access to low and difficult to be reached rectum between the levator ani muscle and the superior margin of EAS.

The abdominal phase of the operation is the same as the abdominal phase of ISR and is described above. The abdominal phase can be performed either laparoscopically or open.

For the perineal phase, the patient is placed in a high lithotomy position and the rectovaginal/prostatic plane is infiltrated with 1 in 300000 adrenaline saline solution. A convex crescentric skin incision is made in the perineum midway between the vagina or the base of the scrotum and the anal verge. The skin and subcutaneous tissue are dissected from the underlying external anal sphincter and transverse perinei muscles, and reflect forwards. In the female, the plane between the posterior vaginal wall and the anterior rectal wall is entered anteriorly to the perineal body. In the male, the rectourethral/prostatic plane is entered similarly, firstly isolating and then dividing bilaterally the rectourethralis muscle, close to the rectum. After dividing the rectourethralis muscle, the anterior rectal

wall is mobilized from the prostate, using both blunt and sharp dissection. At the inferolateral aspect of the prostate, the dissection is performed close to the rectum, avoiding damage to the neurovascular bundles. The perineal dissection is continued cephalad until the plane created from above by the abdominal operator is reached. The rectum is then freed laterally and the specimen is delivered through the perineum. The continuity of GI tract was established with either straight coloanal anastomosis or a short colonic pouch, with protecting ileostomy[55].

Early postoperative outcome

Both in the initial pilot study[55] as well as the latter case report studies[56,57], no mortality was reported. The main postoperative complication was perineal wound infection with an incidence ranging from 15.4% to 60% and subsequent colonic/ileoanal pouch perineal fistulation in some of the patients[55-57]. Anastomotic stricturing occurred in 3 patients in the pilot study[55].

Oncological outcome

Oncological outcome is documented in only two studies[55,57]. In the pilot one[55], only half of the patients (7 out of 14) had rectal cancer, the median DRM was 20 mm (range: 10-37 mm) and the median circumferential resection margin was 5 mm (range: 2-21 mm). No local recurrence was noted within a median follow up of 2 years, but one patient developed distant metastases. In the recent one, no recurrence was documented, but the median follow up was only 11 mo[57].

Functional outcome: QoL

Functional outcome were also documented in only two studies[55,57]. In the pilot one, the median Wexner score after ileostomy closure was 5 in the patients treated with coloanal anastomosis, all patients were continent to solid and liquid stool, with only one patient reporting fragmented evacuation and three patients reporting fecal urgency[55]. In the later study, the average Wexner score was 5.5 after ostomy closure[57]. Both articles showed normal resting and squeezed pressures after the APPEAR. QoL was reported in only one study[55] with the use of SF-36 questionnaire, disclosing no significant changes following the APPEAR procedure after the ileostomy closure.

Conclusion

Compared to ultra-low anterior resection, the APPEAR technique has the advantage of providing greater distal access to the rectum for mobilization and compared to ISR, has the advantage of not disrupting the sphincters[3]. However, the complication rate is high, mainly related to perineal wound infection, while the reports on the oncological and functional outcomes are limited. More studies are needed for evaluation of the technique.

LOCAL EXCISION TECHNIQUES: TRANSANAL ENDOSCOPIC MICROSURGERY AND TRANSANAL MINIMALLY INVASIVE SURGERY

Selection of patients

Both methods are primarily used for local excision of lower, middle and upper rectum benign tumors *via* the anus[58,59].

Literature addresses that Transanal Endoscopic Microsurgery (TEM) can be applied for the resection of other benign rectal and extrarectal masses such as neuroendocrine tumors, retrorectal cysts, masses within the anovaginal septum, as well as for the repair of high rectovaginal fistulas[60], although the experience in these rare indications is limited. TEM has also been effectively used to treat anastomotic strictures, rectal prolapse, high extrasphincteric fistulas and for transrectal drainage of pelvic collections[61].

Current indications for local excision have expanded to include either the treatment of early stage rectal cancer in curative intense or as a palliation in patients with advanced rectal cancer who either refuse radical excision or they are poor surgical candidates[62,63]. Patients with incidentally found carcinoma following endoscopic polypectomy are suitable candidates for local excision, especially in the setting of a sessile polyp or when there is concern about margin positivity[64].

Applications of TransAnal Minimally Invasive Surgery (TAMIS) beyond local excision, have already been defined and they include the repair of rectouretheral fistulae[65], distal rectal mobilization[66], extraction of rectal foreign bodies[67], and most importantly, the use of TAMIS for transanal TME[68].

The right selection of the patients, who will be benefited by local excision in cases of early rectal cancer, is still an obstacle. Endorectal Ultrasound (ERUS) and/or pelvic MRI are mandatory for the preoperative staging of them. ERUS is more sensitive for the determination of the bowel wall depth invasion, while MRI is superior at evaluating mesorectal lymph nodes and the circumferential resection margin[69].

Based on the above imaging findings, NCCN guidelines[1], clearly recommend local excision as the treatment of choice for: (1) mobile/nonfixed rectal tumors, (2) less than 3 cm in size, (3) occuping less than 1/3 of the circumference of the bowel, (4) not extending beyond the submucosa (T1) which are (5) well to moderately differentiated and (6) with low-risk histopathological features. On the other hand, local excision should be avoided in cases of lymphovascular invasion, perineural invasion and mucinous components which are considered as high-risk characteristics, with high lymphnode metastatic potential.

Surgical technique

TEM, was firstly developed in the 1980s by Buess *et*

al[70], for the removal of endoscopically unresectable sessile rectal polyps. The author developed specific surgical rectoscope and instruments to address this problem. This facilitated a new way of operating in the rectum that was very precise and accurate due to its binocular vision and 3D visualization[71].

The equipment consists of a rigid rectoscope fixed to operating table and a unit for carbon dioxide insufflation, suction, irrigation and rectal pressure monitoring. The rectoscope is 4 cm in diameter, available in two main sizes, short (12 cm) and long (20 cm). Which one will be used, depends on the pre-operative location of the lesion in the rectum. The removable faceplate of the rectoscope has ports to facilitate the insertion of the long instruments, the suction required and to accommodate the stereoscope through which the surgeon can see the lesion magnified by six-fold. In recent times this can be connected to a laparoscopic video stack, which some surgeons prefer[72].

For anterior lesions, the patient should be placed prone and for posterior lesions in the lithotomy position[72]. The pneumorectum is maintained at a constant pressure of 10-12 mmHg which is enough for rectal wall distension and exposure of the tumor. The dissection begins by making a dotted line with the monopolar electric scalpel 10-15 mm from the macroscopic tumor margin[73].

For adenomas located within the intraperitoneal portion of the rectum, a careful mucosectomy, avoiding entry into the peritoneum, is indicated[64].

For extraperitoneally located adenomas and for all invasive carcinomas, full thickness resection should be offered as a standard treatment option[64].

Circumferential adenomas in the lower and middle rectum can be resected as complete full thickness segments, followed by an end-to-end anastomosis[64].

Invasive carcinomas in the posterior or lateral wall may be resected with some perirectal fat, often including 1 or 2 adjacent lymph nodes, which can be examined for metastatic spread[64].

With TEM, it is possible to perform local excisions with low risk of perforation at a distance up to 18-20 cm when the tumor is located in the posterior quadrant and up to 15 cm when it is located anteriorly or laterally. The limit for low located lesions is the anal verge itself[73].

The resection bed is usually closed using a running 3-0 polydioxanone (PDS) suture on a small-half needle[64]. If peritoneum is entered, the defect should be always closed, while the resection bed below the peritoneal reflection, may be left open[64]. Finally, the surgical specimen is pinned out and oriented for pathological analysis of the margins[74].

TEM has not become universally adopted by colorectal surgeons due to the considerable cost of the apparatus and the steep learning curve required for the mastering of the technique[75]. These disadvantages prompted surgeons to examine alternative methods for performing transanal surgery.

TAMIS was developed in 2009[76], and it is defined as the use of any multichannel single-port which can be placed transanally, combined with the use of ordinary laparoscopic instruments, such as a laparoscopic camera (preferably a 5-mm, 30° or 45° lens) and a standard laparoscopic carbon dioxide insufflator for performing endoluminal and more recently, extra-luminal surgery[77]. A systematic review[75] of the published studies revealed that eight different types of TAMIS platforms have been used for local excision of rectal neoplasia. Regardless of which platform is used, the principles of TAMIS remain the same and the key advantages to its use are upheld.

Early post-operative outcome

Few deaths have been reported in the literature, mainly related to metastatic disease in the late postoperative period or due to advanced disease in patients in whom palliative TEM had been performed[59,78].

The overall complication rate for TEM has been reported between 6% and 31%, with an equal distribution between benign and malignant disease[79]. Perioperative complications include hemorrhage and intraperitoneal perforation (0%-9%), which both may require conversion to laparotomy[79]. Postoperative hemorrhage has been reported in 1% to 13% of patients. Most resolve spontaneously or conservatively with blood transfusion[64]. The conversion rate was around 5%, mainly related to technical difficulties[74].

Since TAMIS is a fairly a new technique, the appraisal of its results is mainly based on retrospective studies and case reports. Albert *et al*[77], reported a 6% microscopically positive margins on final pathology and a recurrence rate of 4% at 6- and 18-mo follow-up. The largest multicentre series on TAMIS for rectal lesions[80] included 75 patients (low grade rectal adenoma 33%, high grade rectal adenoma 23%, rectal adenocarcinoma 43% and carcinoid tumour 1%). Intraoperative complications occurred in 8% and postoperative morbidity rate was 19%, with only one patient requiring re-intervention.

However, the only systematic review[75] of 390 TAMIS resections published in the English literature, disclosed: a 3.0 cm average size of lesions resected, located within a 7.6 cm mean distance from the anal verge (range: 3-15 cm), an overall margin positivity rate of 4.36%, a tumor fragmentation of 4.1% and an overall complication rate of 7.4%.

Oncological outcome

The ideal goal for the treatment of T1N0M0 rectal cancers should be the maximization of the oncological outcome, with simultaneous minimization of the long-term impact of the treatment on the QoL[81].

Long-term results studies on the oncological outcome following traditional transanal local excision

for T1 tumors, disclosed local recurrence rates higher than 29%[82-84].

On the other hand, the published results on the oncological outcome following TEM remain controversial, since other studies[85] reported favorable results with local recurrence rates lower than 10%, others[86] confirmed the lower local recurrence rates following TEM compared to the transanal local excision (18.5% *vs* 27.7%) but without statistical significance, others[87] stated alarming figures for local recurrence following TEM for T1 rectal tumors, while local recurrence rates as high as 20.5% have also been reported[88].

In an attempt to evaluate further the above findings, both Tytherleigh *et al*[89] in 2008, as-well-as Bach *et al*[90] in 2009, offered possible explanations for these unfavorable results. Both studies made clear that the depth of submucosal invasion (sm level) constituted a strong predictor for recurrence, since sm1 tumors showed low recurrence rates, but sm2-3 tumors showed recurrence rates similar to the T2 lesions[89,90]. Thus, locally excised pT1sm1 tumors without lymphovascular invasion, up to 3 cm in diameter, have a local recurrence rate of less than 5%, while locally excised pT1sm2-3 tumors have a local recurrence rate of up to 20%, similarly to T2 tumors[72].

Apart from the sm level of invasion, tumor differentiation, vascular or perineural invasion, positive resection margins, lymphocytic infiltration, lymph node spread and tumor budding (presence of neoplastic cells below the invasive front), have been proposed as additional dismal prognostic factors for local recurrence[73].

According to the NCCN guidelines[1], the standard treatment for T2N0M0 rectal adenocarcinoma is TME without adjuvant therapy *per se*, since such tumors have a lymph node involvement rate between 12% and 29%[58].

However, literature addresses that for T2 tumors, simple local excision (either transanal or TEM), local excision followed by postoperative CRT, as well as preoperative CRT followed by local excision, have been attempted.

TEM alone is not acceptable treatment option for fit patients with rectal cancer of local stage T2 or deeper[79].

CRT after local excision presented disappointing results, since local recurrence rate has been reported as high as 45%[91].

However, TEM after neoadjuvant CRT for downstaging of advanced tumors has been investigated demonstrating promising results[79].

Lezoche *et al*[92] prospectively randomized 70 patients with T2N0 rectal cancers to either TEM (n = 35) or laparoscopic radical resection (n = 35) after CRT. Patients were restaged after neoadjuvant therapy. Those in the TEM group had significantly better results in terms of hospital stay, blood loss and duration of surgery than those in the radical resection group, although there was no difference in complication rates between the two groups. Oncologic results after TEM and radical resection were comparable in terms of local (5.7% *vs* 2.8%), distant (2.8% *vs* 2.8%) and combined (9% *vs* 6%) recurrence rates, as well as the probability of disease-free survival (both 94%). The above oncological outcome, combined with the shorter hospital stay and the faster return to normal activities in the TEM group of patients, may suggest that TEM is a favorable and acceptable technique for selected T2 patients without nodal involvement or distant metastasis, though more evidence is required.

In their review, Borschitz *et al*[93] included seven studies and 237 patients who underwent local excision for T2-3 rectal tumors after neoadjuvant CRT. The authors addressed that when complete pathological response was achieved (ypT0), local recurrence was 0% and systemic recurrence was 4%, in ypT1 tumors, local recurrence was 2% and systemic recurrence was 7%, in ypT2 tumors, both local and systemic recurrence rose to 7%, while when there was no response (ypT3), the local recurrence was 21% and the systemic recurrence was 12% after local excision.

Similarly, a prospective study[94] of 27 patients with ypT0-2 tumors in the lower rectum after neoadjuvant CRT, treated by TEM, showed a local recurrence rate of 15% within a median follow-up of 15 mo. Lymphovascular invasion was disclosed as the only independent dismal prognostic factor for local failure (P = 0.04), while tumor size, ypT status, T-status downstaging, lateral/radial margins and tumor regression grade, did not reach statistical significance.

Finally, a systematic review[95] comparing the effectiveness of TEM to radical surgery for T1-2 rectal tumors, concluded that the TEM procedure was associated with a higher risk of local recurrence, but was statistically equivalent to radical surgery in terms of overall mortality, overall survival and the risk of distant metastasis. We should state however, that the main bias of this review was that low risk T1, high risk T1, as well as T2 tumors, were indiscriminately enrolled.

Functional outcome

As a result of the dilation of the anal canal by the proctoscope and the prolonged operative time, it has been suggested that damage to the anal sphincter could cause postoperative fecal incontinence[96]. Existing data indeed suggested reduced anorectal manometric pressures (particularly the resting one) in patients who have undergone TEM, directly correlated to the length of the procedure, however this did not change continence scores or other anorectal parameters[97].

In a prospective study of 41 patients, Cataldo *et al*[98] found no deleterious consequences on fecal continence after TEM. They did not find any significant difference between pre- and postoperative mean Fecal Incontinence Severity Index (FISI) score (2.4 *vs* 2.4),

mean FIQL score, number of bowel movements per day (mean 2.4 *vs* 1.5) and ability to defer defecation.

In a recent study of 50 patients, Doornebosch *et al*[99] found significantly improved FISI and FIQL scores after TEM (all $P < 0.05$).

Patients themselves reported improved QoL after surgery[99]. This improvement may be attributed to the fact that rectal lesions and subsequent mucous production contribute to the symptoms of fecal incontinence, which disappear once the lesion is excised. Furthermore, the presence of a large rectal mass may induce a continuous internal anal sphincter reflex, leading to a decreased anorectal function.

Allaix *et al*[100] studied the long-term functional outcomes and the QoL parameters after 5 years follow-up in 93 patients who underwent TEM. Similarly to previous studies, manometric values, such as anal resting pressure, rectal sensitivity threshold, maximum tolerated volume and urge to defecate threshold, declined at 3 mo but returned to preoperative level 12 mo after surgery. Compared to preoperative levels, there were no significant changes in anal squeeze pressure after surgery. Wexner incontinence scores and general QoL scores, which were increased in the early postoperative period, returned to preoperative levels at 5 years.

The functional outcome of TAMIS after rectal polyps resection are reported in only one study[101], showing excellent short-term results and comparable to functional results using the dedicated TEM equipment.

Conclusion

Both TEM and TAMIS are safe procedures. TEM should be used in T1 rectal cancer, with favorable pathologic features. The use of TEM after preoperative CRT is still debatable. Anal function after TEM is improving. Not enough oncological or functional outcomes are available for TAMIS.

TRANSANAL TOTAL MESORECTAL EXCISION

Selection of patients

Transanal Total Mesorectal Excision (TaTME) was developed to overcome technical difficulties associated with laparoscopic TME[68]. Most of the surgeons believe that patients with narrow pelvis, visceral obesity or large tumor diameter, are favored by this technique[102]. The procedure is feasible for mid and low rectal cancers.

In a systematic review[68] of 150 cases, rectal adenocarcinoma was the indication for TaTME, except 9 cases published by Wolthuis *et al*[103].

In their report, Tuech *et al*[102] stated as contraindications for the procedure T4 tumors invading the vagina or the prostate, tumors with no objective response to preoperative CRT as well as tumors invading EAS or levator ani. de Lacy *et al*[104] added as contraindications a BMI over 35, the recurrence and the intolerance of pneumoperitoneum.

Surgical technique

Trananal TME is a new technique that allows the transanal mobilization of the rectum from distal to proximal using a variety of flexible or rigid transanal platforms[68]. The devotees of the technique support that TaTME facilitates radical dissection of the difficult distal part of the TME dissection in a narrow and/or rigid pelvis, allowing clear and safe definition of the tumor-free distal resection margin[105].

TaTME can be performed in conjunction to transabdominal assistance through multiport laparoscopy, mini-laparoscopy or a single-port access[68]. Some authors report that abdominal phase of the operation should be performed first, with the transanal phase to follow, other teams perform the two phases synchronously[102], pure TaTME has also been reported[106,107], while different type of platforms or even robotic TaTME has also been performed[108].

The standardized technique has two phases, abdominal and transanal. Most authors complete the abdominal phase with high ligation of inferior mesenteric vessels and mobilization of the left colon and the splenic flexure. The fecal stream is diverted with a loop ileostomy, unless a permanent stoma is being fashioned[12].

The transanal phase starts after the placement of a self-retaining retractor and the exploration of the rectum. For tumors located up to 3 cm from the anal verge, there is a necessity to be performed an intersphinteric dissection, after sectioning the dentate line with electrocautery. Once the full-thickness rectal wall is completely sectioned, a purse-string suture is placed through the rectum to tightly occlude it. Thereafter, it is necessary for the transanal dissection of the first 4 to 4.5 cm of the anal canal to insert a Transanal Access Platform. CO_2 is insufflated to a pressure of 10 to 12 mmHg, and it is adapted during the progression of the dissection. Once introduced into the presacral plane, the mesorectum is mobilized and the posterior dissection proceeded cephalic in the avascular presacral plane in accordance to TME principles. This plane of dissection is extended medially, laterally, and interiorly to achieve circumferential rectal mobilization. The dissection is performed circumferentially and progressively to avoid retraction of the rectum that could make the division of one side difficult. Finally, the peritoneal reflection is visualized and divided to achieve sigmoid colon mobilization, with both teams collaborating to complete it. The device is removed and the specimen is carefully extracted transanally. The section of sigmoid colon is performed proximal to the vascular pedicle with scalpel. The division of the remaining mesentery and the marginal artery are completed with the specimen exteriorized. A handsewn coloanal anastomosis is then performed

Table 1 Table comparing the different surgical techniques, regarding their oncological outcomes, complication and success rate as well as the ideal patient selection criteria

	Patients selection (inclusion criteria)	Surgical technique	Postoperative outcome		Oncological outcome	
ISR	T1-3 tumors within 30-35 mm from the anal verge	Abdominal phase: high ligation of the inferior mesenteric vessels	Morbidity:	8%-64%	Local recurrence Lymphnode metastases	2.6%-9.5% 2.40%
		Perineal phase: dissection on the anatomical plane between the IAS and the EAS	Mortality	0%-1.7%	Distant metastases CRM (-) 5-yr overall survival	9.3%-14.1% 80.4%-96% 76.4%-86.3%
APPEAR	Was developed to treat patients with malignant or benign disease, needing APR or completion protectomy, if treated with conventional surgery	Abdominal phase, same as TME in LAR	Morbidity:	15.4%-60%	Distal resection margin	Median: 20 mm
	Mostly used in rectal cancer 2-5 cm from anal verge	Perineal phase involves a convex crescentic incision in the perineum, between vagina/scrotum and anus, the dissection continues upwards to the plane made from abdominal phase. The rectum is freed laterally and posteriorly from the perineal aspect and the specimen delivered through the perineum	Mortality	0%	CRM Local recurrence	Median: 5 mm 0%
TEM TAMIS	(1) Are indicated for: mobile/nonfixed tumors, less than 3 cm in size, occupying less than 1/3 of the circumference of the bowel, not extending beyond the submucosa well to moderately differ-rentiated with low-risk histopatho-logical features	Adenomas located within the intraperitoneal portion of the rectum: careful muco-sectomy, avoiding entry into the peritoneum	TEM Morbidity	6%-31% 7.4%-19%	Without neo-adjuvant CRT	
		Extraperitoneally located adenomas: full thickness resection	TAMIS Morbidity	Occasionally	T1sm1: Local recurrence	< 5%
		All invasive carcinomas: full thickness resection	TEM Mortality	Occasionally	T1sm2-3: Local recurrence	20%
	(2) As a palliation in patients with advanced rectal cancer who either refuse radical excision or they are poor surgical candidates	Circumferential adenomas in the lower and middle rectum: complete full thickness resection, followed by an end-to-end anastomosis	TAMIS Mortality		After neo-adjuvant CRT	
	(3) Furthermore, TAMIS is used for the repair of rectoure-theral fistulae, distal rectal mobilization, extraction of rectal foreign bodies, and for transanal TME				T2N0: Local recurrence Distant recurrence Combined recurrence After neo-adjuvant CRT T2-3N0 (ypT0): Local recurrence Systematic recurrence T2-3N0 (ypT1): Local recurrence Systematic recurrence T2-3N0 (ypT2): Local recurrence Systematic recurrence T2-3N0 (ypT3): Local recurrence Systematic recurrence	 5.7% 2.8% 9% 0% 4% 2% 7% 7% 7% 21% 12%

TaTME	Was developed to overcome technical difficulties associated with laparoscopic TME, mainly related to narrow pelvis, visceral obesity or large tumor diameter	Abdominal phase involves high ligation of inferior mesenteric vessels and mobiliazation of left colon and splenic flexure Perineal phase: for tumours ⩽ 3 cm from anal verge, ISR and after, transanal access platform insertion, and CO_2 insufflation. Dissection starts from the presacral plane, the mesorectum is mobilized and the posterior dissection proceeded cephalic in the avascular presacral plane in accordance to TME principles. The dissection continues until peritoneal reflection is visualized and divided to achieve sigmoid colon mobilization. The specimen is extracted transanaly	Morbidity Mortality	22.7%-26% 0	Distal resection margin CRM CRM (-) "Intact" mesorectum "Nearly complete" mesorectum R0 resections achieved No of retrieved lymphnodes	Median: 10 mm Median: 2 mm 92.7%-96.7% 84% 16% 94.6% ⩾ 12

APPEAR: Anterior Perineal PlanE for Ultra-low Anterior Resection of the Rectum; APR: Abdominoperineal resection; CRM: Circumferential radial margin; CRT: Chemo-radio-therapy; EAS: External anal sphincter; IAS: Internal anal sphincter; ISR: Intersphincteric resection; LAR: Low anterior resection; TAMIS: TransAnal Minimally Invasive Surgery; TaTME: Transanal Total Mesorectal Excision; TEM: Transanal Endoscopic MicroSurgery; TME: Total mesorectal excision.

between the proximal sigmoid colon and the distal anorectal cuff[109].

For middle and low rectal tumors, after positioning of a self-retaining retractor, the Transanal Access Platform, is positioned in the anal canal. A purse-string suture is placed through the rectal mucosa to tightly occlude it distally to the lesion. Endoscopic transection of the full-thickness rectal wall is performed and, thereafter, another purse-string suture was placed in the distal rectal mucosa. The mesorectum mobilization is made as previously described. The specimen is exteriorized transanally, the colon is sectioned, a purse-string suture is placed and the anvil is inserted. The rectal anastomosis is performed with a EEA 33 mm circular stapler[109].

Early postoperative outcome

In the only systematic review enrolling 150 cases[68], no mortality was reported. The complications rate was 22.7%, mainly related to infectious complications such as pelvic abscess (n = 6) and anastomotic fistulas (n = 2). In a latter study[102], the postoperative complications rate was 26% and the anastomotic leak rate was 5.3%.

Oncological outcomes

The available on the oncological outcome data of TaTME is currently derived from non-randomized retrospective comparative series, case series and case reports.

The macroscopic quality of TME was documented in all published reports, except in study of Wolthuis *et al*[103] and has been reported as "intact"[110], "adequate"[111,112], "satisfactory"[104,113], or "complete"[11,12,106,107,111,114].

Results from four studies[7,11,12,115], addressed positive CRM in 10 out of 136 patients (7.3%), while all studies, except two[103,113], reported a mean number of retrieved lymphnodes equal to or greater to 12.

A recent study (n = 56)[102], showed intact removal of the mesorectum in 47 cases (84%) and a nearly complete mesorectum in 9 (16%), median number of retrieved lymphnodes per patient equal to 12 (range: 7-29), median radial and distal resection margins of 8 mm (range: 0-20 mm) and 10 mm (range: 3-40 mm), respectively, CRM involvement in 5.3%, achievement of R0 resection in 53 patients (94.6%) and overall survival of 96.4% within a median follow-up period of 29 mo.

A systematic review[68], addressed that an oncological adequate TaTME operation is reproducible, with lower than APR and equal to LAR positive CRM margins and comparable extent of mesorectal excision and lymphadenectomy.

Although more studies are required to be confirmed the above findings, the comparative results published by Velthuis *et al*[7], indicated that TaTME may be associated to a significantly higher rate of completeness of mesorectal excision, compared to laparoscopic TME.

Functional outcome

Functional outcomes are reported only in one study[102]. After the reversal of the ileostomy in 52 out of the 56 enrolled patients, 3 (5.7%) required a colostomy because of severe fecal incontinence, while for the remaining 49 patients without stoma (94.3%), the median Wexner score was 4 (range: 3-12) and among them, 14 (28.5%) had a score greater than 7 and 13 (28%) reported stool fragmentation and difficult evacuation.

Conclusion

TaTME is feasible and safe. The general consensus is that curative TaTME should be performed only when a board approved protocol is available and only by colorectal surgeons with extensive experience in minimally invasive and transanal endoscopic surgery. More studies are needed to evaluate the oncological and functional outcomes of the technique.

SUMMARY

If ISR is applied in T1-3 rectal tumors located within 30-35 mm from the anal verge with or without IAS invasion, is technically feasible, with acceptable morbidity rates, equal oncological outcomes compared to LAP and APR and acceptable QoL, reserving APR for locally advanced tumors (Table 1).

APPEAR is a promising technique, having the advantage of not disrupting the sphincters compared to ISR. However, it carries a significant complication rate, while the long-term oncological and functional outcomes are unknown, since few studies have been published.

TEM and TAMIS should represent the treatment of choice for T1 rectal tumors, with specific criteria according to the NCCN guidelines and favorable pathologic features.

However, if the pathology report discloses depth of submucosal invasion sm2-3 level, we should advised the patient that since the locally excised pT1sm2-3 tumors have a local recurrence rate of up to 20%, they should be treated as suffering from T2 tumors.

The recommended treatment of choice for T2 rectal tumors is TME without adjuvant therapy *per se.*

Alternatively to that, although still debatable, preoperative (neoadjuvant) CRT followed by TEM or TAMIS seems the most promising available therapeutic option for T1sm2-3 or T2 tumors.

TaTMEs should be performed only when a board approved protocol is available and only by colorectal surgeons with extensive experience in minimally invasive and transanal endoscopic surgery.

Finally, apart from selecting the right type of operation, for every specific tumor, in every selected patient, we should also select patients that should avoid surgery. Habr-Gama *et al*[116] proposed the "Watch and Wait" approach for patients achieving complete clinical response (26.8%) after neoadjuvant CRT. With such an approach, the 5-year overall survival and disease free survival rates have been reported as high as 100% and 92%, respectively.

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REVIEW

Anti-angiogenic agents in metastatic colorectal cancer

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Abstract

Colorectal cancer (CRC) is a major public health concern being the third leading cause of cancer mortality in the United States. The availability of better therapeutic options has led to a decline in cancer mortality in these patients. Surgical resection should be considered in all stages of the disease. The use of conversion therapy has made surgery a potentially curative option even in patients with initially unresectable metastatic disease. In this review we discuss the role of various anti-angiogenic agents in patients with metastatic CRC (mCRC). We describe the mechanism of action of these agents, and the rationale for their use in combination with chemotherapy. We also review important clinical studies that have evaluated the safety and efficacy of these agents in mCRC patients. Despite the discovery of several promising anti-angiogenic agents, mCRC remains an incurable disease with a median overall survival of just over 2 years in patients exposed to all available treatment regimens. Further insights into tumor biology and tumor microenvironment may help improve outcomes in these patients.

Key words: Anti-angiogenic agents; Metastatic colorectal cancer; Targeted agents; Conversion therapy; Colorectal metastasectomy

Core tip: Colorectal cancer is a major health concern and a leading cause of cancer mortality worldwide. New innovations have provided improved survival in recent years. In this review, we outline the novel anti-angiogenic agents and their respective roles in metastatic colorectal cancer. In addition to three agents approved by the Food and Drug Administration, several alternative anti-angiogenic agents hold promise for use in the metastatic setting.

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INTRODUCTION

The past decade has seen a significant decline in the incidence rate and cancer mortality in patients with colorectal cancer (CRC) in the United States. The decrease in cancer deaths appears to be due largely to the widespread use of screening colonoscopy and the availability of better treatment options. However, from a public health perspective, CRC remains a major concern, with 136830 new cases estimated to be diagnosed and over 50000 deaths predicted to occur in the United States alone in 2014. Today, CRC is the third leading cause of cancer mortality in the United States, surpassed only by lung cancer, breast cancer in women and prostate cancer in men[1].

Surgery remains the mainstay of treatment in patients with early stage and locally-advanced CRC and should be considered for those with metastatic CRC (mCRC) with liver-only or lung-only metastases. Though only 10%-20% of patients with liver-only metastases are resectable at the time of diagnosis[2], the use of conversion therapy can make up to 61.9% of tumors resectable[3].

Since the discovery of 5-fluorouracil (5-FU) in 1957[4], several chemotherapeutic agents have been approved for the treatment of mCRC, including capecitabine, oxaliplatin, and irinotecan. Insights into the molecular mechanisms of disease led to the discovery of biologic agents targeting tumor vascular endothelial growth factor (VEGF) and epidermal growth factor receptor (EGFR). This review focuses on the anti-angiogenic agents used in the treatment of mCRC.

BIOLOGICAL BASIS OF ANTI-ANGIOGENIC THERAPY

Tumor cells and endothelial cells are inter-dependent for their growth *via* a carefully regulated system[5]. Pre-clinical studies have shown that implanted tumor cells can only grow to a size of 2-3 mm without neovascularization. They can remain dormant for several years or switch to an angiogenic phenotype[6]. Tumor cells with the angiogenic phenotype release growth factors (pro-angiogenic factors) which stimulate endothelial proliferation, migration, and formation of new capillaries. This process is called tumor angiogenesis and leads to tumor perfusion, growth, and metastases[5,7]. Hematopoietic stem cells and circulating endothelial progenitor cells (CEPs), which are bone marrow derived rapidly proliferating cells, are also thought to contribute to tumor angiogenesis[8].

Vascular endothelial growth factor (VEGF) is one of the most extensively studied pro-angiogenic factors. It is produced by normal and certain neoplastic cells (such as CRC cells)[9]. The human VEGF family is primarily composed of 5 glycoproteins (VEGF A, B, C, D, and platelet derived growth factor, or PlGF). These proteins exert their effects by binding to receptor tyrosine kinases (VEGFR1, R2, and R3)[9-11]. VEGF-A is commonly referred to as VEGF or vascular permeability factor (VPF) and is first discovered by Senger *et al*[12].

Tissue hypoxia (*via* hypoxia inducible factor), growth factors (*e.g.*, epidermal growth factor, insulin like growth factor-1), and oncogenes (*e.g.*, c-Src proto-oncogene) increase VEGF expression[9,13,14]. VEGF then exerts its angiogenic effects predominantly *via* VEGFR2; however, the role of VEGFR1 remains unclear[15]. VEGF promotes tumor angiogenesis by increasing permeability of post-capillary venules, which subsequently leads to the leakage of plasma proteins such as fibrinogen and clotting factors into the extracellular matrix (ECM). Fibrinogen is converted to fibrin in the ECM which leads to increased endothelial cell migration and proliferation[16]. VEGF is also an endothelial cell mitogen[13] and causes endothelial cell proliferation by activating members of the MAP kinase and protein kinase C pathways[9]. Other pro-angiogenic factors include hepatocyte growth factor (HGF), axon guidance factors, interleukins (IL-1, 6, 8, and stromal cell derived factor 1), fibroblastic growth factors (FGF 1 and 2), angiopoietins, and pro-angiogenic chemokines[17]. Another important regulator of angiogenesis is the tyrosine kinase with immunoglobulin and epidermal growth factor homology domain 2 (TIE2) expressed primarily on endothelial cells. TIE2 interacts with angiopoietin 1, angiopoietin 2, VEGF, and FGF to cause maturation of immature blood vessels[18].

The use of anti-angiogenic therapy to arrest tumor growth and thereby make these tumors more susceptible to chemotherapy and cell-mediated immunity was first proposed by Folkman[5] in 1971. Angiogenesis inhibitors can be broadly classified into 2 groups, direct and indirect anti-angiogenic agents. Direct angiogenic inhibitors act on endothelial cells of the microvasculature, thus inhibiting their response to angiogenic stimuli. Indirect angiogenic inhibitors on the other hand target pro-angiogenic stimuli either at the level of the ligand (*e.g.*, VEGF inhibition) or at the level of the receptor (*e.g.*, VEGFR inhibition)[19].

ANTI-ANGIOGENIC AGENTS IN THE TREATMENT OF UNRESECTABLE mCRC

Bevacizumab is an IgG1 monoclonal antibody against the VEGF-A ligand that was developed by humanization of the murine anti-human VEGF antibody A.4.6.1[20,21]. It was the first anti-angiogenic agent to be FDA-approved in the treatment of mCRC in combination with chemotherapy[22]. Adverse effects include hypertension, proteinuria, hemorrhage, GI perforation, delayed wound healing, and arterial and venous thromboembolism[23,24]. Hypertension is a common side effect of bevacizumab therapy, with more than half of the patients requiring pharmacologic intervention. It has been hypothesized

Table 1 Bevacizumab in the first-line setting in metastatic colorectal cancer

Ref.	Regimen	PFS (mo)	*P* value	OS (mo)	*P* value
Kabbinavar *et al*[26]; Phase Ⅱ	[1]Bolus 5-FU/LV ± bevacizumab	9 *vs* 5.2 (TTP[3])	NA	21.5[2] *vs* 13.8	NA
Kabbinavar *et al*[27]; Phase Ⅱ	[1]Bolus 5-FU/LV + bevacizumab *vs* bolus 5-FU/LV + placebo	9.2 *vs* 5.5	0.0002	16.6 *vs* 12.9	0.16
Hochster *et al*[35]; Phase Ⅱ (TREE-1)	mFOLFOX6/bFOL/CapeOX	8.7/6.9/5.9 (TTP[3])	N/A[4]	19.2/17.9/17.2 18.2 (overall)	N/A[4]
Hochster *et al*[35]; Phase Ⅱ (TREE-2)	mFOLFOX6 + bevacizumab/bFOL + bevacizumab/CapeOX + bevacizumab	9.9/8.3/10.3 (TTP[3])		26.1/20.4/24.6 23.7 (overall)	
Hurwitz *et al*[22]; Phase Ⅲ	IFL + bevacizumab *vs* IFL + placebo	10.6 *vs* 6.2	< 0.001	20.3 *vs* 15.6	< 0.001
Stathopoulos *et al*[115]; Phase Ⅲ	IFL ± bevacizumab	NA	NA	22 *vs* 25	0.1391
Saltz *et al*[29]; Phase Ⅲ	FOLFOX/CapeOX + bevacizumab *vs* FOLFOX/CapeOX + placebo	9.4 *vs* 8	0.0023	21.3 *vs* 19.9	0.077

[1]Roswell Park regimen: LV 500 mg/m^2 over 2 h and FU 500 mg/m^2 as a bolus midway through the LV infusion; [2]Data presented is on patients who received chemotherapy plus low-dose bevacizumab; [3]Time to progression; [4]Comparison between outcomes of TREE-1 and TREE-2 is not possible as they were sequential cohorts. 5-FU: 5-Fluorouracil; LV: Leucovorin; PFS: Progression-free survival; OS: Overall survival; mFOLFOX6: Three fluoropyrimidine regimens-infusional 5FU/LV; bFOL: Bolus FU/LV; CapeOX: Capecitabine; FOLFOX: 5-FU/LV/oxaliplatin; NA: Not available; N/A: Not applicable.

that VEGF inhibition leads to a decrease in nitric oxide synthase, leading to inhibition of vasodilation. In addition, bevacizumab decreases arteriolar and capillary perfusion leading to increased peripheral vascular resistance and hypertension[25]. The hemorrhage and thrombosis paradox of bevacizumab therapy can be explained by the disruption in hemostasis secondary to VEGF inhibition. VEGF inhibition leads to apoptosis of quiescent endothelial cells, which in turn leads to activation of the extrinsic coagulation pathway. This mechanism lends credence to the prothrombotic properties of bevacizumab. Inhibition of angiogenesis and platelet function are thought to contribute to hemorrhage and impaired wound healing related to bevacizumab therapy. Proteinuria as a consequence of bevacizumab therapy is common and is secondary to renal thrombotic microangiopathy leading to glomerular endothelial injury[25].

Bevacizumab in treatment-naive patients

Bevacizumab has been extensively studied in several clinical trials with favorable results (Table 1). The combination of bevacizumab and bolus 5-FU/leucovorin (LV) chemotherapy was compared to bolus 5-FU/LV alone in treatment-naive mCRC patients in a phase Ⅱ randomized study by Kabbinavar *et al*[26] in the year 2003. The addition of low-dose bevacizumab led to higher response rates (40% *vs* 17%), longer time to disease progression (9 mo *vs* 5.2 mo), and a longer median overall survival (OS) (21.5 mo *vs* 13.8 mo) in these patients[26]. In another phase Ⅱ trial comparing first-line bevacizumab plus chemotherapy (bolus 5-FU/LV) to chemotherapy alone in mCRC patients who were poor candidates for irinotecan therapy, a 3.7 mo progression-free survival (PFS) advantage was noted in the group that received bevacizumab (9.2 mo *vs* 5.5 mo; HR, 0.50; *P* = 0.0002). There was a trend toward a longer median OS in the bevacizumab-containing group; however; this difference was not statistically significant (16.6 mo *vs* 12.9 mo; HR, 0.79; *P* = 0.16)[27].

Subsequently, a large randomized phase Ⅲ trial compared the use of bevacizumab plus irinotecan, bolus 5-FU/LV (IFL) *vs* IFL plus placebo as front-line therapy. The addition of bevacizumab not only conferred a benefit in median OS (20.3 mo *vs* 15.6 mo; HR, 0.66; *P* < 0.001) and PFS (10.6 mo *vs* 6.2 mo; HR, 0.54; *P* < 0.001), but also led to higher response rates (44.8% *vs* 34.8%; *P* = 0.004) and more durable responses (10.4 mo *vs* 7.1 mo; *P* = 0.001)[22]. The results of this trial led to the FDA approval of bevacizumab for use as a first-line agent in mCRC patients in combination with chemotherapy. In a combined analysis of 2 phase Ⅱ (53, 54) and 1 phase Ⅲ study[22], patients in the 5-FU/LV/bevacizumab arm had a statistically significant improvement in median OS (17.9 mo *vs* 14.6 mo; HR, 0.74; *P* = 0.008) and median PFS (8.8 mo *vs* 5.6 mo; HR, 0.63; *P* ≤ 0.0001) when compared to the chemotherapy-only arm (patients receiving 5-FU/LV or IFL)[28]. However, in another phase Ⅲ randomized trial comparing IFL with and without bevacizumab, the addition of bevacizumab did not confer an OS advantage (22 mo in the IFL-bevacizumab arm *vs* 25 mo in the IFL arm; *P* = 0.1391)[28]. With the emergence of combination chemotherapy regimens [5-FU/LV/oxaliplatin (FOLFOX), and 5-FU/LV/irinotecan (FOLFIRI)], subsequent studies focused on testing the efficacy and safety of these regimens in combination with bevacizumab. In a randomized phase Ⅲ study by Saltz *et al*[29], untreated mCRC patients were randomized to receive either bevacizumab or placebo in combination with chemotherapy (FOLFOX-4 or Cape-OX). Though the effect size was small, a PFS advantage was seen in the bevacizumab-containing arm (9.4 mo *vs* 8 mo; HR, 0.83; *P* = 0.0023), however there was no statistically significant difference in median OS between the two groups (21.3 mo *vs* 19.9 mo; HR, 0.89; *P* = 0.077). An interesting observation in this study that the authors effectively point out is the similar median treatment duration of

patients receiving bevacizumab and placebo (approximately 6 mo), in contrast to the significantly longer PFS (as noted above) in the bevacizumab arm. The early discontinuation of bevacizumab (prior to disease progression) probably explains the absence of a survival advantage in the bevacizumab-containing arm. The authors concluded that continuation of bevacizumab until disease progression is critical for a meaningful clinical benefit[29].

A randomized head-to-head comparison of FOLFIRI with and without bevacizumab has not been done to date. However, sufficient evidence to justify the use of FOLFIRI plus bevacizumab in untreated mCRC patients exists. In a pooled analysis on 29 published trials, patients who received FOLFIRI-bevacizumab had a median PFS of 10.8 mo (95%CI: 8.9-12.8) and a median OS of 23.7 mo (95%CI: 18.1-31.6)[30]. In an open-label, phase Ⅳ AVIRI study, patients who received first-line FOLFIRI plus bevacizumab had a PFS of 11.1 mo and a median OS of 22.2 mo[31]. A phase Ⅲ trial of 285 patients compared efficacy of CapeIri plus bevacizumab with FOLFIRI plus bevacizumab. There was no difference in PFS (10.2 mo *vs* 10.8 mo; P = 0.74), or median OS (20.0 mo *vs* 25.3 mo, P = 0.099) between the two groups[32].

After the Gruppo Oncologico Nord Ovest (GONO) group showed that 5-FU/LV/oxaliplatin/irinotecan (FOLFOXIRI) improved response rate (RR), PFS, and OS in treatment-naive unresectable mCRC patients in a phase Ⅲ randomized trial[33], the addition of bevacizumab to FOLFOXIRI was compared to FOLFIRI plus bevacizumab by the same group of investigators. The latter trial was also a phase Ⅲ randomized trial (TRIplet plus BEvacizumab, or TRIBE), which showed that patients receiving triplet chemotherapy (FOLFOXIRI) plus bevacizumab had a longer PFS (primary end point; 12.1 mo *vs* 9.7 mo; HR, 0.75; P = 0.003) and better objective response rate (65% *vs* 53%; P = 0.006) when compared to those receiving FOLFIRI plus bevacizumab. Though patients in the FOLFOXIRI arm had a longer median OS when compared to those in the FOLFIRI arm, this difference was not statistically significant (31.0 mo *vs* 25.8 mo; HR, 0.79; P = 0.054). Not surprisingly, patients who received the triplet chemotherapy regimen plus bevacizumab had a significantly higher incidence of grade 3-4 neutropenia, diarrhea, stomatitis, and peripheral neuropathy when compared to the FOLFIRI plus bevacizumab arm[34].

The Three Regimens for Eloxatin Evaluation (TREE) study was initially designed to evaluate the efficacy and safety of Oxaliplatin (Eloxatin) in combination with three fluoropyrimidine regimens- infusional 5FU/LV (mFOLFOX6), bolus FU/LV (bFOL), and Capecitabine (CapeOX). When the trial was nearing completion of accrual, data on the efficacy of bevacizumab in mCRC began to emerge. The study was therefore modified to include 2 sequential cohorts of patients- the initial cohort of patients who did not receive bevacizumab (TREE-1) and a subsequent cohort of patients who received bevacizumab in combination with one of the above three chemotherapy regimens (TREE-2). The incidence of serious (grade 3/4) treatment related AEs in the first 12 wk of therapy in each of the patient groups in the TREE-2 cohort (primary end point) were 59% (mFOLFOX6/bevacizumab), 51% (bFOL/bevacizumab), and 56% (CapeOX/bevacizumab), with neutropenia, diarrhea, and nausea/vomiting being the most common AEs in each of the treatment groups respectively. The respective incidence of grade 3/4 AEs in the TREE-1 cohort were 59% (mFOLFOX6), 36% (bFOL), and 67% (Cape-OX). The overall median OS was nearly 2 years (23.7 mo) in the TREE-2 cohort, and 18.2 mo in the TREE-1 cohort[35].

Two randomized phase Ⅲ trials (FIRE-3 and CALGB 80405) compared the efficacy of cetuximab *vs* bevacizumab in combination with chemotherapy in previously untreated KRAS WT mCRC patients. The FIRE-3 study randomized patients with KRAS WT exon 2 tumors to receive either cetuximab plus FOLFIRI or bevacizumab plus FOLFIRI as front-line therapy. Though the objective response (CR/PR; primary end point) and median PFS were similar between the two groups, median OS favored the cetuximab-containing group (28.7 mo *vs* 25.0 mo; HR, 0.77; P = 0.017)[36]. The CALGB 80405 trial randomized untreated mCRC patients with KRAS WT (codons 12 and 13) tumors to receive either cetuximab or bevacizumab in combination with chemotherapy (FOLFIRI or mFOLFOX6). The OS (primary end point) and PFS were similar in both groups and the authors concluded that either regimen would be an appropriate option in these patients. It is important to note that in contrast to the FIRE-3 study, most patients (73.4%) in the CALGB 80405 study received mFOLFOX6 as their combination chemotherapy regimen[37].

Bevacizumab as maintenance therapy

Maintenance treatment in advanced CRC for the Treatment of Digestive Tumors (MACRO TTD) was the first randomized phase Ⅲ study undertaken to evaluate the role of bevacizumab alone in the maintenance setting. Patients were randomized to receive either bevacizumab alone *vs* bevacizumab plus maintenance chemotherapy (Cape-OX), after completion of induction therapy (Cape-OX + bevacizumab, or Cape-OX-B). The primary end point was PFS and the prespecified non-inferiority limit of HR for PFS was set at 1.32. After a median follow-up of 29 mo, median PFS in patient receiving maintenance Cape-OX-B *vs* Bevacizumab alone was 10.4 mo and 9.7 mo respectively. The HR for PFS was 1.10 with a 95%CI: 0.89-1.35. The study thus did not confirm non-inferiority of bevacizumab maintenance when compared to Cape-OX-B as the upper limit of the 95%CI of HR for PFS exceeded the pre-specified limit of 1.32. However, there was no statistically significant difference in PFS,

Table 2 Phase Ⅲ trials using bevacizumab in the second-line setting

Ref.	Regimen	PFS (mo)	*P* value	OS (mo)	*P* value
Giantonio *et al*[41]; (E3200)	FOLFOX4 ± bevacizumab	7.3 *vs* 4.7	< 0.0001	12.9 *vs* 10.8	0.0011
Bennouna *et al*[45]; (ML18147)	Chemotherapy ± bevacizumab	5.7 *vs* 4.1	< 0.0001	11.2 *vs* 9.8	0.0062
Masi *et al*[46]; (BEBYP)	Chemotherapy ± bevacizumab	6.8 *vs* 5.0	0.010	14.1 *vs* 15.5[1]	0.043[1]

[1]The lower median OS in the bevacizumab arm was due to intersection of curves; adjusted HR was 0.77 (stratified log-rank *P* = 0.043) and favored the bevacizumab arm. PFS: Progression-free survival; OS: Overall survival.

OS, and response rate between the two arms, with a significantly lower frequency of grade 3-4 sensory neuropathy in the bevacizumab alone group (8% *vs* 26%; *P* < 0.0001)[38].

Subsequently, the role of bevacizumab maintenance therapy in patients who had stable disease/partial response (PR)/complete response (CR) after bevacizumab-containing induction chemotherapy was evaluated in a multicenter retrospective analysis of treatment-naive mCRC patients. The study results favored bevacizumab maintenance over no maintenance therapy (PFS: 13 mo *vs* 8 mo; *P* < 0.0001). An OS advantage was only seen in those patients who received bevacizumab maintenance after they had an objective response to induction chemotherapy[39].

More recently, the role of bevacizumab plus chemotherapy as maintenance therapy was investigated in the phase Ⅲ CAIRO3 trial. After completion of six cycles of Cape-OX-B, patients were randomized to either receive maintenance therapy with capecitabine plus bevacizumab (Cape-B) or receive no further therapy. Irrespective of randomization, patients who had first progression (PFS1) received Cape-OX-B until second progression (PFS2). After a median follow-up of 2 years, maintenance therapy conferred a PFS advantage (PFS1: 8.5 mo *vs* 4.1 mo; *P* < 0.0001; PFS2: 11.7 mo *vs* 8.5 mo; *P* < 0.0001)[40]. In patients with baseline synchronous metastases and resected primary tumor, an OS benefit was noted as well (25 mo *vs* 18 mo; *P* < 0.0001)[40].

An ongoing randomized phase Ⅲ trial (NCT00973609) is evaluating three treatment strategies in mCRC patients. All patients will receive induction (and re-induction) with a 5-FU, oxaliplatin, and bevacizumab-based chemotherapy for a period of 6 mo. Induction therapy will be followed by maintenance therapy with a fluoropyrimidine and bevacizumab (active comparator), or bevacizumab alone (experimental arm) or no maintenance therapy (experimental arm).

Bevacizumab in the second-line setting

A multi-center, randomized phase Ⅲ E3200 study was pivotal in bevacizumab's approval in previously treated mCRC patients (Table 2). This study randomized patients who were previously treated with fluoropyrimidine and irinotecan to received FOLFOX-4 plus bevacizumab (group 1), FOLFOX-4 alone (group 2), or bevacizumab alone (group 3). Patients in group 1 had a longer median OS and a better PFS when compared to patients in group 2 (group 1 *vs* group 2; OS: 12.9 mo *vs* 10.8 mo; *P* = 0.0011; PFS: 7.3 mo *vs* 4.7 mo; *P* < 0.0001) and group 3 (group 1 *vs* group 3; OS: 12.9 mo *vs* 10.2 mo; PFS: 7.3 mo *vs* 2.7 mo)[41].

Bevacizumab beyond progression: The rationale behind continuing bevacizumab despite progression on bevacizumab-containing chemotherapy is that the mechanisms of resistance to cytotoxic chemotherapy and to bevacizumab differ significantly and may not necessarily occur concomitantly[42,43]. Changes in tumor cell biology and genetic instability *via* mutations in tumor suppressor genes or of drug targets, contribute to chemotherapy resistance. As anti-VEGF therapy targets the genetically stable tumor microvasculature, emergence of resistance to bevacizumab requires development of alternative proangiogenic signaling[43]. Thus, it is not unreasonable to assume that progression of disease on a combination treatment regimen (cytotoxic chemotherapy plus bevacizumab) may be secondary to resistance to chemotherapy alone and continuation of bevacizumab beyond progression in combination with a different chemotherapy regimen may be an option. This hypothesis was validated by two large observational studies[42,44] and large phase Ⅲ study (ML 18147)[45]. The BRiTE (Bevacizumab Regimens: Investigation of Treatment Effects and Safety) study was a large observational cohort study undertaken to evaluate the role of bevacizumab continuation beyond disease progression. The study enrolled 1445 mCRC patients who had progression on a first-line bevacizumab-containing treatment regimen. Patients who had received "Bevacizumab Beyond Progression" (BBP: *n* = 642) had a significantly longer median OS when compared to those who discontinued bevacizumab (no-BBP; n, 531) therapy (median OS: 31.8 mo *vs* 19.9 mo; HR, 0.49; *P* < 0.001). As would have been expected, patients in the BBP group had a higher rate of hypertension requiring medication compared to the no-BBP group or to the overall study population (24.6% *vs* 19.2%), however, the risk of serious AEs including arterial thromboembolic events, grade 3 or 4 bleeding, and GI perforation were similar between the two groups[42].

The ARIES study was another observational study that confirmed that findings of the BRiTE study. In this study, a total of 1105 patients survived longer that 2 mo after first progression, and were included in the modified ITT analysis. The median post-progression

survival was higher in the BBP group when compared to the no-BBP group [14.4 mo *vs* 10.6 mo; multivariable HR (95%CI): 0.84 (0.73-0.97)]. Protocol-specified adverse events were higher in the BBP group *vs* the no-BBP group (13% *vs* 8.5%)[44].

In order to validate the results of the BRiTE and ARIES studies, a multinational phase Ⅲ trial (ML18147) randomized mCRC patients with POD within 3 mo of discontinuation of 1st line bevacizumab plus chemotherapy, to receive 2nd line chemotherapy while continuing BBP *vs* chemotherapy alone. A total of 819 patients were included in the ITT analysis. After a median follow-up of 11.1 and 9.6 mo in the chemotherapy plus BBP group and chemotherapy alone group respectively, the median OS (primary end point) significantly favored the bevacizumab containing arm [11.2 mo *vs* 9.8 mo; HR (95%CI): 0.81 (0.69-0.94); unstratified log-rank test, 0.0062] (Table 2). Patients receiving BBP had a higher rate of grade 3-5 bleeding (2% *vs* < 1%), GI perforation (2% *vs* < 1%), and VTE (5% *vs* 3%), but as is evident from the frequency of these AE, the difference between the two arms was not considerable. Neutropenia (16% *vs* 13%), diarrhea (10% *vs* 8%), and asthenia (6% *vs* 4%) were the most common grade 3-5 adverse events and were comparable between the two arms. Also, the rate of arterial thromboembolism was not increased in the BBP group when compared to the no-BPP group. Thus, continuation of bevacizumab beyond first progression in mCRC patients significantly improved median OS without substantially increasing serious AEs[45].

The Bevacizumab Beyond Progression (BEBYP) trial (Table 2) was a phase Ⅲ, prospective, multicenter Italian study that evaluated the efficacy and safety of continuation or reintroduction of bevacizumab after first progression in patients with unresectable mCRC. The sample size was much smaller when compared to the ML18147 trial, but also included patients with POD beyond 3 mo of discontinuation of first-line therapy. PFS was the primary end point and 184 patients were included in the ITT analysis. After a median follow-up of 45.3 mo, the median PFS was noted to be significantly higher in the bevacizumab group when compared to the chemotherapy-only group (6.8 mo *vs* 5.0 mo; HR, 0.70; stratified log-tank $P = 0.010$). PFS benefit persisted when patients were stratified based on the bevacizumab-free interval (≤ 3 mo *vs* > 3 mo). An OS advantage was also noted in the bevacizumab group (adjusted HR, 0.77; stratified log-rank $P = 0.043$), though responses were comparable between the two arms (17% in the chemotherapy arm *vs* 21% in the bevacizumab arm; $P = 0.573$). Consistent with the safety data of the ML18147 trial, grade 3-4 AEs were similar between both arms[46].

Bevacizumab-based chemotherapy in the elderly

The efficacy and tolerability of bevacizumab in the elderly has been studied both in the first- and second-line settings. The BRiTE study was a large observational cohort study of 1953 untreated mCRC patients with 896 patients ≥ 65 years of age. PFS in the elderly patients was similar to their younger counterparts though median OS declined with increasing age[47]. Interestingly however, in another large observational cohort study of 1777 treatment-naive German mCRC patients, those ≥ 75 years of age had a significantly lower PFS and median OS when compared to those < 75 years of age (PFS: 10.5 mo *vs* 8.9 mo; $P = 0.00019$; OS: 25.8 mo *vs* 20.8 mo; $P < 0.0001$)[48]. In a multicenter phase Ⅱ study by the Hellenic Oncology Research Group, the combination of capecitabine, oxaliplatin, and bevacizumab (AVELOX) was proven to be safe and effective in the first-line treatment of elderly patients (≥ 70 years old)[49].

In a pooled analysis of 439 untreated mCRC patients ≥ 65 years old, bevacizumab-based chemotherapy produced a PFS and OS advantage when compared to chemotherapy alone[50]. In another retrospective pooled analysis of 4 RCTs (3 RCTs in the first-line setting and 1 RCT in the second-line setting), the addition of bevacizumab conferred a PFS and OS advantage in elderly patients (≥ 65 and ≥ 70 years old) when compared to chemotherapy alone. Patients receiving bevacizumab had more arterial thromboembolic events; however, there was no increase in ≥ grade 3 adverse events with increasing age[51].

More recently, the safety of bevacizumab-based chemotherapy was studied in a multi-national phase Ⅲ randomized trial (AVEX) in which 280 patients with a median age of 76 years were randomized to receive bevacizumab plus capecitabine *vs* capecitabine alone. Progression free survival favored the bevacizumab-containing arm (9.1 mo *vs* 5.1 mo; $P < 0.0001$). Overall, the percentage of patients who had any grade treatment related adverse events was similar in both groups (84% *vs* 81% in the bevacizumab-containing arm *vs* the chemotherapy alone arm). However, a higher percentage of patients in the bevacizumab plus chemotherapy group had grade 3 or greater treatment-related adverse events when compared to the chemotherapy alone group (40% *vs* 22%). Not surprisingly, bevacizumab-specific any grade adverse effects such as hypertension, proteinuria, and venous thromboembolism were greater in the bevacizumab containing arm[52].

Ziv-aflibercept (VEGF trap): A human recombinant soluble decoy protein that was engineered by the fusion of the second immunoglobulin (Ig) domain of VEGFR1 and the third Ig domain of VEGFR2 with the constant region (Fc) of human IgG1[53]. The drug binds to VEGF-A, VEGF-B, and placental growth factor (PlGF) with high affinity, thus preventing these ligands from binding to their respective endogenous receptors[54]. This leads to tumor growth and angiogenesis inhibition as shown in *in-vitro* and *in-vivo* studies[53]. When compared to bevacizumab, in addition to inhibiting

endothelial cell migration, ziv-aflibercept has a much greater binding affinity to VEGF-A and more potent inhibition of VEGFR1 and VEGFR2 activation[54]. Adverse effects include fatigue, headache, hemorrhage, nausea, diarrhea, hypertension, and proteinuria[55-57].

The efficacy of ziv-aflibercept in mCRC patients was evaluated in a large randomized phase Ⅲ trial (VELOUR). The study included all mCRC patients who progressed after prior oxaliplatin-based therapy for metastatic disease or who relapsed within 6 mo of adjuvant oxaliplatin-based chemotherapy. Prior bevacizumab therapy was not an exclusion criterion, though prior irinotecan therapy was not allowed. Patients who received prior bevacizumab therapy constituted 30.6% of the intent to treat (ITT) population. Patients were randomized to receive FOLFIRI plus ziv-aflibercept (ziv-aflibercept arm) *vs* FOLFIRI plus placebo (control arm). After a median follow-up of 22.28 mo, patients in the ziv-aflibercept arm had a significantly longer median OS (13.50 mo *vs* 12.06 mo; $P = 0.0032$) and PFS (6.90 mo *vs* 4.67 mo; HR, 0.758, $P < 0.0001$) when compared to the control arm. Neither prior bevacizumab use nor ECOG PS had an interaction with treatment for OS or PFS. OS and PFS advantage with ziv-aflibercept *vs* placebo was noted regardless of prior bevacizumab exposure [prior bevacizumab therapy: median OS: 12.5 mo *vs* 11.7 mo; HR (95%CI): 0.862 (0.673-1.104); median PFS: 6.7 mo *vs* 3.9 mo; HR (95%CI): 0.661 (0.399-1.095); no prior bevacizumab therapy: Median OS: 13.9 mo *vs* 12.4 mo; HR (95%CI): 0.788 (0.669-0.927); Median PFS: 6.9 mo *vs* 5.4 mo; HR (95%CI): 0.797 (0.58-1.096)]. Grade 3 and 4 adverse events that were higher in the ziv-aflibercept arm included hypertension, hemorrhage, thromboembolic events (arterial and venous)[56]. In a post-hoc subset analysis of the VELOUR trial, patients with liver-only metastases had a greater OS and PFS benefit from ziv-aflibercept in comparison to patients with no liver metastasis or liver plus other organ metastases. Prior bevacizumab therapy did not have an influence on treatment effect[58]. Ziv-aflibercept in combination with FOLFIRI is FDA approved for use in the treatment of mCRC patients who have progressed through or following a first-line oxaliplatin-based regimen.

Regorafenib: A biaryl-urea compound which functions as an oral multikinase inhibitor of angiogenic (VEGF R1-3, tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domains, or TIE2), stromal (PDGF-β, fibroblast growth factor receptor 1), and oncogenic (RET, KIT, BRAF) receptor tyrosine kinases[18,59]. The safety and efficacy of regorafenib was first demonstrated in humans in a phase Ⅰ dose-escalation study which enrolled 53 patients with advanced and refractory solid tumors. The maximum tolerated dose was determined to be 160 mg daily, with a 3 wk on, 1 wk off schedule every 4 wk. More than half of the patients (66%) had either partial response or stable disease per RECIST criteria. The most common drug-related adverse events noted were voice changes, hand-foot syndrome (HFS), mucositis, diarrhea, and hypertension. Most patients (83%) developed at least 1 treatment related AE. The most frequently observed grade 3 or 4 drug-related AEs were hand-foot skin reaction (HFS), skin rash, hypertension, and diarrhea[60].

Another phase Ⅰ dose-escalation and extended cohort trial enrolled 37 patients with advanced or mCRC refractory to standard therapy and 1 patient with treatment-naive disease who refused standard therapy. Of the 27 patients evaluable for response, 19 had stable disease and 1 had partial response. The median PFS was 107 d (95%CI: 66-161). As in the prior phase 1 trial, most patients (84%) had treatment-related AEs (HFS, skin rash/desquamation, fatigue, fatigue, voice changes, diarrhea), though most of the AEs were grade 3 or lower. HFS was the most common grade 3 or greater treatment related AE. More than half of the patients (66%) required dose reduction or treatment interruption due to AEs with HFS being the most common AE requiring dose reduction[61].

The efficacy and safety of regorafenib in combination with FOLFOX or FOLFIRI chemotherapy in the 1st or 2nd line setting was evaluated in a phase Ib trial of 45 mCRC patients. Of the 38 patients evaluable for treatment response, either partial response or stable disease was noted in 33 patients. Median TTP for the study population was 119 d (FOLFOX group: 116 d; FOLFIRI group: 186.5 d). Most patients (71%) had treatment related AEs that were grade 3 or higher, of which neutropenia was the most common AE. Common any grade AEs included diarrhea, mucositis, neutropenia, HFS, alopecia, and fatigue. Interestingly the area under the curve (AUC) of irinotecan and its active metabolite (SN-38) were significantly higher in cycle 2 when compared to cycle 1 prior. Overall, regorafenib was shown to have acceptable tolerability in combination with chemotherapy in this study[62].

The CORRECT trial was a randomized multinational phase Ⅲ trial evaluating the benefit and tolerability of regorafenib in previously treated mCRC patients after failure of standard licensed therapy. A total of 760 patients were randomized in a 2:1 ratio to receive regorafenib plus BSC or placebo plus BSC. The mean treatment duration was 2.8 mo in the regorafenib arm and 1.8 mo in the placebo arm. Either partial response or stable disease was achieved in 41% of patients in the regorafenib group compared to 15% of patients in the placebo arm ($P < 0.0001$). Median OS was 6.4 mo *vs* 5 mo (HR, 0.77; $P = 0.0052$) and median PFS was 1.9 mo *vs* 1.7 mo (HR, 0.49; $P < 0.0001$) in the regorafenib group *vs* placebo arm, respectively. Though the magnitude of OS benefit with regorafenib *vs* placebo appears small, the HR of 0.77 would imply a 23% reduction in the risk of death during the study period. When stratified based on the primary site

of disease, patients with colon cancer who received regorafenib had a significant OS advantage with an HR of 0.70 and 95%CI: 0.56-0.89, when compared to the placebo arm, however this benefit was not seen in patients with rectal cancer [HR (95%CI), 0.95 (0.63-1.44)]. PFS favored the regorafenib arm in colon, rectal, and colon and rectal cancer subgroups with an HR of 0.55, 0.45, and 0.35 respectively. The apparent lack of an OS advantage despite a PFS advantage in patients with rectal cancer could be attributed to the higher percentage of patients in the placebo group and the smaller proportion of patients in the regorafenib group went on to receive further anti-cancer therapies post-study. Ninety three percent of patients in the regorafenib group *vs* 61% of those in the placebo group had treatment-related adverse events. Hand-foot syndrome, fatigue, diarrhea, hypertension, skin rash or desquamation were the most frequent toxicities that were ≥ grade 3. Hepatotoxicity with elevated liver transaminases and bilirubin (mostly grade 1-2) was noted to be more common in the regorafenib group when compared to placebo. A fatal case of liver injury in a 62-year-old male with liver metastases was noted in the regorafenib arm 43 d after treatment initiation. Health-related quality of life and health outcomes were measured using standard scoring systems and showed no difference in deterioration in the regorafenib *vs* placebo arms. Regorafenib monotherapy appears to be a reasonable option in patients with refractory mCRC who have exhausted all other systemic treatment options[63].

Ramucirumab: A human IgG1 monoclonal Ab against the extracellular domain of VEGFR2, thereby preventing the interaction between VEGF and VEGFR2[64]. The efficacy and safety of Ramucirumab was initially reported in a phase Ⅰ study of 37 advanced solid tumor patients, of whom 6 had a primary CRC (refractory to standard therapy). After at least 12 wk of therapy, three of the six (50%) CRC patients experienced SD for 30 (dose level: 2 mg/kg), 31 (dose level: 4 mg/kg), and 15 wk (dose level: 10 mg/kg) respectively. Overall, 22 patients (60%) developed grade 3-5 AEs, with hypertension, abdominal pain, anorexia, vomiting, increased blood alkaline phosphatase, headache, proteinuria, dyspnea, and deep venous thrombosis being the common serious AE[65]. Subsequently, a phase Ⅱ study enrolled 42 treatment-naive mCRC patients to receive Ramucirumab (at a dose of 8 mg/kg) in combination with mFOLFOX6 every 2 wk. The combination was shown to be efficacious with a median PFS of 11.5 mo and a median OS of 20.4 mo (Table 3). Neutropenia, hypertension, and neuropathy were the most commonly reported serious (grade 3-4) AEs[66]. The benefit of the addition of Ramucirumab to FOLFIRI in the second-line setting was recently evaluated in a large, randomized double-blind phase Ⅲ study (RAISE). The trial enrolled a total of 1072 patients who had POD during or after first line therapy with a combination of bevacizumab, fluoropyrimidine, and oxaliplatin, were randomized in a 1:1 design to receive FOLFIRI plus Ramucirumab *vs* FOLFIRI plus placebo. Patients in the Ramucirumab arm had a longer median OS (primary end-point; 13.3 mo *vs* 11.7 mo; HR, 0.84; log-rank $P = 0.0219$) and a longer PFS (5.7 mo *vs* 4.5 mo; HR, 0.79; log-rank $P = 0.0005$). The commonly reported serious AE (≥ grade 3) included neutropenia, hypertension, diarrhea, and fatigue[67]. Ramucirumab in combination with FOLFIRI is a promising second-line treatment option in patients with unresectable mCRC.

Famitinib: A small molecule multi-tyrosine kinase inhibitor with predominantly antiangiogenic properties[68]. The drug inhibits VEGFR2 and VEGFR3, PDGFR, stem cell factor receptor c-KIT, FMS-like tyrosine kinase-3 receptor (FLT3), and the proto-oncogene tyrosine-protein kinase inhibitor RET[69,70]. The tolerability of famitinib in patients with advanced solid tumor malignancies was evaluated in a phase Ⅰ study of 44 patients, including 7 patients with advanced CRC. The most common grade 3-4 toxicities at occurring in the first 8 wk of therapy dose levels of 24, 25, and 27 mg included hypertension, bone marrow suppression leading to leukopenia, neutropenia, thrombocytopenia, and anemia, HFS, hypertriglyceridemia, and proteinuria. The authors recommended a dose of 25 mg for a phase Ⅱ trial. The efficacy data in patients with advanced CRC was not reported in this study[69]. More recently, the efficacy and safety of famitinib in the third or later line setting was studied in a multicenter phase Ⅱ, randomized, double-blind study of 154 advanced CRC patients. Patients were randomized in a 2:1 design to receive either famitinib or placebo. Patients who received famitinib have a longer median PFS (primary end point; 2.8 mo *vs* 1.5 mo; HR, 0.58; $P = 0.0034$) and a better disease control rate (57.58% *vs* 30.91%; $P = 0.0023$) when compared to the placebo arm. The most commonly reported AEs were predominantly grade 1-2 and included neutropenia, thrombocytopenia, hypertension, proteinuria, and HFS. There was no significant difference in serious AEs between the two arms. Famitinib was thus noted to be efficacious and safe in mCRC patients who have failed second or later line therapies[68]. The results will however require further validation with a phase Ⅲ trial.

OTHER ANTI-ANGIOGENIC AGENTS

Several other antiangiogenic agents have been studied in patients with advanced CRC with disappointing results.

Sorafenib is an orally administered small molecule multi-tyrosine kinase inhibitor which targets the RAF/MEK/ERK pathway in addition to inhibiting several receptor tyrosine kinases including VEGFR2, VEGFR3, PDGR beta, c-KIT, FLT3, and tyrosine kinase colony-

Table 3 Other anti-angiogenic agents

Ref.	Regimen (line of treatment)	Sample size	Objective response (%)	PFS (mo)	OS (mo)	Serious AE (grade 3-4)[5]
Samalin *et al*[73]; Phase Ⅰ/Ⅱ	Sorafenib/irinotecan (NEXIRI) (2nd or later line KRAS mutated)	10 (phase Ⅰ) 54 (phase Ⅱ)	64.9 (DCR)	3.7	8	Asthenia, diarrhea, neutropenia, HFS
Tabernero *et al*[74]; Phase Ⅱb	Sorafenib/mFOLFOX *vs* Placebo/mFOLFOX (1st line)	198	NA	9.1 *vs* 8.7 HR, 0.88 $P = 0.46$	17.6 *vs* 18.1 HR, 1.13 $P = 0.51$	Neutropenia, peripheral neuropathy, HFS
Starling *et al*[116]; Phase Ⅰ	Sunitinib/FOLFIRI (1st line)	37	57.9	NA	NA	Febrile neutropenia neutropenia, anemia, diarrhea, mucosal inflammation, stomatitis, vomiting, lethargy, pyrexia, thrombotic events
Yoshino *et al*[117]; Phase Ⅰ	Sunitinib/mFOLFOX6 (1st line)	12 (6 + 6)[3]	66.7 in each arm	NA	NA	Neutropenia, leukopenia, thrombocytopenia
Saltz *et al*[118]; Phase Ⅱ	Sunitinib (refractory setting)	43 (prior bevacizumab)	2.4	2.2 (TTP; prior bevacizumab)	7.1	Fatigue, diarrhea, nausea, vomiting, and anorexia (most common any grade toxicities)
		41 (no prior bevacizumab)	0	2.5 (TTP; no prior bevacizumab)	10.2	
Tsuji *et al*[75]; Phase Ⅱ	Sunitinib/FOLFIRI (1st line)	71	36.6[1]/42.3[2]	6.7[1]/ 7.2[2]	NR due to early study closure	Neutropenia, leukopenia, thrombocytopenia diarrhea, nausea decreased appetite and fatigue (most common any grade)
Carrato *et al*[119]; Phase Ⅲ	Sunitinib/FOLFIRI *vs* Sunitinib/placebo (1st line)	768	NA	7.8 *vs* 8.4 HR 1.095 one-sided stratified log-rank $P = 0.807$	20.3 *vs* 19.8 HR, 1.171 $P = 0.916$	Diarrhea, stomatitis/oral syndromes, fatigue, HFS, neutropenia, thrombocytopenia, anemia, febrile neutropenia
Michael *et al*[79]; Phase Ⅰ	Vandetanib/mFOLFOX6 (1st or 2nd line)	9 (100 mg/d dose)	44.44	NA	NA	Diarrhea, nausea and lethargy (most common any grade toxicities)
		8 (300 mg/d dose)	NA	NA	NA	
Saunders *et al*[80]; Phase Ⅰ	Vandetanib/FOLFIRI (1st or 2nd line)	11 (100 mg/d dose)	18.18	NA	NA	Diarrhea, nausea fatigue (most common any grade toxicities; were grade 1-2)
		10 (300 mg/d dose)	NA	NA	NA	
Yang *et al*[81]; Phase Ⅱ	Vandetanib/mFOLFOX6 *vs* Placebo/mFOLFOX6	32 (100 mg/d dose)[4] 35 (300 mg/d dose)[4]	NA	NA	NA	Diarrhea, nausea, thrombocytopenia, peripheral sensory neuropathy (most common any grade toxicities)
Van Cutsem *et al*[84]; Phase Ⅲ	FOLFOX 4/Vatalanib *vs* FOLFOX4/placebo (2nd line)	855	NA	5.6 *vs* 4.2 HR, 0.83 $P = 0.013$	13.1 *vs* 11.9 HR, 1.0 $P = 0.957$	Neutropenia, HTN, diarrhea, fatigue, nausea, vomiting, dizziness
Hecht *et al*[85]; Phase Ⅲ	FOLFOX4/Vatalanib *vs* FOLFOX4/placebo (1st line)	1168	NA	7.7 *vs* 7.6 HR, 0.88 $P = 0.118$	21.4 *vs* 20.5 HR, 1.08 $P = 0.260$	Neutropenia, HTN, diarrhea, fatigue, nausea, vomiting

[1]By independent review; [2]Investigator initiated review; [3]Six patients received sorafenib 2 wk on, 2 wk off and another 6 patients received sorafenib 4 wk on, 2 wk off; [4]Progression events (objective/clinical progression/death) in vandetanib 100 mg arm *vs* placebo: 72% *vs* 65% (HR, 1.21; 2-sided $P = 0.53$); vandetanib 300 mg arm *vs* placebo: 77% *vs* 65% (HR, 1.41; 2-sided $P = 0.25$); [5]In study drug containing arm. DCR: Disease control rate; NA: Not available; NR: Not reached; PFS: Progression-free survival; OS: Overall survival; mFOLFOX6: Three fluoropyrimidine regimens-infusional 5FU/LV; HFS: Hand-foot syndrome.

stimulating factor 1 receptor (c-Fms)[71]. Adverse effects include HFS, fatigue and diarrhea[72]. In a phase Ⅰ/Ⅱ trial evaluating the benefit of sorafenib plus irinotecan in previously treated mCRC patients, the combination was shown to be well tolerated in both phases of the trial. In phase 2, an encouraging response rate of 64.9% was noted with a PFS of 3.7 mo and a median OS of 8 mo[73]. However, in a subsequent phase Ⅱb study of 198 treatment naive mCRC patient, the combination of sorafenib and mFOLFOX4 was shown to offer no PFS or OS advantage over placebo (Table 3)[74].

Sunitinib is an oral multi-tyrosine kinase inhibitor targeting VEGFR1, VEGFR2, VEGFR3, PDGFR alpha and beta, FLT3, stem cell factor receptor, colony stimulating factor receptor, and glial cell line-derived neurotrophic factor[75]. The efficacy and tolerability of sunitinib as monotherapy and in combination with chemotherapy was studied without significant benefit (Table 3). Common side effects include fatigue, HFS, diarrhea, mucositis, hypothyroidism, yellow discoloration of skin, and cardiotoxicity[76].

Vandetanib is an antiangiogenic agent that inhibits VEGFR2 and VEGFR3 in addition to targeting EGFR,

and several tyrosine and serine-threonine kinases[77]. Common side effects include diarrhea, rash, dermatitis, nausea/vomiting, hypertension, fatigue, abdominal pain, decreased appetite, and QT prolongation[78]. After early phase trials[79,80] demonstrated safety of vandetanib in combination with chemotherapy in advanced CRC patients, a phase Ⅱ trial randomized patients to receive chemotherapy plus vandetanib *vs* chemotherapy plus placebo[81]. In this study the frequency of progression events - defined as objective or clinical progression or death from any cause- were noted to be higher in the vandetanib containing arm when compared to placebo (vandetanib 100 mg arm *vs* placebo: 72% *vs* 65%; HR, 1.21; 2-sided $P = 0.53$; vandetanib 300 mg arm *vs* placebo: 77% *vs* 65%; HR, 1.41; 2-sided $P = 0.25$)[81].

Vatalinib is an orally active antiangiogenic agent that blocks all VEGFR tyrosine kinase mediated signaling by competitively inhibiting the binding of ATP to the receptor kinase[82]. Adverse effects include lightheadedness, ataxia, nausea, vomiting, and hypertension[83]. Despite a tolerable toxicity profile in phase 1 studies[83], Vatalinib showed no survival advantage over placebo in two phase Ⅲ randomized trials studies in mCRC patients (Table 3)[84,85].

ANTI-ANGIOGENIC THERAPY IN INITIALLY AND POTENTIALLY RESECTABLE mCRC

Carefully selected patients can be cured, if not at least provided with improved survival benefits, with resection of their metastases. Improved 5-year OS after liver resection was found in up to 46% of patients with up to 25% resected patients considered cured[86-90]. The 5-year survival rate of patients treated with pulmonary metastasectomies was found to be 55%-67%[91,92]. The median disease-free survival (DFS) and OS for those who had both hepatic and pulmonary resection has been shown to be 13-19.8 mo and up to 87 mo, respectively[93,94].

Bevacizumab is the only anti-angiogenic agent that has been extensively studied in the setting of resectable (or potentially resectable) mCRC. Small phase Ⅱ studies have shown that when used either as preoperative therapy or as conversion treatment, bevacizumab in combination with Cape-OX or FOLFOX is associated with improved pathologic response, PFS, and OS in these patients[3,95,96]. Additionally, the combination of Cape-OX and bevacizumab rendered 40% of initially unresectable patients resectable in the BOXER (bevacizumab, oxaliplatin, capecitabine in unresectable liver metastases) study. This regimen provided objective responses in 78% of patients (95%CI: 63% to 89%) with 9% of patients (4 patients) achieving complete radiologic responses. These 4 patients remained in remission for 18-30 mo[97]. However, the sample sizes of these studies are too small to draw meaningful conclusions. Furthermore, the similar response rates (38% *vs* 38%; OR, 1.00; $P = 0.99$) between the bevacizumab and placebo arms when added to oxaliplatin-containing chemotherapy, in conjunction with a similar proportion of patients undergoing attempted curative intent metastasectomies (8.4% *vs* 6.1%) in a large phase Ⅲ study by Saltz *et al*[29] argue against the use of bevacizumab in combination with oxaliplatin-based chemotherapy as conversion therapy. Several studies have demonstrated benefit with an irinotecan-containing regimen in combination with bevacizumab. In a retrospective study evaluating histopathologic features of resected liver tissue samples of 42 patients with mCRC who received FOLFOXIRI/Cape-irinotecan (Cape-IRI) with or without bevacizumab in the pre-operative setting, a significantly higher pathological response was noted in patients who received bevacizumab plus chemotherapy *vs* chemotherapy alone (63% *vs* 28%; $P = 0.033$)[98]. In a phase Ⅱ study evaluating the efficacy and safety of preoperative bevacizumab plus FOLFIRI, patients with resectable liver metastases had a median PFS of 14 mo (95%CI: 11-24 mo), median OS of 38 mo (95%CI: 28-NA mo), an objective response rate of 66.7% (95%CI: 49.8% to 80.9%) and an R0 resection rate of 84.6%[99]. Masi *et al*[100] showed a conversion rate to R0 resection of 26% and up to 40% in those with liver-only metastatic disease after treatment with FOLFOXIRI and bevacizumab. Osterlund *et al*[101] also showed that bevacizumab plus cytotoxic chemotherapy was able to convert unresectable patients to resectable candidates in the 1st- and 2nd-line setting. Finally, Loupakis *et al*[34] found a response rate of 53.1% in the FOLFOX and bevacizumab arm compared to 65.1% in the FOLFOXIRI and bevacizumab arm with an odds ratio of 1.64 (95%CI: 1.15-2.35, $P = 0.006$) in the phase Ⅲ TRIBE trial. However, there was no difference in the rate of R0 metastasectomy (12% *vs* 15%, respectively, $P = 0.33$)[34].

More recently, the OLIVIA trial provided further support for use of FOLFOX or FOLFOXIRI with bevacizumab. It also provided further evidence that while FOLFOXIRI with bevacizumab resulted in increased toxicities, it also offered improved resection rates and PFS compared to the FOLFOX and bevacizumab regimen. Thirty-nine patients with initially unresectable disease were assigned to the FOLFOX with bevacizumab arm and 41 patients received FOLFOXIRI with bevacizumab. The overall resection rate was 49% (95%CI: 32-65) and 61% (95%CI: 45-76), respectively. R0 resection was accomplished in 23% and 49% of patients, respectively. Median overall survival was 32.2 mo in the FOLFOX and bevacizumab group. It has not yet been reached in the FOLFOXIRI and bevacizumab group. Median PFS was 11.5 mo (95%CI: 9.6-13.6) in the FOLFOX and bevacizumab group compared to 18.6 mo (95%CI: 12.9-22.3) in the FOLFOXIRI and bevacizumab group.

Table 4 Bevacizumab plus chemotherapy as conversion therapy

Ref.	Regimen	Rate of conversion (%)	Overall response (%)	Median PFS (mo)	Median OS (mo)
Bertolini *et al*[95]; Phase Ⅱ	FOLFOX6 + bevacizumab	61.9	57.1	12.9	22.5
Wong *et al*[97]; Phase Ⅱ	CAPE-OX + bevacizumab	40	78 (95%CI: 63-89)	NA[1]	NA[1]
Nasti *et al*[99]; Phase Ⅱ	FOLFIRI + bevacizumab	N/A	66.7 (95%CI: 49.8-80.9)	14 (95%CI: 11-24)	38 (95%CI: 28 to NA)
Klinger *et al*[3]; Meta-analysis/phase Ⅱ	CAPE-OX/FOLFOX + bevacizumab	N/A	38 *vs* 10 (P < 0.001)	NA[2]	67 (95%CI: 8.4-125.6)[2]
Gruenberger *et al*[96]; Phase Ⅱ	CAPE-OX + bevacizumab	N/A	73.2	NA	NA
Gruenberger *et al*[102]; Phase Ⅱ	FOLFOX/FOLFOXIRI + bevacizumab	49% (FOLFOX), 61% (FOLFOXIRI)	62% (95%CI: 45-77) (FOLFOX), 81% (95%CI: 65-91) (FOLFOXIRI)	11.5 (95%CI: 9.6-13.6) (FOLFOX), 18.6 (95%CI: 12.9-22.3) (FOLFOXIRI)	32.2 (FOLFOX), not yet reached (FOLFOXIRI)
Masi *et al*[100]; Phase Ⅱ	FOLFOXIRI + bevacizumab	26	NA	NA	NA
Loupakis *et al*[34]; Phase Ⅲ	FOLFOX/FOLFOXIRI + bevacizumab	53.1 (FOLFOX), 65.1 (FOLFOXIRI)	12 (FOLFOX), 15 (FOLFOXIRI)	NA	NA
Saltz *et al*[29]; Phase Ⅲ	FOLFOX/Cape-OX + bevacizumab *vs* FOLFOX/Cape-OX + placebo	8.4 *vs* 6.1 P = NA	38 *vs* 38 P = 0.99	9.4 *vs* 8 P = 0.0023	21.3 *vs* 19.9 P = 0.077
Loupakis *et al*[98]; meta-analysis	FOLFOXIR/Cape-IRI ± bevacizumab	NA	63 *vs* 28 P = 0.033	NA[3]	NA
Osterlund *et al*[101]; retrospective analysis	FOLFIRI + bevacizumab	9	42%	8.8	18.4

[1]Though median PFS and OS were not specifically reported by Wong *et al*[97], the 12-mo PFS was 50% (95%CI: 34%-64%) and 12-mo OS was 86% (95%CI: 70%-94%); [2]The OS in this study was not reported as a single parameter given its sample population. Instead, it was reported as a function of tumor regression grade, or TRG. The median OS of 67 mo cited in this table was found in those patients with lower TRGs (histologically with more fibrosis/necrosis than tumor, or major histological response). This OS decreases to 44 mo (95%CI: 14.1-73.8) in those with higher TRGs (histologically with more tumor than fibrosis/necrosis, or no histological response). Though the median PFS was not reported in this study, the 5-year PFS was 34% in lower TRGs and 9% in higher TRGs; [3]Again, the PFS was reported in this study as a function of TRGs. There was a PFS benefit in those with lower TRGs compared to those with higher TRGs such that for every 10 units in the percentage of necrosis, there was a 0.83 HR reduction (95%CI: 0.7-0.99, P = 0.04). NA: Not available; N/A: Not applicable; PFS: Progression-free survival; OS: Overall survival.

Most common grade 3-5 toxicities included diarrhea (14% with FOLFOX, 30% with FOLFOXIRI) and neutropenia (35% and 50%, respectively)[102].

Table 4 summarizes some of the currently available clinical data in this patient population.

Multiple studies have now established that the use of bevacizumab in combination of cytotoxic chemotherapy given preoperatively neither affects the recovery of liver function nor its regeneration. The anti-VEGF activity likely persists after preoperative cessation for at least 6 wk but does not seem to affect postoperative liver recovery. Furthermore, it was found not to increase the rate of complications if discontinued at least 5 wk prior to resection[96,103-111]. In fact, there is evidence that bevacizumab, when added to oxaliplatin-based chemotherapy, may protect against sinusoidal dilatation or sinusoidal obstruction syndrome[3,112,113].

Despite the efficacy of bevacizumab plus chemotherapy in the neoadjuvant setting, it was not found to provide either a PFS or OS benefit when used as adjuvant therapy after liver metastasectomy[114].

CONCLUSION

Anti-angiogenic therapy has assumed a vital role in the management of patients with mCRC. A total of three anti-angiogenic agents are currently approved in the treatment of these patients: bevacizumab, ziv-aflibercept, and regorafenib. The choice of agents differs based on tumor resectability and line of therapy. Patients with potentially resectable liver metastases have been shown to have an improved pathological response with the addition of bevacizumab to neo-adjuvant chemotherapy. Studies have refuted concerns about hepatotoxicity and liver regeneration in patients treated with bevacizumab in the neoadjuvant setting.

Bevacizumab in combination with irinotecan-based chemotherapy has also been used as conversion therapy with a resection rates up to 61% in combination with FOLFOXIRI though at the expense of increased toxicities. In patients with treatment-naive unresectable mCRC, the addition of bevacizumab to cytotoxic chemotherapy achieves better and more durable responses, in addition to an advantage in PFS and OS when compared to chemotherapy alone. The beneficial role of bevacizumab in combination with a fluoropyrimidine in the maintenance setting, and the benefits of continuing bevacizumab beyond progression have been confirmed in multiple studies.

The use of bevacizumab in the first-line setting in patients with KRAS WT unresectable mCRC has been challenged by the FIRE-3 and CALGB 80405 studies, and cetuximab-based chemotherapy appears to be a viable option in these patients. More recently,

two new anti-angiogenic agents were added to the armamentarium of targeted agents approved for use in mCRC. Ziv-aflibercept improved survival when used in the second-line setting in combination with an irinotecan-based chemotherapy in patients who have failed oxaliplatin-based therapy, and Regorafenib improved survival when compared to placebo in the treatment of patients with refractory mCRC. Another antiangiogenic agent, Ramucirumab has shown to improve survival in the second-line setting when used in combination with chemotherapy, and awaits FDA approval.

Despite these advances, mCRC remains an incurable disease with a median OS of approximately over 2 years in patients exposed to all available treatment regimens. Further insights into tumor biology and tumor microenvironment may help improve outcomes in these patients.

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ORIGINAL ARTICLE

Case Control Study

Association of *endothelial nitric oxide synthase* gene T-786C promoter polymorphism with gastric cancer

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Author contributions: Krishnaveni D had searched the literature and isolated genomic DNA from peripheral blood leucocytes; Krishnaveni D, Shravan Kumar P, Uma Devi M and Ramanna M collected blood samples from patients; Krishnaveni D performed the genotyping of genomic DNA; Amar Chand B, Jyothy A and Pratibha N gave suggestions in writing the article; Balakrishna N performed biostatistical review; Venkateshwari A directed and coordinated the study.

Institutional review board statement: The study was reviewed and approved by the Institutional Review Board of Institute of Genetics and Hospital for Genetic Diseases.

Informed consent statement: All the study subjects involved in the present study gave their informed written consent prior to study inclusion.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Data sharing statement: The corresponding author states that Technical appendix, statistical code and dataset in the present research article entitled "Association of *endothelial nitric oxide synthase* gene T-786C promoter polymorphism with gastric cancer" has been submitted to Dryad repository to be made available and provide a permanent, citable and open-access home for the dataset.

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Abstract

AIM: To investigate the role of endothelial nitric oxide synthase -786T > C promoter polymorphism in the etiology of gastric cancer (GC).

METHODS: A total of 150 GC patients and 150 control subjects were included in the study. The information on demographic features was elicited with an informed consent from all the patients and control subjects using a structured questionnaire. *Helicobacter pylori* (*H. pylori*) infectivity status was tested in antral biopsies from all the subjects by rapid urease test following the method of Vaira *et al*. Genomic DNA was isolated from whole blood samples following the salting out method of Lahiri *et al*. Genotype analysis of the rs2070744

polymorphism was carried out by allele-specific polymerase chain reaction method. The genotypes were determined based on the appearance of bands on an agarose gel stained with ethidium bromide under ultraviolet gel documentation with the help of 100 bp ladder. Odds ratios and corresponding 95%CIs were determined using java stat online software.

RESULTS: There was a significant difference in the distribution of *C* allele (*C vs T*; *P* = 0.000, OR = 5.038) in patient group compared to the control subjects exhibiting a fivefold increased risk for GC. When the *T/T* and *C/C* genotypes were compared, there was an enhanced GC risk for individuals with *C/C* genotype (*T/T vs C/C*; *P* = 0.000). Among the demographic factors, smoking and alcoholism were the common risk factors in patients compared to the control subjects (*P* < 0.05). Patients with smoking and alcoholism developed cancer even in heterozygous *T/C* condition (smoking: *P* = 0.020 and alcoholism: *P* = 0.005). Individuals with *H. pylori* infection showed seven fold increased risk for cancer. All the patients with *C/C* genotype revealed a significant association between *H. pylori* infection and GC. Among the patients 2.4% of them revealed familial incidence of GC. No significant difference was noticed between cases and controls with regard to consanguinity (*P* = 0.473).

CONCLUSION: The Present data suggest that eNOS-786 *C/C* genotype and C allele may be considered as potential risk factors in patients with GC.

Key words: Genetics; *Helicobacter pylori*; Nutrition; Oncology; Endoscopy; Gastro duodenal; Nitric oxide; Single nucleotide polymorphism rs2070744; Agarose gel electrophoresis; Allele specific polymerase chain reaction

Core tip: The present study reveals first molecular epidemiological evidence from south Indian cohort for the association of endothelial nitric oxide synthase -786T > C promoter polymorphism with a risk to develop gastric cancer (GC). The *CC* genotype and *C* allele of the -786T > C polymorphism were significantly associated with an elevated risk to GC, probably due to the lowered nitric oxide levels in case of *C/C* genotype which result in tumour proliferation, angiogenesis and metastasis.

Krishnaveni D, Amar Chand B, Shravan Kumar P, Uma Devi M, Ramanna M, Jyothy A, Pratibha N, Balakrishna N, Venkateshwari A. Association of *endothelial nitric oxide synthase* gene T-786C promoter polymorphism with gastric cancer. *World J Gastrointest Oncol* 2015; 7(7): 87-94 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i7/87.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i7.87

INTRODUCTION

Gastric cancer (GC), also known as stomach cancer, is the accumulation of malignant cells that form a tumour in any part of the stomach. In most cases, it is adenocarcinoma, starts off in the mucus producing cells present in the inner most lining of the stomach. On the whole 90% of the stomach tumours are of malignant and 95% of these tumours reported to be adenocarcinomas[1]. GC is defined as a multifactorial disorder resulting from various genetic, epigenetic predisposition and environmental risk factors[2]. The incidence and mortality rates of GC vary by ethnicity and sex. On global scale, GC causes approximately 800000 deaths per year and it is the third leading cause of cancer death worldwide in both males and females[3]. A recent study from Tata Memorial Centre (TMC) on cancer mortality in India has rightly focused GC as the second largest cause of cancer related deaths among Indians[4].

Nitric oxide (NO) is a short lived vasoactive substance of prime importance constitutively produced from L - arginine by the enzyme nitric oxide synthase (NOS)[5]. Four isoforms of NOS have been identified and described as inducible nitric oxide synthase (iNOS), neuronal nitric oxide synthase (nNOS), endothelial nitric oxide synthase (eNOS) and mitochondrial nitric oxide synthase (mNOS)[6]. The *eNOS* gene has a pivotal role in the maintenance of stress balance because of its ability to generate nitric oxide (NO) and this feature of the gene makes it a logical candidate gene for various cancers[7]. Human eNOS is encoded by *NOS3* gene comprised of 26 exons with a total size of 21 kb and mapped on to chromosome 7q35 to 36[8]. The *NOS3* gene transcribes an mRNA of 4052 nucleotides which translates 135-KD protein containing 1203 amino acids[9].

NO is a hydrophobic, highly diffusive and small pleiotropic free radical, acting as a signaling molecule in various inflammatory diseases and cancers[10]. It is reported to cause DNA damage in the course of nitration, deamination, nitrosation, and inhibit sealing activity of DNA ligase, facilitating the accumulation of breaks in DNA and promote tumor proliferation, angiogenesis and metastasis[11,12].

A single nucleotide polymorphism (T > C) rs2070744 due to transition of a thymine to a cytosine at -786 in the promoter region of eNOS was found to reduce the rate of mRNA transcription by 50%, resulting in decreased serum NO levels which can inhibit apoptosis or stimulate tumour proliferation, angiogenesis and metastasis[13]. These effects might depend upon the fact that a mutant allele can bind the replication protein A1, which acts as a gene repressor protein[14]. The -786T > C promoter polymorphism has been reported to play very important role in various cardiovascular diseases, hypertension and diabetic neuropathy[15-17]. The genetic-epidemiological studies examining the association of

T > C promoter polymorphism with gastric cancer (GC) from Indian population were meagre. Hence, the present case-control study was aimed to investigate the association between the eNOS (-786T > C) promoter polymorphism rs2070744 and the risk of GC in south Indian population.

MATERIALS AND METHODS

Study group

The study included 150 endoscopically and histopathologically confirmed GC patients referred to the Department of Gastroenterology, Gandhi Hospital and Indo-American cancer Hospital and Research Centre, Hyderabad over a period of 3 years. A total of 150 healthy control subjects with no family history of gastric ulcer or cancer were selected randomly among the persons having normal upper gastro intestinal endoscopy report. The information on demographic features such as age, gender, dietary habits, weight, consanguinity, familial incidence of cancer, addiction to smoking and alcohol was elicited with an informed consent from all the patients and control subjects using a structured questionnaire. *H. pylori* infectivity status was tested in antral biopsies from all the patients by rapid urease test following the method of Vaira *et al*[18] (1988).

Ethics

The study was reviewed and approved by Ethics Committee of the Institute in order to conduct the experiments on human subjects and the procedures followed were in accordance with the ethical standards of the committee.

Bio specimens

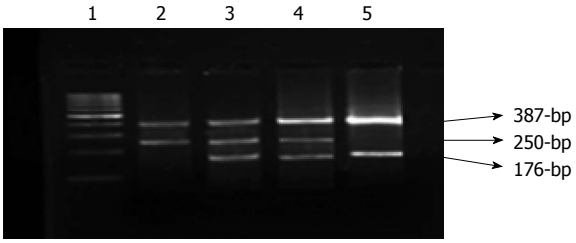
Five millilitres of blood was collected from both patients and control subjects in EDTA coated vacutainers.

eNOS-786T > C rs2070744Genotyping

DNA Isolation: Genomic DNA was isolated from whole blood samples following the salting out method of Lahiri *et al*[19] (1991).

Polymerase chain reaction

Analysis of the eNOS-786T > C promoter polymorphism was carried out by allele -specific polymerase chain reaction method. The oligonucleotide primers used in the reaction were C0: 5′ TTT CTC CAG CCC CTC AGA TG 3′; 2684C: 5′ GGC AGA GGC AGG GTC AGA CG 3′; 2684 T: 5′ CAT CAA GCT CTT CCC TGT CT 3′ and T0: 5′ AGG CCC AGC AAG GAT GTA GT 3′. Amplification was performed in a total volume of 20 μL containing 50 ng genomic DNA, 0.25 μmol/L 2684T and 2684C primers, 0.063 μmol/L T0 and C0 primers, 62.5 μmol/L dNTPs, 1.5 μmol/L $MgCl_2$, and 0.4 U Taq polymerase (Genei Bangalore). After a hot start at 96 ℃, amplification was achieved by 35 cycles at 94 ℃ for 30 s, 60 ℃ for 30 s, and 72 ℃ for 20 s.



Lane 1: 100 bp ladder
Lane 2: Homozygous *TT*
Lane 3 and 4: Heterozygous *TC*
Lane 5: Homozygous *CC*

Figure 1 Gel picture showing various genotypes of endothelial nitric oxide synthase T-786C polymorphism.

Agarose gel electrophoresis

After performing polymerase chain reaction, the amplicons were resolved on a 3% agarose gel stained with ethidium bromide and visualized under ultraviolet gel documentation (Figure 1). The C and T alleles gave a 176 bp and a 250 bp product, respectively, with a 387 bp common product. The genotypes were determined based on the appearance of bands with the help of 100 bp ladder. Ten percent of the samples were taken randomly, subjected to sequencing and no bias was observed in the genotyping. The study revealed similar findings with 100% concordant results.

Statistical analysis

The evaluation of case and control differences in the distribution of alleles and genotypes was carried out by Pearson's χ^2 test of association. Odds ratios (ORs) and corresponding 95%CIs were determined using Javastat 2-way Contingency analysis to measure the strength of association between eNOS-786T > C promoter polymorphism and GC[20]. All statistical tests were two-tailed and *P*-values < 0.05 were considered to be statistically significant. Statistical review of the study was performed by a biomedical statistician.

RESULTS

The present case-control study was conducted on a total of 150 patients and 150 control subjects. Mean age of the patients in the study group was 53 years (Mean ± SD = 53 ± 14 years) and that of controls was 50 years (Mean ± SD = 50 ± 11 years). The demographic characteristics of the study population have been represented in Figure 2. All the GC patients and control subjects were of South Indian origin. The study subjects were classified based on demographic factors such as age, gender, addiction to smoking and alcoholism, consanguinity, *H. pylori* infection, *etc*. We found no significant difference between cases and controls with regard to gender and age. The risk factor profile exhibited that addiction to

Table 1 Distribution of genotype, allelic frequencies and odds risk estimates in patients compared to control subjects

Genotype	Patients (n = 150)		Controls (n = 150)		Odds ratio (95%CI)	P value
	n	%	n	%		
T/T	3	2.00	22	14.67	Reference	
T/C	12	8.00	29	19.33	3.034 (0.67-15.53)	0.136
C/C	135	90.00	99	66.00	10.00 (2.73-43.24)	0.000[a]
T/C + *C/C*	147	98.00	128	85.33	8.42 (2.32-36.2)	0.000[a]
T/T + *T/C vs C/C*	15	10.00	51	34.00	4.636 (2.37-9.17)	0.000[a]
Alleles						
T	18	6.00	73	24.33	Reference	
C	282	94.00	227	75.67	5.038 (2.841-9.026)	0.000[a]

[a]$P < 0.05$.

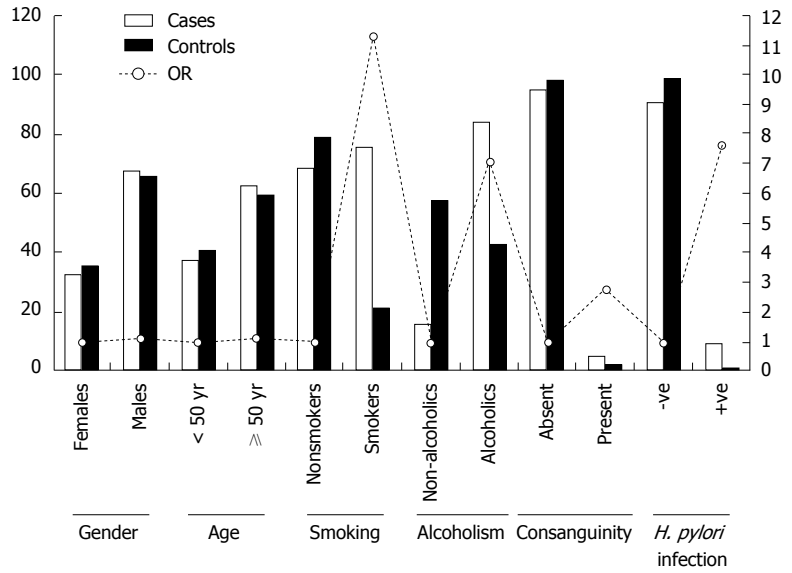


Figure 2 Demographic characteristics and odds risk estimates among gastric cancer cases and controls.

smoking and alcohol were the most common risk factors in patients compared to the control subjects ($P < 0.05$). Individuals with smoking exhibited eleven fold increased risk, where as those with alcoholism revealed seven fold increased risk of developing GC. Individuals with *H. pylori* infection showed 7.6 fold increased risk for cancer. Among the patients 2.4% of them revealed familial incidence of GC. No significant difference was noticed between cases and controls with regard to consanguinity ($P = 0.473$).

The distribution of genotype and allele frequencies of eNOS-786T > *C* promoter polymorphism in patients and controls were given in Table 1. The frequency of *T/T*, *T/C* and *C/C* genotypes in patients were 2%, 8% and 90%, where as in controls the distribution was 14.67%, 19.33% and 66%, respectively. The allelic frequencies were found to be 6% of T and 94% of C in patient group, where as 24.33% and 75.67% in controls, respectively.

The allelic frequencies were compared in order to assess the risk ratio based on the type of allele present in the individuals. There was a significant difference in the distribution of *C* allele (*C vs T*; $P = 0.000$, OR = 5.038) in patient group compared to the control subjects (Table 1) exhibiting a five fold increased risk of GC. Further, we compared *T/T*, *T/C* and *C/C* genotypes against each other in various combinations. When the *T/T* and *C/C* genotypes were compared, there was ten fold enhanced GC risk for individuals with *C/C* genotype (*T/T vs C/C*; $P = 0.000$). In addition, we compared *T/T vs T/C* + *C/C* genotypes as well as *T/T* + *T/C vs C/C* genotypes and found a significant association with the disease, exhibiting 8.4 fold and 4.6 fold increased risk, respectively, in patients compared to the controls (*T/T* + *T/C vs C/C*; $P = 0.000$, OR = 4.636, and *T/T vs T/C* + *C/C*; $P = 0.000$, OR = 8.42).

Table 2 exhibits the genotype distribution based on *H. pylori* infection. The *H. pylori* +ve and -ve individuals in cases and controls were compared separately and observed no association of *T/T* and *T/C* genotypes, but all the patients with *C/C* genotype revealed a significant association between *H. pylori* infection and GC.

Table 3 stratifies the genotype distribution based on addictions like smoking and alcoholism. Individuals

Table 2 Distribution of genotype frequencies in patients and control subjects with regard to *Helicobacter pylori* infection

Characteristic	Controls *n* (%)	Cases *n* (%)	χ^2	OR (95%CI)	*P* value
H. pylori infection					
T/T genotype					
+ve	1 (33.33)	0 (0.00)			
-ve	2 (66.67)	22 (100.00)	1.424	-	0.120
T/C genotype					
+ve	3 (25.00)	1 (3.45)			
-ve	9 (75.00)	28 (96.55)	2.364	9.333 (0.699-267.06)	0.068
C/C genotype					
+ve	10 (7.41)	1 (1.01)			
-ve	125 (92.59)	98 (98.99)	3.887	7.840 (1.008-166.50)	0.027[a]

[a]$P < 0.05$. *H. pylori*: *Helicobacter pylori*.

Table 3 Distribution of genotype frequencies in patients and control subjects with regard to addictions

Characteristic	Cases *n* (%)	Controls *n* (%)	χ^2	OR (95%CI)	*P* value
Smoking status					
T/T genotype					
Smokers	2 (66.67)	4 (18.18)			
Nonsmokers	1 (33.33)	18 (81.82)	1.263	9.000 (0.44-339.56)	0.133
T/C genotype					
Smokers	7 (58.33)	5 (17.24)			
Nonsmokers	5 (41.67)	24 (82.76)	5.080	6.720 (1.21-40.80)	0.020[a]
C/C genotype					
Smokers	104 (77.04)	23 (23.23)			
Nonsmokers	31 (22.96)	76 (76.77)	64.475	11.086 (5.74-21.58)	0.000[b]
Alcoholism					
T/T genotype					
Alcoholics	2 (66.67)	7 (31.82)			
Non-alcoholics	1 (33.33)	15 (68.18)	0.290	4.286 (0.23-145.14)	0.530
T/C genotype					
Alcoholics	10 (83.33)	9 (31.03)			
Non-alcoholics	2 (16.67)	20 (68.97)	7.351	11.11 (1.68-93.07)	0.005[b]
C/C genotype					
Alcoholics	113 (83.70)	48 (48.48)			
Non-alcoholics	22 (16.30)	51 (51.52)	31.385	5.457 (2.86-10.46)	0.000[b]

[a]$P < 0.05$, [b]$P < 0.01$.

having the habit of smoking developed cancer with *T/C* and *C/C* genotypes showing six fold and eleven fold augmented risk of GC, respectively. Similarly, those with alcoholism developed GC with *T/C* and *C/C* genotypes exhibiting eleven fold and five fold enhanced risk of cancer, respectively. Both smoking and alcoholism did not show any association with *T/T* genotype. It is very clear from the Table 3 that association of heterozygous condition with disease indicates single C allele in association with addictions is enough to act as a risk allele for developing the disease (smoking: $P = 0.020$ and alcoholism: $P = 0.005$).

DISCUSSION

Gastric cancer, the fifth most frequent cancer in the world and third most in India, is defined as a multifactorial disorder resulting from interaction among distinctive genetic, epigenetic and environmental risk factors[21]. A single nucleotide polymorphism (-786T > C) in *eNOS* gene promoter rs2070744 demonstrated to play very important role in various cardiovascular diseases, hypertension and diabetic neuropathy and some cancers[7,9].

The polymorphism was found to reduce the transcription rate resulting in decreased serum NO levels which can inhibit apoptosis or stimulate tumour proliferation, angiogenesis and metastasis. The C allele of T > C polymorphism may influence the expression and activity of the NOS enzyme and shown to increase the risk for the development of various diseases[15-17]. There is much more contradiction among the association studies on eNOS T > C promoter polymorphism from divergent ethnic groups. A study by Paradossi *et al*[22] (2004) revealed no significant association of T > C promoter polymorphism with atherosclerosis in Italian population. An association of C allele and *C/C* genotype with coronary artery disease (CAD) was demonstrated in the Iranian population by Khaki-Khatibi *et al*[23] (2013). A hospital-based case-control study by Lu *et*

al[12] (2006) on non-Hispanic white women exhibited a significant association of T > C promoter polymorphism with sporadic breast cancer. In a study by Ghilardi *et al*[24] (2003), the -786T > C polymorphism was found to be associated with vascular invasion in breast cancer[24]. Another study by Jang *et al*[25] (2013) on South Korean population highlighted that TC+CC genotype of the -786T > C polymorphism was significantly associated with an increased risk of colorectal cancer. However, the molecular basis for the significant association of *C/C* genotype of eNOS-786T > C promoter polymorphism remains to be explored.

The present study revealed a statistically significant difference in the distribution of *C/C* genotype and C allele in GC patients compared to control individuals and is in accordance with the report of Ciftçi *et al*[26] (2008). A significantly augmented risk of GC was observed in individuals with *C/C* genotype than those with *T/C* and *T/T* genotypes and it is evident from the literature that *C/C* genotype showed down regulation of eNOS transcription yielding decreased NO levels and ultimately inhibit apoptosis or stimulate tumor proliferation, invasion, angiogenesis and metastasis[13]. It was reported that NO can act as both a pro- and anti-tumorigenic factor. The pro tumorigenic *vs* anti-tumorigenic effect of NO mainly depends on the genetic profile of the individual, cellular microenvironment, the localization and activity of NOS isoforms, and overall NO levels[27]. In some tumor tissues, NO has been found to enhance tumor angiogenesis and induce vasodilatation, thus accelerating tumor growth[28]. In other tumors, including gastric and colon cancer, a decreased amount of NOS protein was demonstrated by immunohistochemistry, and there was a possible relationship between lowered levels of NO and carcinogenesis[29,30]. Previous studies on GC have demonstrated that the expression of the endothelial NOS, neuronal NOS and inducible NOS in the tumor tissue was significantly lower than in normal gastric mucosa and indicates a marked reduction of all three NOS isoforms expression[29,30]. The function of lowered NO levels in tumor development, promotion, progression and metastasis is still obscure. But it is postulated that reduced NO production in tumors help the tumor cells escape programmed cell death and retain the ability to multiply, ultimately contribute to progression and metastatic potential of the tumor[29].

It should be emphasized that from the present study, the demographic factors revealed a significant association of smoking, alcoholism with GC and may be assigned to environmental risk triggering factors present in alcohol and smoke. The present findings can be correlated with earlier studies which had shown that smoking and alcohol were co - operating in increasing GC risk and interpreted based on the fact that cigarette smoke may enhance the risk to develop GC *via* the formation of nitroso amine, a potent carcinogen, where as consuming alcohol had impact on tumor volume doubling time (TVDT) invigorating tumor growth by promoting angiogenesis[31,32].

An important finding from the current study is that there is an interplay between addictions such as smoking, alcoholism and the eNOS genotype which could play a critical role in the etiology of GC. Patients having the habit of smoking or alcoholism develop cancer even with a single C allele showing that only one C allele is sufficient to act as a risk allele for developing the disease. This is in accordance with studies of Wang *et al*[33] (1996) in cardiovascular disorders demonstrating the effect of the eNOS genotype on the risk for the development of disease.

In conclusion, we clearly observed that C allele and CC genotype of the -786T > C polymorphism rs2070744 were significantly associated with etiology of GC and probably due to the lowered NO levels in *C/C* genotype which may ultimately result in tumor proliferation, angiogenesis and metastasis. To the best of our knowledge, present data provides the first molecular epidemiological evidence from south Indian population for the association of *T > C* polymorphism with a risk to develop GC. However, a large confirmatory study involving other populations is warranted to understand the population-specificity and the relative contribution of this polymorphism in the disease phenotype.

COMMENTS

Background

Gastric cancer is a major cause of cancer mortality worldwide. The etiology of a specific cancer may be associated with a set of genetic variants and their interaction with environmental factors. A single nucleotide polymorphism (SNP) occurs when a normally expected nucleotide (Adenine, Guanine, Thymine or Cytosine) in the genome is replaced by other nucleotide. Analysis of SNPs in cancer research has pleiotropic medical implications for health issues, as well as cancer biology. New findings can help in developing targeted therapies for early intervention and management of cancer.

Research frontiers

There has been an increasing focus on the role of SNPs in the development and progression of various cancers and also to assess risk prediction and diagnostics. These SNPs may contribute to differences in disease susceptibility. Analysis and characterization of SNPs as biomarkers in cancer has become the hotspot of current research. The *-786T > C* polymorphism of the endothelial nitric oxide synthase (*eNOS*) gene is one among such important polymorphisms in the etiology of gastric cancer (GC).

Innovations and breakthroughs

The eNOS-786T > C promoter polymorphism was reported to play a role in various cardiovascular diseases. Very limited studies were available on the association of T > C promoter polymorphism with cancer and no studies were done from south Indian population. The results obtained from divergent ethnic groups were contradictory for the association of eNOS T > C promoter polymorphism. A study on atherosclerosis revealed no significant association of T > C promoter polymorphism with the disease in Italian population where as another study on colorectal cancer in the South Korean population highlighted that TC + CC genotype of the -786T > C polymorphism was significantly associated with an increased risk of cancer. Present data clearly revealed an association of C allele and *C/C* genotype with a five fold increased risk of development of GC. Patients with habit of smoking or alcoholism had cancer even with a single C allele showing that only one C allele is able to act as a risk allele for developing the disease. Thus, the present study suggests a strong role of *eNOS* gene rs2070744 promoter polymorphism in modifying cellular micro environment which in turn facilitate tumour development.

Applications

The present study showed that the eNOS -786T > C promoter polymorphism influenced the risk of GC in patients with C allele and CC genotype. The identification of patients with high-risk of GC could help in development of novel treatment strategies and personalized medicine for effective management of the disease.

Terminology

SNP refer to a DNA sequence variation occurs commonly within a population (approximately 1%) in which a normal nucleotide (Adenine, Guanine, Thymine or Cytosine) in the genome is replaced by another nucleotide that differs between members of a biological species or paired chromosomes. Polymerase chain reaction: The polymerase chain reaction is a technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA, generating thousands to millions of copies of a particular DNA sequence and useful in functional analysis of genes, detection and diagnosis of hereditary and infectious diseases and the identification of genetic fingerprints; Agarose gel electrophoresis is a method used in biochemistry, molecular biology, and clinical chemistry to separate a mixed population of DNA or RNA in a matrix of agarose, based on the size of the DNA/RNA fragments.

Peer-review

The authors have written an interesting paper regarding the role of eNOS-786T > C polymorphism in developing GC in south Indian population and found a significant difference in the distribution of C allele in patient group compared to the control subjects exhibiting a fivefold increased risk for GC. Overall the paper is quite educative giving new information in this issue.

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Contents

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EDITORIAL

MINIREVIEWS

CASE REPORT

Contents

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EDITORIAL

Anti program death-1/anti program death-ligand 1 in digestive cancers

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Abstract

Human tumors tend to activate the immune system regulatory checkpoints as a means of escaping immunosurveillance. For instance, interaction between program death-1 (PD-1) and program death-ligand 1 (PD-L1) will lead the activated T cell to a state of anergy. PD-L1 is upregulated on a wide range of cancer cells. Anti-PD-1 and anti-PD-L1 monoclonal antibodies (mAbs), called immune checkpoint inhibitors (ICIs), have consequently been designed to restore T cell activity. Accumulating data are in favor of an association between PD-L1 expression in tumors and response to treatment. A PD-L1 expression is present in 30% to 50% of digestive cancers. Multiple anti-PD-1 (nivolumab, pembrolizumab) and anti-PD-L1 mAbs (MPDL3280A, Medi4736) are under evaluation in digestive cancers. Preliminary results in metastatic gastric cancer with pembrolizumab are highly promising and phase Ⅱ will start soon. In metastatic colorectal cancer (CRC), a phase Ⅲ trial of MPDL3280A as maintenance therapy will shortly be initiated. Trials are also ongoing in metastatic CRC with high immune T cell infiltration (*i.e.*, microsatellite instability). Major challenges are ahead in order to determine how, when and for which patients we should use these ICIs. New radiologic criteria to evaluate tumor response to ICIs are awaiting prospective validation. The optimal therapeutic sequence and association with cytotoxic chemotherapy needs to be established. Finally, biomarker identification will be crucial to selection of

patients likely to benefit from ICIs.

Key words: Program death-1; Program death-ligand 1; Antibody; Digestive cancer

Core tip: Anti-program death-1 and anti-program death-ligand 1 (PD-L1) monoclonal antibodies have been designed to restore T cell activity, since human tumors tend to activate this immune regulatory checkpoint as a means of escaping immunosurveillance. A PD-L1 expression is present in 30% to 50% of digestive cancers and accumulating data are in favor of an association between this PD-L1 expression and response to treatment, which make digestive cancers promising candidates for those breakthrough immunotherapies. We review the ongoing clinical trials and the major challenges ahead of us in order to learn how, when and for which patients we should use these therapeutics.

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TUMOR IMMUNOLOGY

Up until recently, only melanoma and renal cell cancer (RCC) were considered as immunogenic tumors. But in 2012 the results of a phase I study with nivolumab, an anti program death-1 (PD-1) monoclonal antibody (mAb), showed clinical responses in non-small cell lung cancers (NSCLC), thereby introducing the notion that any tumor can respond to the immune checkpoint inhibition strategy[1]. To prevent autoimmunity, to allow peripheral tolerance (during a woman's pregnancy, for instance) or to permit negative feed-back on immune reactions and secure immune system homeostasis, multiple immune checkpoints must be crossed so that immune response can occur and last. Human tumors tend to activate these immune checkpoints as a means of escaping immunosurveillance. That is one reason why new therapeutics called immune checkpoints inhibitors (ICIs) have been designed.

Cancer immunoediting

Cancer immunoediting is currently defined by three E's: elimination, equilibrium and escape[2]. The first phase reflects active immunosurveillance, which facilitates tumor eradication and is mostly mediated by tumor-associated antigen-specific lymphocytes. The second phase refers to the period during which tumor growth is still prevented by the host immune system even though the surviving tumor and its stroma are also shaped by the immune response, which they learn how to downsize. Lastly, the escape phase describes tumor growth notwithstanding an immunologically intact environment due to selection of tumor cell variants during the equilibrium phase.

T cell activation

In order to be activated, a T lymphocyte needs an association of triggering signals. Antigen coupled with major histocompatibility complex recognition is the first step toward activation. A second signal arising from the interaction of co-stimulatory molecules of activation must occur, avoiding T cell anergy. CD28 is the most commonly cited example of co-stimulatory molecules, and it is constitutively expressed on the T cell surface. It binds to B7.1 (CD80) or B7.2 (CD86), which are primarily expressed on activated antigen-presenting cells. B7 molecules also interact with cytotoxic T lymphocyte associated antigen 4 (CTLA-4), which is expressed on T cells. CTLA-4 transmits an inhibitory signal to T cells to prevent early excessive T cell activation. The molecules involved are called immune checkpoint. PD-1 is more widely expressed than CTLA-4 and can be detected not only on T cells but also on B lymphocytes and natural killer cells. Program death-ligand 1 (PD-L1) expression is up-regulated by interferon-γ production, which follows T cell activation. PD-1/PD-L1 interaction allows for negative feedback on the immune response regulating effector T cell responses in peripheral tissues and leads to peripheral T cell tolerance[3,4] (Figure 1). PD-L1 expression is up-regulated on a wide range of cancer cells and tumor-infiltrating immune cells strongly involved in tumor immunosurveillance escape. Several ICIs have been developed so as to prevent those negative regulations of the host immune system.

IMMUNE CHECKPOINT INHIBITORS

To boost immune responses, ipilimumab, an anti-CTLA-4 mAb has been designed and has produced good results in cases of melanoma. Its limiting toxicities are mostly autoimmunity since it seems to upregulate all immune reactions. The PD-1/PD-L1 axis can be targeted by either anti-PD-1 mAbs or anti-PD-L1 mAbs (Figure 2). Anti PD-1 mAbs target PD-1 interactions with both PD-L1 and program death-ligand 2 (PD-L2), while PD-L1 mAbs target interactions between PD-L1 and either PD-1 or B7.1. PD-1 mAbs have been approved for the treatment of unresectable melanoma and NSCLC and their development for bladder cancer and RCC is well-advanced. Targeting of the CTLA-4 pathway has changed the melanoma treatment landscape[5,6] but PD-1/PDL1 axis targeting is also highly promising in multiple tumors[1,7].

Association between PD-L1 expression and treatment response

Several studies have demonstrated an association

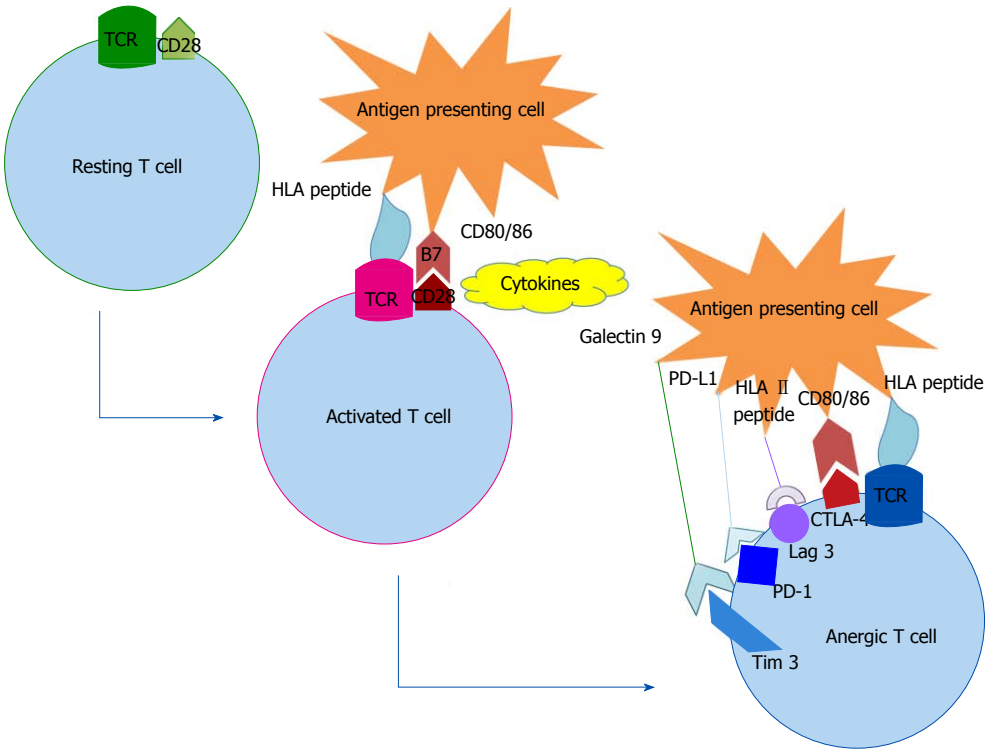

Figure 1 From a resting T cell to an activated or an anergic T cell. To be activated a T cell lymphocyte needs recognition of an antigen coupled with major histocompatibility complex by its specific TCR, adequate cytokines and activation of co-stimulatory molecules such as CD28. An inhibitory signal can instead be transmitted by co-inhibitory molecules (PD-1, CTLA-4, Lag 3, Tim 3…) and lead to T cell anergy. TCR: T cell receptor; CD28: Cluster of differenciation 28; HLA: Human leucocyte antigen; CD80/86: Cluster of differenciation CD80/86; PD-1: Program death-1; PD-L1: Program death-ligand 1; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4.

between pretreatment PD-L1 expression and tumor responses to anti-PD-1/PD-L1 therapies in melanoma, bladder cancer and NSCLC[1,8]. A PD-L1/PD-1 positive tumor should consequently be a good candidate for these treatments. For example, Dong *et al*[9] found 53% of PD-L1 positive colon carcinomas. Later, Droeser *et al*[10] studied PD-L1 expression in 1420 colorectal cancer (CRC). Strong PD-L1 positivity was found in 36% and 29%, respectively in mismatch repair (MMR)-proficient and deficient (dMMR) CRC. dMMR CRC has been associated with high level of tumor-infiltrating lymphocytes (TIL) and a good prognosis[11]. In other digestive cancers, especially in esophageal, gastric and pancreatic cancers, a PD-L1 expression was found in 30%-50% of cases[12-15].

Anti-PD-1 mAbs

Preliminary results are available for two anti-PD-1 mAbs (nivolumab and pembrolizumab) in digestive cancers. Nineteen patients with CRC were enrolled in the phase Ⅰ study of nivolumab, but no efficacy was demonstrated[1]. However, nivolumab is currently being evaluated in multiple digestive cancers both alone and in combination with other ICIs (such as ipilumumab or anti-Lag 3) or with immune system stimulators. A phase Ⅱ clinical trial of nivolumab *vs* nivolumab plus ipilimumab in recurrent and metastatic colon cancer with a stratification between dMMR and pMMR status is ongoing. Pembrolizumab has been evaluated in gastric cancer and preliminary results were presented at the 2014 European Society for Medical Oncology meeting and updated at the 2015 American Society of Clinical Oncology Gastro Intestinal symposium[14]. In this trial, only PD-L1 positive tumors were eligible. Thirty-nine patients were enrolled and 67% had received at least two prior chemotherapy regimens. The overall response rate was 22%. The 6-mo progression-free survival and overall survival rates were 24% and 69%, respectively. Four patients experienced grade 3 to 4 adverse events and one patient died due to treatment-related hypoxia. A phase Ⅱ study will shortly be initiated with pembrolizumab monotherapy or in combination with cisplatin and 5 fluoro-uracil (5FU) in advanced gastric cancer treatment. Pembrolizumab is also currently under investigation in pancreatic cancer and in combination with aflibercept in CRC.

Anti-PD-L1 mAbs

Now focusing on anti-PD-L1 mAbs (BMS936559, MPDL-3280A and MEDI4736) results in digestive cancers, the phase Ⅰ study with BMS936559 enrolled eighteen patients with CRC, fourteen with pancreatic cancer and seven with gastric cancer. None of the gastric cancer patients could be included in the efficacy analysis and no objective response was observed in either CRC or in pancreatic cancer[7]. MPDL3280A showed very promising

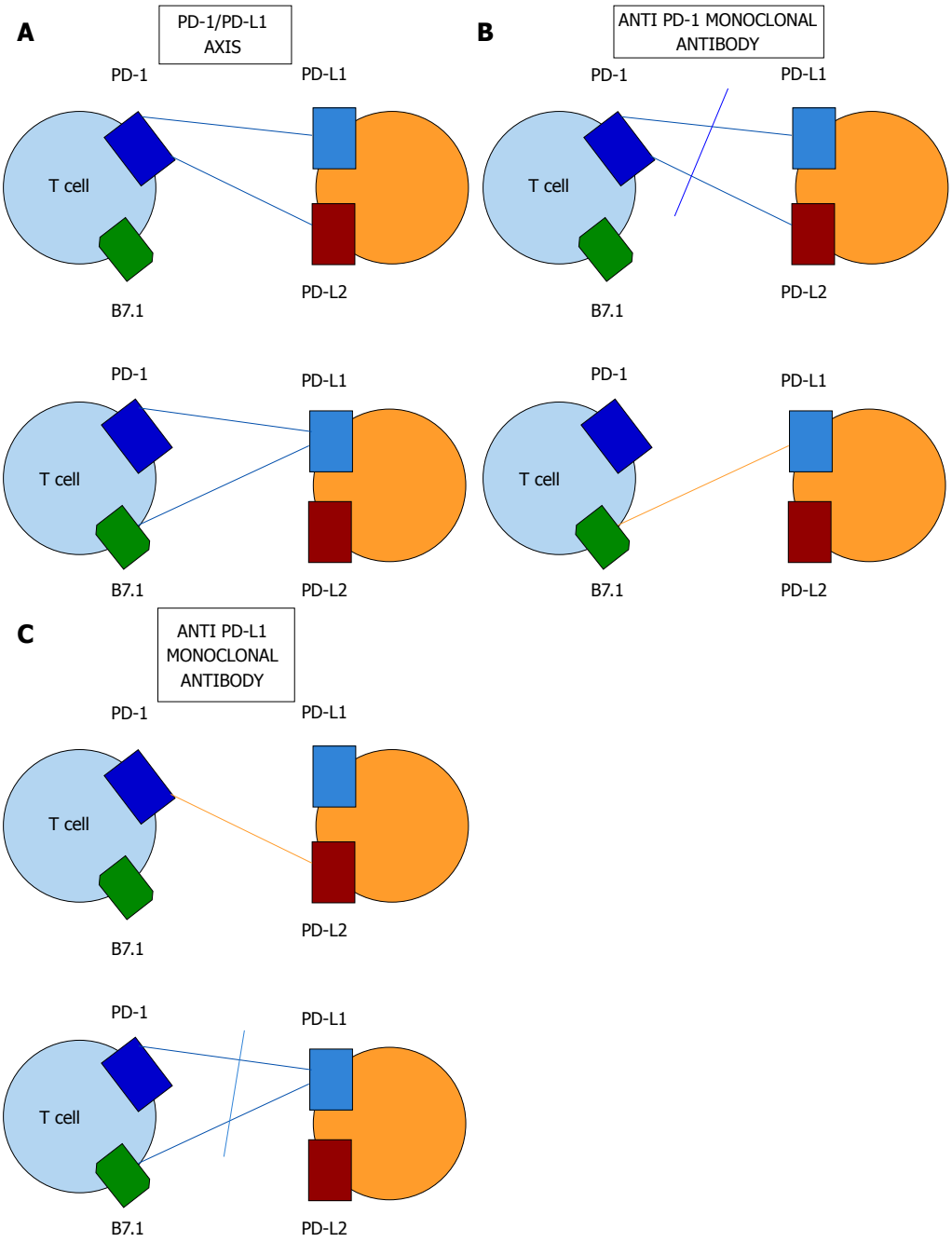

Figure 2 The program death-1 and program death-ligand 1 axis blockade. A: The PD-1 and PD-L1 interactions: PD1 has two ligands called PD-L1 and PD-L2. PD-L1 can interact either with PD-1 or B7.1; B: Anti PD-1 monoclonal antibody blockade prevents PD-L1 and PD-L2 ligation to PD-1 but not the B7.1 and PD-L1 interaction; C: Anti PD-L1 monoclonal antibody blockade prevents PD-1 and B7.1 ligation to PD-L1 but not the PD-1 and PD-L2 interaction. PD-1: Program death-1; PD-L1: Program death-ligand 1; PD-L2: Program death-ligand 2.

results in metastatic bladder cancer[8], NSCLC and RCC[16] but so far no result has been presented in digestive cancer. However, clinical trials are ongoing in combination with immune-modulating therapies (ipilumumab or interferon-α) and in combination with bevacizumab, MEK inhibitor or CD40 agonist. Finally, the MODUL trial is a randomized phase Ⅲ multicenter trial with biomarker-driven maintenance therapy in metastatic CRC first-line treatment (Figure 3). After a four-month FOLFOX plus bevacizumab induction therapy, patients with disease control will be treated by maintenance therapy with 5FU, cetuximab and vemurafenib in *BRAF* mutated tumors or with 5FU, bevacizumab and MPDL3280A in *BRAF* wild-type tumors (the control arm will be 5FU and bevacizumab in both cohorts). MPDL3280A and MEDI4736 are both human IgG1 PD-L1 mAbs whose Fc domain has been engineered to prevent antibody-dependent cell-mediated cytotoxicity (ADCC). Indeed, PD-L1 can be expressed by the tumor-infiltrating immune cells, including T cells and if ADCC was induced, the latter would be killed, which would be counterproductive. The results of the MEDI4736

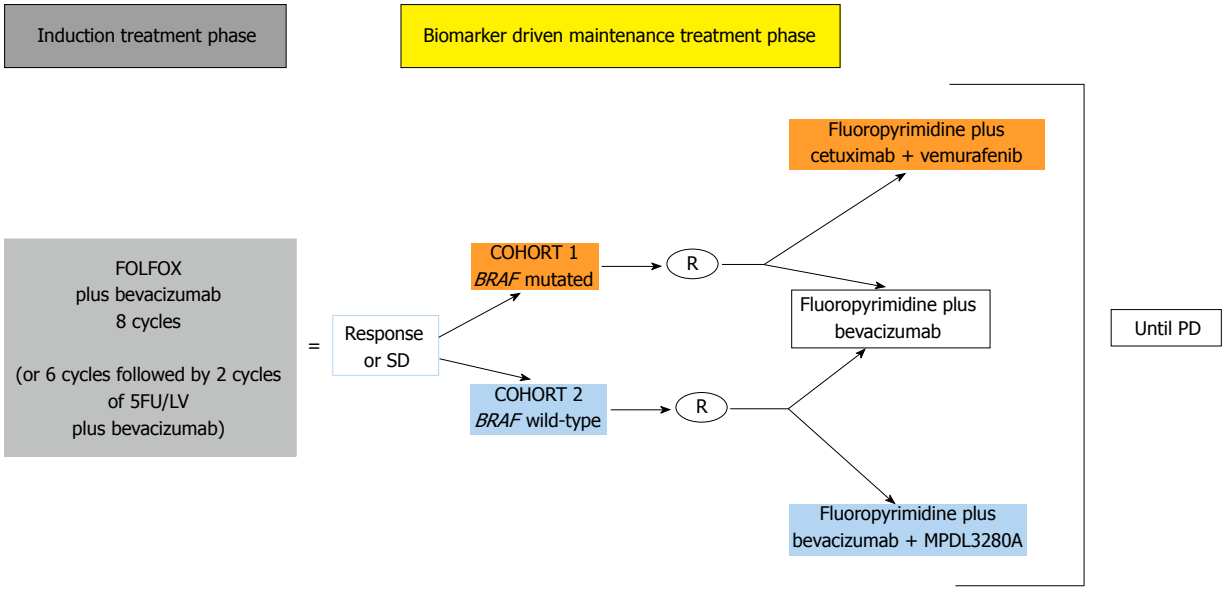

Figure 3 MODUL Phase Ⅲ trial design. 5FU: 5-Fluoro-Uracil; LV: Leucovorin; SD: Stable disease; R: Randomization; PD: Progressive disease.

Table 1 Ongoing anti program death-1 or anti program death-ligand 1 monoclonal antibodies clinical trials in digestive cancers

Monoclonal antibody	Antibody description	Association	Tumors
MPDL3280A	Anti-PD-L1 Engineered Human IgG1[1]	MODUL trial: Phase Ⅲ biomarker driven maintenance therapy	Metastatic colorectal cancer
Medi 4736	Anti-PD-L1 Engineered Human IgG1[1]	None	Immunological subsets of advanced colorectal cancer
Nivolumab	Anti-PD-1 Fully human IgG4[2]	Nab-paclitaxel +/- Gemcitabine	Pancreatic cancer
		GVAX pancreas vaccine + CRS-207	Pancreatic cancer
		None	Squamous cell carcinoma of the anal canal
		Ipilimumab	Recurrent and metastatic colon cancer
		None	Hepatocellular carcinoma
		None	Advanced or recurrent gastric cancer
Pembrolizumab	Anti-PD-1 Humanized IgG4[2]	None	Resectable or borderline resectable pancreas cancer
		None	Advanced gastro-intestinal cancers
		None	Metastatic colorectal cancer with and without microsatellite instability

[1]Engineered Fc domain prevent antibody dependent cell mediated cytotoxicity (ADCC); [2]IgG4 antibody do not induce ADCC. PD-1: Program death-1; PD-L1: Program death-ligand 1; Ig: Immunoglobulin; GVAX: Granulocyte-macrophage colony-stimulating factor–secreting allogeneic pancreatic tumor cells, induces T-cell immunity to cancer antigens, including mesothelin; CRS-207: Live-attenuated L monocytogenes-expressing mesothelin.

multi-arm dose expansion study were presented at the 2014 ASCO meeting and updated at the 2014 ESMO meeting. A disease control rate of approximately 20% was observed across all relevant histology (10 mg/kg every two weeks), especially in hepatocellular carcinoma (19 patients), gastro-esophageal cancer (28 patients) and pancreatic cancer (29 patients)[15]. Tolerance was acceptable with 5.6% grade 3-4 adverse events, and no autoimmunity was reported. A study with MEDI4736 in dMMR CRC and pMMR CRC presenting with high TIL infiltration is scheduled to start.

UPCOMING THERAPEUTIC CHALLENGES

Since ICIs seem as promising in digestive cancer as in other tumors, the same major challenges will be faced. Firstly, since initial progression is not rare, there arises the need for novel criteria to evaluate tumor response to immunotherapeutic agents. As with anti-angiogenic therapies, a tumor burden increase or appearance of new lesions can precede objective response and caution should be used before drawing any conclusion on disease progression[1,6,8,16]. Immune cell

infiltration can explain these features. Recently, immune-related response criteria have been defined and await prospective validation[17]. In any case, progression should be confirmed by a new radiological evaluation four weeks later. Secondly, optimal therapeutic sequences need to be established since most studies have included patients with advanced tumors. As of now no data are available in first-line therapy or in the adjuvant setting, but promising results with ipilimumab in melanoma have been reported[18]. Thirdly, in solid tumors, ICIs will probably need to be combined with chemotherapy, which could cause some problems, given the detrimental effects that chemotherapy can exert on the immune system. Combination with an immunogenic chemotherapy such as oxaliplatin should nonetheless be a good option. Finally, biomarkers are eagerly awaited to enable selection of the patients most likely to benefit from these ICIs. Only 20% to 30% of patients show objective response and in addition to inefficacy, patients are exposed to unnecessary toxicity. PD-L1 expression seems to correlate with clinical outcome but objective responses have been observed in PD-L1 negative tumors. Moreover, definition of a PD-L1 positive tumor needs standardization, given that the threshold of positivity varies between 1% and 5% across different studies and also given that PD-L1 expression can be analyzed either on tumor cells or on tumor-infiltrating cells[16,19]. In melanoma, a predictive model using CD8, PD-1 and PD-L1 positive cells at invasive margins and the tumor center has been correlated with a treatment response but requires prospective validation[20]. In addition, the expression of PD-L1 could be different in primary tumors at the beginning of the disease compared to metachronous metastasis several months later.

CONCLUSION

Many digestive cancers are candidates for the anti-PD-1/PD-L1 axis blockade (Table 1) but we have still got to elucidate for whom, when and how to use them. dMMR CRCs are good candidates due to their high TIL infiltration associated with their high load of frameshift mutations[21]. dMMR CRCs are associated with high-CD8 cytotoxic T cells but also with up-regulation of at least five negative regulatory immune checkpoint molecules (PD-1, PD-L1, CTLA-4, LAG-3, IDO)[22]. One limit to use of ICIs in dMMR CRC could be that it represents only 5% of stage Ⅳ CRCs. Nevertheless, both nivolumab and pembrolizumab are currently being tested in this particular subset.

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MINIREVIEWS

Neoadjuvant or adjuvant therapy for gastric cancer

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Abstract

Currently, there is no international consensus on the best treatment regimen for patients with advanced resectable gastric carcinoma. In the United States, where a limited lymph-node dissection is frequently performed, adjuvant chemoradiotherapy after surgery is the standard treatment. In Europe, intensified perioperative chemotherapy is commonly administered. In Japan and South Korea, postoperative S-1-based adjuvant chemotherapy after surgery with D2 lymph-node dissection is the standard treatment. Several ongoing trials are currently evaluating the optimal sequence of chemotherapy, radiotherapy, and surgery, as well as the place of targeted therapeutic agents in the treatment of advanced gastric carcinoma.

Key words: Radiotherapy; Chemotherapy; Review; Gastric cancer

Core tip: Gastric cancer (GC) treatment is controversy, particularly between Asia and Western countries. In this paper, we have performed a systematic and up-to-date review of resectable GC treatment strategies and discussed different treatment options. We have also discribed ongoing clinical randomized phase 3 trials and future directions in GC treatment.

Quéro L, Guillerm S, Hennequin C. Neoadjuvant or adjuvant therapy for gastric cancer. *World J Gastrointest Oncol* 2015; 7(8): 102-110 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i8/102.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i8.102

INTRODUCTION

Gastric cancer (GC) is one of the most common cancers worldwide, with a total of 989600 new cases diagnosed and 738000 deaths estimated for 2008, which accounted for 8% of total cancer cases and 10% of total deaths from cancer. Over 70% of new cases and deaths occur in developing countries, with the highest incidence rates in Eastern Asia, Eastern Europe, and South America[1]. In the United States, the incidence of GC is

Table 1 Neoadjuvant chemotherapy in gastric carcinoma: Randomized meta-analysis

Studies	Country	Years	Randomization arms	Surgery	Protocol	Patients (*n*)	Overall survival	*P* value	Disease free survival	*P* value
			Neoadjuvant chemotherapy							
Xiong *et al*[16] meta-analysis	-	2014	Chemotherapy	-	-	753	46.6% at 53 mo[1]	0.01	41.1% at 3 yr[1]	< 0.0001
			Surgery alone		-	813	43.7% at 53 mo		27.5% at 3 yr	

[1]Statistically significant result.

approximately 22000 per year and the mortality rate is nearly 11000 per year[2]. The worldwide incidence of GC has declined rapidly over the last three decades in Western countries.

Patients with resectable gastric carcinoma have a poor prognosis with a 5-year overall survival of approximately 20%-30% worldwide, but, in Japan, patients with gastric carcinoma have a better prognosis with a 70% 5-year overall survival rate. This difference is probably because of screening programs for GC in Japan, where the higher incidence of GC results in detection of disease at an earlier stage in approximately 50% of cases. In contrast, gastric carcinoma is usually diagnosed at a later stage in Western countries where there is no such screening program[3]. Moreover, patients with GC in Western countries have more frequently lesions in the upper third of the stomach, whereas patients from Asia have more frequently lesions in the middle or lower third of the stomach; a lesion in the upper third of the stomach has a worse prognosis than a lesion in the lower third[4,5].

Surgical resection remains the cornerstone treatment for non-metastatic GC. In Asia, particularly in Japan and South Korea, gastrectomy with a D2 lymph-node dissection is the standard surgical treatment. In Europe, two randomized trials, performed in the United Kingdom and the Netherlands, have reported little initial benefit from gastrectomy with a D2 dissection compared to gastrectomy with a D1 dissection[6,7]. However, after a 15-year follow-up, the benefit of a gastrectomy with a D2 dissection was confirmed in the Dutch trial in terms of both locoregional recurrence and GC-related death[8]. Gastrectomy with a D2 dissection is now recommended by the National Comprehensive Cancer Network in the United States[9] and the European Society for Medical Oncology in Europe[10].

Resected GC recurs in multiple patterns: locoregional, peritoneal, and distant sites are common modes of recurrence[11,12].

To improve outcomes in patients with locally advanced GC, several strategies in association with surgical resection have been evaluated, such as neoadjuvant chemotherapy, perioperative chemotherapy, adjuvant chemotherapy, and adjuvant chemoradiotherapy.

NEOADJUVANT CHEMOTHERAPY

Several randomized trials have evaluated neoadjuvant chemotherapy before surgery, but have reported conflicting results. To date, four meta-analyses have been published on neoadjuvant chemotherapy for GC[13-16]. The first two meta-analyses were underpowered with only four and five randomized trials analyzed, respectively[13,15]. The third meta-analysis was biased because it included both neoadjuvant chemotherapy and chemoradiotherapy trials[14] (Table 1).

In 2014, Xiong *et al*[16] published a meta-analysis based on results extracted from published trial reports on 1820 patients from 12 different studies. Among these 12 studies, six were from Asia and six were from Western countries. The median follow-up period was 53 mo. The meta-analysis showed that patients treated with neoadjuvant chemotherapy plus surgery had only a marginally improved survival benefit over patients treated with surgery alone, with an odds ratio of 1.32 (P = 0.001). However, the 3-year progression-free survival rate, the tumor down-staging rate, and the R0-resection rate were better in patients treated with neoadjuvant chemotherapy plus surgery, with odds ratios of 1.85 (P < 0.0001), 1.71 (P = 0.0006), and 1.38 (P = 0.01), respectively. Subgroup analyses showed that patients treated with polychemotherapy or *via* an Ⅳ route had better survival, with odds ratios of 1.14 and 1.42, respectively. Subgroup analyses also showed that 5-year overall survival rates of patients treated with neoadjuvant chemotherapy plus surgery were statistically improved in studies conducted in Western countries, with an odds ratio of 1.39 (P < 0.01), whereas similar trials in Asian countries found no significant differences (P = 0.32).

PERIOPERATIVE CHEMOTHERAPY

In locally advanced disease, preoperative chemotherapy may result in tumor downstaging and eradicate micrometastases. Two randomized trials in Western countries have evaluated perioperative chemotherapy in advanced gastroesophageal junction or GC. The United Kingdom Medical Research Council Adjuvant Gastric Cancer Infusional Chemotherapy (MAGIC) randomized trial compared surgery with or without perioperative ECF chemotherapy (epirubicin, cisplatin, infused fluorouracil). A total of 503 patients were enrolled in this trial; most patients had GC (74%), and approximately 50% of patients had a (y)pT3-T4 and 70% had a (y)pN+ tumor[17]. In this study, about 25% and 50% of patients were treated for GC, and received D1 or D2 surgery, respectively. Of the 86% of patients assigned

Table 2 Perioperative chemotherapy in gastric carcinoma: Randomized trials

Studies	Country	Years	Randomization arms	Surgery	Protocol	Patients (*n*)	Overall survival	*P* value	Disease free survival	*P* value
MRC MAGIC trial[17]	United Kingdom	2006	Chemotherapy and surgery	42.5% D2 surgery	ECF chemotherapy	250	36.3% at 5 yr[1]	0.009	34.8% at 5 yr[1]	< 0.001
			Surgery alone			253	23% at 5 yr		24.9% at 5 yr	
ACCORD07/FFCD 9703 trial[18]	France	2011	Chemotherapy and surgery	D2 recommended	5FU-CDDP chemotherapy	113	38% at 5 yr[1]	0.02	34% at 5 yr[1]	0.003
			Surgery alone			111	24% at 5 yr		19% at 5 yr	

[1]Statistically significant result. ECF: Epirubicin, cisplatin, and 5-fluorouracil; 5FU: 5-fluorouracil; RT: Radiotherapy; CDDP: Cisplatin.

to perioperative-chemotherapy and who received preoperative chemotherapy, only 55% also received postoperative chemotherapy. In this study, perioperative chemotherapy improved overall survival, and local + distant control, when compared with surgery alone. Five-year overall survival rates were 36% for patients treated with perioperative-chemotherapy *vs* 23% for those treated with surgery alone (P = 0.009). In the perioperative-chemotherapy group, 14% had local recurrence *vs* 21% in the surgery group. Metastatic progression was also less frequent in the perioperative-chemotherapy group compared to the surgery-only group, at 24% and 37%, respectively (Table 2).

In the French ACCORD07/FFCD 9703 multicenter phase-Ⅲ trial[18], 224 patients with resectable adenocarcinoma of the lower esophagus, the gastroesophageal junction, or the stomach were randomly assigned to receive surgery with or without infused fluorouracil–cisplatin perioperative chemotherapy. In this study, only approximately 25% of the patients had gastric carcinoma; most patients had lower esophageal or gastroesophageal-junction carcinoma (75%). Patients treated with surgery alone had a more advanced tumor than patients treated with surgery plus perioperative chemotherapy. Sixty-eight percent and 80% of patients treated with surgery alone had a (y)pT3-T4 or a (y)pN+ tumor, respectively, compared with 58% and 67% of patients treated with perioperative chemotherapy. Moreover, fewer patients had a R0 resection in the surgery arm compared to the perioperative-chemotherapy arm (74% *vs* 87%, P = 0.004). Of the total, 87% of patients received preoperative chemotherapy as planned but only approximately 50% of patients were able to receive postoperative chemotherapy. Patients treated with surgery and perioperative chemotherapy had significantly better 5-year overall survival and disease-free survival rates than patients treated with surgery alone (38% *vs* 24%, P = 0.02; 34% *vs* 19%, P = 0.003), respectively. In both groups, of the approximately 80% of patients that had a relapse, this was a distant relapse. In multivariable analyses, perioperative chemotherapy was only significantly effective in patients with cancer within the esophagogastric junction, but not for those with GC; however, the gastric subgroup was too small (*i.e.*, 25% of the population) to distinguish between no effect or a small effect.

ADJUVANT CHEMOTHERAPY

Several studies have evaluated adjuvant chemotherapy in GC, but the results are conflicting. Over the past two decades, six meta-analyses have been published regarding the role of adjuvant chemotherapy in GC[19-24]. Five of these six meta-analyses observed improved survival after adjuvant chemotherapy compared to surgery alone[20-24] (Table 3).

In 2010, the Global Advanced/Adjuvant Stomach Tumor Research International Collaboration group published the largest meta-analysis to date, based on individual data from 3838 patients in 17 different studies. Among these studies, four were conducted in Asia and 13 in Western countries. The median follow-up period was approximately 7 years. This meta-analysis reported a small but significant absolute 5.8% benefit to 5-year overall survival (49.6% *vs* 55.3%, P < 0.001) and a 7.4% benefit to 10-year overall survival (37.5% *vs* 44.9%). Adjuvant chemotherapy also improved disease-free survival compared with surgery alone, with an absolute 5.3% benefit at 5 years (48.7% *vs* 54.0%, P < 0.001)[24].

The greatest benefit from adjuvant chemotherapy occurred in the Asian studies. Indeed, the Japanese Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer trial compared surgery with a D2 dissection and either with or without S-1 oral adjuvant chemotherapy in patients with stage-Ⅱ or -Ⅲ gastric carcinoma. This trial enrolled 1059 patients between October 2001 and December 2004[25]. Patients treated with surgery plus adjuvant S-1 chemotherapy had significantly better 5-year overall and disease-free survival rates than those treated with surgery alone (71.7% *vs* 61.1% and 65.4% *vs* 53.1%, respectively). Peritoneum and hematogenous metastases represented approximately 80% of the relapses. All tumor subgroups benefited from adjuvant chemotherapy. However, poor outcomes were observed in patients with stage-ⅢB gastric carcinoma, with a 5-year overall-survival rate of 50.2% in the S-1 group and 44.1% in the surgery-alone group[26]. This observation suggests the need for therapeutic improvement in advanced gastric carcinoma. Because of these results, adjuvant chemotherapy without radiation for GC has now become the standard-of-care in Japan.

The Asian CLASSIC trial compared surgery with a

Table 3 Adjuvant chemotherapy in gastric carcinoma: Randomized trials/meta-analysis

Studies	Country	Years	Randomization arms	Surgery	Protocol	Patients (*n*)	Overall survival	*P* value	Disease free survival	*P* value
ACTS-GC trial[25,26]	Japan	2007	Chemotherapy and surgery	D2 surgery	Oral S1 chemotherapy	529	71.7% at 5 yr[1]	-	65.4% at 5 yr[1]	-
			Surgery alone			530	61.1% at 5 yr		53.1% at 5 yr	
GASTRIC metaanalysis[24]	-	2010	Chemotherapy	-	-	1924	55.3% at 5 yr[1]	< 0.001	54% at 5 yr[1]	< 0.001
			Surgery alone		-	1857	49.6% at 5 yr		48.7% at 5 yr	
CLASSIC trial[27]	South Korea	2012	Chemotherapy and surgery	D2 surgery	XELOX chemotherapy	520	83% at 3 yr[1]	0.049	74% at 3 yr[1]	< 0.0001
			Surgery alone			515	78% at 3 yr		59% at 3 yr	

[1]Statistically significant result. XELOX: Xeloda and oxaliplatin.

D2 dissection either with or without adjuvant combined capecitabine/oxaliplatin (XELOX) chemotherapy in 1035 patients with stage Ⅱ–ⅢB gastric carcinoma[27]. After a median follow-up of 34 mo, 3-year disease-free and overall-survival rates were significantly better in the XELOX plus surgery group than with surgery alone (74% *vs* 59%, $P < 0.0001$; 83% *vs* 78%, $P = 0.0493$, respectively). The most common sites of disease progression were the peritoneum and distant sites (*i.e.*, > 80%).

ADJUVANT CHEMORADIOTHERAPY

In the United States, the SWOG 9008/ Intergroup 0116 trial reported a benefit after postoperative chemoradiotherapy. In this trial, 556 patients with locally advanced gastric adenocarcinoma or cancer within the gastroesophageal junction were randomized to receive surgery alone or surgery plus postoperative radiotherapy associated with 5-fluorouracil/leucovorin chemotherapy[28]. Three-year overall survival was 50% in the chemoradiotherapy group *vs* 41% in the surgery-only group ($P = 0.005$). The 3-year relapse-free survival rate was 48% in the chemoradiotherapy group *vs* 31% in the surgery-only group ($P < 0.001$). This benefit from postoperative chemoradiotherapy was confirmed in an update, published by Smalley *et al*[29] in 2012, with 10-year overall survival of 25.9% *vs* 17.3% for surgery only ($P = 0.0046$) and a 10-year relapse-free survival rate of 21.6% *vs* 14.4% ($P < 0.001$).

Local and regional relapses were significantly less frequent in the chemoradiotherapy group, at 2% and 22% *vs* 5% and 31% in the surgery-alone group, respectively ($P = 0.012$). There were no differences in terms of distant relapses between the two groups (16% and 17%, respectively) (Table 4).

However, several criticisms have been raised regarding this study. Most patients had limited lymph-node dissection and only 10% of patients received a formal D2 dissection (36% had a D1 and 54% had a D0 dissection) and many patients experienced high rates of acute toxicity (54% and 33% of patients had ⩾ grade 3 hematological and gastrointestinal toxicities, respectively). Only 64% of patients completed the protocol treatment in the chemoradiotherapy group: 17% of patients interrupted treatment because of its toxic side-effects and 8% declined further treatment. These high rates of toxicity may be explained by the use of the older 2D radiotherapy technique associated with the 5-fluorouracil Mayo Clinic chemotherapy regimen.

The United States CALGB80101 phase-Ⅲ trial compared 546 patients with resected gastric or gastroesophageal-junction adenocarcinoma who had adjuvant chemoradiotherapy with the 5-fluorouracil Mayo Clinic chemotherapy regimen (SWOG 9008/Intergroup 0116 protocol) *vs* adjuvant chemotherapy with ECF (epirubicin, cisplatin, 5-fluorouracil) followed by chemoradiotherapy with fluorouracil[30]. Seventy-five percent and 69% of patients completed the planned treatments in the ECF and Mayo 5-fluorouracil arms, respectively. Patients receiving adjuvant ECF chemotherapy had lower rates of grade ⩾ 3 diarrhea/mucositis (15% *vs* 7%) and also less grade-4 neutropenia compared to patients receiving the adjuvant fluorouracil Mayo-Clinic chemotherapy regimen (33% *vs* 19%). However, the 3- and 5-year overall survival rates were not significantly improved with ECF compared to fluorouracil (52% *vs* 50% and 44% *vs* 41%, respectively; $P = 0.8$). These results suggest that the intensified chemotherapy in association with adjuvant radiotherapy was better tolerated but was not associated with better outcomes compared to the fluorouracil-based chemoradiotherapy used in the SWOG 9008/Intergroup 0116 protocol. However, a longer follow-up period is needed to confirm these results.

The Korean phase-3 Adjuvant chemoRadiation Therapy In STomach cancer (ARTIST) trial randomized 458 patients with locally advanced gastric carcinoma and who had been initially treated with D2 lymph-node dissection. The trial compared postoperative capecitabine–cisplatin chemotherapy *vs* capecitabine–cisplatin chemotherapy plus chemoradiotherapy with capecitabine. In this trial, it is important to note that 60% of patients had early stages of gastric carcinoma (IB and Ⅱ) and, therefore, had a spontaneously better prognosis than patients with locally advanced-stage carcinoma. Treatment was completed as planned in 75.4% of patients in the chemotherapy arm *vs* 81.7% in the chemoradiotherapy arm.

After a median follow-up of 53.2 mo, there was no difference in 3-year disease-free survival (78.2% in the

Table 4 Adjuvant chemoradiotherapy in gastric carcinoma: Randomized trials

Studies	Country	Years	Randomization arms	Surgery	Protocol	Patients (*n*)	Overall survival	*P* value	Disease free survival	*P* value
INT 0116 trial[28,29]	United States	2001	Chemoradiotherapy and surgery	10% D2 surgery	5FU Mayo clinic/5FU RT	281	50% at 3 yr[1]	0.005	48% at 3 yr[1]	< 0.001
			Surgery alone			275	41% at 3 yr		31% at 3 yr	
Chinese multicentre trial[33]	China	2012	Chemoradiotherapy and surgery	D2 surgery	5FU RT	186	48.4% at 5 yr	0.122	45.2% at 5 yr[1]	0.029
			Chemotherapy and surgery		5FU chemotherapy	165	41.8% at 5 yr		35.8% at 5 yr	
ARTIST trial[31]	South Korea	2012	Chemoradiotherapy and surgery	D2 surgery	Xeloda CDDP/Xeloda RT	230	-	-	74.2% at 3 yr	0.086
			Chemotherapy and surgery		Xeloda CDDP	228	-		78.2% at 3 yr	
CALGB 80101 trial[30]	United States	2011	Chemoradiotherapy and surgery	Not available	ECF/5FU RT	266	52% at 3 yr	0.8	47% at 3 yr	0.99
			Chemoradiotherapy and surgery		5FU Mayo/ 5FU RT	280	50% at 3 yr		46% at 3 yr	

[1]Statistically significant result. 5FU: 5-fluorouracil; RT: Radiotherapy; CDDP: Cisplatin.

chemotherapy arm *vs* 74.2% in the chemoradiotherapy arm; $P = 0.0862$)[31]. However, in a subgroup analysis of 396 patients with positive pathological lymph nodes, there was statistically better 3-year disease-free survival in patients treated with chemoradiotherapy compared to those treated with chemotherapy (77.5% *vs* 72.3%, $P = 0.0365$). There were no significant differences between the two arms in terms of locoregional recurrence or distant metastases (8.3% *vs* 4.8%; $P = 0.353$ and 24.6% *vs* 20.4%; $P = 0.557$, respectively). Due to the lack of events at the time of analysis, the secondary end point for overall survival was not analyzed.

In a Korean observational study, Kim *et al*[32] compared 544 patients who had received postoperative chemoradiotherapy after a curative D2 dissection with 446 patients who had received surgery without any further treatment. In this study, it is important to note that the proportion of patients with advanced-stage carcinoma was significantly greater in the chemoradiotherapy group than in the surgery-only group (stage ⅢA: 34.1% *vs* 26.0%, and stage Ⅳ: 21.9% *vs* 13.9%).

Twenty-five percent of patients treated with chemoradiotherapy did not complete the planed protocol: the main reasons for this were its toxic side-effects (40%) and the patient's refusal to continue (35%). Thirty percent of patients experienced ≥ grade 3 hematological toxicity and 15% experienced ≥ grade 3 gastrointestinal toxicity. After a median follow-up of 66 mo, the 5-year overall survival and relapse-free survival rates were better in patients treated with chemoradiotherapy compared to those treated with surgery only (57.1% *vs* 51%; $P = 0.0198$, and 54.5% *vs* 47.9%; $P = 0.0161$, respectively). Locoregional recurrence rate was significantly lower in patients treated with chemoradiotherapy compared to those treated with surgery alone (14.9% *vs* 21.7%, $P = 0.005$). The occurrence of distant metastases did not differ between the treatment groups (37.7%).

A Chinese randomized trial compared postoperative fluorouracil–leucovorin chemotherapy *vs* intensity modulated radiation therapy plus fluorouracil–leucovorin chemotherapy in 380 patients initially treated with a D2 dissection for locally advanced gastric carcinoma (70% had stage Ⅲ or Ⅳ disease). Five-year overall survival in those that received postoperative radiotherapy was better than for those treated with chemotherapy only, but this difference was not statistically significant (48.4% *vs* 41.8%, $P = 0.122$). The 5-year recurrence-free survival rate in patients receiving chemoradiotherapy was also better (45.2% *vs* 35.8%, $P = 0.029$)[33]. Patients treated with chemoradiotherapy also had less local relapses than those treated with chemotherapy only (15.6% *vs* 24.2%; $P = 0.042$). However, the occurrence of distant metastases did not differ between the treatment arms (24.2% *vs* 26.7%, $P = 0.595$). In this study, multivariate analyses showed that pathological lymph node involvement and TNM stage were both independent prognostic factors.

ONGOING TRIALS AND FUTURE DIRECTIONS

The ongoing CRITICS phase-Ⅲ study (ChemoRadiotherapy after Induction chemoTherapy In Cancer of the Stomach) (NCT00407186) is comparing patients undergoing preoperative epirubicin, cisplatin, capecitabine (ECC) chemotherapy followed by a D1 dissection, with patients receiving postoperative ECC chemotherapy alone, with patients receiving radiotherapy plus concurrent capecitabine + cisplatin[34]. The study plans to accrue 788 patients with gastric carcinoma. The primary endpoint of the study is overall survival; secondary endpoints are disease-free survival, toxicity, health-related quality of life, prediction of response, and recurrence risk, assessed by genomic and expression profiling (Table 5).

Table 5 Ongoing phase-Ⅲ randomized trials

Study	Country	No. registration	Standard arm	Experimental arm	Patients (*n*)
		Neoadjuvant chemoradiotherapy			
EORTC 22114 - 40111 TOP GEAR study	Europe	NCT01924819	ECC/ECF preoperative CT	ECC/ECF preoperative CT and RTCT preoperative	752
		Perioperative chemotherapy			
MAGIC-B/ST03 study	United Kingdom	NCT00450203	ECC perioperative CT	ECC + bevacizumab perioperative CT	1100
PRODIGY trial	South Korea	NCT01515748	S-1 adjuvant CT	Neoadjuvant DOS CT and S-1 postoperative CT	640
		Adjuvant chemotherapy			
ARTIST II Trial	South Korea	NCT01761461	S-1 adjuvant CT (arm 1)	SOX adjuvant CT (arm 2), S-1 and RT adjuvant (arm 3)	1000
		Adjuvant chemoradiotherapy			
CRITICS Trial	The Netherlands	NCT00407186	ECC perioperative CT	ECC preoperative CT and RTCT postoperative	788

CT: Chemotherapy; RTCT: Radiochemotherapy; ECC: Epirubicin, cisplatin and capecitabine; ECF: Epirubicin, cisplatin, and fluorouracil; DOS: Docetaxel, oxaliplatin, and S-1; SOX: S-1 and oxaliplatin.

The international ongoing phase-Ⅱ/Ⅲ European Organisation for Research and Treatment of Cancer (EORTC) 22114–40111 TOP GEAR study (Trial Of Preoperative therapy for Gastric and Esophagogastric junction AdenocaRcinoma) (NCT01924819) is currently testing whether adding chemoradiotherapy to ECF or ECC chemotherapy is superior to ECF or ECC chemotherapy alone for the preoperative treatment of resectable esophagogastric-junction or gastric carcinoma when treated with at least a D1 dissection (D2 dissection recommended). The phase-Ⅱ part of this study is being conducted in 35 medical centers in nine countries: Belgium, France, Germany, Israel, Czech Republic, Slovenia, Spain, Turkey, and Italy, and is planning to accrue 120 patients. The study is designed to demonstrate the efficacy of chemoradiotherapy. The phase-Ⅲ trial plans to accrue 752 patients and will determine whether chemoradiotherapy is superior to chemotherapy in these patients.

The Korean ARTIST Ⅱ phase-Ⅲ trial (Adjuvant chemoRadiation Therapy In STomach cancer Ⅱ) (NCT01761461) plans to accrue 1000 patients with stage-Ⅱ or -Ⅲ gastric or gastroesophageal carcinoma with positive lymph nodes (AJCC 2010), and who are being treated with curative gastrectomy and more than a D2 lymph-node dissection. This three-arm trial is currently comparing surgery + adjuvant S-1 chemotherapy for 1 year, *vs* surgery + adjuvant SOX (S-1 and oxaliplatin) chemotherapy, *vs* surgery + adjuvant SOX (S-1 and oxaliplatin) chemotherapy + radiotherapy. The primary endpoint of the study is disease-free survival.

The United Kingdom MRC MAGIC-B/ST03 study (NCT00450203) is an ongoing phase-Ⅱ/Ⅲ study being conducted in 106 United Kingdom centers, which plans to accrue 1100 patients with histological stage Ib (T1 N1, T2a/b N0), Ⅱ, Ⅲ or stage Ⅳ (T4 N1 or N2) gastric or gastroesophageal-junction carcinoma. This randomized trial is currently comparing standard surgery + ECC (epirubicin, cisplatin, capecitabine) perioperative chemotherapy *vs* standard surgery + ECC perioperative chemotherapy + bevacizumab. Primary endpoints are the safety and efficacy of the phase-Ⅱ trial and overall survival in the phase-Ⅲ trial. Secondary endpoints are response rates to preoperative treatment, surgical-resection rates, disease-free survival, quality of life, and cost-effectiveness. A pilot study within ST03, which is randomizing *HER2*-positive patients to standard ECX with modified ECX plus Lapatinib (Tyverb), will assess the safety and *HER2* positivity rate in 40 patients.

The Japanese JCOG 0501 phase-Ⅲ trial (NCT00252161) plans to accrue 316 patients, from 35 institutions, with type-4 and large type-3 gastric carcinoma and who have undergone a gastrectomy + more than a D2 dissection. The primary endpoint will be overall survival; secondary endpoints will be progression-free survival, response rate, proportion completing treatment, proportion having a curative resection, and adverse events.

The ongoing Korean PRODIGY phase-Ⅲ randomized trial (NCT01515748) plans to accrue 640 patients with resectable advanced GC (T2–3, N+, or T4 tumors). This study is currently testing neoadjuvant DOS (docetaxel, oxaliplatin, S-1) chemotherapy + surgery + adjuvant S-1 chemotherapy for 1 year *vs* surgery + adjuvant S-1 chemotherapy for 1 year. The primary endpoint is progression-free survival; the secondary endpoints are overall survival, stage distribution between the groups assessed after surgery, and R0 resection rate.

Targeted therapy in GO

Several molecular pathways are known to be involved in gastric carcinogenesis, such as HER2, HER3, EGFR, HGFR/c-MET, E-Cadherin, MMP, VEGF/VEGFR, WNT/β-catenin, FGFR and Akt/PI3K/mTOR[35]. Targeted and biological therapies are promising treatments in advanced GC. Combining chemotherapy with a targeted therapy may improve the complete pathological response (pCR) and survival, but also individualize

therapies and reduce toxicities.

HER2 is a transmembrane growth-factor receptor encoded by the proto-oncogene *ERBB2*, which is located on chromosome 17q21. The frequency of HER2-positive GC varies considerably between studies, ranging from 6.0%-36.6%[36].

HER2 overexpression has been shown to predict the response to trastuzumab, a humanized recombinant monoclonal antibody that selectively binds to the extracellular domain of HER2, thereby blocking its downstream signaling. In the randomized ToGA trial, the addition of trastuzumab to cisplatin + capecitabine–fluorouracil significantly improved the objective response rate from 35% to 47% (P = 0.0017), progression-free survival from 5.5 to 6.7 mo (P = 0.0002), and overall survival from 11.1 to 13.8 mo (P = 0.0046)[37].

The ongoing German Herceptin in combination with Fluorouracil, Leucovorin, Oxaliplatin, and Taxotere AIO-STO-0310 multicenter phase-Ⅱ study is currently testing perioperative chemotherapy with 5-FU, leucovorin, docetaxel, and oxaliplatin (FLOT) in combination with trastuzumab in patients with HER2-positive, locally advanced, resectable adenocarcinoma of the gastroesophageal junction or stomach (NCT01472029). The primary endpoint is the rate of pCR. Hofheinz *et al*[38] reported the preliminary results from the first 25 patients at the 2014 ASCO meeting: A pCR was found in 22% of patients and near complete regression (< 10% residual tumor cells) was observed in 24% of patients. The complete resection rate was 90%.

The Spanish NEOHX multicenter phase-Ⅱ study evaluated the efficacy and toxicity profile for perioperative XELOX-T (capecitabine, oxaliplatin, trastuzumab) followed by adjuvant trastuzumab as a monotherapy in patients with advanced resectable stomach or esophagogastric-junction adenocarcinoma that was HER-2+. The primary endpoint was 18-mo disease-free survival. By the end of the study, 36 patients had been included. Preliminary results were reported at the 2013 ASCO meeting: pCR was observed in 19% and complete-resection rate (R0) was observed in 78% of patients. However, the follow-up period was too short for disease-free survival or overall survival to be assessed[39].

The future EORTC randomized phase-Ⅱ trial (INNOVATION) will test neoadjuvant chemotherapy with cisplatin–capecitabine plus trastuzumab *vs* cisplatin–capecitabine plus trastuzumab plus pertuzumab in HER2-positive resectable gastric or gastroesophageal-junction adenocarcinoma (NCT02205047). Pertuzumab is a humanized monoclonal antibody that binds to extracellular dimerization domain Ⅱ of HER2, and inhibits heterodimerization of HER2 with other HER family members, especially HER2–HER3, which is the most potent signaling HER heterodimer. The primary endpoint will be the rate of major pathological response (*i.e.*, < 10% vital tumor cells).

CONCLUSION

Currently, the treatment for locally advanced gastric carcinoma is based on R0 surgical resection with D2 lymph-node dissection. A D1 lymph-node dissection, with at least 15 lymph nodes resected, could also be performed in less experienced centers. Complementary treatment after curative surgical resection in T3 and/or N+ gastric carcinoma should be discussed. Perioperative chemotherapy and adjuvant chemoradiotherapy have significantly improved overall survival compared to surgery alone in Europe and the United States. In Asia, adjuvant chemotherapy, with S-1 or XELOX delivered after surgery + a D2 lymph-node dissection has shown significantly improved survival compared to surgery alone. Ongoing randomized trials are currently testing the efficacy of adjuvant chemoradiotherapy after neoadjuvant chemotherapy; intensified chemotherapy, and targeted therapy plus chemotherapy.

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Inflammation-based factors and prognosis in patients with colorectal cancer

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Abstract

Several parameters for predicting survival in patients with colorectal cancer have been identified, including the performance status, age, gender and tumor-node-metastasis (TNM) stage. Although the TNM stage is important and useful for predicting the prognosis and determining the appropriate treatment, it is well known that the survival time varies widely, even in patients with the same stage of disease. Therefore, the identification of new parameters capable of more precisely predicting patient survival is needed to help select the optimal treatment, especially in patients in the advanced stage of disease. Although the TNM stage reflects the tumor characteristics, cancer progression and survival are not determined solely based on the local characteristics of the tumor, but also the host systemic immune/inflammatory response. Therefore, using a combination of parameters that reflect both tumor characteristics and the host systemic inflammatory status is thought to be important for accurately predicting patient survival.

Key words: Colorectal cancer; Platelet-to-lymphocyte ratio; Prognosis; Glasgow Prognostic Score; C-reactive protein; Neutrophil-to-lymphocyte ratio; Inflammation-based factor; Nutritional Prognostic Index

Core tip: Recently, it has become clear that an elevated systemic inflammatory response is consistently associated with a poor outcome, independent of the tumor stage. The inflammatory response is represented by the levels of serum neutrophils, lymphocytes and platelet s as well as acute-phase proteins and their combinations. These parameters are simple and easy to measure using widely available standardized assays. In this review, we discuss the prognostic value of various inflammation-based factors in patients with colorectal cancer.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common causes of cancer-related death worldwide[1]. Approximately 20% of patients with CRC present with distant metastasis at the time of diagnosis[1], and the survival of patients with unresectable stage Ⅳ CRC is very poor, with a median survival time (MST) of approximately six to eight months among those who receive the best supportive care without chemotherapy[2]. However, due to the development of chemotherapeutic and molecular targeting agents, the survival time has improved dramatically within the last decade, with an MST of 24-30 mo[3-6].

Several parameters for predicting survival in patients with CRC have been identified, including patient characteristics, such as the performance status (PS), age and gender, and tumor characteristics, such as clinicopathological factors and the TNM stage. Although the stage determined according to the Union for International Cancer Control (UICC) TNM classification[7] is important and useful for predicting the prognosis and determining the appropriate treatment, it is well known that the survival time varies widely, even in patients with the same stage of disease. Therefore, the development of a new parameter able to more precisely predict the patient survival required to help select the optimal treatment, especially in patients with advanced disease. It has been reported that many molecular parameters (such as proteins involved in cell cycle regulation, apoptosis and angiogenesis or RAS/RAF mutations) are associated with survival[8-14]. However, measuring these molecular parameters requires sophisticated and expensive laboratory techniques.

It is now recognized that disease progression in cancer patients is determined not only by tumor characteristics, but also the host inflammatory response[15]. Moreover, it has become clear that an elevated systemic inflammatory response is consistently associated with a poor outcome independent of the tumor stage[16-18]. The inflammatory response is represented by the levels of serum white blood cells, neutrophils, lymphocytes and platelets and acute-phase proteins, such as C-reactive protein (CRP) and albumin. These parameters are simple and easy to measure using widely available standardized assays.

Recently, several combinations of these factors, including Glasgow Prognostic Score (GPS), neutrophil-to-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), and prognostic nutritional index (PNI), have also been reported to be useful prognostic factors in various malignant solid tumors, including CRC (Table 1)[19-32].

The aim of this review was to examine the value of various inflammation-based factors as useful prognostic factors in patients with CRC.

CRP LEVEL

CRP is an acute-phase protein synthesized in hepatocytes whose serum level increases in response to inflammatory disease[33,34]. Cancer growth also induces a tissue inflammatory response, and thus increases the serum CRP level. Elevation of the serum CRP concentration reflects a state of hyper-cytokinemia, as the CRP level is upregulated by proinflammatory cytokines, such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)[33,34]. These cytokines have the ability to promote tumor growth and metastasis and play a role in tumor progression.

Many investigators have reported that a high level of serum CRP significantly correlates with poor survival in patients with CRC treated with curative surgery[19-21]. Nozoe *et al*[19] reported that the preoperative elevation of CRP was related to recurrence after curative resection for CRC. Toiyama *et al*[20] reported a correlation between elevated CRP and recurrence in patients with rectal cancer undergoing chemoradiotherapy followed by surgery. We investigated the correlation between serum CRP levels and the prognosis of patients with stage Ⅳ CRC who underwent the palliative resection of their primary tumor[20]. We found that a high preoperative serum CRP level was a convenient marker for identifying the stage Ⅳ CRC patients with a poor prognosis.

GPS

GPS, which is also an inflammation-based factor, is defined according to the presence of an elevated serum CRP level and hypoalbuminemia. Briefly, patients with both an elevated CRP level (> 1.0 mg/dL) and hypoalbuminemia (< 3.5 g/dL) are allocated a score of 2. Patients in whom only one of these biochemical abnormalities is present are allocated a score of 1 and those in whom neither of these abnormalities are present are allocated a score of 0[17,18]. This score has been shown to be a prognostic indicator, independent of the tumor stage, in a variety of gastrointestinal cancers[22-24,35,36]. Sugimoto *et al*[22] examined patients with stage Ⅱ CRC who underwent a curative resection and reported that the cancer specific survival was significantly worse in the patients with a GPS of 2 than in those with a GPS of 1 or 0. Proctor *et al*[35] also reported that a raised GPS was associated with reduced overall survival and cancer specific survival in CRC patients, independent of age, gender and Dukes' stage. Moreover, GPS of 2 has been reported to be an independent significant prognostic factor, even in patients with unresectable stage Ⅳ CRC[23,24]. Ishizuka *et al*[24] reported a correlation between GPS and chemotherapy tolerance and noted that it would be useful for deciding

Table 1 Previously reported correlations between various inflammation-based factors and the prognosis

Inflammation-based factors	Ref.	Year	Timing of measurement	*n*	TNM staging	Treatment	Survival analysis	Summary results
CRP	Nozoe *et al*[19]	1998	Preoperation	120	Ⅰ-Ⅳ	Resection	OS	Positive
	Toiyama *et al*[20]	2013	Preoperation	84	Ⅰ-Ⅲ	Resection and CRT	DFS, OS	Positive
	Shibutani *et al*[21]	2014	Preoperation	144	Ⅳ	Resection and CT	PFS, OS	Positive
GPS	Sugimoto *et al*[22]	2012	Preoperation	166	Ⅱ	Resection	OS	Positive
	Kishiki *et al*[23]	2013	Pretreatment	79	Ⅳ	CT	OS	Positive
	Ishizuka *et al*[24]	2013	Preoperation	108	Ⅳ	Resection	OS	Positive
NLR	Chua *et al*[26]	2011	Pre and post treatment	171	Ⅳ	CT	OS	Positive
	Chua *et al*[26]	2011	Preoperation	674	Ⅰ-Ⅳ	Resection	OS	Positive
	Li *et al*[27]	2014	-	-	Meta-analysis	-	DFS, OS	Positive
OPNI	Nozoe *et al*[28]	2012	Preoperation	219	Ⅰ-Ⅳ	Resection	OS	Positive
	Maeda *et al*[29]	2014	Preoperation	100	Ⅳ	Resection and CT	OS	Positive
	Ikeya *et al*[30]	2014	Pre and post treatment	80	Ⅳ	CT	OS	Positive
PLR, NLR	Kwon *et al*[31]	2012	Preoperation	200	Ⅰ-Ⅲ	Resection	OS	Positive
GPS, NLR	Maeda *et al*[32]	2013	Preoperation	94	Ⅳ	resection and CT	OS	Positive

CRP: C-reactive protein; GPS: Glasgow Prognostic Score; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; CT: Chemotherapy; CRT: Chemoradiotherapy; OS: Overall survival; DFS: Disease-free survival; PFS: Progression-free survival.

the indications for palliative surgery or preoperative chemotherapy.

Neutrophil-to-lymphocyte ratio

The neutrophil-to-lymphocyte ratio (NLR), calculated as the neutrophil count divided by the lymphocyte count, is suggested to be a marker of general immune response to various stress stimuli. Initially, the NLR was described to be correlated with the severity of the clinical course of severely ill patients in the intensive care unit by Zahorec *et al*[37].

Neutrophils play a key role in tumor proliferation, producing a number of ligands that induce tumor cell proliferation and invasion and promoting tumor vascularization by releasing proangiogenic chemokines and other factors[38,39]. Therefore, increased neutrophils may promote tumor growth and metastasis. On the other hand, lymphocytes play a key role in tumor suppression[40]. The function of lymphocytes is to induce cytotoxic cell death and the production of cytokines in cancer cells[40]. A decrease in the number of lymphocytes impairs the host's antitumor immune response and confers a poor prognosis[25]. NLR can therefore be considered as a balance between the pro-tumor inflammation status and the anti-tumor immune status. Although the cut-off values varied between 2.5 to 5 in the previous reports[25-27], emerging evidence shows that an elevated NLR is significantly associated with poor prognosis in patients with CRC. We analyzed 674 CRC patients who underwent surgery and used a receiver operating characteristic curve to determine an appropriate cut-off value[25]. As a result, an NLR > 2.5 was a significant independent predictive factor for cancer-specific survival. With respect to patients with unresectable stage Ⅳ CRC, Chua *et al*[26] examined 349 patients with unresectable CRC who received first-line palliative chemotherapy and reported that the prognosis of patients with an NLR of > 5 was significantly worse than the prognosis of the patients with an NLR of < 5. They also reported that a high NLR resulted in a reduced response to chemotherapy and that the reduction of NLR after one cycle of chemotherapy in a subset of patients resulted in improved survival. Li *et al*[27] performed a meta-analysis of CRC patients and concluded that the NLR is an inexpensive, widely available and reproducible index that is closely associated with survival. Because a peripheral blood cell count is a quick and easy assay to perform, NLR is a useful marker for identifying patients with a poor prognosis and allows for the planning of more frequent surveillance and intensive therapy in patients with unresectable stage Ⅳ CRC.

Platelet-to-lymphocyte ratio

Malignant solid tumors commonly induce a hypercoagulable state, resulting in a predisposition to thromboembolic events[41,42]. Reactive thrombocytosis is induced against a background of hypercytokinemia *via* tumor *vs* host interactions[43]. Among several inflammatory cytokines, IL-6 has an important role in the onset of reactive thrombocytosis, as it is a multifunctional cytokine with a number of physiological actions, stimulating not only CRP up-regulation but also albumin down-regulation in the liver, as well as protein synthesis[44]. Similarly, IL-6 has a cell-proliferative effect, triggering the differentiation of megakaryocytes to platelets in the bone marrow[44]. Hence, it is reasonable that reactive thrombocytosis would be associated with the survival of patients with malignant tumors.

As previously described, lymphocytopenia has shown to be associated with poor survival. Therefore, the platelet-to-lymphocyte ratio (PLR) is also thought to be a powerful prognostic factor in patients with malignant tumors. Indeed, PLR is an independent prognostic factor, in addition to other inflammation-based factors, for pancreatic ductal adenocarcinoma according to Smith *et al*[45], ovarian cancer according to Raungkaewmanee *et al*[46], and CRC accrding to Kwon *et al*[31].

Nutritional Prognostic Index

The inflammatory response has been proposed to be pathogenic with respect to the development of cancer-associated malnutrition[47]. Several studies have reported that patients with advanced gastrointestinal malignancies are often malnourished, and that the preoperative nutritional status is associated with postoperative complications, tumor progression and a poor clinical outcome[48,49]. There are several assessment tools for evaluating the nutritional status, including the malnutrition universal screening tool (MUST), nutritional risk scoring 2002 (NRS2002) and mini nutritional assessment[50,51]. These tools are simple, well-validated and cost-effective and are widely utilized to assess the nutritional status of cancer patients. Onodera's Prognostic Nutritional Index (OPNI) is another such tool and a simple index that can be calculated using only two parameters, the serum albumin level and total lymphocyte count (TLC)[52]. The OPNI is calculated using the following formula: 10 × serum albumin concentration (g/dL) + 0.005 × lymphocyte count (number/mm^2) in the peripheral blood. Albumin is a main component of plasma proteins that preserves the colloid osmotic pressure, and its level reflects the nutritional status. The TLC has also been proposed to be a useful indicator of the nutritional, as well as host inflammatory status. Both albumin and TLC levels are routinely examined in daily clinical practice. Therefore, the OPNI, which reflects the immunonutritional status, is thought to be a useful and convenient index for predicting tumor progression and survival in patients with malignancy.

Regarding the prognosis, Nozoe *et al*[28] reported that the OPNI is significantly correlated with the prognosis of patients with CRC. The above study examined patients who underwent curative surgery. Therefore, we thought to clarify the prognostic value of the OPNI in patients with unresectable stage Ⅳ CRC[29]. Initially, we examined patients who underwent palliative resection of the primary tumor. The result revealed that a low-OPNI is an independent predictor of a worse prognosis, even in patients limited to stage Ⅳ CRC disease. In particular, the MST of the patients with a low-OPNI was 9.5 mo, which was shorter than that reported for patients with stage Ⅳ CRC treated with chemotherapy alone. Therefore, although the necessity of palliative resection in patients with asymptomatic primary tumors and unresectable stage Ⅳ CRC remains controversial, measuring the OPNI may be useful for selecting patients expected to receive a survival benefit associated with palliative resection.

It has been reported that malnutrition results in the loss of lean body mass, an impaired immune function, a reduced rate of response to chemotherapy and poor survival[53]. Therefore, we evaluated the clinical significance of the OPNI among patients with unresectable stage Ⅳ CRC treated with chemotherapy[30]. We collected data from blood tests conducted within one week prior to the start of the first-line chemotherapy and at eight weeks after the first day of chemotherapy. As a result, the overall survival of the patients with a high pretreatment OPNI was significantly (P = 0.005) better than that of the patients with a low pretreatment OPNI; the MST was 37 and 22.8 mo, respectively. Moreover, when we categorized the patients into four groups according to the combination of the pre- and post-treatment OPNI values, only the group who maintained a high OPNI had a better prognosis than the other groups, and a decrease in the OPNI after chemotherapy was associated with a worse survival, even in the patients with a high pretreatment OPNI value. Therefore, it is important to maintain a good nutritional and immune status before and during treatment in patients receiving chemotherapy. It has also been reported that nutritional interventions may improve the immunonutritional system, response to chemotherapy and patient survival[54-56]. Such nutritional interventions should be implemented in order to improve the survival of patients with a low-OPNI.

COMBINATION OF CLINICOPATHOLOGICAL AND INFLAMMATION-BASED FACTORS

The current report of inflammation-based factors is by no means exhaustive, although we wish to provide an overview of the topic in order to help guide the management of CRC patients. Both clinicopathological and inflammation-based parameters are independent powerful prognostic factors; therefore, the user of a combination of these factors may have more precise clinical, prognostic and therapeutic value compared to a single factor.

From the above point of view, Laird *et al*[17] reported that the GPS is similar to the PS in terms of prognostic power and that the combination of these factors may have a potential role in effectively predicting survival.

We investigated the correlation between clinicopathological factors, the GPS, NLR and prognosis in order to identify parameters useful for selecting stage Ⅳ CRC patients with a poor prognosis. As a result, the GPS, NLR, performance status (PS) and extent of distant metastasis were found to be independent predictors of survival[32]. We classified the patients, using a combination of four prognostic factors, into three risk groups: patients without any prognostic factors (the low-risk group), patients with one or two prognostic factors (the intermediate-risk group) and patients with three or four prognostic factors (the high-risk group). There were significant (P < 0.0001) differences in the postoperative cancer specific survival rates among the three groups. The median survival time (MST) was only five months in the high-risk group, compared to 21.5 mo in the intermediate-risk group and 37 mo in the low-risk group. The MST of the high-risk group was five months, which was very short and similar

to that reported for patients with stage Ⅳ CRC who received the best supportive care without surgery or chemotherapy. Therefore, there may be no survival benefit associated with palliative resection in the high-risk group. On the other hand, relatively better survival is expected in the low-risk group. This risk classification is simple and easy to use and may be helpful for determining the optimal treatment for patients with stage Ⅳ CRC.

CONCLUSION

Conventional clinicopathological factors are currently widely- used and important prognostic factors for patients with CRC. However, these factors are not universally helpful for predicting the prognosis in patients within the same stage of disease. Inflammation-based factors are determined based on laboratory data that are routinely recorded in the clinical setting and can be easily estimated prior to treatment.

Although clinicopathological factors reflect the tumor characteristics, cancer progression and survival are not determined solely according to the local characteristics of the tumor, but also the host systemic immune/inflammatory response. Therefore, the application of a combination of these parameters reflecting both the tumor characteristics and host systemic inflammatory status is important for predicting patient survival more precisely and selecting the optimal treatment in patients with CRC.

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CASE REPORT

Gastric carcinoma originating from the heterotopic submucosal gastric gland treated by laparoscopy and endoscopy cooperative surgery

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Institutional review board statement: This study was institutionally reviewed and comprehensively approved by the Kyoto Prefectural University of Medicine.

Informed consent statement: The patients provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

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Abstract

Gastric carcinoma is derived from epithelial cells in the gastric mucosa. We reported an extremely rare case of submucosal gastric carcinoma originating from the heterotopic submucosal gastric gland (HSG) that was safely diagnosed by laparoscopy and endoscopy cooperative surgery (LECS). A 66-year-old man underwent gastrointestinal endoscopy, which detected a submucosal tumor (SMT) of 1.5 cm in diameter on the lesser-anterior wall of the upper gastric body. The tumor could not be diagnosed histologically, even by endoscopic ultrasound-guided fine-needle aspiration biopsy. Local resection by LECS was performed to confirm a diagnosis. Pathologically, the tumor was an intra-submucosal well differentiated adenocarcinoma invading 5000 μm into

the submucosal layer. The resected tumor had negative lateral and vertical margins. Based on the Japanese treatment guidelines, additional laparoscopic proximal gastrectomy was curatively performed. LECS is a less invasive and safer approach for the diagnosis of SMT, even in submucosal gastric carcinoma originating from the HSG.

Key words: Heterotopic submucosal gland; Laparoscopy and endoscopy cooperative surgery; Gastric carcinoma; Gastric submucosal tumor; Less invasive treatment

Core tip: This report describes the rare case of a submucosal gastric carcinoma originating from the heterotopic submucosal gastric gland (HSG) that was safely diagnosed by laparoscopy and endoscopy cooperative surgery (LECS). LECS is a less invasive and safer approach for the diagnosis of submucosal tumor, even in submucosal gastric carcinoma originating from the HSG.

Imamura T, Komatsu S, Ichikawa D, Kobayashi H, Miyamae M, Hirajima S, Kawaguchi T, Kubota T, Kosuga T, Okamoto K, Konishi H, Shiozaki A, Fujiwara H, Ogiso K, Yagi N, Yanagisawa A, Ando T, Otsuji E. Gastric carcinoma originating from the heterotopic submucosal gastric gland treated by laparoscopy and endoscopy cooperative surgery. *World J Gastrointest Oncol* 2015; 7(8): 118-122 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i8/118.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i8.118

INTRODUCTION

Gastric carcinoma is commonly derived from epithelial cells in the gastric mucosa and is very rarely initially diagnosed as a submucosal tumor (SMT). We herein presented a case of submucosal gastric carcinoma originating from the heterotopic submucosal gastric gland (HSG) that was safely diagnosed by laparoscopy and endoscopy cooperative surgery (LECS) and treated by subsequent laparoscopic gastrectomy with D1+ lymphadenectomy. We reviewed the clinical features of this rare tumor and selected successful decision-making using the LECS technique.

CASE REPORT

Patient

The patient was a 66-year-old man who underwent upper endoscopy in a medical checkup, which showed a SMT on the upper gastric body. The patient was referred to the hospital for diagnosis and treatment. Endoscopic re-examination detected a SMT of 15 mm in diameter on the anterior wall of the upper gastric body. The tumor did not have a depression or ulceration (Figure 1A). The results of endoscopic biopsy from the gastric mucosa on the tumor were chronic gastritis with no evidence of malignancy. Barium gastrography showed a smooth elevated lesion of 2 cm in diameter on the anterior wall of the upper gastric body near the esophago-gastric junction (Figure 1B). Computed tomography revealed a 15-mm low density area with calcification in the anterior wall of the upper gastric body and no lymph node or distant metastasis (Figure 1C). Endoscopic ultrasound (EUS) showed an 11.2 mm × 13.5 mm SMT that was derived from the third layer of the gastric wall as a heterogeneous lesion with a mixture of a high echoic lesion, low echoic lesion, and calcification (Figure 1D). The tumor could not be diagnosed histologically, even by EUS-guided fine-needle aspiration biopsy at multiple sites. LECS for gastric local resection was selected as decision-making for a pathological diagnosis and safe removal.

LECS for the SMT

Observations in the abdominal cavity by laparoscopy confirmed no distant or nodal metastasis. The SMT was endoscopically detected on the anterior wall of the lesser curvature of the upper gastric body, but not by laparoscopy. To avoid bleeding, the peripheral branches of the left gastric artery near the tumor were coagulated using a laparoscopic ultrasonically activated device. Endoscopic submucosal resection around the tumor was performed using the endoscopic submucosal dissection technique and seromuscular dissection was performed around the tumor along the line of submucosal resection. The incisional line in the stomach was closed using a laparoscopic stapling device. The resected tumor had negative lateral and vertical margins with normal mucosa (Figure 2A). A pathological examination confirmed that the tumor was a SMT that invaded 5000 μm into the submucosal layer, measured 20 mm × 11 mm × 6 mm, and was a well differentiated adenocarcinoma (Figure 2B). Dilated gastric glands were detected in the submucosal layer (Figure 2C). There was no lymphovascular invasion. Immunohistochemical staining revealed the positive expression of MUC5AC and MUC6, indicating differentiation into the pyloric glands (Figure 2D).

Eighty-four days after LECS, additional laparoscopic proximal gastrectomy with D1+ lymphadenectomy was performed based on the Japanese Gastric Cancer Treatment Guidelines[1]. A pathological examination confirmed no residual tumor cells or lymph node metastasis. The postoperative course was uneventful and the patient is alive without recurrence 1 year after surgery.

DISCUSSION

HSG shows that cystic dilated gastric glands exist in the gastric submucosal layer and has been recognized as a benign condition occurring as a result of repeated mucosal damage[2,3]. HSG was previously described

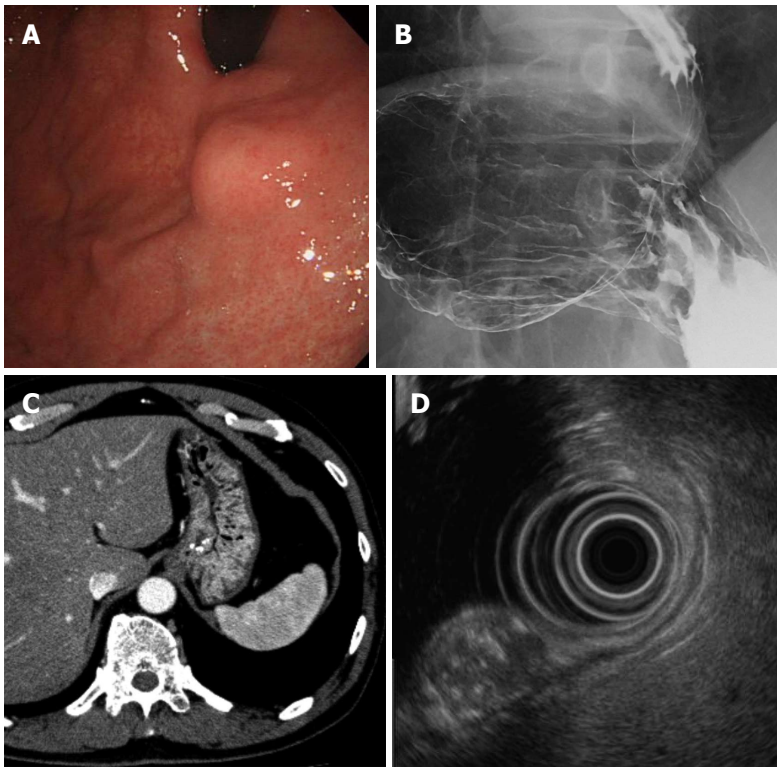

Figure 1 Results of pre-operative examinations. A: Endoscopic findings showing a submucosal lesion of 15 mm in diameter on the anterior wall of the upper gastric body near the esophago-gastric junction. The surface was covered with normal gastric mucosa; B: Barium gastrography showed a smooth elevated lesion of 2 cm in diameter on the anterior wall of the upper gastric body near the esophago-gastric junction; C: Computed tomography revealed a 15-mm submucosal low density area with calcification in the anterior wall of the upper gastric body. No lymph node or distant metastasis was detected; D: Endoscopic ultrasound showed an 11.2 mm × 13.5 mm submucosal tumor derived from the third layer of the gastric wall as a heterogeneous lesion with a mixture of a high echoic lesion, low echoic lesion, and calcification.

Table 1 Previous case reports of gastric carcinoma originating from the heterotopic submucosal gastric gland

Total number of reported cases		*n* 17	(%)
Age		64.1 (45-81)	
Sex	Male	11	65
	Female	6	35
Location	Upper	4	24
	Middle	8	47
	Lower	5	29
Size (mm)		20.5 (8-50)	
Ulceration or depression	Present	13	76
	Absent	4	24
Histological type	Well differentiated	16	94
	Unknown	1	6
Depth of invasion	m	1	6
	sm	14	82
	T2 or more	2	12
Diagnosis by biopsy	Present	6	35
	Absent	11	35
EUS-FNA	Present	2	12
	Absent	15	88
Treatment	EMR	1	6
	EMR and surgical resection	3	18
	Surgical resection	12	71
	LECS + surgical resection	1	6

EUS-FNA: Endoscopic ultrasound-guided fine-needle aspiration biopsy; EMR: Endoscopic mucosal resection; LECS: Laparoscopy and endoscopy cooperative surgery. Note: Ref. [2,6-18].

as a para-cancerous lesion found in 4% of resected specimen from the stomachs of patients with gastric carcinoma, and multiple cancers have been detected in 30% of specimens of gastric carcinoma associated with HSG[4]. However, little is known about the carcinogenesis of HSG itself. Kim *et al*[5] described two cases of early gastric carcinoma arising from HSG that were treated by laparoscopic gastric wedge resection. To the best of our knowledge, there have been no other studies in English concerning gastric carcinoma originating from HSG.

Table 1 shows a summary of 17 previously reported cases, including cases in Japan and our case. Gastric carcinoma originating from HSG occurred more frequently in males and in the middle area of the stomach. Regarding histological findings, the well differentiated type was more common. A study has not yet been conducted on lymph node metastasis from gastric carcinoma originating from HSG. This summary showed that more than 65% of patients could not be histologically diagnosed by biopsy and FNA using EUS before resection.

The recent development of endoscopic and laparoscopic techniques has allowed for less invasive diagnoses and treatments. LECS is a novel and excellent approach for local gastric resection, and was developed by Hiki *et al*[19] as an alternative strategy to laparoscopic wedge resection for gastric SMT. The feasibility and safety of this procedure for gastric SMT have been demonstrated

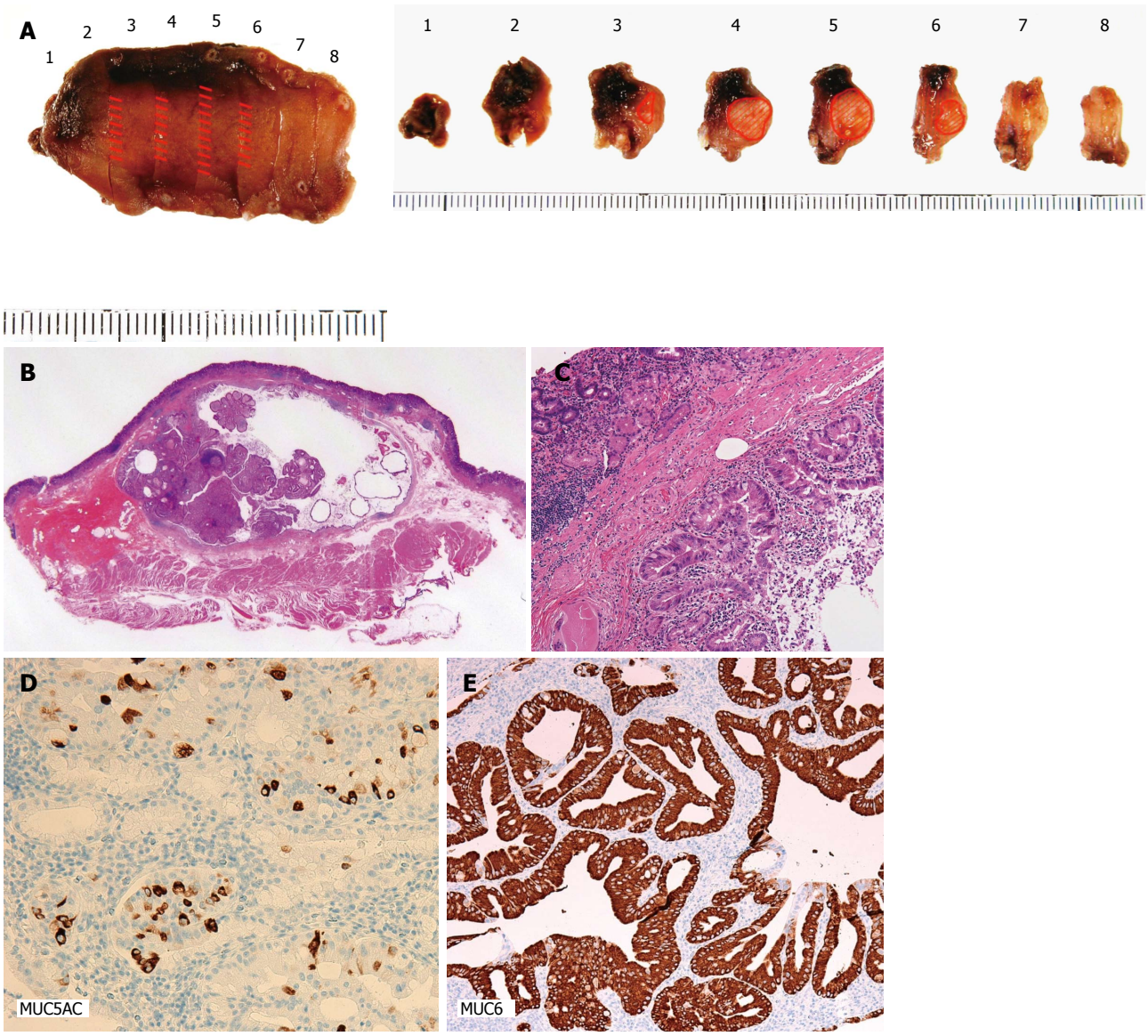

Figure 2 Results of histopathological examinations. A: The resected specimen had negative lateral and vertical margins with normal mucosa; B: A pathological examination confirmed that the tumor was intrasubmucosal (the depth of invasion into the submucosal layer was 5000 μm), measured 20 mm × 11 mm × 6 mm, and was a well differentiated adenocarcinoma; C: Dilated gastric glands were found in the submucosal layer. There was no lymphovascular invasion; D: An immunostaining method showed MUC5AC (+) and MUC6 (+), indicating differentiation into the pyloric glands.

in several studies[20-22]. LECS is now being applied to the treatment of early gastric cancer[23]. The most critical issue associated with its application to gastric cancer is the dissemination of cancer cells into the peritoneal cavity during surgery. Therefore, several methods have been investigated for LECS[24-26]. LECS is a promising approach for the diagnosis of SMT, even in gastric carcinoma originating from HSG.

COMMENTS

Case characteristics

A 66-year-old man who underwent upper endoscopy in a medical checkup, which showed a submucosal tumor (SMT) on the upper gastric body.

Clinical diagnosis

The presented patients had submucosal gastric tumor that could not be diagnosed histologically by endoscopic biopsy.

Differential diagnosis

Gastrointestinal stromal tumor, early gastric tumor, smooth muscle tumor.

Laboratory diagnosis

There were no abnormal findings in laboratory examinations including tumor markers.

Imaging diagnosis

Endoscopic ultrasound and computed tomography showed that the tumor was derived from the third layer of the gastric wall.

Pathological diagnosis

Pathological examination confirmed that the tumor was an intra submucosal tumor that was a well differentiated adenocarcinoma.

Treatment

Laparoscopy and endoscopy cooperative surgery (LECS) for gastric local resection was selected as decision-making for a pathological diagnosis and safe removal.

Term explanation

LECS: Laparoscopy and endoscopy cooperative surgery; HSG: Heterotopic submucosal gastric gland.

Experiences and lessons

Gastric carcinoma originating from the HSG forms a submucosal gastric tumor and is often difficult to diagnose by endoscopic biopsy. If unable to deny malignant disease, resection of the tumor should be considered.

Peer-review

This manuscript described a rare case of submucosal gastric carcinoma originating from the HSG and the authors also described the treatment of the carcinoma by LECS.

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CASE REPORT

Esophageal granular cell tumors: Case report and literature review

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Author contributions: Wang HQ collected the clinical data and wrote the manuscript; Liu AJ revised the manuscript.

Institutional review board statement: The study was approved by Research Ethics Committee of the Third People's Hospital of Hefei and the Chinese People's Liberation Army General Hospital in China.

Informed consent statement: The study participant provided informed written consent prior to the study.

Conflict-of-interest statement: The authors declare that there are no conflicts of interest.

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Abstract

We reported 5 cases of granular cell tumors (GCTs) of esophagus and reviewed the literature. There were 4 females and 1 male with a median age of 43 years and an average age of 44 years. All of the cases had solitary tumors. Tumor size was 0.4-2.5 cm in diameter. Gastroscopy revealed that 2 cases were located in the middle esophagus, 1 case in the upper esophagus, and 2 cases in the distal one. Five cases displayed gray-white, pink, yellow mucosal uplifts of esophagus, 3 cases had smooth surface, 1 case was slightly concave, and the biggest tumor had erosion. Tumor cells were large and polygonal with rich granular and eosinophilic cytoplasm, and small oval nuclei. Cells were arranged in nest or aciniform. Immunohistochemistry and histochemistry staining showed S-100+, neuron specific enolase+, Vim+, CD68+, smooth muscle actin-, Des-, CK-, CD117-, CD34-, Ki67-or $\leqslant$ 5%+. Periodic acid-Schiff reaction and epithelial membrane antigen were both weakly positive. GCTs of esophagus are rare and most of the cases have good prognosis.

Key words: Immunohistochemistry; Granular cell tumors of esophagus; Gastroscope examination

Core tip: Granular cell tumors (GCTs) of esophagus are rare and most of the cases have good prognosis. We reported 5 cases of GCTs of esophagus and reviewed the literature. The report is helpful in comprehensively understanding the characteristics of GCTs and guiding the treatment of this disease.

Wang HQ, Liu AJ. Esophageal granular cell tumors: Case report and literature review. *World J Gastrointest Oncol* 2015; 7(8): 123-127 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i8/123.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i8.123

INTRODUCTION

Granular cell tumors (GCTs) of the esophagus are rare and mostly isolated lesions, usually accidentally discovered by annual endoscopic examination[1,2]. Reports of esophageal GCTs have increased with socioeconomic development and improvement of medical technology in recent years. To explore the clinicopathological characteristics of esophageal GCTs, we reported five cases of esophageal GCTs, including four from the Third People's Hospital of Hefei and one from the Chinese People's Liberation Army General Hospital between 2012 and 2014. The study was approved by the Research Ethics Committee of the Third People's Hospital of Hefei and the Chinese People's Liberation Army General Hospital in China, and consent was obtained from all patients who were enrolled in the study.

CASE REPORT

Some clinicopathological data are shown in Table 1. Case 1 had intermittent heartburn for 3 mo. The patient was diagnosed with superficial gastritis (active phase). She had hepatitis B for > 20 years. Case 2 was accidentally found through physical examination 3 mo ago. Case 3 had dysphagia for approximately 3 mo. Clinicians' first impression was stromal tumor. Case 4 had slight pain behind the sternum for 6 mo. Clinical diagnosis was chronic gastritis with erosion, gastric polyps, and xanthoma of the esophagus. Case 5 complained of acid reflux for 1 mo, with intermittent abdominal distension and belching. Endoscopic ultrasonography revealed a low-echo lesion in the submucosa of the distal esophagus, with integrity of the muscularis propria. Clinicians' first impression was GCT. The patient also had diabetes.

All cases underwent successful endoscopic mucosal resection without complications, using endoscopic electrosurgical snare resection. The lesions were found by gastroscopy and the size, color, topography and peripheral tissue were observed. The motion and position of the lesion were assessed with biopsy forceps. If the lesion was located in the mucosa and submucosa, and $\leqslant$ 3 cm in diameter, trap resection could be used. This involved fixing the position of the tumor by gastroscopy, focusing on the base of the tumor with a snare trap, tightening the snare, cutting the tumor with an electrotome, and stemming the bleeding. Finally the specimen was sent for pathological examination.

Under light microscopy, the tumor was located under the mucosal squamous epithelial basement membrane. The tumor cells were large and polygonal. The cytoplasm was granular and eosinophilic. The nuclei were small, ovoid or slightly irregular with fine chromatin, and some were deviated. Small nucleoli were visible in some cells (Figure 2). Small crack-like blood vessels were observed. In Case 3, a few lymphocytes infiltrated the stroma, and lymph follicles were formed around the tumor. In Case 4, the tumor was located between the mucosal squamous epithelial basement membrane and the submucosa. The polygonal cells were arranged in an aciniform manner. Immunohistochemical and histochemical staining are shown in Table 2 and Figure 3.

The above five cases were all diagnosed with esophageal GCT. Four patients had no recurrence during follow-up of 7-33 mo. One patient was lost to follow-up.

DISCUSSION

GCTs in the esophagus are rare, however, the study of rare diseases has repeatedly led to breakthroughs in our understanding of more common diseases. GCTs in the esophagus mainly occur in the middle age. Most tumors are solitary and benign, and located in the middle and lower esophagus[1-7]. In the present study, There were four women and one man, with a median age of 43 years and average age of 44 years. All of our cases had solitary GCTs. Tumor diameter ranged from 0.4 to 2.5 cm. Two cases were located in the middle esophagus, one in the upper esophagus, and two in the lower esophagus.

The tumors were mostly located in the mucosa and submucosa, and only a few invaded into the muscular layer. The tumor cells were large and appeared polygonal. The cytoplasm was granular and eosinophilic. The cell nuclei were ovoid with fine chromatin and no mitotic figures. The cells were arranged in nest or acinar form[1-6].

GCTs are commonly identified as nonspecific painless masses. Patients with small tumors are often asymptomatic[1,2], and the emergence of clinical symptoms is related to tumor size. When the tumor diameter is > 1 cm, patients may experience dysphagia[1]. Esophageal lesions are often found by chance through gastroscopic examination[1,2,3,6]. Patient complaints are mostly abdominal distension, acid reflux, belching, and loss of appetite[3]. In our study, the patients complained of intermittent heartburn, dysphagia, acid reflux with intermittent abdominal distension and belching, and slight pain behind the sternum. GCT in Case 2 was accidentally found through physical examination.

The color of the tumor surface is usually white-gray, pink or yellow. The tumors show polypoid or nodular uplift without pedicles, and most have a smooth surface[2,6,8]. In the five cases described in this report, the tumors were gray-white, pink or yellow, with mucosal uplifts of the esophagus under gastroscopy. Three cases had a smooth mucous surface, one had slight concavity, and the largest tumor had erosion.

By EUS, GCTs are often located in the mucosal layer or submucosa, as round or circle-like masses, hypoechoic and homogeneous lesions, with clear borders. A few GCTs invade the muscular layer. In EUS images, average grayscale values of GCTs are greater than those of esophageal leiomyoma, which can help with differential diagnosis and improve the accuracy of EUS for the diagnosis of esophageal GCTs[2-4,9]. In the present Case 5, EUS showed a low-echo lesion in the submucosa, with

Table 1 Clinicopathological data of five cases of granular cell tumor

Case	Gender	Age (yr)	Esophageal location	Diameter (cm)	Color and quality	Surface
1	Female	43	Middle	0.5	Gray-white	Smooth
2	Female	32	Middle	0.4	Gray-white	Smooth
3	Male	47	Distal	2.5	Gray-white and pink, fine quality	Erosion
4	Female	42	Upper	0.6	Grayish yellow (Figure 1)	Smooth
5	Female	56	Distal	0.8	Yellow	Slightly concave

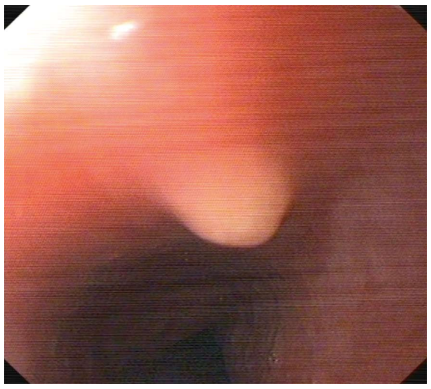

Figure 1 A grayish-yellow uplift was seen in the esophagus 18 cm from the incisor with smooth surface under endoscope in Case 4.

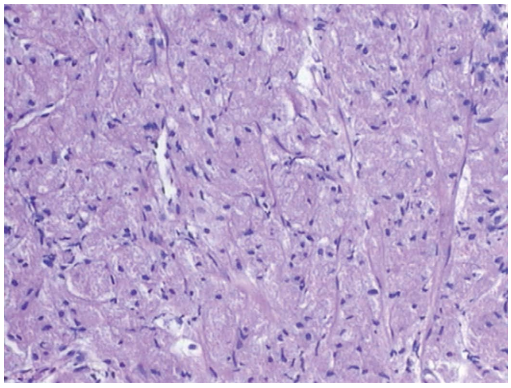

Figure 2 The tumor cells were large and polygonal with granular and eosinophilic cytoplasm, and small oval nuclei. Hematoxylin and eosin staining, × 100.

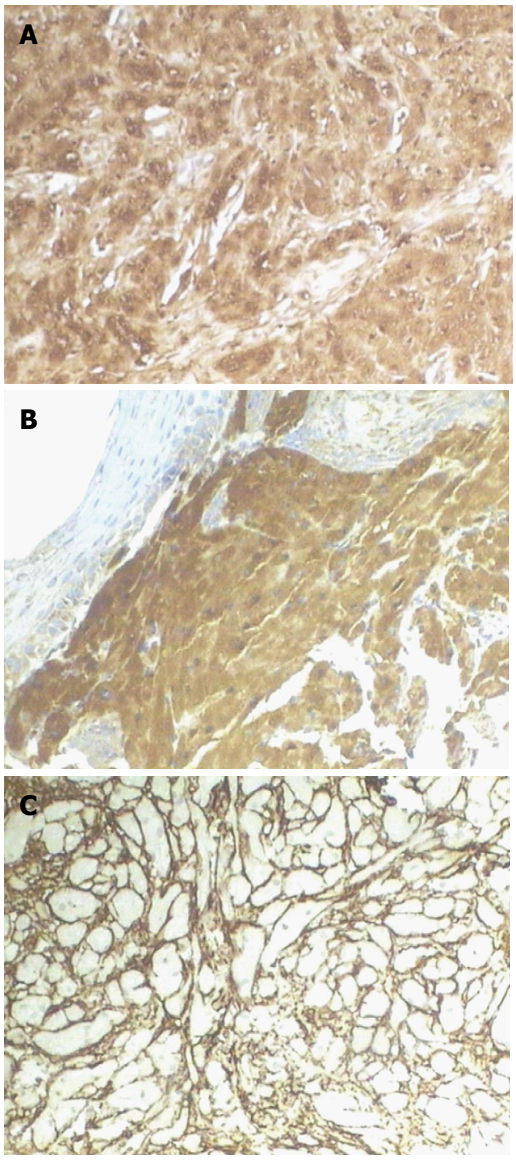

Figure 3 Immunohistochemical and histochemical staining. Envision method, × 100. A: S-100 was strongly positive in tumor cells; B: CD68 was strongly positive in tumor cells; C: CD34 was negative in tumor cells, but the surrounding mesenchymal cells were positive for CD34.

no violation of the muscularis propria layer, which was similar to previously described cases. Palazzo *et al*[10] found that GCTs had three characteristics: (1) tumor size < 2 cm in 95% of cases; (2) a hypoechoic solid pattern in all cases; and (3) a tumor arising in the inner layers in 95% of cases[10].

Immunohistochemical and histochemical staining was positive for S-100, CD68, neuron speoific enolase (NSE) and vimentin. Periodic acid-Schiff (PAS) and epithelial membrane antigen staining was weakly positive. Staining was negative for cytokeratin, desmin, smooth muscle actin (SMA) and CD34 (although surrounding mesenchymal cells were positive for CD34). This was most in accordance with the literature[2,3,6,11].

Differential diagnosis includes the following tumors: (1) Gastrointestinal stromal tumors (GISTs). GISTs are usually located in the submucosa, and are rare in the esophagus. The tumor cells are spindle-shaped or round and arranged in fasciculus, weave or whirlpool shape. Immunohistochemical staining is positive for CD117 and CD34[12]; (2) Leiomyoma. Leiomyoma is composed of moderate spindle cells with eosinophilic cytoplasm. The spindle cells are arranged in beam and/or weave

Table 2 Immunohistochemical and histochemical staining of five cases of granular cell tumor

Case	S-100	NSE	Vim	CD68	Des	SMA	CK	Ki-67	CD117	CD34[a]	EMA	PAS	Dog-1
1	+	+	+	+	-	-	-	-	None[b]	None	None	Weak+	None
2	+	+	+	+	None	-	-	-	-	-	Weak+	Weak+	None
3	+	+	+	+	-	-	-	2%+	-	-	None	Weak+	-
4	+	+	+	+	None	None	-	None	None	None	Weak+	Weak+	None
5	+	+	+	+	None	-	-	5%+	-	-	None	Weak+	-

[a]Surrounding mesenchymal cells were positive for CD34; [b]None means the tissue was too small, and immunohistochemical staining was not possible. CK: Cytokeratin; Des: Desmin; EMA: Epithelial membrane antigen; Vim: Vimentin; NSE: Neuron speoific enolase; SMA: Smooth muscle actin; PAS: Periodic acid-Schiff.

pattern. The nuclei are rod-shaped or cigar-shaped. Immunohistochemical staining is positive for SMA and desmin, and negative for CD34 and CD117. Average grayscale values of esophageal leiomyoma are lower than those of GCTs; (3) Schwannoma and neurofibroma. Schwannoma has a complete capsule. The tumor cells are spindle-shaped or stellate. Typical schwannoma has two kinds of histological structure under microscope: pyknotic Antoni type A and loose Antoni type B. Neurofibroma is composed of thin and long spindle cells with wavy shape and pale cytoplasm. Negative staining for CD68 helps with differential diagnosis; and (4) Xanthoma. Cells are round or polygonal with pale cytoplasm. The nuclei are round, small and moderate, and usually located in the center of the cells. Cells are located in the mucosal lamina propria. Xanthoma usually occurs in the stomach. Cells have a lack of granular cytoplasm, and stain positive for CD68 and negative for PAS.

Benign and malignant GCTs have similar histopathology, and there are no clear histological diagnostic criteria for benign and malignant tumors. The following are suggestive of malignant GCT: rapid tumor growth, > 5 cm in diameter and karyokinesis in > 2/10 high-power fields; tumor cells are spindle shaped, with vesicular nuclei and nucleoli; high ratio of nucleus to cytoplasm with cellular pleomorphism; and tumor tissue necrosis[13]. One study found that > 50% p53-positive cells and > 10% Ki-67 positive cells were significantly correlated with malignancy[13].

In recent years, most investigators have thought that GCT is related to peripheral nerve tissue. Some studies have found that tumor cells are surrounded by nerve bundles, and there is a transition phenomenon from Schwann cells to tumor cells. Immunohistochemistry and ultrastructural analysis show the differentiation of Schwann cells. All the present cases were strongly positive for S-100 and NSE, which suggested the neurogenic origin of GCTs.

Narra *et al*[8] showed that treatment options include endoscopic surveillance, endoscopic resection, and surgery. According to EUS, 11 cases with lesions ≤ 3 cm in diameter without muscular layer invasion underwent endoscopic resection without complications, and another three cases underwent surgical resection[9]. A new technique of submucosal tunnel endoscopic resection was performed in three submucosal cases with lesions ranging from 2 to 3 cm in diameter[9]. The chief complications of gastrointestinal submucosal endoscopic resection are bleeding and perforation[14].

The prognosis of esophageal GCT is good, and recurrence and metastasis are uncommon. Many studies have shown no recurrence and metastasis during follow-up[1,3,8].

COMMENTS

Cases characteristics

Case 1, a 43-year-old woman with intermittent heartburn for 3 mo. Case 2, a 32-year-old woman was accidentally found through physical examination 3 mo ago. Case 3, a 47-year-old man with dysphagia for about 3 mo. Case 4, a 42-year-old woman with slight pain behind the sternum for 6 mo. Case 5, a 56-year-old woman with acid reflux for 1 mo, with intermittent abdominal distension and belching.

Clinical diagnosis

Case 1, superficial gastritis (active phase); Case 2, middle esophageal apophysis; Case 3, first impression was stromal tumor; Case 4, chronic gastritis with erosion, gastric polyps, and xanthoma of the esophagus; Case 5, first impression was esophageal granular cell tumor (GCT).

Differential diagnosis

Gastrointestinal stromal tumor, leiomyoma, schwannoma and neurofibroma, xanthoma.

Laboratory diagnosis

Case 1 had hepatitis B for > 20 years.

Imaging diagnosis

In Case 5, endoscopic ultrasonography revealed a low-echo lesion in the submucosa of the distal esophagus, with integrity of the muscularis propria.

Pathological diagnosis

Five cases were all diagnosed with esophageal GCTs.

Treatment

All five cases underwent successful endoscopic mucosal resection without complications.

Term explanation

GCTs of the esophagus are rare benign tumors.

Experiences and lessons

The prognosis of esophageal GCTs is good, and recurrence and metastasis are rare.

Peer-review

This is an interesting article on the rare tumor of esophagus. The experiments are well designed and described in detail.

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Contents

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EDITORIAL

Fish oils in parenteral nutrition: Why could these be important for gastrointestinal oncology?

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Abstract

By the time a gastroenterology patient is moved to parenteral nutrition, he or she is usually in poor health. All parenteral nutrition formulae contain essential nutrients, avoiding components that could cause an adverse reaction. The lipid component is often provided by a soy extract, containing all the fatty acids considered to be essential in the diet. Several trials have considered parenteral nutrition formulas with added fish oils, high in the long chain omega-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Given the range of biological functions associated with such compounds, especially in reducing inflammatory symptoms, this move would appear rational. However, while data from such trials are often positive, there has been variability among results. Some of this variability could be caused by environmental contaminants in the fish, and/or oxidation of the lipids because of poor storage. The situation is complicated by a recent report that fish oils may counter the effects of platinum chemotherapy. However, this effect associated with a minor component, hexadeca-4,7,10,13-tetraenoic acid. It is suggested that pure DHA and EPA would be beneficial additions to parenteral nutrition, reducing the probability of carcinogenesis and enhancing rational disease management. However, the jury is still out on fish oils more generally.

Key words: Inflammatory bowel diseases; Colorectal cancer; Fish oils; Eicosapentanoic acid; Docosahexaenoic acid

Core tip: Parenteral nutrition formulae contain essential nutrients, in which the lipid component is often provided by a soy extract, containing essential fatty acids. Several trials have considered such formulas with added fish oils, high in the long chain omega-3 polyunsaturated fatty acids, eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA). Such compounds have a range of biological functions, especially in reducing

inflammatory symptoms. However, there has been variability among results of clinical trials, possibly caused by environmental contaminants in the fish, and/or lipid oxidation. It is suggested that pure DHA and EPA, but possibly not fish oils *per se*, would be beneficial.

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PARENTERAL NUTRITION REQUIREMENTS AND FORMULATION

Both enteral and parenteral nutrition become important in the care of hospitalised patients with Inflammatory bowel diseases and many other gastrointestinal (GI) disorders[1,2]. These formulas utilise essential nutrients, including lipids. However, there has been some controversy regarding optimal formulations, especially in regard to the nature of the most appropriate lipids[3]. Soybean has been the basis for the most commonly used formulations, since it is a well-recognised source of the essential omega-6 polyunsaturated fatty acid (PUFA), linoleic acid, and the omega-3 PUFA, alpha-linolenic acid. It also contains the saturated fatty acids, stearic acid and palmitic acid, as well as the monounsaturated fatty acid, oleic acid[2]. Where there seems to be some controversy is whether fish oil, which contains two long chain omega-3 PUFA, eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), adds anything of importance. Many of the controversies raised are in relation to the need for this[4,5]. Unfortunately, however, currently available trials are underpowered to answer some of these controversies. Given the possibility that many of these GI disorders may progress to cancer[6], the questions raised are highly relevant to GI oncology.

LIPID FORMULAE AND RISK OF CANCER INITIATION IN NORMAL SUBJECTS

Shortened telomeres have been related to significantly increased risks of cancer[7]. Thus, there would be significant benefits in having a nutritional formula that increases the length of telomeres, or at least prevents or slows shortening. While there is no evidence that any known lipid formulas may be able to increase length, there are comparative data available for omega-3 (DHA-rich or EPA-rich) formulae, as compared with a formula containing only the omega-6 PUFA, linoleic acid. O'Callaghan *et al*[8] supplemented elderly adults for 6 mo with each of these formulas, and compared the groups in terms of telomere length at the beginning and end of that time. They found preliminary evidence that telomere shortening could be attenuated by either of the omega-3 PUFA-containing formulae, but not by the formula containing only the soy- derived linoleic acid.

LIPID FORMULAE AND PROGRESS OF GI SURGERY

It is difficult to compare all available studies on the effects of added fish oils to the clinical progress of GI surgery, since these are generally small, and not standard as regards to the formulae being compared in the presence or absence of fish oils[9].

Although addition of a fish oil to an olive oil-based parenteral nutrition formula for 5 d had no effects on measures of inflammation, it appeared that GI patients showed a lower risk of infection following surgery as compared with patients nourished by the olive oil formula alone[10].

While inflammation is necessary for responses to external challenges, there is no question but that excess inflammation is detrimental[2,11,12], and plays an important role in the progression of GI diseases towards a cancer phenotype[11]. A number of small studies had compared the effects of soybean oil in various combinations with medium chain triglycerides (MCT) and olive oil suggesting there may be benefits of these combinations, but larger and more systematic studies implied that this effect may not always hold[5,10]. However, the inclusion of fish oil in combination with one of these other oils was shown to have beneficial effects on immune status and inflammatory markers in patients following major GI surgery[2,13].

Wang *et al*[14] compared a fish oil-enriched emulsion to an MCT/long chain triacylglycerol mix in GI surgery patients for 5 d after surgery. Clinical outcomes were comparable across the groups and there were no significant differences in standard measures of inflammation such as C-reactive protein. However, the fish oil formula led to an increase in leukotrienes B5 and B6, along with significant decreases in the pro-inflammatory cytokines, interleukin 6, tumor necrosis factor-alpha and nuclear factor-kappa B. Interleukin 6 in particular has been strongly implicated in the development of colorectal cancer[15]. These effects all implied that inclusion of fish oil in the formula beneficially modulated inflammatory response, reducing the probability of post-surgery infection and subsequent adverse effects including CRC initiation.

LIPID FORMULAE IN COLORECTAL CANCER PATIENTS

In elderly patients after colorectal cancer surgery, Zhu *et al*[16] found that addition of fish oil to the soybean oil-based formula again reduced pro-inflammatory cytokines, reduced infectious complications and incidence of systemic inflammatory responses, and resulted in a shorter hospital stay. In a larger trial of similar lipid mixes, this time in colorectal cancer patients

of varying ages, de Miranda Torrinhas *et al*[17] again found improved post-operative immune responses. Thus, most of the published studies, albeit considering small numbers, suggest beneficial results from adding fish oils to the more standard parenteral nutrition formulas conventionally used.

LIPID FORMULAE IN LIVER DISEASE

A range of isolated case reports have appeared, showing significant changes in problems associated with non-alcoholic fatty liver disease, when fish oils are added to standard parenteral nutrition. For example, Crook *et al*[18] and also Venecourt-Jackson *et al*[19] reported on the successful treatment of parenteral nutrition-associated liver disease in individual adults using a fish oil-based formula. More generally, this area has been reviewed by several authors, including Bouzianas *et al*[20] and Premkumar *et al*[21]. Fish oil formulae have also benefited pediatric oncology patients who have developed liver disease[22], and promoted high rates of resolution of cholestasis[23].

The mechanisms of the fish oil-associated effects on liver disease are almost certainly associated with the EPA and DHA-associated shift towards anti-inflammatory proresolving lipid mediators[24,25].

POTENTIAL PROBLEMS WITH THE USE OF FISH OILS

Despite a generally positive climate, a significant warning has been raised following evidence that addition of fish oil during a cancer chemotherapy regime containing platinum compounds may lead to cancer drug resistance[26]. However, this effect was related to a fairly minor fish oil component, the omega-3 PUFA 16:4(n-3) (hexadeca-4,7,10,13-tetraenoic acid) that, when administered to mice, neutralized chemotherapeutic activity. Although such studies have not been done in humans to this date (and could not ethically be justified), Daenen *et al*[26] found that, when the recommended daily amount of 10 mL of fish oil was administered to healthy volunteers, rises in plasma 16:4 (n-3) levels were observed, reaching up to 20 times the baseline levels. Herring and mackerel contained high levels of 16:4 (n-3), whereas salmon and tuna had very much lower levels. The authors concluded that, until further data become available, it may be desirable to avoid fish oil and fish containing high levels of 16:4 (n-3) on the days surrounding chemotherapy[26].

We have previously pointed to apparently contradictory results of dietary supplementation with oily fish or with fish oils in the development and progression of inflammatory bowel diseases. The pattern which became apparent is that the nature of the results, *i.e.*, whether positive, neutral or negative, largely depended upon the source of the fish (whether polluted or not), or in the case of oils, the degree of purification and protection against oxidation[27]. These data are equally relevant to the case of colorectal cancer. That is, we believe that it may not only be somewhat desirable, but very important to add fish oils to parenteral nutrition therapy. However, it would also appear important that addition of the 16:4 omega-3 PUFA hexadeca-4,7,10,13-tetraenoic acid, or any possibility of formation of this product be avoided.

CONCLUSION

In summing up, there seems good evidence that the classic (usually) soy-based parenteral nutrition formulae may not provide adequate nutritional support, especially when used for patients with GI disorders. Furthermore, these formulae may themselves lead to complications, including liver disease. Fish oil-based formulae have given some extremely good results in most, but not all studies. Part of the reason for this could be environmental contaminants in the original fish source, or oxidation products because of poor storage. It would appear that a good case can be made for a strong EPA and/or DHA component, preferably as purified forms of these fatty acids, becoming an essential part of parenteral nutritional formulae. This would not only protect against the development of colorectal cancers, it would help to avoid the complications of current nutritional therapies in patients who already have the disease.

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REVIEW

Adenosquamous carcinoma of the pancreas: Molecular characterization of 23 patients along with a literature review

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Abstract

Adenosquamous carcinoma of the pancreas (ASCP) is a rare entity. Like adenocarcinoma of the pancreas, overall survival is poor. Characteristics of ASCP include central tumor necrosis, along with osteoclasts and hypercalcemia. Various theories exist as to why this histological subtype exists, as normal pancreas tissue has no benign squamous epithelium. Due to the rarity of this disease, limited molecular analysis has been performed, and those reports indicate unique molecular features of ASCP. In this paper, we characterize 23 patients diagnosed with ASCP through molecular profiling using immunohistochemistry staining, fluorescent *in situ* hybridization, chromogenic *in situ* hybridization, and gene sequencing, Additionally, we provide a comprehensive literature review of what is known to date of ASCP. Molecular characterization revealed overexpression in MRP1 (80%), MGMT (79%), TOP2A (75), RRM1 (42%), TOPO1 (42%), PTEN (45%), CMET (40%), and C-KIT (10%) among others. One hundred percent of samples tested were positive for *KRAS* mutations. This analysis shows heretofore unsuspected leads to be considered

for treatments of this rare type of exocrine pancreas cancer. Molecular profiling may be appropriate to provide maximum information regarding the patient's tumor. Further work should be pursued to better characterize this disease.

Key words: Adenosquamous carcinoma of the pancreas; Molecular profiling; Review

Core tip: This analysis of 23 adenosquamous carcinoma of the pancreas in light of the reviewed literature highlights the potential to identify novel treatments when using a personalized medicine approach to patient tumor characterization.

Borazanci E, Millis SZ, Korn R, Han H, Whatcott CJ, Gatalica Z, Barrett MT, Cridebring D, Von Hoff DD. Adenosquamous carcinoma of the pancreas: Molecular characterization of 23 patients along with a literature review. *World J Gastrointest Oncol* 2015; 7(9): 132-140 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i9/132.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i9.132

INTRODUCTION

Pancreas cancer remains a deadly disease. In 2014 it is estimated that 46420 new cases will occur, along with 39590 deaths, making it the fourth leading cause of cancer deaths in the United States[1]. The most commonly diagnosed pancreas cancer histology is adenocarcinoma, with an incidence of 85% of pancreas malignancies[2]. As shown in Table 1, other pancreas cancer histological subtypes include mucinous cyst adenocarcinoma, adenosquamous, undifferentiated/anaplastic, papillary mucinous, acinar cell, spindle cell, and pancreatoblastoma[2-4].

Adenosquamous carcinoma of the pancreas (ASCP) is a rare entity. Its estimated incidence in the literature is between 0.38% to 10% of all exocrine pancreatic tumors (Table 2)[2,5-19]. ASCP has also been referred to as adenoacanthoma, mixed squamous and adenocarcinoma, and mucoepidermoid carcinoma[20]. The entity was first described in 1907 by Gotthold Herheimer, who referred it as cancroide[20]. Adenosquamous histology is seen in cancers of other organ systems such as lung, esophagus, colon, stomach, salivary glands, and the female reproductive system[20]. Compared to pancreatic adenocarcinoma, which has a poor 5-year overall survival, survival is worse in patients with ASCP[12-15].

The etiology of ASCP is unknown. Most literature reports of this disease have come from Asia. The largest known case study showed that 79% of 415 patients with ASCP were Caucasian[12]. It is unknown if risk factors for the development of pancreatic adenocarcinoma such as chronic pancreatitis, ABO blood group, alcohol use, tobacco use, germline mutations such as BRCA2, PALB2, ATM, and p53 are also risk factors for the development of ASCP[12,21].

In this review, we will discuss the current understanding of ASCP. We have profiled 23 patients with ASCP and will present our findings, along with other molecular analyses reported in the literature. We will also discuss potential treatment strategies specifically targeting ASCP.

Table 1 Frequency of malignant exocrine pancreatic neoplasms

Frequency of malignant exocrine pancreatic neoplasms	
Histological subtype	**Frequency**
Adenocarcinoma	85%
Mucinous cyst adenocarcinoma	2%
Adenosquamous	0.38%-10%
Undifferentiated/anaplastic carcinoma	< 1%
Intraductal papillary mucinous carcinoma	3%
Acinar cell carcinoma	< 1%
Rare subtypes	4%

Rare subtypes include signet ring cell carcinoma, giant cell tumor, cystadenocarcinoma, pancreatoblastoma, spindle cell carcinoma.

Table 2 Incidence of adenosquamous carcinoma of the pancreas

Pancreatic cancer specimens	No. (%) of ASCP	Ref.
15185	81 (0.05)	[2]
5075	46 (0.9)	[6]
264	10 (3.8)	[8]
391	13 (3.4)	[9]
80	8 (10)	[10]
202	6 (3)	[11]
3651	45 (1.2)	[12]
45693	415 (0.9)	[13]
237	7 (2.9)	[14]
406	14 (4)	[15]
1025	46 (4.5)	[16]
24604	95 (0.38)	[17]
635	20 (3.1)	[18]
8372	25 (0.3)	[19]
234	7 (2.9)	[20]

ASCP: Adenosquamous carcinoma of the pancreas.

PATHOLOGY

Normal pancreas tissue has no benign squamous epithelial components[9,15,22]. Various hypotheses have been proposed regarding the histogenesis of ASCP. One theory hypothesizes that squamous metaplasia occurs as a result of ductal inflammation due to chronic pancreatitis or obstruction by an adenomatous tumor, and this process leads to ASCP[5,23]. Another theory, termed the collision theory, suggests that two histologically distinct tumors arise independently in the pancreas and are joined together leading to ASCP[20,23,24]. Finally, the third theory, the differentiation theory, suggests that a primitive pancreatic stem cell

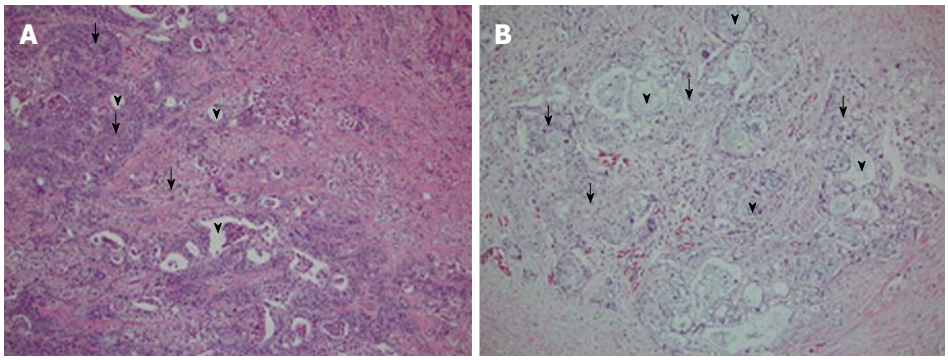

Figure 1 Typical pathology of adenosquamous carcinoma of the pancreas. H and E slides of two patient's tissues, showing the adeno *vs* squamous component (arrowheads = adeno; arrows = squamous component). A: Tissue from head of pancreas; B: Tissue from tail of pancreas; both are G2, moderately differentiated cancers.

differentiates into either squamous or adenocarcinoma or becomes a combination of both[14,22,23]. Despite different hypotheses, there has been no study to elucidate the mechanism of origination of ASCP.

The pathology of ASCP includes the typical squamous carcinoma pattern that is characterized by epithelium with whorls, keratohyalin, or pearls[14,16], as seen in Figure 1. Compared to nuclei of benign squamous cells, the nuclei of malignant squamous cells are hyperchromic and pleomorphic[15,22]. The squamous carcinoma component of ASCP appears to be more focal in the tumor. An interesting histological feature is the finding in several case series of ASCP that the squamous cell carcinoma component is located in the periphery of the tumor, while the adenocarcinoma component is in the center[9,22]. There is a transitional zone where the glandular structure blends into the squamous component[22]. There is an entity descriptive of pure squamous cell carcinoma of the pancreas, but this classification has been debated and is considered to be more secondary to metastasis to the pancreas from a non-pancreas primary carcinoma[15,25,26].

Tumor cell necrosis is frequently seen in patients with ASCP, along with high tumor grade[25,27]. Other unusual reported pathology has included the presence of clear cell and rhabdoid components[27,28]. One pathology case report noted the presence of both osteoclast and giant tumor cells which were scattered individually within the stroma[3]. The presence of osteoclasts is not unique to ASCP, as osteoclasts have been seen in adenosquamous carcinoma of other organs, including the esophagus, gallbladder, and kidney[3,13]. Acantholysis has also been noted[3]. The squamous component of the cancer has been shown to be more likely to demonstrate vascular invasion, but less likely to metastasize to the lymph nodes[16]. One study found that pancreatic adenosquamous carcinoma grows at twice the rate of pancreatic adenocarcinoma[29].

The current guideline to diagnose adenosquamous pancreatic cancer requires the presence of at least 30% of squamous component in the pancreas tumor tissue[18,20,30]. However, this classification system is being debated, due to both the subjective nature of estimating percentage composition and the sampling method of a patient's tumor at biopsy through fine needle aspiration (FNA) *vs* surgical resection. The clinical relevance of the degree of squamous cell differentiation in adenosquamous pancreas cancer is unknown[16,18]. The proportion of squamous differentiation in ASCP did not influence survival in one case series of 38 patients[22]. Some have proposed that presence of any squamous cell carcinoma component in a pancreatic tumor should classify the cancer as adenosquamous[16,26,31].

Prior immunohistochemistry (IHC) analysis on patients with adenosquamous carcinoma have shown the cancer to be positive for cytokeratin (CK) 5/6, CK 7, and p63 and negative for CK 20, p16, and p53[18,32]. IHC positivity for pancreatic adenocarcinoma includes CK7, CK20, mesothelin, cancer antigen 125 (CA-125), and lysozyme[18,33]. The KI-67 index for one patient with ASCP with approximately a 70%-80% squamous carcinoma component was 33%[32].

As in pancreatic adenocarcinoma, *KRAS* mutations have also been observed in ASCP[18,27,31,34]. A molecular study involving analysis for p53, Dpc4/SMAD4, p16, E-cadherin, EGFR protein expression levels, *KRAS* mutational analysis; *p16/CDKN2a* amplification, and HPV DNA detection was carried out on 8 patients with ASCP[27]. The *KRAS* mutations only screened for mutations in codons 12 and 13, which were present in 5/8 of the squamous component of the cancer samples. A homozygous deletion of the *p16* gene was present in 3/8 squamous components. Regarding protein expression in the same patient samples, DPC4 was lost in 5/8 samples, p53 was positive in 5/8 samples, p16 was universally lost, E-cadherin was either reduced or lost in 7/8 samples, and P63 and EGFR were positive in all 8 samples[27]. The lack of protein expression of p16 was particularly interesting since the gene was present in 5/8 patient samples, suggesting other causes of loss of protein expression, such as gene silencing like DNA methylation. There was no HPV DNA detectable in the eight patients tested[27]. HPV status was looked at another analysis of 7 patients, and none of these patients were positive[13]. The lack of positivity of HPV is noteworthy due to its influence in the development of

Table 3 Molecular profiling of patients with adenosquamous carcinoma of the pancreas: Immunohistochemistry analysis

IHC analysis percent positive expression (positive/number examined)																		
MRP1	BCRP	MGMT	TOP2A	TUBB3	PTEN	SPARC	TOPO1	RRM1	cMET	TLE3	TS	ERCC1	PGP	C-kit	PR	AR	ER	Her2
80 (8/10)	80 (4/5)	76 (16/21)	78 (14/18)	38 (3/8)	41 (9/22)	39 (9/23)	38 (8/21)	43 (9/21)	33 (4/12)	42 (5/12)	38 (8/21)	31 (4/13)	11 (2/18)	10 (1/10)	5 (1/21)	0 (0/21)	5 (1/21)	0 (0/22)

IHC: Immunohistochemistry.

Table 4 Molecular profiling of patients with adenosquamous carcinoma of the pancreas: Fluorescence *in situ* hybridization/ chromogenic *in situ* hybridization analysis

FISH/CISH percent positive expression (positive/number examined)				
cMET	EGFR	Her2	TOP2A	ALK
9 (1/11)	0 (0/6)	0 (0/12)	0 (0/2)	0 (0/1)

FISH: Fluorescence *in situ* hybridization; CISH: Chromogenic *in situ* hybridization.

other squamous histology cancers such as the cervix, head and neck, and anus[13].

We have conducted a molecular characterization using a commercially available assay[35]. Twenty-three patients with ASCP were identified and the results of the profiling are presented (Tables 3-5). The median age was 60 years old (range 41 to 86 years old), and 17/23 patients were male. Evaluation of protein expression by IHC analysis revealed the following: DNA topoisomerase2 (TOPO2A) overexpression was prevalent in 78% of the samples, which in some studies of other histologic types indicates sensitivity to agents such as doxorubicin or etoposide. Low expression of ribonucleotide reductase M1, which can indicate sensitivity to gemcitabine, was low in 57% of the patient samples. Low thymidylate synthase expression, found in 62% of patient samples, correlates to sensitivity in some tumor types to fluoropyrimidines such as 5-FU, capecitabine, and pemetrexed. Low expression of excision repair cross-complementation group 1, or ERCC1, is associated with sensitivity to platinum-based therapies in some tumor types and was found to be low in 69% of patient samples. Other positive findings included 10% (1 in 10) positivity of c-KIT, and TOPO1 overexpression in 38% of patient samples. These biomarkers are correlated to sensitivities to imatinib and topotecan/irinotecan, respectively, in some tumor types. The high expression of both MRP1 and BCRP1 at 80% highlights the difficulty of treating ASCP, as these proteins are involved in drug resistance to chemotherapy. FISH/CISH analysis revealed an 11% overexpression of c-MET, an oncoprotein that is increasingly targeted in new drug development. Also, one sample had a mutation in c-MET. Of note, mutation analysis revealed *KRAS* mutations in all sixteen patient samples tested, but none had *EGFR* mutations.

Very little genomic sequencing data is available in the literature on adenosquamous pancreatic cancers. However, a study published examining whole genomic sequencing in eleven patients with advanced cancer included one patient with ASCP[36]. This patient's sequencing included single nucleotide variations (SNV), whole genome sequencing (WGS), and whole transcriptome sequencing (WTS). Some of the variations found included the upregulation of two ligands, transforming growth factor (TGF)-β 1 and TGF-β 2 along with their accompanying receptor, TGF-β receptor type Ⅱ. These growth factors are involved in the epithelial to mesenchymal transition (EMT). Other members of a shared pathway, Lef-1, TCF8, and E2A, were also found to be upregulated. E-cadherin was found to be down-regulated, which is a hallmark of the EMT phenotype[33]. The EMT phenotype has been shown to play a crucial role in cancer cell metastasis along with resistance to chemotherapy and contributing to the formation of cancer stem cells[36]. This patient's tumor did have a mutation in KRAS at codon 12 along with a mutation in *PI3KCA*. The patient's sequencing was done during therapy and upon progression on gemcitabine and cisplatin. The patient was then enrolled on a phase I trial involving a combination PI3K and MEK inhibitor, and experienced a clinical benefit in the form of a dramatic decrease in his pain, along with tumor response[36].

Another genetic analysis done recently looked at 23 patients with ASCP through genomic sequencing and showed a mutation of the *UPF1* gene, which encodes a RNA helicase essential for the highly conserved RNA degradation pathway, nonsense-mediated RNA decay[37]. This mutation was not seen in the adjacent normal tissue of these patient samples. The pathways that UPF1 is implicated in are not all known but appear to be involved in the normal splicing of RNA, affecting such genes as *p53*[37].

IMAGING

While there is not a definitive characteristic appearance of ASCP on computed tomography (CT) imaging, they are usually not well circumscribed[38]. CT imaging of ASCP lesions commonly show the presence of central necrosis within the tumor mass[31,38,39], which is rarely seen in pancreatic ductal adenocarcinoma or in endocrine tumors of the pancreas[40,41]. Another common imaging finding is the propensity for vascular and nerve encasement[38].

A large series looking at ASCP through CT and magnetic resonance imaging showed the presence of frequent intra-tumor necrosis, increased enhancement, and exophytic growth[42]. It is theorized that this pheno-

Table 5 Molecular profiling of patients with adenosquamous carcinoma of the pancreas: Mutated gene analysis (either sanger or next generation sequencing)

Mutated genes percent positive (number found/examined)								
cMET	KRAS	TP53	BRAF	NRAS	SMAD4	cKIT	PIK3CA	EGFR
13 (1/13)	100 (16/16)	50 (4/8)	0 (0/9)	0 (0/9)	25 (2/8)	0 (0/9)	0 (0/11)	0 (0/10)

menon may reflect the presence of the squamous component causing rapid proliferation, as these characteristics are not seen as often in adenocarcinoma of the pancreas[43]. Other unique features noted in imaging evaluation with ASCP are the lack of pancreatic atrophy and mild duct dilatation, which are more common features of pancreatic adenocarcinoma[42]. Like adenocarcinoma of the pancreas, adenosquamous cancers of the pancreas may exhibit the double duct sign[38], which consists of simultaneous dilatation of the common bile and pancreatic ducts[44].

Gallium-67 is an older radioactive tracer that is taken up by some malignancies and infections and has been replaced by PET scans in relation to tumor staging[45]. Intense Gallium-67 uptake, which rarely is detected in pancreatic adenocarcinoma, has been observed in ASCP[45,46]. PET-CT imaging has been reported in a limited number of case reports. One case report noted a patient with localized ASCP to have a standardized uptake value (SUV) of 15.8, which was over 3 times higher than the SUV average for patients with pancreatic adenocarcinoma at their institution[47].

Figure 2 highlights several key imaging findings from patients we have treated with ASCP, including necrosis and mixed morphology. Of note is that in looking at one of our recent ASCP patients, the hypermetabolism that has been previously reported in patients with ASCP was not seen[47].

CLINICAL CHARACTERISTICS

The characteristics of patients with ASCP tend to favor more aggressive features with more node positive disease, more poorly differentiated disease, and more perineural invasion present compared to patients with pancreatic adenocarcinoma[16]. Patients with ASCP present with symptoms similar in nature to patients with pancreatic adenocarcinoma, with abdominal pain, weight loss, back pain, nausea, vomiting, anorexia, and jaundice being the most common presenting symptoms[19,20,38]. As with pancreatic adenocarcinoma, there appears to be an increased risk of deep vein thrombosis[25]. In larger case series, patients are typically male, white, present in their sixth decade of life, and the tumor is located in the head of the pancreas[12,13,20]. Serum lab abnormalities may include elevated bilirubin, elevated alkaline phosphatase, anemia, and elevated carbohydrate antigen 19-9 (CA 19-9)[19,20,25]. Occasionally, patients may also have elevated levels of carcinoembryonic antigen (CEA) or CA-125[38].

Long term survival overall is poor for ASCP. Survival, despite surgical resection, is slightly poorer for patients with ASCP than those with pancreatic adenocarcinoma. Those with ASCP have a 3-year survival rate of 14% with surgery, as opposed to 19% 3-year survival of resected pancreatic adenocarcinoma patients[29,48]. Like patients with pancreatic adenocarcinoma, patients with ASCP tend to present more commonly in advanced stage, with one large analysis through the California Cancer Registry database (CCR) indicating over 50% of ASCP patients presenting in advanced stage[11]. The mean tumor diameter in one series of resected ASCP patients was 46.3 mm *vs* 33.5 mm of adenocarcinoma pancreas patients (*P* value 0.0001)[11]. Comparisons between patients at single institutions and matching for stage have yielded an overall median survival of 6.51 mo *vs* 11.0 mo for ASCP *vs* adenocarcinoma[15]. In another large single institution analysis of patients with ASCP, the median survival of patients with resection was 10.9 mo, which was worse than those with pancreatic adenocarcinoma who underwent resection, which was 17.9 mo[16].

In an analysis of Surveillance, Epidemiology, and End Results (SEER) patients that identified 415 patients with ASCP, the mean age was found to be 66 years old and the tumor more likely to be in the head of the pancreas. Compared to patients with adenocarcinoma of the pancreas, patients with ASCP were more likely to be poorly differentiated (71.4% *vs* 45%), larger (5.7 cm *vs* 4.3 cm), and more likely to have positive lymph nodes (52.8% *vs* 47.1%)[12]. In patients with ASCP, overall 1 and 2-year survival was 21.2% and 10.8% compared to 24.7% and 10.9% in patients with pancreatic adenocarcinoma[12]. Patients with ASCP were found to have a median survival of 4 mo compared to 5 mo in patients with pancreatic adenocarcinoma[12]. Patients with ASCP who underwent resection had worse survival rates than those with adenocarcinoma pancreas cancer who underwent resection. One year and 2-year survival rates of 50.7% and 29% and median survival was 12 mo in patients with ASCP as opposed to 60.1% and 35.8% and median survival of 16 mo in those with adenocarcinoma of the pancreas[12]. After primary resection, recurrence may occur in a number of sites. Common sites of metastases include the liver, lung, retroperitoneum, and development of malignant ascites[16,38].

Several studies have examined various clinical features of survival in patients with ASCP. Lymph node status, tumor size, or resection in patients with ASCP does not impact survival when compared with

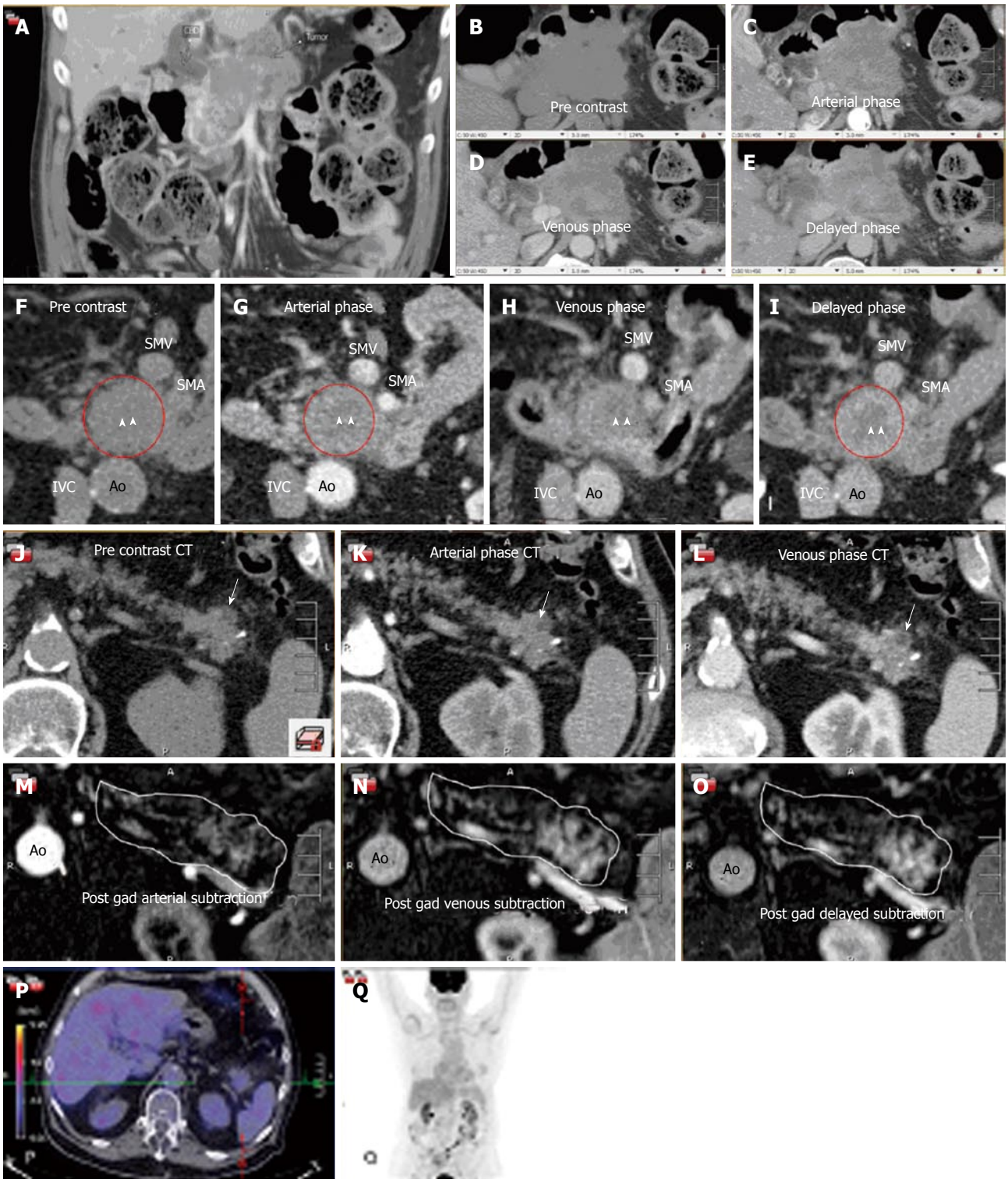

Figure 2 Collection of images from three separate patients with adenosquamous carcinoma of the pancreas. The typical complex enhancing mass and mixed morphology of necrosis and enhancing tissue is demonstrated in this figure. A-E are taken from a four phase contrast enhanced CT (pre-contrast, arterial, venous and delayed images). This type of scanning technique can be helpful to define the tumor and its invasion into surrounding structrures. A-E represent a coronal (A) and axial (B-E) images through a large, infiltrating, necrotic tumor with islands of slow enhancement (B-E). Note the islands of soft tissue enhancement increasing from arterial to delayed phase contrast enhanced CTs. These features are usually signs of very aggressive tumors. In another subject (F-I) there is again a central area of necrosis (arrowheads) surrounded by a ring of slowly enhancing tumor (red circle). Note the relative lack of surrounding soft tissue infiltration compared to the tumor on Panels A-E. Panels J-O are taken from a third subject and are an example of an atypical adenosquamous carcinoma involving the pancreas tail with a slowly enhancing, non-infiltrating lesion both on CT (J-L) and post gadolinium subtraction MRI (M-O). The white outline in Panels M-O outlines the contour of the pancreas with the enhancing lesion seen towards the tail of the pancreas. There is a small focus of necrosis present (arrow), a feature typical of adenosquamous carcinoma of the pancreas. The corresponding FDG PET/CT (P and Q) is unusual in that it shows that this mass is not hypermetabolic unlike most adenosquamous pancreas carcinomas. Ao: Aorta; IVC: Inferior vena caval; SMA: Superior mesenteric artery; SMV: Superior mesenteric vein.

patients with adenocarcinoma of the pancreas[12,16]. Not surprisingly, risk factors for poorer survival of patients with ASCP are those with distant disease, advanced age, and patients unable to undergo surgical resection[12]. In one study, only 40% of patients with ASCP were resectable[12]. A single institution case series from the Mayo clinic showed that patients with an R1 resection still benefited in survival compared to those who did not undergo surgery[49]. Patients from that study that had either an R0 or R1 resection had a median survival of 14.4 and 8 mo respectively, compared to 4.8 mo who received no surgical treatment[49]. Location of the tumor matters, with poorer survival noted if the location was in the body or tail as opposed to the head of the pancreas[48]. This was based on a chart analysis of 39 patients with ASCP and may be accounted for by size of these tumors by location as the ones located in the head were smaller (4.7 ± 1.9 cm) as opposed to the body/tail lesions (7.3 ± 1.8 cm)[48]. The likely reason for poorer survival is that patients with head of pancreas lesions tend to present with obstructive symptoms, which are clinically evident when the lesion is smaller in comparison to body/tail lesions of the pancreas.

It is unclear why patients with ASCP have such a poor prognosis. Due to the small sample size, data to shed light on this disease has been limited. One case series from Voong *et al*[16] looking at patients diagnosed with ASCP and who had undergone surgery showed *via* univariate analysis that only patients who received adjunct chemoradiation had a clinical significant improvement in survival[16]. The patients who received adjunct chemoradiation had a median survival of 13.6 mo as opposed to 8.6 mo for those that did not[16]. Other factors such as age, tumor size, differentiation, margin, node status, type of surgery were not shown to affect survival in this case series[16].

Malignancy associated hypercalcemia, which is a rare phenomenon of exocrine pancreatic carcinoma, has been described in ASCP[50,51]. Of note is that malignancy associated hypercalcemia is more commonly associated with squamous cell carcinomas of the head, neck, lung, and esophagus. Case reports have also described patients with adenosquamous pancreatic cancer having elevated levels of calcium due to elevated levels of parathyroid hormone related protein[50,51]. In both reported cases, hypercalcemia persisted despite bisphosphonate treatment[50,51]. Curiously, hyperglycemia has not been reported with great frequency in ASCP despite being reported in up to 80% of patients with pancreatic adenocarcinoma[52].

MANAGEMENT

Diagnosis of patients with ASCP requires biopsy along with pathology review using criteria of ASCP with at least 30% of the tumor positive for squamous histology. Staging is done in a similar manner as pancreatic adenocarcinoma with guidelines set forth by the American Joint Committee on Cancer (AJCC)/ International Union Against Cancer (UICC). Unresectable disease is designated as Stage Ⅲ and metastatic disease is designated as stage Ⅳ. One issue with diagnosis includes the current standard approach of using endoscopic ultrasound for diagnosing pancreatic cancer and using FNA. In a retrospective review of patients at John Hopkins University and Emory University it was noted that in patients who eventually had a diagnosis of ASCP after surgical resection, two thirds of them (67%) were initially diagnosed as being pancreatic adenocarcinoma only. It is possible for pathologists to misclassify or ignore the squamous cell compartment in pancreatic FNA specimens, which not only leads to underreporting of ASCP but may also miss the diagnosis of malignancy altogether[15].

There are currently no guidelines for treating patients with ASCP. Literature reports often cite treatment regimens similar to adenocarcinoma[48]. Due to its relative infrequency in incidence there have been no published randomized clinical trials specifically targeting patients with ASCP. Treatments in years past have focused on resection of local adenosquamous pancreatic carcinoma using the same guidelines for pancreatic adenocarcinoma. These include pancreaticoduodenectomy (PD), pylorus-preserving PD, distal pancreatectomy, and total pancreatectomy[48]. These techniques are not modified for ASCP and surgical resection remains the best opportunity to achieve long lasting survival[48].

The role of neoadjuvant and adjuvant chemotherapy is unclear, mimicking some questions that continue to be explored in pancreatic adenocarcinoma[48]. Most case reports in the literature have used 5-fluorouracil based therapies for treatment around surgical procedures and have not examined the role of gemcitabine or more robust regimens such as FOLFIRINOX or nab-paclitaxel/ gemcitabine[49]. In a retrospective series of 62 patients identified with pancreatic adenosquamous carcinoma, 14 patients received platinum therapy in the adjuvant setting as opposed to 48 who did not[53]. The patients who received platinum therapy in the adjuvant setting had an overall median survival of 19.1 mo as opposed to 10.7 mo for those who did not (P = 0.011, hazard ratio of survival 0.48)[53].

The role of radiation therapy as an adjunct to resection of ASCP is also unclear[48,54,55]. Two retrospective studies examined adjuvant radiation therapy, but did not show a benefit in overall survival for those that received adjuvant therapy *vs* those who did not. In a previously published literature review of 30 patients who received radiation therapy either intra and/or postoperatively, the 2-year survival rate was 20% and median survival 13 mo[48]. In the patients who did not receive radiation therapy their 2-year survival rate was 9% and median survival period was 6 mo. Despite the differences in survival between the 2 groups, they did not reach statistical significance[48].

CONCLUSION

ASCP is an aggressive variation of carcinoma of the pancreas. Overall it carries a poor prognosis. A study to assess the percentage component of squamous carcinoma in ASCP and associating this with differences in clinical outcome is certainly warranted, but may be difficult to carry out due to the scarcity of this disease and the subjective evaluation needed by pathologists to determine percent squamous in a pancreas carcinoma specimen. Obtaining the proper amount of tissue makes diagnosis difficult and is akin to diagnosing patients with lymphoma by way of FNA: there may be diagnostic inaccuracies depending upon where the sample is biopsied. This role of subjective evaluation also makes interpreting retrospective analysis difficult, such as examining databases like SEER.

There is a need to better characterize the disease beyond traditional pathology analysis. Doing further work characterizing this disease on a molecular level may further elucidate the requirements for classifying pancreatic carcinomas as adenosquamous or adeno. Our work in molecular characterization, while small in sample size, points to the use of novel therapeutic combinations in patients with ASCP, such as epirubicin/cisplatin/5-FU, which may be tested in small clinical trials. Targeting novel pathways such as those affecting the epithelial to mesenchymal change pathway, using agents that target APC, WNT, B-catenin, along with those targeting chromatin remodeling may be worth trying against this disease. Using cell lines derived from ASCP patients and studying them in growth assays and xenograft models may yield clues regarding their response to newer anti-cancer agents in development[54,55]. Understanding the key genetic drivers for this disease may lead to better treatment outcomes since it is clear traditional treatments for pancreatic adenocarcinoma do not translate well to ASCP.

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Proton therapy for pancreatic cancer

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Abstract

Radiotherapy is commonly offered to patients with pancreatic malignancies although its ultimate utility is compromised since the pancreas is surrounded by exquisitely radiosensitive normal tissues, such as the duodenum, stomach, jejunum, liver, and kidneys. Proton radiotherapy can be used to create dose distributions that conform to tumor targets with significant normal tissue sparing. Because of this, protons appear to represent a superior modality for radiotherapy delivery to patients with unresectable tumors and those receiving postoperative radiotherapy. A particularly exciting opportunity for protons also exists for patients with resectable and marginally resectable disease. In this paper, we review the current literature on proton therapy for pancreatic cancer and discuss scenarios wherein the improvement in the therapeutic index with protons may have the potential to change the management paradigm for this malignancy.

Key words: Proton therapy; Pancreatic cancer; Review

Core tip: Radiotherapy is commonly offered to patients with pancreatic malignancies although its ultimate utility is compromised since the pancreas is surrounded by exquisitely radiosensitive normal tissues, such as the duodenum, stomach, jejunum, liver, and kidneys. Proton radiotherapy can be used to create dose distributions that conform to tumor targets with significant normal tissue sparing. Because of this, protons appear to represent a superior modality for radiotherapy delivery to patients with unresectable tumors and those receiving postoperative radiotherapy. A particularly exciting opportunity for protons also exists for patients with resectable and marginally resectable disease.

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INTRODUCTION

Radiotherapy is commonly offered to patients with pancreatic malignancies although its ultimate utility

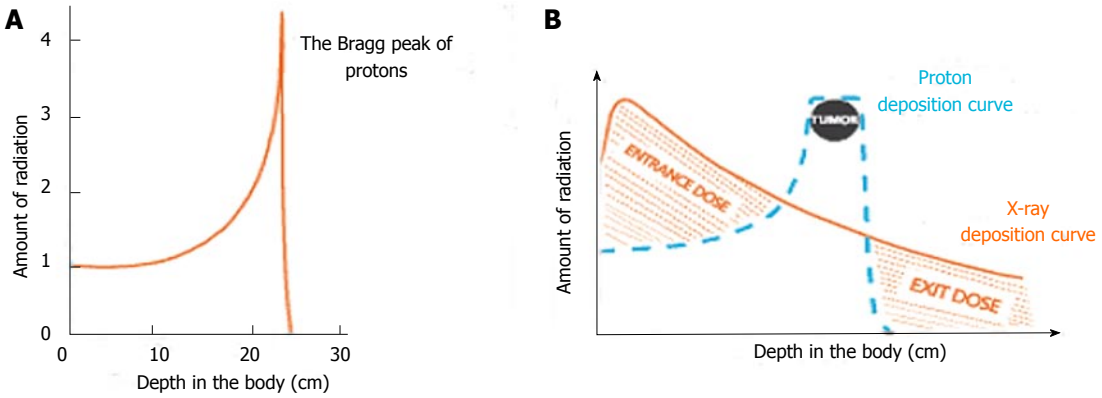

Figure 1 Charged particles like protons travel a finite distance into tissue, as determined by their energy, and then release that energy in a tightly defined region called "Bragg peak" (A). By delivering a range of energies toward the tumor target, a summation of these Bragg peaks allow for the creation of a "spread-out Bragg peak", which conforms to the depth and position of the tumor target (B). Image borrowed from the University of Florida Health Proton Therapy Institute.

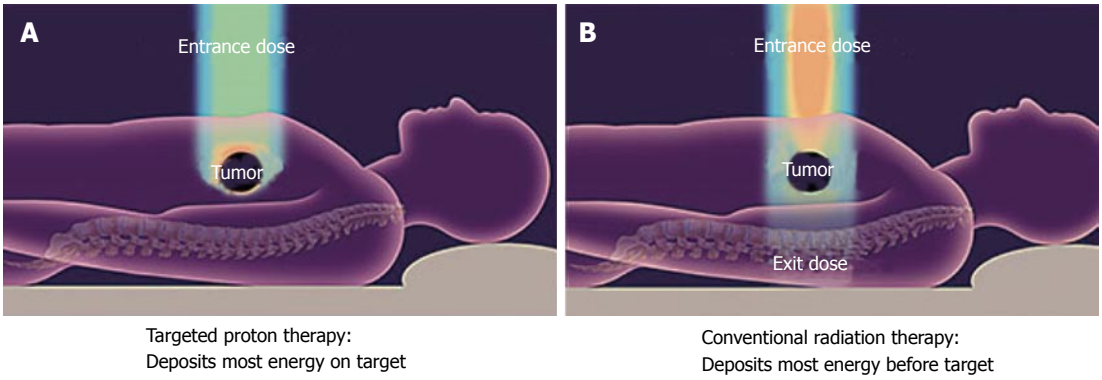

Figure 2 With X-rays, the tumor dose is significantly less than the entry dose and exit dose is delivered beyond the tumor target. With conventional radiotherapy (A) using X-rays (photons), the highest dose is near the point of beam entry into the patient. The tumor dose is significantly less than the entry dose. Also, an exit dose is delivered beyond the tumor target. With protons (B) and other particle therapies, such as carbon ions, the entry dose is low. The highest dose is at the depth of the tumor target and there is no exit dose beyond the target. Image borrowed from the University of Florida Health Proton Therapy Institute.

is compromised since the pancreas is surrounded by exquisitely radiosensitive normal tissues, such as the duodenum, stomach, jejunum, liver, and kidneys. Proton radiotherapy can be used to create dose distributions that conform to tumor targets with significant normal-tissue sparing. Because of this, protons appear to represent a superior modality for radiotherapy delivery to patients with unresectable tumors and those receiving postoperative radiotherapy. A particularly exciting opportunity for protons also exists for patients with resectable and marginally resectable disease. While many surgeons are hesitant to perform major pancreatic operations on patients who have received preoperative X-ray-based radiotherapy, it is possible that the normal tissue-sparing characteristics of protons will allow for more wide-spread adoption of preoperative radiotherapy in the setting of resectable potentially curable disease.

PHYSICS OF PARTICLE THERAPY

Charged particles such as protons travel a finite distance into tissue, determined by their energy, and then release most of that energy in a tightly defined region called the "Bragg peak". By delivering a range of energies directed toward the tumor target, a summation of these Bragg peaks allow for the creation of a "spread-out Bragg peak", which conforms to the depth and position of the tumor target (Figure 1). This process stands in contrast to X-rays for which the highest dose is near the point of beam entry into the patient. With X-rays, the tumor dose is significantly less than the entry dose and exit dose is delivered beyond the tumor target (Figure 2).

With X-ray-based therapies such as intensity-modulated radiotherapy (IMRT), the conformality of the dose distribution around a tumor target is achieved by delivering multiple treatment beams from multiple angles which intersect to create a central high-dose volume. This necessarily results in radiation exposure to virtually the entire cylinder of the abdomen. With protons, because the radiation dose deposition can be modulated along the beam path, fewer beam angles are required to create a conformal dose distribution. As a result, radiation exposure to large volumes of normal tissues is either minimized or eliminated (Figure 3)[1].

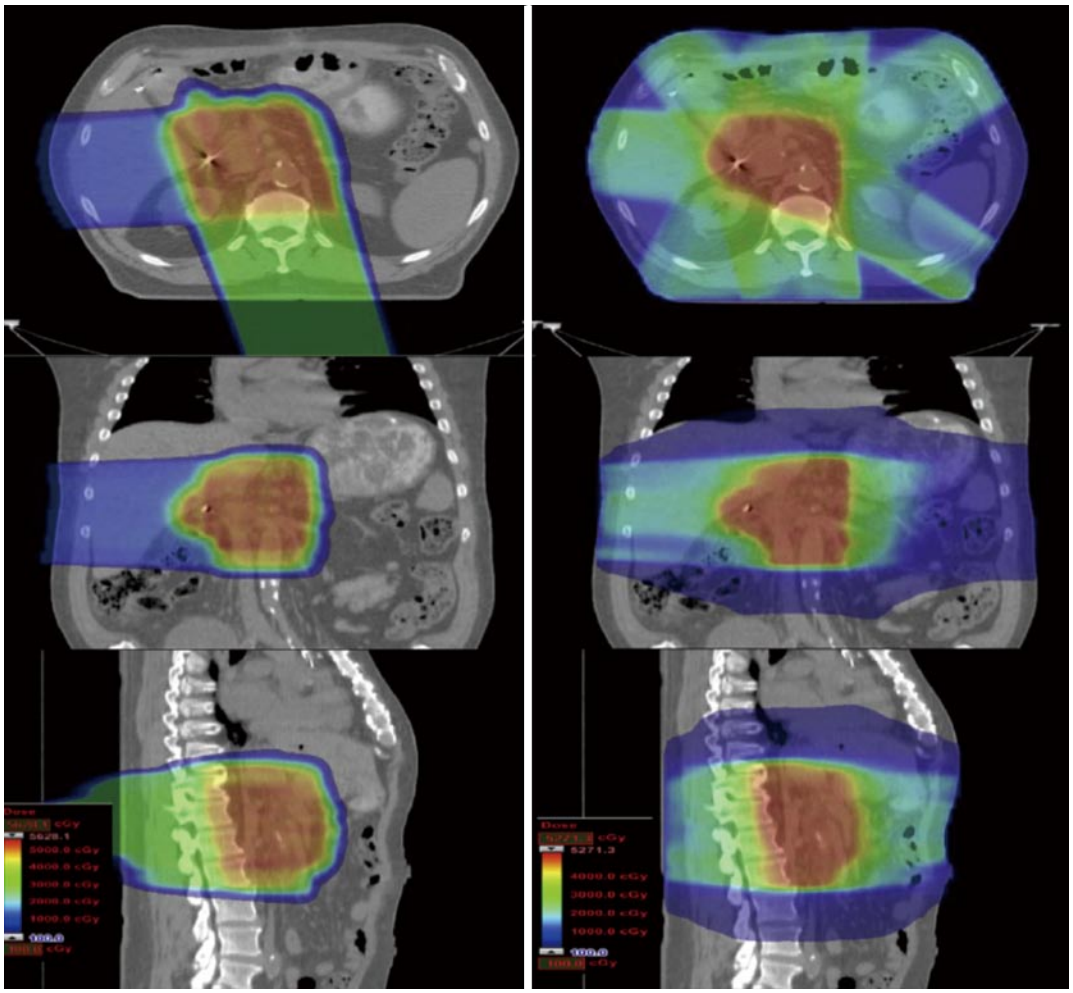

Figure 3 A passively scattered proton plan is shown on the left and an intensity-modulated X-ray therapy plan is shown on the right for a typical patient receiving postoperative radiotherapy for pancreatic cancer. To achieve a conformal dose distribution, the intensity-modulated X-ray therapy plan delivers beams from multiple angles and necessarily irradiates the entire cylinder of the abdomen. With protons, however, because the dose distribution can be modulated along the beam path, significant sparing of sensitive gastrointestinal structures (small bowel and stomach) can be achieved. In the proton plan, 75% of the dose is delivered *via* a posterior field that irradiates the tumor bed but does not exit into the small bowel. The remaining dose is delivered through a right lateral field that also irradiates the tumor bed but does not exit into the stomach.

CONTROVERSIES REGARDING THE ROLE OF RADIOTHERAPY FOR PANCREATIC CANCER

While radiotherapy has historically been offered to patients with unresectable disease or postoperatively to patients with resected disease, several recent studies have questioned its value, suggesting that its toxicity outweighs its potential benefit. The ESPAC-1 trial, using a complicated randomization scheme[2,3], concluded that postoperative radiotherapy was associated with a nominal, but statistically insignificant, survival decrement as irradiated patients demonstrated a 15.5-mo median survival *vs* 16.1 mo for patients receiving chemotherapy alone. While valid criticisms of the ESPAC-1 study have been published[4], chemotherapy alone, without radiotherapy, has been adopted as a standard postoperative approach for resected patients in many centers. For patients with unresectable disease, the recent report of the LAP 07 study (of patients with locally advanced pancreatic cancer) showing a 16.4-mo median overall survival for patients receiving chemotherapy alone *vs* 15.2 mo for the chemoradiation arm[5] has led to further doubts about the utility of radiotherapy in this group of patients. Finally, while some institutions have advocated preoperative X-ray-based radiotherapy for patients with marginally resectable or resectable disease, many surgeons are reluctant to operate on previously irradiated patients, citing concerns about radiotherapy toxicities complicating what is already a complicated operation.

CAN PROTONS IMPROVE THE THERAPEUTIC RATIO?

Considering the above concerns regarding the toxicity-efficacy tradeoffs for X-ray-based radiotherapy, numerous dosimetric and clinical studies have explored the possibility that protons might offer an improved

therapeutic index for pancreatic cancer patients receiving radiotherapy.

Dosimetric studies

Hsiung-Stripp *et al*[6] demonstrated the ability of 130-180 MeV protons to effectively treat unresectable pancreatic cancers. Compared with similarly effective X-ray plans, proton plans significantly reduced doses to the spinal cord (P = 0.003), left kidney (P = 0.025), right kidney (P = 0.057), and liver (P = 0.061). The authors argued that this reduction in normal tissue exposure might allow for radiotherapy dose escalation.

Kozak *et al*[7] demonstrated the dosimetric feasibility of hypo-fractionated proton therapy for neoadjuvant pancreatic cancer treatment using anatomical data from 9 patients. Compared with IMRT, protons offered a significant reduction of dose to the liver, kidneys and small bowel-particularly in the low-dose regions.

Bouchard *et al*[8] compared 3-dimensional (3D) conformal photon radiotherapy with IMRT and protons in the delivery of 72 Gy (RBE) to unresectable tumors. The authors concluded that protons were superior to photons for tumors with anteriorly located small bowel.

Nichols *et al*[9] compared passively scattered protons with intensity-modulated X-ray therapy for 8 patients in the postoperative setting. Patients were treated with a planning target volume dose of 50.4 Gy (RBE). Proton plans offered significantly reduced normal tissue exposure over the IMRT plans with respect to median small bowel V20Gy (RBE) (P = 0.0157), median gastric V20Gy (RBE) (P = 0.0313), and median right kidney V18Gy (RBE) (P = 0.0156). The authors argued that, by reducing small bowel and gastric exposure, protons have the potential to reduce acute and late toxicities of postoperative chemoradiation.

Lee *et al*[10] explored the feasibility of using proton therapy in the neoadjuvant setting to cover a planning target volume including gross disease and regional lymph nodes. Utilizing a field arrangement heavily weighted to a posterior field, the investigators demonstrated the feasibility of expanding the target volume to cover nodal targets without significantly increasing critical normal tissue exposure. The authors argued that treating a similar increase in target volume would be substantially more difficult with X-rays due to normal tissue exposure issues.

Ding *et al*[11] compared passively scattered and modulated scanning proton therapy to a number of X-ray-based strategies including 3D conformal radiation therapy (3DCRT), 5-field IMRT, and 2-arc volumetric modulated radiation therapy. Proton plans demonstrated lower doses to the kidneys, stomach, liver, and bowel.

Thompson *et al*[12] compared proton and IMRT plans in 13 patients with unresectable cancer of the pancreatic head. Both the double-scattered and pencil-beam plans decreased gastric, duodenal, and small bowel dose in the low-dose regions compared to IMRT; however, protons were associated with increased dose in the mid- to high-dose regions.

Clinical studies

Three groups (Massachusetts General Hospital in Boston, Hyogo Ion Beam Center in Japan, and University of Florida) have published preliminary clinical data on the treatment of pancreatic cancer patients with protons.

The group from Massachusetts General Hospital completed a phase 1 study of preoperative short-course chemoradiation confirming the safety of a preoperative dose of 25 Gy (RBE) in 5 fractions over 1 wk with concomitant oral capecitabine at 825 mg/m² twice a day, Monday through Friday, for 10 d followed by surgery. No dose-limiting toxicities were observed. Grade 3 toxicity was noted in 4 of 15 patients. Eleven patients underwent resection. Mean postsurgical length of stay was 6 d with no unexpected 30-d postoperative complications[13]. Of note, a corresponding study of hypofractionated preoperative X-ray-based radiotherapy using the same dose with X-rays was closed early due to toxicities that included intraoperative fibrosis and increased operating room time[14]. A phase Ⅱ trial of proton therapy using the above dose regimen enrolled 50 patients, of whom 47 were eligible for analysis and 37 underwent pancreaticoduodenectomy. Of this cohort, 81% had positive nodes. Local regional failures occurred in 6 of 37 resected patients and distant metastases in 35 of 48. With a median follow-up of 38 mo, the median progression-free survival for the entire group was 10 mo and overall survival was 17 mo. The grade 3 toxicity rate was 4.1%.

Investigators at the Hyogo Ion Beam Center in Japan published the results of an aggressive phase I/II study of chemoradiation for patients with locally advanced pancreatic cancer. All patients received gemcitabine at 800 mg/m² weekly for 3 wk concurrent with proton therapy. Most of the patients received a dose of 67.5 Gy (RBE) in 25 fractions. The initial report suggested tolerability of this regimen[15]; however, a subsequent publication in the gastroenterology literature reported a high rate of upper gastrointestinal complications[16]. Post-treatment endoscopic examinations in 45 of 91 patients revealed radiation-induced ulcers in the stomach and duodenum. While the authors of the second publication suggested that proton therapy for inoperable pancreatic cancer was associated with a high rate of gastric and duodenal ulceration, a subsequent criticism of this study[17] pointed out that the severe toxicity exhibited was more likely due to the extremely aggressive radiotherapy dose offered with full-dose gemcitabine rather than any toxicity unique to proton therapy.

Researchers at the University of Florida published a preliminary report on the outcomes of 22 patients treated with proton therapy and concomitant capecitabine (1000 mg by mouth twice a day) for resected (n = 5), marginally resectable (n = 5), and unresectable/inoperable (n = 12) biopsy-proven pancreatic and ampullary adenocarcinoma[18]. Proton doses ranged from 50.4 Gy (RBE) to 59.4 Gy (RBE). No patient demonstrated any grade 3 toxicity during treatment

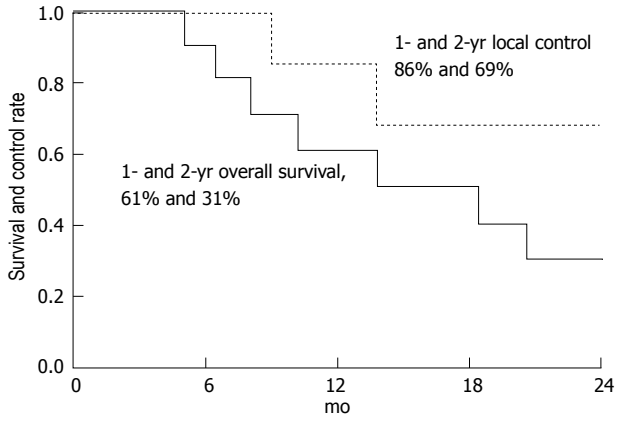

Figure 4 Overall survival and freedom from local progression at 2 years for 11 patients accrued to a phase II clinical trial for unresectable pancreatic cancer. Image borrowed from Ref. [19].

or during follow-up. Three patients experienced grade 2 gastrointestinal toxicity; all 3 of these patients were treated early in the series with fields that included anterior and left lateral components. When field design was modified to deliver the majority of the dose through the posterior field with a lightly weighted right-lateral field, grade 2 gastrointestinal toxicity was eliminated. The median weight loss during treatment was 1.3 kg. Chemotherapy was well-tolerated with a median of 99% of the prescribed doses delivered.

A subsequent publication by the same group reported the outcomes of a phase II clinical trial for patients with unresectable pancreatic cancer[19]. A total of 11 patients were reported. All patients received 59.4 Gy (RBE) at 1.8 Gy (RBE) per fraction over 7 wk with concomitant oral capecitabine at 1000 mg by mouth twice a day on radiation treatment days only. The median follow-up for surviving patients was 23 mo. The 2-year overall survival rate was 31%, the median survival rate was 18.4 mo, and the 2-year freedom from local progression rate was 69% (Figure 4). No patient experienced grade 2 or higher gastrointestinal toxicity. Four patients had an adequate radiographic response to radiation therapy to justify surgical exploration.

RATIONALE FOR PREOPERATIVE RADIOTHERAPY

Of the approximately 49000 cases of pancreatic cancer diagnosed annually in the United States, only 20% of these patients can be considered resectable or "curable"[20]. Unfortunately, the "cure" rate for these patients is only approximately 20%[21]. While many of these patients fail exclusively with distant metastatic disease, a substantial number experience local recurrence after surgery. Published data suggest that the local failure rate after surgery, even with negative margins, is in the range of 50%-80% if these patients do not receive radiotherapy[22,23]. Postoperative radiation therapy, however, has intrinsic limitations in this disease site. For example, postoperative convalescence generally necessitates a 10- to 12-wk window between surgery and initiation of postoperative radiation therapy. In reality, many patients are unable to receive postoperative radiation therapy within a clinically meaningful time frame. Additionally, the dose of postoperative radiation therapy is limited by the fact that a large volume of transposed small bowel is located in the radiotherapy field, making it unlikely that doses above 50 Gy can be safely delivered to these patients - a dose that is unlikely to control anything larger than the smallest microscopic adenocarcinoma deposits. In fact, published studies on patients receiving postoperative radiation therapy after surgery indicate local-regional failure rates ranging from 25%-36%[24,25]. Additionally, published data from respected high-volume centers suggest that patients undergoing extirpative surgery in the modern era for pancreas cancer have a high rate of margin and lymph node positivity. The series published by investigators at Johns Hopkins Medicine (Baltimore, MD) on 905 patients undergoing pancreaticoduodenectomy between 1995 and 2005 indicated a 41% margin positivity rate and a 79% node positivity rate[26]. The series from Memorial Sloan-Kettering Cancer Center (New York, NY) on 625 resections between 2000 and 2009 indicated a 16% margin positivity rate and a 70% node positivity rate[27]. Based on these data it is reasonable to believe that even "resectable" patients would be likely to benefit from preoperative radiotherapy - perhaps even with fields that could cover regional lymph nodes.

PLANNED PREOPERATIVE PROTON THERAPY FOR RESECTABLE OR MARGINALLY RESECTABLE DISEASE

It is possible that proton therapy in the postoperative setting will offer reduced toxicity compared to X-ray-based therapy and thereby improve local control and offer a positive impact on survival. While the results of proton therapy for patients with unresectable pancreatic cancer are encouraging, it is unlikely that this therapy, without meaningful improvements in systemic therapy, can be viewed as a potentially curative intervention.

It may be argued, however, that the best use of particle therapy would be in the preoperative setting for patients with resectable or marginally resectable disease. Preoperative radiotherapy is well-established in the treatment of other gastrointestinal disease sites (such as the esophagus and rectum) and improves local disease control and survival. It is reasonable to infer that a similar benefit could be achieved in the setting of pancreatic malignancy. As stated earlier, the main resistance to the use of preoperative radiotherapy involves concerns about radiotherapy toxicity and its potential to complicate what is already a complicated operation. If proton therapy can be delivered with negligible toxicity so that it does not compromise the

performance of extirpative surgery, proton therapy would represent more than a "kinder/gentler" form of radiotherapy; proton therapy would have the potential to alter the management paradigm for this group of potentially curable patients.

CLINICAL DATA SUPPORTING THE FEASIBILITY OF PREOPERATIVE PARTICLE THERAPY

In addition to the data published by Massachusetts General Hospital regarding the feasibility of surgery after preoperative hypofractionated proton therapy, a report from the University of Florida analyzed the outcomes of 5 patients with initially unresectable disease who unexpectedly achieved enough of a tumor response to justify surgical resection after high-dose conventionally fractionated proton therapy[28]. All patients received 59.4 Gy (RBE) in 33 fractions with concomitant oral capecitabine. Three patients subsequently underwent a laparoscopic standard pancreaticoduodenectomy, 1 underwent open pylorus-sparing pancreaticoduodenectomy, and 1 underwent an open distal pancreatectomy with irreversible electroporation after biopsies of the pancreatic head were negative. Duration of surgery, blood loss, intensive care unit stay, total hospital stay, and readmissions were consistent with historical benchmarks. None of the operating surgeons described fibrosis, anastomotic leaks, or perception that the proton therapy complicated the operation. The fact that surgery could be performed without significant complications after high-dose radiotherapy for patients who are initially unresectable suggests that lower doses of preoperative proton therapy in the range of 50 Gy (RBE) or even higher should not complicate surgery for patients with resectable or borderline resectable disease.

CONCLUSION

Dosimetric studies and early clinical outcomes suggest that particle therapy improves the therapeutic index for pancreatic cancer patients receiving radiotherapy. By reducing or eliminating the gastrointestinal toxicity historically associated with X-ray-based radiotherapy, proton therapy should address the concerns of clinicians who are hesitant to employ radiotherapy in the postoperative setting (based on the ESPAC-1 data) and those who are reluctant to offer radiotherapy to patients with unresectable disease (based on the LAP-07 data).

Arguably, the most exciting potential role for particle therapy is in the neoadjuvant treatment of patients with resectable and marginally resectable disease. These patients are well recognized to suffer a high risk of local and regional failure after surgery - a risk that is only marginally reduced with postoperative X-ray-based radiotherapy. Based on the treatment of other gastrointestinal disease sites (such as the esophagus and rectum) it is reasonable to believe that preoperative radiotherapy would have a greater impact on securing local and regional control than chemotherapy or postoperative radiotherapy. Recognizing that the primary barrier to the adoption of preoperative radiotherapy in this setting is the concern of operating surgeons that the gastrointestinal toxicity of radiotherapy will complicate the procedure, it is possible that the favorable toxicity profile associated with proton therapy will make the oncologically rational intervention (preoperative radiation therapy) technically feasible. If this is the case, proton therapy would indeed result in a change in the management paradigm for patients with resectable and potentially curable pancreatic cancer.

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MINIREVIEWS

Intrahepatic therapy for liver-dominant metastatic colorectal cancer

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Abstract

In patients with metastatic colorectal cancer, the liver is the most common site of metastatic disease. In patients with liver-dominant disease, consideration needs to be given to locoregional treatments such as hepatic arterial infusion chemotherapy, transarterial chemoembolisation and selective internal radiation therapy because hepatic metastases are a major cause of liver failure especially in chemorefractory disease. In this review we provide insights on the published literature for locoregional treatment of liver metastases in metastatic colorectal cancer.

Key words: Colorectal cancer; Liver metastases; Intrahepatic treatment; Chemoembolization; Radioembolisation

Core tip: Thanks to the increased chemotherapeutic options in patients with metastatic colorectal cancer (mCRC), the overall survival has significantly improved the last decade. Liver failure is a common cause of death in mCRC with liver metastases. Therefore in these patients locoregional treatment is a valuable treatment option in order to increase survival. In this review we provide insights on the published literature.

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INTRODUCTION

Although the incidence and the mortality of colorectal cancer (CRC) have decreased over the years in some countries, it still remains one of the most prevalent and the third leading cause of cancer death worldwide[1]. Even with improved screening, the incidence of synchronous and metachronous disease remains high. Approximately half of patients with CRC will develop liver metastases[2]. When mCRC is treated with a combination of chemotherapy (5-FU, oxaliplatin, irinotecan) and targeted agents such as the anti-epidermal growth factor receptor and anti-vascular growth factor

monoclonal antibodies, median overall survivals now extend beyond 24 mo in the clinical trial setting[3]. Hepatic metastases are a major cause of liver failure especially once all chemotherapeutic and/or surgical options have been exhausted. Although surgical resection of liver metastases for curative intent is the treatment of choice, most patients present with unresectable liver-predominant metastatic CRC (mCRC). In these cases, consideration needs to be given to the (often favorable) efficacy and safety of locoregional treatments such as hepatic arterial infusion (HAI) chemotherapy, transarterial chemoembolization (TACE) and selective internal radiation therapy (SIRT), either alone or in combination with systemic chemotherapy.

In this review, we provide further insights on the published literature for the locoregional treatment of liver metastases in patients with mCRC.

HEPATIC INTRA-ARTERIAL CHEMOTHERAPY

There is a compelling argument for HAI chemotherapy in patients with liver-predominant mCRC because of the preferential perfusion of liver metastases (compared with the normal parenchyma) by the hepatic arterial network whereas non tumor liver parenchyma is preferentially perfused by the portal vein. In addition, local intra-arterial treatment circumvents the first-pass effects of the liver, exposing the liver metastases to high concentrations of chemotherapy while at the same time reducing the incidence of unwanted systemic side effects.

The femoral artery is the most common access route. The catheter tip is placed into the hepatic artery at the junction of the gastro-duodenal artery to enable bilobar hepatic infusion. To avoid gastric or duodenal lesions, selective distal embolization is performed of the side branches of the hepatic artery leading to the adjacent organs. Catheter displacement or occlusion remains the most frequently reported complication of HAI chemotherapy use[4].

In the United States, fluorodeoxyuridine (FUDR), a 5-FU derivative, is the most commonly used chemotherapy agent in patients treated with HAI chemotherapy FUDR has the advantage of being rapidly metabolized, with a 94%-99% extraction rate within the liver *via* first-pass metabolism, so enabling high intrahepatic concentrations when given by HAI, but the downside of this HAI chemotherapy is hepato-biliary toxicity which may lead to biliary sclerosis. However, when combined with dexamethasone (Dex), the toxicity of HAI FUDR toxicity is reduced[5]. In Europe, 5-FU is more frequently used which has only a 50% extraction rate in the liver, but systemic blood concentrations of 5-FU are higher than FUDR, making it a more effective against extra-hepatic (micro)metastases. 5-FU is also less hepatotoxic compared with FUDR. Oxaliplatin and irinotecan, the other chemotherapeutic agents active in CRC are also now more commonly used for HAI; although the available data are scant[6-8].

Although its rationale is appealing, the benefit of HAI chemotherapy is unclear because of the lack of large randomized trials. Chemotherapy can be used either as neo-adjuvant therapy for isolated, potentially resectable CRC liver metastases or as adjuvant therapy after complete resection in patients at high-risk of recurrence. In the neo-adjuvant setting, the aim of chemotherapy is to render unresectable liver metastases resectable. It is recognized that classical chemotherapy schedules in combination with monoclonal antibodies can achieve response rates up to 80%[9] but the optimal HAI chemotherapy regimen has yet to be established. In the absence of large phase Ⅲ trials, evidence for the reported improvements in resectability with HAIC in CRC-related inoperable liver metastases is based solely on small phase Ⅱ studies[6,7,10]. In the adjuvant setting after curative hepatectomy, phase Ⅱ studies also provide evidence for lower recurrence rates when HAI chemotherapy is combined with systemic chemotherapy[11,12]; thereby providing proof-of concept but evidence from large phase Ⅲ trials are still needed.

In inoperable liver-only mCRC, HAI chemotherapy might also be used to achieve locoregional control. A study conducted by the Medical Research Council and the European Organization for the Research and Treatment of Cancer, randomly assigned 290 patients with unresectable CRC liver metastases to either HIA with 5-FU and leucovorin (LV) or systemic 5-FU/LV. The study observed no difference between the treatment arms for overall survival (OS) (14.7 mo *vs* 14.8 mo), progression-free survival (PFS) or toxicity[13]. There was, however, a high frequency of catheter-related thrombosis in the HAI chemotherapy arm (36%) resulting a lower proportion of patients receiving the intended six or more chemotherapy cycles compared with systemic chemotherapy (38% *vs* 75%)[13]. Some patients in this trial crossed-over to intravenous chemotherapy, but were still analyzed as HAI in an intention-to-treat manner, thereby making it difficult to draw any definitive conclusions from this trial. In contrast, another study lead by the Cancer and Leukemia Group B (CALGB) randomly assigned 135 patients with inoperable CRC liver metastases CRC liver metastases to either HAI-FUDR/LV/Dex or systemic 5-FU/LV and observed a significant benefit in favor of HAI for both median OS (24.4 mo *vs* 20 mo, $P = 0.0034$) and response rate (47% *vs* 24%; $P = 0.12$)[14]. There was no significant difference in time to progression (TTP) (5.3 mo *vs* 6.8 mo), but the time to hepatic progression was longer in the HAI group (9.8 mo *vs* 7.3 mo), and time to extra-hepatic progression was longer in the systemic group (14.8 mo *vs* 7.7 mo)[14].

More recent studies have also evaluated oxaliplatin and irinotecan for HAI. In a French phase Ⅱ study, 26 patients with inoperable, liver-only mCRC were treated with a combination of HAI-oxaliplatin plus systemic

5FU/LV[6]. Twenty-one patients had been pretreated with one line of 5-FU-based therapy, none had previously received oxaliplatin. The median OS was 27 mo, and response rate reported was 64%, which were comparable to regimens with HAI-FUDR and systemic 5FU-LV. In a second study of HAI-FUDR plus systemic 5FU/LV, the same research group investigated patients who had received more than one line of systemic chemotherapy: either FOLFIRI or FOLFOX or both (percentage of 86%, 77% and 96% respectively). The median OS was 16 mo, response rate 62% (18% down-staged for resection) and median PFS 7 mo. Although the results of these studies are initially promising, the advantage of this approach still needs to be confirmed in a phase Ⅲ study *vs* systemic chemotherapy alone.

TRANSARTERIAL (CHEMO)EMBOLIZATION

TACE, the combination of the injection of a drug and embolic material, has mostly been used in hypervascular tumors such as hepatocellular carcinoma. The use of drug-eluting beads (DEB) enables the controlled release of drug after the beads are trapped in the tumoral circulation. Modern angiographic techniques make it possible to selectively deliver the material to the tumor resulting in minimal release of cytoxic agent(s) into the surrounding tissues.

In mCRC, different chemotherapeutic agents can be used to load the drug eluting beads. A prospective single-center study evaluated 463 patients with chemorefractory, unresectable CRC liver metastases who were treated with TACE at 4-wk intervals[15]. Three TACE regimens were used, either: mitomycin C alone, mitomycin C with gemcitabine, or mitomycin C with irinotecan. Embolization was performed with lipiodol and starch microspheres. A total of 2441 TACE procedures were performed (mean of 5.3 sessions per patient). The median OS in this chemorefractory population was 14 mo, with no significant difference between the different chemotherapy protocols. Disease control was 62.9% [14.7% partial response (PR), 48.3% stable disease (SD)][15]. Another German study also evaluated retrospectively the same chemotherapy schedules in 564 patients in either the neoadjuvant or palliative setting[16]. Like the previous study, no significant differences in OS were observed between the chemotherapy regimens and response rates were also in the same range (16.7% PR, 48.2% SD). Finally disease control rates of 43% were found in another retrospective analysis of 121 patients in the chemorefractory setting with TACE with cisplatin, doxorubicine and mitomycin C[17].

To date, the published experience with chemoembolization using DEB-irinotecan (DEBIRI) has mostly been performed in liver-predominant CRC. DEBIRI was evaluated in a phase Ⅱ study in 82 chemorefractory liver-predominant CRC patients, resulting in very high response rates of 78% at 3 mo post-treatment and a mean PFS of 8 mo[18]. In another study response rates with DEBIRI were 66% and 75% at 6 and 12 mo, respectively and PFS was 11 mo[19]. In both these studies of DEBIRI, the most common adverse event was post-embolization syndrome reported as abdominal pain, nausea and vomiting[18,19]. Usually symptoms were mild and transient; rarely has there been any reports of liver toxicity associated with liver abscess, liver failure or pancreatitis and only when more extensive embolization was performed.

Pharmacokinetic studies evaluating DEBIRI show that plasma levels of irinotecan and its active agent SN-38 were almost undetectable 24 h after administration[20]. Only one small randomized phase Ⅲ study has been performed comparing DEBIRI with systemic chemotherapy (FOLFIRI)[21] in 74 patients with unresectable mCRC without extrahepatic disease, who were refractory to at least two lines of chemotherapy. A survival advantage with DEBIRI was suggested (median OS of 22 mo *vs* 15 mo with FOLFIRI; $P = 0.031$). The DEBIRI group also had a significantly higher objective response rate (69% *vs* 20%)[21].

In conclusion, several studies suggest that TACE can achieve disease stabilization in 40%-60% of patients, but whether this leads to a prolongation of OS relative to systemic chemotherapy is uncertain, since almost no randomized-controlled trials have been performed. Therefore larger randomized trials are needed for comparison with standard intravenous chemotherapy.

SELECTIVE INTERNAL RADIATION THERAPY

Selective internal radiation therapy (SIRT) (or radioembolization) is a form of intra-arterial brachytherapy using resin-based microspheres impregnated with ^{90}Yttrium (^{90}Y) as the radiation source. SIRT using ^{90}Y resin microspheres was approved by the FDA in 2002. ^{90}Y-resin microspheres are delivered into the tumor-feeding arteries of the hepatic arterial circulation and embed permanently in the pre-capillary arterioles of liver tumors where they deliver very high doses of localized radiation (and so minimizing the damage to the healthy liver parenchyma). In general, SIRT is safe and well tolerated with fewer side effects and milder post-embolization syndrome than with observed TACE. However, SIRT is more complex to administer and therefore its use is often restricted to specialized centers. Specific complications are rare, and include gastroduodenal ulceration, pancreatitis, cholecystitis, abscess formation and radiation-induced liver or lung disease.

Approval was based on one randomized controlled trial in which 74 patients with liver isolated CRC metastases were assigned to either HAI-FUDR alone or HAI-FUDR in conjunction with a single administration of SIRT[22]. The study found that compared with HAI, the combination of SIRT and FUDR-HAI led to a significantly better complete response rates (44% *vs* 18%) and

prolonged the median time to progression (16 mo *vs* 10 mo).

Radioembolization has also been compared to intravenous chemotherapy in two prospective randomized-controlled trials[23,24]. The first RCT was a small phase Ⅱ study conducted by Van Hazel *et al*[23] in 21 patients with previously untreated liver-predominant mCRC. Systemic 5-FU/LV preceded by a single SIRT procedure significantly prolonged median OS (29.4 mo *vs* 12.8 mo) as well as time to progression (TTP) (18.6 mo *vs* 3.6 mo) compared with 5FU/LV alone. More recently, a phase Ⅲ study assigned 44 patients with chemotherapy refractory liver-limited metastatic CRC to treatment with 5-FU monotherapy or SIRT during the first cycle of chemotherapy followed by 5-FU monotherapy, until hepatic progression[24]. Cross-over to SIRT was permitted after progression in the 5-FU monotherapy arm. Once again the combination of SIRT and systemic chemotherapy significantly improved TTP (4.5 mo *vs* 2.1 mo), but without any difference in OS between the two arms (10.0 mo *vs* 7.3 mo) primarily due to the cross-over of some patients from 5-FU monotherapy to the SIRT arm following progression studies in which SIRT is added to more modern systemic chemotherapy such as FOLFOX and bevacizumab (SIRFLOX and FOXFIRE study) are now ongoing with initial results from SIRFLOX likely to be presented in 2015.

To date most of the published studies with SIRT are in chemorefractory liver predominant mCRC. A systematic review of twenty studies comprising 979 patients treated with ^{90}Y-resin microspheres revealed a median time to intrahepatic progression of 9 mo and OS of 12 mo[25]. Although this review has several shortcomings such as: the inclusion of multiple observational studies, studies with small sample sizes and the heterogeneity of patients, it still demonstrated that SIRT was safe and an effective treatment for unresectable, chemorefractory mCRC.

CONCLUSION

The management of chemorefractory liver metastases from mCRC is a major challenge and effective treatment options are urgently needed. Both HAI chemotherapy as well as TACE and SIRT appear to be effective in this setting when used in centers with expertise in the technical aspects of these local treatments. However, adequately powered prospective phase Ⅲ studies are still needed. Landmark studies such as SIRFLOX and FOXFIRE with SIRT are expected to help better define the role of these treatments earlier in the course of liver-predominant mCRC.

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Hereditary diffuse gastric cancer: What the clinician should know

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Abstract

Hereditary diffuse gastric cancer (HDGC) is an inherited autosomal dominant syndrome with a penetrance of up to 80% affecting diverse geographic populations. While it has been shown to be caused mainly by germline alterations in the E-cadherin gene (*CDH1*), problematically, the genetic diagnosis remains unknown in up to 60% of patients. Given the important knowledge gaps regarding the syndrome, asymptomatic carriers of *CDH1* mutations are advised for a prophylactic total gastrectomy. Intensive annual endoscopic surveillance is the alternative for carriers who decline gastrectomy. As HDGCs have a prolonged indolent phase, this provides a window of opportunity for surveillance and treatment. Recent findings of other gene defects in *CTNNA1* and *MAP3K6*, as well as further characterization of *CDH1* mutations and their pathogenicity will change the way HDGC patients are counselled for screening, surveillance and treatment. This review will bring the reader up to date with these changes and discuss future directions for research; namely more accurate risk stratification and surveillance methods to improve clinical care of HDGC patients.

Key words: Hereditary diffuse gastric cancer; *CDH1*; *CTNNA1*; *MAP3K6*; Gastrectomy

Core tip: While the incidence of hereditary diffuse gastric cancer remains low, it is an important clinical entity to recognize due to its high pathogenicity and penetrance. The International Gastric Cancer Linkage Consortium has outlined *CDH1* testing criteria and developed clinical utility gene cards to help clinicians manage such patients. Significant progress has been made in recent years and in future, testing of other genes is likely for *CDH1*-negative families. The mainstay of treatment for asymptomatic carriers of *CDH1* pathogenic mutations remains prophylactic total gastrectomy. Future research should focus on better risk stratification and surveillance methods.

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INTRODUCTION

Gastric cancer (GC) is currently the fourth most common cancer and the second leading cause of cancer associated death worldwide[1]. Based on the Lauren classification, at least two main histological types of GC have been identified: intestinal and diffuse[2]. Both histological types have different clinical features and molecular mechanisms[3-8]. Hereditary GCs account for only 1%-3% of GC cases[9], but are important for clinicians to identify as potentially curative interventions are available. One well-characterized syndrome is Hereditary diffuse gastric cancer (HDGC), which was attributed to germline mutations of the E-cadherin gene (*CDH1*) in 1998[10]. The International Gastric Cancer Linkage Consortium (IGCLC) has since established the latest set of clinical criteria in 2010 (listed in Table 1) to guide genetic screening[11].

Only about 40% of probands meeting the 2010 criteria carry *CDH1* germline alterations (often point or small frameshift mutations)[9,12]. Of the remaining 60%, a small percentage is due to *CDH1* deletions not detected by conventional DNA sequencing. More intriguingly, mutations in other genes like *CTNNA1*[13], *MAP3K6*[14], *INSR*, *FBXO24* and *DOT1L*[15] are starting to be identified. However, pathogenicity and penetrance of many newer mutations remain unanswered, creating management dilemmas. These non-*CDH1* mutations published thus far have been summarized in Table 2. Most studies are small and will require validation in consortium-led efforts for us to better understand the longitudinal impact.

CLINICAL HISTORY

Presentation

Similar to other gastric carcinomas, patients with HDGC are often asymptomatic in the early stages and tend to present late with symptoms such as weight loss, abdominal pain, nausea, anorexia, dysphagia, melaena and early satiety. The median age at diagnosis is 38 years, with the range varying greatly from 14-82 years[10,16].

Majority of HDGCs are inherited in an autosomal dominant pattern. It exhibits high penetrance and invasive disease often manifests before age 40. Therefore, one should have a high clinical suspicion when a family history reveals two or more cases of gastric cancer in first or second degree relatives, especially with one case diagnosed before age 50. The lifetime cumulative risk for diffuse GC reaches > 80% in men and women by age 80 years[11].

Other features seen in HDGC familes

There is an association of HDGC with lobular breast cancer (LBC) and it can be the presentating pathology[17]. Data based on 11 HDGC families, estimated the cumulative risk for LBC for female *CDH1* mutation carriers to be 39% (95%CI: 12%-84%) by 80 years of age[18]. Thus, personal or family history of multiple LBCs at a young age should also prompt CDH1 screening even if there is no HDGC. There have also been case reports of colorectal, prostate and ovarian carcinomas in HDGC families although these are rare and of uncertain significance[19-22]. Interestingly, cleft-lip, with or without cleft-palate malformations have been reported in several HDGC families, some of whom have specific *CDH1* splice site mutations[23,24].

Table 1 Clinical criteria for *CDH1* genetic testing (adapted from Fitzgerald *et al*[11])

⩾ 2 diffuse GC cases in 1st or 2nd degree relatives with one < 50 yr of age
⩾ 3 diffuse GC cases in 1st or 2nd degree relatives independent of age
Diffuse GC < 40 yr of age, without a family history
Personal or family history of diffuse GC and lobular breast cancer with one < 50 yr of age

GC: Gastric cancer.

Other relevant hereditary cancer syndromes

It should be remembered that GC can develop in the setting of other hereditary cancer syndromes aside from HDGC. One example would be Lynch syndrome which more often presents with intestinal-type gastric cancers and also has a high lifetime risk of colorectal and endometrial cancer. Other examples include Familial adenomatous polyposis, Li-Fraumeni syndrome, Peutz-Jegher's syndrome (PJS) and Juvenile Polyposis Syndrome (JPS) (Table 3). The lifetime risk of GC in these syndromes varies considerably but is generally lower than that in HDGC.

PATHOPHYSIOLOGY

Genetic susceptibility

E-cadherin is a cell adhesion protein that is required for development, cell differentiation and maintenance of epithelial architecture[6]. Since the E-cadherin gene *CDH1* was identified as a genetic basis for HDGC in 1998, more than 120 *CDH1* germline mutations have been published[25]. The most common germline alterations are small frameshifts, splice-site and nonsense mutations[9]. Of note, only two *de novo* mutations have been reported to date[26,27].

However, newer HDGC-susceptibility genes have been identified (Table 2). In 2012, an alpha-E-catenin (*CTNNA1)* germline truncating mutation was been found in a large Dutch HDGC pedigree[14] although the evidence presented was not definitive given a number of carriers remained cancer-free and other studies have failed to replicate findings[28]. At time of writing, *MAP3K6*[15], *INSR*, *FBXO24* and *DOT1L*[16] have also identified as candidate genes although they remain reports from single families. The insulin receptor (*INSR)* gene mutation is of special interest given insulin signaling has been reported to affect tumour cell invasion capability by modulating E-cadherin

Table 2 Summary of non-*CDH1* germline mutations in hereditary diffuse gastric cancer

Gene	Mutation	Location	Mutation type	Ethnicity	Ref.	Study type	Frequency	Remarks
CTNNA1	c.76delGA	Chr 5: 138117693	Nonsense	No data	[13]	Family study	1/1 family	Results in a framshift after Arg27 (p.Arg27Thr.fs*17)
MAP3K6	c.598G>T	Chr 1: 27690792	Missense	Canada	[14]	Family study and case series	1/1 family 1/115 cases	Likely pathogenic
MAP3K6	c.620T>G	Chr 1: 27690770	Missense	No data	[14]		No data	
MAP3K6	c.2837C>T	Chr 1: 27684750	Silent	No data	[14]		No data	Single nucleotide variant also in Canadian family, likely pathogenic
MAP3K6	c.2872C>A	Chr 1: 27684715	Missense	No data	[14]		No data	
MAP3K6	c.2544delC	Chr 1: 27685238 - 27685239	Nonsense	Portugese	[14]		1/115 cases	
INSR	c.3937 G>A	Chr 19: 7117279	Missense	Finland	[15]	Family study	1/1 family	
FBXO24	c.242G>C	Chr 7: 100187900	Missense	Finland	[15]		1/1 family	
DOT1L	c.3437C>T	Chr 19: 2223326	Missense	Finland	[15]		1/1 family	

Table 3 Comparison of hereditary cancer syndromes

Condition	Genetic pathology	Lifetime risk of gastric cancer	Histological subtype	Other clinical features
Hereditary diffuse gastric cancer	*CDH1* germline and other gene mutations	80%	Diffuse	Association with lobular breast cancer and cleft-lip malformations
Lynch syndrome	Mutations in mismatch repair genes	4.8% in *MLH1* carrier 9% in *MLH2* carrier[58]	Mainly intestinal-type	Lifetime risk of colon cancer 31%-38%, endometrial cancer 34% and ovarian cancer 20%[59]
Familial adenomatous polyposis	*APC* germline mutations	Population risk[60]	No data	Malignant extraintestinal tumours rare < 3% (thyroid, pancreas, medulloblastoma)[61]
Li-Fraumeni syndrome	*TP53* mutations	[1]4.9%[62]	No predominant subtype	Associated with wide range of early-onset cancers. Includes haematological and solid organ cancers: sarcomas, breast, brain, adrenal and lung cancers
Peutz-Jegher's syndrome	*STK11* mutations	29%[63]	No data	Characteristic mucocutaneous pigmentation commonly around mouth and nose High cumulative lifetime risk of any cancer (85%), most commonly colorectal (50%)[58]
Juvenile polyposis syndrome	*SMAD4* or *BMPR1A* mutations	[1]21%[64]	No data	Also at increased

[1]Frequency based on cross-sectional sample rather than lifetime risk from cohort study.

glycosylation[29] and is known to play a role in a variety of cancers[30]. There has also been a reported possibility of an association of early onset gastric cancer with *IL12RB1* mutation carriers[31] although this is mainly of the intestinal-type.

Somatic events

Guilford *et al*[10] has suggested HDGC develops from multiple foci of signet ring cell carcinomas (SRCC) in mutation carriers before 30 years of age. These SRCC, which have been termed "early HDGC"[32], develop after loss of the second *CDH1* allele *via* a 2nd-hit mechanism[33-36]. The same patient may present with distinct 2nd hit mechanisms in different lesions. Promoter methylation is the most common 2nd-hit mechanism in primary HDGC tumours although loss of heterozygosity was found to be the most prevalent in lymph node metastases[37].

Interestingly, other studies are starting to look at oncogenic pathways involved in metastatic progression in HDGC and have found one such candidate driver in a transforming growth factor beta receptor 2 loss-of-function mutation[38].

MANAGEMENT

Diagnosis

The identification of germline mutations in families fulfilling the criteria for HDGC relies on information from pathology reports from at least one proband. A report by Hebbard *et al*[39] on 23 patients who underwent prophylactic total gastrectomy showed 21 of them had evidence of diffuse/signet-ring carcinoma on final standardized pathological evaluation which was not picked up by preoperative endoscopic screening. Thus, for adequate pathological sampling, IGCLC recommends targeting any endoscopically visible lesions as well as random sampling of six biopsies for each of the following anatomical zones: antrum, transitional zone, body, fundus, cardia. This would give a minimum of 30 biopsies[11].

Treatment

Probands often present with advanced stage GC and

treatment consists of palliative chemotherapy (often taxanes, platinum agents or irinotecan), targeted radiotherapy and bypass surgery. While research looks into E-cadherin pathway regulators to increase chemosensitivity to epidermal growth factor receptor inhibitors and cytotoxics[40-42], there are currently no specific targeted therapies for diffuse GCs although there is an ongoing Phase I clinical trial studying everolimus in combination with chemotherapy[43].

As personalized therapy becomes increasingly prominent in cancer care, management of patients with HDGC should involve a multidisciplinary team of geneticists, surgeons and pathologists to address the following aspects of care: (1) genetic counselling and screening for both *CDH1* positive and negative patients. This should include a three-generation family pedigree, analysis of *CDH1*/other candidate gene mutation and translation into lifetime risks of diffuse GC and LBC[11]; and (2) discussion of prophylactic gastrectomy *vs* surveillance.

Guidelines for the clinical management of *CDH1* mutation carriers have been reviewed by the IGCLC (2010) and are outlined in clinical utility cards for HDGC[44]. Figure 1 summarises the management algorithm.

CDH1 missense mutation carriers

It is suggested that these individuals go on to have their mutations assessed for pathogenicity *via* functional *in-vitro* testing (aggregation and invasion assays) and *in-silico* models that have been developed[45]. These techniques have found a significant number of pathogenic missense variants and should be carried out by molecular diagnostic laboratories with appropriate expertise.

CDH1-negative individuals

Mutation screening in the research setting of HDGC families without *CDH1* mutations can be considered. Approaches needed would include high density single-nucleotide polymorphism (SNP) genotyping, non-parametric and parametric linkage analysis, whole exome sequencing as well as aforementioned pathogenicity assessments[14,15].

Surveillance

There is currently no reliable screening test for early diagnosis of diffuse GCs in mutation carriers. While IGCLC guidelines suggest annual endoscopic surveillance in specific settings, it should be known that direct visualization with endoscopy tends to detect lesions late in the disease process[46] and multiple random endoscopic samples often returns false negatives[39]. Other screening methods like chromoendoscopy and positron emission tomography have not been deemed to be consistently effective[47,48].

Prophylactic gastrectomy

Due to the lack of reliably sensitive surveillance methods, prophylactic total gastrectomy should be considered in the early 20s and is usually advised before age 40 for those carrying *CDH1* mutations. Some authors suggest consideration of gastrectomies in *CDH1* mutation carriers at an age 5 years younger than the youngest family member who developed gastric cancer[49].

There are currently no recommendations with regards to prophylactic gastrectomy in *CDH1*-negative individuals. Prospective studies evaluating prophylactic gastrectomy in HDGC have offered the surgery only to *CDH1* positive individuals[50], while a systematic retrospective review of 28 articles on prophylactic gastrectomy found a small sample of 11 *CDH1*-negative individuals who had undergone the gastrectomy before *CDH1* testing all had negative histopathology results for cancer[51].

Patients may refuse or decide to postpone the procedure due to young age, fertility concerns or fear of surgical complications. Fortunately, there have been reports of successful pregnancies post-prophylactic gastrectomy[52] and the youngest known carrier to date to undergo gastrectomy was 16 years of age[53].

ONGOING CHALLENGES

Risk stratification for CDH1-negative individuals

A significant proportion of HDGC families are likely to be *CDH1* negative. Further study to identify other genetic causes is needed before their risk and therefore management measures such as prophylactic gastrectomy can be assessed. As more cases of HDGC are identified, two lines of study are especially valuable. First, pathogenicity and penetrance of new germline mutations need to be documented to improve genetic counselling and decision-making. This is especially so for missense mutations. Second, prophylactic gastrectomy specimens provide material to identify molecular mechanisms that may predict progression from SRCC lesions to HDGC. In particular, elucidating epigenetic mechanisms, such as analysis of hypermethylation of cell cycle or DNA repair genes[54-57], may provide useful insights into possible environmental or pharmaceutical chemoprevention strategies.

Surveillance methods

Better surveillance methods could reduce morbidity by picking up target lesions earlier such that they are amenable to endoscopic therapies. While detection of diffuse GCs has proven difficult and surveillance frequency remains challenging, one paradigm to guide further research would be to assume that microfoci of SRCC will be present in all adult mutation carriers. Thus, rather than trying to detect all microfoci, the aim of surveillance should be geared towards detecting "high risk" SRCC. While this will require further elucidation of mechanisms of carcinogensis, it is plausible to imagine current surveillance methods, combined with genetic data, as a reliable alternative to prophylactic total

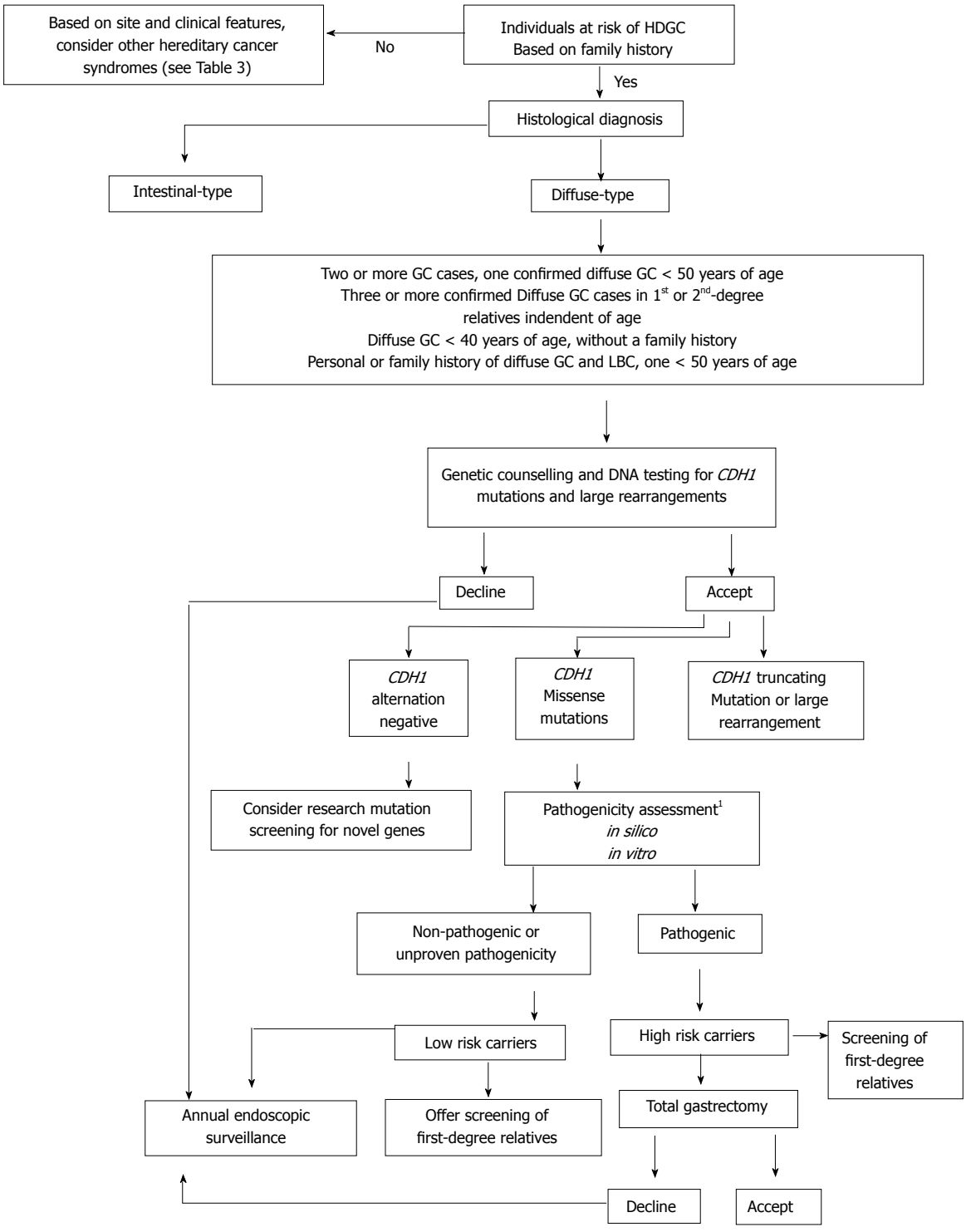

Figure 1 Clinical management of individuals suspected to have hereditary diffuse gastric cancer. Adapted from Pinheiro *et al*[9]. [1]Analyses recommended include: mutation frequency in healthy control population, co-segregation of mutation within pedigree, recurrence of mutation in independent families, in-silico predictions and *in vitro* functional assays[45,65-68].

gastrectomy.

CONCLUSION

While the incidence of HDGC remains low, it is an important clinical entity to recognize because of its high pathogenicity and penetrance. The IGCLC 2010 has outlined *CDH1* testing criteria and developed clinical utility gene cards to help clinicians manage such patients. Significant progress has been made in recent years and in future, testing of other genes is likely for *CDH1*-negative families. The mainstay of treatment for asymptomatic carriers of *CDH1* pathogenic mutations remains prophylactic total gastrectomy. However, it is hoped future research will lead to better risk stratification and surveillance methods to improve clinical

care for patients in terms of screening, prevention and treatment.

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ORIGINAL ARTICLE

Observational Study

Screening for hepatocellular carcinoma by Egyptian physicians

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Abstract

AIM: To assess the practice of Egyptian physicians in screening patients for hepatocellular carcinoma (HCC).

METHODS: The study included 154 physicians from all over Egypt caring for patients at risk for HCC. The study was based on a questionnaire with 20 items. Each questionnaire consisted of two parts: (1) personal information regarding the physician (name, age, specialty and type of health care setting); and (2) professional experience in the care of patients at risk for HCC development (screening, knowledge about the cause and natural course of liver diseases and HCC risk).

RESULTS: Sixty-eight percent of doctors with an MD degree, 48% of doctors with a master degree or a diploma and 40% of doctors with a Bachelor of Medicine, Bachelor of Surgery certificate considered the hepatitis C virus (HCV) genotype as risk factor for HCC development ($P < 0.05$). Ninety percent of physicians specialized in tropical medicine, internal medicine or gastroenterology and 67% of physicians in other specialties advise patients to undergo screening for HCV and hepatitis B virus infection as well as liver cirrhosis ($P < 0.05$). Eighty-six percent of doctors in University Hospitals and 69% of Ministry of Health (MOH) doctors consider HCV infection as the leading cause of HCC in Egypt ($P < 0.05$). Seventy-two percent of doctors with an MD degree, 55% of doctors with a master degree or a diploma, 56% of doctors with an MBBCH certificate, 74% of doctors in University Hospitals and 46% of MOH

hospital doctors consider abdominal ultrasonography as the most important investigation in HCC screening ($P < 0.05$). Sixty-five percent of physicians in tropical medicine, internal medicine or gastroenterology and 37% of physicians in other specialties recommend as HCC screening interval of 3 mo ($P < 0.05$). Seventy-one percent of doctors with an MD degree, 50% of doctors with a master degree or diploma and 60% of doctors with an MBBCH certificate follow the same recommendation.

CONCLUSION: In Egypt, physicians specialized in tropical medicine, internal medicine or gastroenterology with an MD degree and working in a University Hospital are best informed about HCC.

Key words: Hepatocellular carcinoma; Egyptian physicians; Screening; Hepatocellular carcinoma knowledge; Hepatocellular carcinoma management; Hepatocellular carcinoma diagnosis

Core tip: We aim to assess the practice of Egyptian physicians in screening patients for hepatocellular carcinoma (HCC). We included 154 Egyptian physicians caring for patients at risk for HCC, personal information and professional experience of them were analysed. Physicians specialized in tropical medicine, internal medicine or gastroenterology with an MD degree and working in a University Hospital are best informed about HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) considered being the sixth most prevalent cancer and the third most common cause of cancer leading to deaths worldwide[1]. Its annual incidence is increasing worldwide, ranging between 3% and 9% in patients with liver cirrhosis[2]. In Egypt, HCC was reported to develop in about 5% of patients with chronic liver disease[3].

Worldwide, hepatitis B virus (HBV) is considered the major risk factor for the progression of liver cirrhosis to HCC[4]. The relative risk to develop an HCC is estimated to be 100-200-fold higher in HBV-infected patients as compared to non-infected individuals[5]. Integration of HBV DNA into the host genome is considered to be the initiating event for HBV-induced carcinogenesis[6]. In this context, the HBx protein may inactivate the *p53* tumor suppressor gene, resulting in HCC development[7]. While the prevalence of HBV infection in Egypt has been decreasing during the last two decades[3], the prevalence of hepatitis C virus (HCV) infection has increased to an estimated 14% in the general population[8] and was associated with a rising HCC incidence. HCV seems to primarily play an indirect role in HCC development by promoting fibrosis and cirrhosis. However, HCV may also play a direct role in hepatic carcinogenesis through viral gene products inducing liver cell proliferation[9]. In general, promotion of cirrhosis development seems to be the common pathway by which several risk factors exert their carcinogenic effect[9].

Exposure to aflatoxin is an additional risk factor for HCC development through formation of DNA adducts in liver cells affecting the *p53* tumor suppressor gene[7].

As a result, the major hepatological/gastroenterological professional societies worldwide, including the American Association for Study of Liver Disease (AASLD), recommend screening for HCC in high risk patients[10]. Alpha-fetoprotein (AFP) levels and imaging techniques such as ultrasonography are the most common screening modalities used by physicians to detect early HCC[11]. The majority of HCCs are diagnosed in advanced stages, which carries a poor prognosis[12]. Recent curative therapeutic regimens and liver transplantation for early stage HCC encourage physicians to screen high-risk patients[13].

The aim of our study was to assess the practice of Egyptian physicians in screening patients for HCC.

MATERIALS AND METHODS

The study included 154 physicians from different hospitals allover Egypt who care for patients at risk for HCC development. The study included physicians with the following 4 specialties: general practitioners/family medicine, tropical medicine, internal medicine and gastroenterology. The types of health care settings in which the physicians were employed were: primary health care, Ministry of Health (MOH) general hospitals, University hospitals and private hospitals/clinics.

Questionnaire

We designed a 3-page questionnaire with 20 questions for Egyptian physicians to assess their practice in screening patients for HCCs. Each questionnaire consisted of two parts: (1) personal information regarding the physician (name, age, specialty and type of health care facility); and (2) professional experience with patients at risk for HCC development with respect to screening, knowledge about the cause and epidemiology of liver diseases, incl. HCC risk.

Questionnaire distribution

The questionnaires were distributed to Egyptian physicians by personal contact at professional conferences and during seminars. The questionnaires were collected immediately after completion. Doctors were also contacted by e-mail with the questionnaire attached and asked to return the completed questionnaire by

Table 1 Personal data of participating physicians

	n (154)	%
Age (yr)		
24-35	69	45
36-45	43	28
46-65	42	27
Sex		
Male	104	67.5
Female	50	32.5
Specialty		
GP	3	2
Tropical Medicine	78	50
Internal Medicine	48	31
Gastroenterology	4	3
Others	21	14
Highest qualification		
MBBCH	25	16
Msc	49	32
MD	69	45
Others	11	7
Clinical practice		
Primary Health Care	4	3
MOH	51	33
University Hospital	95	61
Private practice	4	3

MOH: Ministry of Health.

e-mail. It was also sent through the Gastrointestinal Club, a group in the Facebook facilitating scientific contacts.

Ethics and consent

The survey was approved by the Faculty's Ethics Committee. Further, permission was obtained from all department heads who had been assured that confidentiality would be maintained and ethical principles would be followed. Before distribution of the questionnaires, the aim of the survey was explained to the potential participants who were encouraged to participate without undue pressure.

Statistical analysis

The data from questionnaires were entered into spread sheets of Microsoft Excel before being transferred to the Statistical Package for Social Sciences (SPSS) software (SPSS Inc., Chicago, IL, United States) version 16 for Windows 7 (Microsoft Corp., Redmond, WA) to be analyzed.

RESULTS

The study included 154 physicians of different age groups, specializations and clinical settings. The aim of the study was to assess the physicians' attitude towards HCC screening, their knowledge regarding different aspects of HCC screening, including screening modalities, as well as awareness of published guidelines.

Personal data of participating physicians

As shown in Table 1, 45% of the physicians were aged between 24-35, 28% between 36-45 and 27% were between 46-65 years; 50% were specialized in tropical medicine, 31% in internal medicine, 3% in gastroenterology, 2% in general practice and 14% in other specialties (Table 1). Regarding their highest qualification 16% had Bachelor of Medicine, Bachelor of Surgery (MB BCh), 32% MSc, and 45% MD degree, and 7% another qualification (Table 1). Regarding their clinical setting 3% of the physicians worked in primary health care, 33% in MOH hospitals, 61% in University hospitals and 3% in private practice (Table 1).

Table 2 Relation of the physicians' age and knowledge of hepatocellular carcinoma epidemiology

	Age (yr)				*P* value
	< 45		⩾ 45		
	n	%	*n*	%	
Recommended HCC surveillance					
Chronic hepatitis B, C and liver cirrhosis	94	84	39	93	0.15
Positive family history	36	32	18	43	0.215
Everyone	19	17	3	7	0.121
Reduction of deaths from HCC by screening					0.419
< 30%	25	22	12	29	
⩾ 30%	87	787	30	71	
Risk factors for liver disease progression					
Age	49	448	14	33	0.242
Regular alcohol consumption	49	44	22	52	0.339
Gender	33	29	17	40	0.194
Obesity, DM	42	37	13	31	0.45
HCV genotype	54	48	32	76	0.002[a]
HBV-HCV co-infection	60	54	18	43	0.236
Leading cause of HCC in Egypt					0.11
HCV	93	83	30	71	
HBV	19	17	12	29	
Causes of death of HCC patients					0.096
Cancer	49	44	18	43	
Liver failure	34	302	19	45	
GI or variceal bleeding	29	25	5	12	

[a]$P < 0.05$ considered statistically significant. HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Knowledge of HCC epidemiology

Relation with physicians' age: Table 2 shows that 76% of doctors older than 45 years and 48% of doctors younger than 45 years think that the HCV genotype is a risk factor for progression of chronic hepatitis C to HCC ($P < 0.05$).

In both age groups there were otherwise no significant differences regarding the physicians' knowledge about HCC epidemiology, people who should undergo HCC surveillance or the number of deaths that can be prevented by adequate HCC screening.

Relation with physicians' specialty: There is significant difference between specialties with respect to patients who should be screened for HCC (Table 3): 90% of physicians in tropical medicine, internal medicine

Table 3 Relation between physicians' specialty and knowledge of hepatocellular carcinoma epidemiology

	Specialty				*P* value
	Specialty A[1]		Specialty B[2]		
	n	%	*n*	%	
People who should undergo HCC surveillance					
Chronic hepatitis B, C and liver cirrhosis	117	90	16	67	0.006[a]
Positive family history	51	39	3	12	0.112
Everyone	15	11	7	29	0.023[a]
Reduction of deaths from HCC by screening					0.903
< 30%	31	24	6	25	
⩾ 30%	99	76	18	75	
Risk factors for disease progression					
Age	54	41	9		0.712
Regular alcohol consumption	63	48	8	33	0.172
Gender	47	36	3	12	0.023[a]
Obesity, DM	50	38	5	21	0.098
HCV genotype	74	57	12	50	0.53
Co-infection	69	53	9	37	0.161
Most common cause of HCC					0.711
HCV	105	81	18	75	
HBV	25	19	6	25	
Cause of death of HCC patients					0.217
Cancer	59	45	8	33	
Liver failure	41	32	12	50	
GI or variceal bleeding	30	23	4	17	

[1]Specialty A (Tropical medicine, Internal medicine, Gastroenterology); [2]Specialty B (General practitioner, Radiology, General surgery). [a]$P < 0.05$ considered statistically significant. HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Table 4 Relation between physicians' qualification and knowledge of hepatocellular carcinoma epidemiology

	Highest qualification						*P* value
	MBBCH		Msc/diploma		MD		
	n	%	*n*	%	*n*	%	
People who should undergo HCC surveillance							
Chronic hepatitis B, C and liver cirrhosis	23	92	51	85	59	85	0.666
Positive family history	8	32	10	17	36	52	0.000[a]
Everyone	4	16	8	13	10	14	0.948
Reduction of deaths from HCC by screening							0.581
< 30%	8	32	14	23	15	22	
⩾ 30%	17	68	46	77	54	78	
Risk factors for progression of the disease							
Age	11	44	21	35	31	45	0.49
Regular alcohol consumption	10	40	26	43	35	51	0.562
Gender	4	16	13	22	33	48	0.001[a]
Obesity, DM	8	32	19	32	28	41	0.525
HCV genotype	10	40	29	48	47	68	0.017[a]
Co-infection	9	36	28	47	41	59	0.098
Leading cause of HCC							0.053
HCV	19	76	43	72	61	88	
HBV	6	24	17	28	8	12	
Cause of death of HCC patients							0.427
Cancer	12	48	25	42	30	43	
Liver failure	7	28	18	30	28	41	
GI or variceal bleeding	6	24	17	28	11	16	

[a]$P < 0.05$ considered statistically significant. HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

and gastroenterology consider patients with chronic HBV or HCV infection and/or liver cirrhosis at risk to develop an HCC as compared to 67% of physicians in other specialties, such as general physicians/family doctors, radiologists or general surgeons ($P < 0.05$). By comparison, 11% of physicians in tropical medicine, internal medicine and gastroenterology think that everyone should be screened for HCC as compared to 29% of general practioners. With respect to gender, 36% of physicians in tropical medicine, internal medicine and gastroenterology consider gender as a risk factor for HCC development compared to 12% of general practitioners ($P < 0.05$).

There were no significant differences with respect to other aspects, such as the number of deaths that can be prevented by HCC screening or the fact that HCC are the leading cause of tumor deaths in Egypt.

Relation with physicians' medical qualification: Table 4 shows that there is a significant difference in awareness regarding HCC risk factors depending on the qualification of the doctors: 52% of doctors with MD degree, 17% of doctors with a master degree or diploma and 32% of doctors with MB BCh think that patients with a family history of HCC should be screened for HCC ($P < 0.05$). There is also a significant difference in knowledge about the risk factors for disease progression depending on the qualification of the doctors: 68% of doctors with MD degree, 48% of doctors with a master degree or diploma and 40% of doctors with MB BCh think that the HCV genotype is a risk factor for progression of the disease; with respect to gender 48% of doctors with MD degree, 22% of doctors with a master degree or diploma and 16% of doctors with MB BCh are aware that gender is the risk factor for disease progression ($P < 0.05$).

There is no significant difference in awareness regarding other aspects, such as the number of deaths from HCC that can be prevented by appropriate screening or the most common cause of death of HCC patients in Egypt.

Relation with hospital setting: Table 5 shows that there is a significant difference in knowledge about HCC risk groups between doctors in different hospital settings: 46% of doctors working in University hospitals and 17% of MOH doctors think that patients with family history of HCC should undergo surveillance ($P < 0.05$). There is also a significant difference in knowledge about the risk factors for disease progression depending on the hospital setting of the doctors: 39% of doctors working in University hospitals and 22% of MOH doctors are aware that gender is the risk factor for disease

Table 5 Relation between hospital setting and knowledge of hepatocellular carcinoma epidemiology

	Type of hospital				*P* value
	University		MOH		
	n	%	*n*	%	
People who should undergo HCC surveillance					
Chronic hepatitis B, C and liver cirrhosis	79	83	54	91	0.141
Positive family history	44	46	10	17	0.000[a]
Everyone	17	18	5	8	0.104
Reduction of deaths from HCC by screening					0.749
< 30%	22	23	15	25	
⩾ 30%	73	77	44	75	
Risk factors for progression of the disease					
Age	43	45	20	34	0.163
Regular alcohol consumption	47	49	24	41	0.287
Gender	37	39	13	22	0.029[a]
Obesity, DM	37	39	18	30	0.288
HCV genotype	55	58	31	52	0.516
HBV-HCV co-infection	50	53	28	47	0.532
Leading cause of HCC					0.011[a]
HCV	82	86	41	70	
HBV	13	14	18	30	
Cause of death of HCC patients					0.493
Cancer	43	45	24	41	
Liver failure	34	36	19	32	
GI or variceal bleeding	18	19	16	27	

[a]*P* < 0.05 considered statistically significant. MOH: Ministry of Health; HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

progression. With respect to the cause of HCC in Egypt, 86% of doctors working in University hospitals and 69% of MOH doctors know that HCV is the leading cause of HCC in Egypt.

There is no significant difference in knowledge with respect to other aspects, such as of the number of deaths that can be prevented by appropriate screening and the most common cause of death in HCC patients.

Knowledge about screening modalities, educational resources and guidelines

Relation with doctors' age: Table 6 shows that there is significant difference in knowledge about the most important investigations for HCC screening, depending on the physicians' age: 58% of doctors < 45 years and 76% of doctors > 45 years of age think that ultrasound (US) is the most important investigation; 16% of doctors < 45 years and no doctor > 45 years think that computer tomography (CT) is the method of choice in HCC screening. Seventy-five percent of doctors < 45 years and 93% of doctors > 45 years think that treating HBV can reduce HCC incidence, while 25% of doctors < 45 years and 7% of doctors > 45 years do not think that treating of HBV can reduce HCC incidence (P < 0.05).

There is no significant difference in other aspects of HCC screening such as screening intervals in high risk groups, knowledge about the existence of guidelines for the management of HCC, the prediction of increased

Table 6 Relation between doctors' age and knowledge about screening modalities, educational resources and guidelines

	Age (yr)				*P* value
	< 45		⩾ 45		
	n	%	*n*	%	
Most important HCC screening					0.037[a]
Physical examination	2	2	1	3	
Alpha fetoprotein	27	24	9	21	
Ultrasound	65	58	32	76	
CT	18	16	0	0	
2nd most important HCC screening					0.175
Physical examination	2	2	0	0	
Alpha fetoprotein	55	49	16	38	
Ultrasound	17	15	4	10	
CT	36	32	22	52	
Angiography	2	2	0	0	
3rd most important HCC screening					0.585
Physical examination	3	3	2	5	
Alpha fetoprotein	21	19	13	31	
Ultrasound	14	12	3	7	
CT	55	49	18	43	
Angiography	8	7	3	7	
Laparoscopy	11	10	3	7	
Screening interval for high risk groups					0.212
3 mo	65	58	29	69	
6 mo or more	47	42	13	31	
HBV treatment reduces HCC incidence					0.014[a]
Yes	84	75	39	93	
No	28	25	3	7	
Familiar with guidelines					0.205
Yes	62	55	28	67	
No	50	45	14	33	
HCV RNA/ALT level are HCC risk factors					0.08
Yes	57	51	28	67	
No	55	49	14	33	

[a]*P* < 0.05 considered statistically significant. HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus; CT: Computer tomography.

HCC risk by elevated HCV RNA and ALT levels and the opinion regarding the second and third most important examinations in HCC screening.

Relation with physicians' medical specialty: Table 7 shows that there is a significant difference in opinion between different medical specialties with respect to the optimal screening interval in high risk groups (P < 0.05): 65% of physicians in tropical medicine, internal medicine and gastroenterology think that the optimal screening interval is 3 mo while only 38% of physicians in other specialties think so; 35% of physicians in tropical medicine, internal medicine and gastroenterology think that the screening interval in high risk groups should be 6 mo or more; 62% of physicians in other specialties share this opinion.

There were no significant differences with respect to other aspects, such as the most important examination in HCC screening, the second and third most important

Table 7 Relation between medical specialty and knowledge about screening modalities, educational resources and guideline

	Specialty A		Specialty B		*P* value
	n	%	*n*	%	
Most important screening for HCC					0.154
Physical examination	2	2	1	4	
Alpha fetoprotein	28	21	8	33	
Ultrasound	82	63	15	63	
CT	18	14	0	0	
2nd most important screening for HCC					0.238
Physical examination	2	2	0	0	
Alpha fetoprotein	64	49	7	29	
Ultrasound	16	12	5	21	
CT	47	36	11	46	
Angiography	1	1	1	4	
3rd most important screening for HCC					0.383
Physical examination	3	2	2	9	
Alpha fetoprotein	27	21	7	29	
Ultrasound	16	12	1	4	
CT	61	47	12	50	
Angiography	10	8	1	4	
Laparoscopy	13	10	1	4	
Screening interval for high risk group					0.010[a]
Every 3 mo	85	65	9	38	
6 mo or more	45	35	15	62	
HBV treatment reduces HCC incidence					0.139
Yes	107	82	16	67	
No	23	18	8	33	
Guidelines in management of HCC					0.991
Yes	76	58	14	58	
No	54	42	10	42	
HCV RNA/ALT risk factors for HCC					0.147
Yes	75	58	10	42	
No	55	42	14	58	

[a]*P* < 0.05 considered statistically significant. HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus; CT: Computer tomography.

Table 8 Relation between highest qualification and knowledge about screening modalities, educational resources and guidelines

	Highest qualification						*P* value
	MBBCH		Msc/ diploma		MD		
	n	%	*n*	%	*n*	%	
Most important screening for HCC							0.023[a]
Physical examination	0	0	1	2	2	3	
Alpha fetoprotein	7	28	13	22	16	23	
Ultrasound	14	56	33	55	50	73	
CT	4	16	13	22	1	1	
2nd most important examination in screening of HCC							0.585
Physical examination	1	4	1	2	0	0	
Alpha fetoprotein	12	48	26	43	33	48	
Ultrasound	2	8	11	18	8	12	
CT	9	36	22	37	27	39	
Angiography	1	4	0	0	1	1	
3rd most important screening for HCC							0.004[a]
Physical examination	1	4	3	5	1	1	
Alpha fetoprotein	3	12	14	23	17	25	
Ultrasound	6	24	2	3	9	13	
CT	12	48	25	42	36	52	
Angiography	1	4	4	7	6	9	
Laparoscopy	2	8	12	20	0	0	
Screening interval for high risk group							0.050[a]
Every 3 mo	15	60	30	50	49	71	
6 mo or more	10	40	30	50	20	29	
HBV treatment reduces HCC incidence							0.441
Yes	20	80	45	75	58	84	
No	5	20	15	25	11	16	
Guidelines in management of HCC							0.000[a]
Yes	13	52	20	33	57	83	
No	12	48	40	67	12	17	
HCV RNA/ALT risk factors for HCC							0.368
Yes	14	56	37	62	34	49	
No	11	44	23	38	35	51	

[a]*P* < 0.05 considered statistically significant. HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; CT: Computer tomography.

examination in HCC screening, the reduction of the HCC incidence by treatment of HBV infection, the existence of guidelines for the management of HCC and the predictive value of elevated HCV RNA and ALT levels for HCC development.

Relation with physicians' highest qualification: Table 8 shows that there is a significant difference of opinion between doctors with different qualifications with respect to the most important investigation in HCC screening ($P < 0.05$): 73% of doctors with MD degree, 55% of doctors with a master degree and diploma and 56% of doctors with MBBCH think that US is the most important screening tool to detect HCC. There is also a significant difference in opinion with respect to the third most important investigation in screening for HCC ($P < 0.05$) as well as with respect to the optimal screening interval ($P < 0.05$): 60% of doctors with a MB BCh, 50% of doctors with a master degree and diploma and 71% of doctors with MD degree think that the screening interval for high risk group should be 3 mo, while 40% of doctors with MB BCh, 50% of doctors with a master degree or diploma and 29% with MD degree think that the screening interval for high risk groups should be 6 mo. Fifty-two percent of doctors with MB BCh, 33% of doctors with a master degree or diploma and 83% of doctors with MD degree know guidelines for the management of HCC patients, while 48% of doctors with MB BCh, 67% of doctors with a master degree and diploma and 17% of doctors with MD used no guidelines for the management of HCC ($P < 0.05$).

There were no significant differences with respect to other aspects, such as the reduction of HCC incidence by treatment of HBV infection and the predictive value of elevated HCV RNA and ALT levels for HCC

Table 9 Relation between health care setting and knowledge about screening modalities, educational resources and guidelines

	Health care setting				*P* value
	University		MOH		
	n	%	*n*	%	
Most important screening for HCC					0.000[a]
0.000[a]	3	3	0	0	
Alpha fetoprotein	19	20	17	29	
Ultrasound	70	74	27	46	
CT	3	3	15	25	
2nd most important screening for HCC					0.799
Physical examination	1	1	1	2	
Alpha fetoprotein	47	49	24	40	
Ultrasound	11	12	10	17	
CT	35	37	23	39	
Angiography	1	1	1	2	
3rd most important screening for HCC					0.001[a]
Physical examination	2	2	3	5	
Alpha fetoprotein	23	24	11	19	
Ultrasound	10	11	7	12	
CT	52	55	21	36	
Angiography	7	8	4	7	
Laparoscopy	1	1	13	22	
Screening interval for high risk group					0.173
Every 3 mo	62	65	32	54	
6 mo or more	33	35	27	46	
HBV treatment reduces HCC incidence					0.011[a]
Yes	82	86	41	69	
No	13	14	18	31	
Guidelines in management of HCC					0.000[a]
Yes	73	77	17	29	
No	22	23	42	71	
HCV RNA/ALT are risk factors for HCC					0.139
Yes	48	51	37	63	
No	47	49	22	37	

[a]*P* < 0.05 considered statistically significant. HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus; MOH: Ministry of Health; CT: Computer tomography.

development.

Relation with hospital setting: Table 9 shows that there is a difference in opinion between doctors in different hospital settings with respect to the most important investigation in screening for HCCs ($P < 0.05$): 74% of doctors working in University Hospitals and 46% of MOH doctors think that US is the most important investigation in screening of HCC; by comparison, only 3% of doctors working in University hospitals and 25% of MOH doctors consider CT as the most important investigation in screening for HCC ($P < 0.05$); 55% of doctors working in University hospitals and 36% of MOH doctors think that CT is the third most important investigation in screening for HCC. Eighty-six percent of doctors working in University hospitals and 69% of MOH doctors think that treatment of chronic HBV infection can reduce HCC incidence while 14% of University doctors and 31% of MOH doctors do not think so ($P < 0.05$). Further, 77% of doctors working in University hospitals and 29% of MOH doctors use guidelines for the management of HCC, while 23% of doctors working in University hospitals and 71% of MOH doctors do not ($P < 0.05$).

Table 10 Relation between physicians' age and hepatocellular carcinoma screening

	Age (yr)				*P* value
	< 45		⩾ 45		
	n	%	*n*	%	
HCC surveillance					0.013
Yes	20	18	15	35	
No	92	82	27	65	
Screening of patients with HCV cirrhosis and SVR					0.661
Yes	94	4	34	81	
No	18	16	8	19	
Screening of patients with hemochromatosis					0.11
Yes	73	65	33	79	
No	39	35	9	21	
No. of incidental HCCs/month					0.087
0	34	30	7	17	
1 or more	78	0	35	83	
No. of HCCs/month					0.193
0	33	29	8	19	
1 or more	79	71	0.000[a]	81	

[a]*P* < 0.05 considered statistically significant. HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HCV: Hepatitis C virus; SVR: Sustained virological response.

There is no significant difference with respect to other aspects, such as the 3rd most important examination in HCC screening, the screening interval for high risk group and the predictive value of elevated HCV RNA and ALT for the individual HCC risk.

Physicians' practice and attitude towards HCC

Relation with physicians' age: Table 10 shows that there is a significant difference of opinion regarding HCC surveillance with respect to the physicians' age ($P < 0.05$): 18% of doctors < 45 years and 35% of doctors > 45 years screen of liver cancer while 82% of doctors < 45 years and 65% of doctors > 45 years do not.

There is no significant difference in opinion regarding other aspects, such as the clinical care of patients with HCV cirrhosis who responded to antiviral therapy or hemochromatosis as well as with respect to number of HCC discovered accidentally per month and the number of HCC patients that physicians care for.

Relation with physicians' medical specialty: Table 11 shows that there is a significant difference in the care for patients with hemochromatosis depending on the physicians' medical specialty ($P < 0.05$): 72% of physicians in tropical medicine, internal medicine and gastroenterology and 50% in other specialties screen patients of hemochromatosis for HCCs while 28% of physicians in tropical medicine, internal medicine and gastroenterology and 50% of general practitioners do not.

Table 11 Hepatocellular carcinoma screening depending on medical specialty

	Specialty				*P* value
	Specialty A[1]		Specialty B[2]		
	n	%	*n*	%	
HCC surveillance					0.193
Yes	32	25	3	13	
No	98	75	21	87	
Screening of patients with HCV cirrhosis and SVR					0.79
Yes	109	84	19	79	
No	21	16	5	21	
Screening of patients with hemochromatosis					0.030[a]
Yes	94	72.3	12	50	
No	36	27.7	12	50	
No. of incidental HCCs/month					0.418
0	33	25	8	33	
1 or more	97	75	16	67	
No. of HCCs/month					0.759
0	34	26	7	29	
1 or more	96	74	17	71	

[a]$P < 0.05$ considered statistically significant. [1]Specialty A (Tropical medicine, Internal medicine, Gastroenterology); [2]Specialty B (General practitioner, Radiology, General surgery). HCC: Hepatocellular carcinoma; SVR: Sustained virological response; HCV: Hepatitis C virus.

Table 12 Hepatocellular carcinoma screening depending on highest medical qualification

	Highest qualification						*P* value
	MBBCH		Msc/ diploma		MD		
	n	%	*n*	%	*n*	%	
HCC surveillance							0.0423
Yes	5	20	10	17	17	25	
No	20	80	50	83	52	75	
Screening of patients with HCV cirrhosis and SVR							0.638
Yes	20	80	52	87	56	81	
No	5	20	8	13	13	19	
Screening of patients with hemochromatosis							0.012[a]
Yes	15	60	35	58	56	81	
No	10	40	25	42	13	19	
No. of incidental HCCs/month							0.000[a]
0	11	44	24	40	6	9	
1 or more	14	56	36	60	63	91	
No. of HCC patients							0.000[a]
0	9	36	29	48	3	4	
1 or more	16	64	31	52	66	96	

[a]$P < 0.05$ considered statistically significant. HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; SVR: Sustained virological response.

There is no significant difference with respect to other aspects, such as HCC screening of patients with HCV cirrhosis with sustained virological response (SVR), the number of HCC cases discovered accidentally per month and the number of HCC patients the physicians care for.

Relation with physicians' highest qualification: Table 12 shows that there is a significant difference with respect to HCC surveillance depending on the highest medical qualification ($P < 0.05$): 20% of doctors with MB BCh and 17% of doctors with a master degree or diploma and 25% of doctors with MD degree screen all patients for HCC while 80% of MB BCh doctors, 83% of Msc doctors and 75% of doctors with MD degree do not. Similarly, 60% of MB BCh doctors, 58% of Msc/diploma doctors and 81% of doctors with MD degree screen patients of hemochromatosis for HCCs ($P < 0.05$), while 40% of MB BCh doctors, 42% of Msc/diploma doctors and 19% of doctors with MD degree do not. There is also a significant difference in the accidental HCC detection per month between the doctors with different medical highest qualification ($P < 0.05$): 44% of MB BCh doctors, 40% of Msc/diploma doctors and 9% of doctors with a MD degree detect less than one HCC per month while 56% of MB BCh doctors, 60% of Msc/diploma doctors and 91% of doctors with a MD degree detect one or more than one HCC per month. Further, there is significant difference with respect to the number of HCC patients cared for by the physician depending on his/her highest medical qualification ($P < 0.05$): 36% of MB BCh doctors, 48% of doctors with Msc/diploma and 4% of doctors with MD degree do not have any HCC patient while 64% of MB BCh doctors, 52% of doctors with Msc/diploma and 96% of doctors with MD degree care for one or more HCC patients.

Relation with hospital setting: Table 13 shows a significant difference in the number of accidentally discovered HCC per month between the physicians' hospital setting ($P < 0.05$): 10% of doctors working in University Hospitals and 54% of MOH doctors do not discover any HCC per month while 90% of doctors working in University hospitals and 46% of MOH doctors discover one or more cases per month. There is also a significant difference with respect to the number of HCC patients that doctors care for depending on the physicians' hospital setting ($P < 0.05$): 9% of doctors working in University hospitals and 54% of MOH doctors do not care for any HCC patient while 91% of doctors working in University hospitals and 46% of MOH doctors see one or more HCC patient in their practice.

DISCUSSION

Knowledge of HCC epidemiology

The results from the questionnaire show that the majority of doctors think that individuals at risk requiring screening for HCC are patients with chronic hepatitis B or C and patients with liver cirrhosis, consistent with the Practice Guidelines from the American Association of the Study of Liver Diseases (AASLD) from 2005 and from the European Association for the Study of the Liver (EASL) from 2001 which recommended HCC surveillance for patients at high risk of developing HCC[8]. Patients at high risk are those with liver cirrhosis and those with chronic HBV infection irrespective of

Table 13 Hepatocellular carcinoma C screening depending on health care setting

	Health care setting				*P* value
	University hospital		MOH		
	n	%	*n*	%	
HCC surveillance					0.178
Yes	25	26	10	17	
No	70	74	49	83	
Screening of patients with HCV cirrhosis and SVR					0.386
Yes	77	81	51	86	
No	18	19	8	14	
Screening of patients with hemochromatosis					0.196
Yes	69	73	37	63	
No	26	27	22	37	
No. of incidental HCCs/month					0.000[a]
0	10	10	31	53	
1 or more	85	90	28	47	
No. of HCCs/month					0.000[a]
0	9	10	32	54	
1 or more	86	90	27	46	

[a]$P < 0.05$ considered statistically significant. HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; SVR: Sustained virological response; MOH: Ministry of Health.

cirrhosis[14,15].

The Cairo Liver Center evaluated in a retrospective study between 2003 and 2008 the effect of surveillance on the early detection of HCC in patients with liver cirrhosis. This cohort was compared to non-screened cirrhosis patients who presented with first symptoms or incidentally. The study clearly showed that surveillance doubled the chance of HCC detection at an early Barcelona Liver Cancer Center (BCLC) stage with a chance for successful loco-regional ablation or liver transplantation. Therefore, the implementation of HCC surveillance in Egypt is recommended[16].

Chronic hepatitis B infection accounts for about 50% of all HCC cases worldwide. At the same time, in approx. Forty percent of patients with chronic HBV infection HCCs develops in a non-cirrhotic liver. Therefore, HCC screening is recommended in all patients of chronic HBV infection[17]. In Egypt, the increasing HCC incidence is due to the high prevalence of HCV infection[10], estimated to be around 14% in the general population[8].

The questionnaire results show that most of doctors agree that more than 30% of deaths can be prevented by HCC screening, consistent with results from a multiple-choice survey study in the United States[18], based on the AASLD Practice Guidelines. The questionnaire asked for an estimate of the proportion of deaths from HCC that can currently be prevented by suitable screening. Most gastroenterologists stated that appropriate screening and surveillance could prevent 20%-50% of deaths[18].

In the United States there was no significant difference of opinion based on the physicians' age, specialty, highest qualification or hospital setting. The questionnaire results indicated that most doctors' know that co-infection, gender, HCV genotype and obesity are risk factors for progression of the liver disease to HCC. This is in line with the data of Crockett *et al*[19] demonstrating that HBV-HCV co-infection is a predictive factor for HCC development. The contribution of the gender to the progression to HCC has also been shown by Buch *et al*[20], demonstrating that the natural history of HCC is different between men and women.

Our results show that the majority of doctors consider chronic HCV infection as the leading cause of HCC in Egypt, reflecting the high prevalence of HCV infection in the general population of around 14%[8] that is responsible for to the increasing incidence of HCCs in Egypt[10].

Our results further show that doctors consider cancer as the main cause of death in HCC patients, followed by decompensated liver cirrhosis and its complications such as bleeding from varices in other HCC patients. This is consistent with the findings of Couto *et al*[21], demonstrating that 57% of patients with unresectable HCC died from cancer progression while 43% died from complications of liver cirrhosis, including sepsis, GI bleeding and renal failure.

Knowledge of screening modalities, educational resources and guidelines

Our questionnaire revealed that 74% of University doctors and 46% of MOH doctors consider US as the most important HCC screening test, consistent with many studies in the United States. This is based on its adequate sensitivity, specificity, its low cost, non-invasive character and wide availability. The effectiveness of US screening for HCCs in the United States depended on the screening frequency, the experience of the examiner and the nature of the patients' liver disease. The sensitivity of US for HCC detection was variable and ranged between 35% and 84%, depending on the expertise of the operator as well as on the US equipment[22].

AFP alone as screening test is no longer considered adequate for HCC screening and surveillance by AASLD and EASL guidelines due to the high rate of false-positive and false-negative results in patients with chronic liver disease. Nevertheless, AFP alone may be used if US is not available[8].

Asked about the second and third choice of screening tests, some doctors favor AFP while others favor CT as the second choice for HCC screening. While CT is an attractive imaging modality for HCC screening because it can detect lesions in cirrhotic livers, allows lesion characterization and contributes to clinical staging, it is expensive and its use as screening test is difficult, especially in countries with limited resources and high HCC prevalence, such as Egypt.

Cost-effectiveness studies of HCC screening revealed that screening European patients with Child-Pugh class A cirrhosis using serum AFP and US every 6 mo costs about 74000 U$ for each HCC detected, while CT alone

every 6 mo costs about 101000U$[23].

With respect to the screening interval in high risk patients our study showed that most doctors consider 3 mo as optimal while some consider 6 or more months as adequate. The 6 mo screening interval for high risk groups has been adopted by many organizations, such as the AASLD, the EASL, the APASL (Asian Pacific Association for the Study of the Liver) and the NCCN (National Comprehensive Cancer Network). The recommendation of the screening interval of 3 mo is based on the estimate that the tumors > 1 cm in diameter may double every 2 mo[24].

With respect to the physicians' age, our study revealed that 93% of doctors older than 45 years and 75% of doctor younger than 45 years think that treatment of HBV infection can reduce the HCC incidence in Egypt, similar to the study of Lok *et al*[25].

It is known that HBV infection is oncogenic, resulting in HCC development also in non-cirrhotic livers. The relative HCC risk of HBV carriers is estimated to be 100-200-fold higher than that of non-carriers[5].

Our questionnaire results show in addition that 93% of doctors' older than 45 years and 75% of doctors younger than 45 years use guidelines in the management of HCC patients while 17% of doctors older 45 and 25% of doctors younger than 45 years do not. The significant difference in the use of guidelines by physicians of different age may be due to the following reasons: most of the older doctors hold a higher medical degree than younger physicians. Further, older doctors had more opportunities to attend medical conferences to update their knowledge. Further, some of them are professors teaching their students the most advanced medical knowledge. The questionnaire results further show that about 71% of doctors in MOH do not know about guidelines for the management of HCC. This may be due to the limited interest of managers and division heads in these hospitals to adapt existing protocols or guidelines appropriate for Egypt as well as the Egyptian government considering other endemic diseases of higher priority with respect to guidelines and screening programs.

Physicians' practice and knowledge about HCC

The questionnaire results clearly show that the majority of doctors do not implement or recommend HCC surveillance according to international guidelines. This may be due to limited information about the benefits and importance of screening programs that allow detecting HCCs at an early, potentially curable stage, resulting in improved patient survival. It also may be due to the unawareness of the Egyptian Ministry of Health and government about the importance of HCC screening among high risk groups which overall my save money, last but not least money that must be spent for the palliative care for HCC patients.

Screening for HCC in Egypt depends on the specialty and qualification of physicians' with general practitioners and family doctors having the lowest rate of practical implementation of HCC screening compared to other doctors. This may be due to the lack of facilities for HCC screening in primary care settings and the limited knowledge of these doctors about the importance of HCC screening among high risk group and about epidemiology of HCCs, being the second most frequent cause of cancer death in Egypt after bladder cancer.

The questionnaire results demonstrate that most doctors screen patients with liver cirrhosis due to chronic HCV infection who responded to antiviral treatment, consistent with a study showing that these patients should still undergo surveillance[26]. A more recent study by Singal *et al*[27] showed that patients with cirrhosis and a SVR had a relative risk for HCC of 0.35 compared to non-responders, resulting in HCC development in 5% of patients with a SVR, warranting regular post-treatment surveillance.

Finally, the answers to the questionnaire show that about 70% of doctors identified one or more HCCs per month. Further, 94% of doctors feel that the HCC incidence in Egypt is increasing while 3% are not sure. In fact, in Egypt the HCC incidence (10-120 cases per 100000 population and year), has nearly doubled from 4.0% in 1993 to 7.2% in 2002 among patients with chronic liver disease[16].

In Egypt, physicians specialized in tropical medicine, internal medicine or gastroenterology, older than 45 years, having MD degree and working in University hospitals are better informed about the HCC epidemiology, the appropriate screening modalities, educational resources and practice guidelines than physicians with other specialties.

COMMENTS

Background

In Egypt, hepatocellular carcinoma (HCC) was reported to develop in about 5% of patients with chronic liver disease. The major hepatological/gastroenterological professional societies worldwide, including the American Association for Study of Liver Disease, recommend screening for HCC in high risk patients. The majority of HCCs are diagnosed in advanced stages, which carries a poor prognosis. Recent curative therapeutic regimens and liver transplantation for early stage HCC encourage physicians to screen high-risk patients. The aim of this study was to assess the practice of Egyptian physicians in screening patients for HCC.

Research frontiers

Screening of HCC is important for early detection and treatment. The study is observational questioner study among Egyptian physicians to assess their knowledge in HCC screening, diagnosis, treatment, and recent guidelines.

Innovations and breakthroughs

The difference to other related or similar studies is that their study conducted among Egyptian physician.

Applications

The study shows the deficient HCC knowledge among Egyptian physicians. It also conclude that physicians with MD degree and those who work in university hospitals having better knowledge than other. Distribution of recent guidelines among physicians is recommended to improve their knowledge.

Peer-review

The manuscript is an interesting and very important study of Egyptian physicians' awareness and screening for HCC.

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Contents

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EDITORIAL

TOPIC HIGHLIGHT

CASE REPORT

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EDITORIAL

Targeted therapies for pancreatic adenocarcinoma: Where do we stand, how far can we go?

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Abstract

Pancreatic adenocarcinoma (usually referred to as pancreatic cancer) is a highly lethal and aggressive malignancy with a disease-related mortality almost equaling its incidence, and one of the most challenging cancers to treat. The notorious resistance of pancreatic cancer not only to conventional cytotoxic therapies but also to almost all targeted agents developed to date, continues to puzzle the oncological community and represents one of the biggest hurdles to reducing the death toll from this ominous disease. This editorial highlights the most important recent advances in preclinical and clinical research, with regards to targeted therapeutics for pancreatic cancer, outlines current challenges and provides an overview of potential future perspectives in this rapidly evolving field.

Key words: Clinical; Cytotoxic chemotherapy; Pancreatic cancer; Preclinical; Targeted agents

Core tip: Expansion of our knowledge regarding the molecular basis of pancreatic cancer has facilitated the development of a significant number of innovative targeted therapies for this lethal disease. Almost all these agents have, nevertheless, failed to produce statistically significant survival benefits when tested in clinical trial settings; therefore, successful clinical translation of preclinical advancements in pancreatic cancer research has yet to be materialized. Future treatment options might include multi-targeted and individualized molecular therapies, ideally guided by patient-specific genomic data, in combination with conventional cytotoxic or other regimens.

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INTRODUCTION

Despite recent advances in our understanding of the molecular mechanisms involved in the development and progression of pancreatic adenocarcinoma and an abundance of preclinical data suggesting the potential value of several targeted agents in treatment of this lethal disease, pancreatic cancer statistics remain grim and nearly the same as they were almost 30 years ago[1-3]. Pancreatic adenocarcinoma - usually referred to as "pancreatic cancer" - currently ranks as the fourth most frequent cause of cancer-related death among males and the fifth among females in the Western world, and is sadly expected to rise to the second leading position within the next decade[3,4]. Median survival is 4 to 6 mo following diagnosis while long term (5-year) survival rates do not exceed 4%-5%, for all stages combined[5]. The only treatment option with a curative potential is surgery, but less than 20% of patients are eligible for this approach, while the survival rates are poor (25%-30%) even among those with localized node-negative disease undergoing complete surgical resection and adjuvant chemotherapy[6].

This dismal clinical record inevitably leads to the following questions: Why have we failed thus far to reduce the death toll from this lethal disease? And, most importantly, what can we do to widen the range of available treatment options and improve their clinical effectiveness?

PRECLINICAL AND CLINICAL DATA: DISCREPANCY PREVAILS

In the preclinical arena of pancreatic cancer research the picture is much rosier; a significant and rather rapidly expanding number of different targeted agents have shown considerable efficacy in controlling growth of human pancreatic cancer cells, both *in vitro* and *in vivo*, and prolonging survival of pancreatic cancer models, as summarized in recent reviews on this topic[5-11]. This rather extensive armamentarium includes, among others, inhibitors of epidermal growth factor receptor (EGFR)[12,13], human epidermal growth factor receptor 2 (HER2)[14,15], vascular endothelial growth factor (VEGF) and VEGF receptors[16], insulin-like growth factor receptor[17-19], KRAS and its downstream effectors (mainly mitogen-activated protein kinase)[20,21], the developmental Wnt, Hedgehog and Notch signaling pathways[22-24], as well as reagents targeting the tumor extracellular matrix/stromal microenvironment or molecules overexpressed in the surface of pancreatic cancer cells (*i.e.,* mesothelin, carcinoembryonic antigen, epithelial cell adhesion molecule, MUC1)[25-29]. Dual-agent and multi-kinase molecular targeting represent additional exciting therapeutic possibilities and are gaining increasing research attention and popularity[30-34]. Alternative approaches, such as targeting the cellular process of autophagy - which plays a key role in the development and progression of malignancy or combined targeting of oncogene-driven signaling pathways and critical energy sources (such as mitochondrial respiration) of the subpopulation of dormant tumor cells surviving oncogene ablation, have also been studied as potential treatment options in pancreatic cancer, but are still in their infancy[7,35,36]. Interestingly, in accordance with increasing data suggesting potential preventive and therapeutic effects of aspirin and non-steroidal inflammatory drugs in gastrointestinal cancers, particularly colorectal cancer[37,38], aspirin is being explored as a targeted therapeutic agent for pancreatic cancer as well[39,40]. As shown in recent preclinical studies, aspirin, either alone or in combination with the antidiabetic drug metformin, may inhibit pancreatic cancer cell growth, counteract desmoplasia and cancer stem cell features and enhance the therapeutic efficacy of cytotoxic agents-such as gemcitabine- in pancreatic cancer by sensitizing pancreatic cancer cells to chemotherapy-mediated cytotoxicity[41-43].

Modified cytotoxic agents, mainly including nab-paclitaxel (paclitaxel conjugated with albumin nano-particles) or other nanovector-based anticancer drugs, such as cationic liposome encapsulated paclitaxel (EndoTAGTM-1) or liposomal doxorubicin, cisplatin and irinotecan, have been recently developed using sophisticated nanotechnology and tested in preclinical studies of pancreatic cancer, with some encouraging results[7,44-49]. These selective drug formulations offer the advantage of improved drug delivery to the tumor tissue and selective targeting *via* binding to tumor-associated receptors or macromolecules, thus positively modulating the pharmacokinetics and therapeutic index of cytotoxic chemotherapy[44]. Nab-paclitaxel, in particular, can bind to SPARC (secreted protein acid and rich in cysteine), an extracellular matrix protein which is frequently overexpressed in pancreatic adenocarcinomas[10,50,51], and, presumably, result in depletion of desmoplastic tumor stroma and an increase in vascularization, thus enhancing transvascular transport and delivery of cytotoxic agents to tumor cells[52].

The overwhelming majority of the abovementioned targeted therapies have, nevertheless, failed to demonstrate any statistically significant efficacy in clinical trials of pancreatic cancer patients; the EGFR and VEGF monoclonal antibodies cetuximab and bevacizumab, respectively, and the multikinase inhibitor sorafenib are representative examples of once-promising targeted agents who failed to produce a statistically significant improvement of survival when used in combination with gemcitabine *vs* gemcitabine alone in phase III randomized trials[53-55]. Hence, successful translation of our otherwise encouraging preclinical achievements into tangible clinical benefit remains an elusive goal. Two notable exceptions, though, leave some room for optimism. Erlotinib, an EGFR tyrosine kinase inhibitor which was United States Food and Drug Administration (FDA)-approved in 2007 for the treatment of advanced pancreatic cancer, is the first targeted agent which

succeeded in producing a significant-albeit modest-survival benefit when administered as an adjunct to gemcitabine, especially among patients experiencing erlotinib-induced skin rash[7,56]; still, given the marginal effect of erlotinib on survival and its unclear therapeutic value in localized, resectable disease this drug has yet to be widely adopted as standard of care in routine clinical practice[8,10]. Based on the results of the recent phase Ⅲ Metastatic Pancreatic Adenocarcinoma Clinical Trial[57] of nab-paclitaxel and gemcitabine combination *vs* gemcitabine alone in 861 patients with metastatic pancreatic cancer, showing a statistically significant survival benefit (as regards overall, progression-free and 1-year survival) in the combinatorial arm, nab-paclitaxel was also approved by the FDA in 2013 to be administered in combination with gemcitabine as first-line therapy for metastatic pancreatic cancer.

CONCLUSION

Considering all available evidence, as summarized above, we should first acknowledge that, although some revolutionary progress has indeed been achieved on the theoretical front, preclinical enthusiasm has been severely tempered by clinical disappointment. The reasons behind this discrepancy remain largely unknown and can only be speculated upon at this point. Resistance of pancreatic cancer to anticancer drugs, including both standard cytotoxic and novel targeted agents, is often attributed to the abundant, dense, fibroinflammatory stroma surrounding pancreatic tumor tissue, which is believed to function as a barrier to efficient delivery of drug formulations to their target tumor cells by restricting blood supply and limiting diffusion of large molecules[10,58,59]. The high genetic heterogeneity and complexity of pancreatic cancer may also explain why targeting a specific mutation in a tumor containing 63 genetic alterations on average -as shown by previous genomic studies[22,60] - or "randomly combining drugs in the hope of achieving a better outcome in an unselected patient population"[10], may be doomed to fail.

Hopefully, the results of ongoing clinical trials on current and emerging targeted therapeutics, including, among others, the anti-EGFR and anti-HER2/neu monoclonal antibodies nimotuzumab (NCT02395016) and trastuzumab (NCT01204372), respectively, the hedgehog inhibitors vismodegib (NCT01195415) and LDE225 (NCT01485744) and agents targeting the Notch pathway, such as the gamma-secretase inhibitor MK-0752 (NCT01098344), may help bridge the gap between preclinical and clinical outcomes. The increasing advances in structural and functional genomics are also expected to further elucidate the key molecular events underlying pancreatic tumorigenesis and identify additional targets for novel agents. Based on data derived from global genomic analyses of pancreatic tumors, previous authors have suggested that agents broadly targeting downstream mediators of critical physiologic functions (such as neo-angiogenesis or cell cycle alterations) may be preferable to agents targeting specific mutated genes[60]. Most importantly, personalized genomic medicine, utilizing patient-specific genomic data for guidance of treatment selection in each individual patient, may not only significantly enhance the clinical efficacy of molecular targeted therapy but also reduce the burden of unnecessary - and potentially harmful-drugs.

As previously commented by Kleger *et al*[7], in a recent review article critically discussing current and future targeted therapies for pancreatic cancer, "smart drugs need smart applications". Indeed, most experts concur that the latter applications should include multi-targeted and, ideally, individualized molecular therapies, in combination with conventional cytotoxic agents or other regimens (such as immunotherapy)[61], guided by reliable biomarkers of treatment response. Increased toxicity resulting from these combinatorial approaches as well as their cost-effectiveness and socioeconomic implications should, nevertheless, be carefully considered and may represent major limiting factors for their widespread use. In a disease as aggressive and lethal as pancreatic cancer, maintaining the highest possible quality of life for as long as possible is the most important target, and expectations should always be based on realistic goals.

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TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Fecal DNA testing for colorectal cancer screening: Molecular targets and perspectives

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Abstract

The early detection of colorectal cancer with effective screening is essential for reduction of cancer-specific mortality. The addition of fecal DNA testing in the armamentarium of screening methods already in clinical use launches a new era in the noninvasive part of colorectal cancer screening and emanates from a large number of previous and ongoing clinical investigations and technological advancements. In this review, we discuss the molecular rational and most important genetic alterations hallmarking the early colorectal carcinogenesis process. Also, representative DNA targets-markers and key aspects of their testing at the clinical level in comparison or/and association with other screening methods are described. Finally, a critical view of the strengths and limitations of fecal DNA tests is provided, along with anticipated barriers and suggestions for further exploitation of their use.

Key words: Colorectal cancer; Screening; Fecal DNA; Cologuard®; Adenoma

Core tip: The molecular DNA targets from genetic and epigenetic alterations hallmarking colorectal carcinogenesis are reviewed here in the context of fecal testing. Also, comparison with other screening methods in terms of limitations, advantages and future perspectives of fecal DNA tests are discussed.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in men and women and accounts for 8% of all cancer-related deaths[1]. The incidence of CRC varies within different geographic locations and racial/ethnic groups. These differences may be related with different dietary and environmental exposures in association with a different genotype-driven susceptibility[2]. Screening for CRC plays a key role in reduction of CRC-related mortality, and the observed decline in the incidence of CRC since the mid-1980s is a striking proof of this effect, along with changes in risk factors[1].

CRC screening may be divided into two main categories: (1) biological sample-based tests, including fecal, blood and urine tests, as well as (2) colon structure-based and image-based tests, including flexible sigmoidoscopy, total colonoscopy, CT colonography and double-contrast barium enema[3,4]. Stool-based tests, including guaiac-based fecal occult blood test (g-FOBT), and the newer ones, fecal immunochemical test (FIT) and stool DNA test are already included in the American Cancer Society recommendations for CRC screening[4].

MOLECULAR RATIONAL FOR FECAL DNA TESTING

The detection of altered DNA from cancerous and pre-cancerous lesions of the colonic mucosa is based on the natural exfoliation of these cells and is further facilitated by their high degree of "integrity" compared to DNA from stools of healthy patients. Accumulating data on key mutations occurring during the early stages of colon carcinogenesis including K-Ras, adenoma polyposis coli (APC), and p53, as well as epigenetic changes such as microsatellite instability (MSI), has guided the targeted development of clinically relevant detection tests[5].

The genetic heterogeneity of CRC is essentially the reason underlying the concept of targeting multiple DNA markers. K-Ras encodes a RAS family protein which is a GTPase involved in many downstream signal transduction pathways[6]. The mutation is found in 13%-95% of CRC patients and is one of the initial mutations in colon carcinogenesis[6]. APC is an important tumor suppressor gene product involved in the Wnt/β-catenin signaling pathway, which in turn is a transcription regulator of several growth-controlling genes, including the oncogene *MYC*[7]. Thus it is not surprising that mutation or inactivation of the APC protein is a driver of inherited (familial adenomatous polyposis) and sporadic forms of CRC, occuring in the early stages of transition from adenoma to carcinoma[7]. Another tumor suppressor gene, *p53* is found deleted or mutated in 30%-60% of CRC tumors[8]. Given its critical role in cell cycle control, apoptosis, and DNA damage response, p53 aberrations ultimately promote the development of increased genomic instability which facilitates transformation of colorectal adenomas to cancer[7].

MSI is a condition of genetic hypermutability within tandem repeats of short nucleotide sequences, the microsatellites, that results from impaired DNA mismatch repair (MMR) and is a frequent event in cancers, including 15% of all CRC[9]. The most common cause of sporadic MSI is epigenetic silencing of *MMR* genes, such as MLH1 due to promoter hypermethylation[7] and there are several MSI markers (BAT25, BAT26, D2S123, D5S346, and D17S2720) for detection of MSI with polymerase chain reaction. The clinical relevance of MSI lies in the fact that patients with MSI positive tumors have better prognosis and longer overall survival compared with non-MSI tumors[9].

Epigenetic methylation of gene promoters is a central mechanism that can promote carcinogenesis in the appropriate context and several preclinical studies have identified hypermethylated genes in stool samples from CRC patients, which are strikingly un-methylated in normal epithelial cells[9]. Characteristic examples include the genes secreted frizzled-related protein (SFRP), vimentin, MGMT, FBN1, and p16[7]. In addition, the panel of methylated genes varies depending on the different stages of carcinogenesis, involving (1) SLC5A8, SFRP1, SFRP2, CDH13, CRBP1, RUNX3, MINT1 and MINT31 from normal colon mucosa to aberrant crypt focus formation; (2) p14, HLTF, ITGA4, p16, CDH1, and ESR1 from aberrant crypt focus to adenoma formation; and (3) TIMP3, CXCL12, ID4, and IRF8 from adenoma to carcinoma formation and metastatic progression of CRC[7].

CLINICAL STUDIES OF FECAL DNA TESTS

An important limiting factor for developing a screening stool test with high sensitivity is the fact that only 0.01% of total fecal DNA is human and the tumor DNA is only a small percentage of the former[10].

K-RAS was the first gene tested for mutations in feces from CRC patients[11-13]. A comparative study assessed gFOBT and a fecal DNA test analyzing a panel of 21 gene mutations[14]. Imperiale *et al*[14] concluded that the multitarget fecal DNA test detected more invasive cancers plus adenomas with high-grade dysplasia than did gFOBT (40.8% *vs* 14.1%) without compromising specificity (94.4% *vs* 95.2%). In a blinded, multicenter, case-control study, with cases including CRC, advanced adenoma (AA), or sessile serrated adenoma $\geqslant$ 1 cm (SSA), an automated multitarget stool DNA assay was able to detect AA with high-grade dysplasia with 83% sensitivity[15]. Another blinded, multicenter, case-control study assessing a similar panel of DNA markers identified 85% of patients with CRC and 54% with AA,

Table 1 Fecal DNA markers for advanced adenoma and colorectal cancer *n* (%)

Ref.	Marker	Sensitivity		Specificity
		CRC	Adenoma > 1 cm	
[12]	Meth BMP3, hDNA, KRAS, APC	67 (91)	21 (78)	85 (85)
[13]	APC, KRAS, p53, long DNA	3 (25)	47 (8)	2246 (96)
[14]	APC, KRAS, p53, long DNA	16 (52)	84 (12)	1344 (94)
[15]	β-actin, KRAS, meth BMP3 and NDRG4, fecal hemoglobin	91 (98)	48 (57)	139 (90)
[16]	KRAS, a actina Meth NDRG4, BMP3, vimentin, TFPI2	214 (85)	72 (54)	264 (90)
[17]	KRAS, NDRG4, BMP3, β-actin, fecal hemoglobin	60 (92)	321 (42)	4457 (90)
[20]	Meth vimentin	9 (41)	9 (45)	63 (95)
[21]	Meth SFRP2	60 (87)	21 (62)	28 (93)
[22]	Meth TFPI2, long DNA	52 (87)	4 (44)	25 (83)
[23]	Meth SFRP2, HPPI, MGMT	50 (96)	15 (71)	23 (96)
[24]	Meth APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1	15 (75)	17 (68)	27 (90)
[25]	Meth vimentin, long DNA	68 (83)	6 (86)	298 (82)
[26]	Meth RASSF2 or SFRP2	63 (75)	25 (44)	101 (89)
[27]	Meth vimentin, MLH1, MGMT	45 (75)	31 (60)	32 (87)
[28]	Meth RARB2, p16INK4a, MGMT, APC	16 (62)	8 (40)	20 (100)

Adapted from Ref.[38]. Copyright 2014 by Baishideng Publishing Group Inc. Adapted with permission. CRC: Colorectal cancer.

without sensitivity differences based on location, but with tumor size affecting detection rates[16].

More recently, Imperiale *et al*[17] reported their results from comparison of fecal DNA to FIT in a huge patient population who had a complete screening colonoscopy (n = 9989). The sensitivity of fecal DNA test including evaluation of KRAS mutations, aberrant NDRG4 and BMP3 methylation, B-actin and a hemoglobin assay was superior to that of FIT (92.3% *vs* 73.8%). However, in addition to a lower specificity of fecal DNA and the lack of comparison with repeated FIT applications over time, a far higher number of patients (n = 689) were excluded due to problematic fecal DNA testing, compared to those who underwent FIT (n = 34)[18].

A systematic review of the literature for studies of biomarkers for early detection of colorectal cancer and polyps since 2007, disclosed overall sensitivities for colorectal cancer detection by fecal DNA markers ranging from 53% to 87%, with varying specificities above 76%[19]. The diversity and combinations of various fecal DNA markers with the corresponding sensitivities and specificities per study[12-17,20-28] are summarized in Table 1.

EVOLUTION OF FECAL DNA TESTING METHODOLOGY AND TECHNIQUES

Initially, the first fecal DNA tests were performed without stabilizing buffers, resulting in low sensitivities[13,14]. Upon incorporation of stabilizing buffers and introduction of more sensitive detection techniques such as the digital melt curve method and beads, emulsion, amplification, and magnetics (BEAMing), the initial detection threshold of 1% of mutated copies was decreased to less than 0.1%[10,12].

Furthermore, implementation of the allele-specific quantitative real-time target and signal amplification (QuARTS) technique led to detection of less frequent mutations, thus improving the sensitivity for AA[12]. Another technique termed fluorescent long DNA (FL-DNA), allows for identification of tumor DNA fragments longer than 150-200 base pairs, given that cancer cells evade apoptosis and subsequent DNA degradation. FL-DNA detects CRC with a sensitivity of 80%[29]. Other advances that have been introduced in different studies include neutralization of bacterial enzymes with EDTA[30], enrichment of the panel of DNA markers (*e.g.,* vimentin gene), and inclusion of hemoglobin detection in the same panel[16,31].

STRENGTHS AND LIMITATIONS OF FECAL DNA TESTS

A major advantage of fecal DNA tests as compared to either FOBT or colonoscopy is the fact that they are not affected by proximal location of tumors[32,33]. Another advantage is the lack of need for purging or dietary changes.

However, the sensitivity of fecal DNA tests appears to be lower for adenomas when compared to CRC detection (Table 1). In addition, although there is evidence of reductions in CRC incidence and mortality from randomized controlled trials of fecal occult blood test (FOBT) screening[34], similar data are lacking for fecal DNA tests.

Other technical difficulties may involve the burden of large volume stool collection and shipping for the patients undergoing screening[31]. In addition, the fact that in the latest study of Imperiale *et al*[17] the DNA tests had over twice as many abnormal results as FIT, with a higher rate of false-positive results implies that more colonoscopies would be needed to further evaluate for CRC in the former arm. Thus, the inevitably higher number of diagnostic testing would increase the costs and risks of screening. Only with the current screening method of gFOBT, 690011 colonoscopies for false positive screening tests result in an additional estimated annual cost of £800000000[19].

Cost-effectiveness *per se* seems to be a major disadvantage of fecal DNA tests as both older and newer studies, particularly based on a Markov model, have concluded that fecal DNA is cost-effective only when compared with no screening, but is essentially dominated by most of the other available screening options, including FOBT and colonoscopy[36,37]. This may necessitate the limitation of number of DNA markers to render their clinical use more reasonable[38].

CURRENT STATUS OF FECAL DNA TESTING (COLOGUARD®)

The United States Food and Drug Administration has recently approved Cologuard® (Exact Sciences Corporation, Madison, WI, United States), a multitarget stool DNA test in CRC screening[39]. The frequency of interval testing was determined to be every 3 years with adequate Medicare coverage[40]. Cologuard® incorporates molecular assays for aberrantly methylated *BMP3* and *NDRG4* gene promoter regions, mutant KRAS and β-actin as well as an immunochemical assay for human hemoglobin. It is based on the recent study of Imperiale *et al*[17] which showed a significantly better sensitivity for cancer detection compared to FIT. Further laboratory-based processing of the samples is necessary, entailing amplification and detection with the use of Quantitative Allele-specific Real-time Target and Signal Amplification (QuARTSTM) technology[41].

FUTURE PERSPECTIVES FOR FECAL DNA SCREENING TESTS

The combined use of screening tests would likely maximize the benefits of different biomarkers for early detection of CRC and adenomas. However, synchronous implementation of these tests in a mass screening program would not fulfill the cost-effectiveness requirement for clinical use.

Thus, there is a need for prospectively designed, systematic evaluations of the most promising fecal tests in a well-defined, large-scale screening population, with standardized sample collection, processing, and storage. This assessment should be combined with sigmoidoscopy or colonoscopy screening and ideally involve repeated testing and longitudinal monitoring of the screened population[19]. Another parameter that merits prospective evaluation is the clinical significance of fecal DNA-positive results in patients with negative colonoscopy results[40].

In the future, Imperiale and colleagues plan to "take this work forward by conducting a post-approval study, which will inform the important issue of test interval, that is, how often does the test need to be repeated". They will also conduct computer simulation studies that will inform comparative effectiveness and cost-effectiveness relative to other screening tests and strategies[42].

Given the high sensitivity for CRC that is unaffected by tumor location and its superior sensitivity over FIT for detection of SSA and AA with greatest risk of progression, Cologuard® may be a good candidate for interval testing after initial colonoscopy. For the same reason, in cases of poor preparation or incomplete colonoscopy, it might represent a convenient follow-up screening test alternative to repeat colonoscopy or other CT colonography, particularly for those patients who are either unable or unwilling to undergo repeat bowel preparation and invasive endoscopy[40].

In an expanding view, fecal DNA testing could be implemented as a screening in CRC predisposing conditions, such as inflammatory bowel disease, playing a role complementary to colonoscopy for early dysplasia detection and surveillance[40,43]. A relevant multicenter validation study has recently been initiated (Government-registered Trial: NCT01819766) and its results are eagerly awaited.

Finally, technological advancements in detection assays of small fragment DNA from stool may render the identification of altered DNA shed from upper GI pre-cancerous and malignant lesions feasible[44-46].

Discussion of screening tests involving non-DNA (*e.g.,* mRNA, miRNA) or non-fecal origin (*e.g.,* blood, urine) biomarkers was beyond the scope of this review. However, it is reasonable to assume that fecal shedding of tumor DNA is an earlier event compared to inner tissue and bloodstream invasion, and is also directly related to the natural, constant process of luminal colonic mucosa exfoliation; thus rendering fecal testing more timely sensitive for the purpose of screening.

Collectively, the accumulation of experience from clinical use of Cologuard® and the numerous ongoing studies on a plethora of biomarkers, as well as further technological advancement of colonoscopy with the full-spectrum endoscopy[47] are expected to further elucidate and expand the landscape of CRC screening research in the coming years, with the hope of further reducing CRC-specific mortality through earlier and accurate detection of pre-cancerous lesions.

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TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Role of retinoids in the prevention and treatment of colorectal cancer

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Abstract

Vitamin A and its derivatives, retinoids, have been widely studied for their use as cancer chemotherapeutic agents. With respect to colorectal cancer (CRC), several critical mutations dysregulate pathways implicated in progression and metastasis, resulting in aberrant Wnt/β-catenin signaling, gain-of-function mutations in K-ras and phosphatidylinositol-3-kinase/Akt, cyclooxygenase-2 over-expression, reduction of peroxisome proliferator-activated receptor γ activation, and loss of p53 function. Dysregulation leads to increased cellular proliferation and invasion and decreased cell-cell interaction and differentiation. Retinoids affect these pathways by various mechanisms, many involving retinoic acid receptors (RAR). RAR bind to *all-trans*-retinoic acid (ATRA) to induce the transcription of genes responsible for cellular differentiation. Although most research concerning the chemotherapeutic efficacy of retinoids focuses on the ability of ATRA to decrease cancer cell proliferation, increase differentiation, or promote apoptosis; as CRC progresses, RAR expression is often lost, rendering treatment of CRCs with ATRA ineffective. Our laboratory focuses on the ability of dietary vitamin A to decrease CRC cell proliferation and invasion *via* RAR-independent pathways. This review discusses our research and others concerning the ability of retinoids to ameliorate the defective signaling pathways listed above and decrease tumor cell proliferation and invasion through both RAR-dependent and RAR-independent mechanisms.

Key words: Colorectal cancer; Retinoid; Vitamin A; β-catenin; Phosphatidylinositol-3-kinase; K-ras; Cyclooxygenase-2; Peroxisome proliferator-activated receptor γ; P53; Phosphatase and tensin homolog deleted on chromosome 10

Core tip: Vitamin A and its derivatives, the retinoids, have been widely studied in many types of cancer for their ability to increase cell differentiation and decrease cell proliferation. This review focuses on the ability of retinoids to affect signaling pathways commonly disrupted in colorectal cancer. We discuss vitamin A metabolism and signaling, how this process becomes aberrant as colorectal cancer progresses, and how treatment with both dietary vitamin A and exogenous retinoids can alter these dysregulated signaling pathways to decrease colorectal cancer cell proliferation and invasion.

Applegate CC, Lane MA. Role of retinoids in the prevention and treatment of colorectal cancer. *World J Gastrointest Oncol* 2015; 7(10): 184-203 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i10/184.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i10.184

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and the second most commonly diagnosed cancer in women worldwide[1,2]. An estimated 1.2 million cases occurred worldwide in 2008, with the highest incidence rates occurring in developed countries including North America, Australia, New Zealand, Japan and Europe[1]. Global trends reflect an overall increase in the incidence of CRC, with the highest increases observed throughout Asia and Europe[1]. About 608700 deaths occurred as a result of CRC in 2008, accounting for 8% of all cancer-related deaths worldwide[1]. Approximately 50% of those patients diagnosed with CRC will experience metastasis to the liver, which is the primary site of CRC metastasis[3]. Risk factors for CRC are both genetic and environmental. A personal or family history of CRC and a personal history of chronic inflammatory bowel disease increase the risk for CRC[4]. Physical inactivity, obesity, smoking, and dietary patterns such as high red and processed meat consumption as well as moderate-to-heavy alcohol use also increase the risk for CRC[4]. Retinoids have long been studied for their effects on organismal development and cellular differentiation, particularly with respect to cancer. Retinoids are currently used as chemotherapies against cancers of epithelial origin, including basal and squamous cell carcinomas. Furthermore, retinoids (whose metabolism is shown in Figure 1) are known to affect signaling pathways frequently altered which result in the development and progression of CRC (Figure 2 and Table 1). CRC is highly influenced by diet, therefore it stands to reason that direct contact with retinoids from supplemented diets or exogenous retinoids administered as medication may have chemotherapeutic effects on CRC tumors.

VITAMIN A METABOLISM

Vitamin A (retinol) and its derivatives, the retinoids, are a group of fat-soluble compounds composed of a similar structure in which a hydrophobic β-ionone ring is joined to a hydrophilic polar moiety by a conjugated tetraene linear chain[5]. Retinol is also able to be synthesized from some types of fat-soluble, antioxidant carotenoids found in fruits and vegetables. While there are several different carotenoid molecules found in plants, only β-carotene, α-carotene, and β-cryptoxanthin have provitamin A activity[6,7]. In the diet, these carotenoids are consumed primarily through carrots, cantaloupes, sweet potatoes, and spinach[6]. Theoretically, cleaving the β-carotene molecule would yield two retinal molecules, each with a β-ionone ring, which can then be converted to two retinol molecules for cellular use[6]. However, this conversion occurs at a much lower rate *in vivo*, with the retinol activity equivalent of β-carotene being much lower than a 1:2 ratio of β-carotene: retinol[6]. Both α-carotene and β-cryptoxanthin only contain one β-ionone ring each and thus have about 50% of the provitamin A activity of β-carotene[6].

Retinol is derived from retinyl esters found in animal sources such as butter, eggs, and meats[8,9]. During digestion in the intestinal lumen, the long-chain fatty acids are cleaved from the retinyl esters *via* hydrolysis, yielding free retinol[10]. The free retinol is then absorbed into the mucosal cells where it is bound by cellular retinol binding protein-Ⅱ (CRBP-Ⅱ), which facilitates the re-esterification of retinol by lethicin retinol acyltransferase (LRAT)[10]. Once re-esterified with long-chain fatty acids such as palmitate, the resulting retinyl esters are incorporated into chylomicrons and secreted into the lymphatic circulation[10]. After draining into the general circulation and transferring their lipid contents into peripheral cells, the remaining chylomicron remnants containing the retinyl esters are taken up by hepatocytes[5]. Depending on bodily needs, the liver either stores the retinyl esters in stellate cells or hydrolyzes the retinyl esters to once again yield free retinol, which binds to retinol binding protein (RBP)[5]. The resulting RBP-retinol complex is released into circulation, where it binds to a small protein, transthyretin (TTR), which prevents the retinol from being excreted by the kidneys[5]. This RBP-retinol-TTR complex circulates in the plasma, until retinol dissociates from the protein complex to enter target cells[11]. The transport of retinol into the cell and its intracellular fate is shown in Figure 1. Because retinol is lipophilic, the molecule can freely diffuse through the plasma membrane of cells[11]. In some cells or during vitamin A deficiency, retinol may be taken up by cells through the RBP receptor, STRA6 (stimulated by retinoic acid 6')[5,11,12]. Cellular uptake of retinol *via* STRA6 is highly preserved in ocular cells, in which the loss of STRA6 leads to visual impairments[13]. However, in STRA6-*null* mice, retinoid homeostasis was only

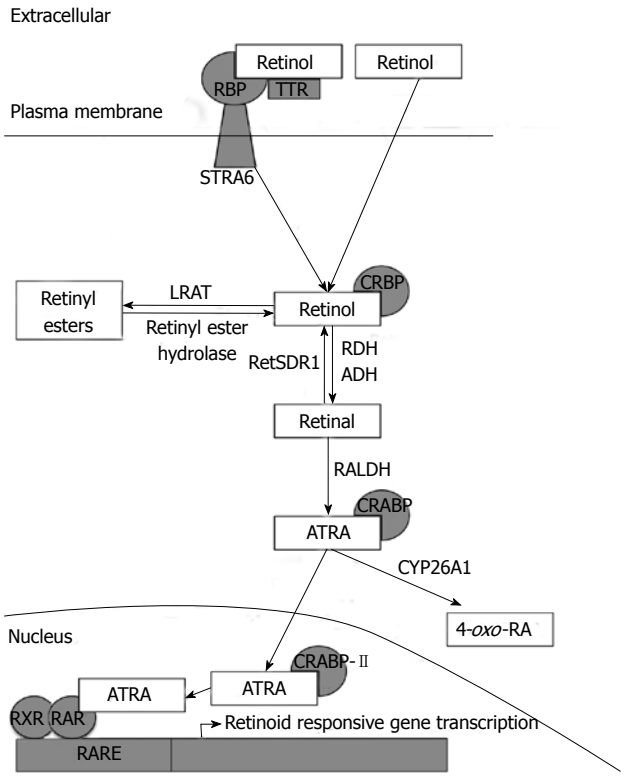

Figure 1 Retinoid metabolism. Vitamin A circulates as retinol bound to RBP and TTR. Retinol can be absorbed into cells *via* STRA6 or diffusion through the cell membrane. Intracellularly, retinol can be stored as retinyl esters or converted to ATRA. ATRA travels to the nucleus where it binds RAR to induce the transcription of retinoid-responsive genes. RBP: Retinol binding protein; TTR: Transthyretin; STRA6: Stimulated by retinoic acid 6; CRBP: Cellular retinol binding protein; LRAT: Lecithin retinol acyltransferase; RALDH: Retinaldehyde dehydrogenase; CRABP: Cellular retinoic acid binding protein; CYP26A1: Cytochrome P450 26A1; 4-*oxo*-RA: 4-*oxo*-retinoic acid; ATRA: *All-trans*-retinoic acid; RXR: Retinoid X receptor; RAR: Retinoic acid receptor; RARE: Retinoic acid response element.

moderately affected, with physiological functions that critically depend on *all-trans*-retinoic acid (ATRA) in both the adult and embryo remaining intact[14]. This indicates that while the receptor functions to assist cells in taking up retinol, STRA6 is not necessary to sustain normal function in cells other than those in the eyes. After diffusion into cells, the internalized free retinol is bound to CRBP or is oxidized to retinal by retinol dehydrogenases (RDH) or alcohol dehydrogenases (ADH) and then to ATRA by retinaldehyde dehydrogenases (RALDH)[5]. ATRA then binds to cellular retinoic acid binding proteins (CRABPs)[5]. CRABP-II shuttles ATRA to the nucleus of the cell, where ATRA serves as a ligand for retinoic acid receptors (RAR).

The RAR and retinoid X receptors (RXR) belong to the nuclear hormone receptor superfamily and are ligand-dependent transcription factors[15]. Each receptor occurs in three subtypes: RARα, -β, and -γ; and RXRα, -β, and -γ. Further, seven different splice variants of RARα (RARα1-7), four different splice variants of RARβ (RARβ1-4), and seven different splice variants of RARγ (RARγ1-7) have been identified[16]. Two different splice variants of each RXR subtype have also been identified that RXRα1 and 2, RXRβ1 and 2, and RXRγ1 and 2[17]. ATRA binds to and activates all subtypes of RAR with a high affinity[15,17]. While the only known retinoid ligand for RXR is 9-*cis*-RA, there has been a general inability to detect this retinoid isomer *in vivo*[18,19]. Recently, 9-*cis*-RA was detected in pancreatic tissue, but the ability of 9-*cis*-RA to act as a ligand for RXR in cells other than pancreatic cells remains controversial[20]. In the absence of ATRA, the RAR/RXR heterodimer binds to RA response elements (RARE) present on DNA promoter regions of ATRA-target genes[21]. The RAR/RXR complex recruits co-repressor proteins, which in turn recruit histone deacetylases (HDAC) to the DNA region[21]. HDAC remove acetyl groups from histone proteins, changing the chromatin structure and negatively regulating gene transcription[21]. By the binding of ATRA, RAR undergoes a conformational change to release inhibitory co-repressor proteins and recruit co-activator proteins, such as histone acetyl transferases, to enhance transcriptional activity[22]. The vast majority of research regarding the ability of retinoids to prevent cancer progression has focused on ATRA and RAR-mediated phenomena. However, as discussed below, cells become resistant to the effects of ATRA on cellular proliferation and differentiation as tumors progress[8,15]. To this end, our laboratory has shown that retinol has non-genomic effects, exclusive of ATRA, such as interference with pathways involving phosphatidylinositol 3-kinase (PI3K) and β-catenin, which play key roles in the progression of cancer[23-29].

ABBERANT VITAMIN A SIGNALING AND METABOLISM IN COLORECTAL CANCER

The luminal side of the colon is an epithelial layer of tissue which is composed of a single sheet of columnar epithelial cells which are folded into finger-like invaginations that are supported by the lamina propria to form a functional unit called a Lieberkuhn's crypt[30]. Different types of epithelial cells line the crypt, including epithelial colonocytes, goblet cells, and endocrine cells[31]. The cells at the bottom of the crypt are stem cells that differentiate into the various epithelial cell types as they move upward to the top of the crypt in a process known as "upward migration"[31]. As the cells migrate upwards, they become terminally differentiated and stop proliferating[31]. Once the cells reach the top of the crypt, they undergo apoptosis and are sloughed off into the lumen[31]. When these cells mutate to retain their proliferative capacity and avoid apoptosis once they reach the top of the crypt, they have the potential to form an adenomatous polyp[31]. These abnormalities may result as a process of inherited genetic mutations, replicative mistakes, or epigenetic changes. If undetected, these polyps may progress into a cancerous lesion[31].

The growth and differentiation of epithelial cells is strongly controlled by retinoid-activated genes. Genes

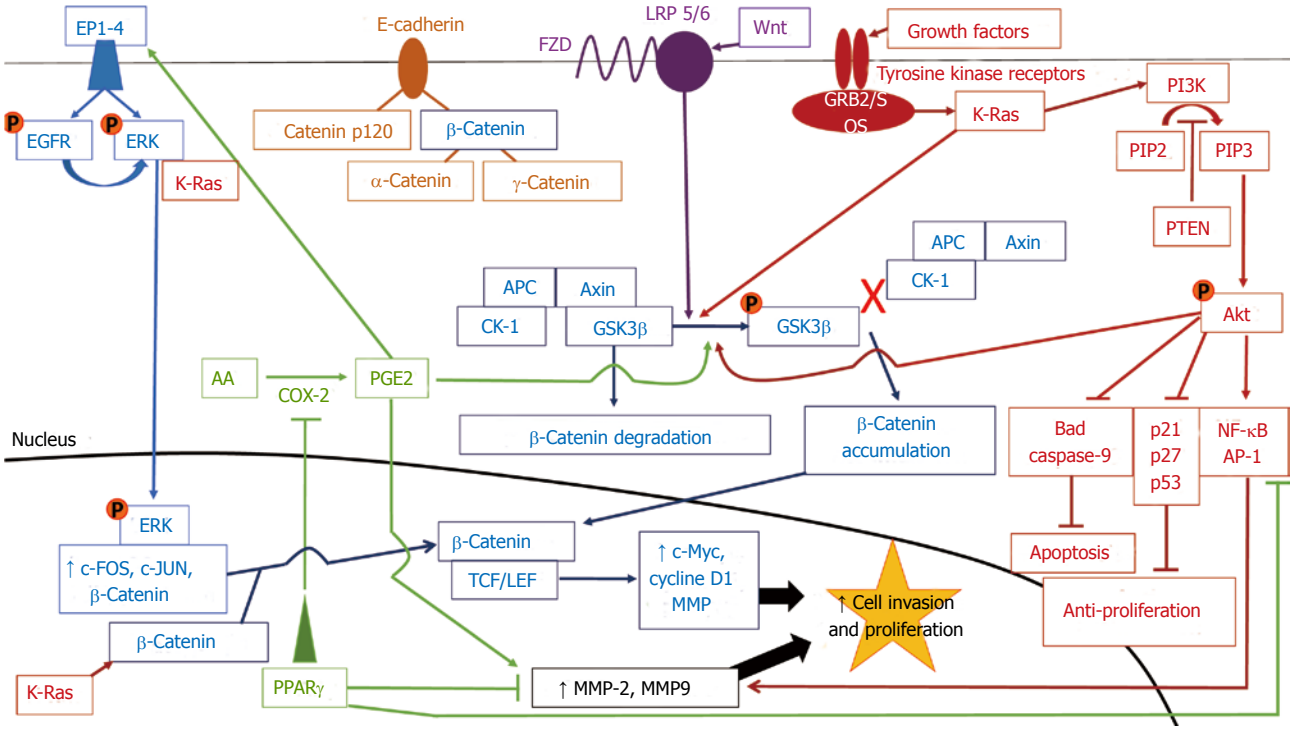

Figure 2 Crosstalk between signaling pathways that lead to colorectal cancer progression. Each pathway is indicated by a specific color. Orange circles represent phosphate groups. β-Catenin is found at the cell membrane, complexed with E-cadherin, in the cytosol, and in the nucleus. Cytosolic β-catenin can be targeted for proteosomal degradation by GSK3β when GSK3β is not phosphorylated and is complexed with APC, Axin, and CK-1. Nuclear β-catenin induces gene transcription when complexed with TCF/LEF transcription factors. Ultimately, all pathways increase the transcription of genes favoring cellular proliferation (c-Myc, cyclin D1) and invasion (MMPs), most *via* increasing β-catenin-mediated gene transcription. CRC: Colorectal cancer; EP1-4: E-prostanoid receptor types 1-4; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal-regulated kinase; K-Ras: Kirsten rat sarcoma viral oncogene homolog; FZD: Frizzled; LRP: Lipoprotein related receptor proteins 5/6; GRB2/SOS: Growth factor receptor-bound protein 2/son of sevenless; PI3K: Phosphatidylinositol-3-kinase; PIP2: Phosphatidylinositol-4,5-bisphosphate; PIP3: Phosphatidylinositol-3,4,5-triphosphate; PTEN: Phosphatase and tensin homolog deleted on chromosome 10; APC: Adenomatous polyposis coli; CK-1: Casein kinase 1; GSK3β: Glycogen synthase kinase 3β; PGE2: Prostaglandin E2; COX2: Cyclooxygenase 2; AA: Arachidonic acid; PPARγ: Peroxisome proliferator-activated receptor γ; TCF/LEF: T-cell factor/lymphoid enhancer factor; MMP: Matrix metalloproteinase; NF-κB: Nuclear factor-kappa B; AP-1: Activator protein 1.

involved in transcription, cell signaling, and tumor suppression contain RAREs in their promoter regions, indicating the importance of ATRA in gene expression[18]. In many epithelial-derived adenomas and carcinomas, the expression of one or more RAR is lost and the cell loses its ability to regulate normal growth[17,32]. This phenomenon is termed "ATRA-resistance". The RARs themselves contain RAREs in their regulatory regions and are thus RA-inducible genes[21,33]. Treatment of patients with premalignant oral lesions with 13-*cis*-RA, a synthetic retinoid, increased the expression of RARβ, which correlated with clinical response, signifying the beneficial effects of retinoid treatment in increasing anti-tumor gene activity in cancers[33,34]. However, the loss of tumor-suppressive RARβ is common in premalignant and malignant tissues and cells, as reviewed in Xu[33]. Loss of RAR has been shown to be partly due to epigenetic changes such as histone modification and DNA methylation becoming aberrant during carcinogenesis, silencing *RAR* gene expression[33,35-38]. The loss of RARβ2 in the HCT-116 colon cancer cell line has been suggested to originate as a result of hypermethylation and the ensuing loss of RARα, which is an upstream regulator of RARβ2[39]. Restoration of RARα by a DNA methylation inhibitor resulted in the re-establishment of RARβ2 expression, indicating a potential role for the combined chemotherapeutic action of DNA methylation inhibitors and retinoids[39]. In contrast, Lee *et al*[32] demonstrated that treatment of RA-sensitive and RA-resistant human colon cancer cell lines with ATRA induced the expression of RARα in all cell lines while only increasing the expression of RARβ in colon cancer cell lines sensitive to RA. Over-expression of RARβ in the RA-resistant colon cancer cell line, DLD-1, resulted in the re-acquisition of RA-sensitivity, inducing growth inhibition and apoptosis in this cell line with ATRA treatment[32]. Over-expression of RARβ in LoVo cells, another RA-resistant human colon cancer cell line, showed similar results in which treatment with ATRA resulted in retinoid-mediated growth inhibition[40].

In addition to the loss of RAR expression and the consequential ATRA resistance, as CRC progresses, colorectal tumor cells appear to lose the ability to produce ATRA[26,41,42] while, at the same time, increasing ATRA degradation *via* the cytochrome P450 enzyme, CYP26A1[43]. Recently, Kropotova *et al*[41] found that all genes involved in ATRA synthesis were decreased in CRC tumors and colorectal cell lines. The researchers also found that ADH IB and IC, the most abundant retinol oxidizing enzymes, exhibited decreased gene

Table 1 Summary of pathways dsyregulated in colorectal cancer and the effect of retinoids on these pathways in both colorectal cancer and other tumor types

Protein	Mutation rate	Result of gene mutation	Response to retinoid treatment
APC	80%[57,65]	Loss of β-catenin degradation[58]; constitutive activation of the Wnt/β-catenin pathway[59]; decreased RDH levels inhibiting formation of ATRA[42]	Not determined
β-Catenin	5%[56]	Loss of β-catenin degradation[56]; constitutive activation of the Wnt/β-catenin pathway[56]; increased CYP26A1 levels resulting in increased degradation of ATRA	Increased degradation of β-catenin *via* RXR-mediated pathway[23,24]
PI3K	30%-50%[77,78]	Activation of Akt and loss of GSK3β function[80,82]; increased cancer metastasis[88], partially through NF-κB activation and increased expression of MMP-2 and -9[87,89,90]; positive cell cycle progression through cyclin D1[105]; loss of cell-cell adhesion by Snail accumulation to repress E-cadherin[106]	Decrease MMP-2 and MMP-9 activity[28]; increase TIMP-1 expression[28]; decrease the phosphorylation of GSK3β, decrease cellular proliferation, and increase the expression of pro-apoptotic proteins in human leiomyoma and myometrial cells[115]; CRBP-I inhibits PI3K/Akt activation in breast cancer cells[116]; inhibit PI3K activity to decrease CRC cell invasion *in vitro* and metastasis *in vivo*[25]
PTEN	20%-40%[80]	Loss of PI3K/Akt inhibition[80]; correlation with tumor aggressiveness and invasiveness[109-111]	Suppression of cellular proliferation and enhanced apoptosis by increasing PTEN expression in smooth muscle cells, neuroblastoma and glioblastoma cells, promyelocytes, leukemia cells, fibroblasts, and breast, endometrial, and hepatocellular carcinoma cells[119-128]
COX-2	80%-90%[134-136]	Increased PGE2 signaling[133,137,138], ERK activation[140], PI3K/Akt signaling through increased EGFR[133,140,141], β-catenin stabilization[142,143], and MMP-2 and MMP-9 expression to promote cellular proliferation[144,145]	Decrease COX-2 expression[146], PGE2, β-catenin levels, and MMP-9[135,144]; inhibition of cell growth[151]; increased apoptosis and RARβ expression[152]
PPARγ	8%[161]	Loss of inhibitory action of gene transcription of pro-survival and growth amplification genes[155,162-165]; increased expression of COX-2[154]	Suppress COX-2 and MMP-7 expression and induction of cell cycle arrest and apoptosis[171]; induce expression of RARβ mRNA in breast cancer cells[175]; increase apoptosis in glioblastoma cells[176]; stimulate PTEN expression in leukemia cells and fibroblasts[121,128]
p53	50%[177,178]	Loss of anti-growth and apoptotic activity; loss of p53/Siah-1-mediated β-catenin degradation[187]	Increase retinyl ester storage through transcription of retSDR1[54]; enhance p53-mediated cell cycle inhibition and apoptosis through activation of AP-2α and p21 in breast cancer cells[192], caspases in keratinocytes[188], Btg2 and CRABP-II in breast cancer cells[191]; STRA6 induction in ovarian cancer cells, fibroblasts, and CRC cells[193]

APC: Adenomatous polyposis coli; RDH: Retinol dehydrogenase; ATRA: *All-trans*-retinoic acid; CYP26A1: Cytochrome P450 26A1; RXR: Retinoid X receptor; PI3K: Phosphatidylinositol-3-kinase; GSK3β: Glycogen synthase kinase 3β; NF-κB: Nuclear factor-kappa B; MMP: Matrix metalloproteinase; TIMP-1: Tissue inhibitor of matrix metalloproteinase 1; CRBP: Cellular retinol binding protein; CRC: Colorectal cancer; PTEN: Phosphatase and tensin homolog deleted on chromosome 10; COX2: Cyclooxygenase 2; PGE2: Prostaglandin E2; ERK: Extracellular signal-regulated kinase; EGFR: Epidermal growth factor receptor; RARβ: Retinoic acid receptor β; PPARγ: Peroxisome proliferator-activated receptor γ; AP-2α: Activator protein 2α; Btg2: Beta cell translocation gene 2; CRABP-II: Cellular retinoic acid binding protein II; STRA6: Stimulated by retinoic acid 6.

expression when adenomas were compared to more advanced carcinomas. Similarly, mRNA levels for RDH-5 and L were decreased in colon tumors and CRC cell lines when compared to normal colon cells[42]. As a result, the CRC cell lines produced only small amounts of ATRA from retinol, a phenomenon our group also observed with the ATRA-resistant CRC cell lines HCT-116, SW620 and WiDR[26]. Loss of adenomatous polyposis coli (APC) function, as seen in the SW620 cell line[44], inhibits RDH expression, the enzyme which converts retinol to retinaldehyde[42]. Interestingly, transfection of APC into an APC-deficient cell line increased the expression of RDH-L and the formation of ATRA, indicating crosstalk between Wnt/β-catenin signaling and retinoid metabolism[42]. To elaborate, APC mediates the proteosomal degradation of C-terminal binding protein 1 (CtBP1). Loss of APC increases the levels of CtBP1. Increased CtBP1, in turn, decreases RDH levels, inhibiting the production of ATRA[45]. Loss of ATRA ultimately leads to less colonocyte differentiation, as ATRA is necessary for epithelial cell differentiation[46]. In fact, homozygous loss of APC causes failed intestinal cell differentiation independent of catenin-mediated gene transcription but dependent upon CtBP1, leading to the hypothetical two-step model of colon adenoma initiation and progression[47]. In this model, APC loss and the resulting increase in CtBP1 leads to adenoma initiation, successive K-ras activation, and the nuclear translocation of β-catenin causing progression to a carcinoma. An incongruity with this model is that administration of ATRA to Apc^{Min} mice, which are heterozygous for a dysfunctional APC mutation, did not prevent tumor formation[48]. Shelton *et al*[43] found that CYP26A1 was increased in tumors from APC^{Min} mice, spontaneous human CRC, and in tumors from patients with familial adenomatous polyposis coli (FAP). These researchers also showed that CYP26A1 expression was dependent upon β-catenin-induced gene expression[43]. Finally, retinoid storage may be altered in cancer. Lecithin retinol acyltransferase (LRAT)

esterifies retinol to retinyl esters, the storage form of vitamin A while retSDR1 converts retinal to retinol. The promoter of the *LRAT* gene is hypermethylated in CRC cell lines and tumors when compared to normal tissue[49]. This hypermethylation would decrease *LRAT* gene expression, potentially decreasing the availability of intracellular retinoids; however, the role of LRAT in cancer progression is controversial with some studies in non-CRC models showing that decreased LRAT levels are protective against carcinogens and correlate with better patient outcomes[50-52]. Proteins in the p53 family have also been shown to affect retinoid metabolism by modulating the expression of retinal short-chain dehydrogenase/reductase (retSDR1). The retSDR1 enzyme is important in regulating retinoid metabolism and storage in many different cell types[53]. Treatment of neuroblastoma cells with physiological concentrations of retinol leads to the accumulation and storage of retinyl esters through the induction of retSDR1 enzyme levels[53]. The overexpression of p53 in the colorectal adenocarcinoma cell line DLD-1 and the CRC cell line HCT-116 yielded a strong induction of both retSDR1 mRNA expression and protein level, even in cells with truncated reporters[54]. The binding of p53 to the retSDR1 promoter was further increased following DNA damage to the cells[54,55]. Importantly, retSDR1 mRNA was shown to be elevated in CRC tumor tissues when compared with healthy samples from the same individuals[54]. These results signify that one mechanism by which p53 acts as a tumor suppressor is by inducing retSDR1 expression in carcinomas to work against tumor progression by supporting retinoid metabolism in these cells[54].

In summary, colorectal tumors often (1) lack RAR, the receptors for ATRA; (2) lose the ability to synthesize ATRA, the RAR ligand, from vitamin A; (3) exhibit increased degradation of ATRA *via* CYP26A1 to 4-oxo-retinoic acid (4-oxo-RA) and (4) may have altered retinoid storage. The regulation of retinoid metabolism is controlled by proteins such as APC, β-catenin, and p53 that play crucial roles in the promotion and progression of CRC as we elaborate below.

THE WNT/β-CATENIN SIGNALING PATHWAY

The Wnt/β-catenin signaling pathway is an important process that regulates the proliferation, differentiation, and motility of cells in normal intestinal epithelium[3,56]. This pathway, and others affecting CRC progression, are shown in Figure 2. During normal intestinal functioning, the APC protein forms a cytoplasmic complex with Axin, another protein present in the cytosol. Both proteins contain binding sites for other members of their functional complex[57]. Together, the APC-Axin complex recruits other functional members, the serine and threonine kinases glycogen synthase kinase 3β (GSK3β) and casein kinase 1 (CK-1)[57]. Together, these proteins form what is known as the β-catenin "destruction complex"[57]. β-catenin, when present in the cytosol, is sequentially bound and phosphorylated by these kinases and thus earmarked for degradation through an ubiquitin-proteasome-mediated pathway[57].

β-catenin performs a dual function in the cell, where it acts as both a transcription factor in the nucleus and as a cell adhesion stabilizer at the cell membrane. When in the cytosol, β-catenin binds to E-cadherin, a transmembrane protein responsible for the formation and maintenance of intercellular adherens junctions formed when epithelial cells come into contact[58]. E-cadherin binds to catenin p120 and β-catenin, which then binds to α-catenin and γ-catenin to anchor E-cadherin to the actin cytoskeleton[58,59]. Together, these proteins form a functional unit termed the E-caderhin-catenin unit (ECCU), in which β-catenin plays the role of an intermediary protein connecting E-cadherin to the α- and γ-catenin proteins that bind to the actin cytoskeleton[58]. The loss of E-cadherin function is thought to occur late in carcinogenesis and leads to the destruction of the ECCU, which causes a loss of the adherens junction and subsequent increase in cell motility and migration[58]. While the function of APC results in the degradation of β-catenin and β-catenin is necessary to form the ECCU, APC and E-cadherin compete for binding of β-catenin and work together to maintain the equilibrium of β-catenin concentration in the cell[58]. Loss of APC function results in E-cadherin saturation and the consequent accumulation of cytosolic β-catenin, which then translocates to the nucleus to enhance the transcription of genes important in cell growth and motility[58,59]. Thus, loss of APC function leads to a disruption in the equilibrium of β-catenin concentration and increased Wnt signaling[58,59]. Similarly, truncation of APC may result in β-catenin binding but not degradation, making β-catenin unavailable for E-cadherin binding[58]. While the over-expression of β-catenin is an important step in early tumorigenesis, later stages of carcinogenesis and loss of tumor differentiation may lead to loss of both β-catenin and E-cadherin expression, leading to the loss of ECCU formation and increased ability to metastasize[58].

Because β-catenin is both degraded and sequestered to the cell membrane during normal APC and E-cadherin function, it is unable to accumulate in the cytosol and translocate to the nucleus, where it binds to proteins of the T-cell factor/lymphoid enhancer factor (TCF/LEF) families[56,57]. If allowed to form a complex with TCF/LEF proteins, β-catenin acts as a transcription co-factor to allow TCF/LEF transcription factors to bind to the regulatory regions of genes regulating cell differentiation, proliferation, and migration such as c-Myc, matrix metalloproteinase-7 (MMP-7), and cyclin D1[3,57,60,61]. Ligand-bound RARs have been shown to compete with TCF in breast cancer cells to decrease β-catenin-mediated gene transcription[62]. In contrast, others have shown that overexpression of RARγ in cholangiocarcinoma cells increases the

nuclear translocation of β-catenin[63], indicating that the effect of RARs on β-catenin varies with tumor type. In phosphorylating β-catenin and thus marking it for ubiquitin-mediated proteasomal degradation, APC and its protein complex constituents act as negative regulators of the Wnt/β-catenin signaling pathway and maintain the homeostasis of intestinal crypt cells and stem cells[3,57,60,64].

Due to its importance in negatively regulating the Wnt/β-catenin signaling pathway, mutations resulting in the loss of APC function are generally thought to be the earliest step in CRC tumorigenesis[56,57]. As a result, APC mutations are found in approximately 80% of human CRCs while mutations involving β-catenin are found in about 5% of all human CRCs[56,57,65]. This APC mutation can be due to an inherited mutation, as in the case of FAP, or due to environmentally-regulated hypermethylation or dysregulation of the *APC* gene[61,66]. In loss-of-function APC mutations, the ability to degrade β-catenin is lost, allowing the Wnt/β-catenin signaling pathway to become constitutively active and upregulate the transcription of oncogenes important in tumor cell proliferation and metastasis[56]. The mutation of the *APC* gene leads to the inability of the APC protein to be exported from the nucleus into the cytoplasm, where APC normally forms a complex with the other proteins involved in the β-catenin destruction complex[61]. The loss of APC results in the increased ability of Wnt proteins to bind to membrane-bound receptors in the Frizzled (FZD) and low density lipoprotein receptor-related families to activate kinases that phosphorylate GSK3β[60,61]. The phosphorylation of GSK3β causes the cytosolic β-catenin destruction complex to become de-stabilized, allowing for the accumulation of β-catenin in the cytosol and its subsequent translocation to the nucleus[60]. When Wnt[66] receptors are not engaged, CK-1 and GSK3β are available to phosphorylate β-catenin to mark it for degradation.

K-RAS MUTATIONS AND CROSSTALK WITH OTHER PATHWAYS

While the APC mutation is found in most colon tumors and is generally regarded to be the earliest step in carcinogenesis, doubt has been placed on its ability to single-handedly cause neoplastic formation. In 30%-50% of CRC tumors, mutation of the *K-ras* gene has also been found, implicating its co-involvement in tumorigenesis[3,60,65,67]. K-ras is responsible for the transduction of mitogenic signals from growth factor receptors on the cell surface to the nucleus[65]. K-ras acts as a molecular switch to regulate the extracellular signal-regulated kinase (ERK) and PI3K/Akt signaling pathways[3]. During K-ras activation, the binding of growth factors to receptor tyrosine kinases causes the recruitment of the growth factor receptor-bound protein 2/son of sevenless (GRB2/SOS) protein complex to the inner cell membrane[60]. This protein complex activates the G-protein Ras (rat sarcoma), resulting in the phosphorylated ERK translocation to the nucleus[60]. In the nucleus, ERK interacts with transcription factors to induce the transcription of target genes such as *c*-FOS and *c*-JUN, which regulate proliferation, differentiation, and apoptosis[60].

Additionally, K-ras activation results in the increased transcription of β-catenin, resulting in the increased accumulation of β-catenin in the cytosol[60]. Mutations of K-ras destroy the GTPase activity of K-ras and fix K-ras in its GTP-bound active forms to permanently activate K-ras and increase ERK signaling[3,60,65,67]. The K-ras mutation interacts with the Wnt/β-catenin signaling pathway by causing the phosphorylation of GSK3β through activation of PI3K[60]. As previously discussed, inactivation of GSK3β leads to de-stabilization of the destruction complex and the resultant stabilization and mobilization of cytosolic β-catenin to the nucleus[60]. Normal activity of GSK3β contributes to negative regulation of both the K-ras and Wnt/β-catenin signaling pathways by phosphorylating K-ras, contributing to its degradation[64]. Thus, GSK3β plays an important role in regulation of both the K-ras and Wnt/β-catenin signaling pathways by degrading key intermediates of each pathway and preventing the transcription of genes important in tumor promotion[64].

K-ras mutations develop after APC loss during progression and metastasis of CRCs, enhancing neoplastic growth[3]. This enhancement of neoplastic growth is achieved by enhanced activation of Wnt/β-catenin signaling[3]. In many cancers, simultaneous activation of K-ras- and β-catenin-dependent pathways are often seen[60]. In human CRC cells and CRC mouse models, gain-of-function K-ras mutations coupled with loss-of-function APC mutations were associated with increased nuclear β-catenin levels and increased size, number, and incidence of tumors when compared to cells or mice with K-ras or APC mutations alone[3]. The resulting tumors displayed an increased migration rate and invasive capability through the increased activity of cyclin D1, which promotes cell cycle progression[3,60]. This evidence results in the theory that carcinogenesis in colon cells requires APC loss with an additional K-ras mutation[3]. Administration of ATRA to mice treated with the carcinogen deoxycholic acid (DCA) decreased colon tumor incidence, but ATRA did not affect the rate of K-ras mutation due to DCA administration[68]. Although we are not aware of any additional research regarding the ability of retinoids to affect K-ras expression or function in CRC, our laboratory and others have shown that retinoids can decrease β-catenin levels and thereby β-catenin-dependent gene transcription as described below.

Table 1 summarizes the effect of retinoids on proteins that affect CRC progression. Although retinoids do not appear to directly alter APC or K-ras activity, they do directly affect β-catenin levels. β-catenin degradation has been shown to be mediated by the activity of three pathways: (1) the APC/GSK3β

pathway; (2) the p53/Siah-1 pathway; and (3) an RXRα-dependent pathway. The RXR-mediated pathway was discovered when Xiao *et al*[69] showed that RXR agonists caused the degradation of RXRα and reduced β-catenin-mediated activation of gene transcription and cell proliferation. Additional work has shown that there is a direct interaction between RXRα and β-catenin[70]. Specifically, in the RXRα-dependent pathway, RXRα binds to nuclear β-catenin and facilitates the transport of β-catenin back into the cytosol where β-catenin is ubiquitinated and degraded by the proteosome. Interestingly, RXRα expression is decreased in advanced CRC when compared to normal adjacent tissue and this decrease is associated with aberrant β-catenin expression[71]. Retinoids increase β-catenin degradation in a variety of tumor types. For example, N-(4 hydroxyphenyl)retinamide (fenretinide) induced the degradation of β-catenin in prostate cancer cells[72] and ATRA decreased β-catenin levels in head and neck cancer stem cells[73]. With respect to CRC, our laboratory has shown that retinol treatment increased β-catenin degradation in ATRA resistant CRC cell lines *via* a RXR-mediated pathway[23,24].

PHOSPHATIDYLINOSITOL 3-KINASE/AKT SIGNALING

The PI3K/protein kinase B (Akt) signaling pathway is another important pathway, the activation of which induces cellular transformation, proliferation, migration, and survival, all of which work together to promote tumor progression[74-76]. Mutations resulting in aberrant activation of this pathway have been implicated in 30%-50% of all human CRCs[77,78]. This dysregulation occurs *via* three mechanisms: (1) activating mutations in exons 9 and 20 on the *PIK3CA* gene; (2) overexpression of Akt itself or activating mutations in the Akt PH domain to increase signaling; and (3) loss of function or expression of the negative regulator phosphatase and tensin homolog deleted on chromosome 10 (PTEN)[79-81]. PI3K belongs to a family of lipid kinases, and is characterized by its ability to phosphorylate the inositol rings of phospholipids on the inner cell membrane[82]. PI3K is present on the cell membrane as a heterodimer, consisting of one of four catalytic p110 subunits and one of two regulatory subunits[80,82]. P110α (PIK3CA) and p110β (PIK3CB) are ubiquitously expressed, with PIK3CA commonly being the more abundant catalytic subunit[82]. PIK3CA and PIK3CB bind to one of two regulatory subunits: p85α or p85β[82]. Class I PI3K enzymes bind Akt *via* pleckstrin homology (PH) domain-containing proteins and are activated mainly by receptor tyrosine kinases, such as those belonging to the epidermal growth factor receptor (EGFR) family, which accept a variety of extracellular signals necessary to stimulate cellular proliferation[80,82]. Once activated, PI3K catalyzes the phosphorylation of membrane-bound phosphatidylinositol-4,5-bisphosphate (PIP2) to generate the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3)[82]. The generation of PIP3 allows for the recruitment of PH domain-containing proteins to the inner plasma membrane[80]. Most notably, the PH domains of 3-phosphoinositide-dependent protein kinase 1 (PDK1) and Akt are drawn together, and PDK1 mediates the phosphorylation of Akt at the threonine 308 site[80,83].

Activating mutations in the *Akt1* gene are rare, occurring in less than 2% of all CRCs[80]. Activating mutations in PDK1 are even rarer, occurring in less than 1% of all CRCs[80]; however, because these proteins are immediately downstream of PI3K, over-activation of PI3K due either to activating mutations of the *PI3K* gene or due to mutations of PTEN, the PI3K inhibitor, ultimately results in the over-activation of Akt. Akt occurs in three isoforms: Akt1, 2, and 3, with Akt1 being most broadly expressed[82]. Akt contains two phosphorylation sites, both of which are required to be phosphorylated for full Akt activation[84]. Phosphorylation of Akt at the threonine 308 site by PDK1 partially activates Akt, whereas full activation requires conjunctive phosphorylation of the serine 473 site by other kinases, such as the mammalian target of rapamycin (mTOR) complex 2 (mTORC2)[83,85]. Full activation of Akt enables Akt to modulate the activity of pathways and expression of genes involved in the regulation of cell survival and proliferation as well as metastasis[86]. As reviewed in Fresno Vara *et al*[82] and Danielsen *et al*[77], Akt prevents the anti-proliferative activities of tumor suppressor genes *p21*, *p27*, and *p53*. Akt also blocks apoptosis in cancer cells by inactivating signals produced by Bcl-2 associated-death promoter (Bad) and caspase-9 proteins, and activates nuclear factor-kappa B (NF-κB), a transcription factor involved in the transcription of genes important in maintaining cell survival and increasing cell invasion[77,82,87]. The mechanism by which Akt activation promotes metastasis is incompletely understood, but elevated Akt phosphorylation has been shown to be correlated with the invasiveness of cancer in human CRC tissues[88]. Specifically, increased levels of phosphorylated Akt are associated with venous invasion of colorectal carcinomas, tumor depth, and the presence of lymph node metastases[88].

One possible mechanism linking Akt activity to cell invasion relies on the activation of NF-κB. NF-κB upregulates the transcription of matrix metalloproteinases (MMPs), which are a class of zinc-dependent enzymes responsible for the degradation of the extracellular matrix[87,89,90]. Specifically, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) belong to a family of gelatinase enzymes that degrade the collagen component of the extracellular matrix[90,91]. Both MMP-2 and MMP-9 are overexpressed in many colon carcinomas when compared with non-cancerous tissue and are associated with increased invasiveness of cancers, advanced tumor stage, and poor survival[87,89,91,92]. Relevant to this review, MMP-9 and MMP-2 have been

shown to be overexpressed in colorectal carcinomas, but not adenomas, indicating their importance in tumor promotion and progression[93]. MMP-2 and -9 are present in the cytosol in inactive pro forms, and cleavage of MMP-2 and -9 by membrane-type matrix metalloproteinases (MT-MMP), such as MT1-MMP, convert inactive pro-MMP-2 and -9 to active MMP-2 and -9[94,95]. This cleavage is inhibited by tissue inhibitors of metalloproteinases (TIMPs), specifically TIMP-1 and -2, which interact with the intermediate (inactive) MMP-9 and -2, respectively, before the proteases are fully activated[94,96]. TIMP-1 expression is regulated by activator protein-1 (AP-1), a transcription factor regulated by the activation of the mitogen-activated protein kinase (MAPK) pathway[90]. Thus, it has been suggested that both PI3K/Akt and MAPK signaling activation must occur simultaneously to regulate MMP-2 and -9 activity and thereby cell invasion[90]. ATRA has been shown to decrease MMP-2 and -9 activity as well as protein and mRNA levels and increase TIMP-1 in a variety of cancers[97-101]. With respect to CRC, our laboratory has shown that treatment of the ATRA-resistant human CRC cancer cell lines HCT-116 and SW620 with retinol resulted in decreased MMP-9 mRNA levels[28]. MMP-2 mRNA levels were decreased in SW620 cells but not in HCT-116 cells[28]. Importantly, the reduction of MMP-2 and MMP-9 mRNA was matched by a reduction in MMP activity[28]. Retinol treatment of HCT-116 and SW620 cells also increased the expression of TIMP-1, potentiating the inhibition of MMP-9 activity in these cells[28].

While TIMP-1 and MMP-2 and 9 expression are regulated by AP-1 and AP-1 activity is in turn repressed by retinoids, this is not thought to be the mechanism by which retinoids affect TIMP-1 and MMP-2 and 9 expression. AP-1 is composed of the proto-oncogenes *c*-JUN and *c*-FOS and its activity is associated with cellular proliferation and invasion[102]. Suppression of AP-1 by 9-*cis*-RA led to the inhibition of cyclin D1 and MMP-2 and 9 in breast cancer cells, however this effect was not matched in SW480 CRC cells, which have low AP-1 activity[102]. Instead, the trans-repressive effects of the cyclin D1 promoter, which contains AP-1 and TCF sites, was independent of the AP-1 site in these CRC cells and required the involvement of a TCF binding element[103]. This data shows that while AP-1 activity is involved in cellular proliferation and invasion, retinoids appear to exert their repressive effects on MMP levels through their interaction with pathways that decrease β-catenin, as β-catenin forms a transactivation complex with TCF/LEF transcription factors. However, promising research involving novel synthetic retinoid derivatives may better target AP-1 for tumor suppression. Um *et al*[104] developed the synthetic retinoid 4-amino-2-(butyrylamino)phenyl-(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)-2,4,6,8-nonatetraenoate (ABPN), which greatly inhibited AP-1 activity in HCT-116 cells. ABPN suppressed *c*-JUN activity, which led to a decrease in MMP-2 expression, by directly affecting AP-1[104].

It is widely accepted that cross-talk between the PI3K/Akt pathway and the Wnt/β-catenin signaling pathway occurs with GSK3β. Activated Akt phosphorylates GSK3β, inactivating GSK3β and causing a loss of function[82]. Without GSK3β to phosphorylate cytosolic β-catenin and mark it for degradation, stabilized β-catenin can accumulate in the cytosol and eventually translocate to the nucleus to act as a co-factor for gene transcription, as discussed previously[82,86]. Additionally, it has been shown that GSK3β phosphorylation of cyclin D1 stimulates cyclin D1 degradation[105]. Therefore, in tumor cells with increased Akt signaling and loss of GSK3β activation, cyclin D1 remains stable and able to positively regulate cell cycle progression[105]. The loss of GSK3β functioning also results in the increased accumulation of Snail, a zinc-finger transcriptional repressor of E-cadherin[106]. Active, unphosphorylated GSK3β binds to Snail and activates its degradation[107]. Loss of GSK3β function by Akt hyperactivation permits Snail to act as a transcription factor to repress E-cadherin transcription, decreasing cell-cell adhesion through E-cadherin loss[106,107]. As discussed, Akt activation also increases NF-κB transcriptional activity, which in turn increases Snail expression in epithelial cells[106]. Alternatively, it has also been proposed that 3%-5% of total cellular GSK3β is stably bound to Axin to form a complex reserved specifically for Wnt signaling[108]. One study conducted in prostate and breast cancer cell lines and *C. elegans* has shown that inhibition of PI3K by the PI3K inhibitor, wortmannin, does not affect GSK3β phosphorylation[108]. Thus, Wnt signaling by PI3K inhibition remains unchanged, refuting the common theory that there is cross-talk between the two pathways[108]. Instead, this evidence suggests that CRC presents with activating mutations in both the Wnt/β-catenin pathway and the PI3K/Akt pathway simultaneously, creating the notion that cross-talk between the two pathways occurs with a common GSK3β protein[108].

PTEN functions as a negative regulator of PI3K signaling by dephosphorylating the second messenger PIP3 to convert PIP3 back to PIP2[109,110]. PTEN exists in the cell as a cytoplasmic protein in an inactive, phosphorylated state[110]. Phosphorylation of PTEN serine and threonine residues stabilizes the protein in a closed state[110]. Upon activation, dephosphorylated PTEN contains an active phosphatase domain[110]. However, this active site leaves PTEN in an unstable conformation susceptible to proteasomal degradation[110]. In this way, the normal negative feedback loop of PI3K signaling and PTEN inhibition can proceed[110]. When active, PTEN is recruited to the plasma membrane where it binds to PIP3 and dephosphorylates the second messenger, inhibiting the downstream Akt signaling[110]. The loss of PTEN expression results in the accumulation of PIP3 at the plasma membrane, resulting in increased recruitment of Akt to the plasma membrane and increased Akt activation[80]. Because of this negative

regulation of PI3K/Akt signaling, PTEN is associated with inhibition of cell cycle progression, induction of cell death, modulation of cell cycle arrest signals, and stimulation of angiogenesis[110].

PTEN mutations and loss of PTEN expression have been shown to occur in a high number of CRCs, with this loss correlating with tumor aggressiveness and invasiveness[109-111]. This correlation might be explained by the involvement of PTEN with maintaining normal cell polarity[109]. Loss of PTEN results in a loss of cell polarity, leading to increased epidermal-to-mesenchymal transition (EMT) of cancer cells and loss of tight junctions[109]. Similarly, reduced expression of PTEN and loss of PTEN are shown to indicate more advanced stages and metastasis of CRC[111]. Loss of PTEN occurs due to loss of chromosomal heterozygosity in CRC tumors with chromosomal instability and is estimated to occur in about 20%-40% of CRCs, while PTEN mutations in tumors without chromosomal instability occur much less frequently, in less than 5% of cases[80,81,110,111]. PTEN expression itself is regulated by peroxisome proliferator activated receptor γ (PPARγ) and p53 activity, both of which are implicated in CRC and will be discussed in further detail later in this review[110].

Due to PTEN interaction with the PI3K/Akt signaling pathway, it has been proposed that loss of PTEN expression and mutations in PIK3CA may work synergistically to increase the activity of both PI3K/Akt and Wnt/β-catenin signaling[79]. However, data obtained from the European Prospective Investigation of Cancer Norfolk Study showed that loss of PTEN expression and PIK3CA mutations occurred independently of one another in CRCs[81]. Further mechanistic studies involving CRC tumors supported these results and showed activating PIK3CA mutations to occur in about 30% of tumors, independent of PTEN loss[80].

As mentioned previously, there is cross-talk between the PI3K/Akt pathway and the Wnt/β-catenin pathway. Investigation into PIK3CA mutations in CRC revealed that in human CRC cells carrying APC mutations and showing constitutive Wnt pathway activation, PI3K inhibition led to no change in the subcellular localization of β-catenin[79]. Interestingly, although the nuclear localization of β-catenin was unaffected by PI3K inhibition, the concentration of β-catenin phosphorylated at the putative Akt serine 552 phosphorylation site was lower in cells in which PI3K activity was inhibited[79]. β-catenin/LEF/TCF-mediated gene transcription was also lower in the PI3K-inhibited cells, resulting in decreased expression of Wnt target genes *c-Myc*, *cyclin D1*, and *LEF-1*[79]. As a component of the β-catenin transcriptional complex, the decrease in LEF-1 expression indicates a further decrease in the transcriptional activity of β-catenin[79]. Taken together, these results demonstrate that the nuclear localization of β-catenin and its transcriptional activity are independent processes, but are linked by PI3K[79].

Interestingly, retinoid treatment in some cancer cell lines has been shown to upregulate the activity of the PI3K/Akt signaling pathway, increasing cell proliferation and invasion to promote tumor growth[112-114]. However, in other cancer cell lines, treatment with retinoids has been shown to inhibit PI3K/Akt signaling[115-118]. These retinoid effects have mostly been shown to be mediated through RAR-mediated pathways involving ATRA binding to receptors[115,116]. Specifically, ATRA has been shown to decrease the phosphorylation of GSK3β, decrease cellular proliferation, and increase the expression of pro-apoptotic proteins in human leiomyoma and myometrial cells[115]. In addition, CRBP-I inhibits PI3K/Akt activation in breast cancer cells through a RAR-mediated pathway by decreasing the heterodimerization of p85 and p110[116]. To our knowledge, our laboratory is the only laboratory to investigate retinoid inhibition of the PI3K/Akt signaling pathway in CRC. Furthermore, because retinoid receptor activity is often down-regulated in CRC, our laboratory studied the effects of retinol, the dietary form of vitamin A, on the PI3K/Akt signaling pathway in human CRC cells exhibiting ATRA-resistance[29]. We have shown that PI3K activity is inhibited by retinol in a dose-dependent manner independent of RAR signaling or inhibition of p85/p110 heterodimerization[29]. We recently showed that it is the ability of retinol to inhibit PI3K activity that confers the ability of vitamin A to decrease CRC cell invasion *in vitro* and metastasis *in vivo*[25]. Specifically, by comparing the effects of retinol treatment on parental HCT-116 cells, expressing one allele of constitutively active PI3K (caPI3K), to mutant HCT-116 cells expressing two alleles of caPI3K, we showed that retinol treatment decreased *in vitro* cell invasion in parental HCT-116 cells, but not in mutant HCT-116 cells[25]. Retinol treatment also decreased total MMP-9 protein levels and active MMP-9 levels in parental HCT-116 cells, while these levels remained unchanged in HCT-116 cells expressing two alleles of caPI3K[25]. Finally, dietary vitamin A supplementation tended to result in a lower incidence of hepatic metastases in mice intrasplenically injected with parental HCT-116 cells but not in mice intrasplenically injected with mutant HCT-116 cells.

More research is needed to determine the mechanism by which vitamin A inhibits PI3K activity in CRC, but one possible mechanism is by the up-regulation of PTEN. Although the effect of retinoids on PTEN activity has not been examined in CRC to our knowledge, retinoids have been shown to alter PTEN activity in smooth muscle cells, neuroblastoma and glioblastoma cells, promyelocytes, leukemia cells, fibroblasts, and breast, endometrial, and hepatocellular carcinoma cells[119-128]. In particular, ATRA treatment of breast cancer cells reduced the methylation of the *PTEN* gene promoter to activate PTEN transcription[122]. Suppression of growth factors by ATRA in hepatocellular carcinoma cells increases PTEN levels and synchronously decreases the presence of phosphorylated Akt[123]. Increases of PTEN and consequent decreases of Akt occur with retinoid treatment of neuroblastoma and glioblastoma cells and of smooth muscle cells as well[119,126,127]. By

increasing PTEN, cellular proliferation is suppressed and apoptosis is induced, perhaps partially through the inhibition of NF-κB transcriptional activity[126,127]. Concurrent activation of PPARγ with retinoid treatment may also be helpful in synergistically reducing carcinogenesis, which will be discussed further in the following section.

CYCLOOXYGENASE-2 AND PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-γ

The use of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin reduces the incidence of CRC and other cancers of the gastrointestinal (GI) tract[129,130]. Chronic NSAID use has been shown to reduce the risk of CRC by as much as 40%-50%, as well as decrease the multiplicity and size of tumors presenting with APC loss[131,132]. These drugs mediate their effects through inhibition of cyclooxygenase (COX) enzymes. COX-2 is an inducible enzyme expressed in the presence of inflammatory cytokines, growth factors, and tumor promoters[133]. In the presence of these factors, COX-2 converts free arachidonic acid to prostaglandin H2 (PGH2), which is the precursor to other prostaglandins, specifically prostaglandin E2 (PGE2)[133,134]. COX-2 over-expression is associated with more aggressive tumors of the GI tract and increased levels of COX-2 mRNA are present in 80%-90% of CRCs[134-136]. This over-expression of COX-2 results in the increased levels of PGE2. Elevated PGE2 is present in high levels in cancer tissues and increases the carcinogenic process by stimulating cell proliferation, suppressing apoptosis, increasing cell motility, and promoting angiogenesis[133,137,138]. The biological effects of PGE2 are mediated by E-prostanoid (EP) G-protein coupled receptor subtypes 1-4 which are present in high levels in CRCs[133,139]. The loss of these EP receptors is associated with decreased PGE2 signaling and decreased cancer malignancy[139]. It should be noted that carcinoma cells that do not display increased COX-2 expression may still receive paracrine signals by PGE2 through EP receptors and thus still exhibit the growth stimulatory effects of PGE2 as well as increased cell motility and activation of ERK signaling[140]. PGE2 binding to EP receptors results in increased phosphorylation of EGFR and the downstream mediator ERK, which induces the expression of *c*-FOS, a gene involved in promoting cell proliferation[133,140,141].

While activation of EGFR contributes to increased PI3K/Akt signaling, COX-2 over-expression also results in the dissociation of GSK3β from the β-catenin destruction complex, leading to the stabilization of β-catenin for translocation to the nucleus[142,143]. PGE2 treatment in human CRC cells led to rapid phosphorylation of GSK3β on its serine 9 residue by Akt, inhibiting the kinase activity of GSK3β[143]. This action was, however, dependent on the loss of APC function in CRC because β-catenin stabilization by PGE2 occurs downstream of APC loss[143]. Inhibition of PGE2 in zebrafish embryos and human CRC cells demonstrating APC loss increased the degradation of β-catenin, with COX-2 knockdown reducing the levels of β-catenin[144]. ATRA treatment of zebrafish embryos and human CRC cells decreased the levels of β-catenin by a mechanism that requires the attenuation of COX-2 expression and subsequent decrease in PGE2 accumulation[144]. β-catenin reduction as a result of ATRA treatment also led to the decreased expression of MMP-9[144]. Furthermore, PGE2 led to the increased expression of TCF-4, a component of the β-catenin transactivation complex, resulting in increased transcription of genes downstream of β-catenin[142]. PGE2 thus leads to the expression of cyclin D1 and vascular endothelial growth factor (VEGF) *in vitro* and *in vivo*, which contribute to the increased formation of intestinal polyps[142]. This effect by PGE2 is synergistically perpetuated by mutated β-catenin[142].

COX-2 over-expression in CRC is also correlated with an increased expression of MMP-2 and MMP-9, both of which contribute to CRC motility and metastasis[145]. Suppression of COX-2 by selective inhibitors in mouse CRC cells decreased proliferation associated with cyclin D1 and inhibited cell migration and motility with an associated decrease in both MMP-2 and MMP-9[135]. This suppression of COX-2 also decreased tumor growth both *in vitro* and *in vivo*, while also slowing liver metastasis[135]. This process may be particularly important when considering metastasis of CRC, as COX-2 expression has been shown to be even higher in metastatic liver tumors[135]. Broad spectrum MMP inhibitors decreased the number of adenomas in mice lacking APC function by decreasing proliferation, inhibiting angiogenesis, and stimulating apoptosis, with a synergistic effect seen when combined with COX-2 inhibitors[145].

Moreover, the lack of a functional APC protein is correlated with the elevated expression of COX-2[146]. APC controls ATRA biosynthesis through the activity of RDH enzymes in human CRC, with this loss of RDH correlating with the increased expression of COX-2[146]. In zebrafish embryos and human CRC cells presenting with a functional loss of APC, this over-expression of COX-2 was attenuated by treatment with ATRA[146]. This attenuation of COX-2 expression was the result of a mechanism involving ATRA inhibition of the levels of CCAAT/enhancer-binding protein (C/EBP) *cis*-acting elements, which are present in the promoter region of the *COX-2* gene[146]. ATRA treatment decreased the expression of C/EBP-β, which leads to the decreased expression of COX-2[146].

The suppression of COX-2 by retinoids has been demonstrated in a variety of human epithelial carcinomas[147-150]. This suppression has been shown to be mediated by a multitude of factors, some of which have been described above, and which also includes a RARα-dependent pathway to limit the amount of CREB-binding protein (CBP)/p300 histone acetyltransferase activity available for AP-1 induction of COX-2[148]. In human CRC

cells, treatment with the retinoid analogue fenretinide decreased COX-2 mRNA and inhibited PGE2 expression, resulting in inhibition of cell growth[151]. Therapy with the selective COX-2 inhibitor celecoxib enhanced the growth inhibitory effects of ATRA in both COX-2-high-expressing HT-29 human CRC cells and COX-2-low-expressing SW480 human CRC cells, resulting in increased apoptosis and elevated RARβ expression through COX-2-independent mechanisms[152]. RARβ2 methylation was inversely associated with COX-2 expression, with increased methylation of RARβ2 in CRC tumors also presenting with high COX-2 expression[153]. These tumors correlated with a worse patient prognosis, proposing the importance of both COX-2 and RARβ2 expression in colorectal carcinogenesis[153]. Overall, COX-2 is over-expressed in CRC tumors, leading to elevated PGE2 and β-catenin and the resulting cellular proliferation and tumor metastasis. Treatment with retinoids inhibits this over-expression of COX-2, suppressing the tumor growth-inducing effects of COX-2.

COX-2 expression is regulated in part by PPARγ. Specifically, the activation of PPARγ decreases COX-2 expression by up to 90% and induces caspase-3-dependent apoptosis in human CRC cells[154]. The *COX-2* gene contains a peroxisome proliferator response element (PPRE) in its promoter, which allows the binding of PPARγ-RXRα heterodimers to inhibit *COX-2* gene transcription[155,156]. PPARγ belongs to the nuclear hormone receptor superfamily of ligand-dependent transcription factors[157]. Ligands existing for PPARγ include prostaglandins, polyunsaturated fatty acids (PUFAs), NSAIDs, and thiazolidinediones (TZDs)[158]. TZDs are a class of PPARγ agonist medications, used in diabetic patients to regulate lipid and glucose metabolism *via* PPARγ activation[158,159]. Upon ligand binding, PPARγ changes conformation to release corepressor proteins and recruit coactivator proteins, such as PPARγ-coactivator-1 (PGC-1)[160]. PPARγ then forms an obligate heterodimer with RXRα, and the resulting heterodimer binds to PPREs in the promoter regions of target genes to regulate expression[156]. In CRC, mutations of PPARγ occur in about 8% of cases, indicating its potential role as a tumor suppressor[161]. Many studies in CRC cell lines and animal models have demonstrated this effect, with PPARγ activation resulting in growth inhibition, apoptotic cell death, and decreased cell invasion[155,162-165]. However, the opposite effect has been observed in mice lacking APC function, with PPARγ activation resulting in tumor promotion[166,167]. In rats fed a high-fat diet, PPARγ and RARβ mRNA expression was suppressed, concomitant with an increase in COX-2 and β-catenin levels and in the number of aberrant crypt foci (ACF)[168]. Supplementing diets with retinyl esters or ATRA attenuated the increases in COX-2 and β-catenin expression and inhibited the formation of ACF[168]. This data indicates that dietary factors, such as lipids and retinoids, are strongly influential in protein expression and tumor formation.

The mechanisms by which PPARγ act on tumor formation are still unknown, yet the evidence presented thus far suggests the importance of PPARγ in tumor growth inhibition. PPRE-independent mechanisms may also be involved, as PPARγ activation has also been shown to interfere with NF-κB and AP-1 to inhibit the transcription of pro-survival and growth amplification genes[157,158,169]. As mentioned, the activation of PPARγ by ligand binding results in the suppression of COX-2 expression in human CRC cells with an ensuing decrease in PGE2 accumulation[156,170]. Additionally, PPARγ agonists lead to a decrease in both MMP-2 and MMP-9 and an increase in TIMP-1 and TIMP-2[156,159]. Treatment with ATRA and synthetic RXR ligands synergistically enhanced this effect, which ultimately led to a decrease in cell proliferation, invasion, and an increase in apoptosis[156,171]. Treatment of HCT-15 cells with ATRA and the TZD rosiglitazone synergistically suppressed COX-2 and MMP-7 expression and induced cell cycle arrest and apoptosis[171]. The growth suppressing effects of PPARγ in CRC have been shown to occur by modulating the transcription of genes regulating cell cycle progression. Treatment of human CRC cells with PPARγ agonists induced apoptosis in cells by halting cell cycling progression and inhibiting the expression of genes such as *cyclin D1* and *c-Myc*[157,158,172]. Adding synthetic RXR ligands to treatment with PPARγ agonists can augment cell growth inhibition and induce terminal differentiation by increasing the interaction of PPARγ and RXRα and their ability to form a heterodimer[169]. However, treatment of human CRC cells with RXR ligands alone does not cause PPARγ-RXRα heterodimer formation in the absence of PPARγ activation[156,172]. Therefore, dual treatment with synthetic rexinoid RXR ligands and PPARγ agonists may work together to inhibit the growth and metastasis of colonic tumors. As synthetic RXR ligands, rexinoids are not true retinoids. True retinoids bind RAR and are the focus of this review. Research regarding PPARγ and retinoids in CRC is lacking, as PPARγ only heterodimerizes with RXRα and not RAR. Yet, expression of RARβ mRNA can be induced by PPARγ activation in other cancers such as lung, breast, liver, and brain cancers[173-176]. ATRA alone and a combination of PPARγ and RXR ligands induced RARβ expression in ATRA-resistant breast cancer cells in the presence of HDAC inhibitors[175]. This induction of RARβ expression was reduced in the presence of a PPARγ antagonist, indicating the involvement of PPARγ/RXR heterodimer activity in RARβ transcription[175]. Treatment of breast and lung cancer cells with PPARγ and RXR ligands also induced apoptosis in these cells[175]. Apoptotic glioblastoma cells showed an increased level of RARβ expression when undergoing apoptosis, and PPARγ agonists induced RARβ mRNA in glioblastoma cells, suggesting that PPARγ activation may mediate apoptosis through RARβ activity[176]. Furthermore, treatment of leukemia cells with a combination of ATRA and the PPARγ agonist, ciglitazone, synergistically increased PTEN levels and

inhibited the growth and proliferation of these cells by inducing cell cycle arrest[121]. Both 9-*cis*-RA and PPARγ activation in fibroblasts stimulated PTEN expression, which led to a decrease in Akt phosphorylation[128]. Because PTEN expression is regulated in part by PPARγ activation, PPARγ ligands have been shown to decrease proliferation of endometrial cancer cells *via* PTEN induction and the inhibition of VEGF secretion[120]. Taken together, this research proposes that retinoid treatment in conjunction with PPARγ activation may be helpful in overcoming ATRA-resistance, inhibiting tumor growth, and promoting cancer cell death in CRC.

P53/SIAH-1 SIGNALING

Mutations of the tumor suppressor gene *p53* are the most common mutations found in human cancers, with p53 absence or mutations present in 50% of CRC cases[177,178]. As a tumor suppressor gene, *p53* is activated in response to genotoxic stimuli in healthy cells, to which p53 responds by arresting cell cycle progression and inducing apoptosis[179]. In healthy cells, p53 suppression is necessary for normal growth and is thus present at low concentrations, its expression is regulated through ubiquitin-dependent degradation most notably by the ubiquitin ligase, MDM2[179]. MDM2 is phosphorylated by kinases such as Akt, after which the activated MDM2 localizes to the nucleus and ubiquinates p53[179]. The ubiquitinated p53 is then exported from the nucleus, where it is degraded in the cytosol to maintain cell proliferative activity[179]. Up-regulation of MDM2 activity and transcription also occurs downstream of other oncogenic pathways to inhibit p53 activity, such as ERK and K-ras signaling[179]. Similarly, *MDM2* is a p53 target gene, creating a negative feedback loop to control p53 expression and activity[179]. In response to genotoxic damage, p53 is activated by kinases, which phosphorylate p53 in its MDM2 binding region, stabilizing p53 and allowing it to accumulate and bind to DNA to induce the transcription of genes such as cyclin kinase-dependent cell cycle inhibitor p21 and pro-apoptotic Bcl-2 associated x protein (BAX)[178-181]. P53 also directly inhibits anti-apoptotic proteins such as B-cell CLL/lymphoma-2 (Bcl-2) and Bcl-2 like isoform 1 (Bcl-xL), which inhibit the release of cytochrome c from the mitochondria to prevent the cell from initiating apoptosis[180]. Silencing of Bcl-2 in CRC cells leads to major p53-mediated apoptosis, demonstrating that Bcl-2 inhibits apoptosis in cells by also inhibiting p53 activity[180]. In CRC cells with mutant p53, transfection with wild-type p53 induces apoptosis and inhibits colony formation *in vitro* and inhibits tumor formation *in vivo*[182].

Missense mutations occur in 80% of all p53 mutations, resulting in a stable protein that accumulates inside the nucleus of tumor cells but lacks its specific DNA-binding activity and, therefore, lacks transcriptional activity[183]. As a result, an accumulation of p53 in the cell is generally thought to be mutagenic, although it is important to distinguish this mutant p53 accumulation in tumor cells from wild-type p53 expression[183]. The accumulation of mutant p53 in CRC patients is strongly correlated with increased metastasis and poor prognosis, further implicating the importance of p53 involvement in cell cycle regulation and stimulation of apoptosis in tumor cells[177]. Most p53 mutations occur in the later stages of adenoma-to-carcinoma progression, after which time many other pathways such as K-ras and the Wnt/β-catenin signaling pathway may already be dysregulated[184]. This point is particularly interesting to consider when looking at p53 involvement in β-catenin degradation. Siah-1 is a p53-inducible protein that binds ubiquitin-conjugating enzymes and targets proteins for degradation to ultimately result in tumor suppression[185]. Specifically, Siah-1 binds to the carboxyl terminus of APC and decreases β-catenin *via* a degradation pathway independent of GSK3β phosphorylation[185]. While Siah-1 does not affect APC levels, Siah-1 influence on β-catenin levels are dependent upon Siah-1 binding to APC[185]. In CRC cells with truncated APC, Siah-1 is unable to decrease β-catenin levels, making this process ineffective in cells expressing APC mutations[186]. Siah-1-mediated degradation of both mutant and wild-type β-catenin in CRC cells was supported by a decrease in TCF/LEF reporter activity and the consequent reduction of β-catenin target genes *cyclin D1* and *c-Myc* to result in cell cycle arrest[185-187]. Increased p53 expression in CRC cells resulted in increased degradation of β-catenin and a decrease in TCF/LEF activity only in the presence of Siah-1, indicating that p53 degradation of β-catenin is dependent on Siah-1 activity[185,187]. Because Siah-1 expression is regulated by p53, the loss of p53 transcriptional activity inhibits Siah-1 expression and activity, preventing the p53/Siah-1 pathway activity to cause β-catenin degradation[187].

In addition to affecting retinoid metabolism and storage, retinoid treatment in many different cell types induces p53 mRNA and protein expression to inhibit cell cycle progression and promote apoptosis[188-193]. ATRA treatment of keratinocytes led to an increase in p53 mRNA and protein levels and a corresponding increase in caspase-3, 6, 7, and 9 enzyme levels, which are responsible for mediating apoptosis[188]. Apoptosis and growth inhibition of mammary carcinoma cells is controlled by RA-induced p53 activity increase, which in turn upregulates the expression of the anti-proliferative B-cell translocation gene, member 2 (Btg2)[191]. Btg2 inhibits cell cycle progression by down-regulating the expression of cyclin D1, and this effect is further augmented by the over-expression of CRABP-Ⅱ, which transports RA to nuclear RAR, to induce the transcription of RA-responsive genes[191]. In murine embryonic stem cells, ATRA caused neural differentiation and apoptosis through increasing p53 mRNA and protein levels to instigate cell cycle arrest[189]. The up-regulation of p21 protein concentration is an important effect of p53 activation as shown in human mammary epithelial cells, of which treatment with 9-*cis*-RA, ATRA,

and fenretinide increases p21 expression and thus, cell growth, in a p53-dependent manner[190]. Furthermore, p21 expression in breast cancer cells and HCT-116 CRC cells is increased by p53 interaction with the tumor suppressor activating enhancer-binding protein-2 α (AP-2α), a RA-inducible gene that regulates apoptosis, cell growth, and differentiation[192]. AP-2α interaction with p53 resulted in enhanced binding to the promoter of p21, which led to cell cycle arrest in these cells[192]. The induction of STRA6, the RBP receptor, by p53 has also been shown to mediate apoptosis in ovarian cancer cells, normal human fibroblasts, and HCT-116 cells expressing wild type p53[193]. Transfection of these with STRA6 increased apoptosis, and inhibition of STRA6 severely compromised p53-induced apoptosis[193]. While the effects of retinoids on p53 expression and activity have not been widely studied with regard to CRC, the known results are summarized in Table 1. In general, retinoid treatment of CRC cells appears to enhance the expression and activity of p53 to further increase tumor suppressor p21 levels, ultimately leading to cell cycle arrest and the initiation of apoptosis.

CONCLUSION

Retinoids decrease signaling *via* the major pathways that promote CRC progression. Ultimately, each pathway is followed to its conclusion, retinoids decrease levels of MMPs, cyclin D1, and other factors that induce cellular invasion or proliferation. Often, β-catenin is an intermediate in these pathways, reflecting the central role of β-catenin in CRC progression. Overall pathway interactions are illustrated in Figure 2, and effects of mutations on CRC progression and the effects of retinoids on these mutated proteins are summarized in Table 1. Because retinoids inhibit critical pathways to decrease CRC progression, dietary vitamin A supplementation or retinoid chemotherapy, alone or in combination with other medications, may prove beneficial for the prevention of the progression and metastasis of CRC.

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TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Treatment of colorectal cancer in the elderly

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Abstract

Colorectal cancer has a high incidence, and approximately 60% of colorectal cancer patients are older than 70, with this incidence likely increasing in the near future. Elderly patients (> 70-75 years of age) are a very heterogeneous group, ranging from the very fit to the very frail. Traditionally, these patients have often been under-treated and recruited less frequently to clinical trials than younger patients, and thus are under-represented in publications about cancer treatment. Recent studies suggest that fit elderly patients can be treated in the same way as their younger counterparts, but the treatment of frail patients with comorbidities is still a matter of controversy. Many factors should be taken into account, including fitness for treatment, the wishes of the patient and family, and quality of life. This review will focus on the existing evidence for surgical, oncologic, and palliative treatment in patients over 70 years old with colorectal cancer. Careful patient assessment is necessary in order to individualize treatment approach, and this should rely on a multidisciplinary process. More well-designed controlled trials are needed in this patient population.

Key words: Colorectal cancer; Surgery; Chemotherapy; Radiotherapy; Elderly; Palliative care

Core tip: With the rise in the incidence of colorectal cancer and in the population > 70 years of age, the need to decide what type of treatment is most appropriate for patients > 70 with colorectal cancer will become more frequent. Age in itself should not be an exclusion criterion for radical treatment, but there will be many elderly patients that will not tolerate or respond well to standard therapies. These patients need to be properly assessed before proposing treatment, and a tailored, individualized approach should be offered in a multidisciplinary setting.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers worldwide, and its incidence is increasing[1]. The choice of treatment is based on several factors, including stage at presentation, location, and the conditions of the patient. Current treatment in general for CRC includes surgery for CRC stage Ⅰ or Ⅱ; surgery followed by adjuvant chemotherapy for stage Ⅲ colon cancer; and in cases of metastatic CRC (mCRC), systemic chemotherapy alone or in combination with targeted biologics. mCRC requires multidisciplinary management, where surgical resection of metastatic disease is considered wherever possible. The treatment of rectal cancer includes surgery alone in stage Ⅰ or short-course radiotherapy or chemoradiotherapy with surgical resection followed by adjuvant chemotherapy in selected stage Ⅱ and Ⅲ patients[2].

Approximately 60% of CRC patients are > 70 years of age at the time of diagnosis, and 43% are > 75[1]. These proportions will likely continue to increase in the near future. Many of these older patients will have problems of frailty and comorbidity that demand careful patient assessment, and, if necessary, individualized treatment approaches[3].

Aging may be defined as a progressive decline in the functional reserve of multiple organ systems. This process is highly individualized, and poorly reflected in chronological age. The treatment of cancer should be based on the assessment of the physiological age, the patient's life expectancy, and tolerance to treatment[4]. Older patients risk being undertreated, and, therefore, presenting a worse oncologic outcome. If they are over treated, however, there is an increased risk of morbidity and mortality[5].

The challenge in this group of patients comes from the physiological heterogeneity of the older patient population, with frequent discrepancies between physiological and chronological age, coupled with the additional complications of coexisting medical conditions and potential psychological and social care issues[6].

The treatment of those at the upper extreme of life often presents significant clinical dilemmas. A critical appraisal is needed of the costs and benefits of treatment, and a better selection of patients who can benefit from available therapies is warranted. There is a paucity of controlled trials including this group of patients, and, therefore, evidence-based decision-making is difficult. Many elderly patients will benefit from radical treatment approaches, but others will not, and in some cases, non-operative "palliative" management should be offered, even though the cancer is "curable". This review aims to focus on the existing evidence to aid in the decision-making process for treatment of CRC in elderly patients.

GERIATRIC ASSESSMENT

The patient's biological age should ideally be established through a comprehensive geriatric assessment in order to aid therapeutic decisions.

There is a paucity of clinical trial data in these patients who, in many cases, have poor functional reserves, major comorbidities, and frailty. In older patients, functional levels vary widely- from robust and able to tolerate cancer treatments to frail and unable to tolerate even minor interventions without life-threatening consequences. At either end of this spectrum, treatment decisions are clear, but the identification of individuals at risk for functional decline and frailty, where interventions or treatment modifications are needed, is where geriatrics could have the biggest impact on oncology[7].

By distinguishing the fit from the vulnerable older patients, treatment can be adjusted to maximize its effectiveness, avoid complications, and better meet the individual requirements of the older patient. When choosing between various treatment options, quality of life and function may be at least as important for the elderly as the cancer-specific or surgical outcome[6].

The main difficulty for individualizing treatment in elderly patients is the capacity to evaluate vulnerability to treatment. Several aspects should be taken into account[8], which include: (1) an estimation of life-expectancy based on functional evaluation and co-morbidities; (2) an estimation of the risk of cancer-related morbidity: a: Tumor stage at diagnosis; b: Risk of recurrence and tumor progression; and c: Tumor aggressiveness; (3) an evaluation of the conditions that could interfere in the cancer treatment and tolerance; a Comprehensive Geriatric Assessment[7] (CGA), which includes: a: undernutrition (recent loss of > 5% weight/body mass index < 19); b: polypharmacy (more than 10 medications); c: social isolation; d: depression; e: cognitive disorder; f: risk of falls; g: side effects of neoplasia: sensory deterioration, urinary incontinence, sexual dysfunction; h: comorbidities (number and severity of co-existing illnesses); and (4) an evaluation of the goals of the patient (what the patient expects from treatment). An important aspect of this evaluation is quality of life (subjective evaluation of life as a whole). The instruments that can be used to measure quality of life include, at least three of the following 10 aspects[9,10]: Pain and other somatic symptoms, functional capacity, social and family well-being, emotional well-being, spirituality, satisfaction with care, future hopes and wishes, sexuality, body image, and social and work-related function.

Elements of the CGA, especially comorbidity, functional status, cognitive dysfunction, and frailty, are consistently associated with adverse treatment outcomes in relation to both toxicity and mortality[11-13].

A complete CGA is time-consuming. For now, it might be beneficial for all elderly patients with cancer

to receive a complete geriatric assessment[14], although recent publications show promise in the use of frailty screening methods to select which patients will benefit from a complete CGA or further assessment: (1) test Timed Up and Go: Patients who require more than 10 s to perform the exercise, need to use their arms to get up, or perform an erroneous trajectory will need a full CGA[15,16]; (2) seven-item physical performance: this test takes 10 min to perform. If the total result is less than 20, a CGA would be beneficial. It has been demonstrated to be more sensitive than the Karnofsky Performance Status in recognising patients with a higher risk of functional decline[16]; and (3) the Vulnerable Elderly Survey 13 (VES-13)[17]: when the scores are equal or above 3 it indicates a higher risk of functional deterioration, and a 4-fold increased probability of death in the next 2 years, and, therefore, a complete CGA is indicated[18-21].

In 2012[22], an algorithm was proposed to evaluate an elderly cancer patient that uses the frailty criteria, the VES-13 scale and the CGA. All patients diagnosed with cancer would be tested using VES-13. If the score is < 3 the patient can receive the standard treatment recommended for adult patients according to tumor stage. If the score is > 3, a full CGA is recommended, and further recommendations can be made according to the possibilities of treatment of the patient's comorbidities or functional dependence; palliative or standard treatment could be recommended.

The concept of frailty is still under construction and has many common aspects with the definition of aging. Fried *et al*[23] criteria include an assessment of weight loss, physical exhaustion, physical activity level, grip strength, and walking speed. Any degree of frailty measured by the Hopkins Frailty Score[24] has been linked to a worse postoperative outcome after surgery for CRC. Core features of frailty include impairments in multiple, interrelated systems, resulting in a reduced ability to tolerate stressors. This is associated with an increase in vulnerability to severe complications with cancer treatment, which translates into an increase in global mortality[25,26].

The CGA should include the following determinations[27]: (1) functional status: Evaluation of dependency in daily activities using scales such as Barthel and Lawron, the TITAN scale, and Karnofsky index. Functional decline in elderly patients is a predictor of short- and medium-term mortality, independent of the disease process[28]; (2) coexisting illness (Comorbiditiy): The Charlson comorbidity index[29] predicts 1-year mortality in patients with comorbidities. Sarcopenia (skeletal muscle depletion) in older patients is related to infection, requirements for rehabilitation following surgery, and length of hospital stay[30]; (3) socio-economic evaluation: the elderly population is at a greater risk of social deprivation[28]. The social situation of the elderly patient should always be evaluated, and the detection of social isolation should lead to the application of the necessary social resources; (4) nutritional status: Mini Nutritional Assessment[31]. An albumin < 2.5 g/dL + CT < 156 mg/dL + weight loss of 10% indicates terminal illness; (5) cognitive status: Mental Status Questionnaire-Pfeiffer and Mini Mental State Examination. The impact of depression and dementia on oncologic treatment is not well known[32,33], but it has been identified as one of the determinant factors in receiving inadequate treatment[34,35]; (6) geriatric syndromes: sleep disturbances, incontinence, risk of falls, *etc*. The presence of geriatric syndromes is an indicator of frailty. An assessment of the cognitive and emotional state is especially important in older cancer patients. Polypharmacy is common in older patients, and the possibility of drug interactions and the delicate clinical situation in a geriatric cancer patient should be considered; (7) surgical risk: The American Society of Anesthesiologists (ASA) classification continues to be one of the most reliable predictors of postoperative morbidity and mortality[34,35]. Multiple studies have shown that the presence of comorbidities increases the risk of postoperative complications, and this is more evident in patients over 70 years of age[35]; and (8) An evaluation of the patient's views on the goals of treatment (what does the patient expect and want?). Optimal treatment of the older adult patient who has cancer starts with a careful delineation of goals through conversation. There is a general tendency to think that geriatric patients do not want to be informed about the diagnosis and prognosis of their disease; however, several studies refute this hypothesis[36,37]. In reality, there does not seem to be any difference with respect to age regarding the wish of cancer patients to receive information[38].

Multidisciplinary cooperation involving oncologists, gastroenterologists, radiotherapists, anesthetists, radiologists, pathologists, and surgeons has become essential in elderly patients. Geriatricians are not typically members of MDTs, but there is clear evidence that older CRC patients should be treated in centers where the expertise is available to provide the most favorable surgical and oncologic treatment and care[21,39].

Balducci[40] studied the role of CGA in the selection of oncologic treatment and divided patients into three groups depending on the severity of frailty symptoms and signs: Type Ⅰ: Functionally independent patient without important comorbidities: these patients would be candidates to receive onco-specific treatment in standard conditions; Type Ⅱ: Functionally dependent patient with two or less comorbidities: these patients could benefit from a modified onco-specific treatment with standard intention; and Type Ⅲ: Partially dependent patient with three or more comorbidities or the presence of a geriatric syndrome: these patients would be candidates for symptom treatment exclusively (palliative care).

SURGERY

There is no consensus about the optimal surgical

management of elderly people, who are a heterogeneous group of patients, ranging from very fit to very frail individuals. This population is undertreated compared with younger patients, with a lower percentage of patients operated on; a lower rate of curative surgery, and more emergency surgery. Elderly patients are generally recruited to clinical trials less often than younger patients and are under-represented in publications about cancer treatment[41].

A comprehensive geriatric assessment is a major consideration when assessing operative risk, treatment decision making, and adapting perioperative care, if surgery is undertaken.

Surgical risk stratification remains one of the most important aspects of management in elderly patients[42]. Age is associated with increased mortality following elective colorectal resection, up to 15.6% in patients > 80 years of age. Elderly patients with higher levels of comorbidity might be expected to have significantly higher rates of complications, longer hospital stays, and higher mortality[43].

Elderly patients deemed to be optimized for surgery through traditional clinical and biochemical markers may still have poor outcomes. The concept of frailty can be used to identify a group of patients for further investigation before surgery[23]. Patients who were positive for frailty had 4 times higher risk of developing major complications (OR = 4.083; 95%CI: 1.433-11.638)[43]. Decreased survival in older (> 75 years) patients post-surgery has mainly been attributed to differences in early mortality[44-48]. The rate of cardiovascular complications increases significantly with age. Pulmonary complications are also twice as common. Postoperative complications are more severe in elderly patients[49-52]. The occurrence of a complication was associated with a significantly increased risk of 6 mo mortality. Overall, 6 mo mortality was 4 times higher in elderly patients than in younger patients (14% *vs* 3.3%; $P < 0.0001$) as was the 1-year mortality rate (20.1% *vs* 5.1%)[53]. Progressive loss of stress tolerance with aging exacerbates the consequences in case of postoperative complications[54]. However, older patients with CRC who survived the first year after surgery had the same overall cancer-related survival as younger patients[53].

Therefore, the focus should be on survival and minimizing postoperative complications during the first postoperative year. Pre-habilitation programs could be of great importance in elderly patients: Correction of malnutrition, optimization of cardiovascular and pulmonary comorbidities, and medication use have been shown to reduce complications after elective surgery in elderly patients and are a promising area of future research[54].

Emergency surgery should be avoided if possible. The presence of obstruction or perforation increases the perioperative mortality rate in older patients. Several studies show the correlation between advanced age, mortality, and emergent surgery. Kurian *et al*[55] reported a postoperative 30 d mortality rate of 28% in emergent surgery compared to only 5% in elective surgery. Morse *et al*[56] found similar outcomes in 39 patients older than 80 in open colectomy for colon cancer. In the same way, Louis *et al*[57] observed the close correlation between advanced age, advanced ASA grade, and emergent surgery, and other authors found that no patients with an ASA grade of 3 or more survived more than 6 mo[58]. Modini *et al*[59] reported a 6 fold higher 30 d postoperative mortality in elderly patients > 80 years of age with respect to others. They noted that although morbidity and mortality rates in elderly patients could be similar to that of younger patients, it would rise up to 9 fold higher in cases of emergent surgery[60,61]. Patients over 70 years of age after emergency surgery have been shown to have a higher rate of postoperative myocardial infarction, and this complication is associated with a 6 times higher rate of mortality in the postoperative period[62]. Other common complications are pulmonary failure, acute renal failure, and sepsis; anastomotic leakage also occurred more frequently in elderly patients after emergency colorectal surgery and presented a significant association with postoperative mortality[63].

A feasible alternative management to emergency surgery for colonic obstruction could be the endoscopic placement of stents, especially in acute left-sided colonic obstruction. Use of these self-expanding metallic stents would provide "extra time" to better study the patient's clinical situation and the tumor-stage, improve the nutritional status, optimize comorbidities, and, in some cases, allow a subsequent elective surgery. Consequently, it is an appealing option either for palliation or as a "bridge" to definitive surgery in the management of left-sided colonic obstruction for elderly patients. Nevertheless, the current data are controversial and the advantages in terms of early morbidity and mortality compared to emergency surgery are not as clear as originally described[64].

Laparoscopic surgery has been shown to reduce postoperative pain, allowing a decreased use of narcotics and opioids, reduced postoperative ileus, and a reduced hospital stay[65]. Furthermore, elderly patients benefit from laparoscopic surgery because it reduces the risk of cardiovascular and pulmonary complications, reduces intraoperative blood loss, and seems to accelerate gastrointestinal recovery. Stocchi *et al*[66] found that the preoperative functional status of patients was more frequently maintained at the time of discharge in elderly patients operated on by laparoscopy. In a randomized trial including 553 patients, Frasson *et al*[65] similarly concluded that laparoscopy should be the first choice in elderly patients operated on for CRC because it increases preservation of functional status, allowing a higher rate of independence during the postoperative period and discharge and a faster postoperative recovery.

However, most trial protocols of laparoscopic surgery for CRC have been biased to exclude or under-

represent the elderly. Decision-making for such patients is, therefore, still based on inadequate evidence[67-69]. Clinical trials on laparoscopic surgery in the older population are lacking: 44% of trial protocols excluded elderly patients. Nevertheless, since a higher systemic inflammatory response to the surgical aggression and lower physiological reserve appear to be the origin of the high postoperative mortality in the elderly patient[70-73], laparoscopic surgery could be beneficial due to its decrease in inflammatory response and lower surgical stress[74-79].

The literature suggests that elderly patients benefit from multimodal rehabilitation programs or enhanced recovery programs after surgery (ERAS) in the same way as younger patients[80]. Initial studies by Senagore *et al*[75] and more recent studies by Keller *et al*[81] and Wang *et al*[82] showed better results in terms of length of stay, readmission rate, and reoperation rates for elderly people using ERAS programs. Elderly patients benefit from the avoidance of bowel preparation, opioid restriction, and early mobilization. There does not seem to be an increased risk of aspiration pneumonitis in elderly patients following early resumption of oral feeding, although overall complications are higher in elderly patients[80].

Delays in discharge of elderly patients can be attributable to inadequate levels of social support or resources in the community, even when the postoperative course has been uneventful. Liaison with elderly care physicians may minimize avoidable hospital stay by optimizing the management of geriatric syndromes and by pre-emptively addressing the psychosocial needs of older patients. Specialized, organized, and coordinated geriatric care in the hospital setting improves outcomes, such as survival and in their own home up to 1 year after surgery[83-85].

In spite of all of the above, the fact still remains that some elderly patients will do very well after curative surgery, and others will not[86,87]. It is quite clear from the literature that the risks and benefits of surgery for CRC in the elderly have not been clearly reviewed[86]. There is, therefore, still no common consensus on how actively we should treat the elderly and when not to push them into unnecessary surgery, which could lead to severe functional impairment and diminished quality of life. Over 74% of patients interviewed in a recent study stated that they would refuse, or be reluctant, to receive treatment leading to severe functional impairment[87]. Life-expectancy, higher rates of 60 d mortality, higher likelihood of impairment of physical and mental function, and the possibility of never returning home and needing permanent residential care, should ideally be considered and discussed with the patient and family before deciding on surgical treatment[88].

RECTAL CANCER

Older patients with rectal cancer undergoing surgery should receive the same treatment as their younger counterparts, but with an adjustment of treatment strategy in the case of comorbidity, limited physiologic reserves, and emergency situations. Complete mesorectal excision is considered the "gold-standard" surgical treatment for rectal cancer, but we continue to look for alternatives to avoid the high rates of postoperative morbidity[89]. Elderly patients are less frequently treated with neoadjuvant radiotherapy or chemotherapy, and non-restorative procedures are more frequently used. Anterior resection is performed less often in elderly patients, although tumor location and stage does not differ[90-92].

Population-based studies clearly show that older patients with rectal cancer are treated less often with RT[90-92]. Fewer older patients are likely to receive preoperative RT with proportionately more receiving palliative RT as an alternative[93]. Older patients with stage II or III rectal cancer who are fit enough for surgery are generally fit enough for preoperative neoadjuvant radiation therapy. Tolerability and response rates are similar to those seen in younger patients. However, Stockholm I and II Trials have shown the distinct negative effects of neoadjuvant radiotherapy in older patients (> 80 years). The incidence of venous thromboembolism, femoral neck and pelvic fractures, intestinal obstruction, and postoperative fistulas was significantly increased after preoperative radiotherapy in this group of patients[90,94].

The aim of rectal cancer surgery in older patients should be not only to avoid local recurrence but also to maintain health and function with a view to optimizing their chances of coping with their treatment. Older patients are keen to avoid a permanent stoma and may accept a higher risk of local recurrence to achieve this. The impact of cancer surgery on quality of life is very important in elderly people. Sphincter function, assessed clinically and if necessary after manometry, is an essential element to consider in the preoperative assessment and the decision-making procedure. The delay of surgery following short-course radiotherapy has also been associated with a decrease in postoperative morbidity.

Rather than age itself, the frailty of patients and preoperative sphincter function determine the operative indication and type of surgery[94,95]. Sphincter preservation in the elderly could give poor functional results with a higher risk of anal incontinence, and the potential effect of a permanent stoma on quality of life should be considered. Age was found as a significant risk factor associated with a decreased likelihood of stoma reversal[95].

Proctectomy in nursing-home residents has been associated with a 1 year postoperative mortality of 51% in patients with a permanent colostomy. Substantial postoperative mortality occurred in the first 6 mo after proctectomy and was significantly higher in elderly populations[96,97].

It has been observed that with neoadjuvant treatment there is a percentage of patients who present a

complete pathological response (pCR), up to 44%[98,99]. There is an increasing interest in a more conservative treatment for these patients. Several authors have proposed a "watch and wait" policy for patients when no residual tumor can be found. In a study published in 2010[100], the authors proposed an analytical decision model comparing the results between empirical radical surgery and observation alone in patients with pCR, and concluded that observation is better than surgery in cases where the ability to detect patients with pCR is higher than 58%, when patients will not have a good quality of life after surgery, or when the risk of recurrence was less than 43% when compared to observation. This study only included patients < 65 years of age, and excluded elderly patients with comorbidity[100].

Following the same working model, Smith *et al*[101] published a study in 2015 evaluating the differences between radical surgery and observation after neo-adjuvant treatment in cases of pCR and divided patients into three groups: Healthy 60-year-old patients, healthy 80-year-old patients, and 80-year-old patients with associated comorbidity. The study concluded that elderly patients, because of their higher surgical risk, obtained the greatest benefit from the "watch and wait" policy and showed an improved survival at 1 year after treatment.

The groups of patients that present a significant tumor regression with neoadjuvant chemoradiation, and especially those with lymph node regression (ypN0), could be candidates for alternative treatments for rectal cancer without needing total mesorectal excision (TME). Transanal endoscopic surgery could be an interesting option in these patients[102,103]. Recent studies have attempted to detect the subgroups of patients with a good response to neoadjuvant treatment where transanal endoscopic surgery could reduce the recurrence rate[104-106]. Habr-Gama *et al*[107] pioneered the decision not to operate on patients with rectal cancer who presented a complete clinical response after chemoradiation. This same group has published a series of "watch and wait" in 70 patients with cT2-4cN1-2 treated with chemoradiation, and of the 47 patients with a complete clinical response, eight (17%) presented an early recurrence and four a late recurrence. All had subsequent radical R0 surgery and were disease-free 56 mo later. This could be an option for patients who are not considered fit for surgery; the difference would be that it does not have to be considered a palliative treatment but a possible standard treatment with a 50% probability of cure in frail elderly patients.

No prospective randomized trials comparing the results of neoadjuvant chemoradiation and local excision include elderly patients, but the results in the general population can be taken into consideration in these patients. A study by Bhangu *et al*[108] analyzed the results of local excision in elderly patients and concluded that local excision achieved the same results as radical surgery in patients with pT1 tumors, the same as in the general population, but decreased survival in pT2. The difference with the general population could be due to the amount of comorbidities present in this group of patients; they would not be candidates for the same type of chemoradiation treatment, and, therefore, the results would not be comparable with those published up to the present time.

However, transanal endoscopic surgery can also be considered as a palliative treatment in patients with comorbidities who are not fit for radical surgery or who refuse a stoma, after carefully considering all options[109].

BIOLOGICAL FEATURES OF CRC IN THE ELDERLY

CRC is related to age, but there are few available data on the genetic differences and alterations in the carcinogenesis process between younger and older patients.

In many studies, younger patients are more likely to have mucinous, poorly differentiated and signet ring tumors, but there are mixed results in terms of prognosis. Several studies have suggested that younger age was a poor prognostic factor[110-112], but others suggested the opposite when adjusting for confounding variables, such as tumor, treatment, and patient factors[113-118].

The most frequently observed somatic mutations in CRC were found in the *APC*, *TP53*, *KRAS,* and *PIK3CA* genes.

A model has been proposed for the carcinogenic process in sporadic CRC, in which normal colonic mucosa would transform into invasive carcinoma. This model, named chromosomal instability pathway (CIN), implicates somatic mutations in a multi-step process, with alterations in different genes in chronological order [APC, Kirsten rat sarcoma (KRAS), Smad2/4, and tumor protein 53 (TP53)]. In a minority of cases of sporadic CRC, approximately 15%, the pathway responsible for the transformation of the colon epithelium is through an inappropriate mismatch repair system (MMR). The system cannot repair the mismatches, resulting in a length variability of DNA microsatellites, called microsatellite instability (MSI). Another proposed pathway responsible for the carcinogenic process is DNA hypermethylation [CpG island methylator phenotype (CIMP)][119,120].

Patients with the same stage of disease have a different natural history and a different prognosis, as a result of the heterogeneity of the process. Some conditions give a more favorable prognosis (MSI, BRAF not mutated) or a worse prognosis (hypermethylation and not MSI). Currently, the only marker applicable to clinical practice is the *RAS* mutation.

In an analysis of 181 patients with CRC, patients were divided into different groups: Those under 50 years of age, from 51 to 70, and over 70. In the

group of patients over 70 years of age, the MSI and BRAF mutations were correlated, but there was no correlation in the group under 50. Mutations in the *KRAS* and *BRAF* genes were more common with age, but no phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) mutations were found. TP53 mutations were more common in older patients. There were no differences in the frequency of phosphatase and tensin (*PTEN*) gene mutations. The conclusions were that older patients had a greater index of genetic mutations, and the incidence of BRAF mutations was higher. CIMP tumors are more common in the older population, who also have a higher rate of KRAS and BRAF mutations. These mutations have treatment implications[120]. TP53 mutation is associated with more advanced stages and vascular and lymphatic involvement[121]. *KRAS* gene mutation is a predictor of resistance to treatment with monoclonal antibody receptor endothelial growth factor (EGFR)[122-124]. BRAF V600E mutation confers worse prognosis[125,126]. A deficiency of the MMR system appears to be a favorable prognostic factor associated with adjuvant treatment in stage Ⅱ CRC[127,128].

CHEMOTHERAPY

The aging process involves an organic functional impairment, with decreased liver and kidney function, decreased bone marrow reserve, increased risk of cardiovascular events, cognitive impairment, other comorbidities, or use of polypharmacy. These conditions favor a greater toxicity with chemotherapy, which results in a diminished quality of life and adherence to treatment. The most commonly used scales to evaluate functional status, such as the Karnofsky performance status or the Eastern Cooperative Oncology Group (ECOG), should be used in the context of a comprehensive geriatric assessment in order to classify the elderly as fit or frail, the latter being more exposed to higher toxicity with chemotherapy, hospitalization, and death.

There is a consensus that frail patients with ECOG PS 3 or 4 or IK less than 60 are not eligible for chemotherapy due to poor benefits and high toxicity; the consensus seems also clear about being more aggressive in fit patients. The challenge is to decide the best treatment for those who are neither fit nor frail[129,130].

Adjuvant treatment

The benefit of adjuvant chemotherapy for stage Ⅲ (node positive) CRC is well established, representing approximately a 30% reduction in the risk of recurrence and a 22%-32% reduction in the risk of death compared with observation alone. Elderly patients are referred to the oncologist less frequently than younger patients, especially those with comorbidities, and when referred they are less likely to be treated with chemotherapy. An update of SEER - Medicare analysis data and three population-based data sets conducted by Sanoff *et al*[131] showed that only 44% of the 5941 patients evaluated received adjuvant chemotherapy within 3 mo of surgical resection for stage Ⅲ CRC.

Since 2001, intravenous 5-fluorouracil modulated with leucovorin (FU/LV) in the adjuvant setting has shown better outcomes than observation, even in elderly patients. A pooled analysis of 3351 patients from seven randomized phase Ⅲ adjuvant chemotherapy trials comparing chemotherapy *vs* surgery alone for stage Ⅱ or Ⅲ colon cancer showed a 29% reduction in the risk of death at 5 years[132]. The benefit was independent of age, and no differences in toxicity were seen with respect to younger patients. Only one study showed a greater proportion of grade 3 or 4 neutropenia (8% *vs* 4%) without increased neurological toxicity, diarrhea, infection, nausea, or vomiting.

Capecitabine (an oral fluoropyrimidine) also proved to be as effective as FU/LV in adjuvant treatment in a subgroup analysis of patients equal to or greater than 70 years of age, with no differences in toxicity by age, although it was more toxic than FU/LV[133,134].

These results are supported by other studies with patients of 80 years of age or more, where there was a higher incidence of grade 3 or 4 toxicity, especially diarrhea (31% *vs* 13%) and hand-foot syndrome[135]. With the MOSAIC trial, oxaliplatin was established as a new adjuvant standard in combination with 5FU/LV plus infusional 5FU short-term and leucovorin (FOLFOX) as compared with 5FU and leucovorin alone in resected stage Ⅲ colon cancer, with a 20% reduction in the risk of recurrence and a 16% reduction in risk of death at 6 years. But the analysis of 315 patients over 70-75 years of age revealed that although there was a survival benefit with fluoropyrimidines, there was no benefit in disease-free survival (DFS), overall survival (OS), or time to recurrence (TTR) by adding oxaliplatin [OS hazard ratio (HR) 1.10, 95%CI: 0.73-1.65] or in patients with stage Ⅱ tumours[136].

The National Surgical Adjuvant Breast and Bowel Project (NSABP) C-07 trial analyzed 2409 patients in stage Ⅱ or Ⅲ treated with weekly bolus of FU and leucovorin with or without oxaliplatin. The results showed that the addition of oxaliplatin to 5FU/LV gave no survival benefit in patients equal to or greater than 70 years of age in stage Ⅱ or Ⅲ colon cancer (n = 396), but a higher grade 4 toxicity (20% *vs* 13%) was found. The benefit in OS was only observed in patients under 70 years of age[137]. In contrast, the N016968 trial, which randomized capecitabine *vs* bolus 5FU and oxaliplatin in stage Ⅲ exclusively, showed an increase in DFS in both populations under or over 65 years of age with an HR 0.8[138].

The Adjuvant CC End Points (ACCENT) database (including seven randomized trials such as MOSAIC, NSABP C-07, and N016968) included 14528 patients in stage Ⅱ or Ⅲ treated with a 5FU combination with oxaliplatin or irinotecan *vs* 5FU alone. The results of the 2575 patients greater than or equal to 70 years of age did not show a benefit in DFS or OS by

adding oxaliplatin to adjuvant treatment (DFS: HR = 0.94; 95%CI: 0.78-1.13; OS: HR = 1.04; 95%CI: 0.85-1.27). They did not consider death from other causes or change in efficacy due to reductions or delays of doses[139]. In contrast to these data, the analysis of Sanoff *et al*[131] with 4060 patients in stage Ⅲ CRC including five cohorts, the largest cohort of the SEER-Medicare database, saw a marginal benefit with no statistically significant difference when adding oxaliplatin. Also, there were more adverse events with oxaliplatin compared with fluoropyrimidine. Among patients older than 75 years of age, more neutropenia (OR = 17.3, 95%CI: 9.8-30.42) and nausea or vomiting were found (OR = 2.14, 95%CI: 1.73-2.65) without differences in diarrhea or hydration[140]. In summary, it seems that the benefit and toxicity of 5FU/LV in the adjuvant setting is similar between young and elderly patients.

Although adjuvant treatment is offered to patients in stage Ⅱ CRC with risk factors (T4, perforation, lymphovascular or perineural invasion, poorly differentiated histology), the benefit of adjuvant chemotherapy for stage Ⅱ is more controversial, and there are no data to ensure which patients are most likely to benefit from adjuvant treatment.

In an attempt to identify the subgroup of patients with stage Ⅱ CRC who may benefit from adjuvant therapy, there have been efforts to find prognostic biomarkers. The deficiency of the MMR system or MSI seems a promising marker. Several studies have found an association between high microsatellite instability (MSI-H) and better prognosis but resistance to treatment with fluorouracil[141].

It seems reasonable to analyze the MMR deficiency in patients with T3 stage Ⅱ to select those who could benefit from treatment with 5FU. Its application has not been validated in clinical practice, and, therefore, clinical decisions to administer chemotherapy should not be based on this analysis. It is not a common occurrence in the metastatic context and does not seem to play a role in the prognostic stratification.

Data from the SEER-Medicare database indicate that adjuvant treatment does not increase the OS in patients over 65 years of age with stage Ⅱ CRC with or without risk factors[142]. In stage Ⅱ patients with risk factors, the chemotherapy options are FU/LV or capecitabine if the patient is capable of adhering to the medication, although no differences were found in the Quasar study. This study showed a marginal benefit in OS of 3.6% in patients greater than or equal to 70 years of age with stage Ⅱ CRC[143]. The lack of benefit in stage Ⅱ does not justify the use of oxaliplatin. The benefit of adding oxaliplatin in patients > 70 years of age in stage Ⅲ CRC is doubtful and is not supported by data from the results of clinical trials, such as MOSAIC and NSABP, even though the elderly population included was very small. It is difficult to establish whether 70 years old is a reasonable cut-off age to safely extrapolate these results or if the decision should depend on the physical and functional status of the patient, not only on the chronological age. In fit elderly patients with stage Ⅲ CRC with a life expectancy of at least 5 years, the benefit of adding oxaliplatin must be discussed. The modified FOLFOX 6 scheme (due to less hematologic toxicity, without bolus if necessary), or XELOX with capecitabine at 1000 mg/m^2, should be considered. If the patient has no serious comorbidity, the full dose should be given. In patients neither fit nor frail with some comorbidity, dose reduction should be considered.

Frail patients with Eastern Cooperative Oncology Group Performance Status 3 or 4 are not candidates for chemotherapy treatment. Therapy with targeted agents is not indicated in adjuvant treatment because of lack of benefit[144].

Treatment in metastatic patients

The goal of palliative chemotherapy in the elderly should be the same as in young patients but with special attention to treatment toxicity. It has been demonstrated in several studies and a meta-analysis that chemotherapy improves the overall survival and time to progression compared to observation. An analysis by Folprecht *et al*[145] of 22 trials showed benefits in OS, progression free survival (PFS), and TTR similar to younger patients (in 629 patients over 70 years of age).

Exposure to the drugs currently available is able to increase the OS, time to response , and the rate of metastatic resection with an average of approximately 24 mo of OS. Even with this data and probably due to toxicity concerns, elderly patients are less likely to be treated with these agents. A population-based study by Ho *et al*[146] reported that less than 50% of elderly patients with mCRC received palliative systemic chemotherapy.

Fluoropyrimidines are the mainstay of treatment and can also benefit elderly patients. Depending on the administration schedule, the toxicity profile is different; diarrhea and leukopenia are more frequent when administered in bolus (24% *vs* 14% and 24% *vs* 10% respectively)[147]. Treatment with capecitabine, because it is administered orally, is perceived to be innocuous, but although it is well tolerated in fit elderly patients, it is still more toxic than 5FU in combination therapy[148-154]. The MRC Focus 2 trial of elderly and frail patients confirmed the higher rate of gastrointestinal toxicity, such as diarrhea, vomiting, and anorexia, with no differences in efficacy[155].

The question is whether a more aggressive regimen is better. There are conflicting data: three phase Ⅲ studies did not observe a survival benefit with combination chemotherapy *vs* 5 FU/LV alone[155-157]. The MRC FOCUS 2 trial included 459 patients who were deemed not fit or too frail for full doses. They were randomized to 5 FU/LV with or without oxaliplatin, or capecitabine with or without oxaliplatin. Approximately 43% were older than 75 years of age, 13% older than 80%, and 29% with a Performance Status of 2. The addition of oxaliplatin improved response rate but not

DFS or OS, and the rate of grade 3 or 4 toxicity was not increased in the oxaliplatin arm, perhaps due to a lower administered dose. Capecitabine and 5FU were equivalent in terms of benefit on PFS (HR = 0.99, 95%CI: 0.82-1.2, *P* = 0.93) or OS (HR = 0.96, 95%CI: 0.79-1.17, *P* = 0.71); however, higher toxicity was observed with capecitabine and, as a consequence, also a lower quality of life.

The combination of irinotecan and 5FU provides the same benefits in the elderly as it does in younger patients, as seen in phase Ⅱ and Ⅲ trials, albeit at the expense of an increased gastrointestinal and hematologic toxicity[158,159]. The tri-weekly administration of irinotecan requires dose reduction in patients over 70 years of age because of an increase in the rates of neutropenia and diarrhea[160].

A phase Ⅲ French study FFCD 2001-02 randomized 282 patients older than 75 with mCRC treated by a first line of palliative chemotherapy with 5FU with or without irinotecan. A geriatric assessment was obtained in 123 (44%). Greater toxicity grades 3-4 (61% *vs* 39%) were observed in the combination arm, and these patients required more hospitalizations or dose reduction. There is no OS data available to justify the increase in toxicity. The study was not designed with sufficient statistical power, so more studies are still needed. IADL dependence and cognitive impairment were established as predictors of greater toxicity[154]. The combination of oxaliplatin and capecitabine (denominated Xelox) is well tolerated, although more toxic as seen in the MRC FOCUS 2 trial[152]. The combination of capecitabine with irinotecan (XELIRI) is more toxic with a high rate of dehydration and asthenia, and it is infrequently used in elderly patients[154-158].

The benefit of the new molecular targets has also been reported in the elderly population[159]. Specifically, bevacizumab (the vascular endothelial growth factor VEGF) increases both PFS and OS, as was observed in a retrospective subgroup analysis and pooled analysis of randomized trials, along with observational cohort studies. A pooled analysis of two randomized trials by Kabbinavar *et al*[160] with 439 patients older than 65 and 276 > 70 years of age, showed an improvement with bevacizumab in PFS of 9.2 mo *vs* 6.2 mo; HR = 0.52: *P* < 0.0001, and OS of 19.3 mo *vs* 14.3 mo, which is statistically significant (HR = 0.7). Another analysis by Cassidy *et al*[161], which included two more phase Ⅲ trials with 712 patients equal to or > 70 years of age and 1142 > 65, confirmed the benefit in OS and PFS with bevacizumab, even though an increased incidence of thrombotic events in patients over 65 years of age was seen (5.7% *vs* 2.5% patients > 65 years, and 6.7% *vs* 3.2% in those > 70 years of age).

The BRITE observational study, which included 896 patients > 65 years of age, also showed better PFS, despite a greater toxicity profile with regard to the incidence of thromboembolic events, that increased with age[162].

The AVEX study, designed to assess the efficacy and tolerability of capecitabine plus bevacizumab *vs* capecitabine alone, included 280 frail patients equal to or greater than 70 years of age. The results showed an increase in PFS (9.1 mo *vs* 5.1 mo) and relative risk (RR) (19.3% *vs* 10%) with no statistically significant difference in OS (21 ms *vs* 17 ms) but more toxic events in the bevacizumab arm (40% *vs* 22%) at the expense of hypertension, hand-foot syndrome, bleeding, and thromboembolic events[163].

In elderly patients, the combination of capecitabine and bevacizumab is effective, but the risk *vs* benefit must be discussed, especially in patients with vascular disease, myocardial infarction, thrombotic events, or severe uncontrolled hypertension in the 6-12 mo prior to the start of treatment.

Aflibercept, another angiogenesis-targeting agent, has demonstrated efficacy in treating mCRC in a recent randomized Phase Ⅲ trial (VELOUR). As a result, it has been approved in combination with FOLFIRI in the second line treatment for metastatic mCRC, supported by an improvement in OS of 13.5 mo *vs* 12.1 mo. The efficacy was similar in the elderly population studied. However, there is no more data available in this population[164]. The most frequently reported adverse events with aflibercept compared with the placebo arm were hemorrhage (2.9% *vs* 1.7%), arterial and venous thromboembolic events (9.7% *vs* 6.8%), grade 3 hypertension (19.1% *vs* 1.5%), and grade 3 or 4 proteinuria (7.9% *vs* 1.2%). Other adverse effects associated with chemotherapy were higher in the aflibercept arm: diarrhea, asthenia, stomatitis, infections (12.3% *vs* 6.9%), palmar-plantar erythrodysesthesia (2.8% *vs* 0.5%), neutropenia (36.7% *vs* 29.5%), and thrombocytopenia (3.3% *vs* 1.7%).

The data on the anti-EGFRs cetuximab and panitumumab in the elderly population are limited. They have been investigated in several trials either in combination or monotherapy in mCRC, with a manageable toxicity profile. Patients with mutations in codon 12 or 13 of the *KRAS* gene should not be treated with anti-EGFR antibody due to lack of benefit. The main adverse effect of these drugs is skin toxicity. The correlation between development and severity of rash with treatment response is unclear. An analysis of EGFR polymorphisms observed that carriers of D994D polymorphism have lower dermatological toxicity than other genotypes, with no difference in PFS or OS and age[165-169]. Mutations in RAS, BRAF, and PIK3CA have also been shown to be associated with resistance to anti-EGFR[170].

Several prospective and retrospective studies have shown no differences in toxicity compared to younger patients and the same clinical benefit. Therefore, these agents should be considered in fit elderly patients[163-169].

The latest drug approved for the treatment of mCRC, the multikinase inhibitor regorafenib, adds a modest increase in PFS without increasing OS. Median overall survival was 6.4 mo with regorafenib *vs* 5.0 mo with placebo (HR = 0.77; 95%CI: 0.64-0.94; one-sided *P* = 0.0052). Adverse events due to treatment

occurred in 465 (93%) patients with regorafenib and in 154 (61%) of those assigned to placebo. The most common adverse events of grade 3 or higher related to regorafenib were hand-foot skin reaction (17%), fatigue (10%), diarrhea (7%), hypertension (7%), and rash or desquamation (6%). There were no differences in toxicity between patients older or younger than 65 years of age in the subgroup analyzed, but there are no available data on efficacy or toxicity in the elderly or frail population[168]. Ramucirumab is a human IgG-1 monoclonal antibody that targets the extracellular domain of VEGF receptor 2. Ramucirumab in combination with FOLFIRI has recently been approved as a second line treatment, after progression with bevacizumab, oxaliplatin, and a fluoropyrimidine. Median overall survival was 13.3 mo for patients in the ramucirumab group *vs* 11.7 mo for the placebo with FOLFIRI group (HR = 0.844, P = 0.0219). The most frequently observed adverse effects grade 3 or worse were neutropenia (38% *vs* 23%), hypertension (11% *vs* 3%), diarrhea (11% *vs* 10%), and fatigue (12% *vs* 8%). The median patient age was 62, and, therefore, there is still not enough data in the elderly or frail population. One of the latest drugs, pending Food and Drug Administration approval, for the treatment of CRC is TAS-102. TAS-102 is an antitumor agent composed of a combination of trifluorothymidine (FTD), a nucleoside that incorporates into DNA and inhibits a variety of genetic functions required for the proliferation of cancer cells, and tipiracil hydrochloride, an inhibitor of thymidine phosphorylase (which degrades FTD) that maintains an effective blood concentration of FTD. Tipiracil protects trifluridine from being broken down when taken orally.

In a Phase 3 study, 800 patients with advanced CRC in refractory to oxaliplatin, irinotecan, fluorouracil, bevacizumab, regorafenib, and anti-EGFR (RAS wild type) were randomized to TAS-102 *vs* placebo. An increase of median overall survival was observed, from 5.3 mo with placebo to 7.1 mo with TAS-102 (HR of death 0.68, P < 0.001). The main grade 3 or higher toxicity was neutropenia (38%) and patients in the TAS-102 group were also more likely than those in the placebo group to have nausea of grade 3 or higher (2% *vs* 1%), vomiting (2% *vs* < 1%), and diarrhea (3% *vs* < 1%). The median patient age was 63. The benefit was seen in patients younger than and older than 65, but data are lacking in elderly or frail patients[171].

In summary, an elderly fit patient may be treated with FOLFIRI and FOLFOX (or XELOX) with or without antibodies, given the high response rate, especially if the treatment is given with neoadjuvant intention prior to surgery for metastases (M1), with certain precautions due to different toxicity profiles. Age by itself should not be a contraindication for M1 surgery. There are more data available for hepatic resections than pulmonary resections[172-176]. Surgical series that include all patients have a median OS of 40% at 5 years after liver resection, with a general perioperative mortality lower than 5%. Fit elderly patients with little comorbidity should be offered chemotherapy with the newer agents that increase the response rate and therefore resectability before surgery.

Two retrospective series of neoadjuvant chemotherapy prior to surgery based on oxaliplatin showed higher response rates as expected. Those who were operated had better recurrence-free survival[176,177].

For those patients unfit or with low IK or PS 2, the treatment may be of benefit if deterioration is related to the oncologic disease, although the benefit is lower and the toxicity higher. The risks or benefit should be evaluated and discussed individually in these patients. Fluoropyrimidine monotherapy or supportive care is probably the best choice in frail patients.

PALLIATIVE CARE

The "frail elderly" may be good candidates for palliative treatment, which can provide a better quality of remaining life. When to begin palliative care is a troublesome question for patients, but when frailty is severe, delivery of palliative care focused on relief of discomfort and enhancement of quality of life is highly appropriate. In addition to symptom management, preservation of functional independence is a major goal of treatment in the elderly. The application of multidisciplinary, team-based palliative approaches is beneficial for treating these patients because of the complexity of their coexisting social, psychological, and medical needs. Although death occurs far more commonly in older people than in any other age group, the evidence base for palliative care in older adults is scarce[178].

CONCLUSION

Older patients with colon or rectal cancer are less likely to receive guideline-recommended therapies. Decisions about cancer treatment in the elderly may be influenced by a number of factors, including pre-existing health problems (comorbidities) and other conditions that might cause the potential risks of surgery, chemotherapy, and radiotherapy to outweigh the benefits of treatment. Risk stratification based on comorbidities and biochemical and physiological markers could help to decide whether to perform surgery, what type of surgery, and the timing of surgery. Physiological rather than chronological age should determine the management of cancer in each individual[5].

Optimal treatment of the older adult patient who has cancer starts with a careful delineation of goals through conversation. Most elderly patients with cancer will have priorities besides simply prolonging their lives. Surveys have found that their top concerns include avoiding suffering, strengthening relationships with family and friends, being mentally aware, not being a burden on others, and achieving a sense that their life is complete[179]. The treatment plan should be comprehensive: cancer-specific treatment, symptom-

specific treatment, supportive treatment modalities, and end-of-life care[180].

The careful assessment of the patient, taking into consideration their functional status, level of frailty, life-expectancy, and wishes, should become an essential and central issue in their management, and choosing the appropriate therapy for each patient within a multidisciplinary process should be the future in the treatment of elderly patients with CRC.

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TOPIC HIGHLIGHT

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Immune cell interplay in colorectal cancer prognosis

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Abstract

The immune response to colorectal cancer has proven to be a reliable measure of patient outcome in several studies. However, the complexity of the immune response in this disease is not well understood, particularly the interactions between tumour-associated cells and cells of the innate and adaptive immune system. This review will discuss the relationship between cancer associated fibroblasts and macrophages, as well as between macrophages and T cells, and demonstrate how each population may support or prevent tumour growth in a different immune environment.

Key words: Colorectal cancer neoplasms; Fibroblasts; Immune system processes; Macrophages; T lymphocytes

Core tip: The outcome of patients with colorectal cancer is influenced by the complex local immune system. Understanding how multiple relationships between immune cells may affect tumour growth or elimination will be key in designing new therapies to treat this disease.

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PERSPECTIVE

Colorectal cancer (CRC) is the second and third most common cancer in women and men, respectively, worldwide[1]. In most cases, the disease occurs sporadically, but can also be caused by genetic predisposition or prior intestinal inflammation. While resection is often curative, approximately 45% of patients still die from the disease.

The recent introduction of successful immunotherapies against cancer, specifically checkpoint blockade antibodies, has increased attention on the immune response to tumours. These new treatments have provided opportunities for the development of new

immune-based therapies for less responsive tumours, such as CRC.

The complexity of the anti-tumour immune response is vast - not only are there multiple cells, these cells interact with each other, and are plastic so can change phenotype and function in response to inflammatory or suppressive signals from the tumour and tumour associated cells[2]. Understanding the relationships between cancer cells and immune cells is critical to understanding and, ultimately, manipulating the tumour immune microenvironment.

The importance of local immunity is particularly true in CRC where the immune response in the gut has been "trained" to ignore commensal microflora, and yet retain the ability to induce an attack against a pathogen. The ability of the gut to do this relies on a series of signals and interactions between bacteria, epithelial cells, and innate cells such as dendritic cells, monocytes and gut resident macrophages. In CRC, there are local adaptive immune cells such as effector T cells likely to have an antitumor effect, and regulatory or inflammatory T cells predicted to have a pro-tumour effect[3].

Recent study of the immune response in CRC has resulted in the development of the Immunoscore, a means of measuring T cell infiltrate into CRCs[4]. The Immunoscore thus far has shown to be predictive of outcome and also superior to other methods for staging patients. Innate immune responses, particularly those involving tumour associated macrophages (TAMs), have been studied and data show that the frequency of these cells infiltrating the tumour can be associated with poor patient outcome, although this is controversial[5].

Immune responses against colorectal tumours can be detected in early stage cancers, indicating that the immune system is capable of recognizing a tumour[6]. However, the tumour produces molecules that inhibit immune cell infiltration, that reduce activity of immune cells, or that change the phenotype of immune cells to a less effective anti-tumour function, ultimately allowing tumour outgrowth[7].

The inflammatory immune environment underlying tumour initiation and progression in CRC has been reviewed extensively[8], although much of the supporting data relies on animal models of colitis-induced cancer[9]. However, colitis-associated cancer accounts for only a small percentage (1%-4%) of CRC cases in humans[10]. The influence of inflammation mediated by immune cells in established familial or sporadic human CRC has been much less studied. In addition, new data demonstrate an impressive complexity of innate and adaptive immune cells[11], suggesting that some associations with cancer progression may have been too simplistic in their interpretation.

This review will concentrate on the networks of innate and adaptive immune cells, and tumour-associated immune cells in established CRC, and how these interactions can influence subsequent patient outcome (Figure 1). Despite recent interest in the immunology of CRC, there are limited experimental data studying the complexity of the immune response and the interactions between cancer cells and immune cells, particularly in humans. We will discuss (1) the interplay between the tumour stromal cells [particularly cancer-associated fibroblasts (CAFs)] and the macrophages infiltrating the tumour; and (2) the interactions between macrophages and T cells and how T cell populations may influence each other. We will attempt to describe the complexity and plasticity of these immune populations and discuss how they can be used to better understand the disease and to predict patient outcomes.

CANCER ASSOCIATED FIBROBLASTS AND TUMOUR ASSOCIATED MACROPHAGES - INNATE CELLS AND TUMOUR PROMOTION

CAFs in CRC

Fibroblasts are a key component of the connective tissue and are found embedded in the extracellular matrix (ECM). Fibroblasts have important roles in tissue homeostasis and remodelling. They produce multiple cytokines and can therefore modulate the immune microenvironment. Fibroblasts found in tumour stroma are referred to as CAFs.

The exact origin of CAFs is not clear. It has been proposed that they are cancer cells that have undergone an epithelial-mesenchymal transition[12]. Other research suggests that fibroblasts mature from fibrocytes that, in turn, have differentiated from monocytes[13] and thus have a similar haematopoietic lineage to macrophages. It is then not surprising that there is significant phenotypic overlap between CAFs and macrophages. CAFs do not express the immune cell marker CD45, however they can express CD68, a marker commonly used to differentiate macrophages[14]. Madar *et al*[15] hypothesised that CAFs were the result of convergent differentiation from any one of multiple pathways within the tumour microenvironment, and that CAF is a description of a functional state rather than a defined lineage.

CAFS may have a direct role in promoting CRC cell growth. Primary CAFs cultured from human colorectal tumours developed into distinct populations, some inducing a pro-migratory effect on CRC cells[16]. These pro-tumour CAFS had a distinct genetic signature with significant prognostic value. In addition, CAFs have been shown to promote metastases in CRC[17].

CAF interactions promoting tumour growth

Because of their role in in tissue homeostasis, CAFs are able to promote tumour growth *via* similar pathways, including *via* inflammatory mediators consistent with the wound healing process. These pathways were reviewed recently[12], so we will discuss the role of CAFs briefly, and focus on their influence on innate immune

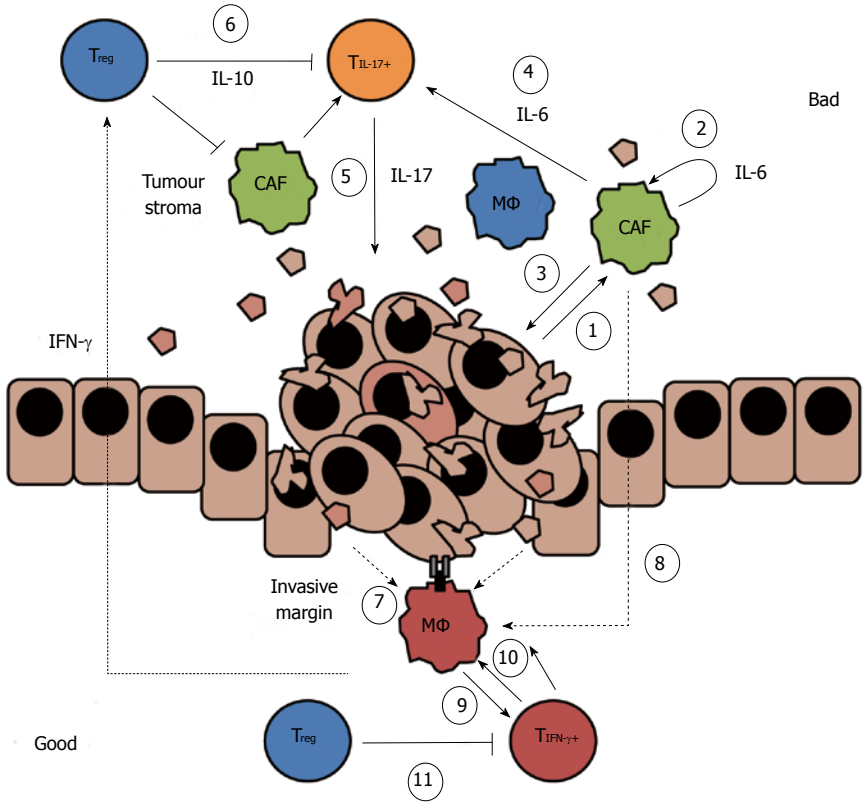

Figure 1 Immune cell interplay in established colorectal cancer. CAFs and macrophages play an important role in promoting tumour progression in the stroma, mediated by IL-6 ("Bad"). Conversely, immune responses at the invasive margin, including macrophage and T cell compartments inhibit tumour growth ("Good"). (1): Unknown factors from colorectal tumours promote IL-6 production from CAFs; (2) IL-6 promotes further IL-6 production from CAFs as well as initiation of VEGF production; (3) IL-6, IL-17, VEGF and ECM modulators produced by CAFs promote growth, angiogenesis and invasion of colorectal tumours; (4) IL-6 produced by CAFs or stromal macrophages promotes T cell differentiation towards an inflammatory IL-17 producing phenotype; (5) IL-17 producing T cells promote colorectal tumour progression and are associated with poorer patient prognosis; (6) Tregs suppress the inflammatory IL-17 response; (7) Macrophages at the invasive margin are associated with improved prognosis; (8) IL-6 produced in the stroma enhances the anti-tumour phenotype; (9) Invasive margin macrophages are primed to induce good effector T cell responses; (10) IFN-γ+ effector T cells are associated with improved prognosis in CRC; (11) Tregs can inhibit effector anti-tumour T cell responses. CAFs: Cancer-associated fibroblasts; IL: Interleukin; VEGF: Vascular endothelial growth factor; ECM: Extracellular matrix.

cells. CAF-derived inflammatory mediators can both promote tumour growth and tumour invasion (Figure 1). An important inflammatory cytokine produced by CAFs in the regulation of wound healing, interleukin (IL)-6, is also associated with disease progression in CRC.

IL-6 in patient serum has been associated with poor patient prognosis in many cancers, including CRC[18]. IL-6 promotes cell survival and supports the production of vascular endothelial growth factor (VEGF) from both tumour and immune cells. VEGF was associated with enhanced tumour progression and poor patient prognosis in CRC[19], likely through its role in angiogenesis[20]. CAFs produced more IL-6 than cancer cells, and CAF-derived IL-6 was increased in the presence of CRC cell lines[21]. In response to greater IL-6 production, CAFs up-regulated production of VEGF, leading to the proposal that the indirect effect of IL-6 on tumour growth *via* CAFs was more important that the direct effect of IL-6 on tumour cells[21].

Other inflammatory mediators produced by CAFs also increase IL-6 production, including IL-1β and TNFα[21]. In patients, high plasma levels of the TNFα receptor, TNFR-2, were associated with an increased relative risk of CRC[22]. Expression of both VEGF[23] and FSTL-1[24] (which enhances inflammatory cytokine and chemokine expression) was increased in CRC-associated CAFs. Chemotherapy, known to cause inflammation as cancer cells are killed[25], resulted in increased numbers of active CAFs in a cohort of CRC patients[26], and enhanced tumour growth in *in vitro* assays.

CAF recruitment of inflammatory cells

Fibroblasts both recruit, and are recruited by, monocytes/macrophages[12]. CAFs have been shown to recruit monocytes to the tumour microenvironment and thus may directly affect the local macrophage compartment. Indeed, Schellerer *et al*[27] showed there were more Intracellular Adhesion Molecule-1$^+$ fibroblasts in tumour tissue than healthy bowel tissue from CRC patients, implying that cancer-associated cells have a higher affinity for monocytic cells. In an *in vitro* human breast cancer model, CAFs produced high levels of the chemokines CCL2 and CCL5 that attracted monocytes[28,29]. The production of these chemokines required IL-6, in a suggested IL-6-CCL2 auto-regulatory cycle[29]. CCL2 and CCL5 were also produced by tumour

cells as well as the recruited monocyte/macrophages, creating a positive feedback loop and generating an inflammatory tumour microenvironment[28].

TAMs in CRC

The prognostic significance of TAMs is controversial, particularly in CRC[30]. Macrophages are myeloid derived cells of the innate immune system. They are potent phagocytes and are involved in clearance of pathogens and cellular debris. They also initiate the adaptive response by functioning as antigen presenting cells (APCs). Macrophages reside in all tissues where they also maintain tissue integrity (reviewed in[31]). The phenotype and ontogeny of tissue resident macrophages varies between tissues. Some are freshly recruited bone marrow-monocyte derived macrophages, whereas others derive from the embryonic yolk sac (reviewed in[32]). In most adult tissue, however, resident macrophages are fetal liver derived. Both the ontogeny and microenvironment of resident macrophages influence their phenotype. As such, resident macrophage populations are often heterogeneous.

The phenotypic diversity of macrophages makes analysis of subpopulations challenging. A great deal of work has been undertaken assessing macrophage subsets using only one or two surface markers to determine function. However, a recent opinion suggests this approach to be misleading, due to the many causes of diversity[33]. Instead, multiple markers must be used to estimate the function of macrophage populations, or, where possible, primary functional data. It has been proposed that minimum reporting standards be introduced to allow better meta-analysis of macrophage data between research groups. This type of approach is paramount when assessing highly plastic macrophages, for example, human macrophages were shown to switch from anti-inflammatory to pro-inflammatory cytokine production within 24 h in response to IFNγ, Granulocyte-Monocyte Colony Stimulating Factor and lipopolysaccharide *in vitro*[34].

The link between macrophage infiltration and prognosis in CRC is still poorly understood. While some studies have shown a positive correlation between macrophage infiltration and patient prognosis, others have shown the opposite[30]. For example, Forssell *et al*[35] demonstrated that a dense macrophage infiltration at the tumour invasive margin was associated with improved patient prognosis, and that macrophage inhibition of tumour spread and growth required direct cell-to-cell contact in an *in vitro* CRC model. In contrast, Kang *et al*[36] demonstrated that intra-tumoural TAM count correlated with parameters of worse disease progression (depth of invasion, lymph node metastasis and stage). Using an *in vitro* co-culture macrophage and CRC cell lines these researchers also demonstrated that macrophages increased cancer cell invasiveness and migration. It may be that the conflicting data relating to the role of macrophages in CRC prognosis is due to inaccuracies of reporting culture conditions or a lack of detailed phenotype[33].

Gut resident macrophages and CRC

Regular interaction between immune cells and microbes in the gut creates an immune environment that must be tightly regulated. Gut resident macrophages provide an important role in regulating this commensal barrier. These particular macrophages have an anergic phenotype; they destroy any bacteria that breach the epithelial barrier but do not initiate an immune reaction against them under homeostatic conditions[37,38].

Unlike most tissue resident macrophage populations, gut resident macrophages are bone marrow derived[32,37]. Newly recruited monocytes undergo a conditioning process, mediated by the gut epithelia, that matures them into the resident anergic phenotype. However, upon acute inflammatory insult, such as that seen in inflammatory bowel disorders, this conditioning process becomes dysregulated, resulting in a mature macrophage population that acquires and maintains migratory and inflammatory characteristics[37,39].

In the context of CRC, monocyte conditioning is unlikely to be modulated only by inflammation, but also factors actively produced by the tumour[40], hypoxic conditions[41] and glucose starvation[28]. As a result, unique macrophage populations will exist depending strongly on the context of the local microenvironment. Hence, describing a homogeneous macrophage population in CRC can be misleading.

TAMs promote an inflammatory pro-tumour environment

It is well documented that TAMs can promote tumour growth, both directly on tumour cells, and indirectly *via* cells in the tumour microenvironment (reviewed in[42]). The human monocytic cell line, THP-1, produced IL-6 in the presence of a colorectal cell line[43], and macrophage-derived IL-6 induced expression of IL-6 by the HT29 CRC cell line[44]. TAMs also upregulated the expression of metalloproteinase (MMP)-2 and MMP-9 on cancer cells, molecules associated with lymph node metastasis[42,45]. TAM-derived IL-6 promoted STAT-3 mediated IL-10 production in CRC cells, a cytokine that has also been associated with poor patient prognosis[46]. In fact, p-STAT3 overexpression in the tumours of CRC patients is significantly correlated with tumour specific mortality[47]. Together, these studies demonstrate that TAMs and CAFs promote an environment to support tumour progression in CRC.

Macrophages have been shown to preferentially migrate to hypoxic regions of tumours[48]. In a mouse model of colitis-associated CRC, repression of hypoxia inducible factor 1 led to decreased macrophage infiltration in tumours[49]. Interestingly, under hypoxic conditions, macrophages can acquire a phenotype similar to that seen in macrophages involved in wound-healing role - a phenotype likely to promote tumour growth. More specifically, human macrophages in hypoxic conditions (0.5% oxygen) up-regulated expression of both VEGF and glucose transporter (GLUT)-1 compared

to normoxia[50]. GLUT-1 is the primary rate limiting glucose transporter in inflammatory macrophages[51]. Using transgenic RAW264.7 macrophages that stably overexpressed GLUT-1, it was shown that high glucose trafficking *via* GLUT-1 promoted a pro-inflammatory macrophage phenotype[51]. It is then possible to hypothesise that under hypoxic conditions such as those in a tumour, macrophages up-regulate GLUT-1 in an attempt to scavenge more glucose in a low glucose environment.

Beyond the production of inflammatory modulators, colorectal tumours also cause barrier defects, which allow for contact between immune cells and microbial products. Myeloid cells showed an increase in production of the inflammatory cytokine IL-23 under inflammatory conditions compared with homeostatic conditions in the APC^{min} mouse model of CRC[52]. IL-23 stimulates and maintains IL-17 production from both tumour cells and T cells. In a mouse model of colitis associated CRC, IL-23- and IL-17-mediated inflammation disrupted the commensal microflora, and created a population of microbes that promoted tumour progression[53]. Furthermore, confocal microscopy of human CRC patient samples revealed that IL-17 production was not limited to T cells, but was also co-expressed with the myeloid cell marker, CD68[54]. These findings indicate that myeloid cells such as macrophages may be capable of producing IL-17 in CRC *in vivo*.

Location of TAMs and influence on CRC prognosis

A high infiltrate of macrophages at the invasive margin of colorectal tumours has been associated with improved patient prognosis[35], and macrophages at the invasive margin of patients with CRC displayed characteristics of an anti-tumour phenotype[55]. These cells expressed the co-stimulatory molecules CD80 and CD86, and apoptotic signalling molecule FasL at greater levels than stromal macrophages. Moreover, macrophages have been closely associated with apoptotic cancer cells along the invasive margin[56] and, using cell lines, CRC TAMs have been observed to be highly phagocytic[57]. In an *in vitro* model of macrophage differentiation, with either human peripheral blood mononuclear cells or murine bone marrow derived macrophages, IL-6 promoted maintenance of the established macrophage phenotype, even when the original cytokine stimuli were removed[58]. Because macrophages themselves also produce IL-6, as well as respond to CAF-produced IL-6, they are especially sensitive to the conditioning signals in their immediate environment. For example, macrophages pre-exposed to IL-4/13, acquired a phenotype characterised by increased IL-10 production in response to IL-6. However, macrophages pre-exposed to IFNγ, acquired a phenotype characterised by production of IL-1β and TNFα in the presence of IL-6. We propose that, in CRC, IL-6 both promotes and inhibits tumour growth *via* uniquely located macrophage populations (Figure 1).

T cells and the anti-tumour immune response

While considerable evidence on the role of T cells in preventing tumour growth in animal models has been acquired over decades, it was not until 2005 that a definitive role for T cells in CRC outcome was shown in patients[59]. Galon *et al*[60] demonstrated, in 2006, that a high infiltrate of $CD3^+$ $CD8^+$ $CD45RO^+$ T cells at the invasive margin and the centre of the tumour was predictive of improved Overall Survival and Disease-Free Survival in a large cohort of people with CRC. Since then, these data have been confirmed by other groups, and have led to the introduction of the Immunoscore to quantify infiltrating T cells in clinical practice[61].

The Immunoscore uses immunohistochemistry techniques to quantify the $CD3^+$ $CD8^+$ T cell infiltrate cell analysis at the centre of the tumour and at the invasive margin in people with CRC[4]. To date, the Immunoscore has proven to provide an accurate staging diagnosis as well as to predict patient outcome[62]. Although the Immunoscore is an improvement on the current staging methods for CRC, its efficacy may be hindered by the interference of T cell subsets that are not associated with good prognosis.

Although it remains clear that the infiltrate of $CD3^+$ $CD8^+$ $CD45RO^+$ T cells is associated with good patient prognosis in CRC, some T cell subsets have been associated with poor prognosis. Specifically, inflammatory $CD4^+$ T cells (Th17 cells), usually measured *via* production of the cytokine IL-17; and regulatory $CD4^+$ T cells (Tregs), often quantified by expression of the transcription factor, FoxP3; have been associated with both good and bad outcomes (reviewed in[63]). In addition, a low ratio of $CD4^+$ to $CD8^+$ T cells is associated with improved outcome[64]. Interestingly, Väyrynen *et al*[65] measured infiltrates of innate cells and adaptive cells in 117 CRC patients and found three parameters associated with Disease Free Survival at 24 mo: High infiltration of $CD3^+$ cells at the invasive margin and high infiltration of $FoxP3^+$ cells at the invasive margin and at the tumour stroma. Taken together, these findings indicate that that $CD8^+$ T cells may be more effective than $CD4^+$ T cells in an anti-tumour immune response, or that beneficial $CD4^+$ T cell subsets are masked by subsets associated with poor outcome[64]. The phenotype of T cells resident in the tumour is controlled by the local cytokine environment, particularly APCs such as macrophages. The efficacy of the T cell response against the tumour is therefore dependent on interactions with other cells (Figure 1).

Effective anti-tumour T cell responses

T cells respond to specific antigens expressed by pathogens or tumours. These antigens are presented by a subset of immune cells, APCs, including dendritic cells and macrophages, but also non-immune cells such as epithelial cells or tumour cells. The T cell infiltrate in CRC is likely to be maximally effective if those cells are specific for tumour antigens.

Nagorsen *et al*[66] used HLA tetramer analysis to show that tumour specific $CD8^+$ T cells in the blood were not correlated with improved clinical outcome in people with CRC or breast cancer, highlighting the need to study the tumour microenvironment. In a separate study, tumour-associated-antigen specific T cells were detected in 30%-40% of patients with CRC[67]. This study also showed that only a small subpopulation of infiltrating T cells could respond to tumour-associated antigens, indicating that not all infiltrating T cells were tumour-specific. Recently, Reissfelder *et al*[68] proposed that a subpopulation of tumour antigen-specific T cells infiltrating the tumours of people with CRC was responsible for the prognostic impact of T cells shown by other studies.

Multiple studies in animals have shown that cytotoxic T cells, *via* IFNγ, perforin and granzymes, can destroy established tumours. Gene cluster analysis of a large cohort of 602 patients with early stage CRC revealed that those patients with high $CD8^+$ and $CD45RO^+$ T cell infiltrates into the tumour also had increased expression of genes associated with anti-tumour responses compared with those patients with low $CD8^+$ and $CD45RO^+$ T cell infiltrates into the tumour[69]. The up-regulated anti-tumour gene signature included genes encoding for granzymes and perforin, as well as effector molecules such as IFNγ and the related transcription factor T-bet. The expression of Granzyme B protein in tumours from CRC patients was also associated with improved survival[70]. These, and many other data, support a role for $CD8^+$ T cells and T cells producing the effector molecules IFNγ and granzymes in eliminating CRC.

Effective T cells must become activated by interactions with APCs presenting antigen in the context of an appropriate cytokine milieu. TAMs were shown to express higher levels of the co-stimulatory molecule, CD80, than tumour stromal cells, indicating that these cells could activate T cells within the tumour[55]. In addition, using a multi-cellular tumour spheroid model, Ong *et al*[71] showed that TAMs up-regulated the expression of CD25 and IFNγ in T cells better than *in vitro* macrophages did. They also showed that the frequency of TAMs in human CRC tumours correlated with the frequency of infiltrating IFNγ-producing T cells *in vivo*. These data indicate that TAMs may be able to promote effector T cell responses within the tumour microenvironment (Figure 1). We propose that effective anti-tumour immunity is determined by TAM-T cell interactions occurring at the invasive margin in CRC.

Th17 cells, inflammation and cancer

Inflammatory T cells [defined here as IL-17-producing (or Th17) cells] are important in antimicrobial responses in the gut (reviewed in[72]). The acquisition of an IL-17-producing phenotype occurs when naïve T cells are activated in the presence of IL-6, IL-1β, TGFβ and IL-23; the maintenance of the phenotype is regulated by these same cytokines. Inflammatory IL-17 responses involve production of cytokines (especially IL-17) that recruit monocytes and neutrophils to sites of inflammation[73]. These innate cells in turn produce the same cytokines to promote ongoing Th17 responses[74].

IL-17 production in CRC has been associated with low Disease-Free Survival and Overall Survival[75] but the exact role of Th17 cells in CRC is not understood. Liu *et al*[54] showed that Th17 induced production of VEGF in CRC cell lines *in vitro*, which decreased T cell production of IFNγ and Granzyme B. This study also showed that in human CRC tumours, high expression of IL-17 correlated with high VEGF expression. VEGF expression has been inversely correlated with $CD8^+$ $CD45RO^+$ T cell infiltrate in tumours of CRC patients[69].

Th17 cells indirectly affect tumour growth via CAFs

CAFs may be activated *via* microbial products that cross the compromised epithelial barrier and promote IL-23 secretion[52], further supporting Th17 responses. Using a mouse model of CRC, Numasaki *et al*[76] showed that tumour cells engineered to express IL-17 led to increased production of angiogenic factors, including VEGF, not only by tumour cells, but also by CAFs. Th17 responses may therefore directly aid in the inflammatory responses of innate cells in CRC.

Th17 cells directly promote tumour growth

Liu *et al*[54] showed that IL-17 was increased in tumour tissue compared to healthy bowel tissue in a cohort of CRC patients, and that it was strongly correlated with overall survival. IL-17 added to human CRC cells *ex vivo* stimulated glucose metabolism by the tumour cells[77]. IL-17 promoted tumour growth through a STAT3-mediated pathway in CRC patients[78]; this result has also been shown in other models of cancer[79]. Together, these data indicate that the presence of intra-tumoural IL-17 may support tumour angiogenesis *via* VEGF and IL-6, and directly promote tumour cell proliferation (Figure 1).

Tregs and IL-10 controlling immunity

Regulatory T cells (Tregs) suppress inflammatory responses in the healthy gut and regulate normal immune responses by inhibiting proliferation and activity of effector T cells. Induced Tregs acquire a suppressive phenotype in the presence of cytokines such as TGFβ; the regulatory phenotype is characterised by up-regulation of the transcription factor FoxP3 and the production of IL-10, amongst other cytokines (reviewed in[80]). Dysregulated immune responses of the gut, for example inflammatory bowel diseases, are often typified by a high infiltrate of Tregs. In the presence of excess inflammatory cytokines from innate and adaptive immune cells, particularly IL-6, Tregs can convert into IL-17 inflammatory cells, or maintain their regulatory function while co-producing IL-17 (reviewed in[81]). Conversely, Treg differentiation can also inhibit the generation of Th17 cells.

In many human cancers an accumulation of Tregs is associated with poor patient outcome, presumably

by suppressing effector T cell responses against the tumour[63]. Controversially, in CRC, Tregs have been associated with both good and poor outcomes for patients[82]. It is possible that because Tregs suppress other T cells, they could impair the function of anti-tumour effector cells as well as pro-tumour inflammatory Th17 cells.

Using a complex library of tumour associated antigen-polypeptides, tumour-antigen specific Tregs were identified in the blood of CRC patients[83] providing evidence that these cells have the potential to inhibit specific anti-tumour immune responses. Therefore, the nature of the tumour immune microenvironment may influence the action of infiltrating Tregs.

Tregs suppress anti-tumour immune responses

Tumour-specific Tregs isolated from ovarian tumours suppressed effector $CD8^+$ T cell production of IFNγ *in vitro* after stimulation with tumour antigen[84]. The infiltrate of Tregs correlated with poor patient prognosis. In CRC patients with recurrent disease, specific T cell responses to the tumour antigens CEA and 5T4 were also suppressed[85]. In the same study, tumour specific Tregs and effector T cells were required to have the same specificity in order for Tregs to suppress the T cell response. Indeed, in an independent study, while tumour-antigen specific Tregs were identified in the tumours of CRC patients, the specificity of the majority of these cells was distinct from that of the effector and memory T cells in the same patients[83]. By depleting Tregs *ex vivo* in culture, only the effector anti-tumour T cells with the same specificity as the Tregs were increased.

The mechanism of Treg mediated suppression in tumour environments is not clear. In a mouse model of transplantable CRC using CMT93 cells, TAMs were able to recruit $CCR6^+$ Tregs to the tumour *via* production of the chemokine CCL20[86]. The infiltrate of Treg cells was associated with tumour development. Similarly, in breast cancer patients, the infiltrate of $CCR6^+$ Tregs into the tumour was inversely correlated with IFNγ production from tumour infiltrating $CD8^+$ T cells[87]. Using flow cytometry, the authors showed that $CCR6^+$ Tregs, but not $CCR6^-$ Tregs were associated with poor survival in breast cancer patients. This leads us to hypothesise that, in CRC, tumour-antigen specific Treg populations are actively recruited to the tumour by TAMs and inhibit the anti-tumour immune response, leading to poor prognosis of patients.

Tregs suppress pro-tumour T cells

Tregs recovered from blood of CRC patients were shown to inhibit the proliferation of Th17 cells sorted from blood and to suppress IL-17 production[88]. It is possible, therefore, that an accumulation of Tregs in the tumour of some CRC patients suppresses the inflammatory Th17 cell response rather than the anti-tumour effector response, leading to improved patient outcome.

Role for IL-10 in regulating tumour immune responses

Tregs are characterised by production of IL-10, a multifunctional cytokine generally believed to support anti-inflammatory immune responses. CRC patients had elevated levels of serum IL-10, and IL-10 remained high in those patients who had recurrent disease following tumour resection[89]. However, it has become clear that treatment of cancer with IL-10 could lead to improved anti-tumour responses (reviewed in[90]). In human CRC, the amount of IL-17 was inversely correlated with the amount of IL-10 produced[91]. Interestingly, it has been shown that IL-10 mediated suppression of IL-17 responses was dependent on type-I IFN signalling[92]. Further, Mumm *et al*[93] showed that IL-10 production induced the production of IFNγ and granzymes from human effector $CD8^+$ T cells *in vitro*. Together these data suggest that IL-10 production from Tregs may, in fact, inhibit pro-tumour inflammatory responses as well as promote anti-tumour immune responses. Phase 1 clinical trials have now begun in advanced solid tumours using recombinant human IL-10 as a therapy (https://clinicaltrials.gov/show/NCT02009449).

CLINICAL RELEVANCE

Experimental limitations

Studying the immune response to CRC is difficult because of the complexity of both the gut immune response and the tumour microenvironment. As with most human studies, much of what has been studied has been observational and compounded by individual patient variation and individual tumour variation. The vast majority of CRC cases in humans are sporadic and the mutations that lead to tumour initiation and progression, and therefore immune responses, differ from person to person. Further, while animal models of CRC have provided useful information, their ability to truly mimic human disease is limited (reviewed in[94]). The two most commonly used models represent colitis-associated CRC (1%-4% of human CRC) or APC^{min} mice representing familial CRC (about 20% of human CRC)[95]. We (and others[96,97]) have developed orthotopic surgical murine models of CRC that result in a tumour immune microenvironment more similar to that seen in sporadic human CRC than other mouse models. It is possible these models may be used to test new immune-based interventions.

Checkpoint blockade in CRC

Two new immune-based drugs have recently been introduced in the treatment of cancer - anti-CTLA-4 (ipilimumab) and anti-PD-L1/anti-PD-1 (nivolumab or pembrolizumab). Both types of drugs act to prevent the tumour-mediated suppression of effector T cell responses, and have been successful in melanoma (reviewed in[98]). However, both checkpoint blockade drugs have shown much less success in CRC[99-102]. The reasons behind this are unclear but it has been

shown that many colorectal tumours do not express PD-L1, the ligand for PD-1. Therefore, if the suppressive effect of PD-L1 on anti-tumour T cells is absent, then therapy targeting the PD-1 pathway is unlikely to be successful[101]. However, it has recently been shown that microsatellite instability (MSI) high CRC tumours (15% of CRC tumours that have mutations in mismatch repair genes and are more immunogenic) expressed more PDL1 than MSI low tumours, indicating that checkpoint blockade may be more successful in the MSI high subset of CRC patients[103]. Clinical trials using anti-PD1 therapy in such a subset of patients are now underway to exploit this possibility.

Adoptive T cell therapy in CRC

Adoptive cell therapy (ACT) has been trialled in CRC to some success. Karlsson *et al*[104] used *ex vivo* T cells (recovered from tumour-draining lymph nodes) of CRC patients as a therapy. No side effects were observed and complete responses were seen in 4 out of 9 patients with metastatic disease. A Phase II trial is currently being undertaken to further test ACT in patients with metastatic CRC (https://clinicaltrials.gov/ct2/show/NCT01174121). The use of genetically engineered tumour-antigen specific T cells has been less successful in CRC. T cells genetically engineered to target carcinogenic embryonic antigen (CEA) caused a measurable decrease in serum CEA levels in 4/4 CRC patients treated but also induced severe colitis in all patients[105], consistent with studies in other cancers. Targeting neo-antigens in tumours and individualising therapy may be the way forward in ACT of CRC.

CONCLUSION

Recent technological breakthroughs have allowed the analysis of single cells, providing enormous amounts of data on the immune system (reviewed in[11]). These data provide novel insights into the function and complex connectivity of immune cells. This new network approach to studying immunology is likely to transform our understanding of the immune microenvironment of individuals with CRC. The immune response to CRC in humans is complex and involves a panoply of cells interacting with each other and the tumour. Patient outcome is unlikely to be accurately predicted by measuring one immune parameter independently. Moreover, any new immune-based therapies will need to take into account the pro- as well as anti-tumour activities of specific innate and adaptive immune cells.

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TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Relationship between intestinal microbiota and colorectal cancer

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Abstract

The human gastrointestinal tract hosts a complex and vast microbial community with up to 10^{11}-10^{12} microorganisms colonizing the colon. The gut microbiota has a serious effect on homeostasis and pathogenesis through a number of mechanisms. In recent years, the relationship between the intestinal microbiota and sporadic colorectal cancer has attracted much scientific interest. Mechanisms underlying colonic carcinogenesis include the conversion of procarcinogenic diet-related factors to carcinogens and the stimulation of procarcinogenic signaling pathways in luminal epithelial cells. Understanding each of these mechanisms will facilitate future studies, leading to the development of novel strategies for the diagnosis, treatment, and prevention of colorectal cancer. In this review, we discuss the relationship between colorectal cancer and the intestinal microbiota.

Key words: Sporadic; Colorectal; Cancer; Intestinal; Microbiota

Core tip: Microbiota's role in providing intestinal homeostasis is not as an audience, but it is active. Both the composition of microbiota and its metabolic activity impact the sensitivity of the host and can cause many pathologies including colorectal cancer.

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INTRODUCTION

Colorectal cancer is the third commonest cancer type

worldwide and causes 600000 deaths every year[1]. Because colorectal cancer patients are frequently asymptomatic in the early phase of the disease, diagnosis at this stage presents a significant clinical challenge. Detection of early stage cancers (stages 1-2) allows curative surgery with a 5-year survival rate of 80%. However, survival rates decrease to approximately 10% for metastatic and late stage tumors[2]. Although there are currently methods for the early diagnosis methods, including computed tomography, colonoscopy, and blood tests, it is expected that evaluation of the intestinal microbiota will prove to be a valuable method allowing earlier diagnosis of colorectal cancer.

In humans, a relationship between cancer and microorganisms has been demonstrated in a number of organs, with the most well-known example being the relationship between *Helicobacter pylori* and gastric cancer and mucosa-associated lymphoid tissue lymphoma[3].

In adults, while the bacterial population in the stomach and small intestine is smaller (10^3-10^4 CFU/g contents), increased concentrations of microorganisms are found in the colon (10^{11}-10^{12} CFU/g contents) compared with the upper gastrointestinal tract. The majority of these microorganisms exist in a favorable symbiotic relationship with humans[3,4]. The intestinal microbiota develops specific to individual variation and environmental conditions beginning at birth[5].

Recently, etiology of colorectal cancer has been shown to be related to genetic mutations, diet, inflammatory processes, lifestyle, and the gut microbiota, with up to 95% of colorectal cancer thought to sporadically develop in individuals with no genetic predisposition[6].

The colonic microbiota is thought to contribute to the development of colorectal cancer by controlling the epithelial cell proliferation and differentiation, synthesizing essential nutrients and bioactive products, preventing the reproduction of pathogenic organisms, and stimulating the immune system[7]. In this review, studies investigating the role of the intestinal microbiota in the development of colorectal cancer development are discussed.

MICROBIOTA OF THE HUMAN INTESTINE

There are 100 billion bacteria in the human intestine with an approximate weight equivalent to 1.5-2 kg. Bacteroidetes and Firmicutes are the major species of the adult intestinal microbiota with the next most frequent species being Actinobacteria, Proteobacteria, and Verrucomicrobia[8].

Normally, colonic bacteria exist in a mutually beneficial symbiotic relationship with humans without adverse effects on the host cells. In situations where this balance is deregulated because of a number of possible causes, the numbers and species of harmful bacteria increase, providing a basis for the development of inflammatory and chronic disease. Changes in the intestinal microbiota have been shown to be associated with obesity, fatty liver, type 1 and 2 diabetes, kidney disease, arthritis, inflammatory bowel disease, and colorectal cancer[9-13]. However, the precise relationship between changes in the microbiota and colorectal cancer has yet to be fully elucidated.

FACTORS INFLUENCING GASTROINTESTINAL MICROBIOTA

The intestinal microbiota is affected by a number of factors, such as antibiotics, diet, and inflammation[4-18]. A number of studies have reported a high degree of similarity in the intestinal microbiota between members of the same family but a low degree of similarity between heterozygous mice despite being housed in the same cage[9,14,19].

The intestinal microbiota of mice fed standard low-in-fat nutrients has been shown to change within a few weeks with particularly great changes in the composition of Bacteroidetes and Firmicutes species. After mice returned to a low-fat diet, a particularly significant reduction in Mollicutes, a species of Firmicutes, was observed[9,20]. Similar changes have observed with diets high in fat, particularly in obese people, genetically obese mice, and obesity-resistant mice[9,14,21]. Transfer of colon microbiota from mice fed a high-fat diet to mice fed a low-in-fat diet has been shown to accelerate tumor growth suggesting diet-induced changes in the colon microbiota may have a synergistic effect with genetic factors on tumor development[22]. Diet-related changes in intestinal microbiota have also been shown to be associated with colorectal cancer[23].

MICROBIAL INFLUENCE ON COLORECTAL CANCER

The relationship between the intestinal microbiota and disease has drawn increased attention in recent years. In particular, recent studies have demonstrated strong associations between the development of colorectal cancer and intestinal bacteria. In these studies, DNA damage caused by superoxide radicals, genotoxin formation, increased T-cell proliferation, and activation of procarcinogenic pathways through a number of receptors have all been shown to contribute to cancer development[24-27].

The enzymatic activation or detoxification of carcinogens, and therefore modulation of their tumorigenic activity, has been shown to be influenced by the intestinal microbiota[24,28-35]. In the 1960s, it was observed that germ-free rats exposed to the glycoside, cyasin, did not develop intestinal tumors. Conversely, germ-free rats directly exposed to methylazoximethanol, a sub-active metabolite of cyasin, did develop intestinal tumors[36]. As the formation of methylazoximethanol depends on bacterial β-glucosidase enzyme activity[36], this study was a potent demonstration of the effect

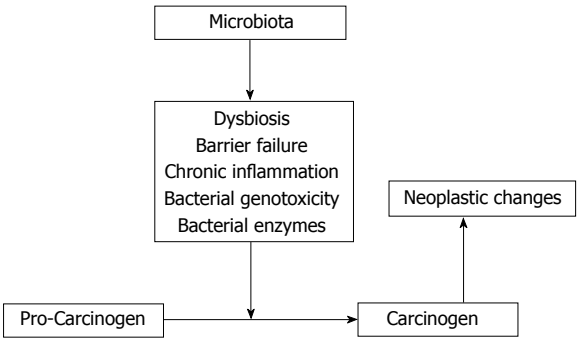

Figure 1 The factors releated to intestinal microbiota promotes neoplasia in the gastrointestinal tract.

of the intestinal microbiota on bioactive carcinogenic compounds. Subsequent research has revealed that the intestinal microbiota converts latent carcinogens to bioactive forms through a number of enzymes, including β-glucuronidase, β-glucosidase, azoreductase, and nitroreductase[37]. Azoxymethane (AOM) is the most frequently used experimental colon carcinogen. AOM is first hydrolyzed in the liver to methylazoximethanol and conjugated to glucuronic acid before bilious excretion into the intestine where it is converted into a highly reactive methyl carbon ion by bacterial β-glucuronidase[34,37,38]. Interestingly, it has been reported that inhibition of β-glucuronidase activity significantly decreases the tumor-inducing potential of AOM in rats[39]. Furthermore, probiotic bacteria, such as Lactobacillus and Bifidobacterium species, have been shown to have anti-carcinogenic effects through the inactivation of microbial enzymes involved in procarcinogenic activation[40]. For example, Lactobacillales, such as *L. Casei* and *L. Acidophilus* suppress β-glucuronidase, azoreductase, and nitroreductase activity[41,42]. This balance between the activation and detoxification of potential carcinogens underlies the activation of host oncogenes and tumor suppressors (Figure 1).

In the study by Boleij *et al*[43] investigating the expression of the Bacteroides fragilis gene (*BFT*) in colonoscopic samples from 49 healthy individuals and 49 colorectal cancer patients, *BFT* gene expression was detected more frequently in samples from colorectal cancer patients. When comparing early and late stage cancer patients, *BFT* gene expression was more frequently detected in late stage cancer patients.

DNA damage and chromosomal instability are early genetic events in the development of colorectal cancer. As with aneuploidy, chromosomal instability is associated with long-term inflammatory bowel disease (IBD) and frequently a precedent event in the subsequent development of colorectal cancer[44-46]. *Enterococcus faecalis* (*E. faecalis*), an intestinal bacteria, has been repeatedly found to induce aneuploidy in colonic epithelial cells in monoassociated interleukin (IL)-10 -/- rats and cause aggressive colitis[47,48]. Inhibitors of reactive oxygen and nitrogen species can prevent aneuploidy induced by *E. faecalis*[49]. These findings demonstrate that intestinal microbiota (particularly specific species) can induce RONS and lead to carcinogenesis.

In intestinal hemostasis, the protective role of the microbiota is thought to be through an effect on epithelial cell proliferation and apoptosis. The main mechanism underlying this effect has been proposed as the conversion of dietary fiber into short chain fatty acids (SCFA), such as acetate, propionate, and butyrate, through microbial fermentation. These SCFAs, particularly butyrate, are readily absorbed easily by the colon and are used as a primary energy source. In addition to significant anti-inflammatory effects[50,51], SCFAs stimulate cell proliferation and differentiation in non-neoplastic normal colon, promote intestinal hemostasis, and the resolution of intestinal injury[51,52]. In addition, SCFAs demonstrate a trans-effect on cancer cells. In particular, butyrate induces apoptosis in colorectal cancer cell lines through a number of mechanisms but predominantly *via* inhibition of histone deacetylase and activation of intrinsic/mitochondrial apoptosis[53-57].

However, SLC5A and GPR109A, the two major receptors of butyrate, provide protection in the early phases of tumorigenesis as they are frequently inactivated in human cancers[58-60]. It is believed that regulation of microbiota species responsible for the production of butyrate will have efficacy in the treatment of gastrointestinal diseases[61,62]. Therefore, probiotics and in-absorbable food are thought to alter the intestinal microbiota leading to a beneficial increase in the production of short chain fatty acids[63].

Although the development of colorectal cancer has not been attributed to any specific microorganism, a number of cancer-promoting bacteria have been identified (Table 1).

In rats, Helicobacter hepaticus increases the development of colorectal cancer related to experimental colitis and spontaneous colorectal cancer[65,67]. Bacteroides fragilis is a widespread intestinal bacteria and a potential cause of spontaneous colon tumorigenesis in rats as an enterotoxigenic variant[26].

Exclusion of opportunist pathogens by colonic bacteria may represent a natural defense against colorectal cancer. Similarly, food containing species of *Lactobacillus* and *Bifidobacteria*, used as probiotics, provide a number of protective benefits against inflammatory bowel diseases[93-95]. Upon colonizing the host and on the condition of the formation of an additional biofilm, probiotic bacteria have been shown to prevent the adhesion and invasion of pathogen types, maintain host tight junction protein structure, decrease host cytokine production, modulate inflammation and immunity, and neutralize carcinogens and toxins[96-100].

Intestinal microbiota have been shown to cause the release of host antibacterial lectins, stimulate antimicrobial host epithelial responses, and deplete subsets of potentially pathogenic bacteria providing a protective role against abnormal immune responses.

In a study by Sobhani *et al*[81] of 179 individuals

Table 1 The relationship between bacterial types and colorectal cancer

Bacteria	Subject of study	Evidence	Ref.
Helicobacter hepaticus	Animal	Augments azoxymethane induced, and spontaneous colorectal cancer in mice	[64-69]
H. hepaticus + *H.bilis*	Animal	Dual infection induces colorectal cancer in mice	[70,71]
H. typhlonius + *H. rodentium*	Animal	Dual infection in neonates induces colorectal cancer in mice	[72,73]
Streptococcus bovis	Human	*S.bovis* bacteremia and endocarditis associated with human colorectal cancer	[74-77]
	Animal	Augments azoxymethane induced colorectal cancer in rats	[78]
	Human	Increased humoral immune response to *S.bovis* antigenRpL7/L12, sassociated with increased risk for colorectal cancer	[79]
Bacteroides fragilis	Animal	Enterotoxigenic *B.fragilis* augments spontaneous colorectal cancer in mice	[26]
	Human	Increased prevalence of enterotoxigenic *B.fragilis* in human colorectal cancer	[80]
	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis	[81]
	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis	[43]
B. vulgatus	Animal	Induces azoxymethane induced, colorectal cancer in mice	[82]
Escherichia coli	Human	Increased mucosa-associated *Escherichia coli* in human colorectal cancer	[83]
Citrobacter rodentium and C. freundii	Animal	Etiologic agent of transmissible murine colonic hyperplasia	[84]
	Animal	Augments spontaneous and 1,2 dimethylhydrazine induced colorectal cancer in mice	[85,86]
Fusobacterium nucleatum	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis	[87]
	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis and 16S ribosomal RNA Gene V3 pyrosequencing analysis	[88]
	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis	[89]
	Animal	16S ribosomal RNA Gene V3 pyrosequencing analysis	[90]
Enterococcus faecalis	Human	Increased in the feces of colorectal cancer patients by quantative PCR analysis	[91]
Furmicutes	Animal	16S ribosomal RNA Gene V3 pyrosequencing analysis	[90]
Akkermansia muciniphila	Human	16S ribosomal RNA Gene V4 pyrosequencing analysis and Gas Chromatography-Mass Spectrometry	[92]
Methanobrevibacterium	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis and 16S ribosomal RNA Gene V3 pyrosequencing analysis in fecal samples	[89]

PCR: Polymerase chain reaction; RNA: Ribonucleic acid; *H. Hepaticus*: *Helicobacter hepaticus*; *H. bilis*: *Helicobacter bilis*; *H. typhlonius*: *Helicobacter typhlonius*; *H. Rodentium*: *Helicobacter rodentium*; *B. vulgatus*: *Bacteroides vulgatus*; *C. freundii*: *Citrobacter freundii*.

undergoing colonoscopy (60 colorectal cancer, 119 normal), significantly greater levels of Bacteroides/Prevotella bacterial DNA were found in patients with colorectal cancer. Further, it was shown that a greater proportion of IL-17 immunomodulatory cells were isolated from patients with colorectal cancer.

In a study by Gao *et al*[88] in 2015 examining colon samples from 30 healthy and 31 cancer patients, distal and proximal colon microbiota from both healthy individuals and cancer patients were evaluated using the 16S RNA V3 sequence. No significant difference was observed between proximal and distal colon microbiota; however, in patients with colorectal cancer, Firmicutes and Fusobacteria were over-represented and Proteobacteria were under-represented. Further, Lactococcus and Fusobacterium were identified more often, and Pseudomonas and Escherichia–Shigella less often, in tissues from patients with colorectal cancer compared to those without cancer[88].

In a study by Zhu *et al*[90] using the 1,2-dimethylhydrazine cancer model, V3 sequences of 16S ribosomal RNA isolated from intestinal microbiota samples from rats with cancer and healthy rats were determined. While Firmucutesin was more frequently observed in rats with colorectal cancer, Bacteroidetes and Spirochetes were less commonly observed. There was no significant difference in the Proteobacteria types between the two groups; however, Prevotella, Lactobacillus, and Treponema were more frequently detected in healthy rats. Furthermore, while Fusobacterium was not observed in healthy rats, it could be identified specifically in cancer rats[90]. In a study of feces samples from healthy individuals and colorectal cancer patients, Akkermansia muciniphila was identified 4 times as often in colorectal cancer patients than healthy individuals[92].

As emphasized in many studies discussed above, intestinal microbiota have a substantial impact on intestinal health through controlling the immune and inflammatory response to individual species of intestinal microbiota, the activation or detoxification of carcinogens, the stimulation of DNA damage and chromosomal instability, dysregulation of the balance between proliferation and apoptosis, and prevention of invasion by pathogens.

CONCLUSION

Although colorectal cancer development is a complex process, recent studies have shown that the microbiota is actively involved.

Recently, we have developed a greater under-

standing of the effect of the microbiota on bowel health and diseases, including esophagitis/Barrett's esophagus, stomach cancer, IBD, and colorectal cancer. However, while a strong relationship between gastrointestinal diseases and the microbiota content is evident, many questions remain unanswered. One of the most clinically challenging issues is to understand how a change in intestinal microbiota will likely impact on the course of disease. Knowledge obtained from dysbiotic microbiota research in germ-free animals and clinical studies involving a variety of intestinal diseases will help provide answers to these important questions. Further, there is currently a lack of data regarding which microorganisms in the microbiota cause disease and are protective.

Continuous improvements in the development of increasingly cost-effective research methods, gene sequencing technology, and high productivity techniques are expected to provide substantial information regarding the healthy and dysbiotic microbiota composition. This information will facilitate functional experiments utilizing cause and effect animal models.

Understanding the relationship between pathology and the microbiota is important; however, the role of microbiota in pathogenesis has yet to be fully elucidated. Therapeutic microbial transplantation has been trialed in metabolic syndrome and also has utility in the treatment of colorectal cancer; however, this technique has many limitations including infection and the promotion of autoimmune disease. Despite this, there is hope that treatments targeting the human microbiota may provide therapies for the prevention and treatment of colorectal cancer in the future.

In summary, the microbiota plays an active role in intestinal homeostasis. Both the composition of microbiota and its metabolic activity have an impact on the host susceptibility to disease and can directly contribute to a number of varied pathologies, including colorectal cancer.

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TOPIC HIGHLIGHT

2015 Advances in Pancreatic Cancer

Management of borderline resectable pancreatic cancer

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Abstract

Pancreatic cancer is the fourth most common cause of cancer death in the United States. Surgery remains the only curative option; however only 20% of the patients have resectable disease at the time of initial presentation. The definition of borderline resectable pancreatic cancer is not uniform but generally denotes to regional vessel involvement that makes it unlikely to have negative surgical margins. The accurate staging of pancreatic cancer requires triple phase computed tomography or magnetic resonance imaging of the pancreas. Management of patients with borderline resectable pancreatic cancer remains unclear. The data for treatment of these patients is primarily derived from retrospective single institution experience. The prospective trials have been plagued by small numbers and poor accrual. Neoadjuvant therapy is recommended and typically consists of chemotherapy and radiation therapy. The chemotherapeutic regimens continue to evolve along with type and dose of radiation therapy. Gemcitabine or 5-fluorouracil based chemotherapeutic combinations are administered. The type and dose of radiation vary among different institutions. With neoadjuvant treatment, approximately 50% of the patients are able to undergo surgical resections with negative margins obtained in greater than 80% of the patients. Newer trials are attempting to standardize the definition of borderline resectable pancreatic cancer and treatment regimens. In this review, we outline the definition, imaging requirements and management of patients with borderline resectable pancreatic cancer.

Key words: Pancreatic cancer; Surgery; Chemotherapy; Radiation; Borderline

Core tip: The diagnosis and treatment of borderline resectable pancreatic cancer (BRPC) remains unclear. The definition of BRPC is not uniform and generally refers to regional blood vessel involvement by the tumor. Recent attempts have been made to standardize the definition of BRPC. Neoadjuvant therapy is recommended in the hopes of obtaining negative surgical margins and consists of chemotherapy and radiation therapy. Data for therapeutic approaches is primarily

derived from single institution retrospective series. In this article, we review the definition, imaging modalities for diagnosis and treatment of patients with BRPC.

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INTRODUCTION

Pancreatic cancer is the fourth most common cause of cancer death in the United States with 48960 incident cases and 40560 deaths estimated in 2015[1]. Despite the recent advances in therapeutic interventions, the 5-year relative survival rate remains approximately 6%. At initial presentation, approximately 50%-55% of the patients are found to have metastatic disease, 20%-25% have locally advanced disease and only 20% have resectable disease[2]. Surgery provides the only curative option with long term survivors. Modern advances in surgical techniques have substantially decreased post-operative mortality and morbidity, especially in high volume centers[3]. Improvement in imaging modalities has led to better delineation of resectable disease and spares patients from unnecessary surgery[4]. Yet, of those patients who undergo potentially curative resections, the 5-year survival remains abysmal at 20%[1].

Despite the fact that the progress has been slow, there has been improvement in systemic therapies for the treatment of pancreatic cancer. Gemcitabine remained the standard of care option for unresectable pancreatic cancer for a long time. Recently, two randomized clinical trials have demonstrated superior efficacy over single agent gemcitabine in the setting of metastatic and locally advanced disease. Conroy *et al*[5] reported a phase Ⅲ trial comparing the combination of 5-fluorouracil, folinic acid, oxaliplatin and irinotecan (FOLFIRINOX) to gemcitabine. The median survival was significantly better with FOLFIRINOX at 11.1 mo compared to 6.8 mo with single agent gemcitabine. The response rates were higher in the combination group as well (31.6% *vs* 9.4%). However, increased grade 3 or 4 toxicities with FOLFIRINOX limits this therapy to highly selected patients. The addition of nab-paclitaxel to gemcitabine has demonstrated improvement in median survival (8.5 mo *vs* 6.7 mo), progression free-survival (5.5 mo *vs* 3.7 mo) and response rates (23% *vs* 7%)[6]. The higher response rates observed with this regimen makes them very appealing for downstaging tumors. Further, since the objective of systemic treatment for borderline resectable pancreatic cancer is the possibility of margin negative surgery and potentially cure, higher toxicities may be acceptable in this group of patients. This is in contrast to patients with metastatic disease where the primary aim is to improve survival by a few months while maintaining a good quality of life.

Involvement of blood vessels by tumor frequently renders the possibility of resection with negative margins problematic in patients with non-metastatic pancreatic cancer. Patients with negative margins have significantly improved survival compared to patients who have gross disease at the resection margin[7]. The term "borderline resectable pancreatic cancer" has no universal definition but, in general, denotes patients with pancreatic cancer that abuts regional blood vessels such that there is a high risk for margin-positive resection[8]. Tumor abutment refers to solid tumor contact of ⩽ 180 degrees of circumference of blood vessel and encasement refers to greater than 180 degree of contact. Unfortunately, the current pancreatic staging system by the American Joint Committee on Cancer (AJCC) does not differentiate this subgroup of patients with those tumors encasing blood vessels termed locally advanced disease. In this staging system, patients with portal vein, superior mesenteric vein or superior mesenteric artery involvement are considered unresectable. All patients with vascular involvement and no metastatic disease are grouped under stage Ⅲ disease.

Staging work up

Pre-operatively, diagnostic imaging is utilized for differentiating pancreatic cancer into resectable, borderline resectable or unresectable disease. The National Comprehensive Cancer Network (NCCN) recommends multidetector computerized tomography (CT) angiography, acquiring thin, preferably sub-millimeter sections using a pancreatic protocol. The images are to be obtained in the non-contrast, arterial, pancreatic parenchymal and portal venous phase contrast enhancement. The multiphasic protocol helps in assessment of vascular invasion of tumors by selective visualization of arterial (superior mesenteric artery, celiac axis, gastroduodenal artery) and venous (superior mesenteric vein, portal vein, splenic vein) structures. Pancreatic protocol CT has an excellent sensitivity (89%-97%) and negative predictive value[9]. However, CT is not very accurate for predicting resectability (45%-79%) as it is not very sensitive to detect small hepatic and peritoneal metastases[9]. Pancreatic magnetic resonance imaging (MRI) can also be used as an adjunct for staging, especially for patients with a contrast allergy. MRI is similar to CT in respect to providing details of tumor anatomy for resectability status but is less widely utilized. The role of positron emission tomography (PET) scan for patients with borderline resectable disease remains unclear. PET scans may help, however, in detecting metastatic disease in addition to CT scans and spare patients from unnecessary surgery[10,11]. Thus, PET scans may be used as adjuncts to CT scans especially in patients with a high risk of advanced disease.

Endoscopic ultrasound (EUS) is a complementary modality to CT scan and is utilized in many centers.

Table 1 Criteria for resectability

	NCCN	AHPBA/ SSAT/SSO	MD Anderson	Intergroup (Alliance)
Celiac artery	No abutment for pancreatic head cancer. For body/tail, ≤ 180° contact	No abutment or encasement	Abutment	Tumor-vessel interface < 180° of vessel wall circumference
CHA	Solid tumor contact ≤ 180° allowing for reconstruction	Abutment or short segment encasement	Abutment or short-segment encasement	Reconstructable short-segment interface of any degree
SMA	Solid tumor contact ≤ 180°	Abutment	Abutment	Tumor-vessel wall interface < 180° of vessel wall circumference
SMV/PV	Solid tumor contact > 180° or contact of ≤ 180° with contour irregularity or thrombosis allowing for safe reconstruction	Occlusion	Occlusion	Tumor-vessel interface ≥ 180° of vessel wall circumference and/or reconstructible occlusion

CHA: Common hepatic artery; SMA: Superior mesenteric artery; SMV: Superior mesenteric vein; PV: Portal vein; NCCN: National Comprehensive Cancer Network; AHPBA/SSAT/SSO: Americas Hepato-Pancreato-Biliary Association/Society for Surgery of the Alimentary Tract/Society of Surgical Oncology.

It is particularly useful for assessment of vascular invasion, especially of the portal vein. EUS is not a good modality for involvement of the superior mesenteric artery. EUS is routinely performed for patients with borderline pancreaticcancer for pathologic diagnosis. Tissue confirmation is not necessary for patients undergoing upfront surgery but should be obtained prior to initiation of neoadjuvant therapy. EUS-guided fine needle aspiration or biopsy is safe and is associated with a low complication rate[12-14]. Further, there is decreased potential for peritoneal seeding compared to percutaneous biopsy.

Staging laparoscopy is performed routinely at selected centers to detect occult metastatic disease, especially peritoneal involvement. It can thus be performed prior to surgery or prior to initiation of neoadjuvant therapy to avoid non-curative surgery and potentially prevent unnecessary complications associated with laparotomy[15]. At some institutions laparoscopy is reserved for patients with a higher chance of metastatic disease, including markedly elevated tumor markers or symptomatic patients. Despite the fact that staging laparoscopy can detect occult disease even in patients who had undergone good quality imaging studies, this procedure is not routinely utilized.

Classification

The definition of borderline resectable pancreatic cancer (BRPC) is not uniform. Some series have included patients based on anatomic imaging criteria for BRPC alone while others include patients with clinical factors. Recently, attempts have been made to clearly define borderline resectable disease and differentiate it from clearly resectable or unresectable disease. Table 1 lists the different classification systems utilized for defining borderline resectable pancreatic cancer including those proposed by the National Comprehensive Cancer Network (NCCN), MD Anderson, Americas Hepato-Pancreato-Biliary Association/Society of Surgical Oncology/Society for Surgery of the Alimentary Tract (AHPBA/SSO/SSAT) and the Intergroup[16-18]. Due to complexities involved in making these distinctions, it is very important that all cases of non-metastatic pancreatic cancer are discussed by a multidisciplinary team in high volume centers.

The NCCN panel has recently updated the guidelines and the definition of borderline resectable pancreatic cancer is included in the Table 1.

Vascular involvement

One of the key concepts for defining borderline resectable pancreatic cancer is the possibility of benefit of surgery in patients with vessel involvement. Vascular reconstruction is frequently the limiting factor during pancreatectomy in these patients. Siriwardana *et al*[19] in 2006 reported outcomes on 1646 patients from 52 studies with portal vein or superior mesenteric vein resections. Median postoperative morbidity was 42% with mortality of 5.9%. Median survival was only 13 mo with 5-year survival of only 7%. This study concluded that pancreatic surgery requiring resection of the portal vein did not improve outcomes. However, this study was limited by relatively older studies from 1996-2005 and heterogeneity of the studies included in the review. Since then, multiple single institution studies from high volume centers have demonstrated similar morbidity, mortality and survival for patients who underwent pancreatic surgery with or without venous involvement[20-24]. Zhou *et al*[25] in 2012 published a meta-analysis of 19 nonrandomized studies comprising 2247 patients. There was no difference in perioperative morbidity, mortality or 5-year survival among patients who underwent pancreatic surgery with or without venous resection. These studies suggest that venous resection with pancreatectomy is safe and feasible and can lead to improvement in long term outcomes. However, the results should be interpreted with caution as there may be publication bias as well as underreporting of morbidity data. Further, studies using National Surgery Quality Improvement Program database and National Inpatient Sample database demonstrated increases in morbidity and mortality with the addition of venous resection to pancreatic resection[26,27]. However, the limitations of these studies include the use of an administrative database, no distinction between venous or arterial resection and the

inability to differentiate between planned and unplanned vascular resections.

There is even limited data for arterial resection during pancreatectomy for pancreatic cancer. Some studies have demonstrated similar morbidity and mortality with the addition of arterial resection to pancreatic surgery[28,29]. However, a meta-analysis including 366 patients from 26 studies demonstrated significantly greater peri-operative morbidity and mortality with arterial resection[30]. This study also found that despite increased complications, patients undergoing pancreatic and arterial resection had improved survival compared to those patients who did not undergo resection. Similar results have been reported in other studies from high volume centers[31,32]. Thus, arterial resection should be limited to highly selected patients.

Treatment

Patients with borderline resectable pancreatic cancer are preferentially treated with neoadjuvant therapy to enhance the potential to facilitate margin negative, or R0, resection. Some patients with micrometastatic disease initially may have progressive disease on subsequent restaging scans after neoadjuvant therapy and thus are spared from unnecessary surgery. These patients would have been unlikely to benefit from pancreatic resection. It is generally acceptable that multimodality treatment is required for this patient population, although some centers have pursued a strategy of neoadjuvant chemotherapy alone[33]. In the adjuvant setting, up to 25% of patients are unable to receive treatment secondary to post-operative complications[34,35]. For these reasons, at some centers, neoadjuvant therapy is recommended even for resectable pancreatic cancer but is not the standard of care at this time[36].

There is no standard of care for the type of neoadjuvant therapy in this patient population. Treatment typically consists of a combination of radiation therapy and chemotherapy. The treatment regimens are usually reported from a single institution experience and are largely retrospective in nature. The chemotherapy regimen, dose and duration of radiation and type of radiation are different in these reports making cross-comparison very difficult. Moreover, the definitions of resectability have not been uniform in these studies. The most commonly cited resectability criteria are similar to the NCCN and MD Anderson anatomic imaging criteria while some studies have classified patients as borderline if they have a marginal performance status for surgery or have findings on imaging indeterminate for metastases.

After neoadjuvant therapy, depending on the case series, approximately 50% of the patients are able to undergo resection. After treatment, the change in tumor size by the Response Evaluation Criteria In Solid Tumors (RECIST) is low, around 10%-20%. RECIST response did not correlate with survival among patients who underwent pancreatic resection after neoadjuvant therapy, suggesting that RECIST criteria is a poor determinant of benefit in these patients[37]. There is the possibility that the tumor near the vessel can be replaced by fibrous tissue which may not be easily discernible on CT scan[38].

There have been four small prospective trials reported in the literature that have evaluated neoadjuvant therapy for patients with borderline resectable cancer (Table 2). Landry *et al*[39] reported the multi-institutional randomized phase II trial comparing two neoadjuvant regimens. Patients in arm A (n = 10), received concurrent gemcitabine and radiation while patients in arm B (n = 11) received induction chemotherapy with gemcitabine, cisplatin and 5-fluorouracil followed by 5-flourouracil based radiation. Three patients in arm A and two patients in arm B underwent resection. The median survival of resected patients was 26.3 mo. These outcomes were consistent with previous retrospective studies[40,41]. The trial was terminated early due to poor accrual. Another phase II trial evaluated the role of neoadjuvant therapy in patients with resectable or borderline resectable pancreatic cancer[42]. Thirty nine patients with borderline resectable disease were identified using NCCN criteria and were treated with gemcitabine and oxaliplatin for two cycles. Radiation was administered with the first cycle of chemotherapy to a total dose of 30 Gy in 15 fractions. Pancreatic resection was performed in 63% of patients and 84% of those patients had R0 resection. The median survival of resected patients was 25.4 mo. Similar results were observed with other small clinical trials[43,44].

The data on clinical outcomes after neoadjuvant therapy for borderline pancreatic cancer is primarily derived from retrospective single institution experience. One of the first restrospective studies from MD Anderson included 160 patients with pancreatic cancer who received pre-operative therapy, including 84 patients who met radiologic criteria for borderline resectable disease[40]. Patients were treated with a variety of neoadjuvant regimens including chemotherapy or chemoradiotherapy with a gemcitabine based regimen being most common. Resection was performed in 38% of the patients with negative margins in 97% of the subjects. The median survival for resected patients was 40 mo and for all patients was 21 mo. In the follow up report, 115 patients who met AHPBA/SSO/SSAT criteria for borderline resectable pancreatic cancer were included[37]. Despite the fact that partial response by RECIST criteria was observed in only 12% of the patients, 70% of the patients underwent resection and only 5% of the patients had positive margins.

Stokes *et al*[41] evaluated capecitabine based chemo-radiation in 40 patients with borderline resectable pancreatic cancer. Patients received external bean radiation in conventional fractionation (50.4 Gy in 28 fractions) or in an accelerated protocol (50 Gy in 20 fractions). Radiation was targeted at the gross tumor as

Table 2 Selected neoadjuvant studies for borderline resectable pancreatic cancer

Ref.	Study type	*n*	Regimen	Resection	R0 resection	Median OS (resected patients)	Median OS (all patients)	Definition
Katz *et al*[40]	Retrospective	84	5-FU, paclitaxel, gemcitabine or capecitabine + RT; Gemcitabine based chemotherapy	38%	97%	40 mo	21	MDA
Turrini *et al*[70]	Retrospective	49	5-FU/cis + RT 45 Gy for 5 wk	18%	100%	24 mo	14 mo	MDA
Chun *et al*[71]	Retrospective	74	5-FU or gem + RT	100%	59%	23	23	Other
Stokes *et al*[41]	Retrospective	40	Capecitabine + RT	46%	75%	23	12	MDA
Katz *et al*[37]	Retrospective	115	Gem followed by gem or 5-FU or capecitabine + RT; Gem or 5-FU or capecitabine + RT	70%	95%	33	22	NCCN
Mellon *et al*[45]	Retrospective	110	GTX X 3 cycles followed by SBRT	51%	96%	19	34	NCCN
Landry *et al*[39]	Randomized phase II	21	Gem + RT; Gem/cis/5-FU followed by 5-FU/RT	24%	100%	26	19.4 mo; 13.4 mo	Other
Lee *et al*[44]	Prospective trial	18	Gem/capecitabine X 3-6 cycles	61%	82%	23	16	NCCN
Kim *et al*[42]	Phase II study	39	Gem/Ox + RT	63%	84%	25	18	NCCN
Motoi *et al*[43]	Phase II study	16	Gem/S1 X 2 cycles	NA	87%	NA	18	MDA
Takahashi *et al*[46]	Prospective	80	Gem + RT followed by Gem	54%	98%	NA	NA	Other

NCCN: National Comprehensive Cancer Network; MDA: MD Anderson; 5-FU: 5-flurouracil; NA: Not available; RT: Radiation therapy.

well as draining lymphatics with a margin ranging from 0.5-2 cm (excluding the para-aortic and porta-hepatis location) utilizing intensity modulated radiation therapy (IMRT) and image guided radiation therapy. Pancreatic resection was performed in 46% of the patients with R0 resection in 87.5% of patients. Accelerated fraction radiation wasn't associated with increased severe toxicities. A report from Moffitt Cancer Center included 110 patients with BRPC treated with induction chemotherapy followed by stereotactic body radiation therapy (SBRT)[45]. The majority of the patients received combination of gemcitabine, docetaxel and capecitabine for 3 cycles. Surgical resection of the tumor was performed in 51% of the patients with R0 resection rate of 96%. Interestingly, 4 (7%) patients had complete pathologic response and a total of 28 (50%) patients had College of American Pathology Tumor Regression Grade 0-1. The median survival for all BRPC was 19 mo.

Radiation type

The neoadjuvant radiation strategies presented above for borderline pancreatic cancer vary greatly from center to center with respect to dose and technique. This ranges from a conventionally fractionated approach all the way to a SBRT approach and everywhere in between. Moreover, some series report the integration of radiosensitizing chemotherapy, consisting largely of continuous infusion 5-flurouracil (5-FU) or gemcitabine.

Standard fractionation has been used in upfront resectable patients with good outcomes and has been adopted at many centers as a strategy for borderline resectable patients[41,46-48]. With standard fractionation, > 90% pathologic response was achieved in 16%-37% and resection rates are around 50%[41,46]. In the report by Stokes *et al*[41], there was a trend for increased survival and a statistically significant increase in > 90% pathologic response in patients that received accelerated fractionation. Takeda *et al*[49] report their results of a phase Ⅰ and Ⅱ trial looking at accelerated hyperfractionation in borderline pancreatic cancer patients. A total of 35 patients were treated with concurrent gemcitabine and accelerated hyperfractionated radiation 1.5 Gy given twice daily to a total dose of 30 Gy (phase Ⅰ) or 36 Gy (phase Ⅱ) targeting the tumor and regional metastatic lymph nodes with a > 1 cm margin utilizing a 4-field technique. No acute grade ⩾ 3 non-hematologic toxicity was observed. Three fourth of the patients underwent surgical resection with all being R0 resections. Greater than 90% pathologic response to neoadjuvant treatment was observed in 23% of patients. Median survival was 41.2 mo in the patients that underwent surgical resection. This, along with the report by Stokes *et al*[41], suggests a benefit in response rates with accelerated fractionation concurrent with chemotherapy.

The radiation dose and volume treated depends on many factors including technique as well as chemotherapy used. Patients treated with the radiation sensitizing chemotherapy agent 5-FU can be treated to a higher dose and a larger volume, targeting the gross tumor as well as draining lymphatics[41]. When concurrent full dose gemcitabine is utilized, caution on the total dose of radiation as well as the volume being treated is indicated. In the prospective trial, only the gross tumor with a 1 cm margin and a total dose of 30 Gy in standard fractionation was used[42].

IMRT and/or SBRT can be used to increase the biologically effective dose and data suggests there may be potential for improved outcomes in the setting of pancreatic cancer not amenable to upfront resection.

The University of Michigan data reporting dose escalation with IMRT (recommended dose of 55 Gy in 25 fractions) in the locally advanced setting with full dose gemcitabine shows promising results as far as toxicity and R0 resection rates[50]. The most recent Radiation Therapy Oncology Group 1201 trial is a phase II trial looking at local *vs* systemic treatment escalation stratified by SMAD4 expression[51]. SMAD4 has been identified and shown to correlate with patterns of failure, either locally destructive failure *vs* metastatic disease in a rapid autopsy study done at John Hopkins[52]. These results will add to the knowledge of dose escalation with IMRT. SBRT along with chemotherapy prior to or after was initially established in locally advanced pancreatic cancer and was shown to be an effective treatment strategy with low rates of toxicity[53-57]. More recently, results from a phase Ⅱ trial reported by Herman *et al*[58], showed that in locally advanced pancreatic cancer patients treated with SBRT (33 Gy in 5 fractions) there were minimal acute and late toxicity (2% and 11%, respectively). The results published by group at Moffitt Cancer Center incorporating SBRT demonstrated that 51% of the BRPC patients underwent surgical resection with 96% being R0 resections[59]. The median dose was 30 Gy (range 28-30) to the gross disease and 40 Gy (25-50 Gy) to the area of vessel abutment. No prophylactic draining lymphatics were in the treatment volume. There were few acute and late grade $\geqslant$ 3 toxicity (7%). With 14 mo of follow up, there were no recurrences in this subset of patients and there was a rate of pathologic complete response of 7%. SBRT allows for escalating and personalizing the dose to each patient based on specific tumor location, vasculature abutment, and proximity to critical normal tissues with no increase in toxicity or peri-operative mortality and allows for the time course from systemic therapy to potential resection to be shorter since the duration of therapy is only one week. No prospective data is yet available in the BRPC setting incorporating SBRT but the available evidence merits further investigation of this novel approach.

Lastly, interest has been generated on the potential of proton therapy to improve outcomes for pancreatic cancer patients. Proton therapy over five days has been successfully integrated with capecitabine for upfront resectable patients on a phase Ⅰ/Ⅱ study with low rates of toxicity[60]. MD Anderson has compared 3-dimentional conformal radiation (3DCRT), IMRT, and passive-scattering proton therapy dose escalation (72 Gy) plans for pancreatic tumors[61]. Overall they found 3DCRT to be inadequate for coverage and IMRT to be more conformal in high gradient dose regions which would be beneficial for dose escalation in patients with organs at risk in close proximity, as seen in pancreatic cancer. Proton therapy had the advantage of a low integral dose but this would not affect dose escalation. Thompson *et al*[62] reported their dosimetric comparison of IMRT, double scattering and pencil beam scanning proton therapy. They found again that proton beam therapy would unlikely result in dose escalation over IMRT. Proton therapy resulted in decreased dose in the low-intermediate dose range but increased dose in the mid to high dose region, with unclear clinical significance.

The optimal technique and dose of radiation therapy is unclear; however, dose escalation with IMRT and/or SBRT show promising results in increasing R0 resection rates with low toxicity.

DISCUSSION

The margin status is very important to the clinical outcomes after pancreatic resection. The goal of the resection is to obtain R0 resection as patients with gross disease at the margins (R2 resection) do not benefit from surgical resection and have similar outcomes as patients without surgery[63-65]. Microscopic disease at the margin (R1 resection) is associated with a poor prognosis but is not consistent across all studies[63,66,67]. The definition of R1 resection has not been uniform in the past which makes interpretation of data from various studies problematic. AJCC criteria define positive resection margins when tumor cells are present at the edge of resected specimen whereas European criteria defines positive margins if tumor cells are present within $\leqslant$ 1 mm of resected margins[68]. The location of margins has prognostic impact as well. In one study, R1 status at the anterior or posterior margins was not relevant for outcomes[69].

Recently, there has been improvement in systemic therapies for metastatic pancreatic cancers that has improved response rates over single agent gemcitabine. The FOLFIRINOX regimen and gemcitabine/nab-paclitaxel combination is associated with response rates of 31% and 23% compared to less than 10% with single agent gemcitabine. These regimens may increase the probability of margin negative resection and the ability to obtain an R0 resection. There are additional toxicities associated with these combination regimens, especially FOLFIRINOX, including neutropenic fever. The Intergroup trial (ALLIANCE A021101) is evaluating neoadjuvant FOLFIRINOX followed by capecitabine based chemoradiotherapy. The dose of 5-FU has been modified to make it more tolerable. Patients who undergo resection will also receive adjuvant gemcitabine. The criteria for resection have been clearly defined through consensus and may become the new standard for resectability.

CONCLUSION

Management of borderline resectable pancreatic cancer continues to evolve. Prior studies have been complicated by low accruing trials, largely retrospective single institution experiences, and different classification criteria, chemotherapy regimens and radiotherapy type and schedule. There is an urgent need to apply uniform criteria for defining borderline pancreatic cancer. The patients should be classified and treated with a

multidisciplinary approach at high volume centers. Patients should undergo a pancreas protocol CT scan and EUS to determine the resectability status. Ideally, these patients should be treated on a clinical trial protocol. The ability to obtain negative margins is of the utmost importance for improving the outcomes of these patients. Newer aggressive chemotherapy regimens may help improve the resectability rate. These regimens followed by SBRT or IMRT may have a role in treatment. Induction chemotherapy followed by chemoradiation is the most commonly utilized approach but is not uniform. Newer trial designs incorporating uniform classification and treatment strategy will help standardize treatment for patients with borderline resectable pancreatic cancer.

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TOPIC HIGHLIGHT

2015 Advances in Pancreatic Cancer

Genomic alterations in pancreatic cancer and their relevance to therapy

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Abstract

Pancreatic cancer is a highly lethal cancer type, for which there are few viable therapeutic options. But, with the advance of sequencing technologies for global genomic analysis, the landscape of genomic alterations in pancreatic cancer is becoming increasingly well understood. In this review, we summarize current knowledge of genomic alterations in 12 core signaling pathways or cellular processes in pancreatic ductal adenocarcinoma, which is the most common type of malignancy in the pancreas, including four commonly mutated genes and many other genes that are mutated at low frequencies. We also describe the potential implications of these genomic alterations for development of novel therapeutic approaches in the context of personalized medicine.

Key words: Pancreatic cancer; Genomic alterations; Signaling pathways; Therapeutic targets; Personalized medicine

Core tip: With the advance of sequencing technologies for global genomic analysis, the landscape of genomic alterations in pancreatic cancer is becoming increasingly well understood. In this review, we summarize the latest knowledge of genomic alterations in pancreatic ductal adenocarcinoma including commonly mutated genes and many other genes that are mutated at low frequencies. We also describe the potential implications of these genomic alterations for development of novel therapeutic approaches in the context of personalized medicine.

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INTRODUCTION

Pancreatic cancer was the seventh leading cause of death in the world in 2012, and is responsible for about 331000 deaths per year[1]. The 5-year survival of pancreatic cancer patients is approximately 5%, and this figure has remained constant in recent decades. Because of the absence of effective methods for early detection and the aggressive nature of this disease, the majority of patients present with locally advanced or metastatic cancer which is not eligible for surgical resection. Chemotherapeutic options for treatment of advanced pancreatic cancer are still limited, and gemcitabine has been the standard chemotherapeutic drug for patients with advanced disease for many years, even though this drug alone provides only a modest survival advantage[2-4]. Since the approval of gemcitabine in United States, many randomized clinical trials have been performed to evaluate combinations of gemcitabine with other drugs, such as 5-fluorouracil (5-FU), cisplatin, oxaliplatin and irinotecan[5], but few of them show a significant survival advantage compared with gemcitabine alone. The combination of gemcitabine with the epidermal growth factor receptor (EGFR) inhibitor, erlotinib, does confer a survival advantage over gemcitabine monotherapy, but the overall survival of patients with advanced disease was extended by only 10 d on average[6]. The combination of gemcitabine with nab-paclitaxel (albumin-bound paclitaxel) was recently shown to be superior to gemcitabine alone, probably because of depletion of tumor stroma, which leads to improved delivery of gemcitabine to tumor cells[7]. Other than gemcitabine-based chemotherapies, 5-FU-based chemotherapeutic regimens have also been evaluated. FOLFIRINOX (folinic acid, fluorouracil, irinotecan and oxaliplatin) improved the median overall survival from 6.8 to 11.1 mo compared with gemcitabine, although significant toxicities associated with this regimen limit its utility in a wide range of patients[8]. It seems that a deeper understanding of the molecular biology of pancreatic cancer is needed to develop novel therapeutic approaches.

In recent years, advances in sequencing technologies have enabled us to perform genome-wide analysis to establish the genetic alterations underlying pancreatic carcinogenesis and progression. In this review, we summarize current knowledge of genomic alterations in pancreatic ductal adenocarcinoma (PDAC), which is the most common type of malignancy in the pancreas, and we discuss their implications for development of novel therapeutic strategies.

GENOMIC ALTERATIONS OF PANCREATIC CANCER

Jones *et al*[9] have shown that PDAC harbors an average of 63 genome alterations, of which the majority are point mutations. Four key genes are frequently altered in PDAC: *KRAS*, *CDKN2A*, *TP53* and *SMAD4*. The most common gene alteration is in *KRAS* (v-ki-ras2 Kirsten rat sarcoma viral oncogene homolog), where mutations occur in codons 12, 13 and 61[9,10]. More than 90% of PDAC contains *KRAS* mutation, and such mutations are also present in about 45% of low-grade pancreatic intraepithelial neoplasia (PanIN) lesions[11,12]. *KRAS* encodes a GTPase that activates various downstream signaling pathways, including the mitogen-activated protein kinase (MAPK) cascades[13]. Mutations in *KRAS* result in constitutive activation. Ras proteins are involved in a variety of cellular functions, including proliferation, differentiation and survival[14,15]. *P16*, cyclin-dependent kinase inhibitor 2A gene (*CDKN2A*) is also inactivated in up to 90% of PDAC, due to intragenic mutation in association with allelic loss, homozygous deletion, or hypermethylation of the gene promoter[16-18]. *CDKN2A* encodes a cyclin-dependent kinase inhibitor that controls G1-S transition in the cell cycle. Mutations in *CDKN2A* are thought to be subsequent to those of *KRAS,* because of the higher prevalence of *KRAS* mutations in early-stage precursor lesions and the fact that most PanIN lesions containing *CDKN2A* inactivation also harbor *KRAS* mutation[19]. *TP53* is one of the most frequently mutated genes in many types of cancer[20-22], and is inactivated in about 75% of PDAC, mainly due to point mutations or small deletions[21,22]. p53 is a transcription factor that determines cell fate by inducing expression of a variety of genes related to cell cycle arrest and apoptosis, and plays an important role as a master regulator of cellular stress responses. *SMAD4* (*DPC4*, SMAD family member 4 gene) is inactivated in up to 55% of PDAC by homozygous deletion or intragenic mutation in association with allelic loss[23]. *SMAD4* encodes a transcription factor that mediates signaling of the transforming growth factor-β (TGF-β) superfamily. *TP53* and *SMAD4* genes are mutated in late-stage precursor lesions, typically in high-grade PanIN[24,25].

In addition to these four frequently altered genes, various other genes are mutated at relatively low frequencies in pancreatic cancer. Jones *et al*[9] reported alterations in genes related to chromatin remodeling (*ARID1A*, *MLL3*). Furthermore, they proposed that core signaling pathways exist in pancreatic cancer (Figure 1), and noted that the pathway components altered in individual tumors may vary widely[9]. Whole-exome sequencing analysis of 99 pancreatic cancers found many significantly mutated genes, including genes

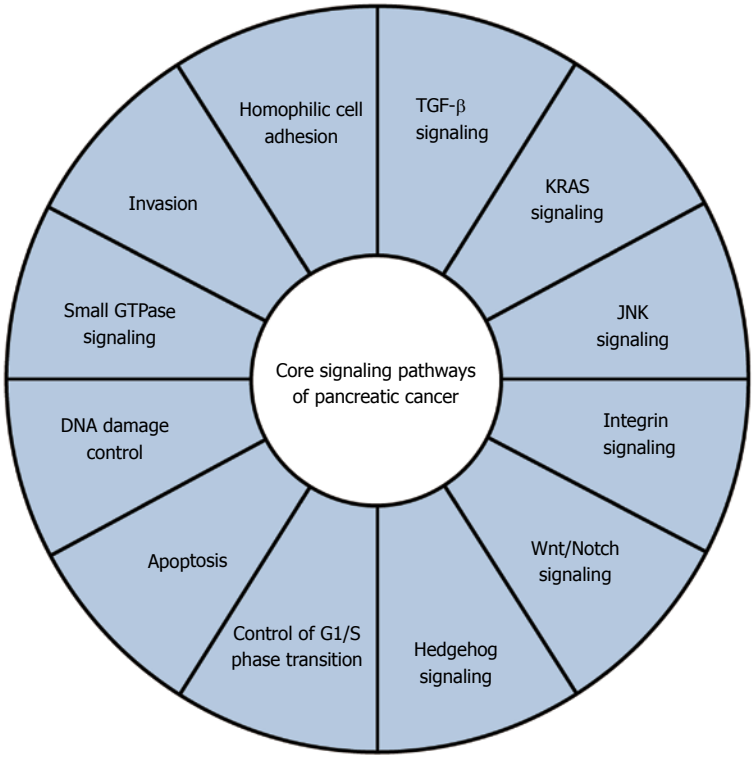

Figure 1 Core signaling pathways of pancreatic cancer. Twelve signaling pathways and cellular processes that are important in pancreatic cancer have been identified based on whole-exome sequencing analysis[9]. Various component genes associated with each pathway are mutated in most pancreatic cancers. Targeting one or more of these pathways, rather than specific gene alterations that occur within a pathway, would be a new strategy for treatment of pancreatic cancer. KRAS: V-ki-ras2 Kirsten rat sarcoma viral oncogene homolog; JNK: C-jun N-terminal kinase; TGF-β: Transforming growth factor-β.

related to chromatin remodeling (*EPC1*, *ARID2*) and DNA damage repair (*ATM*)[26]. In addition to the core signaling pathways mentioned above[9], they identified significant alterations in genes related to the axon guidance pathway, including *ROBO1/2* and *SLIT2*[26]. More recently, whole-genome analysis of 100 PDACs provided a comprehensive picture of the genomic alterations in this disease[27]. In addition to genes known to be important in PDAC (*TP53*, *SMAD4*, *CDKN2A*, *ARID1A* and *ROBO2*), chromosomal rearrangements affecting *KDM6A* and *PREX2* were identified. *KDM6A* is related to chromatin remodeling, and is mutated in renal cell carcinoma and medulloblastoma[28,29]. The RAC1 guanine nucleotide exchange factor, *PREX2*, is mutated in melanoma[30]. Copy number analysis also uncovered a number of amplifications in genomic regions including *KRAS* and *GATA6*[27], in accordance with a previous report[31]. Most importantly, they demonstrated that a small fraction of patients (1%-2%) harbor focal amplifications in druggable genes, including *ERBB2*, *MET*, *FGFR1*, *CDK6*, *PIK3CA* and *PIK3R3*[27].

Some germline mutations are known to be associated with familial clusters of pancreatic cancer. For example, inactivation of *BRCA2*, which encodes a protein involved in DNA damage repair, is related to familial pancreatic cancer. Indeed, *BRCA2* mutation is associated with a 3.5- to 10-fold increased risk of pancreatic cancer, as well as increased risk of breast cancer and ovarian cancer[32,33]. Germline mutations in the Fanconi anemia genes, such as *FANCC*, *FANCG* and *PALB2* (also known as *FANCN*), are also implicated in familial pancreatic cancer[34-37]. In addition, germline mutation of *ATM* has recently been identified in subsets of familial pancreatic cancer[38].

IMPLICATIONS OF GENOMIC ALTERATIONS FOR TREATMENT OF PANCREATIC CANCER

The development of powerful sequencing technologies has led to a detailed knowledge of the human cancer genome, and it has become evident that some types of cancer can be effectively treated by targeted therapies based on their specific gene alterations. Here we discuss potential approaches for gene alteration-based treatment of pancreatic cancer.

The most prevalent oncogenic alteration, in *KRAS*, seems an obvious target for cancer therapy, because mutant KRAS protein has been experimentally demonstrated to play a pivotal role in maintenance of PDAC[39,40]. Activating mutations at *KRAS* codons 12, 13 and occasionally 61 are currently the most common gene alterations in pancreatic cancer. A therapeutic effect of blocking G12D mutant *KRAS* has been demonstrated by using siRNA and a novel siRNA delivery system, both *in vitro* and *in vivo*[41]. Although great efforts have been made to develop small-molecular inhibitors of mutant *KRAS*, no clinically effective antagonist has yet been identified[42]. Instead, some indirect approaches, such as targeting post-transcriptional processes, have been tried. Farnesylation of KRAS allows the protein to associate with the membrane and interact with Ras activating proteins, including Ras-GEFs. Farnesyltransferase is the key enzyme involved in addition of a 15-carbon isoprenoid chain to

KRAS protein. However, despite *in vitro* and xenograft studies[43], farnesyltransferase inhibitors, such as tipifarnib, have proven unsuccessful in combination with gemcitabine[44,45]. This can be attributed to the existence of an alternative post-transcriptional mechanism, geranyl-geranylation, that compensates for inhibition of farnesyltransferase[46]. A dual inhibitor of farnesyltransferase and geranylgeranyltransferase (L-778,123) was tested in a Phase Ⅰ clinical trial in combination with radiotherapy for locally advanced PDAC, and showed acceptable toxicity[47]. Some groups have recently investigated strategies targeting localization of KRAS to the membrane. Deltarasin is a small molecule that binds to the farnesyl-binding pocket of the delta subunit of phosphodiesterase (PDEδ) and inhibits translocation of KRAS to the membrane by blocking the interaction between PDEδ and farnesylated KRAS[48,49]. On the other hand, Salirasib blocks KRAS activation by dislodging the farnesylated protein from the membrane[50]. The results of preclinical and clinical trials suggest that salirasib may be effective[51].

Targeting downstream effectors of KRAS may be an alternative approach to block the KRAS signaling pathway. The MEK/MAPK and PI3K/Akt/mTOR pathways are the principal downstream pathways of KRAS. But, although several MEK inhibitors, such as CI-1040 and PD0325901, have been investigated in clinical trials, they failed to deliver meaningful therapeutic benefit[52,53]. In addition, trametinib, another MEK1/2 inhibitor, was recently tested in combination with gemcitabine for patients with metastatic pancreatic cancer, but failed to improve the clinical outcome[54]. Activation of the PI3K/Akt/mTOR pathway also plays an important role in maintenance of pancreatic cancer[55-57]. An inhibitor of PI3K, LY294002, was reported to induce apoptosis *in vitro* and to inhibit tumor growth *in vivo*[58]. In addition, everolimus, a mammalian target of rapamycin (mTOR) inhibitor, has been reported to inhibit tumor growth *in vivo*[59]. However, everolimus had minimal activity in patients with gemcitabine-resistant PDAC in a phase Ⅱ study[60,61]. It was recently found that tumors with activated *KRAS* and mutant *TP53* did not respond to mTOR inhibition, whereas tumors with *KRAS* activation and *PTEN* loss are responsive to mTOR inhibition[62].

Since the MEK/MAPK and PI3K/Akt/mTOR pathways are both downstream of KRAS, it is possible that inhibition of one pathway induces compensatory activation of the other pathway. Therefore, inhibition of both pathways may have a synergistic effect in treatment of pancreatic cancer[63,64]; thus, simultaneous blockade of MEK/MAPK and PI3K/Akt/mTOR seems to warrant further investigation as a candidate therapy for pancreatic cancer.

In addition to *KRAS*, *CDKN2A*, *TP53* and *SMAD4* are also commonly altered in pancreatic cancer. However, therapeutic approaches targeting these proteins are considered to be difficult for various reasons, including cellular location and multifunctionality. Although a number of therapeutic strategies targeting these genes have been examined for various types of cancer, none has yet been implemented for treatment of pancreatic cancer.

Focusing on signaling pathways in pancreatic cancer may be a better strategy than targeting particular gene alterations for treatment of pancreatic cancer. The core signaling pathways of pancreatic cancer[9] include several druggable pathways. For example, the Wnt/Notch pathway is important, and inhibition of the Notch pathway by inhibiting γ-secretase has been suggested as a potential treatment strategy[65]. The combination of γ-secretase inhibitor MRK003 with gemcitabine has been shown to provide a survival benefit *in vivo*[66]. It has also been reported that pancreatic cancer cells that harbor inactivating mutations of *RNF43* are sensitive to LGK974, a Wnt pathway inhibitor currently in a phase 1 clinical trial[67]. Inhibition of the Hedgehog pathway with a natural hedgehog antagonist, cyclopamine, decreases growth of various types of tumor, including PDAC[68,69]. Clinical use of cyclopamine, however, is problematic because of its side effects and suboptimal pharmacokinetics. A novel, orally bioavailable, small-molecular Hedgehog inhibitor, IPI-269609, has been shown to inhibit tumor initiation and metastasis of pancreatic cancer[70]. Interestingly, blockade of the Hedgehog pathway has also been proposed as a means to target the tumor stroma and improve delivery of gemcitabine *in vivo*[71]. Small-molecular inhibitor Saridegib (IPI-926) was tested in combination with gemcitabine in patients with pancreatic cancer. However, the Phase Ⅰ/Ⅱb trial was stopped because patients receiving the combination had higher rates of progressive disease and lower overall survival in 2012[72].

Although the frequencies are low, mutations of several familial pancreatic cancer-related genes are associated with drug sensitivity. Inactivation of *BRCA2* is found in about 7% of western PDAC patients[32,73]. BRCA2 plays a crucial role in homologous recombination-based DNA damage repair processes[74]. Poly ADP-ribose polymerase (PARP) is an important enzyme in the DNA repair mechanism mediated by BRCA2, and PARP inhibitors induce extreme genome instability and death of *BRCA*-mutated cancer cells[75]. As well as PARP inhibitors, DNA-crosslinking agents such as mitomycin C, cisplatin and carboplatin are also effective for treatment of BRCA-inactivated pancreatic cancer[76]. As *PALB2* encodes a protein that interacts with BRCA2, *PALB2* mutations are expected to disrupt BRCA2-mediated repair of DNA double strand breaks. *PALB2* mutations in PDAC patients confer sensitivity to DNA-damaging agents[77]. Tumors with mutations in *ATM*, another familial pancreatic cancer-related gene, might also be sensitive to PARP inhibitors[78].

Overall, pancreatic cancer is characterized by substantial genomic heterogeneity with numerous infrequently mutated genes[9,26,27]. Although the common mutations in pancreatic cancer, *KRAS*, *TP53*, *CDKN2A* and *SMAD4*, are currently not druggable, stratified therapeutic strategies based on genomic alterations

that occur at low frequency might be beneficial for treatment of pancreatic cancer. Recently, Jones *et al*[79] identified somatic alteration in potentially druggable genes in approximately 20% of PDAC patients. In Australia, the Individualized Molecular Pancreatic Cancer Therapy (IMPaCT) trial screens patients for actionable molecular phenotypes, with the aim of developing personalized therapies for pancreatic cancer[80]. IMPaCT is a randomized phase Ⅱ clinical trial designed to assess standard therapy (gemcitabine) *vs* genotype-guided target therapies in patients with recurrent or metastatic pancreatic cancer. Initially, three subgroups with pre-defined actionable mutations, *i.e., HER2*-amplified (gemcitabine + trastuzumab), DNA damage response-defective (gemcitabine + PARP inhibitor) and anti-EGFR-responsive (gemcitabine + erlotinib), are being tested. This clinical trial was designed so that other arms could be added as novel subgroups or agents are identified. This approach could facilitate development of personalized therapies for pancreatic cancer.

CONCLUSION

Comprehensive genomic studies have provided extensive information on the pancreatic cancer genome, including its heterogeneity and core signaling pathways. These findings should be useful for the development of novel therapeutic strategies. For example, it might be helpful for early detection of pancreatic cancer to identify individuals with a genetic predisposition for the disease, including familial pancreatic cancer-related genes, so that periodic follow-up screening can be performed. Analysis of clonal evolution of pancreatic cancer indicates that it takes more than 10 years from occurrence of the initiating genomic alteration to formation of the parental clone[81]. Thus, there appears to be a substantial time window for early detection. Current sensitive sequencing technologies allow us to detect tumor DNA of various types of cancer in plasma (circulating tumor DNA, ctDNA)[82], and indeed, ctDNA has been detected in plasma from patients with early-stage breast and lung cancers[83,84]. Such an approach could also be applicable to patients with pancreatic cancer. More comprehensive genomic analysis may also be useful for identifying actionable mutations. Furthermore, ctDNA is thought to reflect the genetic heterogeneity of cancer, since it may contain tumor DNA derived from various regions, including metastases. Novel strategies based on genomic information seem likely to revolutionize pancreatic cancer therapy over the next few years, and may ultimately lead to fully personalized medicine.

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CASE REPORT

Paraneoplastic leukemoid reaction in pancreatic cancer: A case report

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Abstract

Paraneoplastic leukemoid reaction is a rare syndrome defined by a leukocyte count exceeding 50 Giga/Liter (G/L), mostly described with progressive lung or renal carcinoma. We report a case of a 68-year-old man with recurrent pancreatic carcinoma presenting a leukemoid reaction with a white blood cell count of 63.87 G/L without identified infectious, iatrogenic or hematologic causes. His overall condition quickly degraded and he died three weeks after the discovery of the leukemoid reaction. This is the first case in French literature of leukemoid reaction in a patient with pancreatic carcinoma with poor prognostic value.

Key words: Leukemoid reaction; Pancreatic neoplasms; Paraneoplastic syndrome; Prognosis

Core tip: Paraneoplastic leukemoid reaction is a rare syndrome which seems to be associated with aggressive tumors, rapid clinical deterioration, and short survival. We report a rare presentation of pancreatic cancer with leukemoid reaction in a 68-year-old man who died three weeks after its discovery. This paper may contribute to clinical practice when encountering such a patient because of its poor prognostic value.

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INTRODUCTION

Carcinoma is the most common (90%) and gravest type of pancreatic tumor with 5-year global survival

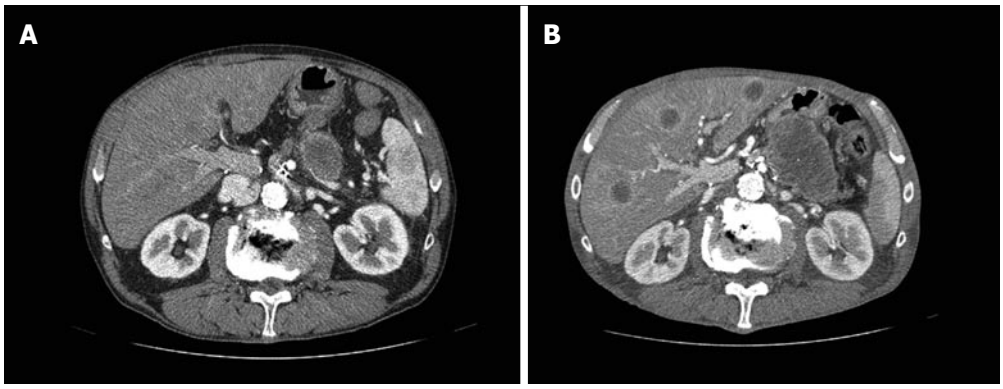

Figure 1 Computer tomography before (A) and after (B) leukemoid reaction. Pancreatic and hepatic evolution.

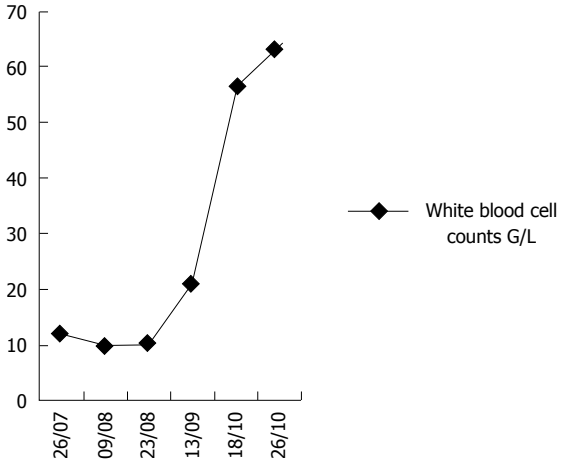

Figure 2 Evolution of white blood cell counts associated with leukemoid reaction. Leukocytosis rapid increase.

rates around 5%. In France, it is the fifth cause of cancer-related deaths and its incidence is increasing fast with approximately 8000 annual new cases. Paraneoplastic syndromes can occur in a minority of cancer cases (less than 10%) and are not directly related to the physical effects of the tumor. Those most frequently associated with pancreatic carcinoma are Trousseau's syndrome, Cushing's syndrome, and the unexplained prolonged fever. They can reveal the disease or arise during progression. They can decline under treatment, even disappear with the cure and reappear in case of relapse. Paraneoplastic leukemoid reaction is defined as leukocytosis exceeding 50 Giga/Liter (G/L). Its diagnosis rests essentially on the exclusion of infectious, hematologic or iatrogenic causes such as growth factor or corticosteroid therapy[1]. This syndrome is most frequently associated with carcinomas, in particular lung and renal[2,3], and is rarely described in cancers of the digestive tract, including pancreatic cancers.

CASE REPORT

We report the case of a 68-year-old man with pancreatic carcinoma, who was diagnosed with paraneoplastic leukemoid reaction in the absence of plausible differential diagnoses.

Our patient was diagnosed with pT2N0M0 carcinoma of the head of the pancreas, discovered by jaundice, and operated by cephalic duodenopancreatectomy. He then received adjuvant chemotherapy with 6 cycles of gemzar. One year later, tumor markers (carbohydrate antigen 19-9 and carcinoembryonic antigen) increased and a positron emission tomography scan detected a local recurrence. Radiological stabilization and a decrease of markers were obtained after 4 cycles of folfox. Therefore, 6 additional cycles were administered.

Follow-up imaging revealed local evolution and hepatic metastases. Tumor marker levels were increased. A new line of chemotherapy was begun with folfiri. After 4 cycles, hepatic (Figure 1) and pulmonary evolution were observed associated with a progressive generalized weakness. Nevertheless, due to the patient's strong insistence on treatment and a relatively stable overall condition, a third line of 5-fluorouracil (5-FU)/cisplatin was considered. During the first cycle, a white blood cell count showed extreme leukocytosis of 63.87 G/L (Figure 2), with neutrophil predominance of 92.7%, associated with a myelaemia of 1%, without abnormal eosinophilia, basophilia or anomaly of the other cell lines (hemoglobin 10.5 g/dL and platelets 207 G/L).

The patient had not received granulocyte colony-stimulating factors (G-CSF) or corticosteroids. Standard infectious investigations found no obvious sign of infection: C-reactive protein was slightly elevated at 138 mg/L, central and peripheral blood cultures as well as urine culture were negative, and a chest radiograph was normal. Moreover, a skeletal scintigraphy was performed and found no evidence of bone metastases. A cytological bone marrow examination showed a massively increased granulopoiesis with predominant neutrophils, complete maturation, without excess of blast cells or other anomalies that might suggest the existence of an acute leukaemia (Figure 3).

Molecular genetic analysis did not find a BCR-ABL fusion gene or a V617F mutation in the *JAK2* gene. The serum level of G-CSF was within normal range (< 40 pg/mL) and interleukin-6 (IL-6) was at 10 pg/mL (reference range: 0-10 pg/mL).

Only one cure of chemotherapy by 5-FU/cisplatin was

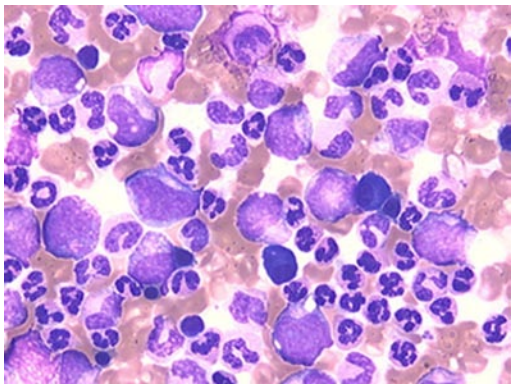

Figure 3 Bone marrow cytology (original magnification × 500). Increased granulopoiesis up to the neutrophils with a complete maturation and without blast cells.

administered, because of the patient's rapid deterioration. He died three weeks after the development of the leukemoid reaction. During this period, leukocyte count remained above 50 G/L.

DISCUSSION

Paraneoplastic leukemoid reaction has rarely been described in cancers of the digestive tract, in particular pancreatic carcinoma, with only four cases found in the literature[4-7]. This seems to be the first case of leukemoid reaction in a patient with pancreatic cancer reported in the French literature.

Making this diagnosis requires eliminating an infection, a treatment with corticoids or G-CSF, and the existence of hematologic neoplasia. This paraneoplastic syndrome has a poor prognostic value without a fast effective anti-tumor treatment, as illustrated by other reviews of the literature. Indeed, it is associated with aggressive tumors, rapid clinical deterioration, and short survival. The mechanism of this reaction is still not formally identified. Some data, concerning essentially lung cancers, suggest a secretion by tumor cells of hematopoietic growth factors such as G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF) inducing extreme leucocytosis[8,9]. Other mechanisms could also be involved in this reaction, in particular the production of pro-inflammatory cytokines in response to tumor progression or necrosis[10,11].

In our case, there was no elevation of G-CSF or IL-6, although serum levels were tested only once because of the fast change in the patient's overall condition. No elevations of these levels were found in other reports, implying the existence of other factors.

Paraneoplastic leukemoid reaction is rarely associated with pancreatic cancer.

The mechanisms, prognosis, and management of this syndrome are poorly understood. More data are needed to conclude.

Leukemoid reaction appears at an advanced stage and may be a prognostic indicator in patients with pancreatic cancer. It is advisable to quickly diagnose the condition, after elimination of other plausible causes, because of its poor prognostic value.

COMMENTS

Case characteristics

A 68-year-old man with pancreatic carcinoma presented a paraneoplastic leukemoid reaction.

Clinical diagnosis

Rapid clinical deterioration with generalized weakness.

Differential diagnosis

Infection, treatment with corticoids or granulocyte colony-stimulating factors and hematologic neoplasia.

Laboratory diagnosis

White blood cell count showed extreme leukocytosis of 63.87 G/L.

Imaging diagnosis

Computer tomography scans revealed progression of local, liver and lung disease.

Pathological diagnosis

Carcinoma of the pancreas.

Treatment

The tumor was treated by cephalic duodenopancreatectomy associated with adjuvant chemotherapy, and three additional lines of chemotherapy for metastatic disease.

Related reports

Poor prognostic value is also illustrated by other reviews of the literature with short survival. The mechanism of this reaction is still not formally identified, but some data suggest a secretion by tumor cells of hematopoietic growth factors or pro-inflammatory cytokines.

Term explanation

Paraneoplastic leukemoid reaction is defined as leukocytosis exceeding 50 G/L.

Experiences and lessons

Paraneoplastic leukemoid reaction is a rare syndrome, infrequently described with pancreatic cancer, which seems to be associated with poor prognostic value.

Peer-review

A very rare complication of pancreatic cancer with very rare occurence in gastrointestinal cancers and pancreatic cancer in peculiar, worth publishing to inform physicians. It is a step forward on the way of clarifying the pathogeny of this syndrome.

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Novel therapy for advanced gastric cancer

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Abstract

Gastric cancer (GC) is a common lethal malignancy. Gastroesophageal junction and gastric cardia tumors are the fastest rising malignancies due to increasing prevalence of obesity and acid reflex in the United States. Traditional chemotherapy remains the main treatment with trastuzumab targeting human epidermal growth factor receptor 2 positive disease. The median overall survival (OS) is less than one year for advanced GC patients; thus, there is an urgent unmet need to develop novel therapy for GC. Although multiple targeted agents were studied, only the vascular endothelial growth factor receptor inhibitor ramucirumab was approved recently by the United States Food and Drug Administration because of its 1.4 mo OS benefit (5.2 mo *vs* 3.8 mo, $P = 0.047$) as a single agent; 2.2 mo improvement of survival (9.6 mo *vs* 7.4 mo, $P = 0.017$) when combined with paclitaxel in previously treated advanced GC patients. It is the first single agent approved for previously treated GC and the second biologic agent after trastuzumab. Even with limited success, targeted therapy may be improved by developing new biomarkers. Immune therapy is changing the paradigm of cancer treatment and is presently under active investigation for GC in clinical trials. More evidence supports GC stem cells existence and early stage studies are looking for its potential therapeutic possibilities.

Key words: Gastric cancer; Novel therapy; Targeted therapy; Immune therapy; Gastric cancer stem cell

Core tip: Advanced gastric cancer (GC) has very poor outcome with chemotherapy remains the main treatment. There is an urgent unmet need to develop novel therapy for GC. Limited success is achieved for targeted therapy after trastuzumab for human epidermal growth factor receptor 2 positive disease. Ramucirumab was recently approved by Food and Drug Administration as a single agent or combined with paclitaxel in refractory advanced GC patients. Immune therapy and GC stem cell research are on the horizon.

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INTRODUCTION

Gastric cancer (GC) is a common malignancy and the second leading cause of cancer death worldwide[1]. In the United States, there were approximately 22220 new cases and 10990 death in 2014[2]. With overweight and obesity being a more serious epidemiologic issue in the United States, gastroesophageal junction and gastric cardia adenocarcinoma have been the fastest rising cancer. Majority of GCs are present at advanced stages with either metastatic or extensive local/regional disease. It is a group of heterogeneous diseases with different anatomy, epidemiology, etiology, pathogenesis, and behavior. Chemotherapy using fluoropyrimidine or platinum as backbone is the main treatment for advanced GCs. The median survival is limited to 7 to 12 mo in clinical trial setting[3,4]. There is an urgent demand for new therapy to improve its treatment and outcome.

DIFFICULTY AND PROGRESS IN TARGETED THERAPY

Targeted therapy has been the main focus in clinical trials, even though majority of the targeted agents were tested in an unselected "off target" patient population and there was a lacking of biomarkers. It has led to the failure of multiple large phase Ⅲ clinical trials in different pathways. Trastuzumab is approved for human epidermal growth factor receptor 2 (HER2) positive GCs. Ramucirumab has recently gained its label as a single agent or in combination with paclitaxel for refractory GCs patients following fluoropyrimidine or platinum containing chemotherapy.

Epidermal growth factor receptor targeting therapy

Epidermal growth factor receptor (EGFR) has been studied extensively. EXPAND and REAL 3 are the two recent phase Ⅲ clinical trials with EGFR antibodies: cetuximab and panitumumab. Both of them failed to show survival benefit and were concerning for worse toxicity in the EGFR inhibitor study arms. In the EXPAND trial, median progression-free survival (PFS) (4.4 mo *vs* 5.6 mo, $P = 0.32$) and overall survival (OS) (9.4 mo *vs* 10.7 mo, $P = 0.95$) favored the chemotherapy only group, overall response rates (RR) were similar 30% *vs* 29%[5]. Grade 3-4 toxicities were substantially higher in the cetuximab-containing regimen than in the control regimen[5]. REAL 3 trial demonstrated inferior OS in the panitumumab study group when compared to control group (11.3 mo *vs* 8.8 mo, $P = 0.013$) with more toxicities[6]. Biomarker was not used to select patient in both studies. Only 6% screened patients were positive for Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation, a potential association of benefit was found in KRAS mutated group although not significant[6]. This result is contrary to KRAS mutated colon cancer[7].

Phosphatidylinositol 3-kinase /Akt/ mammalian target of rapamycin targeting therapy

The phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling pathway was studied with everolimus in 656 previous treated advanced GC patients in a phase Ⅲ trial: GRANITE-1. Primary endpoint was not reached (OS: 5.4 mo *vs* 4.3 mo, $P = 0.12$), even though PFS was improved (1.7 mo *vs* 1.4 mo, $P < 0.001$)[8]. No biomarker was required for this study entry.

HER2 targeting therapy

HER2 overexpression by immunohistochemistry or gene amplification by fluoresecnence *in situ* hybridization was required for patients' recruitment for the phase Ⅲ ToGA trial. This pivotal trial led to trastuzumab approval with all the outcomes better in the study group (median OS: 13.8 mo *vs* 11.1 mo, $P = 0.0046$; PFS: 6.7 mo *vs* 5.5 mo, $P = 0.0002$; RR: 47% *vs* 35%, $P = 0.0017$)[9]. A post-hoc analysis grouped HER2 status and suggested that larger survival benefit in patients with tumor HER 2 IHC 3+ or 2+ and FISH positive group (OS: 16.0 mo *vs* 11.8 mo, $P = 0.036$)[9]. Lapatinib is a dual tyrosine kinase inhibitor (TIK) inhibitor of HER2 and EGFR. It failed to meet OS benefit in two large phase Ⅲ trials: TRIO-013/Logic in the first line and TyTan in the second line settings (TRIO-013/Logic: 12.2 mo *vs* 10.5 mo, $P = 0.35$; TyTan: 11.0 mo *vs* 8.9 mo, $P = 0.1044$)[10,11]. Lapatinib failure in GC trials might partially relate to its EGFR inhibition effect. Pertuzumab is another humanized monoclonal antibody that binds HER2. Its combination with trastuzumab and chemotherapy is established as first line treatment for metastatic HER2 positive breast cancer[12]. This combination is being evaluated in a phase Ⅲ clinical trial for HER 2 positive advanced GCs (NCT01774786). Trastuzumab emtansine (T-DM1) is an antibody–drug conjugate with monoclonal antibody trastuzumab lined to cytotoxic agent emtansine. A randomized phase Ⅲ trial is ongoing with T-DM1 *vs* taxane for previously treated advanced GCs (NCT01641939).

Antiangiogenic pathway targeting therapy

Vascular endothelial growth factor (VEGF) pathway (angiogenesis) is of great interest in advanced GCs with recent success in ramucirumab, although VEGF-A neutralizing antibody bevacizumab did not reach its primary endpoint in phase Ⅲ AVAGAST trial (OS: 12.1 mo *vs* 10.1 mo, $P = 0.1002$; PFS: 6.7 mo *vs* 5.3 mo, $P = 0.0037$; RR: 46% *vs* 37.4%, $P = 0.0315$)[13]. Ramucirumab is a vascular endothelial growth factor receptor 2 (VEGFR2) monoclonal antibody inhibiting VEGF binding. Two pivotal phase Ⅲ clinical trials REGARD and RAINBOW have led to the approval of ramucirumab in 2014 for advanced GCs after progression on fluropyrimidine or platinum containing chemotherapy. In REGARD trial, ramucirumab was compared to placebo in previously treated advanced GC patients. Survival was significant better as a single agent (OS: 5.2 mo *vs* 3.8 mo, $P = 0.047$)[14]. Ramucirumab was investigated in combination with paclitaxel compared to paclitaxel alone in RAINBOW trial. It demonstrated survival benefit again (OS: 9.6 mo *vs* 7.4 mo, $P = 0.017$)[15]. Advanced GC patients in both trials have been treated previously

and the OS benefits were impressive. Ramucirumab has become the standard second line treatment for advanced GC. In the first line setting, ramucirumab was studied together with FOLFOX in a phase Ⅱ trial. It did not add much improvement (PFS: 6.4 mo *vs* 6.7 mo, *P* = 0.89; OS: 11.7 mo *vs* 11.5 mo)[16]. No biomarker has been established for ramucirumab either. A global phase Ⅲ trial RAINFALL (NCT 02314117) is ongoing comparing fluropyrimidine/Cisplatin with or without ramucirumab in HER2 negative advanced GC patients as first line treatment[17]. Apatinib is an oral small molecular TKI of VEGFR-2. In a phase Ⅲ clinical trial of advanced GC patients who failed second-line chemotherapy, the OS was significantly prolonged in the apatinib group when compared to the placebo group (6.5 mo *vs* 4.7 mo, *P* < 0.016; PFS: 2.6 mo *vs* 1.8 mo, *P* < 0.0001; RR 2.84% and 0.00%)[18]. This study further confirmed the efficacy of VEGFR-2 inhibitor for the patients with advanced GC[18]. Regorafenib, an oral multi kinase inhibitor with antiangiogenic effect by VEGFR-2 inhibition, showed PFS benefit over placebo for refractory advanced GC patients in a global phase Ⅱ trial (INTEGRATE, PFS: 11.1 wk *vs* 3.9 wk, *P* < 0.0001; OS: 25 wk *vs* 19.4 wk, *P* = 0.11)[19]. Another phase Ⅱ PaFLO trial (NCT 01503372) examined chemotherapy with or without the antiangiogenic TKI pazopanib as first line in HER2 negative patients. The study did not meet its predefined PFS rate of minimum of 40% at 6 mo (PFS rate: 31.4% *vs* 25.9%). Marginal efficacy in the pazopanib group was observed with median PFS 5.1 mo compared to 3.9 mo in the control group (HR: 0.93, 95%CI: 0.56-1.54)[20].

Mesenchymal-epithelial transition factor receptor/ hepatocyte growth factor targeting therapy

Mesenchymal-epithelial transition factor receptor (c-MET) and its ligand hepatocyte growth factor (HGF) were also evaluated. Rilotumumab is an antibody to HGF, and it was tested in the frontline with chemotherapy in MET-positive advanced GC patients in two phase Ⅲ clinical trials RILOMET-1 (NCT01697072) and RILOMET-2 (NCT02137343) based on the positive phase Ⅱ study[21]. Chemotherapies with or without the drug were examined. These studies have to stop early due to increased fatal adverse events for advanced GC patients. RILOMET-1 study recently reports significantly worse OS in the study group (OS: 9.6 mo *vs* 11.5 mo, HR: 1.37, *P* = 0.016)[22]. Onartuzumab is an antibody against c-MET being studied in combination chemotherapy in advanced GC patients with HER2-negative, MET-positive disease (MetGastric) in the frontline setting (NCT01662869). The study was negative with the addition of onartuzumab to chemotherapy favored placebo group (OS ITT: 11.3 mo *vs* 11.0 mo, *P* = 0.24; OS: MET 2+/3+ 9.7 mo *vs* 11.0 mo, *P* = 0.062)[23].

Poly (ADP-ribose) polymerase targeting therapy

Poly (ADP-ribose) polymerase (PARP) inhibitor in combination with paclitaxel was studied in a second line phase Ⅱ advanced GC study (NCT01063517). The study was enriched for patients with low ATM tumors by IHC based on preclinical data of responsiveness of GC cell lines to olaparib association with low ATM protein level. Of the 124 randomized patients, olaparib plus paclitaxel was well tolerated. Although the primary endpoint of PFS was not met (All patients: 3.9 mo *vs* 3.6 mo, *P* = 0.261; ATM patients: 5.3 mo *vs* 3.7 mo, *P* = 0.35), the OS was statistically significant improved in the study for both all patients and ATM patients (All patients: 13.1 mo *vs* 8.3 mo, *P* = 0.010; ATM patients: NC *vs* 8.2 mo, *P* = 0.003)[24]. A large phase Ⅲ study is ongoing in Asian patients (NCT01924533).

Hedgehog pathway targeting therapy

Hedgehog pathway inhibitor vismodegib combined with FOLFOX was examined in a phase Ⅱ study for advanced GC patients. Hedgehog pathway is over-expressed in GE tumors and pre-clinical data suggested hedgehog inhibitors control tumor growth, cell motility and invasiveness. Median PFS was 11.5 mo *vs* 9.3 mo (*P* = 0.34) and median OS was 12.2 mo *vs* 13.9 mo (*P* = 0.48)[25]. It is another negative trial in an unselected advanced GC population.

Fibroblast growth factor receptor targeting therapy

Fibroblast growth factor receptor (FGFR) pathway is required for driving growth and survival of GC carrying *FGFR2* gene amplification. Dovitinib (TKI258) and AZD4547 are evaluated in this pathway for GCs. Dovitinib is currently being studied as monotherapy or combined with docetaxel in the second or third line setting. One trial (NCT01719549) required patients to have FGFR2 gene amplification and the other two trials (NCT01576380, NCT01921673) were performed in the unselected patient population. The SHINE study (NCT01457846) of AZD4547 monotherapy *vs* paclitaxel for patients with FGFR2 polysomy or gene amplification recently reported to be negative. The PFS was 1.8 mo in the AZD group compared to 3.5 mo in the paclitaxel group[26].

No biomarkers except HER2 are available for clinical practice. The difficulty to identify predictive biomarkers for targeted therapy remains, and warrants further investigation. Majority of the above mentioned large phase Ⅱ or Ⅲ trials were done in unselected patient populations with negative results. The cancer genome atlas project recently proposed to divide GC into four subtypes: Epstein–Barr virus positive tumor, microsatellite unstable tumors, genomically stable tumor, and chromosomally unstable tumor[27]. This classification is based on comprehensive molecular characterization. The advance in technology and understanding of its heterogeneity will potentially lead to identify key targets and pathways for treatments. The laboratory testing to establish positive markers need to be standardized. Future clinical trial design should consider both predictive and prognostic biomarkers to direct targeted therapies.

ERA OF IMMUNE THERAPY

Immune therapy has gained tremendous interest in cancer research and starts a new era for cancer treatment in recent years. Immune checkpoint pathway has made significant progress with several new agents approved for clinical use recently. Suppressing this pathway allows T cell activation and use human immune system to attack tumor cells. High RR and possible durable response have been seen in melanoma and lung cancer with relative low toxicities[28-31]. There are two classes of agents which are under evaluation including inhibitors for cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) and program cell death 1 (PD-1) or its ligand (PD-L1) inhibitors. Multiple agents are in early development and some have been tested in clinical trials. CTLA-4 inhibitors such as ipilimumab (MDX-010) and tremelimumab (CP-675,206) regulate the amplitude of early stage T cell activation. PD-1 and PD-L1 inhibitors such as nivolumab (ONO-4538), pembrolimumab (MK-3475), MEDI4736 and MPDL3280A act on the T cell activity in the peripheral tissues. Seven GC patients were included in a safety study for anti-PD-L1 antibody BMS 936559[32]. Multiple early phase clinical trials are presently ongoing to evaluate their safety and efficacy in advanced solid tumors including GC (for example: NCT01375842, NCT01693562).

CTLA-4 inhibitor tremelimumab was studied in 18 advanced GC patients as a second line treatment. One patient achieved partial response (PR) and four patients had stable disease (SD). Improved survival was observed in patients experiencing a post treatment carcinoembryonic antigen proliferative response (OS: 17.1 mo *vs* 4.7 mo, $P = 0.004$) despite the objective RR was low[33]. Another phase Ⅱ trial of sequential ipilimumab *vs* best supportive care as a second line therapy has completed with results pending (NCT01585987).

PD-1 inhibitor pembrolizumab (MK-3475) demonstrated encouraging results in the phase 1b KEYNOTE-012 study for GC with 67% patients received ⩾ 2 prior therapies. PD-L1+ was used as the biomarker with 65 out of 162 (40%) screened patient being positive, and 39 patients enrolled eventually. ORR was 22% by central review and 33% by investigator review[34]. Median time to response was 8 wk with a median response duration of 24 wk. The 6-mo PFS and OS rate were 24% and 69%[34]. Four patients experienced high-grade drug-related adverse events: peripheral sensory neuropathy, fatigue, decreased appetite, hypoxia, and pneumonitis[34]. This promising result has led to further investigation. A phase Ⅱ KEYNOTE-059 (NCT02335411) study has been launched with pembrolizumab monotherapy or in combination with cisplatin plus 5-fluorouracil for advanced GC. Phase Ⅲ KEYNOTE-061 (NCT02370498) is planned with pembrolizumab *vs* paclitaxel after the first line therapy with platinum and fluropyrimidine. Another phase Ⅲ study with nivolumab (ONO-4538) is recruiting patients with advanced GC (NCT02267343) in Asian countries and PD-L1 positivity was not required.

Combining checkpoint pathway inhibitors are studied in advanced solid tumors with the hope to generate stronger immunogenicity. A phase Ⅰb/Ⅱ study is ongoing to assess the safety and efficacy of PD-L1 inhibitor MEDI4736 in combination with CTLA-4 inhibitor tremelimumab *vs* monotherapy for patients with advanced GC (NCT02340975). Another Phase Ⅰb/Ⅱ study of advanced solid tumor included GC is evaluating nivolumab monotherapy *vs* nivolumab combined with ipilimumab (NCT01928394).

Immune therapy is currently opening a new page for cancer treatment. Harness human immune system to fight for GC may become a reality very soon. Many obstacles and challenges warrant further investigation such as standardization of laboratory testing, biomarkers, tumor immune response criteria, management of immune related adverse events, safety and efficacy of re-exposure.

GC STEM CELL

Hematopoietic stem cell transplant has been well established and widely used in clinical practice to save lives. With more accumulative evidence in recent years, the questionable solid tumor stem cells hypothesis becomes more believable. GC stem cells are thought to be responsible for tumor self-renewal, metastasis, chemotherapy resistance and tumor recurrence[35]. *In vitro* sphere-forming assays and *in vivo* tumor formation in immune-deficient mice have been employed for solid tumor stem cell research. The gastric stem cell was thought to be existed in gastric epithelium initially. Bone marrow derived cells were also identified in mouse models of Helicobacter-induced GC[36,37]. However majority of the studies are still *in vitro* or using mice model[38]. One oral first in class cancer stemness inhibitor called BBI608 was studied plus weekly paclitaxel in a phase Ⅰb trial in refractory solid tumors. Two out of the five refractory GC patients had a partial response (48% and 45% regressions), one had stable disease (25% regression) and two had prolonged stable disease ⩾ 24 wk[39]. A phase Ⅲ clinical trial is ongoing (BRIGHTER: NCT02178956) with this cancer cell stemness inhibitor for previously treated advanced GC patients[40]. One GC patient demonstrated minor regression or SD ⩾ 16 wk in another phase Ⅰ cancer stem cell inhibitor BBI503 trial (NCT01781455)[41].

FUTURE PERSPECTIVE

GC is a common malignancy with poor outcomes. There is an urgent unmet need to improve treatment and outcome for this lethal disease. Understanding the heterogeneous nature of this cancer and incorporate genomic atlas to develop biomarkers as well as newer target agents are important. Develop precision medicine and tailor optimal therapies to individual patient based on

Table 1 Summary of selected targeted agents for advanced gastric cancer

Target	Study agent	Trial	Treatments	Phase	Biomarker	Results primary end point
EGFR	Cetuximab	EXPAND NCT00678535	Arm1: CX + cetuximab Arm 2: CX	Ⅲ	No	Negative PFS: 4.4 mo *vs* 5.6 mo (P = 0.32)
EGFR	Panitumumab	REAL3 NCT00824785	Arm1: EOC+ Panitumumab Arm2: EOC	Ⅱ / Ⅲ	No	Negative OS: 8.8 mo *vs* 11.3 mo (P = 0.013)
mTOR	Everolimus	GRANITE-1 NCT00879333	Arm1: Everolimus Arm2: Placebo	Ⅲ	No	Negative OS: 5.4 mo *vs* 4.3 mo (P = 0.124)
HER2	Trastuzumab	ToGA NCT01041404	Arm1: CF + Trastuzumab Arm2: CF	Ⅲ	Yes HER2	Positive OS: 13.8 mo *vs* 11.1 mo (P = 0.0046)
HER2/EGFR	Lapatinib	TRIO-013/Logic NCT00680901	Arm1: CX + Lapatinib Arm2: CX	Ⅲ	Yes HER2	Negative OS: 12.2 mo *vs* 10.5 mo (P = 0.35)
HER2/EGFR	Lapatinib	TyTAN NCT00486954	Arm1: Paclitaxel + Lapatinib Arm2: Paclitaxel	Ⅲ	Yes HER2	Negative OS: 11.1 mo *vs* 8.9 mo (P = 0.1044)
HER2	Pertuzumab	JACOB NCT0177486	Arm1: CF + Trasuzumab + Pertuzumab Arm2: CF + Trastuzumab	Ⅲ	Yes HER2	Ongoing
HER2	T-DM1	GATSBY NCT01641939	Arm1: Taxane Arm2: T-DM1 2.4 mg/kg once a week Arm3: T-DM1 3.6 mg/kg every 3 wk	Ⅱ / Ⅲ	Yes HER2	Ongoing
VEGF	Bevacizumab	AVAGAST NCT00548548	Arm1: CF + Bevacizumab Arm2: CF	Ⅲ	No	Negative OS: 12.1 mo *vs* 10.1 mo (P = 0.1002)
VEGFR	Ramucirumab	REGARD NCT00917384	Arm1: Ramucirumab Arm2: Placebo	Ⅲ	No	Positive OS: 5.2 mo *vs* 3.8 mo (P = -0.047)
VEGFR	Ramucirumab	RAINBOW NCT01170663	Arm1: Paclitaxel + Ramucirumab Arm2: Paclitaxel	Ⅲ	No	Positive OS: 9.6 mo *vs* 7.4 mo (P = 0.017)
VEGFR	Ramucirumab	RAINFALL NCT02314117	Arm1: CF + Ramucirumab Arm2: CF	Ⅲ	Yes HER2 negative	Ongoing
VEGFR	Apatinib	NCT0152745	Arm1: Apatinib Arm2: Placebo	Ⅲ	No	Positive OS: 6.5 mo *vs* 4.7 mo (P < 0.016), PFS: 2.6 mo *vs* 1.8 mo (P < 0.0001)
VEGFR (multi-kinase)	Regorafenib	INTEGRATE	Arm1: Regorafenib Arm2: Placebo	Ⅱ	No	Positive PFS: 11.1 wk *vs* 3.9 wk (P < 0.0001)
VEGFR, PDGFR c-Kit	Pazopanib	PaFLO	Arm1: FLO + Pazopanib Arm2: FLO	Ⅱ	Yes HER2 negative	Negative PFS rate at 6 mo 31.4% *vs* 25.9% (Did not meet predefined 40%)
MET/HGF	Rilotumumab	RILOMET-1 NCT01697072	Arm1: ECX + Rilotumumab Arm2:	Ⅲ	Yes MET	Terminated due to increased death signal Negative (Detrimental) OS: 9.6 *vs* 11.5 mo (HR 1.37, P = 0.016)
MET/HGF	Rilotumumab	RILOMET-2 NCT02137343	Arm1: CX + Rilotumumab Arm2: CX	Ⅲ	Yes MET	Terminated due to increased death signal
MET	Onartuzumab	METGastric NCT01662869	Arm1: FOLFOX Arm2: FOLFOX + Onartuzumab	Ⅲ	Yes MET+, HER2-	Negative ITT OS: 11.3 mo *vs* 11.0 mo (P = 0.24) MET2+/3+ OS: 9.7 mo *vs* 11.0 mo (P = 0.06)
PARP	Olaparib	NCT01063517	Arm1: Paclitaxel + Olaparib Arm2: Paclitaxel	Ⅱ	Yes ATM	Negative PFS: 3.9 mo *vs* 2.6 mo (P = 0.261) All patients PFS: 5.3 mo *vs* 3.7 mo (P = 0.315) ATM- patients Positive for secondary endpoints OS: 13.1 mo *vs* 8.3 mo (P = 0.010) All Patients OS: NR mo *vs* 8.2 mo (P = 0.003) ATM- patients
PARP	Olaparib	NCT01924533	Arm1: Paclitaxel + Olaparib Arm2: Paclitaxel	Ⅲ	No	Ongoing
Hedgehog	Vismodegib	NCT00982592	Arm1: FOLFOX + Vismodegib Arm2: FOLFOX	Ⅱ	No	Negative PFS: 7.3 mo *vs* 9.0 mo (P = 0.64)
FGFR	Dovitinib	NCT01719549	Dovitinib monotherapy	Ⅱ	Yes FGFR	Ongoing
FGFR	Dovitinib	NCT01576380	Dovitinib monotherapy	Ⅱ	No	Completed, waiting for result
FGFR	Dovitinib	NCT01921673	Docetaxel + Dovitinib	Ⅰ / Ⅱ	No	Ongoing
FGFR/VEGFR	AZD4547	SHINE NCT1457846	Arm1: AZD4547 Arm2: Paclitaxel	Ⅱ	Yes FGFR	Negative PFS: 1.8 (AZD) *vs* 3.5 mo

EOC: Epirubicin, oxalilatin, capecitabine; CF: Fluoropyrimidine, cisplatin; T-DM1: Trastuzumab emtansine; ECX: Epirubicin, csiplatin, capecitabine; CX: Cisplatin, capecitabine; FOLFOX: 5-Fluorouracil, folinic acid, oxaliplatin; NR: Not reached; FLO: 5-FU, leucovorine, oxaliplatin; EGFR: Epidermal growth factor receptor; mTOR: Mammalian target of rapamycin; HER2: Human epidermal growth factor receptor 2; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; PDGFR: Platelet-derived growth factor receptor; MET: Mesenchymal-epithelial transition factor; HGF: Hepatocyte growth factor; PARP: Poly ADP-ribose polymerase; FGFR: Fibroblast growth factor receptor.

information including molecular study results will be the future focus. With the recent breakthrough in immune therapy in other solid tumors and promising early phase clinical trial results in GC, immune checkpoint pathway inhibitors are undergoing evaluation. In order to generate stronger immunogenicity, combining different checkpoint pathway inhibitors or chemotherapy or targeted therapy might be needed. GC stem cell research was initially cluttered with skepticism until more evidence accumulated recently. It is an exciting field warrants further evaluation.

CONCLUSION

Ramucirumab is the second biologic agent after trastuzumab approved with statistically significant but marginal survival benefit for GC patients in spite of multiple negative phase Ⅲ clinical trials of other targeted agents (as summarized in Table 1). Better understanding and use of genomic atlas/biomarkers will potentially lead to development of targeted agents with better efficacy. Immune therapy especially checkpoint pathway inhibition is a promising field and being studied in multiple clinical trials. GC stem cell therapy is finally moving from bench work to early phase clinical investigation. Targeted therapy, immune therapy and cancer stem cell therapy are promising fields and may meet the urgent demand for novel therapy to treat GC in near future.

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TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Autophagy in colorectal cancer: An important switch from physiology to pathology

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Abstract

Colorectal cancer (CRC) remains a leading cause of cancer death in both men and women worldwide. Among the factors and mechanisms that are involved in the multifactorial etiology of CRC, autophagy is an important transformational switch that occurs when a cell shifts from normal to malignant. In recent years, multiple hypotheses have been considered regarding the autophagy mechanisms that are involved in cancer. The currently accepted hypothesis is that autophagy has dual and contradictory roles in carcinogenesis, but the precise mechanisms leading to autophagy in cancer are not yet fully defined and seem to be context dependent. Autophagy is a surveillance mechanism used by normal cells that protects them from the transformation to malignancy by removing damaged organelles and aggregated proteins and by reducing reactive oxygen species, mitochondrial abnormalities and DNA damage. However, autophagy also supports tumor formation by promoting access to nutrients that are critical to the metabolism and growth of tumor cells and by inhibiting cellular death and increasing drug resistance. Autophagy studies in CRC have focused on several molecules, mainly microtubule-associated protein 1 light chain 3, beclin 1, and autophagy related 5, with conflicting results. Beneficial effects were observed for some agents that modulate autophagy in CRC either alone or, more often, in combination with other agents. More extensive studies are needed in the future to clarify the roles of

autophagy-related genes and modulators in colorectal carcinogenesis, and to develop potential beneficial agents for the prognosis and treatment of CRC.

Key words: Colorectal cancer; Autophagy; Gene; Protein; Carcinogenesis

Core tip: This review describes the role of autophagy in cancer, focusing on the involvement of autophagy in colorectal cancer (CRC). Initially, we describe the steps and components of autophagy, and we then further highlight the dual role of autophagy in cancer, where it can potentially act as both a promoter and an inhibitor during the transformation from normal to malignant cell. In particular, we emphasize the major autophagy genes involved in CRC pathogenesis along with autophagy-modulating agents and their modes of action in the context of CRC therapy.

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INTRODUCTION

Despite advances in diagnosis and treatment, colorectal cancer (CRC) remains one of the major causes of cancer death in both sexes worldwide: It is the third most common diagnosed cancer in males and the second most common in females[1]. It is well known that many risk factors, including multiple genes and environmental influences, are involved in malignant transformation. Recent research provides new data regarding the complex mechanisms involved in colorectal carcinogenesis. Among these mechanisms, autophagy is important in the switch from normal to malignant colorectal cells. The involvement of autophagy in cancer appears to be context specific, with evidence suggesting that it can have a dual role in both tumor suppressing and tumor promoting activities. Moreover, autophagy performs important functions in different processes that are connected to carcinogenesis, including inflammation, immune response and genome stability.

Here, we describe the involvement of autophagy in carcinogenesis, with a particular emphasis on CRC. We summarize the components and steps of macroautophagy (herein referred to as autophagy), and we emphasize the conflicting roles of autophagy in cancer, indicating that it has both promoter and suppressor mechanisms during malignant transformations. The second part of this study is focused on the autophagy genes and proteins that are associated with CRC. Finally, the effects of autophagy-based drugs in CRC treatment are discussed.

AUTOPHAGY STEPS AND REGULATION

Autophagy is an evolutionarily conserved catabolic process that is characterized by cellular self-digestion and the removal of excessive, long-lived or dysfunctional organelles and proteins[2]. Autophagy occurs as a physiological process in normal cells at a basal level to assure cellular homeostasis, or as a strategic survival mechanism that recycles energy and nutrients under special conditions. Hypoxia, stress and nutrient deprivation trigger autophagy as a critical adaptive response during starvation[3]. Three morphologically distinct forms of autophagy can be distinguished: macroautophagy, microautophagy and chaperone-mediated autophagy[4]. Macroautophagy is identified by the presence of double membrane vesicles known as an autophagosomes, which engulf cytoplasmic components that include damaged organelles and deliver them to lysosomes for degradation. The other two forms, microautophagy and chaperone-mediated autophagy, involve a direct membrane invagination to engulf damaged proteins and the translocation of soluble cytosolic proteins by chaperone-dependent selection across the lysosomal membrane, respectively[5,6].

Autophagy-related genes (ATGs) play a critical role in facilitating the regulation of well-orchestrated autophagy. To date, thirty-six ATGs have been identified[7]. Autophagosome formation is initiated by unc-51-like kinase (ULK) and class Ⅲ phosphatidylinositol 3-kinase (PI3K) complexes. The ULK complex consists of ATG13, ATG101, ULK1/2 and family-interacting protein FIP200[8,9]. Under normal growth conditions, the mammalian target of rapamycin (mTOR) complex inhibits the formation of the ULK complex, in effect blocking autophagy, and the ULK components are dissociated. Various stimuli (*e.g.*, hypoxia, starvation) inhibit mTOR, allowing the ULK kinase complex to be activated, which initiates the formation of an isolation membrane (Figure 1) called a phagophore[10,11]. The origin of phagophores has not been explained, but the plasma membrane, endoplasmic reticulum, Golgi apparatus and mitochondria are all possible sources[12]. The completion of this critical step is driven by vacuolar sorting protein 34, a class Ⅲ PI3K that is bound to beclin-1, and other ATG proteins (*e.g.*, ATG14), which generate PI3K, the second complex, that catalyzes the production of phosphatidylinositol-3-phosphate[10,13].

Autophagosome elongation and closure steps and the further conversion to a nascent closed autophagosome are controlled by two ubiquitin-like conjugates. First, ATG12 forms a conjugate with ATG5 under the control of ATG7 and ATG10, which have E1 and E2-like enzyme activity, respectively. The resulting ATG12-ATG5

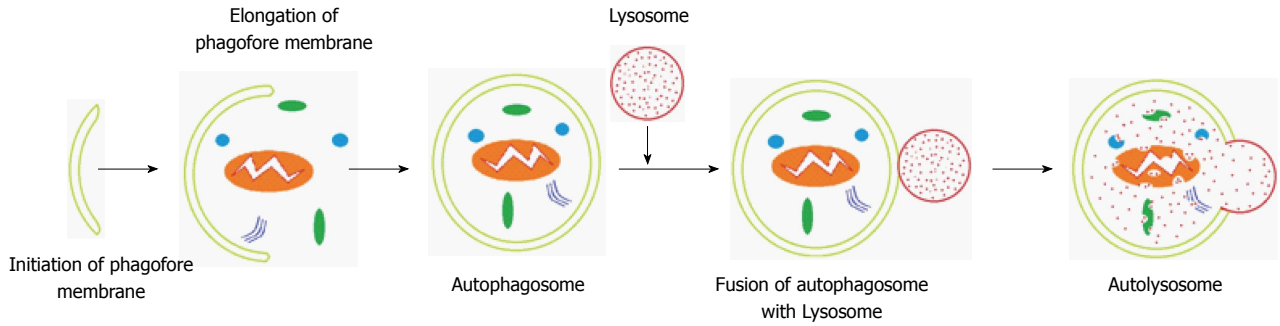

Figure 1 Morphological steps of the autophagy process. Autophagy is initiated with the formation of a phagophore, which sequesters cellular material in a double-membrane vesicle called an autophagosome. The autophagosome fuses with lysosomes to form an autolysosome.

complex interacts with ATG16L1 to form a multimeric ATG12-ATG5-ATG16L1 conjugate that is located on the outer surface of the autophagosomal membrane. It will dissociate from the membrane upon completion of the autophagosome[14,15]. The second ubiquitin-like pathway involves the conjugation of the microtubule-associated protein 1-light chain 3 (LC3-Ⅰ) to the lipid phosphatidylethanolamine (PE) by ATG7 and ATG3, which is an E2-like enzyme, to form the membrane-bound LC3-Ⅱ. LC3 is initially synthesized as a precursor protein, proLC3, and is immediately processed to LC3-Ⅰ by ATG4 through cleavage of its C-terminal amino acid. The membrane-bound form of LC-3, LC-3Ⅱ, is recruited to both sides of the autophagosomal membrane[16,17]. After fusion with lysosomes, LC3-Ⅱ on the cytoplasmic face of the autolysosome can be delipidated by ATG4 and recycled, whereas proteins located on internal surface of the autophagosome are processed for degradation by lysosomal enzymes in autolysosomes. During the maturation process, lysosomal-associated membrane protein 2 and the Ras-related protein Rab-7a facilitate autophagosome fusion with endocytic and lysosomal compartments to form an autolysosome. Autophagic cargo is then degraded through the activity of lysosomal proteases[18-21].

AUTOPHAGY: AN IMPORTANT SWITCH IN CANCER PATHOGENESIS

Autophagy plays crucial roles in the pathogenesis of various human diseases, including cancer, neurodegenerative diseases, infection, and cardiovascular, metabolic, and pulmonary diseases, and aging[22]. The currently accepted hypothesis is that autophagy has dual, contradictory roles in carcinogenesis (Figure 2). First, autophagy is a surveillance mechanism in normal cells, where it acts to protect cells from malignant transformations by removing damaged organelles and aggregated proteins and reducing DNA damage, reactive oxygen species (ROS) and mitochondrial abnormalities. However, autophagy also supports tumor formation by providing access to nutrients that are critical to the metabolism and growth of tumor cells, and by inhibiting cellular death and increasing drug resistance[7,23]. The response of cells to autophagy during cancer metastasis is stage dependent. Autophagy may help to reduce cancer metastasis in the early steps of tumor cell dissemination by promoting inflammatory responses against tumors. Furthermore, autophagy limits tumor necrosis and the expansion of dormant cancer cells into micrometastases, in tandem with impairing oncogene-induced senescence[24]. Autophagy seems to support metastasis during advanced stages of cancer by increasing the survival of detached metastatic cells in the absence of extracellular matrix, and by supporting the dissemination of cancer cells to distant organ sites by triggering tumor cells that lack a connection with the extracellular matrix in the new environment to shift to a dormant state until appropriate conditions occur[24,25].

Autophagy as a suppressor during early stages

Autophagy can prevent the transformation from normal to malignant through several suppressive mechanisms. An appropriate autophagic response is necessary for genome stability and for the clearance of mutagens because it acts to prevent the accumulation of the genetic defects that accompany malignant transformations. Damaged mitochondria and the redox-active aggregates of ubiquitinated proteins are removed by autophagy, resulting in avoidance of the overproduction of highly genotoxic ROS[26]. Inhibition of autophagy switches off this protection and can expose cells to ROS cytotoxicity, which promotes the activation of oncogenes[27,28]. In addition to mitophagy, autophagy supports genomic stability by enabling the discarding of micronuclei that are produced by cell cycle anomalies[29], and it may also promote autophagic cell death, known as type Ⅱ programmed cell death, under certain conditions[30,31].

The impact of autophagy on tumor progression exhibits a significant degree of context dependence[23]. BECN1 gene studies in hormone-related cancers unmasked, for the first time, the possible tumor suppressing role of autophagy[32,33]. There remains significant debate regarding the role of BECN1 as a tumor suppressor due to the proximity of BECN1 to BRCA1, a well-known tumor suppressor gene. Both of these genes are located on

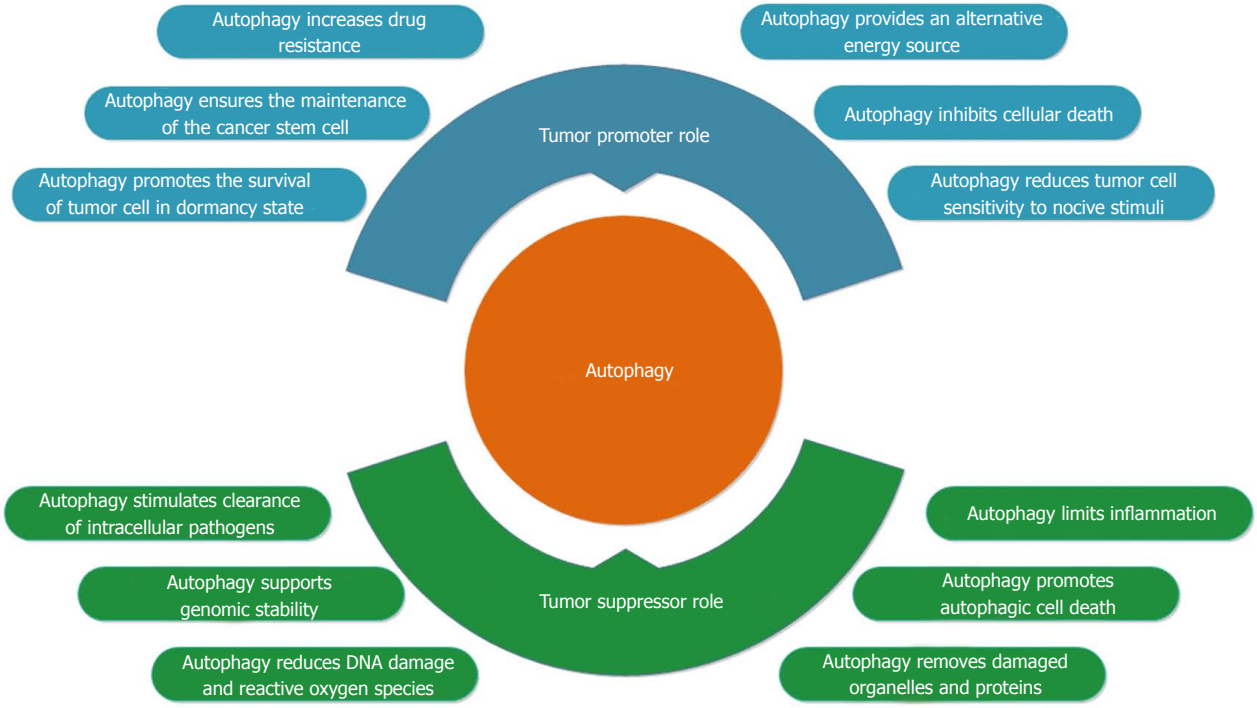

Figure 2 The dual and contradictory roles of autophagy in cancer. Autophagy can potentially act as either a promoter or an inhibitor during the transformation from normal cell to malignant cell. Autophagy supports tumor formation by providing an alternative energy source, increasing drug resistance, inhibiting cell death, promoting the survival of tumor cells in a dormant state and ensuring the maintenance of cancer stem cell compartments. Autophagy protects normal cells from malignant transformation by removing damaged organelles and proteins, reducing DNA damage and reactive oxygen species, supporting genomic stability, promoting autophagic cell death, limiting inflammation and stimulating the clearance of intracellular pathogens.

human chromosome 17q21[34]. The role of autophagy as an important tumor suppressive process that has been demonstrated in murine experiments. Lack of BECN1 gene in embryoid bodies leads to embryonic death[35], and mice with a heterozygotic deletion of BECN1 demonstrate increased susceptibility to tumorigenesis in multiple tissues[36,37]. Similarly, mice deficient for ATG5 and ATG7 died after birth[38,39], while mice with mosaic deletion of ATG5 and liver-specific ATG7-deficient mice developed only benign liver adenomas[40]. Mice lacking autophagy genes ATG5 or ATG7 acquired premalignant pancreatic cancer, while the progression to pancreatic cancer driven by KRasG12D was blocked[41]. ATG7 deletion in a murine model (BrafV600E-induced lung cancer) initially accelerated the proliferation of tumor cells, but at later stages of tumorigenesis it reduced tumor burden, blocked conversion to a more malignant phenotype and increased the life spans of experimental mice[42]. In the absence of autophagy, the advance to cancer can be arrested, resulting in protection from conversion into malignant cells. Progression to a malignant phenotype may require additional genetic alterations[43].

In addition, autophagy is involved in both innate and adaptive immune responses, by which it prevents the establishment and proliferation of malignant cells[44]. Malignant transformation can be stimulated by an inflammatory microenvironment, which contains high amounts of potentially genotoxic ROS as well as various mitogenic cytokines[45]. Autophagy limits inflammation by efficiently disposing of inflammasomes, thereby inhibiting the pro-inflammatory signals that are delivered by some pattern recognition receptors, such as RIG-I-like receptors[46], and limiting the abundance of B-cell CLL/lymphoma 10, a protein that is involved in pro-inflammatory NF-κB signaling[47]. Autophagy ensures a well-coordinated and appropriate response, enabling crucial cells in the immune system to develop properly and to produce interferon, secrete antimicrobial peptides or present antigens to stimulate adaptive immunity. Dying malignant cells may determine innate and/or adaptive antitumor immune responses by recruiting antigen-presenting cells and other cellular components of the immune system. Thus, defects in autophagy may prevent the host immune system from properly recognizing and eliminating premalignant and malignant cells. Moreover, autophagy mediates potent anti-inflammatory effects[48,49].

Autophagy plays a key role in the first line of defense against pathogens and thus has anticarcinogenic effects that combat viral and bacterial infections. A xenophagic response is required for the stimulation of pathogen-specific immune responses and for the rapid clearance of intracellular pathogens[48]. Some of these processes are associated with digestive cancers (*e.g.*, *Helicobacter pylori*, which is associated with gastric carcinoma, or *Streptococcus bovis*, which may cause colorectal carcinoma)[50,51].

Autophagy as a promoting factor during late stages

Autophagy seems to promote malignant progression and resistance to therapy following the initiation of tumor growth[2,27]. As a conserved cellular survival mechanism, tumor cells can use autophagy to provide a backup energy source for survival and expansion[52]. During the progression of tumors, malignant cells are under metabolic stress as a result of a high proliferation rate and exposure to hypoxia, and nutrient deprivation due to inadequate blood supply or selective pressure from therapeutic intervention[53]. Tumor cells usually have a high proliferation rate, which demands more energy and resources than normal cells, and both ATP and metabolites can be obtained by increasing autophagy[54]. Although angiogenesis does occur in tumors, the availability of glucose and glutamine is reduced in some tumor regions due to the leakiness of tumor-associated vessels and continued hypovascularization[55].

Autophagy is activated in the hypoxic areas of tumors, and the inhibition of autophagy by AKT activation or by monoallelic disruption of BECN1 promotes cell death specifically in those regions. These results support hypothesis that tumor cells can use autophagy as a surveillance mechanism under metabolic stress conditions, to provide an alternative energy source for the survival and proliferation of malignant cells[52].

The pro-malignant role of autophagy has been demonstrated in tumor studies in which the inhibition of autophagy was linked to reduced tumor processes. Moreover, down-regulating the expression of essential autophagy proteins impaired tumor growth and led to the accumulation of abnormal mitochondria and reduced oxygen consumption, and autophagy was necessary to support the growth of Ras-driven tumors[56]. However, increased autophagy has also been associated with poor outcomes and short disease-free periods in human pancreatic cancers[57]. *In vitro* studies have shown that the survival of Ras-driven cancer cells requires autophagy and that gaining autophagy results in a marked increase in the survival of malignant cells under conditions of metabolic stress[28]. Inhibiting autophagy by deleting ATG5 prevents the progression of premalignant lesions to cancer in either a p53-independent or p53-dependent manner[41,58]. Furthermore, deletion of ATG7 decreases the tumor growth rate and induces nonmalignant tumor formation. In addition, non-Ras-driven tumoral cell types also need autophagy for survival, and the loss of autophagy has been shown to inhibit malignant tumor development. For example, FIP200 deletion significantly reduced proliferation and suppressed mammary tumor initiation and progression in a mouse model of breast cancer driven by the PyMT oncogene[59]. In a Palb2 knockout mouse model, heterozygous deletion of the autophagy gene BECN1 reduced Palb2-associated mammary tumorigenesis in a p53-dependent manner, indicating that in the presence of DNA damage and oxidative stress, autophagy can support tumor development by suppressing p53[60].

Autophagy can improve the resistance of cancer cells to detachment from the basal membrane, resulting in transformed cells that are less sensitive to therapy-induced cell death. Moreover, this activity sustains the survival of cancer cells that enter a state of dormancy or senescence in response to therapy and ensures the maintenance of the cancer stem cell compartment[23].

Autophagic responses favor the growth and progression of established tumors by reducing their sensitivity to different stimuli that would normally promote their death[61]. KRasG12D-driven pancreatic adenocarcinoma cells that enter a state of dormancy in response to oncogene ablation have recently been shown to activate autophagy to efficiently counteract metabolic stress[62], demonstrating the functional and phenotypic features of cancer stem cells. In addition, mammary cancer stem cells are often characterized by elevated autophagic flux, and their ability to efficiently form tumors *in vivo* appears to rely on autophagy, as tumor formation can be abolished through the genetic inhibition of BECN1 or ATG4A[63,64]. Thus, autophagy may also sustain tumor progression by preserving the viability of the cancer stem cell compartment and/or by promoting the persistence of dormant cancer cells.

Moreover, autophagy is required not only for the emission of immunostimulatory signals by malignant cells succumbing to specific anticancer agents but also for the activation of tumor-targeting innate and adaptive immune responses[49]. Cancer cells that have been isolated from established tumors where autophagy was inhibited were less resistant to exogenous stimuli than their wild-type counterparts[61]. In line with these data, autophagy-deficient tumors are often more sensitive to several chemotherapeutic agents and radiation therapy than their autophagy-proficient counterparts[65,66]. Cancer cells that are exposed to therapeutic interventions can also undergo senescence. Although senescent cells do not proliferate, they may support disease relapse by releasing a wide panel of pro-inflammatory and mitogenic cytokines into the microenvironment[67].

AUTOPHAGY GENE SWITCHES TO CRC

The autophagy machinery involves multiple genes and proteins that have critical functions in complex autophagic pathways, and these genes may be involved in the important switch from normal to colorectal pathology under specific conditions (Table 1).

LC3 gene

The *LC3* gene family encodes three isoforms (*LC3A*, *LC3B*, and *LC3C*) and is the mammalian homologue of yeast ATG8[68]. The isoform *LC3B* is cleaved into the soluble form LC3B-Ⅰ, which is conjugated with PE to generate the lipidated form (LC3B-Ⅱ). LC3B-Ⅱ accumulates specifically on nascent autophagosomes and is one of the most widely and reliably used markers for autophagy[69]. *LC3* was the first autophagy marker proposed to be involved in human CRC[70]. LC3-Ⅱ is overexpressed in CRC compared to normal tissue,

Table 1 Autophagy-related genes in colorectal cancer

Gene/protein	Expression level in colorectal cancer
LC3/LC3-Ⅱ	Higher expression, especially in advanced stages[20]
	Higher expression associated with aggressiveness[71]
	Higher perinuclear expression associated with positive prognosis[77]
	Higher levels in DLD-1 and SW480 CRC lines treated with autophagy inhibitors[72]
	Higher levels in CRC cell lines treated with 5-FU[73]
	Higher levels in CRC cell lines treated with 5-FU and radiotreated[74]
	Lower levels associated with good outcome and treatment response[75,76]
	Negative expression associated with poor clinical outcome and survival[87]
BECN1/ Beclin-1	Higher expression, negatively linked to metastasis[82]
	Higher expression associated with favorable outcome[83]
	Higher expression associated with longer survival in patients treated with 5-FU[84]
	Higher expression associated with a worse survival in patients treated with 5-FU[85]
	Higher expression associated with metastasis and worse prognosis[86]
	Lower levels associated with increased survival in advanced CRC patients treated with cetuximab[75,76]
	Lower levels associated with poor clinical outcome and survival[87]
	Lower levels associated with a good response after chemoradiation in patients with rectal cancer[88]
ATG5	Higher levels associated with lymphovascular invasion[92]
	Lower levels[91]
	Lower expression associated with poor clinical outcome survival[87]
	Lower expression enhanced sensitivity to oxaliplatin[93]
ATG10	Higher expression associated with tumor lymph node metastasis and poor survival[95]
ATG16L1	ATG16L1T300A polymorphism improved overall survival in human CRC patients[116]
BCL2/Bcl-2	Higher levels associated with migration and invasion[105]
	Higher levels associated with resistance to paclitaxel[106]
Bif-1	Lower levels[109]

LC3: Microtubule-associated protein 1 light chain 3; CRC: Colorectal cancer; 5-FU: 5-fluorouracil; Bif-1: Bax-interacting factor 1; BECN1: Beclin 1; ATG5: Autophagy related 5; BCL2: B-cell CLL/lymphoma 2.

especially in advanced stages[20]. Zheng *et al*[71] reported that LC3B-Ⅱ was overexpressed in cancer cells and that autophagy enhanced the aggressiveness of CRC. LC3B expression in the peripheral areas of CRC tissues was correlated with tumor differentiation, growth pattern at the tumor margin, pN and pStage, as well as vessel and nerve plexus invasion. An increased level of LC3-Ⅱ protein was found in DLD-1 and SW480 CRC-derived cell lines that were treated with a combination of autolysosome inhibitors. Association with 3-methyl adenine (3-MA), an inhibitor of PI3K, blocks autophagosome formation and led to increased apoptosis in treated CRC cell lines[72]. The treatment of CRC cell lines with 5-fluorouracil (5-FU) activated the autophagic process as a protective mechanism in cancerous cells, increased LC3-II levels and reduced the rate of apoptosis compared with untreated cell lines, and an increase in the apoptotic rate was induced by adding 3-MA to 5-FU[73]. Similar results were reported by Schonewolf *et al*[74], who reported that both 5-FU treated and radiotreated CRC cell lines showed an increase in autophagy. After adding chloroquine (CQ) to the treatment, these authors reported an increase in the sensitivity of malignant cells to apoptosis. However, in early stages, LC3-Ⅱ expression levels were decreased compared with normal tissue[20]. A low LC3 value has been associated with a good response to treatment and a good survival prognosis, especially in patients with advanced CRC[75,76]. Perinuclear LC3A expression has been shown to be a positive predictor in patients with stage ⅡA-Ⅲ colorectal adenocarcinomas who were treated with only surgery, whereas an increased autophagic response was linked to metastasis and a worse prognosis[77].

BECN1 gene

BECN1, the mammalian orthologue of yeast ATG6, encodes the beclin-1 protein, which exerts its biological activities through three identified structural domains: A Bcl-2 homology domain, a central coiled-coiled domain and an evolutionarily conserved domain[78]. Beclin-1 plays a pivotal role in autophagy as a component of the autophagy class Ⅲ PI3K complex. By interacting with different factors, it regulates autophagy pathways, resulting in the gain (*e.g.*, AMBRA 1, UVRAG) or loss (*e.g.*, Bcl-2) of autophagy. Moreover, beclin-1 dysfunction has been linked to immune disorders, neurodegenerative diseases and cancer[79].

BECN1 plays a controversial role in colorectal carcinomas in that it supports tumorigenesis[80] but may also inhibit CRC cell growth[81]. Higher expression levels of BECN1 have been reported in malignant colorectal tissue than in normal colorectal mucosa[82], with overexpression being especially associated with advanced stages of CRC[75,83-85]. Using immunohistochemistry, Ahn *et al*[80], showed increased BECN1 expression in 95% of colorectal carcinoma samples compared to normal mucosal epithelial tissue, but they found no significant association with invasion, metastasis or stage. High BECN1 expression has been linked to a good prognosis and longer survival in patients with stage ⅢB colorectal carcinoma[83]. Consistent with these findings, an

increased level of BECN1 expression was strongly associated with longer 5-year survival in patients with locally advanced colon carcinomas who were treated with 5-FU chemotherapy for six months after surgery[84]. Overexpression of BECN1 in patients with resected stage Ⅱ and Ⅲ colon carcinomas who were treated with 5-FU-based adjuvant therapy was associated with worse overall survival, supporting a role for autophagy in drug resistance[85]. Moreover, in a meta-analysis, overexpression of BECN1 was associated with a poor prognosis and metastasis in patients with CRC[86]. Furthermore, low levels of BECN1 were correlated with a longer survival in advanced CRC patients who were treated with cetuximab-containing chemotherapy[75,76]. Supporting this hypothesis, a lack of the expression of the autophagy-related proteins LC3B, ATG5 and beclin-1 is associated with poor clinical outcomes and poor survival in CRC patients[87]. Rectal adenocarcinoma patients exhibiting low expression levels of BECN1 were more likely to experience a good response to chemoradiation than patients with increased expression levels of BECN1[88]. Moreover, the expression levels of BECN1 were reduced in a panel of human neoplasms, including brain tumors and gastric and colorectal carcinomas[89].

ATG5 gene

ATG5 protein is encoded by the *ATG5* gene and forms a complex with ATG12 that participates in autophagosome membrane elongation[22]. Mutations in the ATG2B, ATG5, ATG9B, and ATG12 genes have been associated with CRC and gastric cancer[90]. An association between mutations in the ATG5 gene and reduced levels of ATG5 protein expression has been shown in gastrointestinal cancers, including CRC[91]. ATG5 expression was down-regulated in 95% of CRC patients and, interestingly, increased ATG5 expression was associated with lymphovascular invasion[92]. Other research showed that ATG5 is down-regulated in colorectal carcinoma, in both tissue samples and cell lines, and that down-regulation of ATG5 in CRC enhanced sensitivity to oxaliplatin[93]. Heterozygous deletion of ATG5 predisposed mice to intestinal adenoma growth and enhanced the antitumor effect of interferon gamma. In CRC mouse models, treatment with ursolic acid promoted autophagic cell death through a path mediated by ATG5[94].

ATG10 gene

The *ATG10* gene has been mapped to chromosome 5 and encodes an E2 ubiquitin ligase-like enzyme that has essential functions in vesicle elongation, where it catalyzes the conjugation of ATG5 and ATG12[22]. ATG10 was found to be upregulated in CRC tissues and high protein expression of ATG10 was associated with tumor lymph node metastasis and invasion. Moreover, the presence of ATG10 was correlated with poor survival, indicating that ATG10 may be a potential prognostic marker for CRC[95].

AMBRA1 gene

The *AMBRA1* gene encodes the activating molecule in beclin-1-regulated autophagy (Ambra1) protein, which has roles in autophagy, cell growth, cell death, embryonic development and carcinogenesis[96]. AMBRA1 is mutated in a subset of colorectal neoplasms[97].

UVRAG gene

The UV radiation resistance-associated gene (UVRAG) encodes a tumor suppressor protein that induces autophagy by interacting with BECN1. In addition to its function in autophagy, UVRAG is also involved in endocytic trafficking, DNA damage repair and apoptosis[98]. UVRAG, in association with BECN1, supports the maintenance of genomic stability by protecting established CRC cells against radiation-induced DNA damage[99]. UVRAG is heterozygous mutated in a high proportion of gastric and colonic tumors[100,101].

BCL2 gene

The *BCL2* gene encodes the antiapoptotic B-cell lymphoma 2 (Bcl-2) protein, which inhibits autophagy by directly binding to the BH3 domain of beclin-1 and blocking its activity[102]. A recent report suggested that the prosurvival Bcl-2 protein modulates autophagy only indirectly, by inhibiting the apoptosis mediators Bax and Bak[103]. Bcl-2 has been associated with migration and invasion of malignant cells and with the prevention of apoptosis in pT3 CRC patients[104,105]. In addition, the overexpression of Bcl-2 in CRC was correlated with resistance to paclitaxel[106]. Furthermore, the role of Bcl-2 in modulating autophagy has been investigated in different cancer cell lines, including colon carcinoma, where the deletion of the BH4 domain in the Bcl-2 protein in HT29 colon carcinomas was not found to affect tumorigenicity[107].

Bif-1 gene

The *Bif-1* gene encodes Bax-interacting factor (Bif-1), also known as endophilin B1, which is involved in the control of membrane dynamics in cytosolic organelles, such as the Golgi complex and mitochondria, as well as in autophagosomes. Bif-1 induces the formation of autophagosomes and modulates autophagy-enhancing PI3K lipid kinase activity by interaction with beclin-1 through UVRAG[108]. The expression of Bif-1 was found to be reduced in colorectal carcinomas and the loss of Bif-1 suppressed programmed cell death and promoted colon adenocarcinomas. Bif-1 null mice developed normally, with the exception of an enlarged spleen, but they had an increased incidence of spontaneous tumor formation: 82.8% of Bif-1 null mice developed lymphoma compared with 14.3% of their wild-type counterparts[109].

IBD susceptibility genes

Autophagy has also been linked to CRC through inflammatory bowel disease (IBD). In the complex pathogenesis leading to colitis-associated cancer, the

severity of inflammation is a risk factor for CRC[110]. Cytokines released by epithelial and immune cells play an important role, and autophagy can affect the regulation of both inflammation and immune system functions[22]. Autophagy contributes to intestinal homeostasis by ensuring intracellular defenses against microbes, by maintaining the integrity of secretory granules in Paneth cells, and by regulating the inflammasome or mediating antigen presentation[111]. Genome-wide association studies provided the first link between autophagy and IBD by showing that the ATG16L1 T300A polymorphism is associated with an increased risk of Crohn's disease (CD)[112-114]. In addition, IRGM, NOD2, and LRRK2 have been identified as additional markers of CD risk, and autophagy and DAP1 were associated with ulcerative colitis[115]. Recently, the ATG16L1T300A polymorphism was found to improve overall survival in human CRC patients and to enhance the production of type Ⅰ interferon[116].

AUTOPHAGY DRUGS IN CRC

Recent data indicate that only tumors that utilize excessive levels of autophagy, even in nutrient-rich conditions and in the absence of stressful stimuli, respond to autophagy inhibitors *in vivo*[117]. This suggests that only a fraction of cancer patients may benefit from the administration of autophagy inhibitors. Along similar lines, autophagy has been shown to underlie, at least in part, the therapeutic activity of some anticancer regimens[118,119].

Autophagy promotes cancer cell survival under stressful conditions or nutrient deprivation and thus may contribute to chemoresistance. The drugs targeting various autophagy pathways can either induce gain or loss of autophagy. The exaggerated and sustained autophagy that is trigged by anticancer therapies can lead to type Ⅱ cell death in various cancers, including CRC. Increased autophagy in the early stages of cancers can induce protection by suppressing tumorigenesis, necrosis, and chronic inflammation[13]. On the contrary, inhibition of autophagic influx may accelerate the initial steps of tumorigenesis and reduce protein degradation, and as a consequence, the reduced protein turnover might induce the early tumor progression.

In advanced stages, tumor cells use autophagy to survive cellular metabolic stress and to provide essential nutrients to tumor cells that are experiencing ischemia. Therefore, inhibiting autophagy in late-stage cancers can suppress tumor progression by blocking this prosurvival mechanism in nutrient-deprived tumor cells and by preventing protein recycling and cellular growth[120]. On the other hand, inhibition of autophagy can also lead to a decrease in the antitumorigenic activity achieved by promoting non-apoptotic cell death.

This prosurvival autophagy mechanism can be overcome by inhibition. Autophagy-inhibiting compounds include lysosomotropic agents[121]. These agents target acidic compartments, such as lysosomes, but are not specific to tumor cells and therefore have a range of effects on other cells. Lysosomotropic agents cross the lysosomal membrane and are then protonated within the acidic vesicle[122]. This results in an increased pH, which prevents cellular degradation and indirectly inhibits autophagy. Preclinical studies have demonstrated the effects of lysosomotropic agents, including CQ, which include the indirect modulation of late-stage autophagy[123]. Furthermore, CQ inhibits phospholipase A2 and lysophospholipid acylhydrolase, enzymes that are required for the acidification of lysosomes[124].

Treating human colon carcinoma HT29 cells with CQ sensitized mouse colon cancers to antiangiogenic and cytotoxic therapy[93]. Moreover, the combination of CQ and 5-FU displayed a significant advantage over treatment with 5-FU alone in inhibiting tumor growth in colon 26 cells, which are a CRC cell line[125]. A combination of the autophagy inhibitor CQ and vorinostat, a histone deacetylase inhibitor, was shown to significantly reduce tumor growth and induce apoptosis in a colon cancer xenograft model[126]. Notably, the combination of CQ with saracatinib, an inhibitor of Src nonreceptor tyrosine kinase, enhanced apoptotic cell death and resulted in 64% tumor growth inhibition compared with saracatinib alone[127]. Autophagy inhibitors shown synergy with proteasome inhibitors; for example, the simultaneous use of bortezomib and CQ in a colon cancer xenograft model decreased tumor growth to a greater extent than the use of either of these drugs alone[128].

Interestingly, treatment of human HCT-15 colon adenocarcinoma culture cells with B-group soyasaponins induced autophagy and suppressed proliferation through a marked increase in autophagic cell death[129]. In addition to its effects on cell viability and anchorage-independent growth inhibition, the flavonoid quercetin induced autophagic processes in Ha-Ras transformed human colon cells and has been proposed to have anticancer properties[130]. Vitamin D can trigger autophagy by enhancing BECN1 expression and inducing PI3KC3 expression[131]. Cetuximab (an antibody for EGFR) generates autophagy and it is currently used to treat *K-Ras* mutation-negative, EGFR-expressing, metastatic CRC[121]. Moreover, MS-275, a synthetic benzamide derivative of HDAC, promoted Atg7 protein expression and induced autophagy to switch to apoptosis through the modulation of p38 in human colon cancer cells[132].

Curcumin is a natural polyphenolic compound that is isolated from the plant *Curcuma longa*. In addition to apoptosis, curcumin also promotes autophagic cell death type Ⅱ[133] by inhibiting the Akt/mTOR/p70S6K pathway or by activating the ERK1/2 pathway[134]. The proliferation of HT-29 and HCT-15 human colon cancer cell lines was inhibited by curcumin treatment, which arrested the cell cycle in the G2/M phase with no detected apoptosis[135]. Curcumin administered in combination with 5-FU plus oxaliplatin resulted in increased inhibition of growth and enhanced apoptosis

in HCT-116 and HT-29 colon cancer cells compared to each of these drugs alone, and these effects were attained mainly through the attenuation of the EGFR and IGF-1R signaling pathways[136]. The induction of autophagy activation and ROS production was observed in HCT116 human colon cancer cells that were treated with curcumin, and they showed higher mRNA and protein LC3 levels[137].

Autophagy facilitates cancer cell resistance to chemotherapy treatments, and the inhibition of autophagy may resensitize resistant tumor cells to anticancer therapy, thus enhancing the efficacy of the treatment. For example, imatinib induces nonapoptotic autophagic cell death, while the inhibition of autophagy enhances its cytotoxicity, but only at a late stage[138]. Autophagy activation was observed in colon cancer stem cells by analysis of the expression of the intestine-specific transcription factor Cdx1, which plays a crucial role in chemoresistance to paclitaxel[106]. Similarly, autophagy increased resistance to photodynamic therapy-induced apoptosis in CRC stem-like cells[139]. However, this report did not address whether the protective autophagy that was induced in cancer stem cells was due to a drug-mediated response to stress or to the inherent ability of cancer stem cells to maintain a high threshold for autophagy. Suppression of protective autophagy by 3-MA was reported to enhance the therapeutic efficacy of cisplatin and 5-FU in digestive cancers, including colon cancer[140].

Many mTOR inhibitors with effective antitumor activity have been developed. However, they also have downstream effects that include the activation of autophagy, which is linked to prosurvival mechanisms in tumor cells through the recycling of damaged cellular contents. The addition of an autophagy inhibitor could solve this complication by excluding this alternate recovery pathway and sensitizing malignant cells to anticancer therapies[141,142].

Taken together, these observations suggest that autophagy supports the progression of established neoplasms through several mechanisms and that pharmacological inhibitors of autophagy may exert robust antineoplastic effects, at least in some settings.

Future research aimed at exploring the context specific role of autophagy in particular cancer types can provide new opportunities to develop personalized therapeutic strategies based on the regulation of autophagy, and autophagy modulators may become a targetable option for enhancing the efficacy of anticancer therapies used alone or, more likely, in combination with other chemotherapeutic drugs[120].

CONCLUSION

Multiple genes and proteins are involved in the complex steps of autophagy. Recent evidence has suggested that autophagy plays an important role in all stages of carcinogenesis, by influencing initiation, progression and metastatic capacity in tumors. The precise mechanisms that involve autophagy in cancer are not yet defined, and they seem to be context dependent, having both promoting and inhibiting roles. During the first steps of cancer, autophagy may have a suppressive effect, whereas it may alternatively act as tumor promoter during advanced cancer stages. It is necessary to determine how these dual roles of autophagy in CRC are regulated and identify the signals, molecules, and mechanisms that enable autophagy to play a dominant pro-malignant role in one situation and the opposite role in another. The most important research on CRC has been focused on several molecules, mainly LC3, BECN1, ATG5, and these studies have produced conflicting results. Several therapeutic agents that modulate autophagy in CRC have been developed and show promising results supporting their use either alone or, more likely, in combination with other drugs. Further research is required to better understand the relationship between CRC and autophagy, and to produce potentially beneficial agents for the prognosis and therapy of CRC.

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TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Breakthrough therapy for peritoneal carcinomatosis of gastric cancer: Intraperitoneal chemotherapy with taxanes

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Abstract

The effect of chemotherapy on peritoneal carcinomatosis (PC) of gastric cancer remains unclear. Recently, the intraperitoneal (IP) administration of taxanes [*e.g.*, paclitaxel (PTX) and docetaxel (DOC)] during the perioperative period has shown promising results. Herein, we summarized the rationale and methodology for using IP chemotherapy with taxanes and reviewed the clinical results. IP administered taxanes remain in the IP space at an extremely high concentration for 48-72 h. The drug directly infiltrates peritoneal metastatic nodules from the surface and then produces antitumor effects, making it ideal for IP chemotherapy. There are two types of perioperative IP chemotherapy with taxanes: neoadjuvant intraperitoneal and systemic chemotherapy and sequential perioperative intraperitoneal chemotherapy (SPIC). In SPIC, patients receive neoadjuvant IP chemotherapy and the same regimen of IP chemotherapy after cytoreductive surgery (CRS) until disease progression. Usually, a taxane dissolved in 500-1000 mL of saline at ordinary temperature is administered through an IP access port on an outpatient basis. According to phase Ⅰ studies, the recommended doses (RD) are as follows: IP DOC, 45-60 mg/m^2; IP PTX [without intravenous (IV) PTX], 80 mg/m^2; and IP PTX (with IV PTX), 20 mg/m^2. Phase Ⅱ studies have reported a median survival time of 14.4-24.6 mo with a 1-year overall survival of 67%-78%. A phase Ⅲ study comparing S-1 in combination with IP and IV PTX to S-1 with IV cisplatin started in 2011. The prognosis of patients who underwent CRS was better than that of those who did not; however, this was partly due to selection bias. Although several phase Ⅱ studies have shown promising results, a randomized controlled study is needed to validate the effectiveness of IP chemotherapy with taxanes for PC of gastric cancer.

Key words: Taxane; Paclitaxel; Docetaxel; Carcinoma;

Gastric cancer; Intraperitoneal infusions; Cytoreduction surgical procedures

Core tip: Herein, we provided an overview on the recent advances in intraperitoneal (IP) chemotherapy using taxanes (*e.g.*, paclitaxel and docetaxel) for peritoneal carcinomatosis of gastric cancer. In particular, we focus on the rationale of IP chemotherapy with taxanes, treatment methodology, and results of current clinical studies. Intraperitoneally administered taxanes remain in the IP cavity for a long time, and they directly infiltrate the peritoneal metastatic nodule from the surface. Therefore, the repeated intra-abdominal administration of taxanes through an IP access port is needed to increase the antitumor effect of IP chemotherapy.

Yamaguchi H, Kitayama J, Ishigami H, Kazama S, Nozawa H, Kawai K, Hata K, Kiyomatsu T, Tanaka T, Tanaka J, Nishikawa T, Otani K, Yasuda K, Ishihara S, Sunami E, Watanabe T. Breakthrough therapy for peritoneal carcinomatosis of gastric cancer: Intraperitoneal chemotherapy with taxanes. *World J Gastrointest Oncol* 2015; 7(11): 285-291 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/285.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.285

INTRODUCTION

Gastric cancer is the fourth most common cancer worldwide, and it is the second leading cause of cancer-related deaths[1]. Gastric cancer may disseminate along the inside surface of the peritoneal cavity, leading to peritoneal carcinomatosis (PC). PC is the most frequent mode of metastasis and recurrence in patients with gastric cancer. According to the national registry database of Japan, PC accounted for 51% of deaths in 355 patients with non-curable primary gastric cancer[2]. The same database also revealed that PC was the most frequent cause of death in 13002 patients who underwent gastrectomy for primary gastric cancer[2]. Yoo *et al*[3] reported that in 508 patients who underwent radical gastrectomy for gastric cancer, the first recurrence site was the peritoneum (43.9%) and then a local site (32.5%) followed by the liver (16.9%).

Despite recent advances in chemotherapy regimens for gastric cancer, the effect of systemic chemotherapy on PC remains unclear. Clinical trials on methotrexate + 5-fluorouracil (5-FU), FOLFOX-4, and continuous 5-FU for PC of gastric cancer showed that the median survival time (MST) was 5.2-10.6 mo, and the 1-year overall survival (OS) was 16.2%-40.7%[4-7].

In alternative treatment modalities, cytoreductive surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC) has been used for treating PC of gastric cancer. Reportedly, the MST and 1-year survival were 9.2-11.5 mo and 35.5%-48.1% respectively[8-11]. However, CRS + HIPEC should be performed in specialized facilities, because these demanding procedures are associated with a high mortality and morbidity[12].

The intraperitoneal (IP) administration of anticancer drugs is a reasonable method for treating PC, because an IP administered cytotoxic drug acts directly on the peritoneal metastatic nodules at a high concentration. In HIPEC procedures, mitomycin C (MMC) and/or cisplatin (CDDP) dissolved in heated saline at 42 ℃-43 ℃ are usually administered into the peritoneal cavity[13].

Recently, the IP administration of taxanes such as paclitaxel (PTX) or docetaxel (DOC) without heating them at the ordinary temperature during the perioperative period in gastric cancer patients with PC has been performed mainly in Japan. Several clinical trials using IP chemotherapy with taxanes have shown promising results[14-18].

Based on the literature published in the last decade, we summarized the rationale for using IP chemotherapy with taxanes, methodology used for IP chemotherapy, and clinical results of IP chemotherapy in gastric cancer patients with PC.

RATIONALE FOR USING IP CHEMOTHERAPY WITH TAXANES

Taxanes such as PTX and DOC produce cytotoxic effects by inducing excessive polymerization of tubulin and dysfunctional microtubules, which leads to mitotic arrest and cell death[19,20]. PTX and DOC are water insoluble, and for clinical use, they are solubilized with Cremophor EL (Taxol®; Bristol-Myers Squibb Co.) and Polysorbate 80 (Taxotere®; Aventis Pharma SA), respectively.

Since taxanes are hydrophobic, high-weight molecular materials, IP administered taxanes are gradually drained from the peritoneum through lymphatic stomata that open directly into the pleural space[21,22]. In contrast, hydrophilic, low-weight molecular materials such as MMC or CDDP are rapidly absorbed through the peritoneal mesothelial layer and into the capillary vessels.

The area under the curve ratios of the intra-abdominal space to the plasma after IP administration of the drug are about 1000 for PTX, 207-552 for DOC, 10-24 for MMC, and 12-21 for CDDP[23-28]. The prolonged retention of IP administered taxanes within the IP space allows the taxanes to directly penetrate into peritoneal disseminated tumors[23,29-31], which leads to the destruction of peripheral microvessels of tumor nodules[32]. However, the depth of infiltration from the surface of the peritoneal disseminated nodules after the one time IP administration of a taxane is limited[33,34]. In a previous study, we showed that the distance of PTX infiltration reached approximately 100-200 μm from the surface of the tumor[35]. Therefore, to improve the antitumor effects of taxanes against PC, repeated IP administration is necessary.

Table 1 Phase Ⅰ studies on intraperitoneal chemotherapy using taxanes for the treatment of gastric cancer with peritoneal carcinomatosis

Ref.	*n*	Intraperitoneally administered taxanes	Initial dose (mg/m^2)	MTD (mg/m^2)	RD (mg/m^2)	DLT
Kodera *et al*[42]	4	PTX	60	-	-	-
Fushida *et al*[26]	24	DOC	25	60	45	Abdominal pain and diarrhea
Ishigami *et al*[45]	9	PTX	20	30	20	Febrile neutropenia and diarrhea
Fujiwara *et al*[43]	12	DOC	40	-	60	-
Kurita *et al*[44]	18	PTX	40	90	80	Leukocytopenia
Fushida *et al*[16]	12	DOC	35	50	45	Febrile neutropenia and diarrhea

MTD: Maximum tolerated dose; RD: Recommended dose; DLT: Dose-limiting toxicities; PTX: Paclitaxel; DOC: Docetaxel.

From the perspective of pharmacokinetics and tissue penetration, taxanes are ideal drugs for IP chemotherapy. Moreover, even if taxanes are repeatedly administered intraperitoneally, they rarely cause adhesion of organs in the peritoneal cavity because of their antiproliferative effect. Thus, the distribution of IP administered taxanes across the intra-abdominal space is not hampered by drug-induced peritonitis.

METHODOLOGY OF USING IP CHEMOTHERAPY WITH TAXANES

Perioperative IP chemotherapy with taxanes

There are two types of perioperative IP chemotherapy with taxanes for treating PC of gastric cancer: neoadjuvant intraperitoneal and systemic chemotherapy (NIPS)[36] and sequential perioperative intraperitoneal chemotherapy (SPIC)[37]. In NIPS, patients receive 1-6 courses of IP chemotherapy with a taxane as a neoadjuvant therapy; however, they do not receive IP chemotherapy after CRS[17,38,39]. In SPIC, patients receive several courses of IP chemotherapy preoperatively, and they receive the same regimen of IP chemotherapy after CRS until disease progression[14-16].

Peritoneal access port system

In most reported studies, a peritoneal access port system was used for IP chemotherapy. However, this device was not used when patients received a single IP administration during staging laparoscopy[28,39], or if patients received IP administration two times *via* a catheter as neoadjuvant chemotherapy[17]. A peritoneal access port is implanted into the subcutaneous space of the lower abdomen, and a catheter is placed usually in the pelvic cavity. Taxane dissolved in 500-1000 mL of saline at the ordinary temperature is administered through the peritoneal access port. Thus, using this method, taxanes can be repeatedly administered on an outpatient basis.

Complications associated with the port system occurred in 20.6% of 131 patients at our institution[40]. Inflow obstruction and infection were the main complications that occurred in 7.6% and 6.9% of patients, respectively. The median period of IP chemotherapy using the peritoneal port system was 12.9 mo (range, 0.8-61.5 mo). Compared to previous studies on ovarian cancer[41], the course of IP chemotherapy performed at our institution was much longer, but the complication rate was lower.

The use of a peritoneal port system can facilitate IP administration and reduce the patients' burden of receiving IP chemotherapy. Moreover, the device can provide another benefit to patients, because the peritoneal lavage sample, which is essential for evaluating the effect of IP chemotherapy on PC, can be obtained noninvasively through the peritoneal access port.

CLINICAL STUDIES ON IP CHEMOTHERAPY WITH TAXANES

Phase Ⅰ study

The findings from six phase Ⅰ studies on IP chemotherapy with taxanes are summarized in Table 1. PTX was used for intraperitoneally administering agents in three studies, and DOC was used in the other three studies. PTX or DOC was IP administered without other anticancer drugs in two studies[26,42], DOC was IP administered with S-1 in two[16,43], PTX was IP administered with S-1 in one[44], and intravenous (IV) PTX and S-1 was administered in one[45].

The recommended dose (RD) of DOC IP administration was 45-60 mg/m^2. The RD of PTX IP administration was 80 mg/m^2 when PTX was not IV administered, and it was 20 mg/m^2 when PTX was IV administered. Although the RD of 20 mg/m^2 in our phase Ⅰ study was relatively low because we used a combination of IV PTX, the IP PTX concentration remained extremely high for > 72 h.

Dose-limiting toxicities of these phase Ⅰ studies included grade 3 febrile neutropenia, leukopenia, and diarrhea for the PTX IP regimen; and grade 3 febrile neutropenia, abdominal pain, and diarrhea for the DOC IP regimen.

Phase Ⅱ study

The findings of six phase Ⅱ studies on IP chemotherapy with taxanes are summarized in Table 2. PTX was used for IP administered agents in three studies[14,15,39], and DOC was used in the other three studies[16,17,38]. The

Table 2 Phase Ⅱ studies on intraperitoneal chemotherapy using taxanes for the treatment of gastric cancer with peritoneal carcinomatosis

Ref.	*n*	Method	Intraperitoneally administered agents	MST (mo)	1-yr OS (%)	2-yr OS (%)	5-yr OS (%)
Yonemura *et al*[38]	61	NIPS	DOC (40 mg) + CBDCA (150 mg)	14.4	67		
Ishigami *et al*[14]	40	SPIC	PTX (20 mg/m^2)	22.6	78		
Fujiwara *et al*[17]	18	NIPS	DOC (40-60 mg/m^2)	24.6	76	54	
Imano *et al*[39]	35	NIPS	PTX (80 mg/m^2)	21.3	69	46	14
Yamaguchi *et al*[15]	35	SPIC	PTX (20 mg/m^2)	17.6	77	45	
Fushida *et al*[16]	27	SPIC	DOC (35-50 mg/m^2)	16.2	70	33	

MST: Median survival time; OS: Overall survival; DOC: Docetaxel; CBDCA: Carboplatin; PTX: Paclitaxel; NIPS: Neoadjuvant intraperitoneal and systemic chemotherapy; SPIC: Sequential perioperative intraperitoneal chemotherapy.

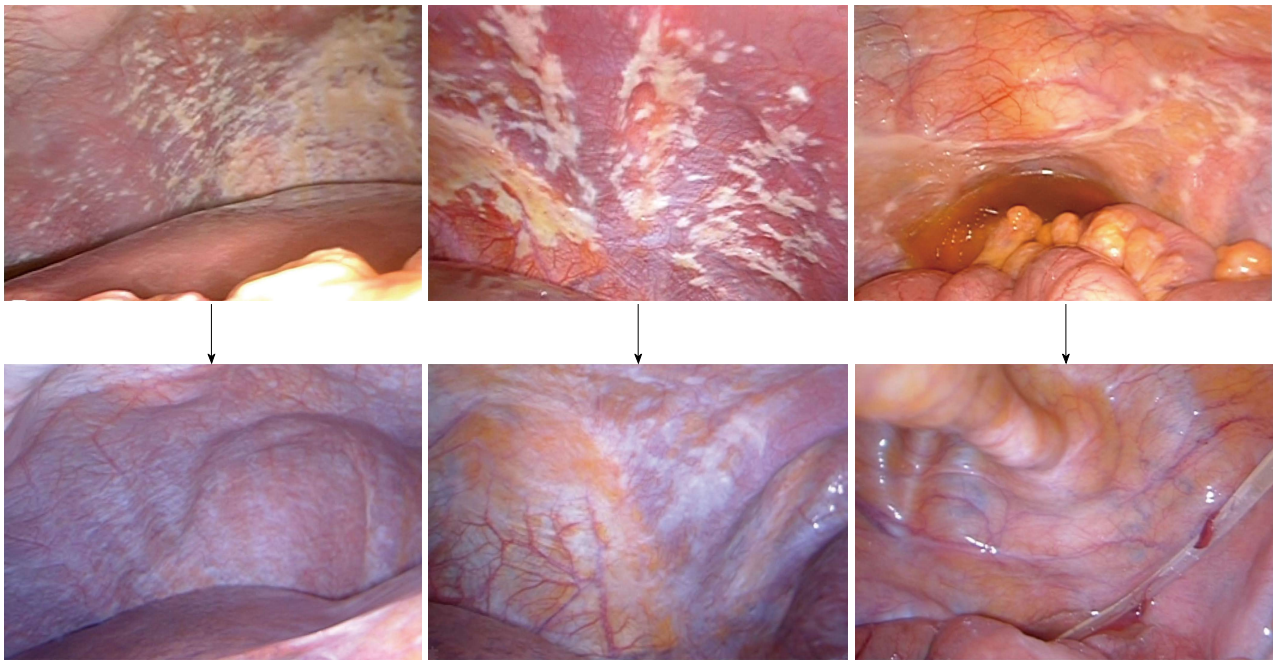

Figure 1 Laparoscopy before and after treatment. Staging laparoscopy (upper) showing peritoneal metastatic nodules in the right subphrenic peritoneum (left), left subphrenic peritoneum (middle), and Douglas pouch (left). The second laparoscopy (lower) revealing that the metastatic nodules have disappeared after 12 courses of the intravenous and intraperitoneal administration of paclitaxel and oral S-1 chemotherapy.

overall response rate among these phase Ⅱ studies ranged from 55%-71%. The MSTs and 1-year OS were 14.4-24.6 mo and 67%-78%, respectively. The main toxicities were hematologic (*e.g.*, anemia, neutropenia, and leukopenia), and the non-hematological toxic effects were relatively mild. Regarding CRS, gastrectomy with D2 dissection was usually performed. In addition to D2 gastrectomy, peritonectomy was performed only by Yonemura *et al*[38]. Post-operative complications, ranging 9%-22%, were reported in four studies[16,17,38,39]. Surgery-related mortality was found in one patient, and the cause of death was sepsis from an abdominal abscess[38].

In three of six phase Ⅱ studies, patients received 1-6 courses of NIPS. The MSTs of patients who underwent CRS after NIPS were 20.4-29.8 mo. In the other phase Ⅱ studies, patients received SPIC. In 2010, we reported on a phase Ⅱ study on SPIC in 40 gastric cancer patients with PC, which included six cytology positive (CY1) and macroscopically negative (P0) patients[14]. Sixteen patients underwent CRS. According to recently updated survival data, the MST was 23.6 mo and the 1-, 2-, and 5-year OS were 78%, 50%, and 18%, respectively.

We performed another phase Ⅱ study with the same regimen in 35 gastric cancer patients with PC[15]. However, in this study, CY1P0 patients were excluded, because they may have a better prognosis compared to macroscopic PC (P1) patients. CRS was performed in 21 patients. Patients with peritoneal cancer index (PCI) scores ≥ 20 had a lower survival rate than those with PCI scores < 20. According to recently updated data, the MST was 18.0 mo, and the 1-, 2-, and 4-year OS were 77%, 42%, and 10%, respectively. The findings from staging laparoscopy and second-look laparoscopy are shown from a representative case (Figure 1).

Fushida *et al*[16] performed a phase Ⅰ/Ⅱ study on SPIC with IP DOC in 27 patients. Fourteen patients underwent CRS and received postoperative IP chemotherapy.

The 1- and 2-year OS of patients who underwent CRS were 92.8% and 62.5%, respectively.

Phase Ⅲ study

In Japan, a randomized, multicenter, phase Ⅲ trial (the PHOENIX-GC trial, UMIN000005930) compared S-1 in combination with IV and IP PTX to S-1 with IV CDDP in 180 gastric cancer patients with P1. This study began in 2011, and the final analysis will be obtained in November 2015.

IP chemotherapy with taxanes combined with CRS

If PC can be controlled by IP chemotherapy with a taxane, gastrectomy as CRS is considered to be a reasonable treatment. Because IP chemotherapy as a localized therapy for peritoneal cavity may not have intensive antitumor effects on primary gastric tumors and metastatic lymph nodes. Other than the aforementioned phase Ⅱ studies, two studies have reported on the treatment results of IP chemotherapy combined with CRS.

Kitayama *et al*[18] treated 64 gastric cancer patients with PC who had malignant ascites with IP and IV PTX combined with S-1. CRS without peritonectomy was performed in 34 patients. After CRS, chemotherapy with the same regimen was continued (*i.e.*, SPIC). The MST of these patients and the 1-year OS were 26.4 mo and 82%, respectively. Those of the 30 patients who did not undergo gastrectomy were 12.1 mo and 26%, respectively.

Yonemura *et al*[46] performed NIPS with IP DOC and CDDP combined with S-1 in 96 patients. After two cycles of NIPS, 82 patients underwent CRS (gastrectomy with D2 dissection and peritonectomy). Complete cytoreduction was achieved in 58 patients. The MST and 1-year OS of patients who underwent CRS was 14.4 mo and 61%, respectively. The MST of patients who underwent complete cytoreduction and those who did not undergo CRS were 21.1 mo and 9 mo, respectively.

In these reports, the prognosis of patients who underwent CRS was better than that of those who did not. However, this survival difference was partly due to a strong selection bias since CRS was performed only in good responders. A randomized controlled study will need to be performed in order to determine the significance of CRS.

DISCUSSION

It is important whether IP chemotherapy with taxanes is needed after CRS. Yonemura *et al*[46] reported that 22 of 61 patients who received NIPS with complete CRS had recurrence in the peritoneum. Fujiwara *et al*[17] suggested that IP chemotherapy may have been needed in their patients, because 8 of 14 patients who had curative surgery following NIPS died from peritoneal recurrence. It is reasonable to consider that IP chemotherapy with a taxane should be continued as long as possible even after CRS to suppress the development of microscopic cancer cells that may still exist in the whole peritoneal cavity. Therefore, we consider that SPIC is better suited for treating PC of gastric cancer.

Another important issue is how the criteria for performing CRS are determined. If patients do not respond to IP chemotherapy, CRS should not be performed. We have performed CRS in patients who have met the following criteria: (1) no distant metastasis, except in the peritoneum; (2) a negative peritoneal lavage cytology; and (3) a second-look laparoscopy reveals that the peritoneal metastatic nodules are reduced. To select eligible patients for CRS more precisely, novel and useful biomarkers that reflect a good response to IP chemotherapy are needed.

Phase Ⅲ studies on IP chemotherapy with taxanes have been reported in the gynecological field, especially for PC of ovarian cancer. IP PTX with systemic chemotherapy for PC of ovarian cancer showed a significant survival benefit[47]. Based on the findings from these phase Ⅲ studies[47-49], the National Cancer Institute has recommended IP chemotherapy in patients with optimally debulked ovarian cancer[50].

Regarding the treatment of PC from gastric cancer, there are promising findings from several phase Ⅱ studies with IP chemotherapy using taxanes. However, it is difficult to draw any definitive conclusions about the overall clinical usefulness of this treatment method until we obtain the findings from the PHOENIX-GC phase Ⅲ trials.

In conclusion, IP administered taxanes remain in the IP cavity for a long period, and they produce antitumor effects by infiltrating peritoneal metastatic nodules from the surface. In addition, repeated IP administration of taxanes through an IP access port before and after CRS seems necessary for improving the effect of IP chemotherapy. Lastly, IP chemotherapy with taxanes for PC from gastric cancer is safe and feasible. Although several phase Ⅱ clinical studies have shown promising results, further randomized phase Ⅲ clinical trials are needed to validate IP chemotherapy with taxanes for gastric PC.

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TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Individualized treatment of gastric cancer: Impact of molecular biology and pathohistological features

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Abstract

Gastric cancer is one of the most common malignancies worldwide. The overall prognosis remains poor over the last decades even though improvements in surgical outcomes have been achieved. A better understanding of the molecular biology of gastric cancer and detection of eligible molecular targets might be of central interest to further improve clinical outcome. With this intention, first steps have been made in the research of growth factor signaling. Regarding morphogens, cell cycle and nuclear factor-κB signaling, a remarkable count of target-specific agents have been developed, nevertheless the transfer into the field of clinical routine is still at the beginning. The potential utility of epigenetic targets and the further evaluation of microRNA signaling seem to have potential for the development of novel treatment strategies in the future.

Key words: Gastric cancer; Molecular biology; Targeted therapy; Personalized medicine; Signaling pathway

Core tip: Advanced gastric cancer remains a frequent malignancy with poor prognosis despite multimodal treatment options. Surgery alone has been demonstrated not to be the optimal strategy and is predominantly limited to cases without distant metastases. About one half of gastric cancer patients cannot be cured. Due to its individual heterogeneity on the molecular level these tumors frequently do not respond to systemic treatment. The implementation of the growing knowledge about the molecular behavior of gastric cancer in the development or improvement of target-specific treatment strategies might be one of the major challenges for the next decades.

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GENERAL CLINICAL ASPECTS

Gastric cancer is still one of the leading oncologic challenges due to its frequent occurrence as well as its poor prognosis[1]. The ongoing improvement of surgical techniques and perioperative care over the past decades have not only extended the repertoire of treatment options with curative intent but also have contributed to the reduction of perioperative morbidity. Thus, currently about 50% of all gastric cancer patients can be treated curatively and the majority of these patients undergo the surgical treatment without severe complications[2]. But still one half of all gastric cancer patients have to be regarded as palliative cases with no chance for long term survival and even the curatively resected patients face an overall recurrence rate of 50%[3].

In view of this development it can be assumed that further evolvement of surgical treatment will not improve tumor-related survival substantially. The molecular biology of the individual tumor might be one important key to a better understanding of the disease and an advancement in the prognosis of gastric cancer patients.

The knowledge about the molecular biology of gastric cancer is of high interest for several reasons: (1) aberrations at the genomic as well as at the proteomic level might be useful as biomarkers for exact classification; (2) molecular markers may further improve and refine tumor staging; (3) knowledge about the individual molecular signature may enable a personalized and target specific treatment; and (4) molecular presentation of the tumor and target specific treatment may lead to an improved prognosis.

UNDERSTANDING OF THE MOLECULAR BIOLOGY: GENERAL CHALLENGES

The understanding of molecular biology of gastric cancer is crucial for the appraisal of its clinical behavior and to control the tumor growth with all its consequences. As in almost all other tumor entities the following characteristics may challenge the establishment of an effective treatment: (1) every individual tumor presents with a unique pattern of molecular variance, comparable with an individual fingerprint; (2) in a certain manner every tumor can be regarded as an autonomous organism which in fact means that tumors do not consist of a homogenous tissue mass but show a regional heterogeneity; (3) over time every tumor changes spontaneously in its molecular biological behaviour; and (4) every tumor reacts in a distinct manner to treatment attempts.

These aspects are basically important in un-targeted treatment approaches as the application of conventional cytostatic substances or surgery but are even more important for target-specific treatment strategies. In view of the multidimensional complexity of molecular tumor biology it becomes clear that it is unlikely to find "the single one agent" to achieve a safe and sustainable tumor control.

CURRENT STATE OF THE ART IN MOLECULAR TARGETED TREATMENT

Growth factors, growth factor receptors and downstream features

Epithelial growth factor: To date, four different types of epithelial growth factor receptors (EGFR) have been identified, also called as ErbB1-4[4]. Once activated, they form homo- or heterodimers and then become internalized within the cell. From there three different pathways (MAP-kinase pathway, STAT pathway and PI3K pathway) can be activated, subsequently leading to the transmission of the signal into the nucleus and specific regulation of gene expression by activated cyclinD1, iNOS, B-myb, COX2 and Aurora kinase 2. With the exception of ErbB2, in addition to the original epidermal growth factors multiple other ligands can bind and activate EGFR: transforming growth factor alpha, epiregulin, amphiregulin and βcellulin. ErbB2 in contrast, can not be activated directly by any growth factor, but can be heterodimerized by other members of the EGFR family[5].

It has been reported that EGFR overexpression occurs in 60% to 70% of gastric cancer cases, however gene amplification seems to be rather uncommon[6,7]. EGFR2 measured by fluorescence *in situ* hybridisation was detected in 22% of gastric cancers[8,9], while it was more frequent in intestinal than in diffuse type gastric cancer according to the Lauren classification (32% and 20%)[9,10]. EGFR overexpression in gastric cancer was related to poorer survival and poorer response to chemotherapy[11].

Due to its central role in epithelial signaling as well as its biological properties EGFR became an interesting target for molecular-based treatment and thus there is now a remarkable variety of EGFR-targeted molecules available.

Three main target points have been proposed: the inactivation of the receptor, the stimulation of antibody-dependent cell cytotoxity and the inhibition of the tyrosine kinase activity by multityrosin kinase inhibitors.

To date, seven monoclonal antibodies targeting EGFR are available: cetuximab, trastuzumab, matuzutumab, panitumumab, nimetuzumab, perluzumab and T-DM1[10].

Cetuximab inhibits the binding of EGF and TGFalpha to EGFR, furthermore it promotes the internalization of the receptor[12]. The application of cetuximab is well established in stage 4 colorectal cancer (with k-ras wild type)[13] and in several head and neck malignancies[14,15].

Several phase 2 and 3 trials showed a positive effect of the administration of cetuximab combined with standard chemotherapy protocols as a first line therapy with response rates up to 58% and 69% in advanced gastroesophageal junction and gastric cancer (overall survival up to 9.5 mo)[10,16]. In contrast, cetuximab in combination with cisplatin or irinotecan as a second line

therapy revealed only a marginal benefit on the overall survival (7.1 mo)[17]. Moreover, cetuximab as a single-agent administration for second line therapy resulted in even lower impact on the overall survival (3.6 to 4 mo) with poor response (9%)[18].

Cetuximab in combination with several cytostatic substances for neoadjuvant chemotherapy showed response rates up to 70%[19,20].

Trastuzumab is known to have a broad variety of molecular effects: Binding to the extracellular part of the her-2/*neu* molecule und thus suppressing the intracellular localised tyrosine kinase activity, antibody dependent cell toxicity (ADCC)[21], activation of natural killer cells, inhibition of angiogenesis and the phosphoinositol-3-kinase signaling pathway (PI3K) as well as cell cycle arrest[22-24]. The administration of trastuzumab as adjuvant treatment has been approved for node positive breast cancer[25].

The most important study with respect to gastric cancer is the ToGA trial. It has been shown that those patients who were positive for the her-2/*neu* receptor (22% of all cases) had a significant improvement in tumor response and overall survival when standard chemotherapy was combined with trastuzumab (47% *vs* 34%, 13.8 mo *vs* 11.1 mo)[26]. An innovative and promising further development of trastuzumab, named T-DM1 is currently undergoing clinical testing. In the T-DM1 molecule the trastuzumab antibody is coupled to maytansine, a microtubule polymerization inhibitor which unfolds its effect after internalization of the antibody-receptor complex within the cytosol[27].

Recently it has been published that *in vitro* the cytotxic effect of trastuzumab on gastric cancer cell lines significantly increased when the cancer cells were pre-treated by incubation with reovirus serotype 3[28].

Matuzutumab is an IgG1 antibody with ADCC. Unlike cetuximab and nimotuzumab it is a fully humanized molecule. Unfortunately, it has been shown that combination treatment of matuzutumab with cytostatic substances is not beneficial for overall survival and response rates[29].

Panitumumab is an IgG2 antibody. It is routinely used in the treatment of metastatic colorectal cancer. The comparison of combined chemotherapy with or without panitumumab yielded disappointing results with a poorer outcome in the the panitumumab group in terms of overall survival and overall response rate (8.8 mo *vs* 11.3 mo and 42% *vs* 46%, respectively). Surprisingly, in the subgroup of patients with severe rash the overall survival of patients who received panitumumab-including treatment was significantly improved (10.2 mo *vs* 4.3 mo)[30].

Nimotuzumab is similar to matuzutumab a fully humanized antibody, known to exhibit ADCC. There is some evidence in the literature that nimotuzumab in combination with cytostatic substances might be effective in squamous cell carcinoma of the esophagus and in glioma. To date, there are two studies available investigating the effect of nimotuzumab plus cytostatic substances in metastatic gastric cancer. In one study, the overall response rate was improved (63% *vs* 50%) with similar progression free survival, the other study showed the progression free survival to be slightly improved with similar response rates (5.5 mo *vs* 3 mo)[10].

Pertuzumab is an inhibitor of homo - as well as heterodimerization of the EGF receptor. Therefore, it seems to be reasonable to combine pertuzumab with different EGF receptor antagonists like trastuzumab. It is also known to exhibit ADCC. The administration of pertuzumab is approved for metastatic breast cancer[31]. The combination of pertuzumab and trastuzumab seems to be effective in advanced gastric cancer with overall response rates up to 86%[32].

Vascular endothelial growth factor: The recruitment of new blood vessels for the supply of the growing tumor with nutrients and oxygen is known to be one of the crucial steps in tumor progression, especially in the development of distant metastases[33]. Although neoangiogenesis in the tumor environment and physiological angiogenesis partly have similar pathways there are remarkable differences in vessel architecture, vascular permeability as well as a different interplay of endothelial cells and perivascular cells. In this context, vascular growth factors play an crucial role. Vascular growth factors are expressed when tissue hypoxia is present. Several other changes can result in vascular endothelial growth factor (VEGF) up-regulation too, *e.g.*, low pH or silenced tumor suppressor genes like p53[34].

To date, we know five important factors of angiogensis: VEGF A-D and placenta derived growth factor. Furthermore, three targets for these growth factors have been detected: vascular endothelial growth factor receptor (VEGFR) 1-3. VEGFR2 seems to be the most important subtype. It is localized on the cell surface of endothelial cells and bone marrow derived endothelial progenitor cells[35]. VEGFR2 binds to VEGF A, C and D, leading to activation of the PI3K signaling pathway as well as MAP kinase signaling pathway[36]. Some of the most important down stream effects are the inhibition of apoptosis, the proliferation of endothelial cells and increased endothelial cell migration[35]. The binding of the mediator molecule to its receptor is substantially increased in the presence of the co-receptors neuropilin 1 and 2. The application of these co-receptors as possible targets for molecular based treatment is currently under development[37].

Overexpression of VEGF and its downstream molecules is common in numerous malignancies. Interestingly, Takahashi *et al*[38] already demonstrated in 1996 that VEGF is more frequently dysregulated in intestinal type than in diffuse type gastric cancer (36% and 16%, respectively). Two different antibodies targeting the VEGF signalling pathway have been shown to be effective and eligible in the treatment of advanced gastric cancer: Bevacizumab and ramucirumab.

Bevacizumab binds to VEGF-A and thus interrupts

the activation of VEGFR1 and VEGFR2[33]. Whereas different phase 1 and 2 trials revealed promising effects of bevacizumab on gastric cancer progression, the results of phase 3 studies were disappointing. Although in the AVAGAST study overall median survival was slightly longer in patients who received bevacizumab plus standard chemotherapy, these results did not reach a statistically significant level (12.1 mo and 10.1 mo, P = 0.1002). Merely progression free survival was significantly longer in the intervention group (6.7 mo and 5.3 mo, P = 0.0301)[39]. The subsequently performed AVATAR study did not show any benefit of treatment with bevacizumab in combination with standard chemotherapy as compared to standard chemotherapy only (median overall survival 10.5 and 11.4 mo, progression free survival 6.3 and 6.0 mo)[40]. Based on these results bevacizumab currently is not routinely used in the treatment of advanced gastric cancer.

Ramuzirumab is a competitive inhibitor of VEGFR2 with a 8fold higher affinity to the receptor as compared to natural ligands[41]. Two phase 3 studies revealed ramucirumab to have positive effects on the containment of gastric cancer progression. The REGARD study investigated the impact of ramucirumab as a second line therapy on advanced gastric cancer. In comparison to the placebo group as well overall survival, disease control rate and overall response rate were significantly better (3.8 mo *vs* 5.2 mo, 49% *vs* 23%, 3.4% *vs* 2.6%). Interestingly, among male patients these effects were even more distinct[42]. The RAINBOW study compared the outcomes after administration of paclitaxel with or without ramucirumab to a similar target audience. Overall survival and disease control rate both were better in the intervention group (9.6 mo *vs* 7.4 mo, 80% *vs* 64%)[43].

In summary, currently ramucirumab seems to be the only one option to treat advanced gastric cancer with a VEGF-R specific antibody.

Platelet derived growth factor receptor: The Platelet derived growth factor (PDGF) family consists of 4 homodimers A-D and the heterodimer AB. Due to its dimeric structure it binds to receptor molecules which subsequently activate each other. Two different subtypes of PDGF receptors have been identified (alpha and β)[44]. Under physiological conditions PDGF is released when platelets are damaged. Furthermore, PDGF signalling is known to play an important role in the embryonic development of kidney, blood vessels, lung and several components of the central nervous system[45,46].

In several aspects the importance of the PDGFs as well as its corresponding receptors have to be regarded as being closely connected with the VEGF system. Whereas activation of VEGF signalling leads to recruitment of new blood vessels, one important downstream effect of PDGF signaling is the maintenance of microvessels. The regulation of the tumor environment - especially activities of fibrocytes and pericytes - as well is partly realized by the PDGF signalling pathway[46].

Up-regulation of PDGF signaling has been demonstrated for prostate cancer, breast cancer, lung cancer as well as colorectal cancer. In gastric cancer it has been shown that PDGF is frequently overexpressed in tumor cells whereas its corresponding receptor is overexpressed in several cell types of the microenvironment. It has been postulated that the tumor cell derived PDGF signal selectively leads to the up-regulation of PDGFR expression in environmental non-tumour cells[46].

To date, there are no PDGF specific antibodies available for clinical use regarding gastric cancer.

Fibroblast growth factor: The fibroblast growth factor family constists of 23 molecule subtypes, targeting four different FGF receptor subtypes. In addition, several co-factors like Klotho-type co-receptors and heparan sulfat proteoglycans are involved in the initiation of the FGF signaling pathway[47]. Binding of the growth factor to its receptors leads to autophosphorylation of the receptor molecule which subsequently activates different signal cascades. Activation of the MAP kinase or WNT signaling pathway terminally regulates the transcription programming, whereas PI3K-AKT, Hedgehog, Notch and noncanonical WNT signaling pathway promote the epithelial-mesenchymal transition. Overall, the FGF signaling is involved in numerous biological processes, such as stemness, anti-apoptosis, proliferation, drug resistance, angiogenesis and invasion[47].

As for many other tumor entities, overexpression of FGF components has been described for gastric cancer, too. The FGFR-2 for instance is known to be up-regulated in 2%-9% of all gastric cancer cases, but is overexpressed in 50% in poorly differentiated and diffuse type gastric cancer[48].

Currently, there are several experimental studies in progress which evaluate the impact of monoclonal antibodies against FGF-19, FGFR-2 and FGFR-3 at the level of animal models.

Hepatocellular growth factor: Under physiological conditions, Hepatocellular growth factor (HGF) and its corresponding receptor MET play a central role in the embryonic development, wound healing and organ regeneration. Therefore, HGF is normally secreted by surrounding mesenchymal cells[49,50]. The physiological HGF signal can be altered by numerous molecular disorders, such as gene amplification, mutation and abnormal gene splicing[51]. Aberrant HGF signaling can be observed in a broad variety of different tumors, among them lung cancer, colorectal cancer, hepatocellular cancer and - as well - gastric cancer. The receptor is activated by receptor dimerization which is induced by binding of HGF. Activation of MAPK and PI3K-AKT signalings are typical subsequent downstream features which lead to cell proliferation, prolonged cell survival and cell mobilisation[52]. Whereas overexpression of MET seems to be a common feature in gastric cancer (22%-24%), gene amplification is infrequent (2%-10%). Aberrant

HGF signaling is related to poorer overall survival[53].

Currently, three different monoclonal antibodies targeting the HGF system are available: onartuzumab, rilotumumab and ficlatuzumab[52].

Onartuzumab has been demonstrated to be beneficial on the level of case reports but did not influence the clinical course in unselected patient populations.

Gastric cancer patients treated with rilotumumab in combination with chemotherapy following the ECX protocol showed a better overall survival as compared with those who received ECX only (5.7% and 4.2%)[54]. Global phase 3 studies dedicated to the impact of onartuzumab and rilotumumab on advanced gastric cancer are currently underway[52].

The benefit of ficlatuzumab combined with chemotherapy has been investigated for non-small cell lung cancer but did not have a statistically significant effect on overall survival[52].

Targeting the growth factor pathways by small molecules

During the last decades two main molecular approaches have been asserted to target growth factor receptors which in fact are complex proteins: Monoclonal antibodies which bind to selected regions on the molecule surface and receptor tyrosine kinase inhibitors (RTKI) which are small molecules. These molecules mimick a metabolite that binds to the active center of the kinase. Two main categories of RTKI can be (more or less) distinguished: RTKIs which bind selectively to one or more related receptor types, and so-called multi-tyrosine kinase receptor inhibitors which have a more pluripotent spectrum of potential receptor targets.

Essentially, RTKI are available for every growth factor receptor. However, clinical outcomes in particular regarding advanced or metastasized gastric cancer show at best moderate improvements in terms of tumor control and survival.

For EGFR gefitinib, erlotinib, lapatinib and dacomitinib have been developed. Gefitinib showed moderate improvement of overall survival in several phase 2 studies. Administration of erlotinib in combination with cytostatic substances led to significant improvement of tumor control in two phase 2 studies. Lapatinib did not show any improvement when administered to patients with advanced, unresectable or metastasized gastric cancer. The benefit of dacomitinib is not clearly evaluated to date[10].

For VEGFR apatinib is a selective inhibitor. Several studies showed a significant improvement for overall and progression free survival in patients with heavily pre-treated unresectable gastric cancer (OS 6.5 mo *vs* 4.7 mo, $P = 0.01$)[12].

Imatinib is a RTKI which targets PDGFR. It is well established in the treatment of gastrointestinal stroma tumors for over 10 years now. A phase 1 study in 2012 showed that imatinib was well tolerated in patients with advanced gastric cancer but did not show significant clinical improvement regarding survival and tumor control. Dasatinib, a novel PDGFR specific molecule is effective in the treatment of chronic lymphatic leukaemia, the benefit of dasatinib in the treatment of solid tumours is currently investigated[46].

For the FGFR family a broad variety of small molecules is presented in the literature: dovitinib, brivanib, intendanib and ponatinib to name only a few. However, none of them is established in the treatment of gastric cancer at present[47].

HGF specific small molecules can be subdivided in three categories: Type 1, 2 and 3.

Type 1 inhibitors are most specific to HGFR, for instance crizotinib. Type 2 inhibitors target a wider spectrum of receptors (AXL, RON, VEGFR2): foretinib, cabozantinib. Type 3 inhibitors bind as well to multiple receptor subtypes and different sites of the respective receptor: tivantinib. For gastric cancer only foretinib reached the level of a phase 2 study but unfortunately without significant benefit on an unselected patient group regarding HGFR expression[52].

Proteinase-activated receptors

Proteinase-activated receptors (PAR) is a subgroup in the family of G-protein-coupled receptors. Receptor activation is realized by specific serine-proteases, such as trypsine and thrombin, which subsequently leads to further activation of the PI3K signaling pathway. Interestingly, one downstream effect of upregulated PAR2 signaling is the trans-activation of EGF receptors with the known subsequent effects. There is some evidence that prostaglandin-2 may inhibit the PAR2 signaling pathway which could be a potential target for specific molecular treatment approaches, but to date there is no PAR-associated treatment introduced in to the clinical routine[55].

Morphogens and embryonic signaling pathways

Sonic hedgehog signaling: The Sonic hedgehog signaling (SHH) signaling pathway is one of the key players in the embryonic development, especially in defining body axes and segmental forming. The SHH signal is transduced within the cell *via* patched (PTCH), a transmembranous receptor which subsequently leads to the activation of smoothened and further to the deactivation of a protein complex which normally abolishes Gli, a nuclear factor that can initiate the expression of components of different other pathways, such as WNT, bone morphogenic protein (BMP) and Transforming growth factor β (TGF-β)[56].

Vismodegib, sonidegib and saredegib are small molecule drugs which inhibit smoothened and thus interrupt the intracellular transmitted SHH signal. Thereby, these molecules mimic the effect of cyclopamine, a naturally occurring SHH inhibitor. The effectiveness of vismodegib in targeted treatment has been described for different tumor entities: With a pilot study on metastatic pancreatic cancer patients it was shown that

vismodgib down-regulates the SHH activity but without statistical significance on survival so far[57]. Vismodegib has been proven as the very first SHH antagonist for the treatment of basal cell carcinoma in 2013[58].

Phase 1 studies to verify the clinical eligibility of sonidegib are currently underway. The evaluation of saridegib is at present in the stage of experimental studies.

Another interesting molecular approach towards SHH signaling might be the application of HMG reductase inhibitors, such as statins. The attachment of a cholesterin residue to the SHH molecule is known to be essential to initiate the SHH signaling pathway by SHH. Although to date there are no clinical trials available which introduced statins to clinical use for certain tumor entities, there is some evidence that statins influence the clinical and biological behavior of malignant tumors. Recently, it has been published that statins significantly decrease cancer-specific mortality, particularly in colorectal, prostate and breast cancer.

WNT signaling

WNT signaling is known to be evolutionary highly conserved. During the embryonic development it is mainly involved in cellular differentiation. But also in adults WNT signaling is indeed important, particularly in the stem cell niches of the gastrointestinal tract. Likewise the SHH signaling pathway, the WNT signal starts by binding of WNT ligands to its receptor frizzled which in turn co-acts with LRP and transduces the signal towards the cytosol. To date four different subpathways have been described. In the classical or also called the canonical WNT pathway a multiprotein complex consisting of Axin, GSK3B and APC is being destabilized. This multiprotein complex normally abolishes β-catenin by phosphorylation. The disintegration of the multiprotein complex in turn leads to an accumulation of active non-phosphorylized β-catenin, which subsequently moves to the nucleus and binds to components of transcription (TCF-LEF complex). Interestingly, WNT signaling is coupled to EGFR signaling by at least two mechanisms: First the activation of EGFR signaling leads to internalization of E-cadherin-β-catenin complexes which in turn promotes WNT-dependent gene expression and second E-cadherin inhibits EGFR signaling by preventing receptor dimerization[59,60].

The following targets have been defined to be eligible to suppress WNT activity: Porcupine (an enzyme that modifies the WNT ligands which is essential for their activity), the frizzled-LRP-dishevelled complex, axin, cyclooxygenase-2, GSK3β and the TCF-β-catenin complex. Different small molecules targeting porcupine are currently under experimental evaluation, most of them act as competitive ligands to porcupine. They are also called "inhibitors of WNT production"[61].

Aberrant WNT signaling is frequently observed in gastric cancer. B-catenin is overexpressed in up to 30% of gastric cancer cases, whereas the loss of APC function occurs in 20% of all gastric cancer cases. SFRP loss, a physiological down-regulation of WNT signaling, is as well frequently to be found in gastric cancer tissue[62,63].

At the moment there is no WNT associated treatment available for clinical routine, in particular not for gastric cancer.

Notch signaling

As another morphogenic signaling pathway Notch is known to be involved in embryonic organ development as well as in adult stem cell niche regulation. Notch promotes its cellular effects *via* regulation of proliferation, differentiation and apoptosis. The basic molecular mechanism is that one membrane-bound ligand (two subgroups: Jagged 1-2 and Delta like 1-4) binds to its receptor which is membrane-bound, too, but is belonging to a different cell. Thereafter the intracellular component of the receptor is cleaved. The Notch intracellular domain then moves to the nucleus and up-regulates expression of several genes, among them c-myc (oncogene), cyclin D1 (cell cycle promotion), p21 (cell cycle arrest) and bcl-2 (apoptosis)[64-66].

Notch activity has been described to be involved in several tumor entities and among them in gastric cancer. Particularly Notch 1, Jagged 1 and DLL 4 were found to be frequently dys-regulated in gastric cancer tissues. Furthermore, there were statistically significant differences in the incidence of their up-regulation when stratifying tumor tissues to the classification according to Lauren as well as tumor location and tumor size[66].

To date, there are no substances available which target at the Notch signaling pathway.

TGF-β and BMP

TGF-β and BMP constitute a super family of morphogens and regulate a broad variety of cellular activities. Up-regulation of the signal cascade may result in antidromic biological effects: At early tumor stages cell differentiation and apoptosis are promoted whereas proliferation is inhibited, leading finally to anti-tumor signals. On the other hand, the up-regulation of TGF-β and BMP in advanced tumor stages may result in the promotion of tumor angiogenesis, cell motility and aberrant interplay with the interstitium[67-69].

Several subtypes of the TGF-β/BMP family are frequently up-regulated in gastric cancer, for instance BMP7 can be verified in 55% of specimen, whereas BMP2 is up-regulated in almost all cases of gastric cancer and BMP4 up-regulation is a frequently occurring event in un-differentiated gastric cancer.

Dalantercept is an inhibitor of BMP9 and BMP10 which has been shown to suppress effectively tumor angiogenesis. It has been proven to be eligible in a phase 1 study and is now under evaluation as a palliative second line treatment for renal cell carcinoma. DMH-1, a novel small molecule which inhibits the intracellular component of BMP-1 has been shown to have anti-tumor effects in the animal model[70].

Nuclear factor κB and interleukin receptors

Nuclear factor κB (NF-κB) as well as interleukin signaling are known to be involved in cancer development and cancer progression. NF-κB can be regarded as a quick time transcription factor that regulates immune reaction as well as proliferation and apoptosis. Extracellular signals like bacterial or viral antigens, interleukin 1β and tumor necrosis factor initiate a signal which enters the nucleus within few minutes. This is realized by storing NF-κB in the cytosol which there is inactivated by forming a complex inhibitor of NF-κB (IκB). IKK, the IκB kinase inactivates IκB, which leads to a NF-κB release. Rapid movement of NF-κB to the nucleus in turn leads to up-regulated expression of different genes like cytokines, chemokines and adhesion molecules.

Upregulated NF-κB signaling in gastric cancer is associated with elevated proliferation, genomic instability and drug resistance.

Two different molecular approaches targeting NF-κB signaling are at the present time available: Phytochemicals: silibinin (Silybum marianum): Prostate cancer; resveratol (red grapes, red wine): Prostate cancer, mesothelioma; catechins (green tea): Prevention against numerous tumor entities.

The abovementioned agents are partly a domain of alternative medicine but not an integral part of the clinical routine. Systematic studies and randomized trials are needed to shed more light on the actual clinical impact of these treatment options.

Denosumab is an inhibitor of RANKL (receptor activator of NF-κB) and thus can down-regulate NF-κB signaling. It has been shown to be effective in giant cell tumor of bone in pre-clinical studies.

To our knowledge currently there is no molecular treatment available targeting the NF-κB signaling pathway in gastric cancer.

Furthermore, there is an abundance of inflammatory-associated molecular markers which are up-regulated in gastric cancer, including those which are associated with significantly poorer survival, such as different interleukins, HIF-1alpha, chemokine receptors as well as matrix metallo proeinases (MMP-3, -7, -9, -11).

Components and regulators of cell cycle

Cell cycle up-regulation is one of the most central mechanisms of tumor cell proliferation and tumor growth. It is strictly regulated by different controlling factors. The cell cycle can be sectioned into different cell cycle phases which only can be entered by passing the respective checkpoints. Under physiological conditions the entry of a cell into the cell cycle needs growth factors, whereas in tumor cells the cell cycle can be started at lower levels of growth factors or even at their complete absence[71,72]. Cyclin D1 and 2 as well as CDK 4 and 6 are the most important factors that promote the entry into the S phase of the cell cycle. Cyclin D1 and 2 are frequently up-regulated in gastric cancer. Furthermore, cyclin D is an important downstream target of different signaling pathways, such as SHH, WNT and Notch. In 15% of gastric cancer cases an up-regulated cyclin E can be observed[62,73,74]. The protein complexes formed by cyclin plus its corresponding CDK are inhibited by different factors, such as p21, which is down-regulated in 60% of gastric cancer cases[75].

Another major cell cycle associated key player is p53, the so-called "guardian of the genome", which is responsible for arresting the cell when DNA is severely damaged. Over 50% of all malignant tumors show a loss of p53, in gastric cancer these are at least 40%. Loss of p53 is known to be particularly frequent in advanced stages of gastric cancer and in those cases when tumor differentiation is low[76,77].

Cell cycle and its regulators are investigated intensively for several decades to find clinical eligible bonds which inhibit cell cycle activity and promote cycle arrest or apoptosis.

Flavipiridol (also known as alvocidib) as well as roscovitin (also known as seliciclib) can be regarded as CDK inhibitors of the first generation, both of them being relatively unspecific.

After promising results of phase 1 studies with inhibitory effects on multiple different CDK subtypes, the clinical outcomes in phase 2 studies were disappointing failing significant clinical activity. After all, there was a measurable clinical activity in some haematological neoplasms, such as chronic lymphatic leukaemia and mantle cell lymphoma.

Roscovitin, a purine based molecule failed to have clinical effects in as well phase 1 and phase 2 studies[78,79].

Dinaciclib as a CDK inhibitor of the second generation revealed remarkable activity on numerous tumor cell lines as well as in several tumor mouse models. In the subsequent phase 1 studies dinaciclib resulted in stable disease in different solid tumors, but again the positive results could not be confirmed with phase 2 studies with the exception of palliative treatment in refractory chronic lymphatic leukaemia, so that now a phase 3 study in this field is underway[78].

The impact of down-regulation of cyclin D1 by using adenoviral vectors is currently explored.

Currently the abovementioned drugs are not approved for clinical use in the treatment of gastric cancer.

SOME FUTURE PERSPECTIVES

Beside the further development of target-specific molecules against components of the abovementioned signaling pathways two categories of molecular tumor biology might be of interest: the clinical importance of micro RNAs and effectors of epigenetic regulation.

MicroRNAs are small molecules without coding function and with a usual length of 18 to 25 nucleotids. To date, more than 2000 different sequences have been detected in the human genome. It is postulated that microRNA molecules are involved in 30% of gene expression. Interestingly they are frequently to be found

at so-called fragile chromosomal sites and typically in intergenic regions. The signature of microRNAs changes from normal tissue to malignant tumor tissue. Micro RNAs can as well be down- and up-regulated.

For example miR-139 has been shown to be frequently down-regulated in gastric cancer. In contrast, overexpression leads to inhibited cell proliferation in gastric cancer cell lines. It seems to be involved in the regulation of the chemokine receptor CXCR4.

The individual signature of microRNAs might be used as a biomarker in predicting the biological behavior of tumors. Furthermore, antagonization of oncogenic microRNAs and the restoration of down-regulated microRNAs with tumorsuppressive activity might be promising targets in the future[80].

To a certain degree, the function of microRNA molecules is associated to epigenetic mechanisms, another challenging future perspective towards better understanding of the molecular biology of gastric cancer. Epigenetics means methylation of the DNA strand as well as different modifications of the histone molecules. DNA methylation is realized by DNMT 1 and 2 which place the methyl residues predominantly at so-called CpG rich regions. Hypermethylation of promoter regions upstream of tumor suppressor genes is a commonly observed phenomenon in different solid tumors. Histone molecules can be acetylated by HAT and deacetylated by HDACs at lysine sites, furthermore lysine as well as arginine sites can be methylated or demethylated. A broad variety of dys-regulated histone modification has been described for gastric cancer, for instance the hyperacetylation of histones neighboring the myc oncogene. Restoration of dyregulated histone and DNA modification might be another promising target to anticancer treatment[81].

Considering the variety of target specific therapeutics in relation to the clinical impact on the population of gastric cancer patients and the individual complexity of the "cancer organism" it becomes clear, that molecular targeted approaches generate their best effects on respective subgroups which harbour the suitable molecular signature. Therefore, the knowledge about the individual presence of molecular markers might become essential and of paramount interest in the future.

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TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Gastric cancer: The times they are a-changin'

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Abstract

Gastric cancer is the third leading cause of cancer death worldwide. Even though during these last decades gastric cancer incidence decreased in Western countries, it remains endemic and with a high incidence in Eastern countries. The survival in advanced and metastatic stage of gastric cancer is still very poor. Recently the Cancer Genoma Atlas Research Network identified four subtypes with different molecular profiles to classify gastric cancer in order to offer the optimal targeted therapies for pre-selected patients. Indeed, the key point is still the selection of patients for the right treatment, on basis of molecular tumor characterization. Since chemotherapy reached a plateau of efficacy for gastric cancer, the combination between cytotoxic therapy and biological agents gets a better prognosis and decreases chemotherapeutic toxicity. Currently, Trastuzumab in combination with platinum and fluorouracil is the only approved targeted therapy in the first line for c-erbB2 positive patients, whereas Ramucirumab is the only approved targeted agent for patients with metastatic gastric cancer. New perspectives for an effective treatment derived from the immunotherapeutic strategies. Here, we report an overview on gastric cancer treatments, with particular attention to recent advances in targeted therapies and in immunotherapeutic approach.

Key words: Targeted therapy; Chemotherapy; Gastric cancer; Immunotherapy

Core tip: Gastric cancer, despite its decrease in West Countries, remains one of the most common malignancies worldwide. The prognosis in the advanced setting is often poor even with a multidisciplinary approach, which aims to increase the patients' survival. The molecular classification of four subtypes of gastric adenocarcinomas (The Cancer Genome Atlas project) allowed a better stratification of patients in clinical trials for targeted

therapies. Biologic agents, modulating the immune checkpoints, seem to be the best promising therapeutic approach, opening new perspective for advanced gastric cancer treatment.

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INTRODUCTION

During these last decades gastric cancer incidence decreased, but it still remains he third most frequent cause of cancer-related mortality worldwide[1,2]. At diagnosis, about half of gastric cancer patients show an advanced disease, with a 5-year survival rate lower than 30%[3,4]. Even though gastric cancer incidence decreased in Western countries, it remains endemic and with a high incidence in Eastern countries. The incidence in Eastern Asia was 24.2/100000; in Latin America and Caribben was 15.8-23.7/100000; in Africa and Northern America there was the lowest incidence (http://globocan.iarc.fr, accessed on 16/01/2015). In the United States the estimated number of new cases of gastric cancer in 2014 overtook 22000 cases[2], with differences among several ethnic groups. In Europe gastric cancer holds the 5th place for male sex and the 6th place for female sex for incidence[5,6].

Gastric cancer can be hereditary and associated to specific mutations[7]. Often Gastric cancer are sporadic and depends on progressive accumulations of genotypic and phenotypic modifications due to different etiological factors such as wrong diets, presence of gastritis, infection by *H. pylori*, smoking, obesity, elevated body mass index (BMI) and reflux[8,9]. Indeed, combinations of smoking, elevated BMI, and reflux may account for almost 70% of total cases[10,11]. Untreated gastritis induces a chronic mucosal inflammation, that causes structural changes of gastric mucosa, leading to metaplastic transformation and structural changes of the glandular tissue, that can undergo to a neoplastic differentiation[9,12].

Many efforts have been done in order to prevent gastric cancer: recognition and treatment of *Helicobacter pylori* (*H. pylori)* infections; diet changes like lower use of salted foods, and the use of refrigerators are factors which contributed to reduce the incidence of gastric cancer[13]. Nonetheless, the incidence of the cancers of gastro-esophageal junction (GEJ) and gastric cardia increased in western country[14]. To explain these epidemiological data there are several interpretations, such as problems related to a correct subdivision among esophageal, junctional and cardia adenocarcinomas, that may have cloud the issue leading to a misclassification[14,15].

MOLECULAR CLASSIFICATION: "THERE'S A BATTLE OUTSIDE AND IT IS RAGING"

The most common classification systems, such as the Laurén and the World Health Organization classifications, are essential for therapeutic decision, but are unable to predict response to targeted therapies. Recent studies on molecular profiling of upper gastrointestinal (GI) tumors increased our knowledge on the biology of gastric cancer and developed a molecular classification, identifying dysregulated pathways in different subgroups of gastric cancer.

The Cancer Genoma Atlas (TCGA) analysis uncovered four main genotypes of gastric cancer based on the molecular characterization of 295 primary adenocarcinomas[16]: Epstein-Barr virus (EBV) positive; microsatellite unstable (MSI); genomically stable (GS); and tumors with chromosomal instability (CIN). The EBV-associated tumors are about 10% of the cancers; they display CDKN2A promoter hypermethylation and in 80% of the cases they have PIK3CA mutations and amplification of JAK2 and CD274 and PDCD1LG2. This subset of gastric cancer can benefit of targeted immunotherapy. MSI tumors represent approximately the 20% of the cases and show mutations in PIK3CA, HER2, HER3, and EGFR. GS gastric tumors represent about 20% of the adenocarcinomas, they show newly described mutations in RHOA, which are relevant to control actin-myosin-dependent cell contractility and motility. Almost 50% of gastric tumors showed CIN, with a marked aneuploidy and focal amplification of receptor tyrosine kinases, such as VEGFA. This subtype is frequently found in GEJ cancer. This study provides a guide to test new agents against new molecular targets specific for a gastric cancer subtype, enabling clinicians to make a better selection of patients for future trials with targeted therapy and immunotherapy in gastric cancer.

SURGICAL TREATMENT

Radical surgery is still the only one curative treatment, but gastric cancer is mostly diagnosed in local advanced or metastatic stage, when the survival still remains poor[17]. Surgical resection for gastric or GEJ cancer combined with D1/D2 lymph node dissection should be performed by experienced team to reduce mortality and morbidity[18]. Surgery with curative intent has to provide free-margin and at least D1 resection combined with removal at minimum of 15 lymph nodes[19]. The extent of lymph node dissection is a significant surgical procedure that specifies the lymph node involvement, because preoperative lymph node staging is considered highly unreliable. The results of many randomized studies have not agreed to demonstrate superiority of D2 resection *vs* the D1 resection; to conclude the standard recommended surgery could be at least D1 resection, while D2 resection could be indicated in some

particular young patients[20-22].

A combine approach of surgery and chemotherapy can improve outcomes of gastric cancer patients, with potentially resectable tumors. The Magic trial conducted in United Kingdom[23] and the ACCORD trial conducted in France[24] showed a statistically significant longer 5-year survival for patients treated with perioperative chemotherapy. Decisions were less clear for adjuvant setting: chemotherapy alone or with radiotherapy should be recommended for patients underwent to a less than optimal lymph node resection, R1 or with lymph node involvement[25].

CYTOTOXIC CHEMOTHERAPY: "YOUR OLD ROAD IS RAPIDLY AGING"

The only treatment for patients with metastatic disease is the systemic chemotherapy. Currently there is no first-line standard single chemotherapeutic regimen but cisplatin based regimens, which able to improve the overall survival (OS) because a cytotoxic combination is superior to a single-agent regimen[26]. The physician's choice of platinum-based doublets or triplets is taken after careful assessment of the patients' performance status. Currently, standard first-line options include FOLFOX [5-fluorouracil (5-FU, oxaliplatin)], S1/cisplatin or 5-FU/cisplatin, DCF (docetaxel, cisplatin, and 5-FU), ECF/EOX (epirubicin, cisplatin/oxaliplatin, and 5-FU/capecitabine). In the platinum-based doublets oxaliplatin could substitute cisplatin, while capecitabine and S1 are equivalent in terms of effectiveness to 5-FU[27,28].

A third drug, usually epirubicin or taxotere, can be added with the aim to obtain a high response rate (RR) and a better control of the disease[29,30].

Although most patients receive a first-line chemotherapy, in clinical practice only less than half of patients progressing after treatment receive a salvage treatment, mostly in western countries. Only recently a second-line chemotherapy has shown to be superior to the best supportive care in advanced disease: Two distinct trials proved that irinotecan and docetaxel, in monochemotherapy, control the metastatic disease[31,32].

It's evident that chemotherapy reached a plateau of efficacy for gastric cancer, thus in an attempt to improve it, getting a better prognosis and decreasing chemotherapeutic toxicity, the combination between cytotoxic therapy and biological agents is useful. Indeed, results of ToGA trial allow to approve the first biologic drug for stomach cancer. Today, trastuzumab is indicated for first-line in patients HER2-positive in combination with 5-FU or capecitabine and cisplatinum[33].

Even more recently, two randomized trials demonstrated that Ramucirumab, a monoclonal antibody directed against VEGFR-2, is effective both alone or in combination with a second line chemotherapy with paclitaxel, in patients with metastatic gastric cancer[34,35].

BIOMARKERS FOR GASTRIC CANCER

Since chemotherapy is not effective in all patients, who are resistant to cytotoxic treatment, it's mandatory to develop new anticancer regimens and to identify biomarkers able to predict the patients' responses to different citotoxic drugs in gastric cancer. One of the molecules currently under investigation is the alpha-1 Microglobulin/Bikunin Precursor (AMBP), because its high level in serum could predict poor response to paclitaxel- capecitabine regimen[36]. Thus AMBP could be a potential biomarker to identify patients who would benefit from this specific chemotherapeutic regimen.

Forkhead box transcription factor 1 (FoxM1) could be an other potential biomarker and target for gastric cancer. Indeed, FoxM1 overexpression is correlated with the pathogenesis of a variety of human malignancies such as breast cancer, non-small-cell lung cancer and ovarian cancer, and it is a critical molecule for chemoresistance to a microtubule-stabilizing anticancer agent as docetaxel[37-42]. FoxM1 overexpression was significantly associated with resistance in chemotherapy of docetaxel in addition to 5-FU, S-1 and cisplatin (CDDP) for patients with advanced gastric cancer[43,44]. Taken together, these results suggest that FoxM1 is involved in the mechanisms of resistance to cytotoxic drugs and its inhibition might be a promising therapeutic strategy for is a pleiotropic protein affecting a wide range of molecular and cellular processes.

Accumulating data, derived by different studies on the role of ANXA2 in tumorigenesis, suggest that ANXA2 is aberrantly expressed in a wide spectrum of tumors, affecting tumor cell adhesion, proliferation, apoptosis, invasion, metastasis and the interaction between immune cells and cancer cells in the microenvironment[45,46]. The expression of ANXA2 in gastric cancer tissue is associated to a poor prognosis[47,48]. A recent study reported that ANXA2 might be a good diagnostic and predictive marker for response to chemotherapy, indeed the chemotherapy-unresponsive patients show higher serum ANXA2 levels than the chemotherapy-responsive ones[49].

Several studies have consistently demonstrated that miRNAs, short noncoding RNA molecules involved in post-translational regulation of gene expression, contribute significantly to human carcinogenesis by modulating the expression of both proto-oncogenes and tumor suppressor genes[50]. Studies on gastric cancer allowed to identify up- and down-regulated miRNAs, which can be associated to clinical-pathological features of gastric cancer[51,52]. Moreover, many data report that the expression of different miRNA patterns is also associated with premalignant stages or even risk conditions to develop gastric cancer, such as *H. pylori* infection[53,54].

TARGETED THERAPY: "FOR THE LOSER NOW, WILL BE LATER TO WIN"

Advances in knowledge of the cancer biology led to the

discover of specific oncogenic signalling pathways of different driver mutations, resulting in the development of many new target agents. The prevalence of genomic alterations in gastric cancer patients has been recently assessed. Indeed, five distinct gastric cancer patient subgroups have been identified, according to the genomic alterations: FGFR2 (9% of tumours), KRAS (9%), epidermal growth factor receptor (EGFR) (8%), ERBB2 (7%) and MET (4%). Therefore, about 37% gastric cancer patients could be treated with anti-RTK/RAS agents[55]. Many new target therapies were tested in clinical trials in gastric cancer patients, but without great results, thus we need further molecular studies to identify right patients for the right drugs.

EGFR1 inhibitors

EGFR is a trans-membrane glycoprotein receptor expressed in about 60% of gastric cancer patients. A meta-analysis on 1600 gastric cancer patients evaluated the survival according to the EGFR expression, showing that positive EGFR expression does not significantly predict the poor survival of gastric cancer[56].

Cetuximab is an immunoglobulin G1 type chimeric monoclonal antibody targeting EGFR. Thanks to the successes achieved by the cetuximab in colorectal cancer, it was also tested in gastric cancer in combination with chemotherapy in phase Ⅱ studies: FOLFIRI[57]; cisplatin plus docetaxel[58]; oxaliplatin plus 5-FU[59,60] with encouraging results regarding ORR in all trials. However, the expected results from the combination of chemotherapy and cetuximab were not confirmed by the phase Ⅲ EXPAND study (cetuximab in combination with capecitabin and cisplatin), that failed both in terms of OS and of progression-free survival (PFS)[61]. The analysis of potential biomarkers such as KRAS mutations, EGFR expression, HER2 expression, did not identify the patients group responsive to cetuximab.

The REAL3 randomised study tested the efficacy of panitumumab in combination with EOX (epirubicin, oxaliplatin, capecitabine). In October 2011, trial recruitment was halted and panitumumab withdrawn because did not show any benefit at interim analysis. In multivariate OS analysis with performance status and disease stage, both *KRAS* mutation and *PIK3CA* mutation were negatively prognostic. No prognostic effect was associated with *HER2* or *PTEN* status, and no *BRAF* mutations were identified[62].

The phase Ⅲ COG trial evaluated Gefitinib *vs* placebo in patients with metastatic esophageal or types Ⅰ/Ⅱ junctional adeno or squamous cell carcinoma, progressing after prior chemotherapy. This study did not improve OS; however, there was significant improvement in PFS, quality of life and palliation of symptoms[63].

Some trials of several novel EGFR agents are still ongoing. The phase Ⅲ ENRICH trial of nimotuzumab in combination with irinotecan in the second-line setting is pre-selecting patients with high EGFR expression (NCT01813253). Finally, before defining EGFR inhibitors as ineffective in gastric cancer, we absolutely identify predictive biomarker for response, in order to avoid repeating the mistakes done with gefitinib in lung cancer[64,65].

HER2 inhibitors

All members of the HER family of receptor tyrosine kinases, whose members include HER1 (or EGFR), HER2, HER3, and HER4, are expressed in gastric cancer. HER2 is a protoncogene encoded by ERBB2 found on chromosome 17. The percentage of gastric cancer patients positive to HER2 ranges from 7% to 42% due to tumor heterogeneity and the different methods and scoring systems used for evaluating HER2[66]. HER2-positivity also depends on histologic type: It is frequent in patients with intestinal histology (34%), rare in those with diffuse-type histology (6%); it also depends on disease site: It's frequent in GEJ (32%) and rare in gastric cancer (18%)[67]. It remains unclear whether HER2 positivity is a negative prognostic factor because there are studies both for and against this hypothesis[68,69]. The ToGA trial is a randomized Phase Ⅲ study which brought to the approval of Herceptin as the only targeted agent for patients with HER2 positive metastatic gastric and GEJ cancer. Three thousand six hundred patients were assessed for HER2 positivity, and the 594 patients HER2-positive were recruited in the clinical trial[33], which evaluated efficacy of anti-HER2 trastuzumab in combination with 5-FU or capecitabine and cisplatin *vs* chemotherapy alone in HER2 patient. Median OS in control arm was 11.1 mo compared with 13.8 mo in experimental arm with a statistically significant increase in RR. Every 3 wk for six cycles, the treatment was administered, whereas trastuzumab was continued every 3 wk until disease progression, or unacceptable toxicity, or withdrawal of consent. One of the most interesting result of this study was that the survival advantage was greatest in patients with IHC 3+ tumors (HR = 0.66, 95%CI: 0.50-0.87), less effective in patients with IHC 2+ tumors (HR = 0.78, 95%CI: 0.55-1.10), and ineffective in those with HER2 gene-amplified, but not protein expressing (IHC 0 or 1+) tumors. Grade 3 or 4 adverse events (AEs) occurred in similar percentages in both arms. Now all patients with advanced or metastatic gastric or GEJ cancer, and suitable for combination chemotherapy with fluoropyrimidine and cisplatin, should be assessed for the expression of HER2 and therefore can be treated with additional trastuzumab.

The phase Ⅲ HELOISE trial, combining trastuzumab with cisplatin and capecitabine (NCT01450696), and the TEX regimen, combining trastuzumab with Taxotere, Eloxatin and Xeloda as treatment for HER2 positive non-resectable cancer (NCT01295086) are ongoing to improve the efficacy of combination chemotherapy. Heloise trial aims to assess whether trastuzumab maintenance is able to increase the gastric cancer patients' survival. The second trial evaluates the safety

and efficacy of three drugs combination in addition to trastuzumab.

Development of resistance to trastuzumab urged investigators to test new drugs target HER2, but not all HER2-targeting agents have had such an unequivocal success.

The dual HER2/EGFR inhibitor lapatinib (Tykerb) is an orally drug. Lapatinib is a very interesting TK1 inhibitor, able to interfere with cell proliferation, to sensitize gastric cancer cells to the irinotecan metabolite SN-38[70] and to have a synergic effect combined with chemotherapy[71].

Lapatinib was evaluated in the first setting in combination with capecitabine/oxaliplatin (LOGiC trial). 545 patients were randomized and 487 had HER2+ centrally confirmed, but combination treatment failed to improve the median OS (12.2 mo *vs* 10.5 mo, HR = 0.91, 95%CI: 0.73-1.12) compared with chemotherapy alone. No correlation was found between intensity of staining for HER2 by IHC and outcomes**.** However, the LOGiC trial did suggest that Asian patients and those under age 60 years might benefit of this combination[72].

The TyTAN trial is a phase Ⅲ study second-line therapy of paclitaxel. Investigators enrolled 261 HER2-amplified Asian patients and they observed statistically significant improvements in OS and PFS among a pre-specified subgroup of patients with strong HER2 positivity. However, addition of lapatinib did not produce any significant benefit on PFS (5.4 mo *vs* 4.4 mo) or OS (11.0 mo *vs* 8.9 mo) with significant gastrointestinal (diarrhoea 20%) and bone marrow toxicity (febrile neutropenia, 7%)[73]. Several other HER2-targeting agents were also evaluated in clinical trials, including trastuzumab emtansine (T-DM1; Kadcyla) and pertuzumab (Perjeta).

T-DM1 is a conjugate molecule that combine a cytotoxic agent with an antibody targeted specific tumor cells. Due to positive results in breast cancer (EMILIA trial)[74], is now ongoing a randomized, multicenter, adaptive phase Ⅱ/Ⅲ study to study the efficacy and safety of trastuzumab emtansine (T-DM1) *vs* taxane (docetaxel or paclitaxel), in patients with previously treated locally advanced or metastatic HER2-positive gastric cancer, including adenocarcinoma of the GEJ (GATSBY trial, NCT01641939). Another phase Ⅰ/Ⅱ study was designed to assess T-DM1 in combination with capecitabine in patients with metastatic gastric cancer (NCT01702558). The ongoing phase Ⅲ JACOB trial is evaluating the combination of pertuzumab, trastuzumab, and chemotherapy (NCT01774786). The combination of two antibodies aims to amplify the trastuzumab antitumor efficacy in HER2-positive patients. Again with the aim of overcoming resistance to trastuzumab, it is also ongoing a phase Ⅱ trial with afatinib, an irreversible panHER TK1 (NCT01522768). A better and more accurate knowledge of the mechanisms of cellular resistance to trastuzumab is essential for the future. Certainly, the intra-tumor heterogeneity in HER2 expression/amplification is very important, but other mechanisms have been implicated as PI3K/Akt pathway, m-TOR inhibitors, MET-inhibitors (when c-MET is overexpressed), overexpression of IGF-1 receptor (IGF-1R), SRC inhibitors. From these pre-clinical studies will emerge the right molecules to be tested in the next clinical trials.

Another HER2-directed strategy is represented by vaccines. Despite the great success of HER2 vaccine strategies in animal models, effective clinical results have not yet been obtained[75].

HER2 vaccines, DNA or peptide-based, are studied mainly for breast cancer, often in combination with other HER2 targeted therapies[76]. Regional treatments are another possible application. Radio-immunotherapy is now evaluating 212Pb immunoconjugates with trastuzumab in intraperitoneal treatment[77].

Angiogenesis inhibitors

Angiogenesis is crucial for tumor growth, thus anti-angiogenic drugs are now a standard of care for many solid tumors of the adult. In gastric cancer VEGF is overexpressed in 40% and VEGFR in 36% of cases. Some studied reported that VEGF overexpression correlates with advanced and aggressive disease[78-80]. We recently showed that even though VEGF serum levels were higher in gastric patients than in controls, they were not correlated to the OS[81].

Bevacizumab is a recombinant humanized monoclonal antibody anti-VEGF-A, a strong driver of angiogenesis in tumorigenesis. Phase Ⅱ studies conducted with bevacizumab in chemotherapy combination, showed encouraging RR, time to disease progression (TTP), and OS[82,83], but not confirmed by phase Ⅲ trials. The phase Ⅲ trial AVAGAST evaluated effects of bevacizumab in combination with cisplatin and capecitabine as a first-line therapy in 774 patients with advanced gastric carcinoma[84]. Addition of bevacizumab failed to improve OS, with median OS 12.1 mo *vs* 10.1 mo, even though it achieved a significant increase in PFS (6.7 mo *vs* 5.3 mo) and overall RR (46.0% *vs* 37.4%). To evaluate the hypothesis that angiogenic markers may be predictive for bevacizumab efficacy, correlations between pre-specified biomarkers (VEGF-A, protein expression of neuropilin-1, and VEGFR-1 and VEGFR-2) and clinical outcomes were assessed too. High plasma VEGF-A levels and low expression of neuropilin-1 showed a trend toward improved OS. These are strong biomarker candidates that aim to predict the response to bevacizumab in gastric cancer patients from non-Asian regions[85]. Moreover, the sub-group analysis by geographical regions, tumor site and histology concluded that the highest survival benefits are for non-Asian patients with distal gastric non-diffuse type cancer (OS 11.4 mo *vs* 7.3 mo).

MAGIC-B trial with bevacizumab in combination with chemotherapy (ECX regimen) in perioperative setting is ongoing[86]. The study results could provide relevant information on antiangiogenic efficacy in the early stages of disease.

In this complex and rather disappointing background,

results of ramucirumab in the treatment of advanced gastric cancer have been published. Ramucirumab (IMC-1121B) is a fully human IgG1 monoclonal antibody direct aganist VEGFR-2. The phase Ⅲ REGARD trial was conducted to assess efficacy and safety of ramucirumab as second-line treatment *vs* supportive care in advanced gastric cancer. Three hundred and fifty-five patients were enrolled. Ramucirumab significantly improved OS (OS 5.2 mo *vs* 3.8 mo) and PFS (2.1 mo *vs* 1.3 mo), with good tolerability. Most frequent grade 3-4 AEs were hypertension (7.3% in experimental arm *vs* 2.6% in placebo arm), anemia (6.4% *vs* 7.8%), abdominal pain (51.% *vs* 2.6%), ascites effusion (4.2% *vs* 4.3%), asthenia (42.% *vs* 3.5%), hyponatremia (3.4% *vs* 0.9%) and anorexia (3.4% *vs* 3.5%). No grade 4 hypertension has been observed[34].

The phase Ⅲ RAINBOW was conducted in 665 patients with the aim to evaluate efficacy and safety of ramucirumab plus paclitaxel combination in second-line treatment in advanced gastric cancer patients. The study reached its primary objective of increasing OS, indeed the combination resulted superior in median OS (9.7 mo *vs* 7.3 mo), median PFS (4.4 mo *vs* 2.8 mo) and RR (28% *vs* 16%). Hypertension, fatigue and neutropenia were the most frequent toxicities in experimental arm, whereas febrile neutropenia had comparable incidence.

Gaining the results of ramucirumab in second-line, we would have expected a good success also in first-line. However, the study combination of FOLFOX6 plus ramucirumab has not demonstrated to increase OS and PFS in patients with metastatic gastric cancer (23%), GEJ (31%) and esophageal (46%). 168 patients were enrolled, median PFS 6.4 mo *vs* 6.7 mo, OS 11.7 mo *vs* 11.5 mo. Addition of RAM to FOLFOX6 showed PFS difference at 3 mo and improved disease control rate (DCR); longer PFS in RAM *vs* placebo was observed in gastric/GEJ cancer patients[87].

Apatinib is a tyrosine kinase inhibitor (TKI) agent targeting VEGFR-2 (VEGFR). A phase Ⅱ randomised trial tested apatinib *vs* placebo in 144 pre-treated gastric cancer patients. Apatinib was taken orally in two different ways: 850 mg once and 450 mg twice a day. Median OS times were 2.50 mo (in the placebo arm), 4.83 mo (apatinib 850 mg once a day arm) and 4.27 mo (apatinib 450 mg twice a day arm). Median PFS times were 1.40 mo, 3.67 mo, and 3.20 mo, respectively. The differences between apatinib and placebo groups were statistically significant for both PFS ($P < 0.001$) and OS ($P < 0.001$ and 0.0017).Toxicities were tolerable and manageable[88]. The multicenter, randomized, double-blind, placebo-controlled phase 3 trial tested Apatinib 850 mg, po, qd, 28 d as one cycle or matching placebo. The study was planned to enroll 270 cases, stratified to the number of metastatic sites ($\leqslant 2$ or > 2). Median overall survival (mOS) was significantly prolonger in the apatinib group compare with in the placebo group. The results confirmed the efficacy and safety of apatinib in the patients with advanced gastric cancer[89].

Sunitinib and sorafenib are multi-target TKIs also studied in order to suppress angiogenesis in gastric cancer. Phase Ⅱ open-label randomized trial evaluated the combination of sunitinib plus docetaxel *vs* docetaxel monotherapy in second-line treatment in 107 patients with metastatic gastric cancer. Sunitinib arm was associated with a significantly higher ORR (41.1% *vs* 14.3%), but there was no significant difference in TTP (3.9 mo *vs* 2.6 mo) [90].

Sorafenib targets BRAF, VEGF, and PDGFR[91]. Combination of sorafenib plus chemotherapy (docetaxel and cisplatin) was assessed in a phase Ⅱ trial, first-line setting, in 44 patients with metastatic gastric cancer. The combination demonstrated a PFS of 5.8 mo, median OS of 13.6 mo, and ORR 41%; grade 3-4 EAs toxicity was neutropenia[92].

Pazopanib is an oral second-generation multitargeted TKI, which showed antiangiogenic and antitumor activity. There are two phase Ⅱ trials now ongoing in order to evaluate efficacy and safety of pazopanib as first-line treatment in metastatic gastric cancer. The first one, a phase Ⅱ PaFLO trial, wants to examine FLO (5-FU, leukovorin and oxaliplatin) + pazopanib used in combination for advanced gastric cancer (ClinicalTrials.gov Identifier: NCT01503372). The second one, a phase Ⅱ non-randomized open label trial, evaluates Pazopanib in combination with Capecitabine and Oxaliplatin in patients with advanced gastric cancer. The primary end-point is RR, the second end-points are PFS, OS and metabolic response rate by PET-CT (ClinicalTrials.gov Identifier: NCT01130805).

Hepatocyte growth factor-mesenchymal-epithelial transition factor axis

Mesenchymal-epithelial transition factor (c-MET) is the TK receptor of hepatocyte growth factor (HGF)[93]. c-MET expression or amplification was documented in many solid tumors and was correlated with poor prognosis in gastric cancer too. IHC analysis in gastric cancer specimens showed c-MET expression in 65% of cases with high-intensity staining in about 20% of cases[94]. However, the real activation of c-MET mutations and its resulting amplification, is a rare event: c-MET amplification occurs in 5%-10% of cases[95]. This discrepancy between expression and amplification of c-MET has important consequences when we design clinical trials with HGF-c-MET pathway inhibitors.

Rilotumumab (AMG 102) is human monoclonal antibody (IgG2) against HGF. A phase Ⅱ double-blind randomized study, evaluated the efficacy and safety of rilotumumab with ECX regimen in gastric cancer patients in first-line treatment. Rilotumumab associated to chemotherapy improved the median PFS from 4.2 to 5.6 mo, and the OS from 8.9 to 11.1 mo. In the rilotumumab plus ECX arms, the most common adverse observed eventswere: neutropenia, anemia, peripheral edema, thrombocytopenia, and deep vein thrombosis[96]. MET protein levels and gene copy

numbers were measured in archival tumor samples by immunohistochemistry (IHC) and fluorescence *in situ* hybridization, respectively. Rilotumumab in combination with ECX improved the median OS from 5.7 to 11.1 mo in patients with gastric tumors with high MET expression.

The RILOMET-01 phase Ⅲ trial evaluated the efficacy and safety of Rilotumumab + ECX in MET-pos by IHC, previously untreated G/GEJ cancer. Primary endpoint was OS. 609 patients were randomized, but the study was stopped early bacause an imbalance in deaths (data cutoff: Nov 2014). OS, PFS and ORR were statistically worse in the exsperimental arm. The subgroup with higher percentages of cells with ⩾ 1+ MET expression does not seem to benefit with ramucirumab. PK and MET biomarker analyses are pending, thus we don't know whether they will offer any answers to this failure[97].

Onartuzumab is a humanized, monovalent (one-armed) monoclonal antibody against MET. One phase Ⅲ trial (randomized multicenter double-blind placebo-controlled studies), currently ongoing (but it's not recruiting participants) is evaluating the efficacy and safety of onartuzumab (MetMAb) in combination with mFOLFOX6 in patients with metastatic HER2-negative and Met-positive adenocarcinoma of the stomach or GEJ (NCT01662869).

Crizotinib is a small MET kinase inhibitor. Phase Ⅰ study showed promising activity in c-MET amplified gastric cancer patients[98].

Tivatinib is a selective non-ATP competitive small-molecule inhibitor of c-MET. Phase Ⅱ single-arm study evaluated the efficacy of tivantinib monotherapy in Asian patients with previous treatment for MGC (ARQ-197). Tivantinib was administered orally daily. The primary end-point was the DCR. Thirty patients were enrolled and no objective responses were observed, and DCR was 36.7%. There was not relationship between efficacy and gene amplification of c-MET, expression of c-MET, p-MET and HGF[99]. New clinical trials with c-MET inhibitors were restricted to patients defined as a "MET positive" to identify selected patients for a special genetic/molecular profile. However, the HGF/c-MET axis is involved in multiple pathways that operate at different levels[100]. The anti-HGF compounds may not be sufficient to complitely inhibit HGF/c-MET axis[101]. Hereafter it will be necessary to define with much more precision what "MET positive" gastric cancer means.

m-TOR inhibitors - PI3K pathway inhibition

m-TOR regulates angiogenesis, cellular metabolism, proliferation, and cell growth. Its activation is done through the PI3K pathway (*via* Akt/protein kinase B and tuberous sclerosis complex). In gastric cancer, mTOR and p-mTOR (its activated form) overexpression were respectively 50.8% and 46.5%. Overexpression of total mTOR protein significantly correlated with tumor differentiation, T1/T2 tumors, and stage Ⅰ/Ⅱ/Ⅲ disease. p-mTOR overexpression significantly correlated with lymph node metastasis and all stage disease[102].

Everolimus is an oral m-TOR inhibitor, approved for the treatment of renal cell carcinoma, breast cancer, and progressive NET of pancreatic origin. A phase Ⅱ study, in 53 patients with previously treated metastatic gastric cancer, reported a median PFS of 2.7 mo and OS of 10.1 mo. Common grade 3/4 AEs included anemia, hyponatremia, increased gamma-glutamyltransferase, and lymphopenia. Grade 1/2 pneumonitis was reported in 15.1% of patients[103]. Another phase Ⅱ trial assessed the efficacy and safety of combination regimen of capecitabine plus everolimus in patients with refractory gastric cancer who have failed at least two cytotoxic regimens. Forty seven patients were enrolled in this trial. Everolimus in combination with capecitabine achieved an ORR of 10.6% and a DCR of 48.9%, with respectively a median PFS and OS of 2.3 mo and 5.1 mo[104]. The phase Ⅲ GRANITE-1 evaluated everolimus or BSC plus placebo in 656 previously treated advanced gastric cancer patients. The results of this trial showed median OS of 5.39 mo in the everolimus arm and an OS of 4.3 mo in the placebo arm, with an advantage in PFS statistically significant but clinically irrelevant (1.7 mo *vs* 1.4 mo)[105]. Phase Ⅲ study in advanced gastroesophageal adenocarcinoma patients comparing everolimus combined with paclitaxel *vs* paclitaxel alone (NCT01248403) is ongoing.

IGF family

The IGF family plays an important role in growth and metabolism. Deregulation of IGFs/IGF-1R system promotes metastases diffusion, proliferation and invasion in gastric cancer. A number of antibodies targeting IGF-1R have been studied. Ganitumab (AMG 479) and figitumumab (CP 751) have been evaluated in phase Ⅰ study in patients with solid tumors, including gastric cancer. They showed promising results[106].

PARP inhibitors

PARP inhibitors (Poly-ADP-Ribose-Polymerase) have been studied in breast cancer with a know history of deficient BRCA1/2. The activity of PARPS inhibitors is improved in presence of drugs that cause double-strand breaks in DNA such as platinum compounds.

Olaparib activity has been proven in a phase Ⅱ trial with paclitaxel (Bang YJ Im SA J ClinOncol 2013 31(sup). The study failed to increase the PFS, but it improved OS. A randomized phase Ⅲ with paclitaxel in gastric cancer patient second-line is ongoing (NCT019245337).

IMMUNOTHERAPY: "…AND KEEP YOUR EYES WIDE"

Until few years ago, the more validated hypothesis was that epithelial tumors originate from tissue stem cells. A large intra-tumoral heterogeneity exists and cancer stem cells are part of it, indeed they are in the primary tumors, but they also disseminate to different

organs, remaining dormant or originating metastases and often are responsible to chemo-resistance[107,108]. To date, it's evident that tumor growth depends on the interactions among cancer cells, microenvironment and immune system cells. Tumor and cancer stem cells express receptors for antigens on specific cell type, thus determining the capability of one tumor to metastasize to a specific organ, such as for breast, lung and prostate cancer which commonly metastasize to bone[109-112]. The importance of tumor microenvironment in promoting cancer progression is even more recognized, because its cellular components release a series of factors which constitute a favourable soil for cancer cell homing and growth[113,114]. Looking at the immune system, a variable number of immune cells infiltrate tumors: mast cells, lymphocytes, macrophages and myeloid derived suppressor cells (MDSCs), with a deep impact on tumor progression[115]. For instance, MDSCs are a heterogeneous population of immature myeloid cells driving the progression of cancer disease by suppressing both the innate and adaptive immune response. Indeed they suppress CD4 and CD8 T cell populations, and promote the activation and expansion of regulatory T cells, which mediate immunosuppression[116-118].

A strong rationale exists to adopt the immunotherapy for gastric cancer, because inflammation has been recognised as an hallmark of cancer[119] and gastric cancer, particularly the upper GI tumors are an inflammatory-mediated disease[120]. Here we will describe the last frontiers of immunotherapy in gastric cancer treatment, but a comprehensive overview of immunotherapy in gastric cancer has been recently published my Murphy *et al*[121].

Encouraging results derive from the combination of cellular immunotherapy and chemotherapy, that improves the quality of life and might prevent the recurrence in patients with advanced gastric carcinoma[122]. The TCGA network identified elevated programmed death ligand-1 (PD-L1) expression in the EBV subtype in gastric cancer[16]. PD-1 is an immune checkpoint, involved in tumor suppression and in tumor microenvironment, because it regulates T cell pathways. New frontiers of immunotherapy are focalized on targeting the immune checkpoints, in order to remove inhibitory pathways that block an effective T cell response against the tumor[123]. Two antibodies against PD-1 (Pembrolizumab and Nivolumab) have been approved in 2014 form United States Food and Drug Administration. The checkpoint therapy could be useful for gastroesophageal cancer, which express PD-L1 in 18% to 42 % of cases[124]. Phase Ⅱ and phase Ⅲ clinical trials involving either single agent PD-1/PD-L1 inhibition or combined with CTLA-4 inhibitors (ipilimumab) are ongoing. In KEYNOTE-012 trial 39 patients PD-L1-positive with advanced gastric cancer received pembrolizumab, which showed a positive anti-cancer activity with an objective response of 22.2%, the median time to response was 8 wk (range 7-16 wk), with a median duration of response of 24 wk (range 8+ to 33+ wk). At 6 mo, 24% of patients showed no signs of disease progression, and 69% remained alive; the median PFS reached 1.9 mo. The most common AEs included fatigue (17.9%), decreased appetite (12.8%), hypothyroidism (12.8%), and arthralgia (10.3%). Four patients showed severe AEs associated with pembrolizumab, particularly, one of these patients died for treatment-associated hypoxia[125]. The OS data were presented at 2015 ASCO Annual meeting: The 6-mo OS rate was 69%. These results support the ongoing development of pembrolizumab for gastric cancer[126]. The phase Ⅱ KEYNOTE-059 study will soon be initiated to evaluate pembrolizumab as monotherapy or in combination with cisplatin and 5-FU in patients with advanced gastric or GEJ adenocarcinoma[127].

On May 2015 the phase Ⅲ KEYNOTE-061 study started. This is a Randomized trial of Pembrolizumab *vs* Paclitaxel in Advanced Gastric or GEJ adenocarcinoma patients who progressed after first-line therapy with platinum and fluoropyrimidine (NCT02370498).

In the near future, ipilimumab and nivolumab, two immunostimulatory monoclonal antibodies with antineoplastic effects, might offer new therapeutic options for patients with advanced gastric cancer[128]. In particular, Nivolumab, a fully human IgG4 anti-PD-1 monoclonal antibody, resulted active and generally well tolerated in patients with advanced solid tumors in a phase Ⅰ trial[129,130]. A Japanese randomized phase Ⅲ study started in october 2014 to evaluate Nivolumab (ONO-4538) *vs* BSC in patients with unresectable advanced or recurrent GC patients (NCT02267343).

CONCLUSION: "...AS THE PRESENT NOW, WILL LATER BE PAST"

Gastric cancer is one of the most common causes of cancer death in the world. Healing can only be guaranteed by an optimal surgery and still in the early stages of the disease. However, especially in Western countries, diagnosis is too late and the survival of patients with metastatic disease rarely exceeds 12 mo of diagnosis.

The multidisciplinary approach is always mandatory: The perioperative treatment, when indicated, has shown to be effective in increasing the survival of these patients and, in advanced disease, the total care by nutritionist, surgeon and oncologist has positive impact on the quality of life of these patients.

Chemotherapy in metastatic disease is the only chance of cure, but brings with it side effects also important and poor response rates. "... Your old road is rapidly aging" sang Bob Dylan (www.bobdylan.com), but it is true that at the moment that is the way we know best. Perhaps times are changing. As for lung and colorectal cancer, the targeted therapies are revolutionizing the clinical practice, but we also learned that to achieve maximum efficacy of these new molecules we have to change tumors classification.

New drugs and new classification: the genomic and molecular classification given by TCGA network will help

us to characterize with greater precision our patients. "... There's a battle outside and it is raging" but we will be armed with new knowledge.

Some clinical trials have led to the registration of drugs such as trastuzumab and ramucirumab. For EGFR inhibitors, lapatinib or everolius, the phase Ⅲ studies represented a setback.

However, the key is still patients selection on basis of molecular tumor characterization. Gefitinib in lung cancer reminds us "... for the loser now, will be later to win".

Which is the best cytotoxic combination for target therapies? Which is the best setting for using the new molecules? We do not know yet. In deed, it's possible that gastric cancer during progression disease and under evolutionary pressure of cytotoxic treatment can transform molecularly into a different phenotype.

Moreover, ethnic differences may cause different responses to the same molecules. Even this finding will lead to a personalized cancer medicine.

Finally, immunotherapy opens a vast and fascinating scenery for gastric cancer treatment. Some etiological factors such as viral and bacterial infections *via* EBV and *H. pylori* suggests that gastric cancer can be treated with new drugs such as immunotherapy checkpoint inhibitors.... And keep your eyes wide.

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TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Clinical significance of MET in gastric cancer

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Abstract

Chemotherapy has become the global standard treatment for patients with metastatic or unresectable gastric cancer (GC), although outcomes remain unfavorable. Many molecular-targeted therapies inhibiting signaling pathways of various tyrosine kinase receptors have been developed, and monoclonal antibodies targeting human epidermal growth factor receptor 2 or vascular endothelial growth factor receptor 2 have become standard therapy for GC. Hepatocyte growth factor and its receptor, c-MET (MET), play key roles in tumor growth through activated signaling pathways from receptor in GC cells. Genomic amplification of *MET* leads to the aberrant activation found in GC tumors and is related to survival in patients with GC. This review discusses the clinical significance of MET in GC and examines MET as a potential therapeutic target in patients with GC. Preclinical studies in animal models have shown that MET antibodies or small-molecule MET inhibitors suppress tumor-cell proliferation and tumor progression in *MET*-amplified GC cells. These drugs are now being evaluated in clinical trials as treatments for metastatic or unresectable GC.

Key words: MET; Gastric cancer; Genomic amplification; Immunohistochemistry; Clinical trial

Core tip: MET protein overexpression or *MET* gene amplification was associated with tumor progression and survival in gastric cancer (GC), although the definition of MET overexpression remains to be standardized. In preclinical studies, MET antibodies or small-molecule MET inhibitors suppressed cell proliferation and tumor progression in *MET*-amplified GC cells. Therefore, MET-targeting therapy is promising, and MET overexpression might be a useful biomarker of the response to chemotherapy inhibiting MET. Some clinical trials of MET inhibitors were conducted in metastatic GC, but sufficient benefits have not been demonstrated yet.

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INTRODUCTION

Gastric cancer (GC) is the fourth most common cancer, with 989600 cases newly diagnosed in the world in 2008, accounting for about 8% of all newly diagnosed cancers[1]. The effectiveness of chemotherapy remains very limited in patients with unresectable or metastatic GC, and overall survival (OS) was 10 to 13 mo in patients who received combination chemotherapy with multiple cytotoxic agents[2,3].

Receptor tyrosine kinases (RTKs) are growth factor receptors associated with various physiological responses to embryogenesis and homeostasis. RTK activity is strictly regulated in normal cells, although dysregulation or constitutive activation of RTKs has been found in various types of cancer cells[4]. Aberrant or oncogenic activation of RTKs augments tumor-cell proliferation, anti-apoptosis, vascularization, metastasis, and resistance to anticancer agents. RTKs are the most intensively pursued target molecules for anticancer drugs, because tumor cells with activated RTK signaling pathways are sensitive to appropriate RTK inhibitors[5]. Trastuzumab, a monoclonal antibody against p185 human epidermal growth factor receptor 2 (HER2), was first used clinically to treat GCs with HER2 overexpression. However, only 12% of patients who received trastuzumab had tumors that overexpressed HER2 in that trial[6]. Ramucirumab is a monoclonal antibody against vascular endothelial growth factor receptor 2 (VEGFR2). Second-line treatment with ramucirumab significantly prolonged survival in two phase Ⅲ trials in GC[7,8]. Many inhibitors of RTKs have been investigated to identify potential targets for the treatment of GC.

Proto-oncogene c-MET (MET), a member of the RTK family, is a known hepatocyte growth factor (HGF) receptor that is encoded by the *MET* gene. MET has a primary single-chain precursor protein made of alpha and beta subunits, the latter of which contains a cytoplasmic kinase domain and a docking site[9]. Binding of HGF to the extracellular domain activates the kinase activity that phosphorylates the tyrosines at the carboxy terminal docking site. Phosphorylated MET (p-MET) can recruit a variety of proteins, including growth factor receptor-bound protein 2 (GRB2), GRB2-associated binding protein 1 (GAB1), phospholipase C (PLC)-gamma, SRC, and SHP2, and activates downstream signaling molecules such as phosphatidylinositol-3-kinase (PI3K)/AKT and extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathways[10,11]. Similar to other RTKs, MET plays key roles in tumor survival, growth, angiogenesis, and metastasis. The aberrant signaling of MET by overexpression or gene amplification has been detected and correlated with tumor progression or patients' survival in GC[12-15]. Alternative activation of the MET pathway is considered an important mechanism causing resistance to treatments targeting HER family members[16,17]. Unfortunately, a phase Ⅲ study of rilotumumab, an HGF monoclonal antibody inhibiting MET pathway, has been recently discontinued because of high treatment-related mortality. However, inhibition of MET must undoubtedly be an important treatment for GC.

In this article, we reassess the clinical significance of MET in GC and summarize currently available results of preclinical studies and clinical trials of MET inhibitors.

CLINICAL OUTCOMES OF MET EXPRESSION IN GC

Protein expression on immunohistochemistry

Studies examining the relation between MET protein expression and clinical outcomes in GC specimens are summarized in Table 1. MET protein expression on immunohistochemistry (IHC) is predominantly detected in cytoplasm of tumor cells, but is also found in the cell membrane[12,18-20]. Lee *et al*[12] assessed membranous MET expression according to a standardized technique, similar to that used to evaluate HER2 expression. MET expression was observed even in stromal cells in tumors[18]. Moreover, MET overexpression was more frequently detected in dysplasia and precancerous gastric lesions than in intestinal metaplasia[21].

MET overexpression has frequently been found in intestinal type or differentiated type cancers[12,14,22,23], although one study reported a correlation with diffuse type[13]. Retterspitz reported that MET was overexpressed in 51% (45 of 88) of diffuse type tumors[24]. MET overexpression has been significantly associated with tumor invasion depth[12,13,23], lymph-node metastasis[12,13,19,20,25,26], distant metastasis[12,13,25], tumor stage[12,20,23,26], and recurrence[14], although several studies found no relation to any clinicopathological factors[24,27,28]. MET overexpression correlated with liver metastasis only in stage Ⅳ disease[29]. Some studies showed that MET overexpression was an independent prognostic factor that was significantly related to poor survival[12-14,19,20,25,26,30-32].

In one study, p-MET was detected in 59% (72 of 121) of GC tumors and was significantly associated with lymph-node metastasis, disease stage, and outcomes[20]. In another study, however, only 7% (2 of 30) of tumors overexpressed p-MET in spite of the fact that 63% (24 of 38) overexpressed MET[22]. In another study using a new technique, collaborative enzyme enhanced reactive-immunoassay, p-MET was detected in 24% (103 of 434) of GC tumors, including 31% of intestinal type, 24% of diffuse type, and 0% of mixed type[33].

Gene expression

Studies assessing *MET* gene expression are summarized

Table 1 MET protein expressions on immunohistochemistry and clinical outcomes in gastric cancer

	n	Definition of overexpression	%	Relation to clinicopathological factors	Relation to survival	Ref.
Usual IHC	495	2+/3+, > 10%	22	Intestinal type, recurrence	Worse[3]	[14]
	170	Cytoplasmic, 2+/3+	13	ND	ND	[38]
	121	⩾ 5%	66	N, stage	Worse	[20]
	114	> 30%	74	NA	Worse[3]	[30]
	98	Intensity and extensity scoring system	59	N, M	Worse	[25]
	50		78	NA	NA	[28]
	38	2+/3+, ⩾ 25%	63	Intestinal type	ND	[22]
	94[1]	⩾ 50%	50	NA	NA	[24]
	121[2]	Any staining	98	Liver metastasis	ND	[29]
TMA	438	Membranous, 2+/3+, > 10%	24	T, N, M, stage, intestinal type	Worse	[12]
	436	Intensity and extensity scoring system	44	T, N, M, diffuse type	Worse[3,4]	[13]
	215	Cytoplasmic, > 10%	69	NA	NA	[27]
	212	2+/3+	12	ND	Worse[3]	[32]
	182	Intensity and extensity scoring system	66	N, intestinal type, differentiated type	Worse	[19]
	163	Cytoplasmic 2+/3+ ⩾ 10%, and positive > 75%	4	ND	Worse[3]	[31]
	124	Cytoplasmic, 3+	71	T, stage, intestinal type	ND	[23]
	114	Intensity and extensity scoring system	82	N, stage	Worse	[26]
	35		43	ND	Likely worse	[18]

[1]Limited to diffuse or mixed type; [2]Only stage IV; [3]An independent prognostic factor on multivariate analysis; [4]Only IHC3+. IHC: Immunohistochemistry; TMA; Tissue micro array; T: Tumor invasion depth; N: Lymph-node metastasis; M: Distant metastasis; ND: Not described; NA: Not associated.

Table 2 *MET* mRNA expressions and clinical outcomes in gastric cancer

	n	Overexpression		Relation to clinicopathological factors	Relation to survival	Ref.
		Cut-off value	%			
Tumor	100	Value determined by nonparametric receiver operating characteristics	11	M	Worse	[34]
	100	ND	24	ND	ND	[43]
	45			N, stage, differentiated type	ND	[35]
	43	Value of mean + 2 SD in noncancerous tissue	70	NA	ND	[36]
	15			Intestinal type	ND	[22]
Serum	52	Detected	62	T, N, M, stage, recurrence, v	Worse	[37]

T: Depth of tumor invasion; N: Lymph-node metastasis; M: Distant metastasis; v: Venous invasion; ND: Not described; NA: Not associated.

in Table 2. *MET* mRNA expression in GC tissue has been reported to significantly correlate with lymph-node metastasis, distant metastasis, and disease stage[34,35], although one study found no clinical significance[36]. Higher levels of *MET* mRNA expression were frequently detected in intestinal or differentiated type cancers[22,35]. Serum *MET* mRNA expression in peripheral blood has been detected and was significantly associated with tumor progression and short survival[37].

Studies of *MET* gene alterations are summarized in Table 3. On fluorescence *in situ* hybridization (FISH) or silver *in situ* hybridization, *MET* gene amplification was detected in 3.4% to 7.1% of tumors[12,32,38]. In a study of esophagogastric adenocarcinoma, *MET* amplification was observed in 2.2% (10 of 460) of patients[39]. However, overexpression has been defined according to two patterns, *i.e.*, both amplification and high polysomy, or amplification alone. Gene amplification has been found to be significantly related to distant metastasis and tumor stage[12,39]. On copy number assay using reverse transcription polymerase chain reaction (RT-PCR), *MET* gene amplification was observed in 1.5% to 30% of tumors, although the definition of *MET* amplification somewhat differed among studies[15,18,40-42]. In a study using single nucleotide polymorphism array, *MET* amplification was detected in 3% to 4% of patients[43,44]. Wang *et al*[43] reported that *MET* amplification was found in 7% (3 of 41) of intestinal type cancers, but not in other types.

In many studies using FISH or RT-PCR, patients with *MET*-amplified tumors had significantly poorer survival than those with non-amplified tumors[12,15,18,32,39,41,42]. Only a Japanese study, with the lowest incidence of gene amplification, reported no relation of *MET* amplification to survival or any clinicopathological characteristic[40].

Gene mutation

A mutation of *MET* exon 14 coding for the juxta-membrane domain with a regulatory site was detected, and all other mutations were found in MET exons 16 to 20[45]. *MET* exon 2 skipping was found in 30% (82 of 272) of GC cases and was associated with increased *MET* gene expression. In addition, novel variants of *MET* exon 18 and/or 19 skipping were observed in 42% (47

Table 3 *MET* gene alterations and clinical outcomes in gastric cancer

	n	Definition of positive expression	%	Relation to clinicopatho-logical factors	Relation to survival	Ref.
FISH	460[1]	GA	2.2	Stage	Worse	[39]
	196	GA	6.1	ND	Worse	[32]
	170	GA or HP	15 (GA7.1 HP7.6)	ND	ND	[38]
SISH	381	GA or HP	19 (GA3.4, HP16)	Intestinal (HP), M (GA), stage (GA)	Worse[2] (GA)	[12]
RT-PCR	472	> 4 copies	21	NA	Worse[2]	[33]
	266	> 4 copies	1.5	NA	NA	[40]
	216	⩾ 5 copies	10	Unknown	Worse[2]	[41]
	128	⩾ 4 copies	30	T, stage	Worse[2]	[42]
	45	⩾ 7 copies	7	ND	Worse	[18]
SNP array	193	GA	4	ND	ND	[44]
	100	GA	3	ND	ND	[43]
Polymorphism analysis	34 (tumor)	Any alterations	59	T, N, M	ND	[47]
	34 (serum)	Any alterations	41	N, M	ND	[47]

[1]Esophagogastric adenocarcinoma; [2]An independent prognostic factor on multivariate analysis. FISH: Fluorescence *in situ* hybridization; SISH: Silver *in situ* hybridization; RT-PCR: Reverse transcription polymerase chain reaction; SNP: Single nucleotide polymorphism; GA: Gene amplification; HP: High polysomy; ND: Not described; NA: Not associated; T: Tumor invasion depth; N: Lymph-node metastasis; M: Distant metastasis.

of 272) of GC patients[46]. In another study, alterations of the *MET* gene were detected in both cancer tissue and peripheral blood of GC patients, and such alterations significantly correlated with tumor depth, lymph-node metastasis, and distant metastasis[47]. *MET* polymorphism (A/G or G/G genotype of *MET* rs40239) was significantly associated with favorable survival in a Japanese cohort, although no significant association was found in American or Austrian cohorts[48].

PRECLINICAL STUDIES OF MET INHIBITORS FOR GC

Several GC cell lines (Hs746T, GTL16, MKN45, SNU5, SNU620, HSC58, 58As9, and 58As1) have *MET* amplification and were used in preclinical studies of MET inhibition.

Selective tyrosine kinase inhibitors for MET

Volitinib (HMPL-504/AZD6094) is a small, potent adenosine triphosphate (ATP)-competitive tyrosine kinase inhibitor (TKI) of MET. Volitinib showed higher antiproliferative activity against GC cell lines with gains of *MET* gene copy number (SNU5, Hs746T, SNU620, GTL16, *etc.*) than against those without such gains (MKN1, MKN74, AZ521, KATO Ⅲ, AGS, *etc.*). The expressions of p-MET, phosphorylated AKT (p-AKT), and phosphorylated ERK (p-ERK) were down-regulated by volitinib in Hs746T cells. In a GC patient-derived tumor xenograft model with *MET* amplification, volitinib inhibited tumor growth; furthermore, the antitumor activity of volitinib was enhanced by concurrent treatment with docetaxel[38].

SU11274 is a small molecule TKI of MET. SU11274 blocked HGF-induced epithelial-mesenchymal transition, inducing down-regulation of Snail-2 and vimentin and up-regulation of E-cadherin in MKN45 cells, but not in non-amplified GC cells (MKN74). SU11274 suppressed proliferation of tumor cells regardless of the presence of HGF and also inhibited migratory potential. In a mouse model of peritoneal dissemination established from MKN45, SU11274 reduced the numbers and sizes of peritoneal tumors[34]. SU11274 treatment combined with SN38 synergistically suppressed proliferation of GC cells (side population cells of OCUM-2M) and tumor volume in a xenograft model[49].

PHA-665752 is a specific TKI for MET. In GTL16 cells, PHA-665752 inhibited growth in soft agar as well as cell proliferation and induced apoptosis regardless of the presence of HGF. PHA-665752 treatment decreased expression of MET-dependent signaling pathways, including p-MET, p-AKT, p-ERK, phosphorylated focal adhesion kinase (p-FAK), p-PLC-gamma, or phosphorylated signal transducer and activator of transcription, in GTL-16 or MKN45 cells[50,51]. Inhibition efficacy was higher in MKN45 cells than in non-amplified GC cells (MKN1, MKN28, and AGS)[51]. PHA-665752 significantly inhibited an increase in tumor volume in a GTL16 xenograft model[50]. PHA-665752 induced autophagy, and combined treatment with PHA-665752 and an autophagy inhibitor acted synergistically in GTL16 cells[52]. Furthermore, PHA-665752 restored growth inhibition in GC cells (SNU216) resistant to lapatinib (anti-EGFR and HER2)[16].

SGX523 is a selective, ATP-competitive MET inhibitor. Tyr 1248 is essential for high-affinity binding of SGX523 to MET. SGX523 inhibited p-MET and downstream signal pathways (p-GAB1, p-AKT, and p-ERK) in GTL16 cells. SGX523 inhibited tumor growth in a GTL16 xenograft model[53].

BAY-853474 is a highly selective, ATP-competitive MET inhibitor. It suppressed tumor growth in an Hs746T xenograft model and reduced plasma biomarkers, such as soluble MET ectodomain and IL-8[54].

KRC-408 is a small-molecule TKI that inhibits MET by occupying the ATP binding site. KRC inhibited p-MET and its constitutive downstream effectors (p-AKT, p-MEK,

p-ERK, phosphorylated mammalian target of rapamycin (mTOR), and p-p70S6K in MKN45 cells. KRC-408 induced apoptosis as represented by increased levels of caspase-3 and PARP. MKN45 cells in G2/M phase accumulated and those in S phase decreased after KRC-408 treatment. KRC-408 significantly delayed tumor growth in an MKN45 xenograft model, accompanied by decreased expression of p-MET, p-AKT, p-ERK, and CD34[55].

AMG 337 is a small-molecule ATP-competitive TKI of MET. Treatment with AMG 337 affected the viability of only two GC cell lines (SNU5 and Hs746T). Administration of AMG 337 resulted in dose-dependent antitumor efficacy in MET-amplified GC xenograft models[56].

Multikinase TKI

Crizotinib (PF-2341066) is an ATP-competitive, small-molecule TKI of MET and anaplastic lymphoma kinase. Crizotinib inhibited GTL16 cell growth and induced apoptosis in GTL16 cells. Crizotinib treatment reduced p-MET expression and inhibited tumor growth in a GTL16 xenograft model. These effects were accompanied by a decrease in tumor mitotic index (Ki67 expression), induction of apoptosis (caspase-3 expression), and a reduction in microvessel density (CD31 expression)[57]. Crizotinib induced apoptosis and reduced expression of p-AKT and p-ERK in *MET*-amplified GC cells (SNU5, HSC58, 58As9, and 58As1), but not in non-amplified GC cells (MKN28 and MKN1). Crizotinib treatment up-regulated the expression of a proapoptotic member of the Bcl-2 family (BIM), whereas it down-regulated the expression of members of the inhibitor of apoptosis protein (IAP) family, such as survivin, X-linked IAP, and c-IAP1. Crizotinib exhibited marked antitumor activity in 58As9 and SNU5 xenografts, but not in other xenografts derived from non-amplified GC cells (AZ521 and MKN28)[58]. In another study, crizotinib effectively inhibited the growth of *MET*-amplified GC cells (SNU620, SNU5, Hs746T, and GLT16) or MET-overexpressed GC cells (SNU638). MET-positive patient-derived GC xenografts responded to crizotinib and showed down-regulation of p-MET, p-AKT, and p-ERK[32].

Forenitib (GSK1363089) is an ATP-competitive multikinase inhibitor of MET, RON, AXL, tunica internal endothelial cell kinase 2 (TIE2), and VEGFR2. Forenitib inhibited the growth of MKN45 cells and FGFR2-amplified GC cells (KATO-Ⅲ) more strongly than that of non-amplified GC cells (MKN1, MKN7, and MKN74). Foretinib suppressed phosphorylation of EGFR, HER3, and FGFR3 *via* MET inhibition in MKN45 cells, while it inhibited phosphorylation of EGFR, HER3 and MET *via* FGFR2 inhibition in KATO-Ⅲ cells[59].

Cabozantinib (XL184) is an ATP-competitive, small-molecule multikinase inhibitor against MET, VEGFR2, and RET. SNU5 and Hs746T cells markedly responded to cabozantinib[60].

S49076 is a potent ATP-competitive multikinase inhibitor of MET, AXL/MER, and FGFR1-3. S49076 decreased p-MET expression and cell viability in GTL16 cells. S49076 down-regulated p-MET, p-AKT, and phosphorylated p70S6K and inhibited tumor growth in a GTL16 xenograft model[61].

T-1840383 is a potent inhibitor that targets MET, VEGFR1-3, RET, RON, RSE, TIE2, and TRKA. T-1840383 inhibited tumor growth in association with reduced p-MET, p-AKT, and p-ERK expression in an MKN45 xenograft model. In a peritoneal dissemination model generated from GC cells (NUGC4 expressing luciferase), T-1840383 treatment significantly prolonged survival in mice[62].

MK-2461, an ATP-competitive multitargeted inhibitor of activated MET, FGFR2, and platelet-derived growth factor receptor, potently inhibited the phosphorylation of three tyrosine residues of MET (Y1003 in the juxtamembrane domain, and Y1349 and Y1365 in the COOH-terminal docking site) in GTL16 cells. The antiproliferative potencies of MK-2461 were higher in GC cells with amplification of *MET* or *FGFR2* (GLT16, SNU5, SNU16, KATO Ⅲ) than in non-amplified GC cells (MKN74, AGS, SNU1, *etc*.). In GTL16 xenograft models, MK-2461 effectively suppressed MET signaling and tumor growth[63].

Other drugs

K252a is a potent small molecule inhibitor of the TRK family and reduced MET-driven proliferation in GTL16 cells. After K252a treatment, GTL16 cells lost the ability to form lung metastases in mice[64].

Oridonin, a diterpenoid isolated from the plant Rabdosia rubescens, has been used in traditional Chinese medicine for the treatment of human cancer, such as esophageal and prostate carcinomas. Oridonin potently inhibited MET phosphorylation and MET-dependent cell proliferation in SNU5 cells. Oridonin inhibited tumor growth and down-regulated p-AKT, p-ERK, p-c-RAF in an SNU5 xenograft model. Expression levels of Ki67 and CD31 on IHC also decreased in that model[65].

Resistance to MET inhibitors

HER kinase activation has been shown to play a role in the acquisition of resistance to MET inhibitor in GC cells. Phosphorylation of EGFR and HER3, which are activated *via* MET-driven receptor cross-talk, were suppressed by a MET inhibitor (PHA-665752) in GTL-16 and MKN-45 cells. However, EGF or heregulin-beta1 (HRG) treatment activated MET-independent EGFR or HER3 and restimulated PI3K/AKT or MEK/MAPK pathway. EGF or HRG treatment increased expression of cyclin D1, which had been reduced by a MET inhibitor, and promoted the cell cycle from arrest phase to synthetic phase. Therefore, combined treatment with an MET inhibitor plus an MEK or AKT inhibitor suppressed cell proliferation that had been promoted by HER family activation[66]. In the other study, activation of HER family members induced resistance to MET inhibitor.

Table 4 Development of MET-targeting agents for gastric cancer

Type	Agent	Other targets	Phase	Line	Combined therapy	Results or status	Ref.
MET selective non-ATP competitive TKI	Tivantinib (ARQ197)	None	Ⅱ	2nd/3rd	None	No CR/PR Median PFS 1.4 mo	[72]
MET-selective ATP-competitive TKI	AMG 337	None	Ⅱ	Any	None	Ongoing	[74]
			Ⅰ	2nd/3rd	None	1 CR and 4 PR in 10 patients with MET -amplified tumor	[73]
Multitargeted ATP-competitive TKI	Foretinib (GSK1363089)	VEGFR2, RON, AXL, TIE2	Ⅱ	1st (95%)	Docetaxel, Cisplatin	No CR/PR Median OS 7.4	[75]
	Crizotinib (PF-2341066)	ALK	Ⅰ			Tumor shrinkage in 2 patients with PFS 3.5 and 3.7 mo	[39]
MET mAb	Onartuzumab (MetMab)	None	Ⅲ	1st	mFOLFOX	Ongoing	[77]
HGF mAb	Rilotumumab (AMG 102)	None	Ⅲ	1st	ECX	Suspended	[79]
		None	Ⅲ	1st	CX	Suspended	[80]
		None	Ⅱ	1st	ECX	Median PFS 4.2 mo Median OS 5.6 mo	[78]

ATP: Adenosine triphosphate; TKI: Tyrosine kinase inhibitor; mAb: Monoclonal antibody; VEGFR: Vascular endothelial growth factor receptor; ALK: Anaplastic lymphoma kinase; TIE: Tunica internal endothelial cell kinase; CR: Complete response; PR: Partial response; RFS: Relapse-free survival; OS: Overall survival; FOLFOX: Folinic acid + fluorouracil + oxaliplatin; ECX: Epirubicin + oxaliplatin + capecitabine; CX: Oxaliplatin + capecitabine.

GTL16 cells that had acquired constitutive activation of EGFR by EGFR-L858R mutation did not respond to anti-MET treatment, such as MET silencing or MET inhibitor (PHA-665752). mRNA levels of HER family members significantly increased in the resistant GTL16 cells[67]. Qi *et al*[68] reported two mechanisms of resistance to the MET inhibitors PHA-665752 and PF-2341066. One mechanism was the activation of EGFR signaling. In GC cells acquiring resistance to MET inhibitors, EGFR signaling (EGFR, AKT, and ERK) was activated *via* an increase in transforming growth factor alpha. The other mechanism involved a gene mutation in the MET activation loop (Y1230). That mutation destabilizes the autoinhibitory conformation of MET on structural analysis and abrogates interaction with the inhibitor[68]. Increased copy numbers of *MET* or *KRAS* and increased expression of p-ERK or p-AKT were detected in GTL16 cells resistant to the MET inhibitor PHA-665752[69]. In addition, a novel *SND1-BRAF* fusion was detected in GTL16 cells that were resistant to the MET inhibitor RF-04217903 and was proven to be responsible for the resistance[70].

CLINICAL STUDIES OF MET INHIBITORS IN GC

Published and ongoing clinical studies of MET inhibitors in GC are summarized in Table 4. Tivantinib (ARQ197) is a non-ATP-competitive, selective MET inhibitor. In a phase Ⅰ trial in 51 patients with GC, 14 patients had stable disease (SD) for 4 mo or longer, and circulating endothelial cells decreased in 58% (25 of 43) of patients. Tivantinib decreased p-MET, MET, and phosphorylated focal adhesion kinase and increased terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick-end labeling (TUNEL) staining in tumor biopsy specimens[71]. In a phase Ⅱ study of tivantinib as second- or third-line therapy in GC, no objective response was observed in the 30 patients enrolled; the disease control rate was 37%, and median progression-free survival (PFS) was only 43 d. Tivantinib seemed to have modest antitumor efficacy and mild toxicity. As for adverse effects, severe (grade 3 or higher) neutropenia and anemia were most common, each occurring in 13% (4 of 30) of the patients[72].

Recently, favorable outcomes of treatment with ANG 337 have been reported in a phase Ⅰ study in 10 patients with MET-amplified esophago GC[73]. One patient had a complete response, and 4 had partial responses, even when ANG 337 was given as second-line or subsequent chemotherapy. An ongoing phase Ⅱ study is expected to explore whether the levels of MET amplification and expression or the presence of mutation in tumor specimens correlates with the response to AMG 337[74].

Foretinib lacked efficacy against metastatic GC in a phase Ⅱ study enrolling 74 patients. The best response was SD in 23% (10 of 44) of patients who received intermittent dosing and 20% (5 of 25) of those who received daily dosing. Only 4% (3 of 67) of the patients had *MET* amplification in tumor specimens, and one of them had SD. OS was 7.4 mo with intermittent dosing and 4.3 mo with daily dosing. Severe (grade 3 or higher) treatment-related adverse events occurred in 44% (21 of 48) of the patients who received intermittent dosing and 35% (9 of 26) of those who received daily dosing. Elevated aspartate aminotransferase levels (10%) and fatigue (15%) were the most frequent adverse events in patients who received intermittent dosing and daily dosing, respectively. Plasma levels of MET, HGF, VEGFR2, and VEGF-A were measured at baseline and during treatment, but these markers did not correlate with response[75].

Crizotinib was administered to 4 patients with *MET*-amplified esophagogastric adenocarcinomas in part of a phase Ⅰ study. Two patients had tumor shrinkage (16% and 30%) with PFS of 3.5 and 3.7 mo, respectively[39].

Onartuzumab (formally called MetMAb and PRO 143966) is an anti-MET receptor monoclonal antibody. In a phase Ⅰ clinical trial, one patient with metastatic GC had a complete response for approximately 2 and a half years[76]. A phase Ⅲ study of onartuzumab combined with modified FOLFOX (5-fluorouracil + leucovorin + oxaliplatin) is ongoing[77].

Rilotumumab (AMG 102) is a monoclonal antibody against HGF. In a phase Ⅰb/Ⅱ study of rilotumumab combined with epirubicin, cisplatin, and capecitabine (ECX) as first-line chemotherapy, 121 patients were randomly assigned to treatment (40 to rilotumumab 15 mg/kg; 42 to rilotumumab 7.5 mg/kg; 39 to placebo). Median PFS was significantly longer in both rilotumumab groups combined than in the placebo group (5.7 and 4.2 mo, respectively). The response rate was 39%, and the disease control rate was 80% in the combined rilotumumab group. MET status was evaluated on IHC in that study, and MET positivity was defined as at least 25% membrane staining of tumor cells at any intensity. In the MET-positive group, median OS was much longer in the combined rilotumumab group than in the placebo group (10.6 mo *vs* 5.7 mo). In the MET-negative group, patients had better survival than those in the MET-positive group, and rilotumumab was not significantly effective. As for adverse effects, severe (grade 3 or higher) venous thromboembolism occurred in 20% (16 of 81) of the patients[78]. However, the management of thromboembolism might be the most critical issue. Two phase Ⅲ trials of rilotumumab plus ECX and rilotumumab plus cisplatin and capecitabine have been suspended because of increased treatment-related mortality[79,80].

CONCLUSION

Many studies have suggested that MET protein overexpression or *MET* amplification plays a critical role in the progression of GC and negatively affects survival in patients with GC. However, the criteria used to define overexpression of MET protein have differed among many studies, and the assessment of MET protein expression is unlikely to be standardized as strictly as that of HER2 or EGFR. It remains unclear whether staining intensity of the membrane or the cytoplasm of tumor cells should be assessed. Differences in staining intensity associated with the use of different antibodies and different IHC procedures used to assess MET expression remain a problem that must be solved before techniques for assessing MET status can be standardized. The use of different assessment techniques by different investigators is another problem. The evaluation of p-MET expression might provide the most objective measure of MET status; however, the fact that different antibodies recognize different phosphorylated sites might be a major obstacle to the standardization of techniques for assessing p-MET expression. On the other hand, *MET* amplification on FISH may be appropriate for standardized assessment, similar to *HER2* amplification. Several studies have used consistent criteria to define *MET* amplification on FISH, and it is more objective assessment than that of protein expression on IHC, although the cost- and time-effectiveness of gene analysis may be poor. Deng *et al*[44] reported that *MET* amplification was mutually exclusive from amplification of other genes, such as *EGFR*, *HER2*, *FGFR2*, and *KRAS*. Therefore, MET-targeting therapy is considered a promising treatment for GC with *MET*-amplification as well as GC with amplification of other RTKs.

Preclinical studies have suggested that MET inhibitors are most promising against *MET*-amplified or MET-overexpressed cancers. Various MET inhibitors have been developed and studied in clinical trials; however, several trials showed insufficient efficacy and unexpected outcomes. These results might have been caused by lack of identification of specific biomarkers. Methodological differences in the evaluation of MET status remain an important problem in conducting clinical trials. In an ongoing study of monoclonal antibodies of MET, patients with MET expression on IHC are being recruited[77]. As mentioned above, the assessment of MET protein expression on IHC remains to be standardized. The same procedure for assessment of MET status on IHC is needed for clinical studies. Many TKIs of MET have produced favorable results in *MET*-amplified GC in many preclinical studies, and AMG 337 and crizotinib were effective in some patients with *MET*-amplified GC in preliminary clinical studies[39,73]. MET TKIs thus may be a promising treatment for patients with *MET*-amplified GC.

Resistance to MET inhibitors is another critical issue. Several lines of evidence from preclinical studies suggest that activation of the HER family is involved in resistance to MET inhibitors, and treatment against HER family pathways may overcome this issue. Owing to the diversity of RTKs, treatment with a multitargeted TKI or combined therapy with single-targeted TKIs might be a promising approach to enhance efficacy. However, potential benefits of treatment with multiple inhibitors of RTKs have yet to be demonstrated in clinical trials in GC.

MET is considered a promising target in GC, although the results of phase Ⅲ trials of rilotumumab have been disappointing. It is essential to identify specific subgroups of patients most likely to benefit from treatment with MET inhibitors. Future studies should attempt to define biomarkers that would optimize the selection of patients who respond to MET inhibitors.

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TOPIC HIGHLIGHT

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Polymorphisms in mucin genes in the development of gastric cancer

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Abstract

Gastric cancer (GC) is the third leading cause of cancer-related death worldwide. In areas of high prevalence, such as Japan, South Korea and China, most cases of GC are related to *Helicobacter pylori* (*H. pylori*), which involves well-characterized sequential stages, including infection, atrophic gastritis, intestinal metaplasia, dysplasia, and GC. Mucins are the most abundant high-molecular-weight glycoproteins in mucus, which is the first line of defense and plays a major role in blocking pathogenic factors. Normal gastric mucosa shows expression of MUC1, MUC5AC and MUC6 that is specific to cell type. However, the specific pattern of MUC1, MUC5AC and MUC6 expression is changed in gastric carcinogenesis, accompanied by *de novo* expression of secreted MUC2. Recent studies have provided evidence that variations in these mucin genes affect many steps of GC development, such as *H. pylori* infection, and gastric precancerous lesions. In this review, we focus on studies of the association between polymorphisms in mucin genes and development of GC. This information should be helpful for the early detection, surveillance, and treatment of GC.

Key words: Gastric cancer; *Helicobacter pylori*; Genetic polymorphism; Mucin; Risk; Association study; Atrophic gastritis

Core tip: *Helicobacter pylori* (*H. pylori*) infection is the single most important risk factor in the development of gastric cancer (GC), however the etiology of GC involves host and other environmental factors. Genetic and biological evidence highlights the important roles of variations in mucin genes in the development and progression of GC. In this review, we summarize studies

of the association between polymorphisms in *MUC1*, *MUC5AC*, *MUC6* and *MUC2* and development of GC, which should be helpful for the early detection, surveillance, and treatment of GC.

Wen R, Gao F, Zhou CJ, Jia YB. Polymorphisms in mucin genes in the development of gastric cancer. *World J Gastrointest Oncol* 2015; 7(11): 328-337 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/328.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.328

INTRODUCTION

Although gastric cancer (GC) incidence and mortality rates are declining in most countries, it is still the fifth most common cancer and the third leading cause of cancer-related death worldwide[1]. Epidemiological studies have shown that a high intake of salt, tobacco smoking, and *Helicobacter pylori (H. pylori)* infection increase the risk of GC[2-4]. In areas of high prevalence of GC, such as Japan, Korea and China, most cases of GC are related to *H. pylori*. GC is the result of a long complex multifactorial and multistep process that involves well-characterized sequential stages. The initial lesion is inflammatory and is usually caused by *H. pylori* infection, which results in chronic superficial gastritis. The following pathological model of GC progression includes atrophic gastritis, intestinal metaplasia, dysplasia and GC[5,6]. *H. pylori* infection is the most important risk factor for GC and it was classified as a class Ⅰ carcinogen by the World Health Organization in 1994, nevertheless, the etiology of GC also involves host and other environmental factors. This is demonstrated by the fact that only 1%-3% of patients with *H. pylori* infection develop GC[7,8]. The hypothesis that genetic susceptibility or predisposition plays an important etiological role in GC is supported by many case-control studies and genome-wide association studies (GWASs)[9-14].

H. pylori initiates colonization of the gastric mucosa by crossing the gastric mucus layer and adhering to the gastric epithelium[15]. Mucus is the first line of defense and plays a major role in blocking pathogenic factors, and mucins are the major components in mucus and are responsible for its biochemical and biophysical properties[16]. The mucin family comprises 21 members. The mucins are high-molecular-weight glycoproteins characterized by a heavily O-glycosylated tandem repeat region rich in proline, threonine and serine, which is encoded by a variable number of tandem repeats (VNTRs)[17-20]. Mucins are categorized into two subgroups according to their physiological and structural characteristics: membrane-bound, such as MUC1, and secreted, including MUC2, MUC5AC and MUC6[17]. *In situ* hybridization and immunohistochemistry have demonstrated the cell-type-specific expression of mucins in epithelial tissues[21,22]. Normal gastric mucosa shows cell-type-specific expression of MUC1, MUC5AC and MUC6[21-23]. Apical MUC1 is expressed in the gastric mucosa in the superficial and foveolar epithelium and mucous neck zone cells[24]. Secreted mucin MUC5AC is detected in the superficial epithelium, whereas MUC6 is found in the deep glands[25,26]. This specific pattern of MUC1, MUC5AC and MUC6 expression is changed in gastric carcinogenesis, accompanied by *de novo* expression of secreted MUC2[26-30]. Recent genetic and biological evidence highlights the important roles of variations in these mucin genes in the development and progression of GC. In this review, we focus on studies of the association between polymorphisms in *MUC1*, *MUC5AC*, *MUC6* and *MUC2* genes and development of GC (Table 1). Details of the studied single nucleotide polymorphisms (SNPs) in mucin genes are described in Table 2.

POLYMORPHISMS IN *MUC1* IN THE DEVELOPMENT OF GC

MUC1 is a highly polymorphic membrane-associated mucin that is often aberrantly expressed in cancer[31]. *MUC1* gene is located on chromosome 1q21 and contains a highly conserved VNTR of 20 amino acids, varying from 25 to 125 repeats, depending on the allele[32]. In recent decades, some studies were performed to investigate the potential roles of genetic variations in *MUC1* in gastric carcinogenesis, but most of them were focused on the VNTRs, with inconsistent results. Costa *et al*[33] observed that polymorphism in the *MUC1* VNTRs influenced the binding of *H. pylori* to gastric cells. Vinall *et al*[28] reported that small *MUC1* VNTR alleles were correlated with *H. pylori*-associated gastritis in European populations. Two studies from Portugal (which has the higher risk of GC in Europe) showed that small *MUC1* VNTR alleles were significantly associated with gastric carcinoma[34], as well as chronic atrophic gastritis and incomplete intestinal metaplasia, which are two well-established precursor lesions of GC[35]. However, another study from Denmark indicated that small *MUC1* VNTR alleles are more frequent in the Danish population (which has the lower risk of GC in Europe) than in Portugal[36].

GWASs have recently been important in identifying potential genetic variations related to cancer susceptibility. In 2010, Abnet *et al*[37] conducted a GWAS in 1625 patients with GC and 2100 controls. They identified a significant SNP of rs4072037 A/G in the *MUC1* gene for GC. The A allele was correlated with increased susceptibility to GC in Chinese patients during initial scanning, however, this association was not maintained in the second phase, or when the results of the two phases were combined. A GWAS on GC in Japan revealed the top 10 SNPs that were significantly related to the diffuse type of GC, which included two located in chromosome 1q22[38]. Subsequently, Saeki *et al*[39] performed high-density mapping to explore the

Table 1 List of association studies between polymorphisms in mucin genes and development of gastric cancer

Gene	Ref.	Population	Disease	Study design	Sample (case/control)	Polymorphism	Association
MUC1	Vinall *et al*[28]	European	*H. pylori* related gastritis	Case–control study	57 gastritis patients	VNTR	Yes
	Carvalho *et al*[34]	Portuguese	GC	Case–control study	159/324	VNTR	Yes
	Silva *et al*[35]	Portuguese	CAG, IM	Case–control study	174 patients	VNTR	Yes
	Abnet *et al*[37]	Chinese	GC	GWAS	1625/2100	rs4072037	Yes
					Replication: 615/1202		No
					Combined: 2240/3302		No
	Saeki *et al*[39]	Japanese	DGC	Case–control study	606/1264/	rs4072037, rs2070803	Yes
		Japanese			304/1465	rs4072037, rs2070803	Yes
		South Korean			452/372	rs4072037, rs2070803	Yes
	Xu *et al*[40]	Chinese	GC	Case–control study	138/241	rs4072037	Yes
	Jia *et al*[43]	Polish	GC	Case–control study	273/377	rs6427184	Yes
				(tag SNP approach)		rs4971052	Yes
						rs4276913	Yes
						rs4971088	Yes
						rs4971092	Yes
						rs4072037	Yes
	Jia *et al*[43]	Polish	*H. pylori* infection	Case–control study	320/57	rs6427184	No
				(tag SNP approach)		rs4971052	No
						rs4276913	No
						rs4971088	No
						rs4971092	No
						rs4072037	No
	Zhang *et al*[44]	Chinese	GC	Case–control study	1681/1858	rs4072037	Yes
	Palmer *et al*[45]	Caucasian	GC	Case–control study	596/587	rs4072037	Yes
	Li *et al*[46]	Chinese	GC	Case–control study	300/300	rs2070803	Yes
	Zhang *et al*[47]	Chinese	Non-cardia GC	Case–control study	288/281	rs4072037	No
				(tag SNP approach)		rs2990245	No
						rs9628662	No
						rs9426886	No
	Zhang *et al*[47]	Chinese	*H. pylori* infection	Case–control study	122/159	rs4072037	No
				(tag SNP approach)		rs2990245	No
						rs9628662	No
						rs9426886	No
	Frank *et al*[48]	German	CAG	Case–control study	533/1054	rs4072037	No
	Marín *et al*[49]	Spanish	GCPLs	Case–control study	387 patients	rs3814316	No
				(tag SNP approach)		rs9426886	No
						rs1045253	No
	Sun *et al*[50]	Hispanic American	GC	Case–control study	132/125	rs4072037	No
	Duan *et al*[51]	-	GC	Meta-analysis	4220/6384	rs4072037	Yes
	Zheng *et al*[52]	-	GC	Meta-analysis	6580/10324	rs4072037	Yes
	Mocellin *et al*[42]	Asian	DGC	Meta-analysis	7279 subjects	rs2070803	Yes
MUC5AC	Jia *et al*[43]	Polish	GC	Case–control study	273/377	rs1541314	No
				(tag SNP approach)		rs2014486	Yes
						rs2075859	No
						rs2672785	No
						rs2735733	Yes
						rs7118568	No
						rs868903	Yes
						rs4963049	No
	Jia *et al*[43]	Polish	*H. pylori* infection	Case–control study	320/57	rs1541314	No
				(tag SNP approach)		rs2014486	No
						rs2075859	No
						rs2672785	No
						rs2735733	No
						rs7118568	No
						rs868903	No
						rs4963049	No
	Zhou *et al*[61]	Chinese	Non-cardia GC	Case–control study	288/281	rs3793966	No
				(tag SNP approach)		rs7118568	No
						rs868903	No
						rs3793964	Yes
						rs3750919	No
						rs5743942	No
						rs4963062	No
						rs885454	Yes
						rs6578810	No
						rs11040869	Yes
						rs7118481	No
						rs7105198	No

	Zhou *et al*[62]	Chinese	*H. pylori* infection	Case–control study (tag SNP approach)	122/159	rs3793966	No
						rs7118568	No
						rs868903	No
						rs3793964	No
						rs3750919	No
						rs5743942	No
						rs4963062	No
						rs885454	No
						rs6578810	No
						rs11040869	No
						rs7118481	No
						rs7105198	No
	Wang *et al*[63]	Chinese	GC	Case–control study	230/328	VNTR	Yes
MUC6	Nguyen *et al*[68]	-	*H. pylori* infection	Case–control study	92/68	VNTR	Yes
	Garcia *et al*[69]	Portuguese	GC	Case–control study	157/376	VNTR	Yes
	Kwon *et al*[70]	South Korean	GC	Case–control study	470/1103	VNTR	Yes
	Jia *et al*[43]	Polish	GC	Case–control study (tag SNP approach)	273/377	rs1128413	No
						rs4077293	No
						rs7483870	No
						rs7943115	No
						rs11602663	No
						rs11605303	No
						rs10902076	No
						rs2071174	No
						rs11245936	No
						rs10794359	No
						rs7112267	No
						rs12574439	No
						rs7119740	No
						rs11601642	No
	Jia *et al*[43]	Polish	*H. pylori* infection	Case–control study (tag SNP approach)	320/57	rs1128413	No
						rs4077293	No
						rs7483870	No
						rs7943115	No
						rs11602663	No
						rs11605303	No
						rs10902076	No
						rs2071174	No
						rs11245936	No
						rs10794359	No
						rs7112267	No
						rs12574439	No
						rs7119740	No
						rs11601642	No
	Marín *et al*[49]	Spanish	GCPLs	Case–control study (tag SNP approach)	387 patients	rs4076950	No
						rs7481521	No
						rs11246384	No
						rs6597947	No
						rs9794921	No
	Frank *et al*[48]	German	CAG	Case–control study	533/1054	rs7481521	No
MUC2	Jeong *et al*[72]	South Korean	GC	Case–control study	455/457	VNTR	Yes
	Marín *et al*[49]	Spanish	GCPLs	Case–control study (tag SNP approach)	387 patients	rs10902073	Yes
						rs10794281	Yes
						rs2856082	No
						rs2071174	Yes
						rs7396030	No
						rs11245936	No
						rs7944723	Yes
						rs6421972	No
						rs10794293	Yes
						rs11245954	No
						rs7480563	No
						rs7126405	No
						rs3924453	Yes
						rs4077759	Yes
	Frank *et al*[48]	German	CAG	Case–control study	533/1054	rs2856111	No
						rs11825977	No

CAG: Chronic atrophic gastritis; DGC: Diffuse gastric cancer; GCPLs: Gastric cancer precursor lesions; *H. pylori*: *Helicobacter pylori*; IM: Intestinal metaplasia; SNP: Single nucleotide polymorphism; GC: Gastric cancer.

Table 2 Description of the studied single nucleotide polymorphisms in mucin genes

Gene	Chromosome	SNPs	Wild alleles	Mutated alleles	Contig position[1]	Location[2]
MUC1	1q21	rs4072037	A	G	12007689	T22T
		rs2070803	C	T	12000652	3′ flanking region
		rs6427184	A	G	11965720	3′ flanking region
		rs4971052	C	T	11968955	3′ flanking region
		rs4276913	A	G	11974610	3′ flanking region
		rs4971088	T	A	11985820	3′ flanking region
		rs4971092	T	C	11986883	3′ flanking region
		rs2990245	T	C	12043084	5′ flanking region
		rs9628662	T	G	12051963	5′ flanking region
		rs9426886	T	A	11994691	3′ flanking region
		rs3814316	C	T	11992655	3′ flanking region
		rs1045253	T	C	12046857	5′ flanking region
MUC5AC	11p15.5	rs1541314	G	A	1182293	3′ flanking region
		rs2014486	A	G	1177573	3′ flanking region
		rs2075859	C	T	1169258	3′ flanking region
		rs2672785	C	T	1165711	3′ flanking region
		rs2735733	C	T	1180410	3′ flanking region
		rs7118568	C	G	1162850	3′ flanking region
		rs868903	T	C	1161460	3′ flanking region
		rs4963049	A	G	1155197	3′ flanking region
		rs3793966	C	T	1221718	3′ flanking region
		rs3793964	C	T	1220752	3′ flanking region
		rs3750919	G	A	1211601	3′ flanking region
		rs5743942	C	T	1232798	3′ flanking region
		rs4963062	G	A	1245411	3′ flanking region
		rs885454	C	T	1162161	3′ flanking region
		rs6578810	T	G	1209349	3′ flanking region
		rs11040869	G	A	1203382	3′ flanking region
		rs7118481	G	C	1267108	3′ flanking region
		rs7105198	G	C	1086133	5′ flanking region
MUC6	11p15.5	rs1128413	C	T	950694	3′ flanking region
		rs4077293	C	T	936522	3′ flanking region
		rs7483870	C	T	916019	3′ flanking region
		rs7943115	G	A	913885	3′ flanking region
		rs11602663	C	T	960778	Intronic
		rs11605303	G	A	978110	5′ flanking region
		rs10902076	G	C	1006044	5′ flanking region
		rs2071174	C	T	1013712	5′ flanking region
		rs11245936	G	A	1026266	5′ flanking region
		rs10794359	C	T	991715	5′ flanking region
		rs7112267	C	T	996981	5′ flanking region
		rs12574439	G	C	997948	5′ flanking region
		rs7119740	C	G	1000419	5′ flanking region
		rs11601642	C	A	1002509	5′ flanking region
		rs4076950	C	T	955021	Intronic
		rs7481521	G	A	967811	V619M
		rs11246384	C	T	970448	Intronic
		rs6597947	G	T	977029	5′ flanking region
		rs9794921	G	T	979867	5′ flanking region
MUC2	11p15.5	rs10902073	C	A	1000934	5′ flanking region
		rs10794281	C	T	1003149	5′ flanking region
		rs2856082	C	G	1011562	5′ flanking region
		rs2071174	C	T	1013712	5′ flanking region
		rs7396030	C	T	1025368	Intronic
		rs11245936	G	A	1026366	G832S
		rs7944723	C	G	1039802	P1832P
		rs6421972	G	A	1042586	I2154T
		rs10794293	C	T	1045031	Intron
		rs11245954	A	G	1047170	V2459V
		rs7480563	G	A	1047741	T2524P
		rs7126405	G	A	1049388	Q2653P
		rs3924453	G	A	1051898	3′ flanking region
		rs4077759	C	T	1052068	3′ flanking region
		rs2856111	T	C	1015747	L58P
		rs11825977	A	G	1015920	V116M

[1]Based on contig NT_004487.20 for *MUC1* gene, and contig NT_009237.19 for *MUC5AC*, *MUC6* and *MUC2* genes; [2]SNP location relative to each gene in the region. SNPs: Single nucleotide polymorphisms.

susceptibility locus of GC at chromosome 1q22 and reported that two SNPs of rs2070803 and rs4072037 were significantly related to susceptibility to diffuse GC in Japan, and the results were validated in other Japanese and Korean studies. SNP rs4072037 is located in exon 2 of the *MUC1* gene and controls alternative splicing at the boundary between exons 1 and 2[39-41]. This SNP affects promoter activity and disrupts the physiological function of MUC1[41,42]. The rs4072037 G allele is correlated with higher VNTRs and the A allele with lower VNTRs[41]. However, the VNTRs are unlikely to be the causal polymorphism for GC susceptibility because the TRs are not translated in normal or malignant gastric epithelial cells[39]. This suggests that the VNTRs are a tagging polymorphism for other genetic variations, such as rs4072037, related to risk of gastric carcinogenesis. It is particularly interesting that rs4072037 A is a major allele in Chinese, Japanese and Korean populations, which have a high incidence of GC, but a minor allele in Caucasians, who have a low incidence of GC. SNP rs2070803 G/A is downstream of the *MUC1* and *TRIM46* genes and its functional effects are unknown. *MUC1* is located downstream of the *TRIM46* gene. These two genes are part of a cluster, which also includes *KRTCAP2*, *THBS3*, *MTX1*, *PKLR* and *HCN3*, located in a region of strong linkage disequilibrium (LD) and are transcribed in opposite directions[42]. *TRIM46* is not expressed in gastric mucosa[39], therefore, SNP rs2070803 might also be a tag for variants in other genes located in this LD region, such as *MUC1*, which are involved in gastric carcinogenesis.

In addition to GWASs, the association of *MUC1* SNPs with GC has been investigated in many case-control studies using a candidate gene approach. An association study in China showed that patients with rs4072037 AA genotype had a significantly increased risk of GC[40]. Jia *et al*[43] conducted a population-based, case-control study in the Polish population. Each of the tested tag SNPs (including rs6427184, rs4971052, rs4276913, rs4971088, rs4971092 and rs4072037) across the *MUC1* region had significant associations with increased risk of GC. This association remained significant after adjusting for multiple tests, which also demonstrated that rs4072037 AA genotype was related to increased risk of GC. However, the study showed that *MUC1* tag SNPs were not associated with *H. pylori* infection, suggesting that the effects of *MUC1* polymorphisms on risk of GC are not mediated by *H. pylori* infection. The association between rs4072037 A allele and increased GC risk was further replicated in Chinese and Caucasian populations[44,45]. Another study demonstrated that rs2070803 GA/AA genotypes were protective against GC, with > 50% risk reduction in Chinese individuals[46]. However, other studies have shown conflicting results. A case-control study conducted by our group showed that four tag SNPs (including rs4072037) in *MUC1* were not associated with the risk of non-cardia GC, or *H. pylori* infection in the Han population in Northwest China[47]. Another study showed no association between rs4072037 and risk of chronic atrophic gastritis, a well-defined precursor of GC in the German population[48]. Marín *et al*[49] reported that three tag SNPs (rs3814316, rs9426886 and rs1045253) in *MUC1* were not associated with precursor lesions of GC in a high-risk area of Spain. Another study demonstrated that rs4072037 was not associated with GC risk in Hispanic Americans[50]. To clarify the current limited and conflicting evidence, and to establish the true impact of *MUC1* variations on gastric carcinogenesis, several meta-analyses have been performed. Duan *et al*[51] conducted an analysis of 10 case-control studies comprising 4220 cases and 6384 controls. They found that rs4072037 G allele was associated with a decreased risk of GC progression, especially in Asians. This result is consistent with the study of Zheng *et al*[52] of 6580 cases and 10324 controls, which suggested the involvement of *MUC1* rs4072037 polymorphism in gastric carcinogenesis among Asian individuals. A further meta-analysis showed that the rare rs2070803 A allele was associated with reduced risk of diffuse-type GC[42]. All the evidence suggests that *MUC1* polymorphisms, such as rs4072037, are promising biological markers for predicting GC risk, especially in Asian populations.

POLYMORPHISMS IN *MUC5AC* IN THE DEVELOPMENT OF GC

MUC5AC is a major secreted mucin in healthy gastric mucosa and is the major receptor for *H. pylori* in the human stomach. BabA and SabA adhesins on *H. pylori* bind to Lewis B blood group antigens on MUC5AC, facilitating colonization[53-55]. In chronic *H. pylori* infection, normally expressed MUC5AC and MUC5AC-producing cells may gradually decrease[56,57]. *MUC5AC* is located on chromosome 11p15.5[58], which often has loss of heterozygosity in patients with GC[59,60]. Studies on the association between *MUC5AC* polymorphisms and GC development are limited at present. Jia *et al*[43] investigated the relationship between eight tag SNPs of *MUC5AC* and GC in a Polish study. The three tag SNPs rs868903, rs2014486 and rs2735733 in the 3' flanking region of *MUC5AC* were related to the risk of GC. Their minor allele homozygotes were significantly associated with increased risk of GC. However, none of the eight tested tag SNPs were associated with risk of *H. pylori* infection. Our group also performed a case-control study to evaluate the association of 12 tag SNPs of *MUC5AC* with risk of non-cardia GC in the Han population in Northwest China. We observed that three tag SNPs, rs3793964, rs11040869 and rs885454, were significantly associated with the risk of non-cardia GC. The minor allele homozygotes of rs3793964 and rs11040869, as well as the heterozygote of rs885454 had a protective effect on risk of non-cardia GC[61]. These three tag SNPs are all located in the 3' flanking region of *MUC5AC*. The discrepancies between the

two studies may have been due to racial differences in variant frequencies. However, few biological studies on genetic variations in *MUC5AC* have been reported. Similarly, our results also suggested that polymorphisms of *MUC5AC* gene were not associated with the risk of *H. pylori* infection, suggesting *MUC5AC* polymorphisms are involved in other processes besides bacterial binding in developing GC[62]. Wang *et al*[63] conducted a case-control study in the Chinese population, which reported that some variations in an upstream repetitive region of *MUC5AC* were associated with GC susceptibility and progression. Their findings highlight the importance of *MUC5AC* polymorphisms in risk of GC.

POLYMORPHISMS IN *MUC6* IN THE DEVELOPMENT OF GC

The secreted mucin, MUC6, is highly expressed in normal gastric mucosa. One study has shown that MUC6 has antimicrobial properties against *H. pylori*. Unique glycan residues on MUC6 inhibit biosynthesis of major cell wall component cholesteryl-α-D-glucopyranoside[64]. MUC6 is aberrantly expressed in response to *H. pylori* infection[65], and MUC6 expression is lower in GC compared with normal mucus[66]. *MUC6* is also located on chromosome 11p15.5, which is a region rich in recombination[59]. *MUC1* and *MUC6* have a large number of VNTRs[67]. Several studies have focused on the relationship between VNTR polymorphisms of *MUC6* and GC development. In one of these, small VNTR alleles of *MUC6* gene were associated with increased risk of *H. pylori* infection[68]. Others showed that small *MUC6* VNTR alleles were more frequent in patients with GC than in healthy blood donors[69], and short rare *MUC6* minisatellite 5 alleles had an effect on susceptibility to GC by regulating gene expression[70]. However, Jia *et al*[43] investigated the relationship between *MUC6* polymorphisms and GC, using a tag SNP approach. Fourteen of the tag SNPs tested across the *MUC6* region were not associated with risk of GC or *H. pylori* infection. The authors inferred that VNTR polymorphisms had many alleles, which might have divided the study population into several classes, thus making statistical analysis difficult. Similarly, Marín *et al*[49] observed that five tag SNPs in *MUC6* were not associated with GC precursor lesions. Furthermore, Frank *et al*[48] investigated the association between polymorphism in *MUC6* and the risk of chronic atrophic gastritis, using a candidate SNP approach. However, there was no association between the putative functional SNP rs7481521 (*MUC6* V619M) and chronic atrophic gastritis. Further studies are needed to elucidate the roles of *MUC6* polymorphisms in the gastric carcinogenesis pathway.

POLYMORPHISMS IN *MUC2* IN THE DEVELOPMENT OF GC

Normal gastric mucosa shows little or no expression of MUC2. However, in intestinal metaplasia and GC, the level of MUC2 is increased[27,29,30]. MUC2 might be activated by proinflammatory cytokines expressed after *H. pylori* infection, leading to its overexpression[71]. *MUC2* gene is clustered on chromosome 11p15.5 with *MUC5AC*, *MUC5B* and *MUC6*[58]. Only three studies have evaluated the relationship between *MUC2* polymorphisms and development of GC. Jeong *et al*[72] reported that the short rare minisatellite 6 alleles of *MUC2* gene are associated with GC. Marín *et al*[49] have investigated the association of 14 tag SNPs in *MUC2* with evolution of GC precursor lesions in 387 patients with 12.8 years follow-up. According to the diagnosis at recruitment and after follow-up, the patients were divided into three groups, that is, those with no change in lesions, progression of lesions, and regression of lesions. The results indicated that three SNPs (rs10794293, rs3924453 and rs4077759) at the 3′ moiety in *MUC2* were associated with a decreased risk of lesion progression. In contrast, another four SNPs (rs10902073, rs10794281, rs2071174 and rs7944723) at the 5′ moiety were significantly associated with lesion regression. The association of SNPs with GC precursor lesions was stronger in patients with *H. pylori* infection. However, it was also shown that functional SNP rs11825977 (V116M) in *MUC2*, which might influence *MUC2* mRNA expression[73], as well as the potentially functional SNP rs2856111 (L58P), were not associated with the risk of chronic atrophic gastritis[48].

CONCLUSION

GC is the third leading cause of cancer mortality and a serious global problem. Many studies have tried to identify the factors responsible for GC, but the exact sequence of molecular events involved in the development of GC remains unclear. In areas of high GC prevalence, most cases are related to *H. pylori* infection, and GC develops through several stages, including infection, gastric atrophy, intestinal metaplasia and dysplasia. There is a lot of evidence to support the key role of mucins in development of GC. This review focused on studies of the association between polymorphisms in mucin genes and development of GC. The strength of such an association varied among the studies. The diversity in study populations and lifestyle, as well as sample size may account for this inconsistency. For example, functional SNP rs4072037 in *MUC1* gene may affect the development of GC, but the effects seem to be stronger in Asian populations. Future association studies need global collaboration to expand sample size and identify more susceptibility polymorphisms. However, lifestyle factors should be taken into account to ensure accurate and significant results. Such studies will identify useful biomarkers for early detection of GC, with the potential for better disease prevention through selective treatment and surveillance of individuals harboring high-risk genetic profiles.

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Immunotherapeutic approaches in biliary tract carcinoma: Current status and emerging strategies

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Abstract

For biliary tract carcinoma (BTC), complete surgical resection of tumor is only feasible in a minority of patients, and the treatment options for patients with unresectable or metastatic disease are limited. Advances in cancer immunology have led to identification of tumor-infiltrating immune cells as indicators of prognosis and response to treatment in BTC. This has also facilitated development of immunotherapy that focuses on enhancing the immune system against biliary tumors. This includes peptide- and dendritic cell-based vaccines that stimulate *in-vivo* immune responses against tumor-specific antigens. Adoptive immunotherapy, which entails the *ex-vivo* expansion of tumor-infiltrating immune cells for subsequent reintroduction, and cytokine-based therapies have been developed in BTC. Clinical studies indicate that this type of therapy is generally well tolerated. Combination therapy with dendritic cell-based vaccines and adoptive immunotherapy has shown particularly good potential. Emerging strategies through discovery of novel antigen targets and by reversal of tumor-associated immunosuppression are expected to improve the efficacy of immunotherapy in BTC. Collaborative efforts by integration of targeted immunotherapeutics with molecular profiling of biliary tumor will hopefully make a positive impact on advancing towards the goal of developing precision treatment of patients with this highly lethal disease.

Key words: Adoptive immunotherapy; Cancer vaccines; Biliary tract carcinoma; Cholangiocarcinoma; Gallbladder carcinoma; Immunotherapy; Precision treatment

Core tip: Advances in cancer immunology have led to development of novel therapeutics that focuses on enhancing the immune system against biliary tract cancer. These include peptide- or dendritic cell-based vaccines, adoptive immunotherapy, and immunostimulatory cytokines. Immunotherapy is generally well tolerated with good potential for developing into treatment. The efficacy of immunotherapy may be improved by

reversal of tumor-associated immunosuppression and through discovery of novel antigen targets. Integration of targeted immunotherapeutics with molecular profiling of biliary tumor is expected to make a positive impact on advancing towards the goal of developing precision treatment of patients with this highly lethal disease.

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INTRODUCTION

Cholangiocarcinoma and gallbladder adenocarcinoma are the most common primary malignancies of the biliary tract. Collectively referred to as biliary tract carcinoma (BTC), these diseases are a cause of substantial morbidity and mortality. Each year in the United States alone, approximately 11000 patients are diagnosed with BTC and 3700 lives are claimed by the disease[1].

Until recently, the treatment options available to patients with BTC primarily involved surgery, radiation, and systemic chemotherapy. Complete surgical resection is potentially curative, but it can only be achieved in the 10% of patients who present with localized disease without vascular invasion[2]. Patients with BTC that is locally advanced, metastatic, or recurrent are typically offered single agent or combination chemotherapy, depending upon performance status. Typical regimens consist of gemcitabine, 5-fluorouracil, and platinum-based agents[3]. Despite these interventions, clinical outcomes in BTC are generally poor. Fewer than 5% of patients with cholangiocarcinoma[2] and 13% with gallbladder cancer[4] survive longer than two years following diagnosis.

Advances in cancer immunology and immunotherapy have facilitated the development of additional treatment options that bring new hope to patients with BTC. This new generation of therapeutics seeks to strengthen the patient's immune system in combating malignancy, typically by priming it against tumor-specific antigens. Such treatments are more selective against malignant cells and therefore tend to be less toxic than traditional chemotherapy. Furthermore, by exerting an antitumor effect indirectly through the immune system rather than *via* direct activity against malignant cells, these therapeutic approaches can produce durable responses that persist long after the drug itself has been metabolized.

In this article, we concisely review cancer immunology as it relates to malignancies of the biliary tract. The immunotherapeutic approaches that are being investigated for use in BTC will be described, along with the data from clinical trials that have been completed thus far. We will also discuss ongoing clinical trials and emerging strategies for immunotherapy in BTC.

CANCER IMMUNOLOGY IN BILIARY TRACT CANCER

Focusing and enhancing the antineoplastic effects of the immune system as treatment for BTC has only recently become a subject of concerted investigation. Evidence suggests that at the earliest stages of tumor development, the host immune system is capable of both detecting and controlling the disease. Over time, however, this generates evolutionary pressure that favors the proliferation of cancer cells that are less immunogenic or otherwise capable of suppressing the host immune response[5-9]. Despite this, there often persists a small cohort of immune cells that remain able to identify and invade the tumor. The characteristics of this immune infiltrate are of prognostic value in a variety of malignancies, including BTC[10,11]. The frequency and clinical significance of tumor infiltration by the cellular mediators of the host immune response is summarized in Table 1.

Tumor infiltration by the innate immune system

The innate immune system, consisting of the complement cascade, natural killer (NK) cells, granulocytes, and phagocytes, mounts an initial non-specific defense against infections and malignancy. The frequency of tumor infiltration by the cellular components of the innate immune system is highly variable. While fewer than half of biliary tumors are penetrated by NK cells[12,13] or mast cells[13], macrophages are observed in the majority of BTC[13].

Despite correlating with outcomes in a host of other malignancies[16-20], infiltration of BTC by the innate immune system appears to be of little clinical significance. Neither the presence of intratumoral NK cells nor mast cells is correlated with clinical outcomes[12]. The density of tumor-infiltrating macrophages, however, appears to increase as lesions progress from pre-malignant precursors to invasive malignancy and later to metastatic disease[13]. This is believed to be the result of activated macrophages releasing pro-inflammatory and pro-angiogenic cytokines that facilitate tumor growth. These include tumor necrosis factor-α, vascular endothelial growth factor A, and granulocyte macrophage colony-stimulating factor[21,22].

Tumor infiltration by the adaptive immune system

The adaptive immune response is initiated by the consumption of foreign material by antigen presenting cells, most often dendritic cells. After processing the antigen for presentation, dendritic cells migrate to lymph nodes where they stimulate the proliferation of antigen-specific lymphocytes and recruit $CD4^+$ T-helper cells. Activated $CD4^+$ cells release cytokines that induce the differentiation of B-lymphocytes into antibody-releasing plasma cells, and activate cytotoxic $CD8^+$

Table 1 Cellular mediators of innate and adaptive immune system in biliary tract carcinoma

Cell type	Frequency of infiltration	Clinical significance	Ref.
Natural killer cells	19.1%-33% overall 20% of ICC, 21% of ECC, 16% of GBC	No correlation with disease stage, grade, or survival	[12,13]
Mast cells	2% of ICC, 2.5% of ECC, 8.5% of GBC	No correlation with survival	[13]
Macrophages	87% of ICC, 70% of ECC, and 71% of GBC	Associated with more advanced disease	[13]
Dendritic cells	Not determined	Associated with improved survival	[12,14]
$CD4^+$ helper T-lymphocytes	43% of ICC, 30% of ECC, and 34%-51% of GBC	Associated with reduced probability of metastases and improved survival in ECC	[12,13]
$CD8^+$ cytotoxic T-lymphocytes	46% of ICC, 49%-55% of ECC, and 38%-51% of GBC	Associated with reduced probability of metastases and improved survival in ECC	[12,13,15]
B-lymphocytes /plasma cells	4.5% of ICC, 6.7% of ECC, and 10.1% of GBC	Associated with improved survival	[13]

ECC: Extrahepatic cholangiocarcinoma; GBC: Gallbladder carcinoma; ICC: Intrahepatic cholangiocarcinoma.

T-lymphocytes (CTL). After clearing the antigen, both $CD4^+$ and $CD8^+$ T cells may differentiate into memory T-cells that organize an expedited secondary immune response if the offending antigen is encountered again. It is these memory cells that form the physiologic basis for vaccination.

Like the innate immune system, there is considerable variability in the frequency of tumor infiltration by cells of the adaptive immune system. Although the exact percentage of BTC that contains dendritic cells is not clear, their presence appears to be nearly universal in both GBC[12] and cholangiocarcinoma[14]. Approximately 30%-50% of BTC is infiltrated with $CD4^+$ or $CD8^+$ T-lymphocytes[12,13]. Tumor infiltration by B-lymphocytes or plasma cells is seldom observed[13], which may be attributed to the tendency for these cells to rarely migrate outside of lymph nodes.

Tumor infiltration by the cellular mediators of the adaptive immune response is generally correlated with improved outcomes in BTC. The presence of dendritic cells[12,14], $CD4^+$ T-cells[12], $CD8^+$ T-cells[12,15], or plasma cells[13] within a biliary tumor is predictive of improved OS. This trend towards more favorable prognosis is consistent with findings in other malignancies, such as colorectal[23] and esophageal carcinoma[24]. Though it has not been reported in BTC, the subset of $CD3^+$ T-cells in colorectal cancer suggests that these cells are possibly involved in vitamin D-mediated immunoprevention[25].

IMMUNOTHERAPEUTIC APPROACHES IN BTC

While the endogenous immune response is initially successful in slowing the growth of BTC, the malignancy eventually becomes capable of evading the immune system. This occurs through intense evolutionary pressure that confers a survival advantage to cancer cells that lack foreign antigens, secrete immunosuppressive substances, or otherwise limit the effectiveness of the host immune system[5-9]. Several approaches for potentiating or redirecting the immune response to BTC are being investigated. Vaccines based upon either peptides or dendritic cells seek to sensitize the immune system against tumor-specific antigens. The extraction, amplification, and reintroduction of a patient's own tumor-infiltrating immune cells *via* adoptive immunotherapy is being evaluated. Treatment using immunostimulatory cytokines has been attempted.

Targets of vaccination

Through the controlled presentation of a particular antigen, vaccination primes the immune system to respond swiftly and accurately to repeat exposures in the future. This occurs, in part, through the production of memory T-cells that orchestrate this secondary response. As a result, the effectiveness of vaccination is a function of both the immune system's strength and the selection of a proper target antigen. Ideally, the target should be highly specific to malignant cells and strictly conserved within the tumor. This ensures that collateral damage to normal tissues will be minimized, while also reducing the likelihood that an antigen-negative cancer cell will arise to repopulate the tumor.

One antigen that largely fulfills these criteria is Wilm's Tumor protein 1 (WT1)[10], a transcription factor that is normally involved in urogenital development. This protein also functions as a tumor suppressor through interactions with platelet derived growth factor receptor, epithelial growth factor receptor, c-MYC, and B-cell lymphoma 2[26]. Approximately 68%-80% of biliary tumors harbor mutations of WT1[26]. While the clinical significance of mutated WT1 in BTC remains unclear, similar mutations are known to correlate with poor prognosis in testicular cancer[27], breast cancer[28], and squamous cell carcinoma of the head and neck[29].

Another potential target for immunization is the glycoprotein, mucin protein 1 (MUC1)[10]. Consisting of a large and heavily glycosylated extracellular domain, MUC1 forms the hydrophilic barrier that is characteristic of BTC and other types of adenocarcinoma. This mucinous shell repels hydrophobic chemotherapeutics and obstructs immune cells, while also allowing the tumor to immerse itself in growth factors[30]. MUC1 is over-expressed in 90% of gallbladder carcinoma[31] and 59%-77% of cholangiocarcinoma[31-34]. Excessive production of MUC1 in BTC is typically indicative of more

advanced disease[32] and impaired OS[31-33].

Peptide-based vaccines and personalized peptide vaccination

Peptide-based vaccines are among the most investigated class of cancer immunotherapy. The vaccine typically contains one or more antigens that are heavily expressed by malignant cells and often emulsified in Freund's adjuvant to increase immunogenicity. The goal of immunization is to stimulate mass-production of memory lymphocytes that can generate a strong secondary immune response against cancer cells that bear the particular antigen.

The efficacy of any single peptide-based vaccine is intrinsically limited, however, by the heterogeneity of BTC. Although the overall expression of certain antigens, such as WT1 and MUC1, is often increased within biliary tumors, the distribution of these antigens is non-uniform. While some cells over-express the antigen, there are often others from which it is entirely absent. Furthermore, the tenacity with which the immune system responds to these antigens varies widely between patients, even among those with similar HLA types[35]. This is due, in part, to differences in the number of lymphocyte precursors that are maximally sensitive to the particular antigen[36].

Personalized peptide vaccination seeks to overcome these limitations by immunizing patients against multiple antigens simultaneously. While it is likely that a tumor will harbor cells that lack any single antigen, the odds are exponentially less that any single cell will lack each of 3 to 4 antigens that are individually quite common. This has the additional benefit of theoretically counteracting the pressure of selection for tumor cells that lack the target antigens[35]. To bypass individual differences in sensitivity to particular antigens, it is possible to measure the frequency of antigen-sensitive CTL precursors within each patient. They may then be vaccinated against only the antigens to which they will most likely respond[36].

Dendritic cell-based vaccines

Similar to their peptide-based counterparts, dendritic cell-based vaccines expose the immune system to an antigen with the goal of generating memory lymphocytes that will produce a robust secondary immune response. Rather than simply introducing a peptide that requires subsequent processing and presentation to the adaptive immune system, these vaccines contain dendritic cells that are already loaded with antigen. These vaccines may be prepared against a particular antigen or more generally against a tumor lysate. While the latter approach stimulates the immune system against a larger number of antigens and theoretically produces a greater antitumor response, it may also carry a risk of autoimmunity. While the use of dendritic cells-based vaccines against BTC remains in its infancy, the success of sipuleucel-T in treating prostate cancer[37] demonstrates the promise that these therapeutics may someday fulfill.

Adoptive immunotherapy

Unlike the treatments described previously, adoptive immunotherapy is not intended to produce an *in-vivo* immune response. Instead, a patient's own tumor-infiltrating lymphocytes are extracted, modified, and induced to clonally proliferate *ex-vivo.* This expanded population of tumor-specific immune cells is then reintroduced, and they migrate back to the tumor and continue to combat its growth. The effectiveness of this treatment may be further increased by depleting the patient's existing lymphocyte population with cytotoxic chemotherapy in advance of returning the grafted lymphocytes. This is believed to prolong the lifespan of the transplanted cells.

Immunostimulating cytokines

The cytokine, interleukin-2 (IL2) is a potent anti-neoplastic agent due to its ability to stimulate the proliferation and cytotoxic effects of $CD8^+$ T-lymphocytes[38-40]. Administering IL2 as a monotherapy or in combination with adoptive immunotherapy is an effective treatment for certain malignancies, such as melanoma[41,42] and renal cell carcinoma[42,43]. Treatment with IL2 is associated with a substantial side effect profile that includes nephrotoxicity, extravasation of fluid secondary to increased vascular permeability, and rarely transient myocarditis[40,41].

CLINICAL STUDIES OF IMMUNOTHERAPY IN BTC

Each type of immune-based approach described above has been evaluated for therapeutic efficacy in patients with BTC. Many of these agents have been studied as monotherapy as well as in combination with traditional chemotherapy or targeted therapeutics. The completed clinical trials of immunotherapy in BTC are described below and the compiled data are summarized in Table 2.

Peptide-based vaccines

To date, most clinical studies of immunotherapy in BTC have focused on peptide-based vaccines, often targeted against WT1 or MUC1. This type of treatment is generally well tolerated; however it appears to exert only a modest anti-neoplastic effect when administered as monotherapy.

Vaccines against WT1 are often administered in combination with gemcitabine based chemotherapy. Preclinical studies suggest that gemcitabine upregulates the expression of WT1, thereby theoretically enhancing the effect of immunization[53]. In a phase I trial, anti-WT1 vaccination and gemcitabine were administered to patients with unresectable gallbladder cancer, cholangiocarcinoma, or pancreatic adenocarcinoma[44]. This regimen increased the number of WT1-specific

Table 2 Trials of immunotherapy in biliary tract carcinoma

Immunotherapy	Treatment regimens	Phase	*n*	Types of BTC	OS (mo)	PFS (mo)	Ref.
Peptide-based vaccine (WT1)	Peptide vaccine + gemcitabine	I	25	Pancreatic, GBC, ICC, ECC	9.3	--	[44]
Peptide-based vaccine (WT1)	Peptide vaccine monotherapy	I	9	Pancreatic, CC	--	--	[45]
Peptide-based vaccine (NUF2, CDH3, KIF20A)	Peptide vaccine triple therapy	I	9	GBC, ICC, ECC	9.7	3.4	[46]
Peptide-based vaccine (LY6K, TTK, IGF2BP3, DEPDC1)	Peptide vaccine quadruple therapy	I	9	GBC, ICC, ECC	12.3	5	[47]
Peptide-based vaccine (Many)	Personalized peptide vaccination +/- chemotherapy	II	25	GBC, ICC, ECC	6.7	--	[48]
Dendritic cell-based vaccine (MUC1)	Dendritic cell vaccination +/- chemotherapy +/- radiotherapy	I / II	12	Pancreatic, CC	26	--	[49]
Dendritic cell-based vaccine (WT1, MUC1)	Peptide vaccine +/- chemotherapy	--	65	GBC, ICC, ECC	--	--	[50]
Dendritic cell-based vaccine, adoptive immunotherapy	Surgery + dendritic cell vaccine + T-cell transfer *vs* surgery alone	--	36	ICC	31.9	18.3	[51]
Interleukin-2	Induction cisplatin + gemcitabine, consolidation capecitabine + radiation, and maintenance IL-2 + 13-cis-retinoic acid	II	54	Pancreatic, GBC, CC	> 27.5	16.2	[52]

CC: Cholangiocarcinoma; OS: Overall survival; PFS: Progression-free survival; WT1: Wilm's tumor 1; NUF2: Cell division cycle associated protein 1; CDH3: Cadherin 3; DEPDC1: DEP domain containing 1; ECC: Extrahepatic cholangiocarcinoma; GBC: Gallbladder cancer; ICC: Intrahepatic cholangiocarcinoma; IGF2BP3: Insulin-like growth factor-II mRNA binding protein 3; KIF20A: Kinesin family member 20A; LY6K: Lymphocyte antigen 6 complex locus K; MUC1: Mucin 1.

lymphocytes in circulation, but it did not improve clinical outcomes or increase toxicity over that which is expected from gemcitabine monotherapy. At the present time, a phase Ⅱ study of WT1 vaccination as an adjunct to combination chemotherapy with gemcitabine plus cisplatin is underway[53]. This study aims to establish the 1-year OS rate for patients receiving treatment.

Similar to WT1, peptide-based immunization against MUC1 is well tolerated but it lacks definite proof of clinical efficacy. In a phase Ⅰ trial of nine patients with advanced stage cholangiocarcinoma or pancreatic adenocarcinoma, monotherapy with peptide-based vaccines against MUC1 produced only a single instance of stable disease[45]. Despite failing to influence outcomes, vaccination did generate a robust anti-MUC1 IgG response in 78% of patients with negligible toxicity. In the future, vaccination against MUC1 could fill a niche in addition to gemcitabine or fluorouracil-based chemotherapy. This is because preclinical studies have found that these agents increase the expression of MUC1 in cholangiocarcinoma cells[53]. Further research is indicated to determine the safety and efficacy of such regimens.

The prospect of combination therapy with multiple peptide-based vaccines has been explored. Triple therapy with vaccines against cell division cycle associated protein 1 (NUF2), cadherin 3 (CDH3), kinesin family member 20A in patients with GBC, ICC, and ECC was investigated in a phase Ⅰ clinical trial[46]. This treatment stimulated peptide-specific T-cell responses in all patients and 55% achieved stable disease. A four vaccine regimen against lymphocyte antigen 6 complex locus K (LY6K), TTK protein kinase, insulin-like growth factor-Ⅱ mRNA binding protein 3, and DEP domain containing 1 has also been tested in a phase Ⅰ trial of nine patients with BTC[47]. Peptide specific T-cell responses were generated in 78% of patients receiving this regimen and clinical responses were observed in 67%. In both trials of combination therapy with peptide-based vaccines, the presence of an injection site reaction correlated with OS[46,47]. This underscores the reliance of this treatment upon provoking a strong immune response to generate an anti-tumor effect. Aside from these local dermatologic reactions, treatment-associated toxicity was minimal.

The efficacy of combination vaccination may be refined by individualizing the process by which targets are selected. This approach of personalized peptide-based vaccination was assessed in a phase Ⅱ trial of 25 patients with either gallbladder adenocarcinoma or cholangiocarcinoma[48]. Patients received as many as 4 of 31 possible vaccines in addition to systemic chemotherapy, if their performance status could support such treatment. This regimen produced stable disease in 80% of patients and negligible toxicity beyond that which is typically associated with chemotherapy.

Dendritic cell-based vaccines

Immunotherapy with antigen-pulsed dendritic cells is exceptionally well tolerated, and it appears to be efficacious against BTC. In a combined phase Ⅰ / Ⅱ trial, 12 patients with BTC or pancreatic adenocarcinoma received an anti-MUC1 dendritic cell-based vaccine following tumor resection and, in some instances, chemoradiation[49]. A median OS of 26 mo was observed, while 33% of patients survived longer than 50 mo without evidence of disease recurrence. While this study was not designed to differentiate between durable responses that occur due to vaccination and those that arise from complete surgical resection, it is conceivable

Table 3 Ongoing clinical trials of immunotherapy in biliary tract carcinoma

Agent	Treatment regimen	Phase	Estimated date of completion	Sponsoring Institution	Identification number
Cytokine induced killer cells	Cytokine induced killer cell monotherapy	Ⅰ / Ⅱ	May, 2016	Siriraj Hospital	NCT01868490
Tumor infiltrating lymphocytes	Tumor infiltrating lymphocytes + IL-2 + cyclophosphamide + fludarabine	Ⅱ	December, 2019	National Cancer Institute	NCT01174121
Poly-ICLC	Cyclophosphamide + radiation therapy + TACE + poly-ICLC	Ⅰ / Ⅱ	July, 2014	Rutgers, the State University of New Jersey	NCT00553683

IL-2: Interleukin-2; Poly-ICLC: Polyinosinic-polycytidylic acid polylysine carboxymethylcellulose; TACE: Transcatheter arterial chemoembolization.

that the combination of adjuvant chemotherapy, radiation therapy, and immunotherapy eliminated microscopic residual disease after surgery.

In another trial, dendritic cell-based vaccines against WT1 and/or MUC1 in combination with chemotherapy was evaluated in 65 patients with unresectable, metastatic, or recurrent BTC[50]. This regimen was well tolerated and 15% of patients had stable disease following 6 mo of treatment. Although the response rate did not differ between patients who were vaccinated against one or both targets, the correlation between post-immunization fever and improved survival does suggest the responses generated by this regimen may be at least partially attributed to immune activation.

Adoptive immunotherapy

Direct transfer of cellular immunity *via* adoptive immunotherapy has also been investigated for use in BTC. In a study of 36 patients with intrahepatic cholangiocarcinoma, surgery alone was compared to surgery followed by combination adoptive immunotherapy with tumor-lysate pulsed dendritic cells and transfer of activated T-cells[51]. Patients who received adjuvant immunotherapy experienced nearly double the OS of those treated with surgery alone with minimal toxicity. Among the 16 patients who produced the largest injection site reaction, median OS was 95.5 mo.

Similar durable and dramatic responses to combined immunotherapy with dendritic cell-based vaccines and activated T cell transfer have been described in case reports of patients with cholangiocarcinoma[54] and gallbladder cancer[55]. Anecdotal evidence also suggests that combining T-cell based adoptive immunotherapy with cetuximab may have activity against malignant ascites and peritoneal carcinomatosis due to metastatic cholangiocarcinoma[56].

IL2 maintenance therapy

The use of IL2 as a maintenance therapy was explored in a multicenter phase Ⅱ trial of 54 patients with pancreatic adenocarcinoma or BTC[52]. These patients initially received 3 cycles of combination chemotherapy with cisplatin and gemcitabine as induction therapy. Patients who remained progression-free were subsequently treated with concurrent capecitabine and radiotherapy as consolidation, followed by maintenance IL2 and 13-cis-retinoic acid. The progression-free survival (PFS) and overall survival (OS) for all patients enrolled in this study was 6.8 and 12.1 mo, respectively. Outcomes were notably better when considering only the subset of patients who were able to complete the entire course of treatment, however, with median PFS of 16.2 mo and OS that had not yet been reached after a median follow-up of 27.5 mo. Further investigation will be needed to determine whether this differential survival is truly due to a response to treatment, or if those patients simply had more indolent disease independent of therapy.

ONGOING CLINICAL TRIALS OF IMMUNOTHERAPY IN BTC

Currently, several clinical trials of immunotherapy in malignancies of the biliary tract are ongoing and as listed in Table 3. These studies utilize different immunotherapeutic approaches. In one study, cytokine induced killer cells are employed as monotherapy. In another study, adoptive transfer of tumor-infiltrating lymphocytes is combined with IL2 and chemotherapy. In attempt to reverse systemic immunosuppression, the immunomodulatory agent, polyinosinic-polycytidylic acid polylysine carboxymethylcellulose, is used in combination with chemotherapy and radiation therapy. In those two studies involving chemotherapy, low-dose metronomic cyclophosphamide is used to eliminate the immunosuppressive regulatory T lymphocytes (T_{reg}) and prevent tumor-associated angiogenesis.

CONCLUSION

Immunotherapy in BTC has been under active investigation and tremendous opportunities exist for developing it into a safe and effective treatment of patients with this disease. Clinical studies indicate that this type of therapy is generally well tolerated. The efficacy of immune-based treatment of BTC is improving as the complex interactions between the immune system and biliary tumors are better understood. Combination therapy with dendritic cell-based vaccines and adoptive immunotherapy has shown particularly good potential. Several directions for future investigation of immunotherapy that may improve the clinical

outcomes of patients with this disease are described as follows.

Preliminary studies suggest that the distribution and types of immune cells that infiltrate biliary tumors may be used to predict the likelihood that an individual tumor will respond to a particular chemotherapy regimen[57]. Further characterizing these associations could be clinically beneficial, as it would provide a physiologic basis for selecting therapy as an adjunct to the current paradigm that relies upon tumor histology and stage. On the other hand, application of mass spectrometry and genomic sequencing to discover new antigens[58] may help facilitate development of novel strategies for targeted immunotherapy in BTC. Furthermore, evidence suggests that increased inflammatory signaling *via* IL6 is associated with reduced response to vaccination[36,48]. The hypothesis that addition of the IL6 receptor antagonist tocilizumab enhances the effects of vaccination remains to be tested.

Besides, tumor evasion of the immune system is often mediated by cytotoxic T-lymphocytes associated antigen 4 (CTLA4) or the interaction between programmed cell death 1 (PDCD1, also known as PD1 or CD279) and its ligand (PDCD1LG1, also known as PDL1 or CD274)[9]. It will be important to investigate the potential of blocking these immunosuppressive pathways with monoclonal antibodies in conjunction with the currently used immunotherapeutic approaches in BTC. The anti-CTLA4 antibody ipilimumab has shown great promise in other malignancies such as melanoma[59], but it has not yet been studied in BTC. Similarly, pembrolizumab and nivolumab, monoclonal antibodies that target PD1/CD279 signaling have been found to improve anti-tumor T-cell response and induce tumor regression in subsets of patients with melanoma, renal cell carcinoma, and non-small-cell lung cancer[8,60,61]. Preclinical studies suggest that immunohistochemical analysis for PDL1/CD274 in biliary tumors may help identify the patients who are likely to benefit from such therapeutics[62].

The synergistic relationships between cytotoxic chemotherapy and immunotherapy deserve further investigation for treatment of BTC. In one study, gemcitabine, which is a mainstay of treatment in BTC, was found to enhance cell-mediated immunity *via* increased expression of HLA on malignant cells[63]. Platinum-based agents have a similar effect on HLA expression, while also reducing PDL2/CD273-mediated suppression of antigen-specific T-lymphocytes[64]. It is plausible that the addition of gemcitabine and cisplatin to immunotherapy could further improve the treatment responses.

Ultimately, the goal is to combine the advances in cancer immunotherapy with those of targeted therapy and molecular profiling to develop precision treatment for improving the clinical outcomes of patients with this highly lethal disease.

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Current status of familial gastrointestinal polyposis syndromes

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Abstract

Because of the rarity of familial gastrointestinal cancer-predisposing syndromes, their exploration in literature is not extensive. In this review, an update of the clinicopathological and molecular criteria of gastrointestinal familial polyposis syndromes with potential malignant transformation is performed. In addition, a guide for screening and surveillance was synthesized and a distribution of gene mutations according to the specific syndromes and geographic distribution was included. The following inherited polyposes syndromes were analyzed: familial adenomatous polyposis, the hamartomatous familial polyposes (Juvenile polyposis, Peutz-Jeghers syndrome, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, hereditary mixed polyposis syndrome, Gorlin syndrome, Birt-Hogg-Dube syndrome, neurofibromatosis type I and multiple endocrine neoplasia syndrome 2B), Li-Fraumeni syndrome, and MUTYH-associated adenomatous polyposis. For proper medical care, subspecialization of gastroenterologists, pathologists, and geneticists in the field of familial diseases should be introduced in the medical curriculum.

Key words: Inherited polyposis syndromes; Hereditary cancer; Stomach; Intestine; Colorectal

Core tip: In this review the clinicopathological and histological aspects of inherited polyposes syndromes of the gastrointestinal tract are explored in detail. In addition, a guide for surveillance is proposed.

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INTRODUCTION

The familial cancer-predisposing syndromes of the

gastrointestinal tract are heterogeneous groups of diseases with the lifetime risk of gastrointestinal cancer generally low but their associated morbidities should be very attentively examined for developing specific programs of familial screening. Because these syndromes are relatively rare in the daily activity, management of their diagnosis and therapy is difficult.

These syndromes include, in particular, the following inherited polyposes syndromes: familial adenomatous polyposis (FAP), hamartomatous polyposis syndromes (Juvenile polyposis, Peutz-Jeghers syndrome, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, hereditary mixed polyposis syndrome, Gorlin syndrome, Birt-Hogg-Dube syndrome, neurofibromatosis type Ⅰ, and multiple endocrine neoplasia syndrome 2B), Li-Fraumeni syndrome, and MUTYH-associated adenomatous polyposis. They are usually diagnosed from the stomach to the rectum, the esophagus and anal canal being only secondarily involved[1-30]. Although Cronkhite-Canada- and Proteus syndrome[22] are also polyposis syndromes of the gastrointestinal tract, they do not present familial predisposition and are not included in this paper.

In this review, an update of clinicopathological criteria used for diagnosis of the inherited cancer-predisposing syndromes of the gastrointestinal tract and identification of eligible families was performed, followed by revision of criteria of screening and surveillance in the daily practice. A synthesis of data regarding the molecular profile of hereditary syndromes and their geographic particularities are synthesized in Table 1, based on our experience and literature data[1-36].

CLINICOPATHOLOGICAL AND MOLECULAR FEATURES

FAP

FAP is a rare autosomal dominant syndrome (1:8300 live births), that is characterized by the presence of hundreds to thousands of adenomatous polyps scattered throughout colorectal mucosa[36] (Figure 1). It is produced through mutations of the adenomatous polyposis coli (*APC*) gene that was firstly described in 1991[1]. The risk for rectal adenocarcinomas is 87% up to 45 years of age and rise by 100% in older ages, but other colorectal segments can also be affected[1,28]. FAP-related colorectal cancer (CRC) represent < 1% of all CRC cases[36].

Other extracolonic associated lesions include small bowel, periampullary and gastric adenomatous polyps, adrenal adenomas and carcinomas[32]. The lifetime risk of occurrence of duodenal polyps is almost 100%[28]. The second and third portion of duodenum, including the periampullary region, are more predisposped to present adenomas[28].

Regarding the stomach, the adenomatous polyps were reported to occur in 12%-84% of patients with FAP but less than half of them are focally dysplastic and below 1% present malignant transformation[2,3]. They are located mostly in the antrum, followed by gastric fundus[2,28]. However, fundic gland polyps can also occur sporadically not only within FAP[2]. The reported incidence of sporadic fundic gland polyps is about 1%-2% of all middle-aged healthy females who underwent upper endoscopy, more rare in males (30% of all cases) while the familial ones are usually multiple, occur at younger ages, and have an equal gender distribution[3]. Microscopically, the fundic gland polyps consist of cystically dilated oxyntic glands lined by parietal cells, chief cells, and neck cells, with apical mucin bubbles[2,4,5]. Dysplasia occurs in the covering neck cells and/or foveolar epithelium and dysregulation of epithelial proliferation is immunohistochemically (IHC) proved by loss of the normal inverse topographic distribution of Ki-67 proliferation marker and the cyclin-dependent kinase inhibitor p21 (WAF1/CIP1)[2,4-6]. In these cases, for unknown reasons, a more increased risk for gastric intestinal-type adenocarcinomas have been reported in Japanese and Korean populations (four-fold) while no significant risk, when compared with the general population, was encountered in the Western countries (two-fold)[2,4-6]. Although FAP syndrome is not rare in Romanian patients, we did not have cases with associated gastric lesions (personal communication).

Gardner's syndrome is a variant of FAP characterized by APC mutation-related gastrointestinal polyps and associated osteomas, dental abnormalities (supranumerary teeth), epithelial and mesenchymal tumors of the skin (epidermoid cysts, lipoma, fibroma, leiomyoma), desmoid tumors (most frequently in the abdominal wall or intra-abdominal), congenital hypertrophy of the retinal pigment epithelium and tumors of the thyroid gland[28,32,34]. Congenital hypertrophy of the retinal pigment epithelium is the commonest extracolonic manifestation of FAP that occurs in 70%-80% of patients[28]. It is characterized by occurrence of gray-brown round lesions in the retina, the clinical significance being not known yet[28].

In Turcot's syndrome, the FAP is associated with tumors of the central nervous system, especially medulloblastoma[32].

The attenuated *FAP* (AFAP) is a less severe form of FAP that is characterized by predominance of proximally located polyps of the colon (10-99 adenomatous polyps), a later age of onset and a lower risk (lifetime cumulated risk < 70%) for developing CRC[7,32].

MUTYH-associated polyposis

It is an autosomal recessive syndrome produced through mutations of the mutY homolog (*MUTYH*) gene that was firstly described in 2002 in three members of a British family[27,28,35]. MUTYH-associated polyposis (MAP) is clinically similar to the AFAP, being characterized by the early-onset of multiple adenomatous polyps of the colorectal segments (10-99 adenomatous or serrated polyps), with risk for malignant transformation,

Table 1 The molecular profile and geographic particularities of inherited gastrointestinal cancer-predisposing syndromes[1-36]

Name of the syndrome	Mutated genes	Type of mutation	Geographic particularities
FAP	*APC*: Exon 15 - first half (54% of patients with FAP)	Classic phenotype: mutations between codons 178 and 309, and between 409 and 1580 (exons 5-8 and 9-14) Germline truncation (C > T), especially at codons 1309 and 1061: Nonsense mutations (28%) Small insertions (10%) Small deletions (46%)	NS
	APC: Chromosome arms 5q, 8p, 17p and 18q	LOH	NS
	β-catenin: Exon 3 (15%)	NS	NS
	APC/*β-catenin* (28%)	NS	NS
	K-ras: Codon 12 (3%) - associated mutation	GGT to TGT/GTT	NS
Gardner syndrome	*APC*: Long arm of chromosome 5	Interstitial deletion	NS
	APC: Patients with congenital hypertrophy of the retinal pigment epithelium	Truncating mutations between codons 311 and 1465	NS
	APC: Patients with desmoid tumor	Downstream codon 1400 (1445-2011)	NS
	APC: Patients with gastro-duodenal adenomas	Mutations at the 3′ before codon 1395 and between codons 564 and 1493	NS
	APC: Patients with hepatoblastomas	Mutations at the 5′ to the mid region between codons 141 and 1751	NS
	APC: Patients with thyroid tumors	Mutations between codons 140 and 1309	NS
AFAP	*APC*	Somatic G:C→T:A	NS
	APC: Exons 3 and 4 (5′ end of the gene), exon 9, and the very 3′ end of the gene beyond codon 1595	Truncating mutation	NS
	APC: Variants	Missense mutations I1307 K N1026S E1317Q	I1307K: almost exclusively in Ashkenazi Jewish descendents - detected in 6% of all family members, with 10%-20% lifetime risk of developing CRC N1026S: Identified in one Spanish AFAP family (all members) E1317Q: NS
MUTYH-associated polyposis	*MUTYH*: Located on the chromosome 1p34.3-p32.1, contains 16 exons	Germline biallelic inactivation	Absent in Asia (Japan, Taiwan, South Korea)
	MUTYH variants	Missense mutations: p.Y179C - exon 7 (c.536A > G; p.Tyr179Cys) p.G396D - exon 13 (c.1187g > A;p.Gly396Asp)	Specific for Eastern, Southern, and Central Europe, North America, European inhabitants from Canada, and Sephardi Jews Absent in Finland, India, Pakistan, Tunisia, Singapore, and Ashkenazi Jewish
		Missense mutation p.Ala385ProfsX23 p.E410GfsX43 Missense mutation p.Y104X Missense mutation p.E480X Heterozygous mutations	Specific for Northern Europe Specific for Tunisia Specific for Pakistan Specific for India Asia (Japan, Taiwan, South Korea): p.Arg19; p.Arg109Trp; p.Gly286Glu Southern Europe: p.Glu480del Pakistan: p.Tyr104 India: p.Glu480
	K-ras: Codon 12 - associated mutation (64%), usually in patients with sessile serrated adenomas	c.34G > T	NS
Juvenile polyposis syndrome (pure type)	*MADH4*/*SMAD4*/*DPC4*: Chromosome 18q21.1 (30%)	NS	NS
	BMPR1A: Chromosome 10q23 (20%-30%)	Large deletions	NS
	Other genes (49%)	NS	NS
	ENG: exons 11, 12		
	PTEN: chromosome 10q23.3		
Juvenile polyposis + hemorrhagic telangiectasia	*MADH4*/*SMAD4*/*DPC4*: Chromosome 18q21.1	NS	NS
	STK11: Chromosome 19p13.3 or 19q13.4 (50%-94%)	NS	NS
Peutz-Jeghers syndrome	*TGF-β*	NS	NS
	PTEN: Chromosome 10q23.3		

Peutz-Jeghers syndrome	*ALK1/ACVRL1*	NS	NS
+ primary pulmonary hypertension		NS	NS
Cowden syndrome	*PTEN*: Chromosome 10q23.3 (13-85%)	Nonsense mutations missense mutations frameshift mutations Large deletions	NS
Bannayan-Riley-Ruvalcaba syndrome	*PTEN*: Chromosome 10q23.3 (60%-65%)	NS	NS
Hereditary mixed polyposis syndrome	*BMPR1A*: Chromosome 10q23	NS	NS
	GREM1	NS	NS
Li-Fraumeni syndrome – classic type	*p53*: Exons 4-9 (23%-50%)	NS	NS
Unclassified/ unexplained polyposis syndromes (50%)	*PTEN*: Chromosome 10q23.3	Nonsense mutations missense mutations frameshift mutations	NS
	Other genes: *BMPR2*, *ACRV1*, *SMAD1*, *SMAD2*, *SMAD3*, *SMAD5*, *SMAD7* (22%)	NS	NS

FAP: Familial adenomatous polyposis; BMPR: Bone morphogenetic protein receptor; CRC: Colorectal cancer; ENG: Endoglin; FAP: Familial adenomatous polyposis syndrome; LOH: Loss of heterozygosity; NS: Non-specified; TGF: Transforming growth factor.

Figure 1 Macroscopic aspect of the colonic mucosa in a 43 years old male with classic Familial adenomatous polyposis.

and infrequent extracolonic manifestations[25-28]. The phenotype of MAP is less severe than classic FAP[36]. In some of the cases, MAP-related CRC can be developed without the polyposis background, the differential diagnosis with Lynch syndrome being difficult[35].

Juvenile polyposis syndrome

It is a rare autosomal dominant hereditary syndrome (1:100000-160000 live births) characterized by identification of 1-100 hamartomatous polyps throughout the gastrointestinal tract, mostly in the colorectal segments, diagnosed in young patients[8-12]. Microscopically, these polyps are covered by normal columnar epithelium and present mucus-filled tortuous dilated glands lined by columnar epithelium in the lamina propria; the dense stroma is edematous and rich in inflammatory infiltrate predominantly composed of plasma cells[8,11,13]. The clinical diagnosis is based on at least one of the following Jass's modified criteria[6,12]: (1) Multiple juvenile polyps throughout the gastrointestinal tract; (2) At least five colorectal juvenile polyps; or (3) Any number of juvenile polyps identified in patients with a family history of juvenile polyps. These polyps can present malignant transformation, the lifetime risk being about 34%-38% for colorectal segments and 21% for stomach[9,10,12]. Juvenile polyposis-related gastric cancers are rather produced through *SMAD4* than *BMPR1A* mutation genes[12]. Association with hereditary hemorrhagic telangiectasia also known as Osler-Weber-Rendu syndrome have been reported in about 20% of the cases; protein-losing enteropathy can also be associated[9,13].

Peutz-Jeghers syndrome

This syndrome is a rare autosomal dominant inherited disorder (1:8300-200000 live births) associated with a lifetime hazard for cancer up to 93%, which occurs as a consequence of a germline mutation in the *STK11* gene[12,14-16]. It is characterized by familial gastrointestinal hamartomatous polyposis and 1-5 mm mucocutaneous melanic spots around the mouth, in the buccal mucosa, on the fingertips and toes, and, infrequently, on the eyelid and sole of the foot[16]. The spots occur in first years of life; the skin spots spontaneously disappear at puberty but mucosal spots remains visible per life[16].

Regarding the polyps, the upper jejunum is most frequently involved (78%), followed by colon and stomach (24%)[15-19]. Solitary gastric polyps can occur rarely, less than 30 cases being reported to 2012[17]. Microscopically, the gastrointestinal hamartomatous polyps, that can undergo focal or total malignant transformation, are characterized by hyperplastic mucosal glands with periglandular proliferation of smooth muscle fibers[16,17]. Arborizing pattern of smooth muscle proliferation is characteristic[15,16]. In solitary polyps of the stomach, it was suggested that the branching of

the muscularis mucosae are not so well developed in the subsequent layers[15,17]. Gallbladder, bronchi, urinary bladder, and the ureter can also present hamartomatous polyps with similar histological architecture and further possible malignization[12].

Multiple synchronous or metachronous colonic and extra-colonic carcinomas of different organs like breast (54%), pancreas (36%), stomach (29%), ovary (21%), small bowel (13%), or other organs (cervix, uterus, testes, lung, appendix), can be associated in the same patient or his first-degree relatives, with a cumulative risk over 90%[12,15-18]. Associated lymphomas and sex-cord tumors were also encountered[16].

For a final diagnosis, one of the following criteria should be filled[12,14-19]: (1) At least two histologically proved Peutz-Jeghers polyps; (2) At least one histologically proved Peutz-Jeghers polyp in a patient with specific mucocutaneous spots; (3) Identification of at least one Peutz-Jeghers polyp in a patient with at least one relative with confirmed diagnosis of Peutz-Jeghers syndrome; and (4) Specific mucocutaneous spots in a patient with at least one relative with confirmed diagnosis of Peutz-Jeghers syndrome.

Cowden syndrome

It is an autosomal dominant hereditary syndrome that occur in 1:200000 live births (more frequent in Asian population). It is characterized by synchronous or metachronous tumors in multiple organs that occur in one patient or in members of his family. This familial gastrointestinal hamartomatous polyposis occurs as a result of mutations in the phosphatase and tensin (*PTEN*) gene.

The clinical diagnosis is based on the following International Cowden Cosortium major criteria, modified by the National Comprehensive Cancer Network Cowden syndrome[9,12,14,19,20]: macrocephaly (75%-97% of the cases - 58 cm for women and 60 cm for men), multiple (at least 3) gastrointestinal hamartomas including ganglioneuromas but excluding hyperplastic polyps (50%), dysplastic ganglyocytomas of the cerebellum associated with seizures, tremors, and disorders of coordination (Lhermitte-Duclos syndrome), breast cancer (37%), nonmedullary (follicular) thyroid carcinoma (16%), endometrial cancer, and macular pigmentation of the glans penis. The mucocutaneous lesions are considered as pathognomonic (major criteria) only if the following associations are identified[12,20]: At least three trichilemmomas (at least one being biopsically proved), at least three acral keratoses, at least three mucocutaneous neuromas, or oral papillomas (at least three without biopsy or at least one biopsically proved). The minor criteria are presence of benign lesions of the breast (fibrocystic change, benign epithelial tumors), thyroid (multinodular goiter, adenoma, papillary carcinoma), single lesion of the gastrointestinal tract (adenoma, lipoma, hamartoma), at least three lipomas, testicular lipomatosis, malformations or tumors of the urogenital tract, vascular malformations, and mental retardation (IQ $\leqslant$ 75)[12,19,20]. Recently, the autism spectrum disorders, colon/renal cancer, and esophageal glycogenic acanthosis (at least three) were included in the minor criteria[12]. For a final diagnosis, the following associations are necessary: at least three major criteria [at least one being macrocephaly, Lhermitte-Duclos syndrome (in adults), or gastrointestinal hamartomas], two major and three minor, or three minor criteria[12,19,20]. Absence of one of the associated criteria allows the diagnosis of the "Cowden syndrome-like family"[19].

Gastrointestinal hamartomas occur in 50% of patients with Cowden syndrome, being currently considered the second most common feature, after macrocephaly[19]. The estimated lifetime risk for malignancy at the age of 70 is 85% for any cancer, 77%-85% for breast and 35%-38% for thyroid cancer, 33% for renal cancer, 28% for endometrial, 7%-15% for CRC and 6% for melanoma[12,15,20,21]. Gastric malignancy is rarely associated, 1/100 patients with Cowden syndrome being affected[20].

Other hamartomatous polyposis syndromes

Besides Cowden syndrome, *PTEN* gene mutations were described in patients with Bannayan-Riley-Ruvalcaba- and hereditary mixed polyposis syndrome[7,12].

Bannayan-Riley-Ruvalcaba syndrome is an autosomal dominant disorder characterized by hamartomatous polyps of the small intestine and colon (25% of the cases) along with genital spots, macrocephaly, subcutaneous/visceral lipomas including lipomatosis of the glans penis, hemangiomas, and mental retard[7].

In some cases, identification of the specific genetic syndrome is very difficult, the recommended diagnosis being hereditary mixed polyposis syndrome. In this category, association of atypical juvenile polyps, hyperplastic polyps, sessile serrated adenomas, and adenomatous polyps can be associated with increased risk for CRC[7].

Other very rare familial hamartomatous syndromes that can include hamartomatous polyps of the gastrointestinal tract are the following[7,12]: Gorlin syndrome (consequence of *PTCH1* mutations), characterized by hyperkeratosis of palms, soles, and jaw, skeleton abnormalities, macrocephaly, frontal bossing, and associated medulloblastoma and basal-cell carcinomas; multiple endocrine neoplasia syndrome 2B (consequence of *RET* mutations), characterized by neuromas of the lips and tongue, and associated pheochromocytoma and medullary thyroid cancer; neurofibromatosis type Ⅰ (consequence of *NF1* mutations), characterized by café au lait spots, axillaries and inguinal freckling, and associated neurofibromas, gliomas, malignant peripheral nerve sheath tumors, and tumors of the breast; and Birt-Hogg-Dube syndrome (consequence of *FLCN* mutations), characterized by spontaneous pneumothorax and associated fibrofolliculomas of the skin, and renal tumors.

Li-Fraumeni syndrome

It is an autosomal dominant hereditary cancer syndrome characterized by mutations in the *p53* gene that determines occurrence of leukemia, carcinomas of the breast and adrenal glands, brain tumors, sarcomas of the soft tissues and bone, *etc*[19-21,23-26]. The classic Li-Fraumeni syndrome criteria of eligible families include one family member diagnosed with sarcoma before 45 years of age, a first-degree relative with any type of cancer before 45 years of age, and a first/second relative with any cancer diagnosed before 45 years of age or a sarcoma at any age[19,20]. Similar to Cowden syndrome, absence of one of the associated criteria allows the diagnosis of the "Li-Fraumeni syndrome-like family"[19,23,24].

Gastric carcinoma, preponderantly located in the proximal stomach, is reported to occur in about 2%-5% of carriers with *p53* mutations at the median age of 36 years, ranging between 12 and 74 years[24]. Association of early-onset gastric carcinoma and CRC can involve in 10%-28% of the families with classic Li-Fraumeni syndrome, but carcinomas of the lung, melanomas, lymphomas, and germ cell tumors have also been reported[24]. The incidence of Li-Fraumeni-related gastric cancer is higher in Asian population (Japan and South Korea), when compared with people from United States, being supposed that *p53* mutation could enhance the carcinogenic effect of *H. pylori*[24].

GENETIC COUNSELING AND CRITERIA FOR SURVEILLANCE

In patients with *FAP* and *FAP-variants* including *Gardner syndrome, Turcot syndrome,* and *AFAP,* the main goal of surveillance is to detect the CRC in early stages[28], combining molecular and clinical approaches[33].

The clinico-genetic screening should be performed in all first degree relatives of a patient with FAP and should be started, when it is possible, from the mid adolescence[28].

The genetic screening consists in attentively examination of the *APC* gene, according to the particularities presented in Table 1, after a proper genetic counseling of the patient who should be asked for the informed consent. The gold standard method is the full sequencing of the *APC* gene, to examine all the 15 exons[28]. The mutation cluster region (mutational hotspot of *APC* gene) is the 5'part of exon 15 from codon 1250 to 1464[28]. If no mutations are detected, the current guidelines recommend to continue testing for large gene rearrangements[28,35].

From colonoscopy point of view, it is worthy noticing that the small polyps are mostly limited to the recto-sigmoid at the time of adolescence and only thereafter increase in size and number[28]. However, because half of patients develop adenomatous polyps before puberty and 95% by 35 years, sigmoidoscopy screening is recommended starting at age 12-14 years old and performed every two years in mutation carriers. Identification of adenomas is an indicator for annually total colonoscopy, with biopsies from the suspect areas, until colectomy will be performed, depending on the individual endoscopic features[1,28]. Profilactic colectomy is recommended for multiple ulcerated polyps larger than 1 cm that shows high-grade dysplasia[28]. The type of resection depends on the patient's age and personal decision, number and extension of polyps, and also by the macroscopic aspect of the tumors[28].

At risk family members carrying germline mutations near codon 1300 can present early-onset CRC in their childhood and colonoscopy surveillance should also begin before puberty[32,33]. On the other hand, if the carrying germline mutations suggest risk for AFAP, screening should be carried out every two years from the age of 18-20 years, with focused attention on identification of the right-sided distribution of adenomas. Once adenomatous polyps are identified, endoscopic polypectomy followed by annually total colonoscopy is recommended, followed by colectomy in case of large ulcerated polyps with high-grade dysplasia[28,32]. Postoperative endoscopic follow-up is necessary in patients with rectal remnant, to detect the possible carcinoma of the ileo-anal pouch[28].

For classic FAP, flexible sigmoidoscopy remains the standard of care, whereas in patients with FAP variants the proximal colon should also be explored through total colonoscopy. Modern imagistic methods such as capsule endoscopy and/or entero-CT-scan or entero-MRI can also be used for complex investigations. Because duodenal cancer is the second cause of death of patients with FAP, with 5% lifetime risk[28], gastrointestinal endoscopy is recommended to be carried out every 5 years after identification of the colorectal polyps[28].

Besides the risk for gastrointestinal cancer, the protocol of surveillance should also take into account the extraintestinal manifestations, including papillary carcinoma of the thyroid (the third commonest tumor in patients with FAP, with a risk of about 160 times higher than in general population, and a male to female ratio of 1:17), pancreatic carcinoma but also the central nervous system tumors and neuroblastomas[14,28], based on the genetic particularities shown in Table 1.

Annually thyroid palpation, eventually completed by cervical ultrasonography, is recommended starting at the age 25 years[28,36]. Because patients with FAP present 1000-fold increased risk developing desmoid tumor, compared to the general population[34], diagnosis of such tumors, mostly in the abdominal wall, should be followed by a total colonoscopy, especially in young people. Although benign, due to highly recurrence rate, desmoids tumor represents one of the main causes of death of patients with FAP[28].

For patients diagnosed with *MAP,* the surveillance is identically to those used for AFAP. The colonoscopy surveillance begins at 18-20 years old being carried out every two years and annually after adenomas detection. Upper endoscopy is also recommended every five years

starting at the age of 25-30 years old, to explore the duodenal segments[28,36]. Screening for extra-intestinal manifestations is not recommended. Biallelic *MUTYH* gene mutations should be suspected and explored in patients with colorectal polyposis diagnosed before the age of 50 years, especially in associated serrated adenomas. In first degree relatives the two most common mutations, p.G396D and p.Y179C, should be determined. Identification of at least one of the two missense mutations should be follow up by full gene sequencing[28]. Sequencing should also be done in non-Caucasian suspected patients, focusing on the specific geographic and ethnic particularities shown in Table 1.

For juvenile polyposis syndrome, annual upper and lower endoscopies are recommended to be performed in the *MADH4/SMAD4* carriers by the mid-teens or at the time of initial symptoms, most of the cases being diagnosed around the age of 40 years[8-13]. Modern imagistic methods such as capsule endoscopy and/or entero-CT-scan or entero-MRI can also be used[37].

In the bioptic specimens of gastrointestinal polyps, loss or partial loss of the epithelial expression of SMAD4 protein, with or without retained stromal expression, can be a first sign of suspected *SMAD4* mutation[11]. Proctocolectomy or subtotal colectomy should be considered in patients with multiple polyps, severe symptoms, and/or history of familial CRC, but a specific guideline does not exist[12]. According to the British Society of Gastroenterology and Association of Coloproctology for Great Britain and Ireland, in asymptomatic family at-risk members, including the proved *SMAD4/ BMPR1A* mutations, every 1-2 years colonoscopy is recommended from age 15-18 years until age 70 years and gastroduodenoscopy from the age of 25 years[12,29].

In *SMAD-4* mutation-carriers, investigation for a possible associated hereditary telangiectasia is also recommended[13]. Because severe gastrointestinal bleeding can be associated in these syndromes, long-time intravenous using of low doses of the antiangiogenic (anti-VEGF) drugs such as bevacizumab (2 mg/kg per course, every 3 wk) have been recently proposed[30]. Identification of a pulmonary associated vascular malformation and a dilated thoracic aorta is mandatory to avoid bleeding complications[12].

Decreased SMAD4 expression can also activate the transforming growth factor-β and, as a consequence, breast epithelial malignant proliferation can occur, as in one of the previously reported cases[31]. Duodenal and pancreatic tumors can also occur in these patients[14].

In patients with Peutz-Jeghers syndrome, surveillance for tumors of the colorectum, small intestine, breast, pancreas, and sex-cord tumors should be performed[12,14]. Endoscopic examination of the gastrointestinal tract is recommended to be performed every 3 years beginning from the age of 18 years (and every 1-2 years after the age of 50 years) while suspicion for breast cancer should be excluded based on annual ultrasound examinations from the age of 25-30 years completed by annual mammography from the age of 50 years[12,15]. In symptomatic children, periodic gastrointestinal endoscopy should be done[12]. In patients with Peutz-Jeghers syndrome, the capsule endoscopy proved to have a higher diagnostic sensitivity than the Barium-contrast X-Ray and entero-MRI but the size and location of polyps are difficult to be evaluated[37].

No guidelines for screening of other cancers have been implemented to date.

For Cowden syndrome, being known that breast cancer and thyroid cancer occurs in 25%-50% of females and 3%-10% of all patients, respectively, a personal and familial cancer surveillance for these associated malignancies and also for endometrial cancer in females would be necessary[12,19]. Currently, the gastrointestinal tract surveillance is not routinely recommended below 50 years of age, although an earlier endoscopic colonic and gastric surveillance beginning at the age of 30-35 years with follow-up every 1-2 years was recently suggested, especially for Asian population[20]. However, annual mammogram and vaginal ultrasound with endometrial sampling should be done from age 30 years for women and biannual colonoscopy and renal ultrasound examination from age 35-40 years in both males and females are recommended in the most recent studies[12]. Annual thyroid examination should begin from age 18 or 5-10 years before the earliest thyroid tumor in the family[12].

For the other previously nominated hamartomatous polyposis syndromes, the childhood surveillance should take into account the gastrointestinal and extra-gastrointestinal complications such as bleeding, severe anemia, intussusception, whereas the adults should be examined to detect malignancies in early stages, similar to patients with Cowden syndrome[7,12].

In patients with Li-Fraumeni syndrome, although germline *p53* mutations can be identified in the family members, it is difficult to establish the rules of surveillance, because tumors can occur in every organs[19]. In these "p53 families", screening program is recommended to begin at earlier ages including investigations for breast, colorectal, and gastric cancer detection[19]. However, the guidelines of the National Comprehensive Cancer Network Surveillance recommend colonoscopy as part of the surveillance protocol in these carriers[20].

Because some of the inherited polyposis syndromes remain unexplained/unclassified, the genetic screening should take into account, after a meticulous histological examination, a minimal number of gene mutations, respectively the genes *SMAD4, BMPR1A, STK11,* and *PTEN*[14]. The surveillance protocol should also take into consideration the other nontumor complications such as intussusceptions, ileus, gastrointestinal hemorrhage, and anemia[21].

CONCLUSION

Despite the well-conducted screening programs worldwide, the accurate diagnosis of inherited cancer-

predisposing syndromes of gastrointestinal tract remains difficult. Lack of experience of both gastroenterologists and pathologists, due to rare occurence of these syndromes, increases the difficulty. Subspecialization in the field of familial malignancies and founded of specialized medical centers in this field is essential for future proper medical care.

Because of geographic and ethnic particularities of gene mutations, national and international guidelines of screening and surveillance in these risk families should be elaborated. Development of the IHC markers that could predict specific gene mutation is a cheaper method that can be routinely used to detect these familial cases. Although rare, association of multiple tumors in the same patient is a time- and money-consuming management, the reason why a proper screening and surveillance could benefit both the patient and medical care system.

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ORIGINAL ARTICLE

Observational Study

Colorectal cancer screening in an academic center compared to the national average

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Abstract

AIM: To investigate if the increased emphases on training and education on current colorectal cancer (CRC) screening guidelines has resulted in improved national CRC screening rates in an internal medicine training program, and to determine if the doctor's post graduate year (PGY) level of training affected CRC screening rates.

METHODS: We conducted a cross sectional study of every patient who presented to the outpatient clinic of New York Methodist Hospital, Brooklyn, NY, over the span of six continuous weeks in 2011. A questionnaire was integrated into every patient's medical interview that helped determine that patient's current CRC screening status, screening mammography status if applicable, Papanicolaou smear status if applicable, and current pneumococcal vaccination status. At the same time, patient demographics were also obtained. All of the questionnaire data was collected at the end of each medical visit and was compiled by a designated researcher. After all the data points were collected, it was ensured that the patient has been seen by his or her continuity care resident at least twice in the past. Data was then compiled into a secure, encrypted database to then be analyzed by our statistician.

RESULTS: Data from 547 consecutive clinic visits were obtained. Of these, we reviewed 483 charts that met all of the inclusion criteria and did not meet the exclusion criteria. The data was then analyzed for differences between PGY levels, patient's sex, race, and educational level. The study population consisted of 138 men and 345 women. 35 patients were white (7.40%), 174 were black (39.79%) and 264 were Hispanic (55.81%). Our CRC screening rates were: 66% for PGY-1's, 72% for PGY-2's and 77% for PGY-3's. There was no statistical difference noted between the three groups ($P \leqslant 0.05$) or was there any difference sex, insurance status or educational level. Overall CRC screening rate was 72% which was not different from the New York State average ($P < 0.05$). There was a statistically significant higher rate of CRC screening amongst Hispanics 76% ($P = 0.034$) and in people within the ages of 70-79, 82% ($P = 0.015$).

CONCLUSION: Patients that are followed by internal medicine residents at our urban outpatient teaching clinic did not receive higher rates of CRC screening nor did rates of screening vary with their PGY level.

Key words: Screening; Colorectal cancer; Post graduate year; Colorectal cancer; Residency; Urban

Core tip: It is assumed that greater seniority and experience amongst medical residents can equal improved colorectal cancer screening percentage in an outpatient academic center. We not only compare screening rates between different post graduate years but also compare the medical resident's screening rates to the national average.

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INTRODUCTION

Despite established screening guidelines, national colorectal cancer (CRC) screening rates vary between 54%-75% of the at risk population[1]. CRC is the third leading cause of cancer-related deaths in the United States when men and women are considered separately, and the second leading cause when both sexes are combined[2]. CRC is expected to cause approximately 49700 deaths during 2015[2]. The American Cancer society estimates that there will be 93090 new cases of colon cancer and 39610 new cases of rectal cancer in 2015[2]. When diagnosed early, CRC is typically curable. Screening guidelines have been developed to help reduce the mortality of CRC. For a person without increased risk factors, starting at the age of 50 years, it has been generally accepted that a colonoscopy every 10 years, flexible sigmoidoscopy (FS) every 5 years or annual fecal occult blood test (FOBT) would be considered a sufficient screening technique[3].

Despite these screening strategies and increased efforts by governing bodies to increase awareness of CRC screening in both the medical community and general public, in 2010 only 54.1%-75.2% of the United States population responded that they were "up to date" with their CRC screening, with the state of New York averaging 69%-75.2%[1].

It is assumed that clinical guidelines are observed and followed more often in an academic training setting like a residency program due to the fact that there is more emphasis on education in an academic setting and the medical residents are under constant supervision. However, we have observed that a majority of resident training involves acute disease management in the inpatient setting and little research has attempted to assess the quality of ambulatory education and resident competence especially for disease prevention and health maintenance[4].

We assessed the CRC screening rates at New York Methodist Hospital in 2010 and compared them to the 2010 New York state screening rates as recognized by the Center for Disease Control (CDC). Furthermore, we wanted to try to recognize possible barriers to CRC screening in our community hospital and try to identify ways that we could improve our CRC screening rates. We felt it was important to ascertain if current efforts to educate physicians in training are effective and to help identify ways to improve education efforts.

MATERIALS AND METHODS

Ambulatory care resident education

The New York Methodist Hospital internal medicine residency program is a traditional, accredited 3 year program consisting of both inpatient and ambulatory based training. At the time of this study there were 106 medical residents providing longitudinal care for patients in the ambulatory clinic. All resident physicians provide patient care in the ambulatory clinic two half days every week throughout all three years of their training. Additionally, residents do 4 to 5 mo solely of ambulatory care without any inpatient responsibilities. During those 4 to 5 mo, residents have a weekly morning rotation in the clinic's gastroenterology clinic and work under the supervision of board certified gastroenterologist. Formal lectures addressing preventive care cancer screening are interspersed throughout the academic year including one lecture focused on colorectal cancer screening in the average risk patient. Throughout their training, residents are given monthly exams; in two of which the primary focus is to test the resident's knowledge on primary prevention and screening strategies.

Table 1 Study population breakdown

Population	Number of patients	Percentage of patients
PGY-level		
PGY-1	170	35.20%
PGY-2	160	33.13%
PGY-3	153	31.68%
Sex		
Female	345	71.43%
Male	138	28.57%
Race		
Blacks	174	36.02%
Whites	35	7.25%
Hispanics	264	54.66%
Other	10	2.07%
Highest educational level		
Elementary school	28	5.80%
Middle school	63	13.04%
High school	186	38.51%
College or University	43	8.90%
Unknown	163	33.75%
Insurance type		
Medicare/Medicaid	288	59.63%
Private Insurance	32	6.63%
Unknown	163	33.75%
Age of patient (yr)		
50-59	179	37.06%
60-69	177	36.65%
70-79	90	18.63%
80-89	34	7.04%
90-99	3	0.62%

PGY: Post graduate year.

Study population

A cross sectional study was taken from patients who received their care at the internal medicine clinic of New York Methodist Hospital over a 6 wk period. Residents were given a questionnaire and integrated it into their clinical data gathering during the patient's clinic visit session. Data was collected after every clinic encounter throughout the six weeks. Exclusion criteria included patients under the age of 50, patients with an increased risk for developing colorectal cancer (family or personal history of adenomatous polyps, CRC, or polyposis syndromes) patients who had previous CRC screening in last 5 years and patients who have been followed by an internal medicine resident for less than 8 mo and had less than 2 clinic visits in which the patient had been seen by their designated resident.

Data collection

Data from 547 consecutive office visits in the internal medicine resident ambulatory clinic over a span of 6 wk was collected. Four hundred and eighty-three of those charts met the inclusion criteria and were selected and reviewed in further detail. The investigators confirmed that there had been a minimum of two clinic visits with their assigned medical resident. Data recorded included patient demographics, patient's level of education, type of medical insurance, data on the use of screening colonoscopy (SC), fecal occult blood testing (FOBT), FS, and other preventative health measures such as influenza vaccination, screening mammography and Pap smear. For the purposes of this study, only the data relevant to CRC screening was analyzed. A patient's CRC screening was considered "up to date" if it met any of the following criteria: (1) the patient has had a SC within the last 10 years;(2) the patient has had a screening FS within the last 5 years; and (3) a FOBT within the last 12 mo. These screening modalities are readily available at our institution and generally accepted as appropriate screening tools[3]. FS, though a well-accepted screening modality, was not included in our survey as the procedure is not offered at our institution. Finally, the data was also then stratified between the resident's level of training (PGY1, PGY2, and PGY3). This study received IRB approval; IRB reference No. 518027.

Statistical analysis

Data was analyzed using the binomial test and the χ^2 distribution test. The binomial statistical test was used to compare the medical resident's screening rate to the New York state's 2010 CDC average of 70.1% and to determine if insurance status, patient's level of education, race, age or sex influenced the results. The χ^2 distribution test was used to determine if there were any statistical differences between the post graduate year level of training, age groups, sex, educational level, insurance status, or race. Statistical significance was defined as $P = 0.05$.

RESULTS

Four hundred and eighty three patients were considered appropriate for inclusion into the study. Table 1 depicts our patient characteristics. The study population consisted of 138 men with a mean age of 63.5 years (range, 50-88 years) and 345 women with a mean age of 64.17 years (range, 50-92 years). Thirty five patients were white (7.40%), one hundred and seventy four were black (39.79%) and two hundred and sixty four were Hispanic (55.81%). Two hundred and twenty nine (47.41%) responded that they had a high school education or above, ninety one (18.84%) responded that their educational level was below high school level and one hundred and sixty three (33.75%) did not provide their educational level. Table 2 depicts our statistical findings. The overall CRC screening rate at our hospital was 72%. We did not observe statistical difference between the CRC screening rates of our hospital compared to the 2010 United States or New York state screening rates as provided by the CDC[1] ($P = 0.05$). There was no observed statistical difference between the screening rates of PGY-1's, PGY-2's, and PGY-3's ($P = 0.096$), sex, insurance status or educational level. There was a statistically significant higher rate of CRC screening amongst Hispanics of 76% ($P = 0.034$) and in people within the ages of 70-79 years of 82% ($P = 0.015$).

Table 2 Statistical analysis comparing our colorectal cancer screening rates to the 2010 New York State screening rates as determined by the Center for Disease Control

Variable	Screening rate	*P* value	*P* value of the χ^2 distribution test comparing variability within groups
PGY-level			
PGY-1	0.66	0.3	
PGY-2	0.72	0.735	0.096
PGY-3	0.77	0.061	
Age of patient (yr)			
50-59	0.64	0.07	
60-69	0.77	0.58	
70-79	0.82	0.015[1]	0.006[1]
80-89	0.61	0.255	
90-99	0.67	1	
Sex			
Female	0.7	0.953	0.33
Male	0.75	0.26	
Race			
Black	0.68	0.508	
Hispanic	0.76	0.034[1]	0.023[1]
Other	0.8	0.733	
White	0.54	0.063	
Highest educational level			
College	0.72	0.869	
Elementary	0.75	0.682	
High School	0.74	0.336	0.888
Middle School	0.72	0.888	
Undisclosed	0.69	0.73	
Insurance type			
Medicare/Medicaid	0.73	0.245	
Private insurance	0.72	1	0.514
Undisclosed	0.68	0.607	
Overall screening rate	0.72	0.48	

[1]Statistical significance is defined as $P = 0.05$. New York State screening rate was standardized to a base rate of 0.701 for comparison. Data was analyzed by binomial statistical analysis. PGY: Post graduate year.

DISCUSSION

Our study did not support the assumption that CRC screening would be offered more frequently at an institution with a residency training program when compared to the state and national average screening rates which include non-teaching outpatient practices. There was a numerical difference between the screening rates of PGY-1 compared to PGY-3 (11%) however statistical significance, possibly due to function of power, was not achieved. Willett *et al*[5] had similar findings in 2005 when they compared PGY-1 and PGY-2 residents in their adherence rates to national guidelines for outpatient preventive health services and found no difference between the two groups for breast and colon cancer screening amongst others.

Despite didactics, emphasis on practicing evidence based medicine, and importance of implementing preventative measures with the use of well accepted screening measures CRC screening in our internal medicine residency training program was still found to be comparable to the national and state average CRC screening rates.

Prior studies have indeed shown poor CRC screening rates amongst internal medicine residents[6]. Numerous studies have elucidated the deficiency in knowledge of and compliance with CRC screening recommendations amongst internal medicine residents[6-9]. Our study however is unique in that we were able to compare the rates of CRC screening at an outpatient clinic of an urban teaching program to state and national rates which include non-teaching practices.

These results highlight the important fact that though we expect and anticipate that teaching programs ingrain the importance of screening and prevention in medicine, for reasons unknown, either fail to do this or just do not seem to reflect this in clinical training practice. If well accepted and proven screening techniques such as CRC screening are not offered more so by physicians in training who are assumed to be "up-to-date" with current screening guidelines and practices through their mandated hours of didactics, this raises the concern that perhaps there needs to be a change in the way both residents and their mentors are trained.

In the future, it is vital that efforts be made to improve education amongst physicians in training regarding CRC guidelines and the importance of CRC screening. A prior study by Gennarelli *et al*[10] showed that knowledge of CRC screening guidelines amongst medical professions is low for both average and high risk patients. Internal medicine residents in our program like most others receive weekly didactics in the form of lectures by attending physicians, fellows, and visiting professors averaging approximately 7 h/wk however these lectures span a wide variety of topics and are not focused on primary prevention or screening. Perhaps physicians in training would benefit from a teaching series focused specifically on preventative measures and screening techniques. A retrospective chart review done by Borum showed that internal medicine residents who had increased exposure to and reinforcement of surveillance recommendations through lectures and required documentation as well as formal FS training adhered to guidelines far more than other resident physicians[7].

Additionally, now that medical records are for the most part transitioning to electronic records across the country, clinical prompts incorporated into the standard outpatient note template may help as a reminder tool for physicians who have adequate knowledge of the topic but for the sake of time and other factors may not necessarily remember to ask their patients regarding their screening status. Seres *et al*[11]. showed that clinical prompts are superior to evidence based lectures when it comes to improving physician CRC screening rates.

Another aspect that must be considered is the patient's role in compliance with recommended screening. 1.5% of our patients had refused CRC screening when offered in the past and it is unknown if they were educated regarding the potential long term consequences of their decision. Residents in training

should learn early on the importance of patient education in both disease prevention and treatment. The realm of primary prevention and screening is one in which patient education regarding the importance of screening and potential dire outcomes of lack of screening become vital. Perhaps implementing use of patient educational tools such as easy-to-read brochures and pamphlets explaining current rates of CRC and screening modalities effect on prevention will help patient's make more educated decisions when it comes to screening. Rowe *et al*[12] even implemented use of an educational video while patients were waiting to be seen by residents.

In assessing the need for further investigations and future direction we will review the limitations of our study. Generalizability of our study, which included only residents from our primarily categorical internal medicine residency program, and if our findings are representative of other residency programs especially those which include family medicine or primary care tracks is of concern. Another limitation of the study is that it was conducted over the span of 6 wk and may not be an adequate representation of overall practice. In addition, the patient population was not a good representation of the different races; with 54.66% of patients were Hispanic and 7.25% Whites, this may explain the perception of higher screening rates in Hispanics as compared to Whites.

COMMENTS

Background

Routine screening has been proven to be an effective tool at preventing colorectal cancer (CRC). Many efforts have been put forth to educate medical professionals on proper CRC screening. The authors investigate if current efforts on CRC screening education are producing improved CRC screening rates.

Research frontiers

The Center for Disease Control has been providing a big push in CRC prevention. Current studies center on methods of improving education towards not only patients but health care providers as well.

Innovations and breakthroughs

This is the only article comparing the screening rate of medical residents compared to the national average and one of the few manuscripts comparing the screening rates between post graduate years (PGY).

Applications

The study results demonstrate that there is no appreciable difference between PGY or compared to the national average. This exposes potential weaknesses in current educational strategies and opens up some proven ideas that may help increase CRC screening rates.

Peer-review

This is a well-designed observational study that was tailored to minimize selection bias. The results can be applied to family medicine and internal medicine training programs alike.

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ORIGINAL ARTICLE

Prospective Study

Tumor neoangiogenesis detection by confocal laser endomicroscopy and anti-CD105 antibody: Pilot study

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Abstract

AIM: To evaluate neoangiogenesis in patients with colon cancer by two fluorescently labeled antibodies on fresh biopsy samples imaged with confocal laser endomicroscopy (CLE).

METHODS: CLE is an imaging technique for gastrointestinal endoscopy providing *in vivo* microscopy at subcellular resolution. An important question in validating tumor angiogenesis is what proportion of the tumor vascular network is represented by pre-existing parent tissue vessels and newly formed vessels. CD105 (endoglin) represents a proliferation-associated endothelial cell adhesion molecule. In contrast to pan-endothelial markers, such as CD31, CD105 is preferentially expressed in activated endothelial cells that participate in neovascularization. Thus, we evaluated CD105 and CD31 expression from samples of ten patients with primary rectal adenocarcinoma, using a dedicated endomicroscopy system. A imaging software was used to obtain the Z projection of the confocal serial images from each biopsy sample previously combined into stacks. Vascular density and vessel diameters were measured within two 50 μm x 475 μm rectangular regions of interest centered in the middle of each image in the horizontal and vertical direction. The results were averaged over all the patients and were expressed as the mean ± SE.

RESULTS: The use of an anti-CD105 antibody was found to be suitable for the detection of blood vessels in colon cancer. Whereas anti-CD31 antibodies stained blood vessels in both normal and pathologic colon equally, CD105 expression was observed primarily in malignant lesions, with little or no expression in the vessels of the normal mucosa (244.21 ± 130.7 vessels/mm^3 in only four patients). The average diameter of anti-CD105 stained vessels was 10.97 ± 0.6 μm in tumor tissue, and the vessel density was 2787.40 ± 134.8 vessels/mm^3. When using the anti-CD31 antibody, the average diameter of vessels in the normal colon tissue was 7.67 ± 0.5 μm and the vessel density was 3191.60 ± 387.8 vessels/mm^3, while in the tumors we obtained an average diameter of 10.88 ± 0.8 μm and a vessel density of 4707.30 ± 448.85 vessels/mm^3. Thus, there were more vessels stained with CD31 than CD105 ($P < 0.05$). The average vessel diameter was similar for both CD31 and CD105 staining. A qualitative comparison between CLE *vs* immunohistochemistry lead to similar results.

CONCLUSION: Specific imaging and quantification of tumor microvessels are feasible in human rectal cancer using CLE examination and CD105 immunostaining of fresh tissue samples.

Key words: Rectal cancer; Neoangiogenesis; Confocal laser endomicroscopy; Panendothelial markers; Anti-CD105 antibody

Core tip: We evaluated CD105 expression from fresh tissue samples of human rectal adenocarcinoma, using confocal laser endomicroscopy (CLE). While vessels marked with fluorescent CD31 were visible in both normal and malignant tissue, CD105 was predominantly expressed in tumor lesions, having reduced affinity for normal rectal mucosa. Our data showed that CLE using CD105 antibody for tumor vascular network imaging is feasible and that CD105 represents a more specific marker for rectal cancer neoangiogenesis than panendothelial markers. To our knowledge, this is the first study to report the use of fluorescently-labeled CD105 antibody in conjunction with CLE in patients with rectal tumor.

Ciocâlteu A, Săftoiu A, Pirici D, Georgescu CV, Cârțână T, Gheonea DI, Gruionu LG, Cristea CG, Gruionu G. Tumor neoangiogenesis detection by confocal laser endomicroscopy and anti-CD105 antibody: Pilot study. *World J Gastrointest Oncol* 2015; 7(11): 361-368 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i11/361.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i11.361

INTRODUCTION

Tumor neoangiogenesis, defined as the neo-formation of blood vessels from pre-existing microvessels, represents an attractive target for both imaging and therapeutic strategies. It is thought that neovascularization is first activated by an "angiogenic switch" during premalignant phases of carcinogenesis, before tumors emerge (Folkman *et al*[1]; Bolontrade *et al*[2]; Huss *et al*[3]). An important question in validating tumor neoangiogenesis is what proportion of tumor vascular network is represented by pre-existing *vs* newly formed vessels. In this respect, new imaging and diagnostic techniques which differentiate tumors vascularization at different stages are desired[4].

Antihuman panendothelial cells antibodies are used to identify all types of blood vessels in a given tissue sample, irrespective of being mature or immature. Commonly used panendothelial markers such as CD31, CD34 or von Willebrand factor detect the parent vessels as well as the tumor vasculature, but they are not always expressed in all tumor blood vessels. Moreover, these antibodies seem to have a higher affinity for large than for microvessels[5].

Endoglin (CD105) is a co-receptor for various TGF-β family members and therefore a target for tumor vasculature[6]. The role of endoglin and the indispensable role for the TGF-β signaling pathway in developmental angiogenesis has been studied on genetically modified mice[7-9]. Unlike all other markers, endoglin mediates direct pro-angiogenic effects of TGF-β on endothelial cells and is specifically overexpressed in tumor vessels, on proliferating endothelial cells, at sites of active angiogenesis. Its expression has also been associated with metastasis and patient survival[6,10,11]. Recent reports suggest that elevated plasma levels of endoglin in patients with colorectal cancer correlate with poor prognosis (Li *et al*[7]; Duff *et al*[12]). As a result, endoglin

Table 1 Patient characteristics

Patient	Gender	Age	Tumor grading	Preoperative stage	RT	CTX
1	F	67	G1	T3N0M0	No	No
2	M	65	G2	T3N0M0	Neoadj	No
3	M	47	G2	T3N0M0	Neoadj	No
4	M	66	G2	T4N0M0	Adj	Adj
5	M	54	G2	T3N0M0	No	No
6	M	67	G1/G2	T3N1M0	Neoadj	Neoadj
7	F	80	G1 + Mucinous areas	T3N0M0	Neoadj	Neoadj
8	F	78	G2	T3N2M0	Neoadj	No
9	M	59	G1	T3N1M0	No	No
10	M	69	G1/G2	T3N0M0	Neoadj	No

RT: Radiotherapy; CTX: Chemotherapy; Neoadj: Neoadjuvant therapy; Adj: Adjuvant therapy; F: Female; M: Male.

could represent a valuable tool for the diagnosis, tumor vasculature visualization and targeted treatment of solid cancers[4].

Since endoglin is highly and specifically expressed on tumor endothelial cells, in the present study we hypothesized that it could be used as an appropriate marker to assess the vascularization of a tumor.

Confocal laser endomicroscopy (CLE) gained an important role in the study and real-time histopathological diagnosis of various gastrointestinal diseases, such as celiac disease, Barrett esophagus, microscopic colitis, inflammatory bowel disease, and recently Clostridium Difficile associated colitis[13]. Recent meta-analyses performed to determine the diagnostic accuracy of CLE in the detection of colorectal neoplasia showed high sensitivity and specificity of the method[14,15].

Recently, we have used CLE to assess tumor vasculature by fluorescence labelled antibodies targeted against endothelial markers[16,17]. In the present feasibility study, we used CLE to compare the selective expression of fluorescently labeled anti-CD105 antibodies in newly-formed vessels to fluorescently labeled anti-CD31 total vessel staining, and the gold standard of histopathology. More specifically, we aimed to answer the following questions: (1) Can the use of CLE in association with CD105 offer a more adequate quantitative and qualitative analysis of newly formed vessels than the commonly used panendothelial markers in human rectal cancer? and (2) Can this method be used *in vivo* for a rapid characterization of tumor microvascularization?

MATERIALS AND METHODS

Subjects

The current study was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004) and approved by the local Ethics Committee. All the patients included read and accepted the written informed consent prior to study entry.

Tissue specimens from ten patients 47-80 years old (mean age of 65.2 ± 9.9 years), with histologically diagnosed rectal cancer, were collected during colonoscopy before undergoing surgical resection or neoadjuvant therapy to avoid artifacts (*e.g.*, false positive resulted from fibrosis or inflammation increased in case of radio-chemotherapy). Fresh tissue samples from these patients were immediately processed for both CLE and immunohistochemistry assessment.

The ten patient population contained stage Ⅱ-Ⅲ (according to AJCC staging system) rectal adenocarcinomas without metastatic spread.

The main clinical signs the patients presented at admission in the hospital were alternating diarrhea and constipation, accelerated intestinal transit, recent constipation, unintended weight loss, rectal bleeding, abdominal pain or discomfort. Only three patients accused rectal bleeding as a single symptom, also confirmed by the physical examination (digital rectal examination). Seven patients had nonspecific findings for the laboratory tests such as moderate elevated hematological values of erythrocyte sedimentation rate (three patients), slightly elevated white blood cells count (two patients) and moderate anemia (two patients). Two patients presented slightly elevated values of both tumor markers CEA and CA19-9, while three of them had only slightly elevated CEA value. Computed tomography scan excluded the presence of metastases in all ten patients and described rectal wall thickening in four cases. Histological examination findings from endoscopic samples are summarized in Table 1.

CLE

The biopsy samples collected with a standard colonoscope (CFQ160ZL, Olympus, Tokyo, Japan) were processed following a standardized protocol. During the endoscopic procedure, for every patient, six biopsies were taken from tumor, avoiding the ulcerated areas (paired biopsies for CLE assessment, standard immunohistochemistry and histopathological examination, respectively), as well as four biopsies from macroscopically normal surrounding tissue samples (paired biopsies for both CLE processing and standard immunohistochemistry). The biopsies were immersed immediately in 10% neutral buffered formalin for histopathological analysis, as well as in saline solution

for the *ex vivo* immunohistochemical processing. Samples from saline solution were thoroughly washed and incubated for one hour in the dark, at 37 ℃, with Alexa-Fluor 488-labeled anti-CD31 (PECAM) antibody (mouse anti-human IgG1, Exbio, Prague, Czech Republic) or respectively FITC-labeled anti-CD105/ Endoglin antibody (mouse anti-human IgG2a, Exbio), diluted as 1:15 and 1:5 in saline with 1% bovine serum albumin (BSA, Sigma-Aldrich, Munich, Germany). Afterwards, the excess antibodies were washed away in saline and the samples were immediately visualized in CLE imaging to assess the microvascularization *ex vivo* up to a maximum depth of 250 μm. CLE images were acquired using Pentax EC-3870 CIFK, Tokyo, Japan, a dedicated endomicroscopy system with an excitation wavelength of 488 nm and with a maximum laser power output of ≤ 1 mW at the surface of the tissue[16,17].

To assess both endothelial markers more accurately, we used the color overlay function in the ImageJ image processing software (National Institutes of Health, United States). This software was used to obtain the Z projection of the confocal serial image stacks from each biopsy sample (60-250 images per biopsy sample). The vascular density and the vessel diameters were measured from the Z projections within two 50 μm × 475 μm rectangular regions of interest (ROI) centered in the middle of each image in the horizontal and vertical direction as before[17].

Statistical analysis

The results were averaged over all the patients and were expressed as the mean ± SE. We used unpaired two-tailed Student's *t*-test, with the level of significance set at $P \leq 0.05$ to evaluate the variation of CD105 expression *vs* CD31 expression in microvessels from the normal mucosa tissue and from the rectal tumors.

Immunohistochemistry

To confirm the role of CD105 *vs* CD31 in tumor neo-angiogenesis, adjacent samples from the same patient were processed for immunohistochemistry, for normal and tumor samples as described previously[16,17]. Briefly, after formaldehyde fixation and paraffin embedding, 4 μm tissue sections were sliced from these blocks, deparaffinized, re-hydrated and processed for antigen retrieval by microwaving for 20 min in citrate buffer pH 6. Endogenous peroxidase was next blocked utilizing 1% H_2O_2 for 30 min, and the false antigenic sites were further blocked by incubating the slides in 5% skimmed milk (Bio-rad, München, Germany). Paraffin-certified antibodies were next incubated alternatively on the slides overnight at 4 ℃ (rabbit anti-human CD105 polyclonal antibody diluted as 1:50, LabVision, Fremont, CA, United States; and mouse anti-human CD31, IgG1, clone JC70A, Dako, Glostrup, Denmark). Next day the sections were washed in saline, signal amplified with a multi-species polymeric HRP system (EnVision, Dako), and finally vessels were visualized by adding the 3-3' diaminobenzidine substrate (DAB, Dako). Afterwards, the sections were counterstained with Hematoxylin and 3-4 hotspot high vessel density areas were captured using a Nikon Eclipse 55i microscope equipped with a 5 Megapixel CCD color camera (Nikon, Tokyo, Japan). There were selected images from the regions with the highest vascular density ("hot-spots"- according to Weidner *et al*[18]). Under constant illumination conditions, images were obtained using the 40 × objective, and saved as uncompressed TIF files using the Image ProPlus AMS 6 software (Media Cybernetics Inc., Bethesda, Maryland, United States). The contour for each microvessel was drawn separately with a dedicated hand tool in Adobe Photoshop software, and these ROI were filled with black RGB color and saved as layers. Images were brought back in Image ProPlus and after distance-to-pixel calibration, they were utilized for automated measurements. Total vascular area, and total vessel count were normalized to 1 mm^2 and automatically measured, considering a total area of the field of 36527.48 μm^2. Inflammatory plasma cells or tumor cells picking up the signal have been excluded from this interpretation by two pathologists (DP and CG).

RESULTS

Targeted anti-CD31 antibodies expression on the confocal laser images

To analyze CD31 expression in rectal cancer, we evaluated tumor rectal cancer tissue and normal rectal mucosa for the vascular morphometric assessment. The CD31 antibody stained blood vessels in both normal and tumor rectal mucosa. In normal mucosa, the average diameter of vessels was of 7.67 ± 0.5 μm and the vessel density was 3191.6 ± 387.8 vessels/mm^3. In the tumor sample, we obtained an average diameter of 10.88 ± 0.8 μm and a vessel density of 4707.3 ± 448.8 vessels/mm^3 (Figure 1A and B).

Targeted anti-CD105 antibodies for CLE imaging of normal colorectal tissue and tumor microvasculature

In the CLE samples that were fluorescently labeled with both CD31 and CD105 antibodies, the typical tumor vasculature pattern was observed, with tortuous, dilated and branched vessels, but the expression of CD105 in tumor tissue was generally lower compared to CD31 vessel staining (Figure 1C and D).

Staining for CD105 was low or absent in normal mucosa (244.21 ± 130.7 vessels/mm^3 in only four patients), whereas the microvascular network was visualized using CD31 as a control on samples from the same patients. The average diameter of anti-CD105 antibody stained vessels was 10.97 ± 0.6 μm in tumor tissue, and average density was 2787.4 ± 134.8 vessels/mm^3.

Next we analyzed the relationship between the vascular expression with CD31 and CD105 in colorectal

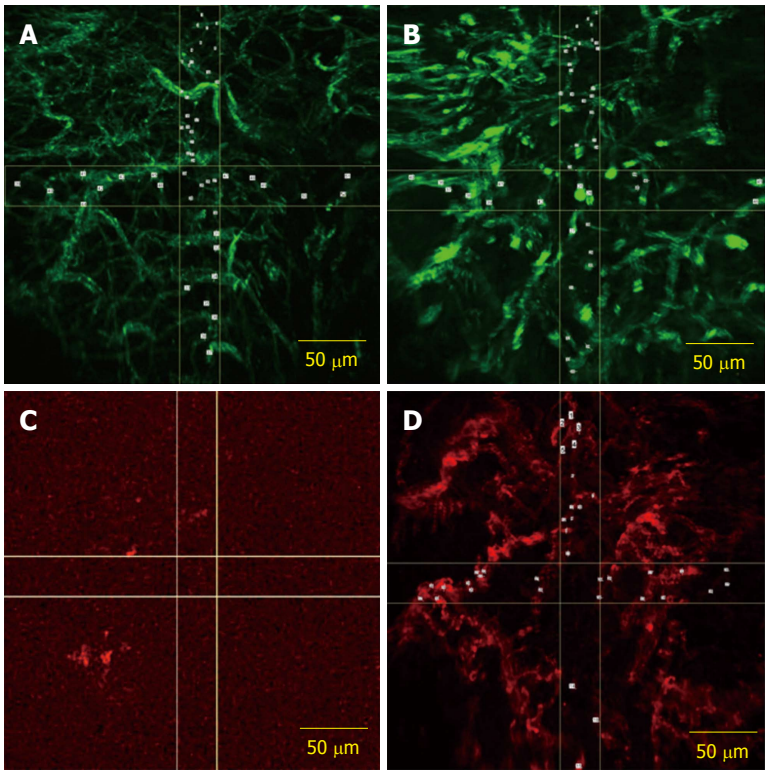


Figure 1 Confocal laser endomicroscopy. A: CLE images with AF488 anti-CD31 antibodies expression on vascular network from both normal; B: Tumor rectal mucosa; C: CLE image showing low expression of the fluorescently labeled anti-CD105 antibodies in normal rectal mucosa; D: Image from the same patient showing microvessels in rectal adenocarcinoma visualized by using CD105 staining as a specific endothelial marker. CLE: Confocal laser endomicroscopy.

Table 2 Quantitative results of vascular parameters from confocal laser endomicroscopy images

		CD31	CD105	*P*-value
Vascular Diameter (μm)	Normal Tissue	7.67 ± 0.5	3.46 ± 1.5	0.01
	Tumor	10.88 ± 0.8	10.97 ± 0.6	0.9
Vascular Density (vessels/mm^3)	Normal Tissue	3191.6 ± 387.8	244.21 ± 130.7	< 0.001
	Tumor	4707.3 ± 448.8	2787.4 ± 133.8	0.001

tumors. There were more vessels stained with CD31 than CD105 (P = 0.0006 for vascular density) in tumor. The average vessel diameter was similar for both CD31 and CD105 staining (P = 0.018 in normal samples, and P = 0.932 in malignant tissue).

The vascular density and the average diameter in tumor samples were significantly higher than the control in the 3D confocal reconstruction and in immunohistochemistry images. This fact was demonstrated by using both markers. In contrast, CD105 expression in colorectal tissues from the same patients was strongly enhanced in tumor vessels suggesting detection of the endoglin is an indication of angiogenesis particularly in malignant disease (Table 2).

Immunohistochemistry results

The CD105 and CD31 vascular expressions were studied in normal rectal mucosa and rectal cancer specimens. The immunohistochemical analysis revealed that the samples from normal tissue showed low detectable CD105 expression. CD105 was rarely expressed in normal mucosa, while in tumor specimens, CD105-positive vascular endothelial cells were clearly identified (Figure 2).

In normal tissue images CD31-stained we measured an average of 202.9 ± 91.8 vessels/mm^2, with a significantly lower density of 56.5 ± 35.1 vessels/mm^2 for the vascular network stained with CD105 (P = 0.00017). The intratumoral MVD average was about 298.04 ± 132.6 vessels/mm^2 on CD31 stained images and on CD105 images - 205.7 ± 100.06 vessels/mm^2 (P = 0.048) (Figure 3).

The values for the vascular area when using the panendothelial marker CD31 were 3.4% ± 1.3% in normal rectum and 9.4% ± 3.3% in tumors (P < 0.001). On CD105 stained sections, the total vascular area was 1.3% ± 1.4% in healthy tissue and 6.9% ± 3.1% in malignant tissue (P < 0.001).

DISCUSSION

Rectal cancer is one of the cancers which can benefit from antiangiogenic therapy with high chances of curability when the treatment is applied at an early stage. To date, no appropriate tissue biomarkers exist for staging, prediction or monitoring of the clinical response to a therapeutic intervention (*e.g.*, antiangiogenic therapy).

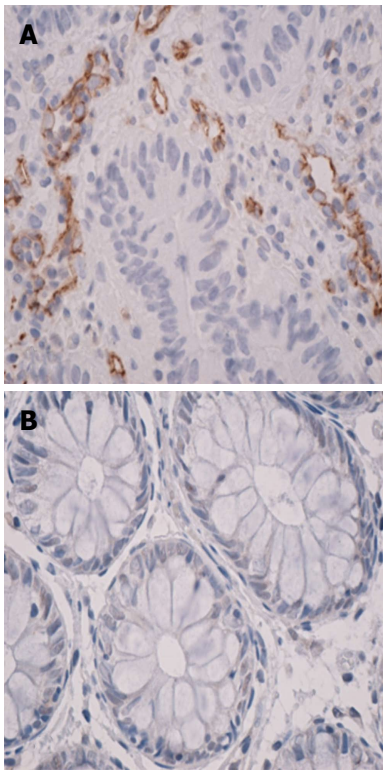
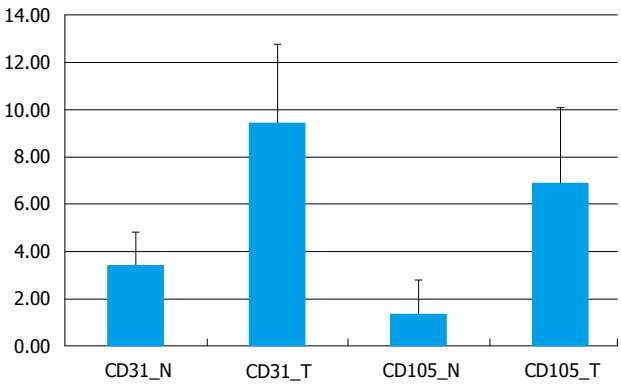


Figure 2 Immunohistochemistry on CD105 stained sequential sections from rectal cancer tissue samples (magnification 40 ×), CD105-positive vascular endothelial cells were clearly identified by their brown staining (A) and normal rectal mucosa displays the absence of endoglin expression (B).



CD31_N- MVD in normal mucosa stained with anti-CD31 antibodies
CD31_T- MVD in tumor mucosa stained with anti-CD31 antibodies
CD105_N- MVD in normal mucosa stained with anti-CD105 antibodies
CD105_T- MVD in tumor mucosa stained with anti-CD105 antibodies

Figure 3 Graphic representation of vascular density (microvessel density) obtained from CD31-immunostained images and CD105-immunostained images of normal mucosa in comparison with tumor mucosa (vessels/mm^2). MVD: Microvessel density.

Beyond its already presumed roles (higher affinity for microvascularization, prognostic role), recent *in vitro* studies suggested that endoglin targeting could improve treatment and could reverse resistance to bevacizumab in some refractory cancer patients[19].

We hypothesized that the use of fluorescently-labeled CD105 antibodies will be suitable for identifying microvessels specific to tumor tissue. Indeed, while vessels marked with fluorescent CD31 were visible in both normal and malignant tissue, CD105 was predominantly expressed in tumor lesions, having reduced affinity for normal rectal mucosa. Thus, specific imaging and quantification of tumor microvessels were feasible using CLE examination and CD105 immunostaining of samples.

Our study proves that fluorescently labeled endoglin antibodies stained intensively intratumoral vessels, whereas vessels in non-neoplastic tissue did not or weakly expressed CD105. These results are consistent with previous observations that endoglin reacts specifically with angiogenic endothelial cells from the malignant tissues[5]. Though, the endoglin expression on macroscopically normal mucosa in four of the patients could be explained by either the existent inflammation, or the tumor spread to normal surrounding tissue.

Endoglin, as a specific marker for activated endothelium, mainly reacts with fresh or frozen tissue, while its activity in paraffin-embedded specimens is dependent on fixation[17]. In the present study, a qualitative comparison between the two methods (CLE *vs* IHC) lead to similar results. The major advantage of the CLE method is time efficacy and less artifacts in comparison to common IHC regarding the processing techniques[20].

Due to CD105 specific overexpression in malignant vessels, the endoglin antibodies for tumor imaging have the potential of becoming an optimal target for anticancer treatment, to improve rectal cancer diagnosis and to monitor the therapy[4]. As there are already studies regarding tumor aggressiveness and the prognostic value of vascular density on IHC when using anti-CD105 antibodies, CLE opens the possibility of applying CD105 targeted therapy, which until now was only tested *in vitro* and on animal models, to *in vivo* human subjects. Its luminal distribution on newly formed vessels makes CD105 readily accessible for the antibodies and, consequently, an interesting candidate for CLE *in vivo*[11].

CLE monitoring of the relationship between endothelial presence of CD105 and survival of patients would be of great interest. In our group of patients, we observed an inter-patients variation in MVD endoglin expression in tumor tissue. On one hand, this could be related to the tumor grading or staging, as an increase in MVD was demonstrated by using CD105 during progressive stages of colorectal carcinogenesis[21]. On the other hand, reduced endoglin expression could also be caused by a decreased tumor vascularization in endoglin haploinsufficiency cases[22]. There are also differences in reactivity to endothelial cells depending on tumor localization[22-24]. However, in colorectal cancer, other studies showed that, with cancer progression, endoglin signaling was lost in most of the epithelial cancer cells which became refractory to the TGF-β growth inhibiting properties[25-29]. All these factors could lead to differences in diagnostic, prognostic and therapeutic efficacy.

To our knowledge, no other studies using fluorescently-labeled CD105 with CLE imaging in patients with rectal cancer have been reported prior to this study. A larger number of patients is needed to study the correlation between MVD and tumor differentiation grade and stage, with great potential for CD105 staining combined with CLE analysis to provide a more reliable evaluation of the angiogenetic status of patients with colorectal cancer. Other studies are needed to investigate if the same CLE method could be applied to other tumor types.

In conclusion, our data showed that CLE using CD105 targeted antibodies for tumor vascular network imaging is feasible and, moreover, that this proangiogenic molecule represents a more specific marker for rectal cancer neoangiogenesis than commonly used panendothelial markers.

COMMENTS

Case characteristics

The main clinical signs the patients showed were alternating diarrhea and constipation, accelerated intestinal transit, recent constipation, unintended weight loss, rectal bleeding, abdominal pain or discomfort.

Clinical diagnosis

Only three patients accused rectal bleeding as a single symptom, also confirmed by the physical examination (digital rectal examination).

Differential diagnosis

Other common digestive diseases such as hemorrhoidal disease, inflammatory bowel disease or irritable bowel syndrome were excluded.

Laboratory diagnosis

Seven patients presented nonspecific laboratory tests findings such as moderate elevated hematological values of erythrocyte sedimentation rate (three patients), slightly elevated white blood cells count (two patients) and moderate anemia (two patients); two patients presented slightly elevated values of both tumor markers CEA and CA19-9, while three of them had only slightly elevated CEA values.

Imaging diagnosis

Computed tomography scan excluded the presence of metastases in all ten patients and described rectal wall thickening in four cases.

Pathological diagnosis

Histological examination of endoscopic samples revealed moderately differentiated adenocarcinoma (G2) in five cases, well differentiated adenocarcinoma in two cases (G1), mixed subtypes in three cases (G1/G2- two cases, G1 with mucinous areas - one case).

Treatment

Tissue samples from patients with histological diagnosis of rectal cancer were collected during colonoscopy before undergoing surgical resection or neoadjuvant therapy.

Term explanation

Immunoendoscopy: Targeting markers of angiogenesis in association with confocal laser endomicroscopy (CLE) examination; Panendothelial markers: Present equal staining intensity in both small and large vessels and comparable reactivity in both frozen and paraffin sections, with obvious disadvantages regarding antigen specificity and sensitivity. They can identify all types of blood vessels in a given tissue sample, irrespective of being mature or immature.

Experiences and lessons

Specific imaging and quantification of tumor microvessels are feasible in human rectal cancer using CLE examination and CD105 immunostaining of fresh tissue samples. A larger number of patients is needed to study the correlation between MVD and tumor differentiation grade and staging, with great potential for CD105 staining combined with CLE analysis to provide a more reliable evaluation of the angiogenetic status of patients with colorectal cancer. CLE monitoring of the relationship between endothelial presence of CD105 and survival of patients would be of great interest.

Peer-review

The manuscript has original results. This is an interesting study on "Tumor neoangiogenesis detection by confocal laser endomicroscopy and anti-CD105 antibody: Pilot study". The research is limited to a small number of patients and, for this reason, this study should be considered pilot.

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CASE REPORT

Does St. John's Wort cause regression in gastrointestinal system adenocarcinomas?

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Informed consent statement: All study participants, or their legal guardians, provided informed written consent prior to case reports enrollment.

Conflict-of-interest statement: We declare that we have no conflict of interest.

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Abstract

St. John's Wort (SJW) is an old herb which has long been consumed widely for its anti-inflammatory, antiviral, and anti-depressive properties. Here we present a detailed clinical evaluation of three cases (two colon and one duodenal adenocarcinoma) with remarkable and intensive lymphoplasmocytic host reaction, at the basal part of tumor, intensive fibrosis, giant cells, plasma cell increase in lymph nodes and few giant cells in germinal centers in resection specimens. The observation of similar host reaction in those tumors having otherwise usual appearance was interesting. None of the cases received neoadjuvant chemoradiotherapy or additional treatment before surgery but only SJW. These cases are presented to increase the awareness about such cases. Further research is needed to reveal the possible effect of SJW, which has long been consumed for different treatment purposes, on human tumors.

Key words: St. John's Wort; Adenocarcinoma; Giant cell

Core tip: St. John's Wort (SJW) is a well known herb that was used in treatment of many diseases during centuries. In this article we offer a perspective about the anti-tumoral effect of SJW with possible mechanisms and pathological data in three gastrointestinal cancer cases, where usage of SJW was identified in history questioning because of tumor regression and intensive inflammatory host reaction following pathological examination.

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INTRODUCTION

St. John's Wort (SJW) is a substance widely used for its anti-inflammatory, antiviral, antidepressant and anticancer effects[1-3]. It contains two active compounds: Firstly, hyperforin is responsible for anti-depressant activity and has supplied to be also a good inhibitor of leukocyte elastase, exerting forceful inhibition of *in vitro* tumor cell chemoinvasion and reduction of neovascularization and metastasis formation *in vivo*[4]. Secondly, hypericin is responsible for photocytotoxic effects *in vivo* and *in vitro*. The *in vivo* and *in vitro* photodynamic activities of hypericin as a photosensitizer mainly to induce a very potent anti-tumoral effect[5]. Also, the anti-retroviral feature of hypericin has beeen demonstrated *in vitro* and in animal models[6].

CASE REPORT

Case 1

A fifty-nine years old male patient has undergone colonoscopy for anemia evaluation, which revealed a tumoral mass in the cecum. The histological diagnosis of the biopsy was adenocarcinoma and no distant metastasis was detected in further clinic radiological investigation. Right hemicolectomy was performed and a pathological examination of surgical material revealed a cecal ulcero-vegetative mass which was 7 cm × 6 cm × 5 cm in size. The tumor invaded through muscularis propria to subserosal fat tissue and was consistent with a moderately differentiated adenocarcinoma. Notably, it showed fibrosis and inflammatory cell infiltration in the transitional zone between deep intestinal layers and normal mucosa, which was easily detectable even under low magnification (Figure 1A). Under higher magnifications, inflammatory cell infiltration was rich in plasma cells and lymphocytes. scattered eosinophils, polymorphonuclear leucocytes and few giant cells were also noted focally (Figure 1B). The inflammatory reaction and fibrosis were surrounding the tumor, as if they were trying to prevent the penetration of the tumor into deep tissue. Most of these lymphocytes were T lymphocytes and showed cytotoxic T cell ($CD8^+$) phenotype on immunohistochemical examination (Figure 1C). CD20 and CD4 stains were almost negative. Plasma cells were stained positive with CD138 and polytypic with kappa/lambda. Two of 18 lymph nodes dissected from mesentery showed few tumor cells located in subcapsular sinuses while no gross metastasis was detected. Notably, germinal centers of some lymph nodes had giant cells and increased number of plasma cells in inter-follicular areas (Figure 2A and B). Giant cells were CD68 positive on immunohistochemical examination (Figure 2C). These features were suggestive of changes developed secondary to neoadjuvant chemotherapy/radiotherapy, but the patient's past medical history did not reveal such treatment. His detailed medical history was taken and when he was also asked for the usage of some alternative treatments, he mentioned usage of SJW for other complaints such as diabetes, dyspepsia. He has been consuming SJW tea in the morning for five years, then he had used SJW oil regularly (one teaspoon in the morning) for two years and he has been using it regularly (one teaspoon in the morning and evening) for the last three years. Medical records of the patient revealed that he had chemotherapy for six months after surgery (FOLFOX-4 protocole once every 14 d) and no recurrence or metastasis were detected during two years of follow up.

Case 2

A fifty-eight years old female patient has undergone colonoscopy for anemia evaluation, which revealed a tumoral mass in the transverse colon. No distant metastasis was detected and the patient had undergone colectomy. On macroscopic examination of colectomy specimen, an ulcerovegetative tumor infiltrating all layers of intestinal wall was detected, measuring 3.5 cm × 2.5 cm × 2 cm in size. Microscopic examination revealed moderately differentiated adenocarcinoma with mixed inflammatory cell infiltration rich in lymphoplasmocytes on the background (Figure 3). Eosinophils were also prominent with a few giant cells. Fourteen lymph nodes, dissected from mesentery, were reactive. However, one of the lymph nodes had an increased number of plasma cells and giant cells in germinal center of the follicle. Immunohistochemical characteristics were similar to that of the first case. Based on the experience of the morphology of the first case, the patient was also asked for usage of alternative treatments. To our surprise she has also mentioned usage of SJW oil (one teaspoon in the morning on an empty stomach) for 1.5 mo. Her medical records revealed that she has refused chemotherapy and followed-up without treatment. No recurrence or metastases were detected during the first six months of follow-up period.

Case 3

A duodenal mass was detected in a 73 years old male patient with the complaints of abdominal pain and weight loss. The biopsy was reported as adenocarcinoma. Since there was no distant metastasis, surgery was recommended. Although, he initially refused surgery he agreed to an operation three months later. On his second admission to hospital it was seen that the tumor size had somewhat reduced during this three months period. When a detailed medical history was taken, it was also revealed there was daily use of SJW oil of one teaspoon for the last three months. On macroscopic examination, an ulcero-vegetative ampullary tumor was observed measuring 3.8 cm × 2.5 cm × 2.5 cm in size, involving all layers of duodenum and infiltrating the pancreas.

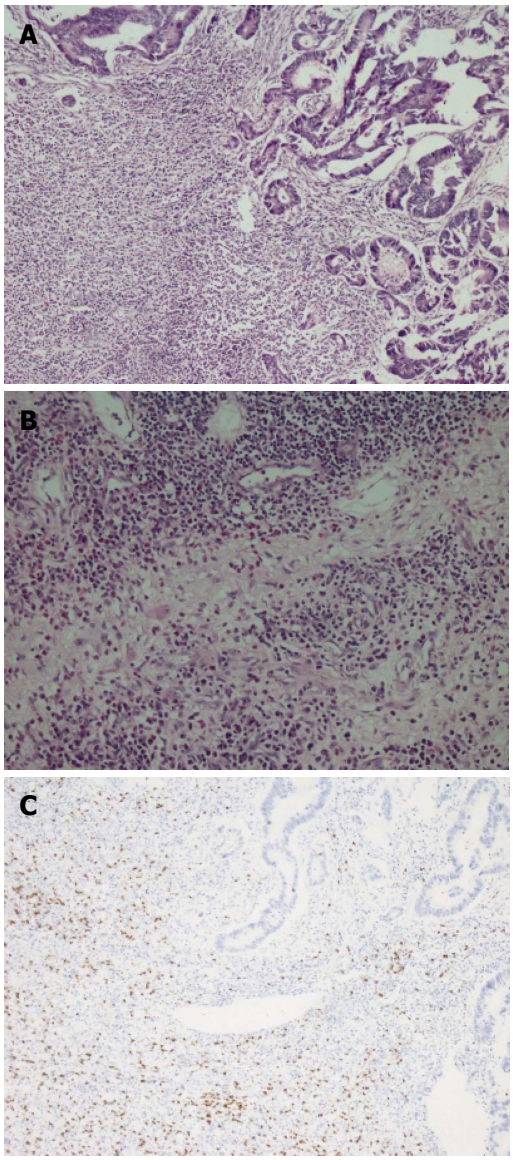

Figure 1 Adenocarcinoma. A: Adenocarcinoma showing fibrosis and inflammatory cell infiltration in the tumor base (HE × 10); B: Inflammatory cell infiltration consisting of plasma cells, lymphocytes, eosinophils and PNLs was seen in these areas (HE × 20); C: Inflammatory cell infiltration observed in the basis of tumors was rich in CD8 positive T lymphocytes (anti-CD8, × 5).

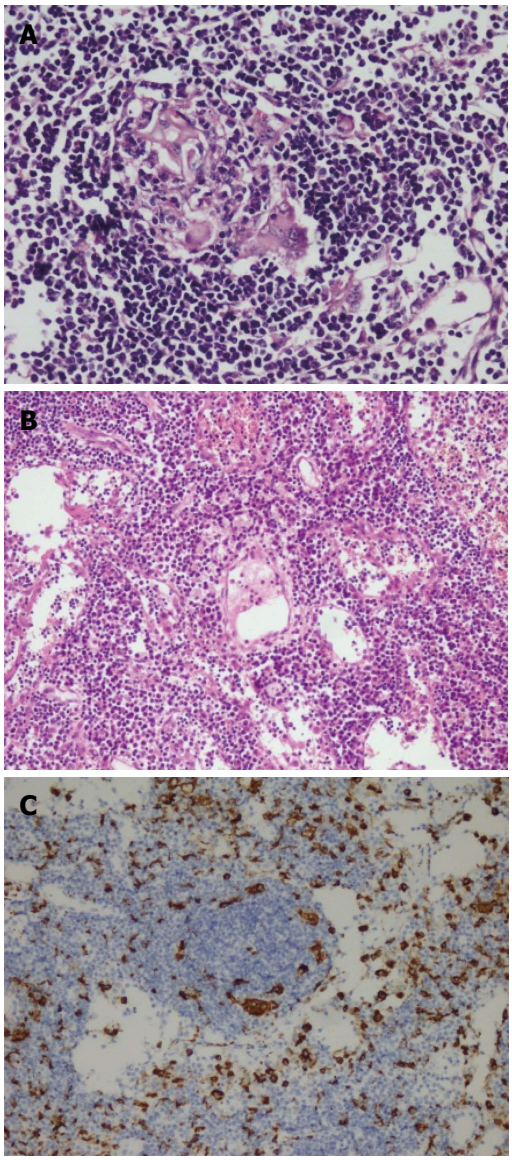

Figure 2 Germinal centers of some lymphoid follicles had giant cells and increased number of plasma cells in inter-follicular areas. A: Giant cells were detected in germinal centers of some lymph nodes (HE × 20); B: Interfollicular areas of some lymph nodes had increased number of plasma cells (HE × 10); C: Giant cells were stained with CD68 immunohistochemically (anti-CD68 × 10).

Areas showing the characteristics of moderately differentiated adenocarcinoma and mixed inflammatory cell infiltration rich in PNLs were observed. Similar to the previous two cases, eosinophils were also present and most prominent in the basilar parts of these areas (Figure 4A). The most common lymphocytic component was again CD8 positive T cells immunohistochemically (Figure 4B). Giant cells were seen in all layers, being more prominent in the areas in the vicinity of serosal surfaces (Figure 5A and B). These cells were stained with CD68 immunohistochemically (Figure 5C). Additionally, extensive perineural infiltration and intra-lymphatic tumoral thrombi were present. Four of 12 lymph nodes dissected from surrounding adipose tissue showed metastasis. The patient died due to anastomosis leakage and bleeding complications after surgery.

DISCUSSION

Hypericum perforatum, known as SJW, is a plant of the genus Hypericum and a herb with antidepressant feature and effective anti-inflammatory characteristics as an arachidonic acide/5-lipoxygenase inhibitor and COX-1 inhibitor[7]. In many countries, its drug form is available and sold out as an over the counter drug without prescription. It is most commonly used for the treatment of depression. Hyperforin is responsible for anti-depressant activity. The hyperforin constituent of SJW is TRPC6 receptor agonist and therefore, it causes noncompetitive reuptake inhibition of monoamines (especially, dopamine, norepinephrine, and serotonin), gamma-aminobutyric acid and glutamate[8]. Hyperforin

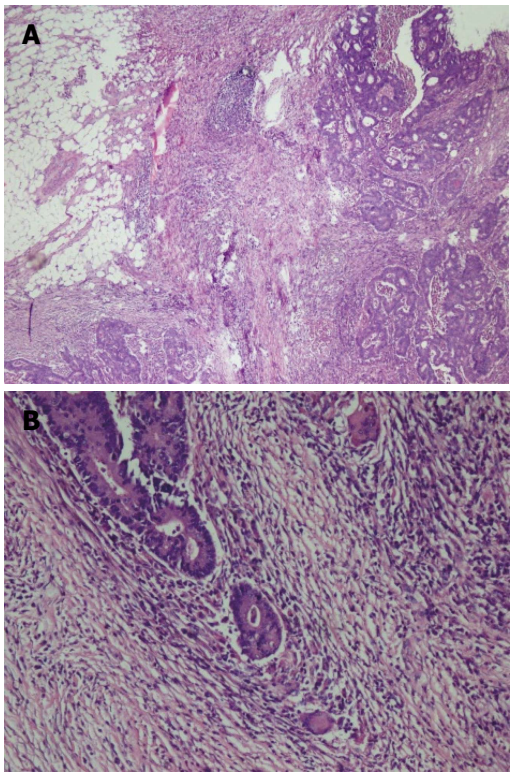

Figure 3 Moderately differentiated adenocarcinoma with mixed inflammatory cell infiltration rich of lymphoplasmocytes, eosinophils and few giant cells (A and B) (HE × 5, HE × 20).

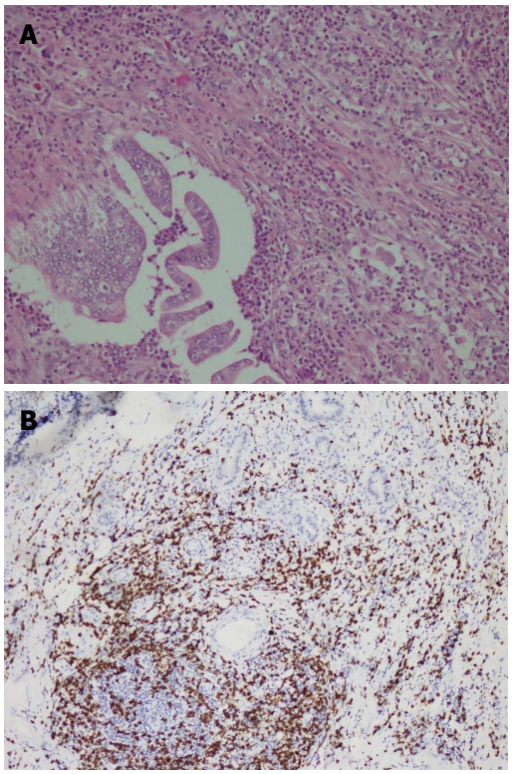

Figure 4 Adenocarcinoma showing mixed inflammatory cell infiltration rich in eosinophils and T - lymphocytes. A: Moderately differentiated adenocarcinoma showing mixed inflammatory cell infiltration rich in eosinophils and T - lymphocytes (HE × 20); B: The most prominent cellular component on immunohistochemical examination was CD8 positive T - lymphocytes (CD8 × 10).

inhibits reuptake of these neurotransmitters by increasing intra-cellular sodium ion amounts. Furthermore, SJW is known to downregulate the β_1 adrenoceptor and upregulate postsynaptic 5-HT_{1A} and 5-HT_{2A} receptors which are serotonin receptor[9]. A 2008 Cochrane review of 29 clinical trials inferred that it was superior to placebo in cases with major depression[10]. With respect to the National Center for Complementary and Integrative Health of the National Institutes of Health, it "may help some types of depression, though the evidence is not definitive"[11]. Hyperforin is also an anti-inflammatory complex with anti-angiogenic, antibiotic, and neurotrophic estates[12]. Moreover, it prevents neutrophil activation of matrix metalloproteinase-9 (MMP9) mobility and recruitment. Anti-proliferative and anti-metastatic feature has also been associated to down-regulation of NF-jB and its regulated molecules for example survivin and MMP9[13].

Hypericin is a photosensitive compound synthesized by SJW, and possesses properties suitable for photodynamic therapy (PDT). PDT is a carcinoma treatment methodology abusing non-toxic photosensitizer specifically localized in tumor tissue and its targeted activation with light. Thus, it leads to reactive oxygen kinds production and causes photochemically caused cell death[14]. The response to PDT depends on the photosensitizer's features, the illumination circumstances and the oxygenation conditions of the tissue[15]. It was also observed that hypericin blocks cell cycle at G2/M control point in colon cancer cell culture[16] Another colon cancer cell culture study showed re-localisation of apoptosis-inducing factor on the nucleus after hypericin treatment. Thus the anti-tumor effect of hypericins likely resulted from its apoptosis stimulating effect and its anti-proliferative effect by decreasing Ras protein[17].

Besides its many benefits there are also some studies in the literature showing its undesired adverse effects. Development of hepatotoxicity, cirrhosis and alteration of dosage properties and bioavailability of some drugs are some of its important adverse effects[18]. SJW has been displayed to cause a lot of drug interactions. Its effects are due to cytochrom P4503A enzyme activation and P-glycoprotein. This drug metabolizing enzyme induction effects in the raised metabolism of some drugs, such as indinavir, cyclosporine and oral contraceptives leading to reduced plasma density and possible clinical impact[19]. The main constituent thought to be responsible is hyperforin.In an other study it has been shown that the amount of intestinal and hepatic cytochrome P4503A and intestinal P-glycoprotein are increased by the short term usage of SJW in humans and rats[20]. Bone marrow necrosis, orofacial dystonia and radiation recall dermatitis are reported as less often adverse effects[21-23].

In an experimental study by Martarelli *et al*[24], on hormone independent human prostate cancer cells, it was shown that Hypericum perforatum extract decreased tumor cell proliferation by inhibiting serotonin

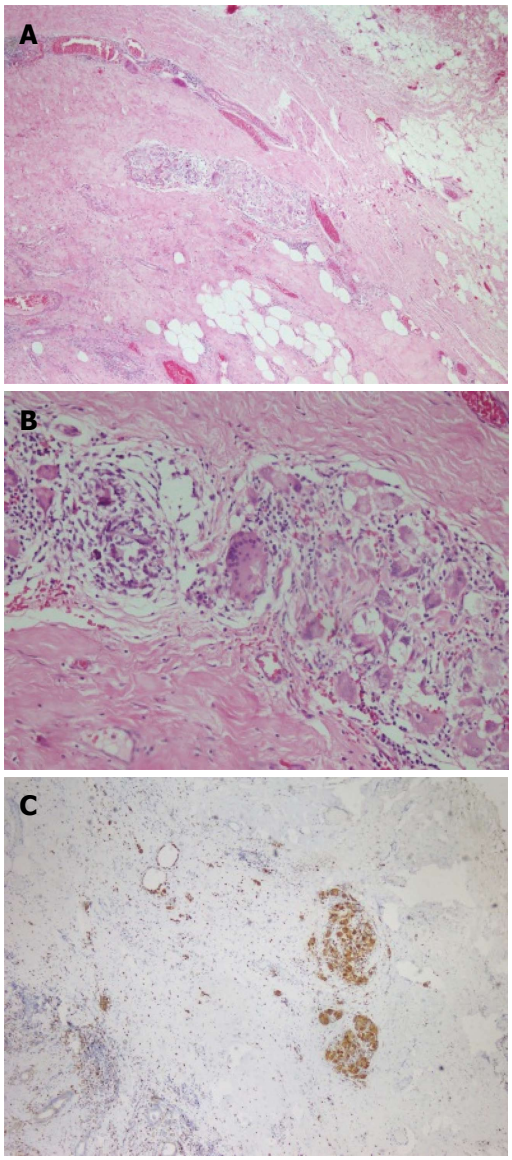

Figure 5 Giant cells in the areas beneath serosal surface and stained with CD68 immunohistochemically. A, B: Giant cells were seen in the areas beneath serosal surface (HE × 5, HE × 20); C: CD68 positivity in giant cells (anti -CD68 × 10).

reuptake and showed cytotoxic effects. In addition, it decreased frequency of local lymph node metastasis when compared to the control group[24]. There are experimental studies on the effects of SJW on colon, bladder and prostate carcinomas. In an experimental study by Dongre *et al*[25], the effect of Hypericum hookerianum on carcinomas was evaluated and it was found that serum neutrophil, lymphocyte, eosinophil, hemoglobin and erythrocyte values were closer to normal range when compared to control group[25]. In our cases, neutrophils and histiocytes-giant cells were more prominent early in the course (2nd and 3rd cases), while plasma cells, histiocytes and lymphocytes (cytotoxic CD8+) took over during chronic usage (1st case). Similar to the study by Dongre *et al*[25], morphological properties of our 2nd and 3rd cases may be due to acute effects (15 d) of Hypericum. In our case with long term SJW use, extensive host reaction and tendency to form barrier against tumor were remarkable and we interpreted it as a morphological sign of its anti-tumor response. Although the exact mechanism of these events is unknown, it may be a result of a chain of events triggered immunologically.

The aim of this presentation is not recommending SJW as a substitute for cancer treatment. The observations presented herein reflect the histological findings of only three cases and not enough to make a precise conclusion on its effects. We don't know yet either whether all cases using SJW present similar morphology or whether any other substances also induce a similar tumor-host reaction. We present these cases only to share our observations and draw attention to its possible effects on human tumor-host interaction. Further dedicated research is needed to unveil these questions.

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COMMENTS

Case characteristics

The authors present a detailed clinical evaluation of three intestinal adenocarcinoma cases which used St. John's Wort (SJW).

Clinical diagnosis

Patients have undergone colonoscopy for anemia, abdominal pain and weight loss evaluation, which revealed a tumoral mass in the colon and duodenum.

Pathological diagnosis

Biopsy and resection materials of all three cases were evaluated morphologically and immunohistochemically. Inflammatory cell population was rich in plasma cells and lymphocytes. In patients that used SJW in early stages polymorphonuclear leucocytes were significant. In patient those who used SWJ for long periods fibrosis and lymphoplasmositic cell infiltration was remarkable. Lymphocytes stained predominantly CD8 positive phenotype immnuhistochemically. Plasma cells were found to be kappa/lambda polytypic nature.

Treatment

Case revealed that he had chemotherapy for six months after surgery (FOLFOX-4 1 protocole once every 14 d).

Experiences and lessons

The aim in this study is not about to recommend usage of SJW. The authors only want to indicate their awareness of SJW usage after pathologic examination. The authors thing that these pathologic features might flash the benefits of SJW that had been discussed for ages.

Peer-review

This manuscript reports the clinico-pathological findings of three adenocarcinoma cases treated with SJW.

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EDITORIAL

Multimodality treatment of recurrent pancreatic cancer: Mith or reality?

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Abstract

Pancreatic adenocarcinoma is the fourth cause of cancer-related death in the United States. Surgery is the only potentially curative treatment, but most patients present at diagnosis with unresectable or metastatic disease. Moreover, even with an R0 resection, the majority of patients will die of disease recurrence. Most recurrences occur in the first 2-year after pancreatic resection, and are commonly located in the abdomen, even if distant metastases can occur. Recurrent pancreatic adenocarcinoma remains a significant therapeutic challenge, due to the limited role of surgery and radio-chemotherapy. Surgical management of recurrence is usually unreliable because tumor relapse typically presents as a technically unresectable, or as multifocal disease with an aggressive growth. Therefore, treatment of patients with recurrent pancreatic adenocarcinoma has historically been limited to palliative chemotherapy or supportive care. Only few data are available in the Literature about this issue, even if in recent years more studies have been published to determine whether treatment after recurrence have any effect on patients outcome. Recent therapeutic advances have demonstrated the potential to improve survival in selected patients who had undergone resection for pancreatic cancer. Multimodality management of recurrent pancreatic carcinoma may lead to better survival and quality of life in a small but significant percentage of patients; however, more and larger studies are needed to clarify the role of the different therapeutic options and the optimal way to combine them.

Key words: Multimodality treatment; Pancreas; Pancreatic neoplasms; Pancreatectomy; Tumor's recurrence

Core tip: Different therapeutic options are available for the treatment of patients with pancreatic adenocarcinoma recurrence, even if only few data have been reported in the Literature on their effective benefit for patients' outcome. In this work we present the current English Literature about this issue, the possible indications for the different therapeutic options and the available data on patients' outcome.

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INTRODUCTION

Pancreatic adenocarcinoma is the fourth most common cause of cancer-related death among men (after lung, prostate, and colorectal cancer) and women (after lung, breast, and colorectal cancer) in the United States[1]. The incidence of pancreatic adenocarcinoma has been increasing in United States while mortality rates have remained largely unchanged[1]. Surgery is the only potentially curative treatment for pancreatic cancer (PC), with a median survival after pancreatic resection of 12.6 mo[2]. There are no effective screening strategies for this tumor and most patients present at diagnosis with unresectable or metastatic disease. Moreover, the majority of patients who undergo surgical resection will die of disease recurrence, with a 3-year disease-specific survival of only 27%[3]. In fact, even after an R0 resection, most patients will experience a cancer recurrence, either as isolated local recurrence, hepatic metastasis or peritoneal dissemination[4]. Most recurrences occur within 2 years of surgery, and are mainly located in the abdomen[5], even if lung and bone metastases can also occur. Recurrent PC remains a significant therapeutic challenge, due to the advanced stage and the limited role of surgery and radio-chemotherapy. So, nihilistic attitude is frequent among clinicians towards PC relapse. In other primary malignancies, such as colorectal cancer, neuroendocrine carcinomas, renal cell carcinoma, resection of recurrent disease can be curative in selected patients[6-8]. On the other hand, surgical management of recurrent PC is usually unfeasible because tumor's relapse typically presents as unresectable, multifocal disease with an aggressive growth[5]. Therefore, treatment of patients with recurrent pancreatic adenocarcinoma has historically been limited to palliative chemotherapy or supportive care. Despite the extremely high rate of tumor relapse, no evidenced-based guidelines for post-surgical follow-up exist. Standard surveillance usually includes clinical examination, serum Carbohydrate Antigen 19-9 (CA 19-9) determination and radiological studies [*i.e.*, ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI) and chest X-ray]. The National Comprehensive Cancer Networks (NCCN) guidelines for follow-up after surgery recommend a physical examination, CA 19-9 determination and CT scan of the abdomen and pelvis every 3-6 mo for 2 year and then annually[9]. However, the value of follow-up in detecting early recurrence and its impact on survival or quality of life of patients has not been clearly determined. Moreover, no treatment has had any strong impact on recurrent PC to date, so the need for a close follow-up is argued. In fact, if an earlier identification of tumor relapse can give indication for further investigational studies, there are no available data showing that earlier recurrence's treatment leads to better patients outcome[9]. However, detection of recurrence in asymptomatic patients has been shown to significantly improve survival in comparison to symptomatic patients[10]. So, detection of asymptomatic relapse may facilitate investigational studies for appropriate treatments. On the contrary, it has been reported that increasing the frequency and intensity of postoperative follow-up (*i.e.*, CT scan) increases cost but not produces survival advantage[11]. According to ESMO Guidelines[12], due to the impossibility of cure a pancreatic recurrence, "a follow-up schedule should be discussed with the patient and designed to avoid emotional stress and economic burden for the patient". Only few data are available in the Literature about this issue, even if in recent years more studies have been published to determine whether treatment after recurrence have any effect on patients outcome. Recent therapeutic advances have demonstrated the potential to improve survival in selected patients, but more and larger studies are needed to argue the role of the different therapeutic options and the optimal way to combine them. So, in order to improve the management of patients with recurrent pancreatic tumor after initial resection, some crucial points have to be considered: (1) which is the best method to follow and detect as soon as possible tumor's relapse? (2) is there a place for surgery in recurrent PC? (3) which is the best treatment for tumor's recurrence, and how to combine different therapeutic strategies?

DETECTION OF RECURRENCE

Post-surgical surveillance of PC include serum Carbohydrate Antigen 19-9 (CA 19-9) determination and radiological studies. CA 19-9 is the only biomarker for pancreatic adenocarcinoma approved by FDA and the most widely studied[13]. The estimated sensitivity and specificity of CA 19-9 for the diagnosis of PC are respectively 71%-81% and 83%-90% (cut-off level of 37 U/mL)[14,15]. A part from its diagnostic utility, CA 19-9 has also a role in predicting cancer recurrence after surgical resection and it is routinely used in post-surgical follow-up of resected patients. Preoperative CA 19-9 levels have been investigated as predictors of tumor recurrence. Sugiura *et al*[16] found that a preoperative CA 19-9 value $\geqslant$ 100 U/mL was a significant predictor of early recurrence and of a poor prognosis after resection for pancreatic adenocarcinoma. After a curative surgical resection, CA 19-9 levels are expected to decrease and return to a normal range. CA 19-9 postoperative elevations precede clinical/radiological evidence of recurrence by 2-6 mo[17]. Some studies have investigated the correlation of postoperative CA 19-9 levels and the rate of recurrence. Hata *et al*[18] found a statistical relationship between postoperative CA 19-9 > 37 U/mL and the rate of disease recurrence. Patients

with postoperative elevated CA 19-9 had an overall recurrence rate significantly higher than patients with normalized postoperative CA 19-9. In the experience of Park *et al*[19] post-treatmnet CA 19-9 and normalization of postoperative CA 19-9 were independent prognostic markers both for disease-free and overall survival. However, the utility of CA 19-9 is limited by the fact that it is not expressed in 5%-10% of population and that it can be falsely elevated in the presence of biliary obstruction[20]. In recent years other gene and molecular biomarkers have been investigated in the early detection of PC recurrence. Mataki *et al*[21] investigated the role of blood circulating tumor cells (CTCs) as an early predictor of tumor relapse after PC curative resection. In particular Carcinoembryonic Antigen (CEA) mRNA expression using RT-PCR was evaluated in blood samples of 53 PC resected patients. CEA mRNA sensitivity and specificity were respectively 75% and 94% in predicting tumor recurrence[21]. Further studies are needed to find accurate and feasible biomarkers for predicting early disease recurrence. Contrast-enhanced CT scanning is the standard radiological study performed in post-surgical follow-up of PC. However, differentiation of post-treatment recurrent or residual tumor from fibrosis or post-surgical alterations is difficult with conventional imaging techniques. After pancreaticoduodenectomy for PC, postoperative changes in the areas around the common hepatic artery and proximal superior mesenteric artery are commonly recognized[20]. These sites are also common areas of tumor recurrence, and it may be a diagnostic problem to differentiate postoperative alterations from recurrent disease[22]. Postoperative complications (cholangitis, pancreatic or biliary fistula, abdominal fluid collections) can contribute to the development of fibrosis or post-surgical alterations[23]. Since fibrosis is present in both adenocarcinomas and postoperative changes, the enhancement pattern may not be helpful, because both benign and malignant recurrent tissue may show delayed contrast enhancement[24]. Therefore, differential diagnosis between postoperative change from recurrence is difficult on a single CT study. Moreover, a reactive mesenteric lymphadenopathy can be present for years after surgery, and it is impossible to differentiate from lymph node metastases: only a progressive increase in lymph node size or the association with a recurrent mass can suggest the presence of lymph node metastases[25]. Recently some Authors have demonstrated the usefulness of PET/CT for restaging and detection of recurrence of PC[26,27]. Kitajima *et al*[27] analyzed forty-five patients previously treated for PC underwent PET/CT for suspected recurrence. The sensitivity of PET/contrast-enhanced CT in detecting local recurrence, abdominal lymph node metastasis, and peritoneal dissemination were 83.3%, 87.5%, and 83.3% respectively[27]. PET detects tumor relapse earlier compared with CT, and influences treatment strategies in a significant percentage of patients. In a previous work, we studied the role of 18-FDG PET in detecting tumour relapse after PC resection in a series of 72 patients[28]. In that study, FDG-PET showed tumor recurrence in 28 patients with negative or inconclusive CT, enabling chemoradiotherapy to be started in 15 patients and the resection of recurrent disease in six[28]. Moreover, preoperative maximum standardized uptake value (SUV) seems predictive of PC recurrence in the early post-operative period[29]. Okamoto *et al*[29] studied SUV values obtained in preoperative FGD-PET and compared them between patients with and without PC recurrence within the first six postoperative months. They found that preoperative SUV was higher in the recurrence group of patients and that a high preoperative SUV was an independent risk factor for early tumor relapse after surgery. Thus, FDG-PET may play a crucial role in predicting and detecting postoperative tumor relapse after PC resection. The ideal timing for postoperative FDG-PET is not well defined, but it may be suggested to perform it 4-6 mo after surgery and at least 1.5 mo after any adjuvant therapy[28].

THE ROLE OF SURGERY FOR RECURRENT PC

Different patterns of recurrent PC have been described: locoregional recurrence (lymph node metastases, tumor relapse in the bed of pancreatic resection, tumor recurrence in the pancreatic remnant), distant metastases (liver, lung, bone) or peritoneal dissemination. Hepatic metastases seems to have a worse prognosis when compared to local recurrence[30,31]. Surgery for recurrent PC has been usually limited to solve gastrointestinal or biliary obstruction, being the morbidity and mortality expected for this kind of surgery high and the benefit for patients unclear. Re-resection of PC relapse is reported only as single case reports or in small series. Therefore, the clinical outcome of patients undergoing surgery for PC recurrence is not known. Even if PC recurrence has commonly be considered a systemic disease, several cases of isolated local recurrence have been reported[32]. Redo surgery for local recurrence (Table 1[4,33-39]) can consists in different surgical approaches, such as local dissection of lymph nodes, exeresis of soft tissue on the pancreatic bed or completion pancreatectomy of the remnant pancreas[4]. Strobel *et al*[32] reported a series of 105 patients undergoing operative expiration for suspected isolated local PC recurrence. Among these patients, 57 isolated local recurrence were intraoperatively confirmed and 41 resections were performed. Patients with confirmed isolated local recurrence had a longer median survival compared to patients with intraoperative finding of metastases (16.4 mo *vs* 9.4 mo)[32]. Moreover, a significantly longer survival was observed in the resected patients compared with the subgroup without resection due to local irresectability[32]. Lavu *et al*[33] reported a series of 11 patients (6 histologically proven) who underwent completion pancreatectomy for recurrence:

Table 1 Review of recent works on redo surgery for ductal adenocarcinoma local recurrence

Ref.	Year	*n*	DFI (mo)	Site of recurrence	Surgery	Associated procedure	Morbidity	Mortality	SPR	OS
Dalla Valle *et al*[36]	2006	1	18	1 panc remnant	1 RP	Distal gastrectomy, segmentary resection of transverse colon, splenectomy, extended lymph node dissection	0	0	24	42
Kleeff *et al*[35]	2007	12	13	8 local 2 local + stomach 2 local + mesentery	11 resection 1 partial gastrectomy	4 IORT 1 right hemicolectomy	NA	NA	13	NA
Koizumi *et al*[37]	2010	2	83 28	2 panc remnant	2 RP	/	NA	0	10 8	93 36
Lavu *et al*[33]	2011	8	27.5	8 panc remnant	8 RP	1 subtotal gastrectomy	(2/8) 25%	0	15	74
Thomas *et al*[34]	2012	7	41.1	1 abdominal wall 5 panc remnant 1 resection bed	2 resection 5 RP	NA	NA	0	NA	79.3
Kobayashi *et al*[38]	2012	1	36	1 panc remnant	1 RP	Partial pancreas autotransplantation	0	0	20	
Boone *et al*[39]	2013	10	25.3	3 resection bed 2 pancr remnant, small bowel 1 pancr remnant, colon 1 pancr remnant, small bowel, stomach 3 stomach	3 resection pancreatic bed mass 4 RP 2 partial gastrectomy 1 SBR	3 SBR 1 partial gastrectomy + splenectomy 1 partial colectomy	NA	0	32.4	59.1
Miyazaki *et al*[4]	2014	11	32	11 pancr remnant	11 RP	1 celicac resection + total gastrectomy 1 portal vein resection	(3/11) 27%	0	25	78.2
Total		62	27.5 (me)				/	0	17.5 (me)	66.55 (me)

DFI: Disease free interval (from primary pancreatic resection); RP: Repeat pancreatectomy; SBR: Small bowel resection; SPR: Survival post-reoperation; OS: Overall survival after initial pancreatectomy; NA: Not applicable; me: Median; IORT: Intraoperative radiation therapy.

The median survival after redo surgery was 32 mo with no postoperative mortality. Miyazaki *et al*[4] published a series of 11 patients undergoing repeated pancreatectomy for isolated local recurrence in the remnant pancreas: Survival after initial pancreatectomy was better in the repeated pancreatectomy group when compared to patients with unresectable recurrence (78.2 mo *vs* 20.3 mo). Thomas *et al*[34] published a series of 21 patients undergoing reoperation for pancreatic recurrence. Patients were selected for surgery according to the recurrence pattern: Patients with carcinomatosis or multiple sites of recurrence were excluded, while local recurrence, one single site of distant recurrence and regional recurrence (as a solitary abdominal wall implant) were considered for surgery[34]. In this series, patients with an initial disease-free interval > 20 mo had a longer median survival than those who did not. Kleeff *et al*[35] reported a survival benefit in patients with a longer disease free interval from primary resection longer than 9 mo. Some studies reported surgical metastasectomy of isolated liver and lung metastases after surgical resection of primary PC (Table 2[34,35,39,40]). Arnaoutakis *et al*[40] published a series of 9 patients undergoing metastasectomy of solitary lung metastasis, with a longer overall survival (51 mo *vs* 23 mo) in comparison to patients who did not receive surgery. The majority of these studies consists of small series of patients, without a true control group, so a general recommendation on redo surgery for PC recurrence cannot be given. However, the available data indicate a potential survival benefit after resection in selected patients. The low morbidity and mortality rates after reoperation reported in the published studies underline the feasibility of this kind of surgery in high volume centers. A careful patients selection plays a crucial role for considering re-resection of pancreatic recurrence. In fact, selecting patients with indolent surgical disease may be the key to give a survival benefit. In particular, patients with a good performance status, with a solitary surgically resectable location of recurrence, and with a relatively long disease free interval from primary pancreatic resection seem to benefit from redo surgery. Moreover, in re-resection for isolated local recurrence an R0 resection must be the goal to obtain a favorable prognosis. Regarding lung metastases, even if it seems that surgical resection in selected patients may be considered therapeutical options, more studies are needed to verify the true survival benefit in these patients. Another issue to focus on may be quality of life: Surgical re-resection could be considered not only for prolonged survival purpose, but also for symptoms palliation. Finally, surgery for recurrent PC has to be embedded in multimodality treatment of these patients, together with preoperative treatment, adjuvant or

Table 2 Review of recent works on redo surgery for ductal adenocarcinoma metastatic recurrence

Ref.	Year	*n*	DFI (mo)	Site of recurrence	Surgery	Associated procedure	Morbidity	Mortality	SPR	OS
Kleeff *et al*[35]	2007	2	15.5	2 liver	1 left hemihepatectomy 1 right hemihepatectomy	/	NA	NA	23.5	NA
Arnaoutakis *et al*[40]	2011	9	34	9 lung	10 lung resection	/	1 AF	0	18.6	51
Thomas *et al*[34]	2012	14	52.4 (LR) 7.6 (LiR)	1 brain 6 liver 7 lung	4 RFA 10 resection	/	NA	0	NA	92.3 (LR); 32.5 (LiR)
Boone *et al*[39]	2013	12	34.35 (LR) 17 (LiR) 7.6 (Ovary)	6 liver 5 lung 1 ovary	4 liver resection 2 RFA 5 lung resection 1 hysterectomy + BSO	2 RFA	NA	0	20.1 (LR) 13.9 (LiR) 12.7 (ovary)	70.8 (LR) 29.8 (LiR) 20.3 (ovary)
Total		37	25.5 (me)	14 liver 21 lung 1 brain 1 ovary				0	18.6 (me)	41.75 (me)

DFI: Disease free interval; SPR: Survival post-reoperation; OS: Overall survival; LR: Lung recurrence; LiR: Liver recurrence; AF: Atrial fibrillation; RFA: Radiofrequency ablation; BSO: Bilateral salpingo-oophorectomy; NA: Not applicable; me: Median.

palliative treatment. More studies are needed to define the clinical outcome of pancreatic re-resection, in combination with other therapeutical modalities.

CHEMORADIOTHERAPY

Limited information is available regarding the importance of chemoradiation applied in local or distant recurrence of PC. In 2006, Wilkowski *et al*[41] published a series of 18 patients with local metastases after surgical treatment of PC and treated with chemioradiotherapy. Five patients treated with Gemcitabine had a longer mean survival compared to four untreated patients (22.3 mo *vs* 6.6 mo). This was the first study suggesting that chemoradiotherapy could be an effective option in recurrent PC. In 2003 an open phase Ⅰ study on the feasibility of a combination therapy consisting of 5-FU/leucovorin plus oxaliplatin and irinotecan (FOLFIRINOX) for the treatment of patients with metastatic solid tumors was published[42]. The study showed anti-tumor activity in two patients with PC. Later Ⅱ phase trials specifically addressed patients with advanced and metastatic PC, showing promising results[43,44]. The randomized phase Ⅲ PRODIGE trial evaluated FOLFIRINOX versus gemcitabine alone in patients with metastatic PC and good performance status: A dramatic improvement in both median progression-free survival and median overall survival in favour of the group receiving FOLFIRINOX was seen[45]. Very recently, a phase Ⅲ clinical trial showed the efficacy of the combination nab-paclitaxel and gemcitabine to improve overall survival compared to gemcitabine alone for metastatic PC[46]. Limitation to these chemotherapy regimens is mainly due to their significant toxicity (neutropenia, thrombocytopenia, sensory neuropathy). Therefore, a balance between side effects and the significant but limited benefit offered by these chemoterapic regimens must be done together with the patient and his family. According to NCCN guidelines for recurrent PC, chemoradiation can be considered in patients with local recurrence only[47]. For patients with metastatic disease (with or without local recurrence), treatment decisions are influenced by the time interval between the end of adjuvant therapy to the diagnosis of metastases. If the interval time is less than 6 mo, an alternative chemotherapy option can be administered[47]. If it is greater than 6 mo, both previously administered systemic therapy and an alternative systemic regimen can be considered[47]. Recommended systemic regimens are the same as for second-line therapy in metastatic disease: Gemcitabine or gemcitabine-based combination therapy for patients previously treated with fluoropyrimidine-based therapy or fluoropyrimidine-based therapy for patients previously treated with gemcitabine-based therapy[47]. Conventional radiotherapy shows unsatisfactory local control because therapeutic radiation dose to the pancreatic tumor is limited by the sensitivity of surrounding tissues[48]. The cyberknife system, used since 2001 to liver radiation in any human radiosensitive tumor, seems to overcome this problem[49]. With the assistance of PET and CT Scan, Cyberknife offers a stereotactic boost of radiation alone or in combination with conventional radiation therapy. Although survival is determined primarily by a systemic control, local control is an important factor contributing to quality of life (pain control, prevention of gastric outlet obstruction)[50]. One more therapeutic option is given by radiofrequency ablation (RFA). RFA has shown to improve survival in patients with locally advanced unresectable PC[51,52]. Some studies have focused on the role of RFA in the treatment of liver metastases from PC. Park *et al*[53] performed RFA on 34 patients with liver metastases from PC: In oligometastatic patients they found an improved survival after RFA compared to patients without liver metastases and no treatment. Available data on chemoradiotherapy, cyberknife and RFA are few and derives from small series of patients. Larger randomized trial are needed in order to define the effective benefit of such therapeutic regimens, the best timing to start treating a patient and the best way to combine the different therapeutic options.

CONCLUSION

Even if few data are available in the Literature, multimodality approach to PC recurrence seems to offer a good palliation in a significant percentage of patients. Radical resection of recurrent tumor may be achievable in very selected patients who had undergone pancreatectomy for PC. Prolonged survival is possible in this subset of patients comparing to those receiving chemoradiotherapy or supportive care. Moreover, the combination of standard therapies (*i.e.*, chemoradiotherapy, surgery) with new treatment modalities (*i.e.*, RFA, Stereotactic radiotherapy, electroporation) may open a new window on an otherwise devastating disease. An accurate follow-up is thus warranted in order to improve the management of recurrent tumor. More studies are needed in order to better define clinical outcome of patients, timing for therapeutical approach and the way to combine surgery with other therapeutic options.

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EDITORIAL

Complete mesocolic excision: Techniques and outcomes

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Abstract

Complete mesocolic excision (CME) for the treatment of colon cancer was first introduced in the West in 2008. The first aim of this procedure is to remove the afflicted colon and its accessory lymphovascular supply by resecting the colon and mesocolon in an intact envelope of visceral peritoneum, which holds potentially involved lymph nodes. The second component of CME is a central vascular tie to remove completely all lymph nodes in the central (vertical) direction. In its original iteration, CME was performed via laparotomy, although many centers preferentially perform laparoscopic surgery, with its associated benefits and similar oncological outcomes, as the standard treatment for colonic cancer. Here, we present the surgical techniques for CME in open and laparoscopic surgery, as well as the surgical, pathological and oncological outcomes of the procedure that are available to date. Because there are no randomized control trials comparing CME to "standard" colon surgery, the principles underlying CME seem anatomical and logical, and the results published from the Far East, reporting an 80% 5-year survival rate for Stage III cancer, should guide us.

Key words: Colon cancer; Complete mesocolic excision; Laparotomy; Laparoscopic colectomy; Surgical technique; Oncological outcome

Core tip: This review presents the most recent knowledge in the field of complete mesocolic excision (CME) for colon cancer treatment and provides key points in both open and laparoscopic surgical techniques, surgical and pathological outcomes, and oncological outcomes of the procedure. The conclusion makes clear that in the absence of randomized control trials comparing CME to "standard" colon surgery, the principles underlying CME seem anatomical and logical, and the favorable long-term results published from the Far East for Stage III colon cancer disease should guide us in the future.

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INTRODUCTION

The years after the introduction of total mesorectal excision led to a major improvement in the survival rate of rectal cancer. Since its introduction, the five-year survival rate has increased from 45%-50% to 75%, and the local recurrence rate has decreased from 30% to 5%-8%[1]. The technique is based on the principle that dissection in the mesorectal plane produces an intact fascial-lined specimen, which contains all the blood vessels, lymphatic vessels, and lymph nodes through which the tumor may disseminate[2,3].

The embryological planes, however, are not narrowed to the rectum and mesorectal layers but continue to the sigmoid and descending colon on the left side, running behind the pancreas and around the spleen, and include the duodenum with the head of the pancreas, the cecum and ascending colon with the mesenteric root on the right side and the lymphatic drainage accompanying the arteries[4]. Thus, the surgical principles of total mesorectal excision have been extrapolated to colonic resection and complete mesocolic excision (CME), which was introduced by Hohenberger *et al*[4]. However, the principles of CME have not been adopted in a widespread manner[5], and the survival rates for colon cancer now lag behind those of rectal cancer[6].

We aim to describe the technique and the outcomes of the CME procedure. The purpose of the CME is to remove the afflicted colon and its accessory lymphovascular supply by resecting the colon and mesocolon in an intact envelope of visceral peritoneum. The mesocolon is situated within two layers of the visceral fascia[7]. This envelope holds potentially disseminated lymph nodes and, by removing it intact, the risk of cancer cells spilling into the peritoneal cavity is minimized. The second component of CME is a central vascular tie to remove completely all lymph nodes in the central (vertical) direction[8].

SURGICAL TECHNIQUE

Open surgery

In open surgery, a "lateral-to-medial" approach is generally performed. For right-side colon cancers, the dissection commences laterally by identifying the lateral peritoneal fold[9] an embryonic fusion plane that facilitates mesofascial and retrofascial separations. Anatomically and histologically, there is a single fascial layer separating the overlying mesocolon from the underlying retroperitoneum (Toldt's fascia). The dissection continues medially in the mesofascial interface. The mesenteric root up to the origin of the superior mesenteric pedicle is mobilized, and the dissection continues over the duodenum and pancreatic uncinate process to allow complete access to the superior mesenteric vein, as well as to the medially and inferiorly located superior mesenteric artery[10]. In their original description, Hohenberger *et al*[4] added a duodenal kocherization at this point, but that is not routinely performed[4]. Continuing medially, the small intestinal mesentery, ileocecal junction, right colon, right mesocolon and mesenteric confluence are fully mobilized and entirely intact from the underlying fascia and retroperitoneum[11]. The autonomic nervous plexus is identified and preserved[10].

After the complete mobilization, the ligation of the supplying vessels follows. Initially, the ileocolic and the right colic vessels (if present) are divided at their origin from the superior mesenteric vessels[4,10,12]. Sharp dissection is then carried out centrally along the superior mesenteric artery, ensuring clearance of all associated lymph nodes[10]. To expose the middle colic vessels, the lesser sac is entered by breaching the omentum caudal to the gastroepiploic arcade[10]. For cecal and ascending colon cancers, only the right branch of the middle colic vessels is divided[4,10,13]. The surgeon needs to be aware of the gastrocolic vein and the loop of Henle because the peri-pancreatic venous vascularity is subject to variability. The transverse mesocolon dissection is continued vertically to meet the dissection along the superior mesenteric vascular pedicle, producing a rectangular specimen with an intact mesocolic envelope containing all central lymph nodes[14]. At that point, the colon is divided at the level of the middle colic vessels[4].

For neoplasms of the hepatic flexure or proximal transverse colon, the lesser sac is entered by dividing the right gastro-epiploic artery and continuing vertically to the transverse colon. The middle colic artery is divided at its origin from the superior mesenteric artery, while the middle colic vein is divided at its junction to the gastrocolic trunk or the superior mesenteric vein[4,9,10,13]. The right gastro-epiploic artery may need to be divided at its origin to allow the retrieval of the peri-pancreatic lymph nodes[4,10]. Some authors[8] advocate for the dissection of the lymph nodes in the lateral 10 cm of the right gastro-epiploic vascular curvature, including the sub-pyloric and over the pancreatic head lymph nodes. For hepatic flexure cancers, the colon is resected near to the splenic flexure[4].

For cancers situated to the left of the middle colic artery, lymph nodes along the inferior aspect of the left pancreas, as well as lymph nodes along the left gastro-epiploic arcade, may be resected[8]. If lymph nodes over the pancreatic head are potentially involved, these nodes should be dissected off the pancreatic head with central ligation of the right gastroepiploic artery. The superior pancreaticoduodenal artery is usually preserved. The surrounding autonomous nervous plexus must be preserved to avoid the risk of functional consequence, *e.g.*, diarrhea[4].

For left colon cancers, the "lateral-to-medial" dissection begins at the lateral peritoneal fold and continues in the mesofascial interface. After the whole mesocolon of the descending and sigmoid colon is dissected, the ureter and the vesicular or ovarian vessels are recognized and left behind. The greater omentum is separated from the transverse colon and the lesser sac is fully exposed, and the two layers of

the transverse mesocolon are divided at the lower edge of the pancreas[4]. The splenic flexure is mobilized when needed. For cancers of the descending colon, ligation of the ascending branch of the left colic artery and dissection of the lymph nodes at the origin of the superior mesenteric artery, without damaging the superior hypogastric plexus, is advocated. For cancers located in the middle of the descending colon down to the sigmoid colon, the root of the inferior mesenteric vessels below the pancreas is divided. Colon is divided proximally, between the left transverse colon and the distal descending colon, depending upon the site of the tumor, while transection distally is always in the upper third of the rectum[4,8].

Laparoscopic surgery

In laparoscopic surgery, a "medial-to-lateral" approach is preferable. In a laparoscopic right hemicolectomy, the mesocolon is incised along the mesenteric axis close to the superior mesenteric vein. The ileocolic vessels are divided as close as possible to the superior mesenteric vein[15]. After exposing the mesocolic interface, a wide separation between the pancreatic head and the transverse colon is achieved. Dissection then proceeds along the superior mesenteric vein, exposing the gastrocolic trunk of Henle. Next, the middle colic artery is identified as it rises from the superior mesenteric artery and is severed at the root of its right branch. This is accompanied by lymph node dissection, taking care to preserve the left branch of the middle colic artery. Simultaneously, the middle colic vein is identified and severed at the root of its right branch. Next, an anterior-to-median approach is performed by dissecting the right side of the greater omentum. The fusion fascia is detached between the omentum and transverse mesocolon and the hepatic flexure is mobilized[16]. The accessory middle colic veins are carefully dissected, accompanied by lymph node dissection, and the transverse mesocolon is dissected below the lower edge of the pancreas, uncovering the superior mesenteric vein. The specimen is extracted by a mini-laparotomy, and an extracorporeal anastomosis is performed[15-17].

In a laparoscopic left hemicolectomy, the procedure starts by retracting the sigmoid mesocolon anteriorly, and the visceral peritoneum on the base of the sigmoid mesocolon is incised at the level of the sacral promontory. The incision continues upward to the ligament of Treitz, and the origin of the inferior mesenteric artery is exposed and divided 1 cm from the aorta. The inferior mesenteric vein is divided below the inferior border of the pancreas. The mesocolic interface is entered and the dissection continues from medial to lateral. Laterally, the sigmoid loop is mobilized by incising along the lateral peritoneal fold. When mobilization of the splenic flexure is required, a medial approach is used. Retracting the transverse colon anteriorly, the root of the transverse mesocolon is dissected onto the body and tail of the pancreas, entering the lesser sac. Then, the dissection moves toward the base of the distal transverse colon and the descending colon. The posterior attachments of these structures are divided. The lateral attachment is freed up to the spleen and the phrenocolic ligament. The splenic flexure is fully mobilized after the omentum is separated from the colon. The distal division of the colon is performed intracorporeally using a linear stapler. The proximal division is performed extracorporeally after dividing the mesocolon up to the chosen site. The specimen is generally extracted through an incision at the level of the umbilicus. Anastomosis is performed intracorporeally using a circular stapler device, which is passed transanally[13,18].

The laparoscopic management of colon cancer close to the flexures and in the transverse colon is still controversial. Many centers use the open approach for these tumors as the standard treatment[19]. Others, for hepatic flexure or proximal colon transverse cancers, perform an extended right hemicolectomy with central ligation of the middle colic and right gastroepiploic vessels, removal of subpyloric lymph nodes, and colon stapling proximal to the splenic flexure[17].

OUTCOMES

To date, the vast number of available studies evaluating CME is retrospective. In a small number of series, CME has been compared to "standard" or "traditional" colon surgery. The problem with "standard" colon surgery is that the surgical technique depends on the individual surgeon and the presence of radical lymph node dissection. It is becoming increasingly evident that differences in oncologic outcomes reported among surgeons are directly related to the differences in the techniques used[18].

Surgical and pathological outcomes

West *et al*[20] reported that specimens from colon cancer resections from Erlangen, Germany, where CME and central venous ligation are routinely applied, are more often in the correct anatomical (mesocolic) plane (92% *vs* 40%, $P < 0.0001$) and have higher number of lymph nodes harvest (median 30 *vs* 18, $P < 0.0001$) compared to standard specimens from Leeds, United Kingdom.

A similar inter-institutional comparison was performed by the same authors[21] among six Danish hospitals where "traditional" surgery was performed and Hillerod Hospital, where surgeons attended a surgical educational training program in CME. As anticipated, the resection specimens from the latter center were characterized by a larger mesenteric surface (144.6 cm^2 *vs* 87.1 cm^2, $P < 0.0001$) and an increased lymph node harvest (median 28 *vs* 18, $P < 0.0001$).

Bertelsen *et al*[15] described how the induction of CME in Hillerod Hospital in 2008 has influence the surgical and pathological outcomes. They reported that the length of the vascular ligation increased from 7.1 to 9.6 cm ($P < 0.0001$), and the mean number

of harvested lymph nodes increased from 24.5 to 26.7 (P = 0.0095). However, the plane of mesocolic resection, the rate of R0 resection and the risk of complications were equivalent, while the median length of hospitalization increased from 4 to 5 d (P = 0.04).

The most recent retrospective population study[22] also reported a statistically significantly greater lymph node harvest in CME compared to non-CME (36.5 *vs* 20.9 P < 0.0001) groups of patients. In addition, 82% of the CME group was dissected in the mesocolic plane, compared to 60% of the non-CME group (P < 0.0001). The CME group in this population study was also from Hillerod Hospital.

Galizia *et al*[12] reported that the number of the harvested nodes and the length of the vascular ligation were significantly better in the CME group (P < 0.01). Moreover, a higher number of tumor deposits were harvested, thus allowing chemotherapy in those newly upstaged patients.

In a systematic review[23], CME resections had a weighted mean R0 rate of 89.9% compared to 86.7% for standard resections.

Interestingly, studies comparing dissection planes in specimens from "standard" and CME resection concluded that the rates of mesocolic and R0 resections were equivalent in the two techniques[15,22], supporting the argument that the majority of trained colorectal surgeons perform mesocolic resection. Thus, CME represents an appealing appellation for an already-practiced technique[24,25].

Oncological outcomes

West *et al*[26] showed that meticulous mesocolic plane surgery is associated with a 15% greater 5-year overall survival rate compared with cases where defects in the mesocolon reached into the mascularis propria.

A Norwegian retrospective study[27], compared colon cancer survival between one hospital that used the CME approach and two other centers that used the "standard" approach. Investigators included only Stage Ⅰ and Ⅱ colon cancer for analyses. In the two groups, there were no significance differences between the T stage (P = 0.171). The authors observed a better 3-year overall survival rate (88.1% *vs* 79.0%, P = 0.003) and disease-free survival rate (82.1% *vs* 74.3%, P = 0.026) in the CME group of patients, while the cancer-specific survival rate was 95.2% in the CME group *vs* 90.5% in the standard group (P = 0.067). Multivariate Cox regression analysis disclosed age, operative technique and T category as independent prognostic factors for both overall and disease-free survival.

Galizia *et al*[12] compared colon cancer recurrence and survival before and after the introduction of CME in 2008 in the same Italian center. Interestingly, there was no local recurrence in the CME group but there was in 21% of the standard group, while distant metastases occurred with similar frequencies (13.3% and 13.7%, respectively). We should mention, however, that significantly more early stage cancer patients were enrolled in the CME group.

Shin *et al*[18] reported a study of 168 patients with Stage Ⅱ and Stage Ⅲ colon cancer treated by laparoscopic CME. A remarkable 89.6% 5-year survival rate was reported.

In the most recent retrospective population study from Demark[22], 364 patients who underwent CME were compared to 1031 patients who were treated with non-CME colectomies. For all patients, the 4-year disease-free survival rate was 85.8% after CME and 75.9% after non-CME surgery (P = 0.0010). Multivariable Cox regression analysis showed that CME surgery was a significant, independent, favorable predictive factor for higher disease-free survival rates for all patients (HR = 0.59; 95%CI: 0.42–0.83) and also for patients with UICC Stage Ⅱ (HR = 0.44; 95%CI: 0.23-0.86) and Stage Ⅲ disease (HR = 0.64, 95%CI: 0.42-1.00). After propensity score matching, the disease-free survival rate was significantly higher after CME, irrespective of UICC stage, with a 4-year disease-free survival rate of 85.8% after CME and 73.4% after non-CME (P = 0.0014). In the same study, overall survival was not significantly higher in the CME group compared to the non-CME group. The authors believe that this may be due to the relatively short follow-up, the improved surgical outcomes for resection of lung and liver recurrences, or advances in chemotherapy for patients with non-resectable recurrences[22].

In a systematic review[23] of 5246 patients, the weighted mean local recurrence rate and the 5-year overall and disease-free survival rates were 4.5%, 58.1% and 77.4%, respectively, with a mean follow-up of 60 months. In the same review of 22 papers on CME, there were overall survival rate (58.7% *vs* 53.5%), disease-free survival rate (77.4% *vs* 66.7%) and local recurrence rate (4.5% *vs* 7.8%) advantages in the CME group.

The improved outcome after CME is likely related to resection in the mesocolic plane[17,19,25] and to high ligation of the tumor-feeding vessels[4,25]. It is unclear which of the two components of CME is more important. We believe that complete removal of an intact meso-colonic envelope (complete mesocolic excision), along with central vascular ligation and apical node dissection, is essential for improving the outcomes.

CONTROVERSIES REGARDING CME

There is a great deal of discussion and debate regarding whether the CME concept is new. The CME technique was introduced in the West in 2008, but Japanese, Chinese, Korean and Taiwanese surgeons have used D3 lymphadenectomy resections for colon cancer for decades. They D3 lymphadenectomy is defined as the dissection of the paracolic, intermediate and central lymph nodes, a procedure equivalent to CME[19].

CME is a more extensive operation than a standard procedure. Originally CME was described as an open procedure[4], although many centers prefer performing

laparoscopic surgery, with its associated benefits[28-31] and similar oncological outcomes[8], as the standard treatment for colonic cancer.

A small comparison study between laparoscopic and open CME approaches concluded that laparoscopy offers specimens of similar quality to the open CME approach in terms of lymph node harvest, rate of achievement of an intact mesocolic plane, and distance from high tie to tumor and high tie to nearest bowel wall in proximal right- and left-sided resections. However, for transverse and hepatic flexure tumors, the open CME group had better outcomes in distance from tumor to high tie and nearest bowel wall to high tie compared to the laparoscopic group[13]. Similar oncological results were found in a prospective study from Norway that compared laparoscopic to open CME. The 3-year overall survival rate (80.4% *vs* 88.2%, $P = 0.152$) and disease free survival rate (74.8% *vs* 80.0%, $P = 0.405$) were similar[32].

A recent Korean study comparing the outcome of laparoscopic right to open right CME showed a better 5-year overall survival rate in the laparoscopic group compare with the open group (77.8 *vs* 90.3%, $P = 0.028$) and a similar 5-year disease-free survival rate (71.8% *vs* 83.3%, $P = 0.578$)[33].

For proximal right and left sided tumors, laparoscopic CME can be performed with safety and good oncological outcome. However, for tumors located near the flexures or in the transverse colon, the open approach is still the standard[22].

CME is a longer operation[19,34], which may lead to increased morbidity, but it does reduces the efficiency of an operating theater and influence the health economy[19]. The duration of surgery remains one of the largest obstacles for laparoscopic CME[34]. The operative duration learning curve reveals an initial duration of approximately 250 min, which is more than double the duration reported for a conventional laparoscopic right hemicolectomy performed by experienced laparoscopic surgeons[35].

Even though CME is a more extensive procedure, mortality and complication rates are in acceptable ranges. In a systematic review[22], overall morbidity, 30-d mortality and re-operative intervention for vascular complications were 19.4%, 3.2% and 1.1% respectively and mean blood loss was 150 mL, all comparable to the reported contemporary series for "standard" resections[36,37] However, unusual complications, such as chyle leakage[18], duodenal injury[28] and major vascular injury[38] have been reported.

CONCLUSION

There are no randomized control trials comparing CME to "standard" colon surgery. The concept of CME and the new anatomical characteristics of the mesocolon, as described by Culligan *et al*[5], offer a great opportunity to re-evaluate colon cancer surgery. The principles underlying CME are anatomical and logical, and the results published from the Far East, reporting an 80% 5-year survival rate for Stage Ⅲ disease[18], should guide us.

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TOPIC HIGHLIGHT

2015 Advances in Gastric Cancer

Neo-adjuvant chemo(radio)therapy in gastric cancer: Current status and future perspectives

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Abstract

In the last 20 years, several clinical trials on neo-adjuvant chemotherapy and chemo-radiotherapy as a therapeutic approach for locally advanced gastric cancer have been performed. Even if more data are necessary to define the roles of these approaches, the results of preoperative treatments in the combined treatment of gastric adenocarcinoma are encouraging because this approach has led to a higher rate of curative surgical resection. Owing to the results of most recent randomized phase III studies, neoadjuvant chemotherapy for locally advanced resectable gastric cancer has satisfied the determination of level I evidence. Remaining concerns pertain to the choice of the optimal therapy regimen, strict patient selection by accurate pre-operative staging, standardization of surgical procedures, and valid criteria for response evaluation. New well-designed trials will be necessary to find the best therapeutic approach in pre-operative settings and the best way to combine old-generation chemotherapeutic drugs with new-generation molecules.

Key words: Gastric Cancer; Neo-adjuvant treatment; Chemotherapy; Radiotherapy

Core tip: Owing to the results of the most recent

randomized phase III studies, neoadjuvant chemotherapy for locally advanced resectable gastric cancer has satisfied the determination of Level I evidence. Remaining concerns pertain to the choice of the optimal therapy regimen, strict patient selection by accurate pre-operative staging, standardization of surgical procedures, and reliable criteria for response evaluation. New well-designed trials will be necessary to identify the best treatment plan in pre-operative settings and to understand how to combine the conventional chemotherapeutic drugs with new-generation molecules.

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INTRODUCTION

Currently, gastric carcinoma remains one of the most widespread tumors in the world[1]. Surgical resection has a relative role in cancers with early lymphatic diffusion, distant metastasis or peritoneal involvement. Thanks to the introduction of screening protocols in high-incidence nations such as Japan and Korea, almost 50% of patients with gastric cancer receive an early diagnosis[2]. However, this screening approach has not shown a cost-effective advantage in lower-incidence countries like Europe and North America. As a consequence, most gastric cancers in the West are already in a locally advanced stage and with lymphatic spread at diagnosis[3]. Many attempts have been made to improve patients' survival, tailoring the extent of surgery and adding the administration of pre-operative and/or post-operative treatment.

In the last twenty years, large-scale randomized trials have demonstrated the efficacy of three different multimodal approaches: Adjuvant chemoradiation treatment (Unites States INT-0116 trial)[4], adjuvant single-drug chemotherapy (Japanese ACTS-GC trial)[5] and perioperative three-drug combination chemotherapy (European MAGIC trial)[6]. After the publication of the results of these trials, standard treatment in patients with locally advanced gastric cancer is no longer based on surgery alone, and the goal of an R0-resection is not exclusively a surgical target. In this review, we discuss the rationale and the state of the art of preoperative neoadjuvant therapy in light of recent evidence and new perspectives.

NEOADJUVANT TREATMENT: THEORETICAL RATIONALE AND LIMITATIONS

Preoperative treatment (*i.e.*, neoadjuvant treatment) has led to higher rates of curative surgical resection in many solid tumors such as rectum and breast carcinoma. In gastric adenocarcinoma, the mainstay of therapeutic treatment still remains surgical resection, and neoadjuvant therapy appears to be justified by similar advantages[7].

Theoretical rationale

Biological rationale: (1) neoadjuvant treatment gives the chance to downstage and downsize the primary gastric tumor and to reach a more probable curative R0 resection; (2) the use of chemo or radiotherapy before surgery provides the theoretical advantage of treating an "untouched" neoplasia (lack of treatment-induced resistance) with intact vessels and without fibrotic remodeling of the tumor bed following surgery; and (3) pre-operative systemic therapy targets micrometastases, being administered when there is an high growth fraction of the cells and the total tumor volume is relatively low for gastric cancer.

Upfront randomization and feasibility: Randomized clinical trials studying adjuvant therapy in gastric cancer may be not representative of the entire curatively operated population because poor patient compliance is often seen after surgery. In addition, due to frequent dose reductions and treatment delays, it is harder to demonstrate a reliable advantage for the treatment arm. Conversely, randomized studies of neoadjuvant systemic treatment allow an appropriate randomization without significant pre-selection and with greater feasibility.

Monitoring: In contrast to adjuvant treatment, which is administered on the basis of clinical trial results with no chance to demonstrate its efficacy on an individual basis, the efficacy of pre-operative treatment can be assessed during its administration. Thus, therapy can be adjusted according to patient response.

Limitations

Pre-operative staging: While adjuvant therapy is based on the pathologic staging performed at the time of the resection of a given tumor, pre-operative treatment is based on clinical staging. In gastric cancer, as discussed earlier, the decision of whether to perform neoadjuvant therapy, based only on clinical staging, remains difficult.

Delayed surgery: The concept of "delayed surgery" is a relatively new entry among the therapeutic options available for gastric carcinoma. Several studies have shown that delaying surgical treatment in favor of preoperative systemic therapy does not reduce the benefits of a postponed but potentially curative resection and that it does not worsen surgical outcomes. However, there is a small number of cases in which the possibility of disease progression during adjuvant therapy persists, and this is the only justification for an

Table 1 Pre-operative chemotherapy in non-resectable gastric cancer

Ref.	Regimen	No. patients	Stage	R0 resection (%)	Median survival (mo)
Wilkie *et al*[8]	EAP	34	NR	44	24
Plukker *et al*[9]	5-FU + MTX	20	NR	40	22
Rougier *et al*[10]	5-FU, P	30	NR	60	16
Kelsen *et al*[11]	FAMTX, IP 5FU-P	56	NR	61	15
Melcher *et al*[12]	ECF	27	R-NR	58 (R pts) 10 (NR pts)	10
Gallardo-Rincón *et al*[13]	P-ELF	60	NR	8,7	10
Cascinu *et al*[14]	EAFPLG	82	NR	45	17

NR: Non-resectable; EAP: Etoposide, doxorubicin, cisplatin; IP: Intraperitoneal; ECF: Epirubicin, cisplatin, 5-FU; R: Resectable; P-ELF: Cisplatin, etoposide, leucovorin, 5FU; EAPFLG: Epi-doxorubicin, 5-FU, cisplatin, leucovorin, glutathione; 5-FU: 5-fluorouracil.

unwillingness to carry out a multimodal preoperative treatment in gastric cancer. Actually, patients who progress while on chemotherapy are unlikely to benefit from resection and can be spared radical surgery. The long therapy developmental time period for neoadjuvant treatment in gastric cancer over the last thirty years partially explains some of the skepticism about this treatment option[7].

Contraindications: Preoperative treatments are contraindicated in obstructive or hemorrhagic cancers. In particular, the lesions of the cardia or the prepyloric areas can already be completely obstructive at the time of diagnosis. In those situations, upfront surgery is the best treatment, but preoperative systemic therapy could be considered, *e.g*., making a jejunostomy for enteral feeding or using parenteral feeding. Rarely does a gastric neoplastic lesion bleed acutely; however, it can be dramatic, and in this case, direct salvage surgery is the only chance.

As explained above, the feasibility, randomization, facility, biological rationale and monitoring represent several potential advantages that make neoadjuvant treatment an interesting path to consider for investigation and patient management. For this reason, many authors over the last 30 years have reported experiences with neoadjuvant treatments in locally advanced gastric cancer (preoperative chemotherapy, preoperative radiotherapy or both).

PRE-/PERI-OPERATIVE CHEMOTHERAPY

Investigation of the efficacy and possible uses of chemotherapy (CT) in patients with advanced gastric cancer began in the late 1970s, but the first encouraging results were not reported until the early 1990s when two independent studies in patients with non-resectable gastric cancer demonstrated that chemotherapy treatment enabled subsequent surgical resection in 40%-50% of patients, with an increase in total median survival of 18 mo compared with unresected patients[8,9]. Following these encouraging preliminary results, neoadjuvant chemotherapy protocols were introduced not only for patients with non-resectable disease (Table 1)[8-14] but also for patients with potentially resectable, locally advanced gastric cancer (Table 2)[6,11,15-27]. Nevertheless, the interpretations of the results of these pioneer studies were limited by their methodological drawbacks. These limitations included the heterogeneous criteria used in the recruitment of patients, such as the inclusion of patients with locally advanced gastric cancer and patients with cancer of unclear stages, and the absence of a clear distinction between resectable and non-resectable cancers. Moreover, other causes of bias in these first trials included the use of different chemotherapeutic protocols, non-standardized surgery or surgery of questionable quality, and the absence of accurate response criteria.

Randomized controlled trials

The first randomized controlled trial of exclusively neoadjuvant chemotherapy for gastric cancer dates back to 1993 and was conducted by the Dutch Gastric Cancer Group[21]. In this trial, cardia tumors were not included, and the chemotherapeutic protocol was based on 5-fluorouracil, doxorubicin, and methotrexate (FAMTX) because at that time, it was the gold standard of treatment for gastric adenocarcinoma. The study was prematurely stopped because an interim analysis demonstrated that using FAMTX as neoadjuvant chemotherapy did not provide the goal of a 15% increase in curative resection. This trial contained many biases, though, such as the use of inappropriate staging procedures, with optional use of CT or laparoscopy, or the inadequacy of lymph node dissection. In this trial, a 36% rate of tumor progression during therapy was found in patients treated with neoadjuvant chemotherapy. Moreover, there was a decreased rate of curative resections (56% *vs* 62%) and a reduction in the median survival rate of treated patients *vs* untreated patients (18 mo *vs* 30 mo). The results of this trial were discouraging, even if the observed differences were statistically insignificant[28].

Since the late 1990s, rigorous European phase Ⅲ trials have been designed and performed to demonstrate the efficacy of neoadjuvant therapy, but in some cases, the selection criteria of patients were too strict, leading to premature cessations due to low patient accrual (EORTC 40954 and SWS-SAKK-43/99 trials)[25,27].

Table 2 Peri-operative chemotherapy in resectable gastric cancer

Ref.	Phase	Selection criteria	Study arms	No. of patients	R0 resection (%)	Pathologic CR (%)	Median survival (mo)
Ajani *et al*[15]	II	M0 Resectable + EGJ	EFP × 2 + surgery + EFP × 3	25	72	0	15
Leichman *et al*[16]	II	M0 resectable	PFL × 2 + surgery (IP FUDR + IP cisplatin × 2)	8	88	8	> 17
Kang *et al*[17]	III RCT	M0 Loc. advanced	EFP × 3 + surgery + EFP × 3-6 *vs* surgery + EFP × 3-6	107 (53 + 54)	79 *vs* 61	8	43 *vs* 30
Ajani *et al*[18]	II	M0 resectable	EAP × 3 + surgery + EAP × 2	48	90	0	16
Rougier *et al*[19]	II	M0 Loc. advanced + EGJ	FP × 6 + surgery	30	78	0	16
Kelsen *et al*[11]	II	M0 Loc. advanced	FAMTX × 3 + surgery + IP FP + F	56	77	NS	15
Crookes *et al*[20]	II	M0 resectable + EGJ	PFL × 2 + surgery (IP FUDR +IP cisplatin × 2)	59	71	9	52
Songun *et al*[21]	II RCT	T2-T4; M0	FAMTX × 3 + surgery *vs* surgery alone	56 (27 + 29)	75 *vs* 75	NS	18 *vs* 30
Schuhmacher *et al*[22]	II	III-IV; M0 + EGJ	EAP + surgery	42	86	0	19
D'Ugo *et al*[23]	II	T3-4 any N; T ⩽ 2 N+; M0	EEP × 3 or ECF × 3 + surgery + EEP × 3 or ECF × 3	34	82	3	> 28
Cunningham *et al*[6] (MAGIC trial)	III RCT	Resectable GC (II-IV); M0 + adenocarcinomas EGJ	ECF × 3 + surgery + ECF × 3 s *vs* surgery alone	503 (250 + 253)	74 *vs* 68	NS	18 *vs* 30
Ychou *et al*[24] (ACCORD trial)	III RCT	Resectable GC + adenocarcinomas EGJ	FP × 2-3 + surgery + FP × 3-4 *vs* surgery alone	224 (113 + 111)	84 *vs* 73	NS	NS
Schuhmacher *et al*[25] (EORTC trial)	III RCT	Loc. advanced GC T3-T4N × M0	PFL × 2 *vs* surgery alone	144 (72 + 72)	81.9 *vs* 66.7	NS	> 36
Kinoshita *et al*[26]	II	Schirrous resectable	S-1 × 2 + surgery	55	80.8	0	NS
Biffi *et al*[27]	III RCT	T3-4 any N or any T N1-3 M0 + EGJ	TCF × 4 + surgery *vs* surgery alone	69 (34 + 35)	85	11.7	NS
Yoshikawa *et al*[30],	II RCT	T2–3/N+ or T4aN0 + EGJ	SC × 2 + surgery *vs* SC × 4 + surgery *vs* PC × 2 + surgery *vs* PC × 4 + surgery	83 (21 + 20 + 21 + 21)	NS	10 10	NS

EL: Exploratory laparotomies; R0: Curative (R0) resections; CR: Complete response; EFP: Etoposide, fluorouracil, and cisplatin; GC: Gastric cancer; IP: Intraperitoneal; FUDR: 5-fluoro-2'-deoxyuridine; RCT: Randomized controlled trial; EAP: Etoposide, doxorubicin, cisplatin; FP: Fluorouracil, cisplatin; F: Fluorouracil; NS: Not stated; EEP: Etoposide, epirubicin, cisplatin; TCF: Docetaxel, cisplatin, fluorouracil; SC: S1, cisplatin; PC: S, cisplatin.

Schuhmacher *et al*[25] reported data from the European Organization for Research and Treatment of Cancer 40954 phase III trial (EORTC) comparing neo-adjuvant cisplatin, folinic acid, and infusional fluorouracil (PLF protocol) with surgery alone in patients with locally advanced adenocarcinoma of the stomach. Unfortunately, this study had to be stopped before its conclusion because of poor accrual following the inclusion of only 144 patients instead of the 360 patients initially expected. Patients assigned to chemotherapy received 48-d cycles of neo-adjuvant biweekly cisplatin, weekly L-folinic acid and 5-fluorouracil (5-FU) for 2 cycles. Only 62.5% of patients assigned to the chemotherapy arm completed 2 cycles of treatment. Median follow-up was approximately 4 years. Pre-operative chemotherapy reduced tumor size and nodal involvement compared to surgery alone. Progression-free survival had a hazard ratio of 0.76 but was not statistically significant (95%CI: 0.49-1.16; P = 0.2). The hazard ratio for overall survival was 0.84 in favor of chemotherapy, though it was not a statistically significant finding (95%CI: 0.52-1.35; P = 0.466). The rate of R0 resection was higher in the group that received neo-adjuvant chemotherapy compared to those undergoing primary surgery (81.9% *vs* 66.7%; P = 0.036).

Although not statistically significant, patients undergoing preoperative CT showed a higher rate of postoperative complications than patients treated with primary surgery (27.1 *vs* 16.2%; P = 0.09). In addition, postoperative death was more common among patients who underwent neoadjuvant chemotherapy (4.3% *vs* 1.5%).

Only the MAGIC trial (started in the United Kingdom in 1994) and the FFCD 9703 trial (started in France in 1996) have been completed[6,24]. The MAGIC trial is presently the most recognized landmark study for perioperative CT. Between 1994 and 2002, 45 centers in the United Kingdom, Europe and Asia recruited patients with resectable gastric cancer and adenocarcinomas of the esophagogastric junction (EGJ)[6]. Patients were randomized in two arms. In the first arm, patients underwent surgery associated with perioperative chemotherapy (n = 250), based on three cycles of neoadjuvant and three cycles of adjuvant epirubicin cisplatin and continuous 5-fluorouracil (ECF). In the second arm, patients underwent surgery only (n = 253). Only 49.5% of the patients who underwent

pre-operative treatment also received the full courses of the planned post-operative CT because of poor performance status, complications or compliance issues in the post-operative period. Median follow-up was approximately 4 years. The group of patients who underwent perioperative treatment had a higher rate of curative resection (79% *vs* 70%; $P = 0.03$), smaller tumors (T1-T2: 51% *vs* 36%; $P = 0.002$) and lower nodal involvement (N0-N1: 84% *vs* 70%; $P = 0.01$). Overall, survival and progression-free survival were significantly increased in patients receiving perioperative CT compared with patients treated by surgery only (HR = 0.75; $P = 0.009$ and HR = 0.66; $P < 0.001$). The 5-year survival rate was 36% for patients receiving perioperative CT and 23% for patients treated by surgery only.

A retrospective study from the United Kingdom on a series of 66 patients undergoing perioperative CT according to the MAGIC protocol confirmed the benefit in terms of disease-free survival for patients receiving neo-adjuvant as well as adjuvant treatment compared with patients who did not undergo post-operative CT[29].

The results of the French FNLCC ACCORD 07 FFCD 9703 trial confirmed data in favor of the establishment of perioperative CT for patients with resectable gastric cancer and esophageal adenocarcinoma[24]. Only 25% of the patients in this study had gastric cancer, while the remaining patients had esophageal or EGJ tumors. The chemotherapeutic regimen consisted of 2 or 3 pre-operative cycles and 3 or 4 post-operative cycles of 5-fluorouracil and cisplatin. A total of 224 patients were randomized to receive pre-operative CT ($n = 113$) or primary surgery ($n = 111$). The median follow-up was 5.7 years. The R0 resection rate was 84% in the chemotherapy group compared to 74% in the surgery group ($P = 0.04$). Overall, survival and disease-free survival were significantly prolonged after CT (HR = 0.69; $P = 0.02$ and HR = 0.65; $P = 0.003$, respectively). The 5-year survival rates were 38% in the CT- and 24% in the surgery-only arm.

More recently, early results from a Japanese phase Ⅱ randomized study (the COMPASS trial) have shown a high rate (approximately 10%) of complete pathologic response after neo-adjuvant chemotherapy with four cycles of S1/cisplatin or paclitaxel/cisplatin regimens without a marked increase of toxicities[30].

Meta-analyses

To date, four meta-analyses regarding neo-adjuvant chemotherapy have been published. The first systematic review and meta-analysis was published by Wu *et al*[31] in 2007, which included only 4 randomized controlled trials, and concluded that neo-adjuvant chemotherapy should not be used routinely in clinical settings until further results are available. Similarly, the second meta-analysis performed in 2008 by He *et al*[32], which included 5 randomized controlled trials, concluded that evidence for the efficacy of neo-adjuvant chemotherapy on gastric cancer was weak and that pre-operative treatment should not be recommended as a regular treatment for gastric cancer.

In 2010, Li *et al*[33] conducted the third meta-analysis that included randomized and non-randomized controlled trials. This study showed a marginal improvement in the 3-year overall survival rate for patients who received perioperative chemotherapy (OR = 1.27; 95%CI: 1.04-1.55) in addition to surgery. Furthermore, this study found that perioperative chemotherapy was more beneficial in improving overall survival in later-stage (pT3-4) gastric cancer *vs* earlier stage (pT1-2) (OR = 1.91; 95%CI 1.24-2.96).

The small number of studies included within the meta-analyses from Wu *et al*[31] and He *et al*[32] and the non-randomized controlled trials included in the meta-analysis by Li *et al*[33] may together compromise the reliability of the results of those meta-analyses.

The most recent meta-analysis was published in 2014 by Xiong *et al*[34] and has provided, by its strict inclusion criteria as well as its population subgroup and regimen-subgroup analyses, the most robust evidence so far on neo-adjuvant chemotherapy. This review concluded that while neo-adjuvant chemotherapy offered a marginal survival benefit over the control group with an OR of 1.32 (95%CI: 1.07-1.64, $P < 0.01$), it significantly improved the 3-year progression-free survival (OR = 1.85; 95%CI: 1.39-2.46; $P < 0.0001$), tumor down-staging rate (OR = 1.71, 95%CI: 1.26-2.33; $P < 0.0006$) and R0 resection rate (OR = 1.38; 95%CI: 1.08-1.78; $P < 0.01$) of patients with advanced gastric cancer.

Finally, a Cochrane Systematic Review conducted by Ronellenfitsch *et al*[35] in 2013 on perioperative chemoradiotherapy *vs* primary surgery for resectable adenocarcinoma of the stomach, gastroesophageal junction, and lower esophagus. The findings showed an absolute improvement in survival of 9% at 5 years for patients undergoing perioperative chemotherapy. This benefit was evident 18 mo after surgery and was maintained for 10 years. The odds of a R0 resection in patients treated with perioperative CT were 1.4 times higher than in untreated patients. Moreover, in subgroup analyses, the authors demonstrated a higher survival benefit for patients with tumors of the EGJ compared to other sites.

PRE-OPERATIVE RADIO(CHEMO)THERAPY

Following the results of the SWOG 9008/INT-0116 trial, the use of a preoperative combination of chemotherapy and radiotherapy garnered increased interest[4]. There have been several pivotal randomized single center studies on preoperative radiotherapy. In the trial performed by Zhang *et al*[36], 317 patients with adenocarcinoma of the cardia were randomly assigned to preoperative radiotherapy followed by surgical resection *vs* surgery alone. This study showed a

Table 3 Pre-operative chemo-radiotherapy in gastric and esophagogastric junction cancer

Ref.	Phase	Selection criteria	Study arms	No. of patients	R0 resection (%)	Pathologic CR (%)	Median survival (mo)
Zhang *et al*[36]	RCT	EGJ	40 Gy EBRT + surgery *vs* surgery alone	370 (171 + 199)	89.5	0	5-yr OS 30% *vs* 20%
Shchepotin *et al*[37]	RCT	M0 resectable and unresectable	Surgery alone *vs* 20 Gy EBRT *vs* 20 Gy EBRT + Hy	293 (98 + 100 + 95)	NS	NS	5-yr OS 21.3%
Skoropad *et al*[38]	RCT	M0 resectable + EGJ	20 Gy EBRT + Hy + 20 Gy IORT *vs* surgery alone	122 (59 + 53)	66	0	16
Safran *et al*[39]	Phase I	Unresectable M0	45 Gy EBRT+ Paclitaxel	27	NS	11	2-yr OS 35%
Lowy *et al*[40]	Phase I	T > 2, Any N, M0	45 Gy EBRT, 5-FU	24	75	11	NS
Ajani *et al*[41]	Phase II	T > 2, Any N	5FU, LV, P + 45 Gy EBRT, 5FU	33	70	30	34
Ajani *et al*[42]	Phase II	M0 resectable + EGJ	FP, paclitaxel + 45 Gy EBRT, 5FU	41	78	20	> 36
Allal *et al*[43]	Phase I	T3-T4, N+	FP, Leucovorin + 31.2–45.6 Gy EBRT	19	NS	5	5-yr OS 35%
Ajani *et al*[44]	Phase II	M0 resectable	FP, LV, P + 45 Gy EBRT, 5FU, cis	49	63	26	23
Stahl *et al*[45] POET trial	Phase III RCT	EGJ	PFL × 3 + 30 Gy + cisplatin/ etoposide + surgery *vs* PFL × 2, 5 + surgery	126 (62+64)	72 *vs* 69	15.6 *vs* 2.0	33.1 *vs* 21.1
Van Hagen *et al*[48] CROSS trial	Phase III RCT	Esophageal or EGJ cancer	Carboplatin + paclitaxel + 41.1Gy + surgery *vs* surgery alone	366 (178 + 188)	92 *vs* 69	29 (CRT + surgery)	49.9 *vs* 24

R0: Curative (R0) resections; CR: Complete response; GEJ: Gastro-esophageal junction; RCT: Randomized controlled trial; EBRT: External beam radiotherapy; IORT: Intraoperative radiotherapy; Hy: Hypertermia; FP: Fluorouracil and cisplatin; LV: Leucovorin; NS: Not stated; OS: Overall survival.

significant five-year survival advantage for patients who received neoadjuvant radiation treatment compared to patients treated with surgery alone (30.1% *vs* 19.8%, respectively), in addition to an improvement in the rate of complete curative resection after radiation therapy (80% *vs* 62%). Another single center trial was a three-arm study, performed in Ukraine from February 1984 to May 1986[37]. That study enrolled 293 patients with gastric cancer and then randomized by envelope assignment into three groups: (1) radiotherapy and subsequent surgery; (2) radiotherapy combined with local hyperthermia and subsequent surgical resection; or (3) only surgery. This study demonstrated a five-year survival rate of 30.1% for surgery alone, 44.7% for radiotherapy followed by surgery, and 51.5% for radiation therapy combined with hyperthermia and subsequent surgical resection: The last multimodal treatment was demonstrated to be significantly more effective than surgery alone ($P < 0.05$). Moreover, in this study, an advantage in using radiotherapy and surgery *vs* surgical resection alone was demonstrated, but it was statistically insignificant. Skoropad *et al*[38] reported the 20-year follow-up results of a randomized trial on pre-operative radiotherapy (given at a dose of 20 Gy) compared to surgery alone. No significant difference in overall survival was detected between the two treatment groups. Published phase Ⅱ studies have confirmed the efficacy of chemo-radiotherapy (CRT) in terms of complete pathological response (up to 30% in some series) and increased long-term survival without an increase in morbidity or mortality (Table 3)[28,36-44].

Safran *et al*[39] showed that patients treated concurrently with paclitaxel and radiotherapy had an overall response of 56%, with 11% of the sample achieving complete response (3 cases) in patients with local-regional unresectable gastric cancer. The 2-year progression-free and overall survivals were 29% and 31%, respectively. Lowy *et al*[40] performed a pilot study of preoperative chemo-radiotherapy (combined with IORT) in patients with a diagnosis of gastric tumor, which used a staging protocol based on the results of computed tomography, endoscopic ultrasonography, and staging laparoscopy to determine the possibility of surgical resection. Twenty-four patients with a potentially resectable disease, but who had a poor prognosis tumor (T2 or higher at EUS), were treated with 45 Gy of external-beam radiation at 1.8 Gy/d and 5 d/wk with continuous-infusion 5-FU (300 mg/m^2 per day). All but one patient were able to complete the treatment. The radiation field included the entire stomach and regional lymph nodes. A restaging CT scan was performed at 4 to 6 wk after neoadjuvant therapy and before surgery. Nineteen patients (83%) were treated with a spleen-preserving D2 gastrectomy after the end of chemo-radiotherapy, and the surgical resection was accompanied by IORT (10 Gy). Two patients (11%) showed a complete pathological response.

In 2004, Ajani *et al*[41] treated patients with two courses of 5-FU, folinic acid, and cisplatin (P), following those with 5FU-potentiated radiotherapy (45 Gy). This study enrolled 34 patients with localized gastric adenocarcinoma, and 85% of them underwent surgical resection after neoadjuvant chemo-radiotherapy, without an increase in postoperative complications. Thirty percent of patients showed a complete pathologic response, while 24% showed a partial response. The

Table 4 Currently recruiting trials for pre and peri-operative chemo(radio)therapy

Country	Title	Phase	Study arms	Trial registration
United Kingdom[51]	Chemotherapy with or without bevacizumab or lapatinib to Treat Operable Oesophagogastric Cancer (ST03)	II/III	ECX + bevacizumab *vs* ECX *vs* ECX + lapatinib	NCT00450203
United Kingdom[52]	A study of bevacizumab in combination with capecitabine and cisplatin as first-line therapy in Patients With Advanced Gastric Cancer (AVAGAST)	III	Bevacizumab + ECX *vs* ECX	NCT00548548
Multicenter study (24 countries)[53]	ToGA study - A study of herceptin (trastuzumab) in combination with chemotherapy compared with chemotherapy alone in patients with HER2-positive Advanced Gastric Cancer	III	Trastuzumab + fluorouracil/ capecitabine + cisplatin *vs* fluorouracil/capecitabine + cisplatin	NCT01041404
Australia and New Zealand	Trial of Preoperative Therapy for Gastric and Esophagogastric Junction Adenocarcinoma (TOPGEAR)	II/III	Epirubicin + cisplatin + 5-fluorouracil + 45 Gy *vs* Epirubicin + cisplatin + 5-fluorouracil	NCT01924819
Netherlands[54]	Randomized phase III trial of adjuvant chemotherapy or Chemoradiotherapy in Resectable Gastric Cancer (CRITICS)	III	ECC + surgery + ECC *vs* ECC + surgery + 45 Gy + capecitabine + cisplatin	NCT00407186
China	Peri-operative chemotherapy with ECX or XP in the treatment of advanced gastric cancer	III	Epirubicin + cisplatin + capecitabine *vs* capecitabine + cisplatin	NCT01558947
Korea	DOS regimen as neoadjuvant chemotherapy in advanced gastric cancer (PRODIGY)	III	Docetaxel + oxaliplatin + tegafur *vs* surgery only	NCT01515748
China	Peri-operative chemotherapy with ECX (epirubicin + cisplatin + capecitabine) or XP (capecitabine + cisplatin) in the treatment of advanced gastric cancer: A randomized, multicenter, parallel controlle	III	Epirubicin + cisplatin + capecitabine *vs* capecitabine + cisplatin	ChiCTR-TRC-11001319
Ireland	MAGIC *vs* CROSS Upper GI. ICORG 10-14	III	MAGIC regimen *vs* CROSS protocol	NCT01726452

AVAGAST: The Avastin in gastric cancer; ECX: Epirubicin, cisplatin and xeloda; TOPGEAR: Trial of preoperative therapy for gastric and esophagogastric junction adenoca rcinoma; CRITICS: Chemoradiotherapy after induction. chemotherapy in cancer of the stomach; XP: Xeloda and cisplatin; DOS: Docetaxel, oxaliplastin and S1; MAGIC: Medical research council adjuvant gastric infusional chemotherapy; CROSS: Chemoradiotherapy for oesophageal cancer followed by surgery study; ICORG: Ireland cooperative oncology research group.

overall median survival duration was 33.7 mo, but in patients who reached a complete response, the median survival time was 64 mo. For those with a partial response, the median survival duration was 12.6 mo ($P < 0.05$). The results from this trial demonstrate that patients with cancers responding to treatment can achieve a substantial survival benefit. Similar results were obtained by the same authors in two subsequent studies using a different combination of chemotherapeutic drugs[42-44].

The German POET trial[45] compared neo-adjuvant chemotherapy with neo-adjuvant chemo-radiotherapy in patients with locally advanced adenocarcinoma of the lower esophagus or gastric cardia. Patients were randomly allocated to one of two treatment groups: induction chemotherapy (15 wk of cisplatin, fluorouracil, leucovorin) followed by surgery or chemotherapy (12 wk of cisplatin, fluorouracil, leucovorin) followed by 3 wk of chemo-radiotherapy (30 Gy, cisplatin/etoposide) followed by surgery. The median length of survival was 33.1 mo for patients in the chemo-radiotherapy arm and 21.1 mo for those in the chemotherapy arm.

Although the study was closed early due to low accrual and no evidence of a significant survival benefit for chemo-radiotherapy, the results suggest a survival advantage for pre-operative chemo-radiotherapy compared with pre-operative chemotherapy. Based on this study, most European guidelines consider neo-adjuvant or perioperative chemo-radiotherapy as an alternative to chemotherapy in adenocarcinomas of the EGJ[46,47].

A recent multicenter, randomized phase III trial investigated the role of neo-adjuvant chemo-radiotherapy in the treatment of esophageal or EGJ cancer (CROSS trial)[48]. Patients with resectable tumors were randomly assigned to receive surgery alone (n = 188) or CRT (carboplatin, paclitaxel, 41.4 Gy in 23 fractions) followed by surgery (n = 178). Seventy five per cent of the patients had adenocarcinoma. Patients treated with CRT had a higher R0 resection rate than patients treated with surgery alone (92% and 69%, $P < 0.001$), and 29% of patients showed a pathological complete response (23% in patients with adenocarcinoma and 49% in patients with squamous-cell carcinoma). The median overall survival duration was 49.9 mo in patients undergoing CRT associated with surgical resection and 24 mo in patients undergoing surgery only ($P = 0.003$). Post-operative complications and in-hospital death rate (4% in both) were similar in both arms.

Based on the results of this trial, pre-operative

chemo-radiation is now the preferred approach for localized adenocarcinoma of the EGJ in the United States, whereas chemotherapy is regarded as an alternative, but less preferred option.

Recently, Kumagai *et al*[49] conducted a meta-analysis regarding chemo-radiotherapy in resectable gastric and gastro-esophageal junction cancer. Eighteen studies were collected, from which data were available from 14. In this meta-analysis, pre-operative chemo-radiotherapy as well as chemotherapy for resectable gastric and gastro-esophageal cancers were associated with a significant survival benefit compared to surgery alone. Due to the lack of studies comparing pre-operative chemotherapy and chemo-radiotherapy in this study, the comparison between the two regimens was performed in adjusted indirect form. Despite this methodological bias, pre-operative chemo-radiotherapy showed a trend towards better long-term survival.

ONGOING TRIALS

Many answers are expected from ongoing trials that are exploring ways of improving pre-operative treatment strategies for resectable gastric cancer[50-53] (Table 4).

In the field of neo-adjuvant chemotherapy, an ongoing phase Ⅱ/Ⅲ British trial (ST03) is actively recruiting localized gastric and EGJ tumor patients and comparing perioperative epirubicin, cisplatin and capecitabine (ECX) with or without bevacizumab (ECX-B)[50]. This chemotherapeutic protocol is based on the demonstrated beneficial effect of bevacizumab in the treatment of advanced gastric cancer (AVAGAST trial). The preliminary results of phase Ⅱ about safety showed that chemotherapy is feasible with acceptable toxicity (specifically gastrointestinal perforation rates and cardio-toxicity) with no negative impact on surgical outcomes[51].

The findings of the ToGA-study, which revealed the beneficial effects of trastuzumab for human epidermal growth factor receptor 2 (HER2)-positive advanced gastric and GEJ cancers in combination with a platinum-based chemotherapy[52], gave rise to studies investigating the HER2 positivity in advanced gastric cancer with bulky N2 or N3 nodal disease with possible implications in a neo-adjuvant setting (JCOG2005-A).

Several ongoing trials are currently investigating the role of a neo-adjuvant chemotherapeutic protocol. Most of these trials come from Eastern countries (JCOG 001, JCOG 0405, JCOG 0210, JCOG 0501, JCOG 1002, Kyoto trial, PRODIGY trial) and are recruiting patients with advanced and marginally resectable gastric cancer (T3-4, large type 3 gastric cancer, linitis plastica, and bulky N2-3 + tumor).

At the same time, ongoing trials are evaluating the role of radiotherapy in the setting of pre- and perioperative treatment. For instance, the TOPGEAR phase Ⅱ/Ⅲ trial is examining the addition of a neo-adjuvant chemo-radiotherapy strategy to perioperative chemotherapy in patients with resectable adenocarcinoma of the stomach or EGJ. Patients are randomized to receive three cycles of ECF alone or chemo-radiotherapy (two cycles of ECF followed by 45 Gy or radiation with concurrent 5-FU). Following surgery, both groups receive three cycles of ECF. Part Ⅰ of the trial (phase Ⅱ component) will recruit 120 patients with the aim of demonstrating the efficacy and safety of pre-operative CRT. The second part (phase Ⅲ component) will recruit 632 additional patients. The primary endpoints are the pathological complete response rates and overall survival.

The CRITICS trial (Chemoradiotherapy after Induction Chemotherapy in Cancer of the Stomach) is a phase Ⅲ study that randomizes between pre-operative chemotherapy (three courses of epirubicin, cisplatin, capecitabine; ECC) and gastric surgery followed by post-operative chemotherapy (three courses of ECC) or chemo-radiotherapy (45 Gy in 25 fractions; concurrent capecitabine and cisplatin)[53]. The MAGIC *vs* CROSS Upper GI. ICORG 10-14 trial are randomizing patients with adenocarcinoma of the esophagus and EGJ in neo-adjuvant and adjuvant chemotherapy according to the MAGIC regimen *vs* neo-adjuvant chemo-radiation according to the CROSS protocol in order to assess 2- and 3-year patient survival, clinical and pathological response rate, tumor regression grade, and disease-free survival.

INTERNATIONAL GUIDELINES AND AREAS OF UNCERTAINTY

The above-mentioned collection of study data led neoadjuvant therapy to be included into several national and international guidelines for gastric cancer management, but significant differences exist among different countries. Both United States and European guidelines[54,55] consider pre-operative chemotherapy as the preferred pathway for ⩾ T2 and/or N ± gastric cancer reaching the "level 1" of recommendation in the National Comprehensive Cancer Network Consensus. Similarly, pre-operative chemoradiation is the favorite approach to manage esophagogastric junction cancer in American guidelines.

In contrast, neoadjuvant therapy was still considered investigational by the last edition of the Japanese Gastric Cancer Association guidelines while researchers await the results of dedicated ongoing trials[56].

This probably reflects the well-known diversity of gastric cancer epidemiology and pathology between the West and East, which leads to different treatment approaches.

Considering that locally advanced cancers are more frequent than earlier stages in the United States and Europe, the current guidelines suggest the use of neoadjuvant therapy in a majority of gastric cancers. Although potentially beneficial, this wide application of pre-operative treatment requires proper patient selection to avoid its potentially dangerous overuse[57].

Several studies have reported that a tumor's response may depend on different factors such as the tumor site, grading and Lauren's histotype[58,59], and a recent large retrospective study demonstrated that survival was mainly influenced by the disease stage after neoadjuvant chemotherapy, rather than the clinical stage at presentation[60].

In particular, signet-ring cell cancer seems to be less responsive to pre-operative chemotherapy as shown in some large European retrospective studies[61,62].

Interestingly, the subset analysis of the final report of the trial evaluating the role of adjuvant radiotherapy after D2 gastrectomy showed that chemoradiation significantly improved DFS in patients with intestinal-type cancer, but there was no benefit in diffuse histotype[63]. A French phase Ⅱ/Ⅲ multicenter trial evaluating the role of neoadjuvant therapy in resectable signet ring cell gastric cancer is currently ongoing (NCT01717924; clinicaltrial.gov) and will probably help clarify this issue.

Basically, in the last two decades, Western surgeons have tried to extend the overall lower survival rate of their gastric cancer patients - compared with Eastern patients - adding pre- and/or post-operative multimodal therapy to surgery. However, "high quality" surgery is still the cornerstone of the management of gastric cancer, and D2-lymphadenectomy has been recently introduced as the standard surgical procedure also in the Western Countries[54,55]. So far, the most evidence on neoadjuvant therapy comes from studies including patients generally treated by inadequate surgery, considering that in the MAGIC and FFCD trials, a D0-1 lymphadenectomy was the more common procedure[6,24]. Therefore, we could argue that pre-operative therapy could fill the survival gap of a limited surgery.

An emerging and unresolved question regards the management of gastric cancer in elderly patients. The incidence of gastric cancer increases with advancing age[64], and the elderly population is dramatically growing due to increased life expectancy, especially in developed countries[65]. Several studies have demonstrated that age alone is not a contraindication for surgery[66-69], but there are limited data on the role of perioperative therapy in older patients. The recent review by Ronellenfitsch *et al*[35] reported no survival advantage from adding pre-operative therapy in elderly patients, but several important issues such as under-representation of older patients in clinical trials, heterogeneity of elderly definitions and non-specific analyzed end-points may have significantly affected the interpretation of the current available data[70,71]. Further specifically designed studies and reliable biologic indicators of real functional status are needed to properly select older patients for multimodal treatment.

CONCLUSION

In gastric cancer, radical surgery (R0-resection), defined as the complete surgical resection of all the tumor cells in the tumor bed, is considered the best chance of a cure. However, distant and loco-regional failure rates in radically resected patients with positive lymph nodes or involvement of the serosa make this definition somewhat contradictory. Currently, the tailoring of the treatment, both in terms of the extent of surgical resection and of the administration of pre- and post-operative therapies, represents the major goal. In the last ten years, three pivotal studies from three different areas of the world (United States, Europe and Japan) have shown that combined treatments can lead to a better prognosis for patients with resectable gastric cancer. Multimodal treatments aim to obtain an improvement in prognosis by means of a truly complete curative surgery (R0 resection) with minimal morbidity and mortality. In gastric cancer, surgical research has always proceeded slowly, and standardization is still far from being settled. Geographical differences in epidemiology and therapeutic approaches and the absence of a surgical gold standard have diverted attention from the development of an ideal multimodal approach.

More data are necessary to define the role of neo-adjuvant chemo-radiotherapy in the field of gastric cancer treatment. The results of pre-operative chemotherapy in the multimodal treatment of gastric adenocarcinoma are undoubtedly encouraging. Modern and unsolved concerns regarding the choice of the optimal chemotherapy regimen, the role of radiotherapy, a reliable pre-operative staging protocol for accurate patients selection, standardization of surgical procedures, and reliable criteria for response evaluation amid new well-designed trials will be necessary to identify the best treatment plan in the pre-operative setting and to understand how to combine the conventional chemotherapeutic drugs with new-generation molecules.

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REVIEW

Targeted therapy for advanced gastric cancer: A review of current status and future prospects

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Abstract

In the West in particular, the vast majority of gastric cancer (GC) patients present with advanced-stage disease. Although combination chemotherapy is still the most important component of treatment for these patients, it confers a modest survival advantage. Recently, increased knowledge of the key molecular signaling pathways involved in gastric carcinogenesis has led to the discovery of specific molecular-targeted therapeutic agents. Some of these agents such as trastuzumab and ramucirumab have changed the treatment paradigm for this disease. In this paper, we will summarize the current clinical status of targeted drug therapy in the management of GC.

Key words: Gastric cancer; Targeted therapy; Angiogenesis; Epidermal growth factor; Treatment

Core tip: Systemic chemotherapy confers a modest survival advantage in patients with advanced gastric cancer. The new therapeutic agents that target various inter- and intracellular signaling transduction pathways offer the promise of improved clinical outcomes in selected patients. The success of some of these agents has changed the treatment paradigm for advanced gastric cancer. We herein discuss the current and potential future roles of targeted therapy in the management of this malignancy.

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INTRODUCTION

Gastric cancer (GC) is a very aggressive tumor and is currently the third leading cause of cancer-related deaths in both sexes at the world level (8.8% of the

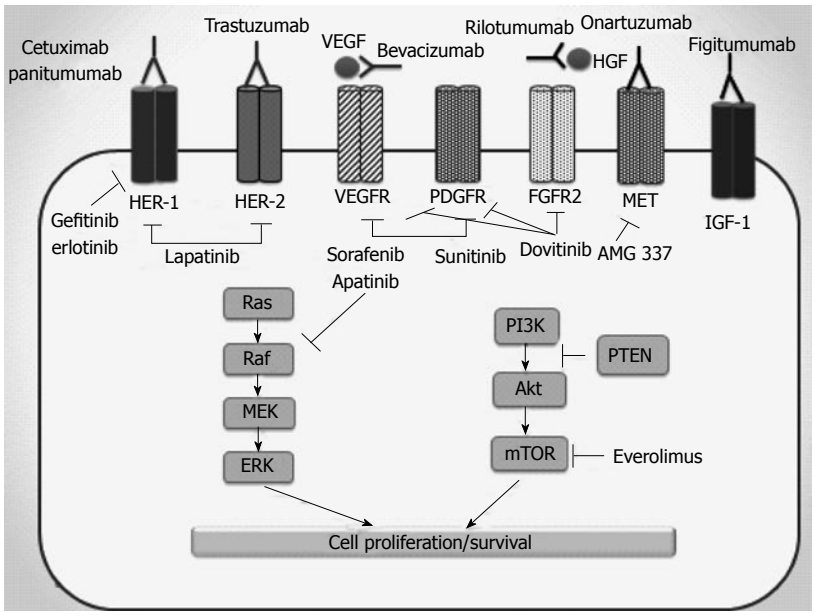

Figure 1 Molecular targets and relevant drugs in metastatic gastric cancer. HER: Human epidermal growth factor receptor; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor; PDGFR: Platelet-derived growth factor receptor; HGF: Hepatocyte growth factor; FGFR2: Fibroblast growth factor receptor 2; IGF-1: Insulin-like growth factor 1; Raf: GTPase Raf; Ras: GTPase Ras; MEK: MAP kinase; ERK: Extracellular-signal-regulated kinase; PI3K: Phosphatidylinositol 3-kinase; PTEN: Phosphatase and tensin homolog; AKT: Protein kinase B; mTOR: Mammalian target of rapamycin.

total)[1,2]. At initial diagnosis, a significant proportion of Western GC patients (65%) are found to have unresectable disease or distant metastases. In Japan and South Korea, where nationwide government-sponsored screening programs have been established, still up to 80% of patients who undergo a curative resection for GC develop locoregional or distant recurrence[2,3].

The clinical management of patients with advanced GC remains one of the most challenging tasks in clinical oncology. Until recently, systemic chemotherapy alone has been the mainstay of treatment for these patients[4]. However, the disease often exhibits relative resistance to chemotherapeutic agents, and a satisfactory response is achieved only in a minority of the patients[5,6]. In addition, although systemic chemotherapy can substantially increase symptom control and improve the patient's quality of life, its long-term results are still not satisfactory and unfortunately many patients die less than a year after starting therapy[5,6].

Thus, there is undoubtedly a need to develop more effective treatment strategies for this formidable disease. As in other solid tumors, the uses of targeted agents that block vital inter- and intracellular signaling pathways have recently emerged as a strategy for the treatment of advanced GC[7-12]. Significant advances in our understanding of the underlying biologic processes of GC have recently expanded the number and range of potential therapeutic targets. Targeted agents may be used either alone or in combination with anti-neoplastic agents for patients with both chemotherapy-naïve and chemotherapy-refractory disease. Some of these, such as trastuzumab and ramucirumab have been shown to have significant therapeutic activity and a good safety profile, have changed the treatment paradigm, and are therefore currently licensed in the United States and Europe as part of the management of patients with GC.

In this review, we will outline well-established targeted treatments for GC and discuss novel agents currently in development as well as some directions for future research.

Anti-epidermal growth factor receptor therapies

The epidermal growth factor receptor (EGFR) belongs to the ErbB family of receptor tyrosine kinases (RTK), which contains four closely related members: ErbB1 (HER1 or EGFR), ErbB2 (Her2/neu), ErbB3 and ErbB4[13,14]. EGFR activation by one of its ligands initiates diverse downstream signaling pathways including the RAS/RAF/MAP kinase and PI3K/Akt/mTOR signaling networks. Both pathways play a vital role in several critical cellular processes including proliferation, growth, survival, motility, and tissue invasion[13,14].

EGFR overexpression has been correlated with more aggressive tumor behavior and a worse clinical results in patients with GC, suggesting that EGFR is therapeutic target for this aggressive malignancy[13,14]. The current therapeutic strategies targeting EGFR include neutralizing monoclonal antibodies (moAbs) directed against the extracellular receptor domain and small molecule tyrosine kinase inhibitors (TKIs) of the intracellular tyrosine kinase domain (Figure 1).

Cetuximab and panitumumab are engineered antibodies that bind to EGFR with higher affinity compared to its natural ligands[15,16]. Several phase II clinical trials have tested the feasibility of adding cetuximab to different chemotherapy regimens including 5-FU/folinic acid (LV)/irinotecan, 5-FU/LV/oxaliplatin (FOLFOX), docetaxel/cisplatin, capecitabine/cisplatin,

Table 1 Summary of completed phase III trials of targeted agents in the treatment of advanced gastric and gastroesophageal adenocarcinoma

Author/trial	Line of treatment	Target	Agent	Treatment	ORR (%)	PFS (mo)	OS (mo)
Lordick *et al*[22] (2013)/ EXPAND	First	EGFR	Cetuximab	Cisplatin/capecitabine ± cetuximab	30 *vs* 29 *P* = 0.77	4.4 *vs* 5.6 *P* = 0.32	9.4 *vs* 10.7 *P* = 0.95
Waddell *et al*[23] (2013)/REAL-3	First	EGFR	Panitumumab	EOX ± panitumumab	46 *vs* 42 *P* = 0.42	6.0 *vs* 7.4 *P* = 0.068	8.8 *vs* 11.3 *P* = 0.013
Bang *et al*[32] (2010)/ ToGA	First	HER2	Trastuzumab	Cisplatin/capecitabine or 5-FU ± trastuzumab	47 *vs* 35 *P* = 0.0017	6.7 *vs* 5.5 *P* = 0.0002	13.8 *vs* 11.1 *P* = 0.0046
Hecht *et al*[34] (2013)/ LoGIC	First	EGFR/ HER2	Lapatinib	CAPOX ± lapatinib	53 *vs* 40 *P* = NA	6.0 *vs* 5.4 *P* = 0.1	12.2 *vs* 10.5 *P* = 0.35
Ohtsu *et al*[37] (2011)/ AVAGAST	First	VEGF-A	Bevacizumab	Cisplatin/capecitabine ± bevacizumab	46 *vs* 37.4 *P* = 0.03	6.7 *vs* 5.3 *P* = 0.037	12.1 *vs* 10.1 *P* = 0.1002
Shen *et al*[39] (2015)/ AVATAR	First	VEGF-A	Bevacizumab	Cisplatin/capecitabine ± bevacizumab	40.7 *vs* 33.7 *P* = 0.348	6.3 *vs* 6.0 *P* = 0.47	11.4 *vs* 10.5 *P* = 0.55
Bang *et al*[35] (2014)/TyTAN	Second	EGFR/ HER2	Lapatinib	Paclitaxel ± lapatinib	27 *vs* 9 *P* < 0.001	5.4 *vs* 4.4 *P* = 0.13	11.0 *vs* 8.9 *P* = 0.1044
Fuchs *et al*[41] (2014)/ REGARD	Second	VEGFR-2	Ramucirumab	BSC + ramucirumab or placebo	3.4 *vs* 2.6 *P* = 0.76	2.1 *vs* 1.3 *P* < 0.0001	5.2 *vs* 3.8 *P* = 0.0473
Wilke *et al*[43] (2014)/ RAINBOW	Second	VEGFR-2	Ramucirumab	Paclitaxel + ramucirumab or placebo	28 *vs* 16 *P* = 0.0001	4.4 *vs* 2.9 *P* < 0.0001	9.6 *vs* 7.4 *P* = 0.017
Ohtsu *et al*[52] (2013)/ GRANITE-1	Second or third	mTOR	Everolimus	Everolimus or placebo	4.5 *vs* 2.1 *P* = NA	1.7 *vs* 1.4 *P* < 0.001	5.4 *vs* 4.3 *P* = 0.124

ORR: Overall response rate; PFS: Progression-free survival; OS: Overall survival; EGFR: Epidermal growth factor receptor; EOX: Epirubicin, oxaliplatin and capecitabine; HER2: Human epidermal growth factor receptor 2; 5-FU: 5-fluorouracil; CAPOX: Capecitabine and oxaliplatin; NA: Not available; VEGF-A: Vascular endothelial growth factor A; VEGFR-2: Vascular endothelial growth factor receptor 2; mTOR: Mammalian target of rapamycin.

and capecitabine/oxaliplatin for chemotherapy-naïve advanced GC patients[17-20]. In these trials, overall response rates ranged from 41% to 69%, median progression-free survival (PFS) varied from 5 to 8.5 mo, and median overall survival (OS) varied from 9 to 16.6 mo. A randomized phase II clinical study (CALGB 80403/ ECOG 1206) evaluated three different conventional chemotherapy regimens (Epirubicin, cisplatin and 5-FU *vs* irinotecan and cisplatin *vs* FOLFOX) in combination with cetuximab. Response rates were 58%, 38%, and 51%, respectively, and median OS was 8.6 and 10 mo, respectively. Cetuximab combined with FOLFOX was found to be the least toxic of the three[21].

Unfortunately, these promising initial outcomes were not verified in the phase III EXPAND trial[22]. In this study, 904 previously untreated metastatic GC and gastro-esophageal junction (GEJ) cancer patients were randomly allocated to receive chemotherapy (cisplatin and capecitabine) with or without cetuximab[22]. No differences in clinical outcome were found between treatment groups, and the primary and secondary efficacy endpoints were not met; the median PFS and OS were 4.4 mo (95%CI: 4.2 to 5.5 mo) and 9.4 mo (95%CI: 8.3 to 10.6 mo), respectively in the combined therapy group compared with 5.6 mo (95%CI: 5.1 to 5.7 mo) and 10.7 mo (95%CI: 9.4 to 11.3 mo), respectively in the chemotherapy-alone group (P = 0.32 and P = 0.95 for PFS and OS, respectively). The addition of cetuximab also did not increase the overall response rate, which was 30% and 29% with or without cetuximab, respectively (Table 1).

Similarly, the phase III REAL-3 trial was performed to determine the effects of adding panitumumab to a combination chemotherapy regimen of epirubicin, oxaliplatin, and capecitabine (EOX) in patients with advanced esophago-gastric adenocarcinoma[23]. In this trial, patients were randomly allocated to receive EOX or a modified EOX plus panitumumab. Disappointingly, adding panitumumab to EOX chemotherapy resulted in worsened OS [8.8 mo compared with 11.3 mo for the EOX regimen (HR = 1.37; P = 0.013)]. A trend toward a shorter PFS was also seen in patients receiving panitumumab (6.0 mo *vs* 7.4 mo, HR = 1.22; P = 0.068). The panitumumab-containing arm was associated with an increased rate of grade 3-4 diarrhea (17% *vs* 11%), rash (11% *vs* 1%), mucositis (5% *vs* none), and hypomagnesaemia (5% *vs* none) but reduced rate of neutropenia (13% *vs* 28%).

Lastly, other novel humanized IgG1 anti-EGFR moAbs including matuzumab and nimotuzumab have also been investigated as first- or second-line treatment for advanced GC, and have also failed to generate a strong efficacy signal[24-26]. The small molecule EGFR TKIs have not been extensively studied in the treatment of advanced GC largely due to their limited activity in this setting[27,28]. Why EGFR-targeting strategies have failed to be successful in this disease in spite of lack of activating KRAS mutations and in spite of good biologic rationale remains a mystery.

Anti-HER2 (ERBB2) therapy

As previously mentioned HER2 is another member of the ERB family of receptor tyrosine kinases[29]. Overexpression and amplification of the HER2 is detected in 10%-38% of GC patients[30]. Although the association between HER2 status and prognosis

in GC still controversial, the results of some clinical studies have suggested that patients with HER2 negative disease have a more favorable prognosis than those with HER2 positive disease[29,31]. Perhaps one of the most convincing data supporting the clinical benefits of targeted therapy in advanced GC come from the phase Ⅲ ToGA study[32]. This landmark study investigated the addition of trastuzumab, a moAb that binds to the extracellular ligand binding domain of the HER2 receptor, to combination chemotherapy (cisplatin and either capecitabine or 5-FU) in patients with previously untreated HER2 overexpressing [defined as HER2 fluorescence in situ hybridization (FISH) positive or immunohistochemistry (IHC) 3 positive], and advanced gastric or GEJ cancer. Over 3000 patients were screened for the study. Among the 594 enrolled patients, 296 received chemotherapy alone and 298 received chemotherapy plus trastuzumab. Patients receiving the combined therapy achieved improvement in all measures of efficacy including OS (13.8 mo *vs* 11.1 mo; HR = 0.74, *P* = 0.0046), PFS (6.7 mo *vs* 5.5 mo; HR = 0.71, *P* = 0.0002), and overall response rate (47% *vs* 35%, *P* = 0.0017). A post hoc subgroup analysis of the study demonstrated that the patients with strongly HER-2 positive tumors (defined as IHC2+/FISH+ or IHC3+) derived significant OS benefit from the addition of trastuzumab to chemotherapy (16 mo *vs* 11.8 mo, HR = 0.68). Moreover, the tolerability of the combination was good and there was no significant difference in the incidence of grade 3 or 4 side effects between the treatment groups. Based on these results, trastuzumab was approved in the Unites States and European Union for use in the first-line treatment of HER2-overexpressing locally advanced or metastatic GC.

Pertuzumab is a new moAb that binds to the extracellular ligand binding domain of HER2 and blocks its dimerization with other HER-family receptors[31]. When used together, the combination of pertuzumab plus trastuzumab provide a more comprehensive blockade of HER signalling than either agent alone. Therefore, the JACOB phase Ⅲ study is currently recruiting participants to evaluate the effectiveness of pertuzumab in addition to trastuzumab plus chemotherapy (cisplatin plus capecitabine or 5-FU) in chemo-naïve patients with HER2-overexpressing advanced gastric or GEJ cancer (NCT01774786).

Trastuzumab emtansine (T-DM1) is a newly developed HER2-targeted antibody–drug conjugate that links trastuzumab to a highly potent maytansine-derived anti-microtubule drug (DM1)[33]. After binding the trastuzumab moiety to HER2 receptors on the tumor surface, T-DM1 is internalized by endocytosis and degraded in lysosomes, resulting in release of DM1-containing cytotoxic catabolites[33]. A phase Ⅱ-Ⅲ trial (NCT01641939) is now investigating the effectiveness of T-DM1 compared with taxanes (docetaxel or paclitaxel) in patients with metastatic HER2-positive GC who develop progression of disease following first-line chemotherapy.

Lapatinib is an oral small-molecule tyrosine kinase inhibitor of EGFR and HER2 that blocks their tyrosine kinase activities. Two phase Ⅲ trials were performed to explore the effectiveness of lapatinib in first- and second-line treatment of advanced GC. The LoGIC Ⅲ trial investigated the efficacy of lapatinib when administered in combination with capecitabine plus oxaliplatin (CAPOX) as first-line therapy[34]. In total, 545 patients whose tumors overexpressed HER-2 were assigned to receive CAPOX plus lapatinib or placebo. No significant difference in survival between the two treatment arms was detected. Median OS and PFS in the chemotherapy + lapatinib group were 12.2 and 6 mo, respectively, compared to 10.5 and 5.4 mo in the control group. Similarly, in the phase Ⅲ TyTan trial conducted in Asia, 430 patients with advanced GC who had experienced failure of fluoropyrimidine and cisplatin-based chemotherapy and exhibited FISH-confirmed HER2 amplification received lapatinib plus weekly paclitaxel or weekly paclitaxel alone[35]. Although, the addition of lapatinib to paclitaxel extended the primary endpoint of OS from a median of 8.9 mo to 11.0 mo, this improvement failed to reach statistical significance (*P* = 0.1044). The further subgroup analysis revealed a statistically significant benefit in both OS and PFS from the addition of lapatinib to chemotherapy in patients with HER2 IHC3+ tumors and in Chinese patients.

Targeting angiogenesis pathways

Angiogenesis is necessary for tumors to grow beyond a certain size, survive or spread. Vascular endothelial growth factor (VEGF) and its receptors (VEGFR1, VEGFR2 and VEGFR3) are important players in the development of this process. Binding of the ligand VEGF-A to VEGFR-2 triggers a signaling cascade leading to endothelial cell proliferation, migration, new vessel formation, and sustained angiogenesis[24]. Therefore, inhibition of the VEGF signaling has become a useful clinical maneuver in the treatment of several types of cancer.

Anti-VEGF moAb: Bevacizumab is a fully human moAb targeting VEGF-A[36]. The potential role of this drug in the management of patients with metastatic GC was evaluated in the phase Ⅲ AVAGAST and AVATAR trials. The AVAGAST trial was global, randomized, placebo-controlled trial conducted for evaluation of the benefits of bevacizumab when added to first-line capecitabine and cisplatin chemotherapy in 774 metastatic GC patients[37]. The trial did not show any significant improvement in OS in the bevacizumab cohort. Median OS was 12.1 mo with bevacizumab plus chemotherapy and 10.1 mo with placebo plus chemotherapy (HR = 0.87; 95%CI: 0.73 to 1.03; *P* = 0.1002). Despite this, both median PFS (6.7 mo *vs* 5.3 mo; HR = 0.80; 95%CI: 0.68 to 0.93; *P* = 0.0037) and overall response rate (46.0% *vs* 37.4%; *P* = 0.0315) were significantly increased by the addition of bevacizumab *vs* placebo. Preplanned subgroup analysis

of the study also demonstrated geographical differences in the therapeutic effectiveness of bevacizumab treatment. A survival benefit for bevacizumab was demonstrated in patients recruited from North America and Latin America centers (median, 11.5 mo *vs* 6.8 mo for placebo-chemotherapy; HR = 0.63; 95%CI: 0.43 to 0.94), whereas patients recruited from Asia centers seemed to have no obvious benefit (HR = 0.97; 95%CI: 0.75 to 1.25). Subsequently, the study investigators identified plasma VEGF-A levels and degree of tumor neuropilin-1, a co-receptor for VEGF-A, expression as potential predictive biomarkers of bevacizumab efficacy[38]. A negative OS correlation was found in patients with low expression of tumor neuropilin-1 (HR = 0.75; 95%CI: 0.59 to 0.97) compared to those with high expression (HR = 1.07; 95%CI: 0.81 to 1.40; interaction $P = 0.06$). Of note, these findings were significant only in non-Asian patients.

AVATAR, a study similar in design to AVAGAST, was performed in Chinese patient population with advanced GC[39]. It was again demonstrated that the addition of bevacizumab to chemotherapy consisting capecitabine and cisplatin in this specific patient population did not improve OS (11.4 mo in the placebo arm *vs* 10.5 mo in the bevacizumab arm, HR = 1.11; $P = 0.55$).

Ramucirumab is a novel humanized IgG1 moAb that selectively binds to the extracellular ligand binding domain of VEGFR-2 and blocks VEGF-induced angiogenic signaling[40]. In theory, this has the advantage of blocking signaling from VEGF isoforms other than VEGF-A. Its efficacy and safety in advanced GC was evaluated in two international, phase Ⅲ, randomized, double-blinded and placebo-controlled studies. In the REGARD trial, a total 355 advanced gastric or GEJ cancer patients progressing after first-line platinum- or fluoropyrimidine-based combination chemotherapy were randomized to receive best supportive care (BSC) plus either ramucirumab or placebo[41]. Ramucirumab was given intravenously every 2 wk at 8 mg/kg and the median treatment duration was 8 wk. Patients receiving ramucirumab had a significantly improved median OS (5.2 mo *vs* 3.8 mo; HR = 0.776; $P = 0.0473$) and PFS (2.1 mo *vs* 1.3 mo; HR = 0.483; $P < 0.0001$) than patients receiving placebo. The 12-wk PFS rate was 40% for ramucirumab group and 16% for placebo group. Additionally, the overall response rate (3.4% *vs* 2.6%) and disease control rate (49% *vs* 23%) were also higher in the ramucirumab group compared to the placebo group ($P < 0.0001$). Ramucirumab had an acceptable toxicity profile. The most frequently recorded grade 3 or higher side effects in patients receiving ramucirumab were hypertension, anemia, abdominal pain, ascites, fatigue and hyponatremia. After presentation of these results, ramucirumab was approved for the second-line therapy advanced GC in the United States. Interestingly, these results are quite similar to those achieved with chemotherapy in the second-line setting[42].

The RAINBOW study tested ramucirumab in combination with paclitaxel in metastatic GEJ or gastric adenocarcinoma patients who experienced disease progression after first-line platinum- and fluoropyrimidine-based chemotherapy[43]. In this study, 665 patients were randomly assigned to receive ramucirumab or placebo plus paclitaxel. OS was defined again primary endpoint for efficacy. Median OS for patients received ramucirumab plus paclitaxel was 9.6 mo, compared to 7.4 mo for those received paclitaxel alone (HR = 0.807; 95%CI: 0.678-0.962; $P = 0.0169$). Median PFS was 4.4 mo and 2.9 mo, respectively (HR = 0.635; 95%CI: 0.536-0.752; $P < 0.0001$). The objective response rate was higher in the combination arm compared to paclitaxel alone arm (28% *vs* 16%, $P = 0.0001$). Ramucirumab was relatively well tolerated. As expected, grade 3 or higher side effects were somewhat more frequent among patients receiving ramucirumab plus paclitaxel greater with combination treatment and included neutropenia, leukopenia, hypertension and fatigue. The RAINBOW study showed that an effective second-line treatment may improve the duration of survival in metastatic GC, and it is the only study to date to demonstrate a 2-mo improvement in OS in this setting. Therefore, ramucirumab is the first anti-angiogenic agent to demonstrate activity for advanced GC, and now approved both as monotherapy and in combination with paclitaxel for this malignancy.

Anti-VEGF TKI: Apatinib is an orally administered TKI that selectively binds to VEGFR-2 and inhibits VEGF-induced endothelial cell proliferation and migration. As a result, it leads to a significant decrease in tumor microvessel density[44]. In a phase II trial conducted in China, apatinib was shown to increase PFS and OS in patients with metastatic GC progressing after 2 or more previous lines of chemotherapy[45]. Data from a phase Ⅲ trial presented at the 2014 ASCO Annual Meeting confirmed the effectiveness of apatinib in this setting[46]. This trial included 273 patients with advanced GC who experienced disease progression after second-line treatment. Patients were randomly assigned to receive apatinib or placebo. The primary endpoint, median OS, was significantly longer in the apatinib group than in the placebo group (195 d *vs* 140 d; HR = 0.71; 95%CI: 0.54-0.94; $P < 0.016$). The apatinib group also had a better median PFS than the placebo group; 78 d *vs* 53 d, respectively (HR = 0.44; 95%CI: 0.33-0.61; $P < 0.0001$). Therefore, apatinib provides a new promising treatment option for advanced GC, although one which overlaps with ramucirumab in both degree of activity and mechanism.

Two multi-targeted kinase inhibitors that share VEGF receptors as targets are sunitinib and sorafenib. Both of these agents have been tested in GC and have shown some signs of efficacy, but have not progressed to advanced trials[47-49]. Given the modest activity and the toxicity profiles of these two agents, it is unlikely that they would supplant ramucirumab at this time and are no longer being studied in GC.

The mTOR pathway: The mTOR (mammalian target of rapamycin) is an essential cellular signaling pathway that has a crucial role in the regulation of cell growth, survival, proliferation, metabolism, and angiogenesis[50]. Everolimus, an orally administered rapamycin analog, is the only mTOR inhibitor that has been evaluated in advanced GC[51]. Phase Ⅱ trials documented that it can produce stabile disease in a significant portion of patients with chemo-refractory advanced GC. Despite these promising data, in the phase Ⅲ GRANITE-1 trial, everolimus failed to demonstrate any significant improvement in OS compared to BSC alone[52]. In this study, advanced GC patients who had progressive disease after first- or second-line cytotoxic chemotherapy were randomized to receive everolimus treatment (10 mg/d) or matching placebo in conjunction with BSC. Median OS was 5.4 mo for patients receiving everolimus and 4.3 mo for patients receiving placebo (HR = 0.90; 95%CI: 0.75 to 1.08; *P* = 0.124). Another phase Ⅲ trial (AIO-STO-0111) is now investigating the efficacy of everolimus when given in combination with paclitaxel in GC patients progressing following prior chemotherapy regimen.

Targeting the hepatocyte growth factor/c-MET signaling pathway

A transmembrane protein-tyrosine kinase receptor c-MET and its ligand, hepatocyte growth factor (HGF) control many vital cellular events such as cell proliferation, survival, motility, invasion and angiogenesis[53]. C-MET overexpression has been detected in 18%-82% of GC patients, with genetic amplification of the CMET occurring in only 2%-3% of cases[54]. Patients with c-Met overexpressing tumors may have poorer survival, and the prognostic effect of overexpression seems to be independent of disease stage[53]. Therefore, c-MET has been recognized as potentially significant therapeutic target in GC.

Rilotumumab is a fully humanized IgG2 moAb that selectively binds HGF and prevents its binding to the MET receptor[53]. The results of a phase Ib/Ⅱ study of rilotumumab in combination with platinum-based chemotherapy in patients with locally advanced or metastatic GC have demonstrated the potential therapeutic value of drugs that target the c-MET pathway in this disease[55]. In the phase Ⅱ part of this study, 121 patients were randomized to ECX regimen plus placebo (*n* = 39) or ECX plus either 7.5 mg/kg (*n* = 42) or 15 mg/kg (*n* = 40) rilotumumab. Median PFS was 5.1 mo (2.9-7.0) in the rilotumumab 15 mg/kg group, 6.8 mo (4.5-7.5) in the rilotumumab 7.5 mg/kg group, 5.7 mo (4.5-7.0) in both rilotumumab groups combined, and 4.2 mo (2.9-4.9) in the placebo group. The HR for PFS compared with placebo was 0.69 (80%CI: 0.49-0.97; *P* = 0.164) for rilotumumab 15 mg/kg, 0.53 (80%CI: 0.38-0.73; *P* = 0.009) for rilotumumab 7.5 mg/kg, and 0.60 (80%CI: 0.45-0.79; *P* = 0.016) for combined rilotumumab. Rilotumumab was generally well tolerated by patients, with common side effects including neutropenia, anemia, thrombocytopenia, peripheral edema, and deep vein thrombosis. The association between MET expression and clinical outcomes was also evaluated in this trial. MET expression was found to be prognostic for shortened OS in the placebo group (5.7 mo *vs* 11.5 mo). In the subgroup of patients with increased MET expression, median OS was longer in patients receiving rilotumumab than in those receiving placebo (10.6 mo *vs* 5.7 mo). However, no survival benefit was seen with the addition of rilotumumab to chemotherapy among MET-negative patients.

Based on these data, the RILOMET-1 [a multicenter, randomized, double-blind, placebo-controlled phase Ⅲ study of rilotumumab (15 mg/kg) plus ECX regimen as first-line therapy for metastatic MET-positive gastric or GEJ adenocarcinoma] and the RILOMET-2 trial (a multicenter, randomized, double-blind, placebo controlled phase Ⅲ study of rilotumumab plus cisplatin and capecitabine regimen as first-line therapy for Asian patients with metastatic MET-positive gastric or GEJ cancer) have been conducted. Unfortunately, the RILOMET-1 study has been reported as negative via press release (AMGEN press release), with final presentation of data pending at an upcoming meeting.

Onartuzumab is an Escherichia coli-derived humanized monovalent moAb against MET that specifically binds to the MET receptor and blocks HGF-MET binding[56]. Shah *et al*[57] have presented the results of a phase II trial that compared FOLFOX plus onartuzumab *vs* FOLFOX plus placebo in patients with metastatic gastroesophageal adenocarcinoma. The primary endpoint of the trial was not met (6.77 mo in onartuzumab arm *vs* 6.97 mo in the placebo arm, HR = 1.08; 95%CI: 0.71-1.63). In MET-positive patients, PFS was 5.95 mo for patients receiving onartuzumab *vs* 6.8 mo for those in the placebo arm (HR = 1.38; 95%CI: 0.60-3.20). Serious adverse events, including neutropenia, thrombocytopenia, peripheral edema, and pulmonary embolism also occurred more frequently in patients on onartuzumab (55% *vs* 40%).

The phase Ⅲ MetGastric trial will assess the effectiveness and toxicity of onartuzumab in combination with modified-FOLFOX6 chemotherapy in patients with metastatic HER2-negative and MET-positive gastric or GEJ adenocarcinoma[58]. In this study, enrolled patients will receive the chemotherapy with either onartuzumab or placebo, and patients who have not progressed after 12 cycles of treatment will continue with either onartuzumab or placebo until evidence of disease progression or intolerable toxicity.

Targeting programmed cell death-1 receptor and its ligand

Programmed cell death-1 (PD-1) is a cell surface and immune inhibitory receptor expressed on a variety of immune cells, especially cytotoxic T cells. Two distinct ligands for PD-1 were identified: Programmed death ligand 1 (PD-L1) and PD-L2[59]. While PD-L2 is expressed mainly on macrophages and dendritic cells, PDL-1 is expressed exclusively by tumor cells and their

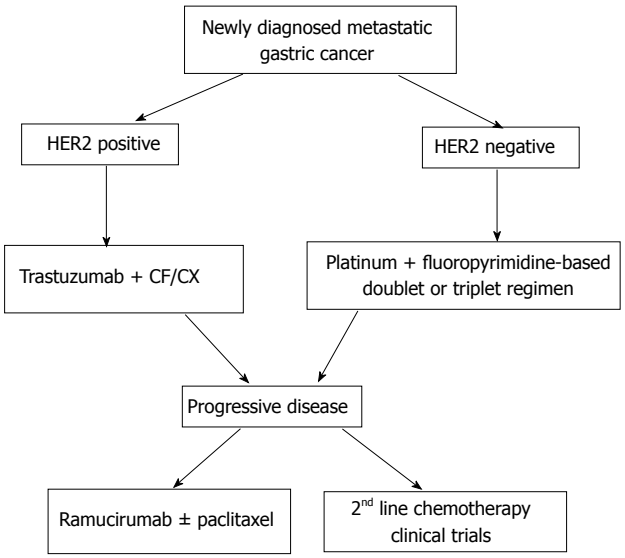

Figure 2 Proposed targeted therapy algorithm for advanced gastric cancer. CF: Cisplatin plus 5-Fluorouracil; HER: Human epidermal growth factor receptor; CX: Cisplatin plus capecitabine.

microenvironment[60]. Tumors that express PD-L1 often tend to be aggressive and carry a poor prognosis[61]. Tumor cells utilize the PD-1/PD-L1 pathway to evade immune-cell attack. Activation of this pathway in tumor cells blocks T-cell-mediated cytotoxicity and allows tumor cells to continue to proliferate[59-61]. Drugs targeting PD-L1 pathway may stimulate antitumor immunity, especially (although not exclusively) in PD-L1 positive tumors.

At the 2014 European Society for Medical Oncology meeting, data on safety and tolerability, and preliminary anti-tumor efficacy of pembrolizumab in advanced GC patients were presented by Muro *et al*[62] (KEYNOTE-012 study). This drug is a selective and humanized moAb that blocks interaction between PD-1 and its ligands PD-L1 and PD-L2. Muro *et al*[62] enrolled 39 patients with PD-L1 positive advanced GC: 19 from Asia-Pacific, 20 from rest of world. Sixty-seven percent of these patients had received more than 2 chemotherapy lines. Pembrolizumab was administered 10 mg/kg once every 2 wk for up to 24 mo in the absence of intolerable toxicity or disease progression. The overall response rate was 31.6% in patients in the Asia-Pacific region and 30% in patients from rest the world. Median duration of response has not yet been reached at the time of initial presentation, but ranged from 8+ to 20+ wk. Four patients developed grade 3-5 drug-related adverse events including peripheral sensory neuropathy, fatigue, decreased appetite, hypoxia, and pneumonitis (n = 1 each). One treatment-related death was recorded due to hypoxia. The authors of the study have concluded that pembrolizumab treatment seems to have and acceptable safety and tolerability profile and it provides encouraging clinical antitumor activity in chemo-refractory disease. On the basis of these promising preliminary data, phase II KEYNOTE-059 study will be initiated to evaluate pembrolizumab as single agent or in combination with cisplatin and 5-FU in patients with metastatic PD-L1 positive gastric or GEJ adenocarcinoma.

Recent analysis from the Gastric Cancer Genome Atlas Project: The Cancer Genome Atlas is a large-scale effort coordinated by the United States National Cancer Institute to extensively characterize the genetic and epigenetic landscape of human cancers. The group has reported on the analysis of 259 untreated primary gastric cancers. This analysis proposed dividing gastric cancer into 4 molecular subtypes: EBV driven, microsatellite unstable (MSI high), genomic stable and chromosomal unstable tumors. This molecular subtyping highlights important targets within these groups for further study, and potentially allows for patient enrichment that could result in higher chance of positive trial results. For example, EBV driven tumors are characterized by high rate of PIK3CA mutations, where drugs targeting the Pi3K pathway are available in clinical trials[63]. Additionally, EBV-positive gastric cancers preferentially overexpress CD274 and PDCD1LG2 (PD-L1 and PD-L2) that were discussed above[64]. These are currently being evaluated as predictive biomarkers for immune checkpoint inhibitor activity[65,66]. In addition, this subgroup has significant promoter hypermethylation, such that evaluating hypomethylating agents such as azacitidine, decitabine and others in clinical development might represent a promising strategy.

The MSI-high genotype is associated with high mutational rate, representing a wealth of antigens that could be recognized by the immune system[67,68]. This genotype has been proposed to be responsive to checkpoint inhibitors, and clinical trials are ongoing (NCT01876511, NCT02060188) addressing response to checkpoint inhibitors in MSI high gastrointestinal cancers.

Other mutations that have been reported (KRAS, P53, APC, and CTNNB1) are still challenging to target and are the subject of numerous reviews. Knowledge of frequency of mutation of these genes, however, provides impetus for further basic research. For example, cell cycle regulators could have better chance of activity in P53 mutant tumors[69,70]. Lastly, the WNT/beta catenin pathway is currently a focus of much preclinical and clinical research[71].

CONCLUSION

Gastric cancer has long represented one of the most difficult gastrointestinal malignancies to treat. Encouragingly, recent progress with targeted therapies offers hope for patients with advanced GC, and expands the therapeutic armamentarium considerably against this formidable disease. As these therapies continue to be developed, we must focus on determination of predictive markers, and preferably co-develop drugs with these markers. The mechanisms underlying primary or acquired resistance to targeted agents also should be clarified in order to help further drug development.

We propose a treatment algorithm that is consistent with current National Cancer Center Network guidelines (version 3, 2015) and that integrates targeted therapies into the management of advanced GC (Figure 2). The addition of trastuzumab to a first-line chemotherapy doublet (cisplatin and capecitabine or 5-FU) is now considered standard of care for patients with HER2 positive advanced GC. The results of the phase III JACOB trial are awaited with great interest to see if the combined use of trastuzumab and pertuzumab can improve clinical outcome. Anti-angiogenic therapy has failed to meet the expectations as first-line treatment. But second-line treatment with ramucirumab or apatinib now represents a good alternative for chemo-refractory GC patients for whom the options are still are quite limited. Other targeted agents currently under evaluation in clinical trials including inhibitors of m-TOR, c-MET, IGFR, and FGFR pathways can help expand our treatment repertoire in the future against advanced GC. Lastly, knowledge gained from detailed molecular profiling of gastric cancers gives us a roadmap for future basic and clinical research.

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REVIEW

Minimally invasive surgical approach to pancreatic malignancies

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Abstract

Pancreatic surgery for malignancy is recognized as challenging for the surgeons and risky for the patients due to consistent perioperative morbidity and mortality. Furthermore, the oncological long-term results are largely disappointing, even for those patients who experience an uneventfully hospital stay. Nevertheless, surgery still remains the cornerstone of a multidisciplinary treatment for pancreatic cancer. In order to maximize the benefits of surgery, the advent of both laparoscopy and robotics has led many surgeons to treat pancreatic cancers with these new methodologies. The reduction of postoperative complications, length of hospital stay and pain, together with a shorter interval between surgery and the beginning of adjuvant chemotherapy, represent the potential advantages over conventional surgery. Lastly, a better cosmetic result, although not crucial in any cancerous patient, could also play a role by improving overall well-being and patient self-perception. The laparoscopic approach to pancreatic surgery is, however, difficult in inexperienced hands and requires a dedicated training in both advanced laparoscopy and pancreatic surgery. The recent large diffusion of the da Vinci® robotic platform seems to facilitate many of the technical maneuvers, such as anastomotic biliary and pancreatic reconstructions, accurate lymphadenectomy, and vascular sutures. The two main pancreatic operations, distal pancreatectomy and pancreaticoduodenectomy, are approachable by a minimally invasive path, but more limited interventions such as enucleation are also feasible. Nevertheless, a word of caution should be taken into account when considering the increasing costs of these newest technologies because the main concerns regarding these are the maintenance of all oncological standards and the lack of long-term follow-up. The purpose of this review is to examine the evidence for the use of minimally invasive surgery in pancreatic cancer (and less aggressive tumors), with particular attention to the oncological results and widespread reproducibility of each technique.

Key words: Pancreatic cancer; Pancreatic adenocarcinoma; Neuroendocrine pancreatic tumors; Laparoscopic; Robotic; Da Vinci

Core tip: Laparoscopic and robotic surgeries for pancreatic cancer are very promising for reducing the frequent complications that occur after open surgery. Nevertheless, the oncologic long-term results remain the cornerstone of any procedure. Most of the studies revealed a lack of evidence for long-term benefits and few comparisons with alternative options.

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INTRODUCTION

The actual incidence of pancreatic cancer [(pancreatic ductal adenocarcinoma (PDAC)] is not high worldwide, ranging from 1 to 9 new cases per 100000 inhabitants. Unfortunately, the reported mortality is almost equivalent to the incidence, illuminating the high number of affected patients who will die within a few months of the diagnosis[1] (Figure 1).

The best chance for a cure is still represented by a curative surgical resection[2]. Other pre-neoplastic lesions [*i.e.*, intraductal papillary mucinous neoplasm - (IPMN)] and borderline neuroendocrine tumors often require a resection *via* a surgical approach[3,4].

Despite the histologic subtype, neoplasms growing in the pancreas can be managed through a minimally invasive approach, but the widespread adoption of such techniques is still limited. According to a large, nationwide, American database, only less than 5% of hepato-bilio-pancreatic procedures were reported to be carried out by a minimally invasive approach[5].

There is a myriad of possible explanations for the limited use of minimally invasive surgery (MIS) compared to other approaches. Firstly, the major pancreatic surgeries [*i.e.*, pancreaticoduodenectomy (PD)] require multiple complex reconstructions, with a high incidence of severe post-operative complications. Simpler resections [*i.e.*, distal pancreatectomy (DP) or enucleation] can bring with them the development of life-threatening fistulas or postoperative severe pancreatitis.

Moreover, an important group of published studies demonstrated a subspecialized training among surgeons as well as a caseload centralization, drastically reduced mortality and failure to rescue after a life-threatening complication occurred[6-8]. Similarly, the need for advanced laparoscopic or robotic skills requirements and expensive technical facilities required for minimally invasive pancreatic surgery is reserved to only a few subspecialized centers[9]. Lastly, the oncologic accuracy, rather than the feasibility, remains the cornerstone of pancreatic surgery for cancer[10].

However, the poor oncologic prognosis of patients affected by PDAC represents an important incentive to adopt some minimally invasive operation that is able to minimize the perioperative morbidity and mortality. Indeed, the traditional benefits of MIS over open surgery are the reduction of pulmonary complications, infections, pain, length of stay and cosmetic result. Many researchers confirmed the utility of MIS in decreasing the pro-inflammatory and immunologic response to surgical trauma that is associated with a superior oncologic result[11,12].

Interestingly, a survey within patients and medical personnel found some preference towards laparoscopic procedures when dealing with pancreatic benign disease and a preference towards open surgery in cases of cancer[13]. Nevertheless, most of the minimally invasive pancreatic procedures failed to reach a sufficient level of evidence-based efficacy to enable a routine application.

The aim of this review was to focus on the MIS (laparoscopy, robotic, hybrid) to manage malignancies and borderline neoplasms arising from the pancreas. Endoscopic and percutaneous maneuvers, although recognized as a great help when dealing with pancreatic neoplasms, did not represent the core of the article and were treated marginally.

A web-based search of MEDLINE (through PubMed and Ovid) and Cochrane databases was updated to April 2015. Many cross-matched manual references were also included. Randomized controlled clinical trials (RCTs) or meta-analyses were considered a priority. Data arising from more recent, English-written, multi-centric, international studies and those with long-term follow-up and oncologic results were also considered of major interest.

MINIMALLY INVASIVE PD

PD is a highly demanding surgical operation, even in the hands of skilled surgeons with specific training. The most challenging steps include pancreatic, biliary and gastroenteric reconstructions that can lead to leakages, perioperative complications and mortality. Most of these operations are carried out to treat malignancy, although more restrictive indications could be IPMN, neuroendocrine tumors or borderline lesions.

As in other gastrointestinal districts, many efforts have been made to limit the destructive impact of this kind of surgery through a minimally invasive approach.

Gagner *et al*[14] described the first laparoscopic PD (LPD) in 1994, but less than 500 operations have been reported in the literature since then[15-18] due to many unresolved issues.

First of all, the limited incidence of pancreatic tumors compared to colorectal cancer reduced the number of the centers with sufficient caseload. Second, the reconstructive steps and the vascular dissection

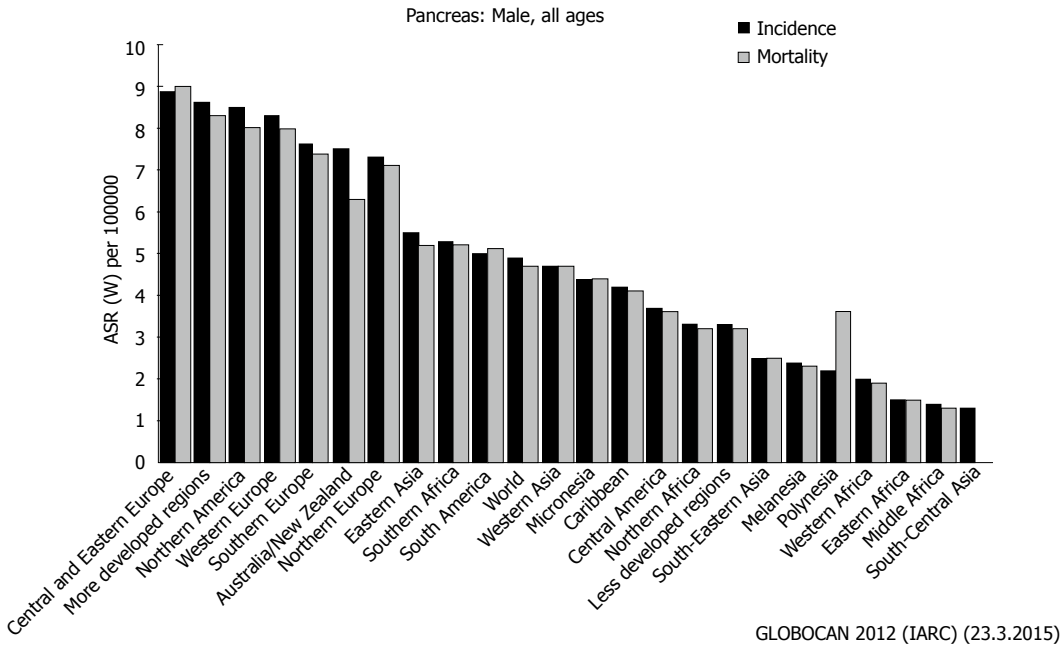

Figure 1 Incidence and mortality of pancreatic cancer worldwide[1].

are very complex and difficult to be achieved by the laparoscopic route whereas the benefits are still under discussion.

Croome *et al*[19] in a recent paper comparing 108 LPD and 214 open PD (OPD) cases well matched for pathologic parameters, reported a shorter length of hospital stay in the LPD group (6 d *vs* 9 d, $P < 0.001$). The other perioperative outcomes, including leakages, were similar. Interestingly, the authors found an earlier starting of adjuvant therapy and a longer progression-free survival in the LPD patients, although the overall survival was similar between the two groups. From a speculative point of view, the prolonged interval between surgery and the beginning of adjuvant chemotherapy may affect the overall survival.

Conversely, Dokmak *et al*[20] retrospectively compared 46 LPD to 46 OPD cases, matched for demographic data, associated comorbidities, and underlying disease. Patients in the laparoscopic group had a lower BMI, a softer pancreas, longer operating time (342 min *vs* 264 min; $P < 0.001$), more grade C pancreatic fistula (PF) (24% *vs* 6%; $P = 0.007$), bleedings (24% *vs* 7%; $P = 0.02$), and revision surgery (24% *vs* 11%; $P = 0.09$). According to these results, the authors concluded that LPD was not to be recommended on a routine basis.

In one updated review by Boggi *et al*[21] including 25 selected articles, a total of 746 minimally invasive PD cases were collected. Of these, pure LPD was used in only 386 patients (51.7%), robotic assistance in 234 (31.3%), laparoscopic assistance in 121 (16.2%), and hand assistance in five (0.6%). LPD was associated with some better perioperative parameters (*i.e.*, blood loss and operative times) compared to robotics and hybrid approaches. Conversion to open surgery was required in 64 LPD (9.1%). No differences were noted in conversion rate, incidence of PF, morbidity, and mortality when comparing results from larger ($\geqslant$ 30 LPD) and smaller ($\leqslant$ 29 LPD) series. Interestingly, pure laparoscopy was employed in half of the whole cohort, while PDAC amounted only to 30% of the entire specimen. These two findings suggested how the laparoscopic approach was indicated in selected cases in the hands of skilled surgeons with wide technologic facilities available, including robotics.

In recent years, the use of robotic systems is gaining momentum as a valuable operative option in the field of pancreatic surgery. Indeed, robotics has emerged as a most interesting and promising innovation, improving the high-demanding surgical procedures, such as PD, with encouraging results[22-25].

With PD in particular, several limitations of standard laparoscopy have been partially overcome by robot-assisted surgery. The major benefits of the surgical robot are a magnified intraoperative imaging, an increased range of motion within narrow and deep spaces, and the enhanced surgical dexterity, affording optimal control during surgical dissections and reconstructions.

It is now more than 10 years since the first large series of robotic general surgical procedures was reported[26], including eight robotic PDs (RPD). The intervening years have seen RPD gaining relatively large distribution worldwide, and more than 350 robotic PDs have been made available in the literature in the last five years[22-32].

Despite the lack of evidence, based solely on retrospective analyses, the use of the robotic platform has already shown several potential advantages over both open surgery[22,33,34] and standard laparoscopy[24,34].

For example, Giulianotti *et al*[27] in 2010, published a cohort of 60 RPD with a rate of PF of 31.3% and only one reoperation. Another single-surgeon experience[32] reported 34 patients operated by RPD with a mean duration of surgery of 597 min and an extra cost of more than 6000 euros. However, the early outcomes were good, with a 0% 30-d mortality and a global 55% morbidity rate. The crucial point of the number of harvested nodes and the negative margins status were also highly comparable to that of open surgery.

In the largest series available in the current literature[24], 132 RPD were followed for postoperative complications in the first 90 d. The 30-d and 90-d mortality were 0.8% and 2.0%, respectively, with a percentage of important complications of 14% and 6% (grade C PF rate of 4%).

Several other non-randomized studies and meta-analyses[21-24,27-31] comparing laparoscopic, robotic and open resections showed comparable complication rates (including PF), mortality and adequacy of lymph nodes yield. Notably, wound infections, hospital stay, blood loss, transfusion rate and R1 resections were significantly lower in patients who underwent minimally invasive resections, with several observations supporting the potential advantages of robotics over conventional laparoscopy[32-34].

Unfortunately, data from the inherent knowledge still fail to provide definitive conclusions concerning the actual role of MIS in performing PD. Further investigations are strongly required, with a special need for randomized analyses comparing robotics and standard laparoscopy. Nonetheless, robot-assisted surgery seems to offer potential advantages in favoring the application of MIS for the treatment of pancreatic neoplasms.

MINIMALLY INVASIVE DP

DP is considered a less challenging operation for the surgeon, with a minor impacting postoperative recovery for the patient compared with PD. The reason is found in the lack of multiple anastomoses, including the potential life-threatening pancreatic remnant pancreaticojejunostomy or pancreaticogastrostomy. Therefore, minimally invasive DP has been widely accepted in the worldwide surgical community.

Gagner *et al*[35] published the first laparoscopic DP (LDP) in the mid-nineties, to manage neoplasms with a borderline behavior (*i.e.*, neuroendocrine tumor). Since then, many retrospective experiences and less comparative series had been published, with the LDP becoming almost the gold-standard approach to both malignant and borderline-benign (mostly) lesions arising from the body-tail of the pancreas[10,36].

Unfortunately, there are some important discrepancies between the literature and ongoing surgical practice. For example, according to a survey conducted on a nationwide database during the period 1998-2009[37] and sampling 20% of United States hospitals, only 5% of DPs were carried out using a minimally invasive approach. However, a similar study regarding 2003-2009[9], found that LDP was utilized in 15%-27% of patients, although many postoperative parameters and the overall costs favored the laparoscopic route. A third[38], more recent (years 2005-2013) cohort study from 17 expert centers in the United States reported that LDP was superior to open distal pancreatectomy (ODP) regarding postoperative morbidity and length of hospital stay. However, only 64 (10%) patients of a total 633 had undergone LDP.

A possible explanation of these surveys could be the presence of few specialized environments with the available expertise and facilities to address pancreatic diseases, although a specific training could improve both the use and outcomes of LDP.

Obviously, the greater the experience, the lesser the patient selection, including complex patients, in maintaining the same postoperative morbidity[39]. Conversely, other authors suggested continuing a careful patient selection for laparoscopy to guarantee the reduction of blood loss and postoperative stay[40].

Nevertheless, many of the available reviews on LDPs include only retrospective case-series with short-term follow-up, different techniques and confusing data reporting[41,42]. One of the largest comparative series was that published by Jayaraman *et al*[43] from the Memorial Sloan Kettering Center on a total of 343 distal pancreatectomies during the 7-years study observation. One hundred seven (31%) of the 343 patients were approached laparoscopically, with a high conversion rate of 30%. However, the LDPs resulted in better outcomes (27% *vs* 40% of postoperative complication; $P = 0.03$), reduced blood loss, and shorter hospital stay (median 5 d *vs* 7 d; $P < 0.0001$), compared to standard operated controls. However, the operative times were longer (median 163 min *vs* 194 min; $P < 0.0001$), and the specific incidence of pancreatic leaks was similar in the two groups (15% *vs* 13%; P = NS).

Kooby *et al*[44] collected data from eight centers performing ODP and LDP, matching patients for age, American Society of Anesthesiologists score, tumor size, and diagnosis. The final analysis included 667 DPs, with 159 (24%) attempted laparoscopically. The conversion rate was 13%. In the final comparison (200 ODP *vs* 142 LDP), the authors reported no differences in the positive margin rates, operative times, or leak rates (18% *vs* 11%; $P = 0.1$). However, LDP had lower blood loss (357 mL *vs* 588 mL; $P < 0.01$), fewer complications (40% *vs* 57%; $P < 0.01$), and shorter hospital stays (5.9 d *vs* 9.0 d; $P < 0.01$).

Vijan *et al*[45] compared 100 matched patients undergoing LDP to an equal cohort undergoing ODP with similar demographic characteristics, but larger tumor size in the ODP group. The LDP group experienced decreased blood loss (171 mL *vs* 519 mL; $P < 0.001$) and shorter duration of hospital stay (6.1 d *vs* 8.6 d; $P < 0.001$). Conversely, they reported no differences in the operative time, pancreatic leak rate (17%), 30-d morbidity (34% *vs* 29%; $P = 0.45$), and

30-d mortality (3% *vs* 1%; $P = 0.62$).

According to an economic perspective, the cost-effectiveness of LDP *vs* ODP was also reported due to the cumulative reduction of hospital stay (5 d *vs* 7 d; $P < 0.001$)[46].

A recent, very impressive, review[47] of all studies comparing LDP and ODP collected data from 29 observational studies (3701 patients overall) to conduct a rigorous meta-analysis. The conclusion was that LDP was superior in terms of blood loss, time to first oral intake, and hospital stay.

Another review by Pericleous *et al*[48] selected only four comparative articles with an above average quality (none was a RCT) reporting LDP to have longer operative time but the reduced length of postoperative stay. Another more recent meta-analysis[49] found 18 comparative studies including more than 1800 patients. LDP was found to reduce blood loss, length of hospital stay, and overall complications.

Although the morbidity related to a distal PF is less dangerous than the morbidity that occurs after PD, the crucial issue of how to reduce its incidence is still unresolved[50]. Many systematic reviews of comparative retrospective studies conclude that the real incidence of fistula after LDP and open surgery are similar, with the stapled or anastomotic closure being the preferred methods despite the access route[51].

Interestingly, a specific analysis[52] of the prognostic factors related to pancreatic remnant leaks, conducted in a comparative matter between 439 OLP and 254 LDP, reported how patients with a body mass index (BMI) $\leqslant$ 27, without adenocarcinoma, and with a pancreatic specimen length $\leqslant$ 8.5 cm had significantly higher rates of PF after OLP than after LDP.

Unfortunately, many of the published series reported different surgical indications for LDP, including PDAC, IPMN and neuroendocrine tumors; these last two are able to be managed more conservatively or tolerate a suboptimal oncological adequacy. Nevertheless, when dealing with PDAC, the minimum prerequisite is to maintain the same oncological outcomes of open surgery, including the overall survival and the disease-free survival. Surrogate parameters, such as the number of harvested lymph nodes and the negative margins of resections, should also be taken into consideration.

A recent paper by Shin *et al*[53] was specifically targeted to compare LDP and ODP in 150 patients operated on for PDAC after using unmatched and propensity score-matched analyses. The oncologic adequacy was considered a primary endpoint whereas the postoperative recovery was marginal. LDP was associated significantly with a shorter median postoperative time to restarting diet and a shorter hospital stay in both matched and unmatched analysis. Interestingly, the 5-year survival rates, the length of surgery, the number of harvested lymph nodes, the resection margin status, and the incidence of PF were all similar.

Another retrospective study[54] reported no evidence of oncological detriment of patients with PDAC and operation by LDP, when cohorts were adjusted for factors affecting selection of operative technique.

A review by Fischer *et al*[55] included only studies reporting pancreatic laparoscopic resections for confirmed malignancies, and the author concluded that LDP (but not LPD) achieved the same rates of margin-positive resections and numbers of retrieved lymph nodes without different long-term survival. Alternatively[56], another study concluded that, due to a lack of statistically powered studies, LDP might not be advised for aggressive tumors. Another group from the United Kingdom[57] reported that LDP, although increasing, should be reserved to benign to low grade malignancies.

Spleen preservation, when indicated in the case of IPMN or less aggressive neuroendocrine tumors, should be the preferred strategy because it leads to a reduction in both blood loss and postoperative complication[58-61]. The recent advent of robotic assisted distal pancreatic resection (RDP) should, potentially, resolve many of the major issues of pure laparoscopy, including the preservation of the spleen[27,62]. Some retrospective series reported the spleen left in situ after a preoperative decision in more than 95% of cases[63,64], compared to inferior percentages (< 90%) achieved by both an open or laparoscopic approach[65].

Moreover, when dealing with PDAC, a more radical dissection and regional lymphadenectomy allowed by the robotic instrumentations should be of some help[36,66,67]. Lastly, the conversion rates seem to decrease with the robotic assistance (0%-18.3%)[24,68] with respect to laparoscopy; this represents an indirect proof of better feasibility or a superior control of bleeding.

One of the first large statistical studies was published by Giulianotti *et al*[27] and colleagues in 2010, with 134 robot-assisted pancreatic operations, including 46 RDPs. Conversion, morbidity and mortality rates for the whole series were 10.4%, 26% and 2.2 %, respectively. The rate of PF was 20.9% after RDP. Only one patient was re-operated on.

The largest series was published by Zureikat *et al*[24] on 250 robotic pancreatic resections, 83 of which were RDP. The 30-d mortality was 0.8%, the rate of Clavien-Dindo grade 3 (or more) complications were 6%, and the type C PF was only 4%. The mean operative time was 257 for RDP.

A very intriguing paper by Daouadi *et al*[69] retrospectively compared 94 LDP with 30 RDP patients well matched for age, sex, race, ASA score, and tumor size. Postoperative length of hospital stay and rates of PF, blood transfusion, and readmission were not significantly different. However, patients in the RDP group had less conversions than the LDP group (16%, $P < 0.05$) and reduced risk of blood loss. Moreover, the percentage of PDAC that was approached robotically was higher (43%) than laparoscopically (15%) ($P < 0.05$), but the oncological outcomes were superior for the RDP, with higher rates of margin negative resection and improved lymph-node clearance ($P < 0.0001$).

A meta-analysis by Zhang *et al*[63], which included

seven trials, merged the data of 137 robotic and 203 open pancreatectomies. Many of the analyzed parameters, including morbidity, redo surgery, resection margins, blood loss and length of hospital stay, had a trend favoring the robotic procedures, but none of them reached statistical significance. Conversely, the length of surgery was demonstrated to be significantly shorter in the open group, whereas fistula formation and mortality rates were similar.

MINIMALLY INVASIVE TOTAL PANCREATECTOMY

Traditionally, total pancreatectomy (TP) is a rarely performed procedure due to its high mortality and morbidity[70]. Nevertheless, the number of TP has been increasing over the years due to the higher number of multifocal pathologies discovered during advanced imaging[71]. In high volume centers, TP makes up 6.7% of all pancreatic procedures[72].

The surgical indications include multifocal neuroendocrine tumors, diffuse IPMN, renal cell metastasis, and MEN-1 syndrome[73-75]. In approximately 20% of cases, the decision to perform a TP is made intraoperatively for PDAC with persistent positive margins in frozen sections[70,74,75] or in the case of fragile pancreatic stump with unacceptable anastomotic risk[76]. TP for chronic pancreatitis has been abandoned with the advent of more efficacious medical management[71].

Post-operative endocrine insufficiency is the most concerning sequel, despite the great improvements in insulin regimen management. Other improvements have been made for the treatment of exocrine deficiency[71].

Similar to PD and DP, a minimally invasive (laparoscopic and robotic) approach to TP has been proposed in recent years. Obviously, the indications for minimally invasive TP are the same as for open surgery.

However, only a few small case-series reporting laparoscopic TP are available in current literature. Nevertheless, preliminary anecdotal papers report laparoscopic TP (LTP) to be safe and feasible, although technically demanding. Morbidity and mortality rates were low after LTP[77,78].

The robotic technique may overcome the intrinsic limits of pure laparoscopy and may provide some advantages compared to open surgery, including spleen preservation[75]. Giulianotti *et al*[74] reported safety and feasibility of this procedure allowing acceptable perioperative morbidity and shorter hospital stays. Globally, morbidity and mortality rate ranges up to 70% and 16%, respectively, with consistent differences according to the surgical indication[75].

Boggi *et al*[79] published a case-matched study comparing 11 robotic TP (RTP) and 11 open TP (OTP). There was no conversion to open surgery in the RTP group. The operative time was longer and the blood loss was lower for RTP, whereas morbidity was similar (lower severity in the RTP group). The length of stay was similar between the two groups, but the robotic patients experienced faster recovery and lower pain. Interestingly, lymph-node collection was higher for the robotic group (45 *vs* 36, not statistically significant).

In the series of 10 RTPs published by Zureikat *et al*[75], there was one conversion to laparotomy, no 90-d mortality, a 20% Clavien Ⅲ-Ⅳ complication rate and only one readmission within 90 d.

MINIMALLY INVASIVE PARENCHYMA PRESERVATION AND UNCOMMON PANCREATIC RESECTIONS/ ENUCLEATIONS

Due to the routine use of high resolution imaging techniques, diagnosis of small benign or low-grade malignant lesions of the pancreas has increased in the last years, leading to a higher number of proposed resections. Nevertheless, major pancreatic resections are still at risk of potentially life-threatening complications, even if performed through a minimally invasive approach.

From this perspective, much effort should be attempted to spare healthy tissue and to avoid unnecessary pancreatic anastomosis in non-frankly malignant tumors. Pancreatic enucleation (PE) and central pancreatectomy (CP) are the most frequent proposed operations, whereas duodenum-preserving pancreatic head resection (DPPHR), pancreatic head resection with segmental duodenectomy, inferior head resection, dorsal pancreatectomy, pancreatic head excavation, middle-preserving pancreatectomy[80] and resection of the uncinate process[81,82] are less popular, very rare alternatives. All of these procedures had been proven to be safe and feasible with low mortality and recurrence rates[83].

The selective indications for these conservative operations include cystoadenomas, pseudopapillary neoplasms, non-invasive branch-type IPMN, endocrine tumors[84-87] and isolated metastasis from renal cancer[82].

One major drawback is the high complication rates mostly related to PF[80] that were mostly grade A or B (slightly higher rate of severe grades after CP compared to PE) and managed conservatively[83].

Parenchyma-sparing resections performed in a minimally invasive fashion would be the ideal procedure for those patients. The introduction of new instruments and growing experience make laparoscopic techniques broadly used even in conservative pancreatic laparoscopic resections. For example, the laparoscopic ultrasonography probe is a powerful tool to accurately find the lesion and its correlation with vessels and the pancreatic duct, thus overcoming the absence of any tactile sense[87-89].

Moreover, the robotic assistance may overcome some limitations of laparoscopy itself with a dedicated

Table 1 Authors' recommended approach to pancreatic procedures

Type of procedure	Open surgery	Laparoscopic surgery	Robotic	Level of evidence[1]
Distal pancreatectomy	Standard/accepted	Being standard	Pioneeristic	LE 2
Pancreaticoduodenectomy	Standard	Pioneeristic	Pioneeristic	LE 2
Total pancreatectomy	Standard	Pioneeristic	Pioneeristic	LE 4
Enucleation	Accepted/standard	Standard/accepted	Pioneeristic	LE 3

[1]Oxford Centre for Evidence-Based Medicine. Levels of Evidence Working Group. "The Oxford 2011 Levels of Evidence". Available from: URL: http://www.cebm.net/index.aspx?o=5653.

flexible probe that has been developed to replace standard laparoscopic ones. This integrated robotic probe is moved by the console surgeon and allows reproducing all of the movements of open surgery. Lastly, the ultrasound screen is seen in picture-in-picture mode.

In his systematic review, Beger *et al*[83] reported the results of PE in 838 patients (22.5% of them underwent minimal-invasive surgery) demonstrating an overall morbidity rate of 41.3% (with a 9.6% of severe complications), a PF rate of 36.7%, a reoperation rate of 4.7% and a mortality rate lower than 1%. Zhang *et al*[90] collected data from 119 patients, which showed 0% exocrine insufficiency, no worsening of diabetes after surgery and 2.8% new-onset endocrine insufficiency. Cardiac impairment and operative time longer than 180 min were found to be independent risk factors for PF.

Unfortunately, no RCTs comparing open PE (OPE) and laparoscopic pancreatic enucleation (LPE) are available in the current literature, although many case series and retrospective comparative studies reported feasibility, safety and effectiveness of the minimally invasive approaches, with lower blood loss and length of hospital stay[85,88,91-93]. The conversion rate ranges from 20% to 33%[88]. Overall, the morbidity is similar between the OPE and LPE groups, but major complications are more frequent in the open group[89]. The incidence of PF after LPE ranged from 13% to 38%[92], which is lower than the rate after OPE. The long-term results of LPE are still lacking.

In his systematic review including 101 patients treated with a LPE, Briggs *et al*[94] reported a conversion rate ranging from 10% to 44% and a morbidity rate ranging from 22% to 67% without significant differences in morbidity and mortality rates compared to open pancreatic surgery.

Interestingly, pancreatic robotic enucleation seems to be both safe and feasible with lower intraoperative blood loss, better perioperative outcome, mortality rates less than 1% and shorter hospital stays compared with open surgery. However, rigorous trials matching robotics, laparoscopy and open surgery are still lacking[24].

CP is performed more rarely (less than 3% of pancreatic resections in high volume centers)[95,96]. Indications for CP include tumors up to 5-6 cm in size arising from the pancreatic neck or body, which are in proximity to the pancreatic duct and are not suitable for PE. Many options for proximal stump are possible, including staple or suturing techniques, but none have proven a real superiority over another[96-99].

The indications for minimally invasive CP are equivalent to that for open CP. However, laparoscopic CP (LCP) remains controversial due to the difficulties in pancreatic reconstruction. Preliminary results show its safety and feasibility[82,100,101].

Again, the robotic platform was reported to overcome some of the limitations of a pure laparoscopic approach. Nevertheless, only a few small case-series of robotic central pancreatectomies (RCP) reported the same high rate of PF and longer operative times, but faster recovery compared to open surgery[98,102,103].

Zureikat *et al*[24] reported the results of 13 cases of RCP with a conversion rate of 15%, no perioperative mortality, but a 92% PF rate. Abood *et al*[104] reported a PF rate of 22.2%, and R0 surgery in all nine patients with no endocrine or exocrine impairment. Kang *et al*[102], in his retrospective match-compared study of five patients treated robotically and ten patients treated with open CP, demonstrated no significant differences in overall complication rate, perioperative mortality and length of hospital stay. The intraoperative blood loss was significantly lower in the robotic group and operation time was longer compared to the open procedure.

Interestingly, Machado *et al*[105] performed a review on 22 cases of LCP versus 27 RCP cases. The study showed low blood loss, PF rate of 46%, no mortality, no recurrence at a mean follow-up of 19.6 mo and no exocrine or endocrine deficiency. Chen *et al*[101] reported the results of LCP performed in 10 cases. The incidence of PF was 20% (grade A), and there was no recurrence (median follow-up of 22.9 mo) of either exocrine or endocrine pancreatic insufficiency.

Resection of the uncinate process and DPPHR are very rarely performed procedures, and only a few cases reports describing any laparoscopic approach are available in the literature. Most of these cases had a high rate of PF[82]. In a very inclusive review, Beger *et al*[83] reported the results of 431 DPPHR cases, demonstrating a rate of severe complications of 11.5%, a PF rate of 20%, a reoperations rate of 1.8% and mortality lower than 1%.

CONCLUSION

Patients affected by PDAC are still expected to die a

few years after surgery (if indicated), with the overall survival slightly increased by a regimen of perioperative adjuvant radio-chemotherapy. Borderline neoplasm and even pre-cancerous lesions require complex management, which often includes a surgical approach with some potential life-threatening complications.

A strong effort to minimize those complications and to enhance the recovery after surgery could be a great help to those patients (Table 1).

In this view, the minimally invasive surgical approach (laparoscopic and robotic operations) to pancreatic neoplasm leads to many benefits, including recovery, cosmetic results, early access to adjuvant therapies and psychological implications. Unfortunately, most of the articles published and reviewed were flawed by a weak statistical power (heterozygous methods, facilities and devices employed, insufficient case load), and many reported conflicting results. A possible explanation is the extreme weight of technologic equipment and expertise needed to develop a minimally invasive pancreatic cancer program in addition to the low incidence of such pathology. However, a strategic centralization of pancreatic malignancies together with more rigorous scientific data reporting should be mandatory in future years.

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REVIEW

Colorectal cancer diagnosis: Pitfalls and opportunities

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Abstract

Colorectal cancer (CRC) is a major health problem in the Western world. The diagnostic process is a challenge in all health systems for many reasons: There are often no specific symptoms; lower abdominal symptoms are very common and mostly related to non-neoplastic diseases, not CRC; diagnosis of CRC is mainly based on colonoscopy, an invasive procedure; and the resource for diagnosis is usually scarce. Furthermore, the available predictive models for CRC are based on the evaluation of symptoms, and their diagnostic accuracy is limited. Moreover, diagnosis is a complex process involving a sequence of events related to the patient, the initial consulting physician and the health system. Understanding this process is the first step in identifying avoidable factors and reducing the effects of diagnostic delay on the prognosis of CRC. In this article, we describe the predictive value of symptoms for CRC detection. We summarize the available evidence concerning the diagnostic process, as well as the factors implicated in its delay and the methods proposed to reduce it. We describe the different prioritization criteria and predictive models for CRC detection, specifically addressing the two-week wait referral guideline from the National Institute of Clinical Excellence in terms of efficacy, efficiency and diagnostic accuracy. Finally, we collected information on the usefulness of biomarkers, specifically the faecal immunochemical test, as non-invasive diagnostic tests for CRC detection in symptomatic patients.

Key words: Colorectal cancer; Colonoscopy; Primary health care; Faecal immunochemical test; Diagnostic yield; Diagnostic accuracy; Risk stratification; Open endoscopy unit; Practice guidelines; Health plan implementation

Core tip: In this review, we summarize the pitfalls in the diagnostic procedure for colorectal cancer (CRC) in symptomatic patients. We collected the available information concerning the value of symptoms as predictors of CRC and the factors involved in the delay of CRC diagnosis, including those related to the patient, to the physicians and to hospital delay. In this way, we review the currently available sets of appropriateness criteria for colonoscopy in symptomatic patients, the prioritization criteria and predictive models for CRC detection and, finally, the role of available biomarkers in

the evaluation of symptomatic patients.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related death[1]. In Western Europe, it is the seventh leading cause of death, the fourth leading cause of loss of life expectancy, and it is associated with an elevated consumption of resources[2,3]. The stage of the tumour at the time of treatment is considered the most important predictor of survival. Thus, in Europe, survival is 93% after 3 years for Duke stage A tumours, but it is only 16% after 3 years for stage D tumours[4]. Two strategies are widely used to improve CRC prognosis and to optimize the health resources consumed: Population-based CRC screening programs and early diagnosis strategies in symptomatic patients[5-7]. Population-based screening programs in asymptomatic patients have been demonstrated to reduce the incidence and mortality rates of CRC in two ways: Removing preneoplastic lesions with polypectomy and diagnosing a higher proportion of CRCs at an early stage[8-10].

On the other hand, the early diagnosis of CRC in symptomatic patients remains a problem. It is a complex process that begins when the patient detects the first symptoms until a diagnostic procedure is performed, undergoing a consultation with a general practitioner, a referral to the specialist, and the waiting period for diagnostic procedures, such as colonoscopy. All this contributes to the perception that delay in CRC diagnosis is a multifactorial problem[11]. In the general population, lower abdominal symptoms are very common and are a frequent cause of visits to the general practitioner. The issue is that symptoms are usually very vague and non-specific, with a poor sensitivity for CRC. In most cases, these symptoms are produced by benign, self-limiting illness, contributing to the patient's delay in seeking help and the practitioner's delay in referring the patient to a specialist. Moreover, the growing demand for colonoscopy has become a significant problem, as endoscopic resources are limited; these waiting periods also delay the diagnosis of CRC. Computed tomography (CT) colonography could be an alternative, especially in elderly patients with poor specific symptoms such abdominal pain or weight loss[12]. However, the referral rate for additional tests after CT-colonography must be reduced to avoid the potential to increase anxiety and overall cost[13]. For these reasons, as colonoscopy is the gold standard for CRC investigation, several risk classification scores based on symptoms have been developed to determine which patients are most at risk of having CRC and thus to reduce the delay between the initial consultation and the colonoscopy[6,7,14-16].

The objective of this article was to review the pitfalls and missed opportunities in the process of CRC diagnosis in symptomatic patients. First, we evaluated the evidence concerning the value of symptoms as predictors of colorectal neoplasia. We showed the effect of delayed diagnosis on CRC prognosis as well as the factors related to this delay. This includes factors related to the patient, to the first attending physician (most likely in a primary setting), and, finally, to the hospital delay as a result of the waiting period before colonoscopy. We analysed the available sets of criteria for colonoscopy diagnosis in symptomatic patients, along with the prioritization criteria and the predictive scores for CRC diagnosis and their diagnostic yield for CRC. Finally, we explored the usefulness of the available biomarkers to determine the types of patients who can benefit the most from a colonoscopy.

VALUE OF SYMPTOMS

In the general population, abdominal symptoms account for up to 10% of consultations with general practitioners[17]. Most of these symptoms are related to chronic functional conditions (irritable bowel syndrome, chronic constipation) or anorectal benign lesions that do not benefit from colonoscopy evaluation[18,19]. In clinical practice, it is common to perform a colonoscopy in patients with bowel symptoms due to the suspicion of CRC[20]. In fact, many practice guidelines suggest that colonoscopy should be performed for bowel symptoms, but the importance and value of symptoms as indicators of CRC is not well established. While some reports suggest that symptoms may be useful in identifying CRC, others have found no such association[21-24]. Moreover, few of these studies are recent and the perception of symptoms may have changed since the early studies were conducted.

Recently, several meta-analyses have analysed the risk of detecting CRC according to the symptoms reported. Ford *et al*[22] performed a systematic review and meta-analysis to assess the diagnostic accuracy of alarm features in predicting CRC. They included fifteen studies evaluating 19443 patients, with a pooled 6% CRC prevalence. CRC diagnosis was based either on colonoscopy (8), sigmoidoscopy (1), double contrast barium enema (1), or both lower endoscopy or barium enema (5). They included 1 population-based study, 11 secondary healthcare level studies, 2 primary healthcare level studies and 1 mixed levels study. In summary, the pooled sensitivity of the symptoms was poor (5% to 64%), but specificity was 95% for dark red rectal bleeding and abdominal mass (Figure 1). It is remarkable that both positive and negative likelihood ratios (PLR and NLR) lie close to 1; thus, the presence or absence of symptoms does not significantly modify

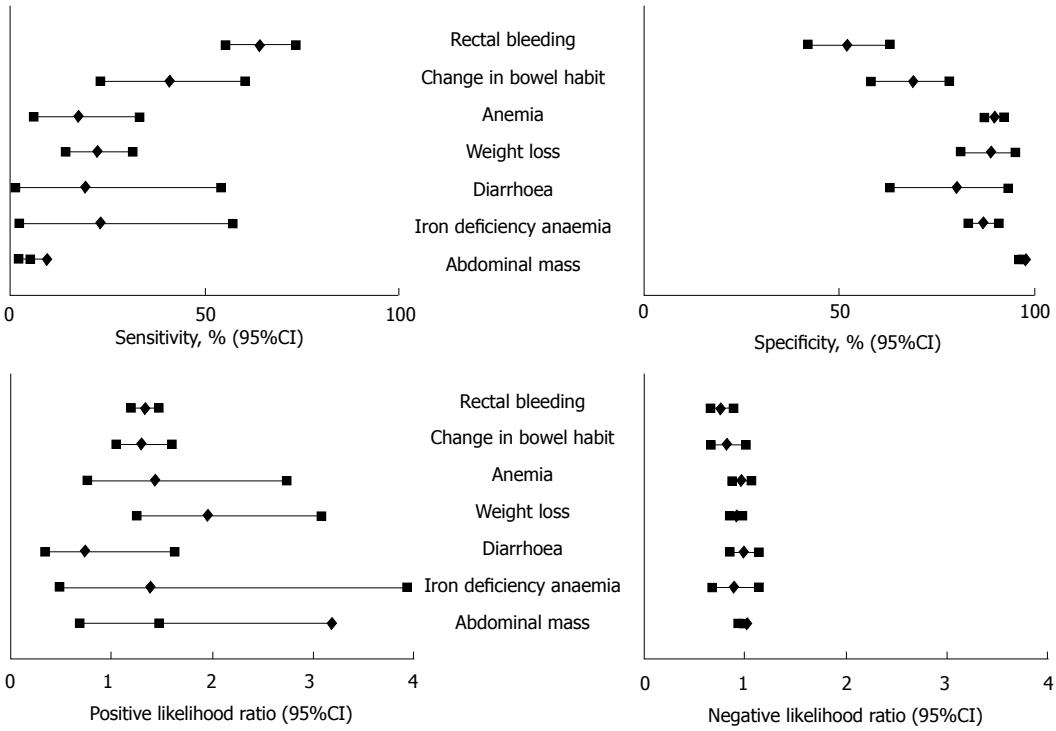

Figure 1 Diagnostic accuracy of symptoms for colorectal cancer detection. Adapted from Ford *et al*[22].The results are expressed as the median (%) and 95%CI.

the probability of CRC detection.

Astin *et al*[23] performed an additional systematic review and meta-analysis to identify the risk of CRC in patients reporting a symptom to a primary care provider. They included 23 studies that recruited 81464 participants. They analysed both single and paired symptoms. Positive predictive values (PPVs) for rectal bleeding from 13 papers ranged from 2.2% to 16% with a pooled estimate of 8.1%, and PLR ranged between 1.09 and 10.13 with a pooled estimate of 5.31. Pooled PPV estimates for other symptoms were: Abdominal pain (three studies), 3.3%; and anaemia (four studies), 9.7%. For rectal bleeding accompanied by weight loss or change in bowel habits, pooled PLRs were 1.9 and 1.8, respectively. Conversely, the PLR was one or less for abdominal pain, diarrhoea, or constipation accompanying rectal bleeding. The authors concluded that the investigation of rectal bleeding or anaemia in primary care patients is warranted, irrespective of whether other symptoms are present.

Additionally, Jellema *et al*[24] performed a meta-analysis to summarize the available evidence concerning diagnostic tests that might help primary care physicians to identify patients with an increased risk for CRC among those consulting for non-acute lower abdominal symptoms. The tests evaluated included signs, symptoms, referral criteria, blood and faecal tests. With respect to symptoms (Table 1), sensitivity ranged between 13% and 51% and specificity ranged between 59% and 89%. As a result, the risk of detecting a CRC was not modified significantly between those patients with symptoms (PPV ranging between 6% and 14%) and those without any of the symptoms evaluated (1-negative predictive value, NPV, ranging between 3% and 10%). In contrast, the variable age (> 50 years) was more sensitive than any of the symptoms (91%), although the specificity was lower (36%), significantly modifying the risk of CRC detection between patients older and younger than 50 years (10%, 2%).

Therefore, the value of symptoms for CRC detection is very poor. Symptoms alone are not adequate to establish a suspicion of CRC and they must be synthesized with other variables, such as demographic variables and analytical data.

DELAY IN CRC DIAGNOSIS

The period of time from the first symptoms until a final diagnosis is achieved can vary. In a recently published study, the median interval between symptoms and diagnosis was 128 d with a wide interquartile range (57.5-257.5). This interval was due to the delay from the first symptom until the initial consultation (19 d, interquartile range 3-83) and the delay in health service (66 d, interquartile range 25-159) (Figure 2)[25]. There is a controversy regarding the association between diagnostic and therapeutic delay and the prognosis of CRC. In fact, there seems to be a lack of association between diagnostic delay, CRC survival and stage[26], suggesting that, in CRC, the symptomatic phase is only a small component of the natural history of the disease. When colon and rectal cancer are analysed separately, an opposite association exists. For the colon, a greater delay is associated with an earlier stage at diagnosis,

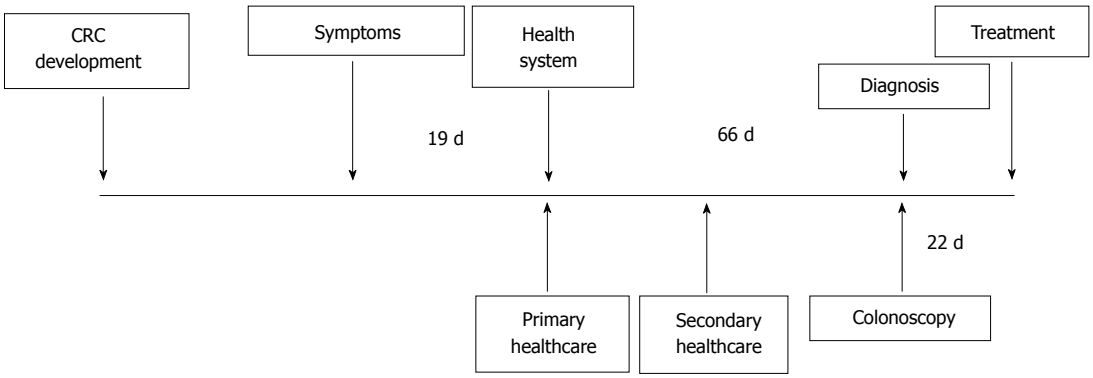

Figure 2 Distribution of delay intervals in colorectal cancer diagnosis (in days). Adapted from Esteva *et al*[25]. CRC: Colorectal cancer.

Table 1 Summary of findings (sensitivity, specificity, predictive values) for diagnostic tests for colorectal cancer detection evaluated by at least four primary diagnostic studies

Index test	Sensitivity	Specificity	PPV	1-NPV
Age (> 50)	91%	36%	10%	2%
Sex (male)	62%	55%	13%	3%
Family history	16%	91%	6%	4%
Weight loss	20%	89%	9%	6%
Abdominal pain	35%	59%	5%	7%
Rectal bleeding	44%	66%	7%	4%
All bleeding, dark blood	35%	85%	14%	5%
All bleeding, mixed with stool	51%	71%	6%	3%
Change in bowel habits	52%	61%	9%	4%
Diarrhoea present	20%	73%	6%	10%
Constipation	13%	72%	6%	9%
Two week rule positive	92%	42%	14%	3%
Iron deficiency anaemia	13%	92%	13%	8%
Faecal occult blood test positive				
Chemical	75%	86%	28%	1%
Immunological	95%	84%	21%	0%

The results are expressed as medians or pooled estimates. Adapted from Jellema *et al*[24]. PPV: Positive predictive value; NPV: Negative predictive value.

and for the rectum, a smaller delay is associated with an earlier stage[26]. This could be explained because rectal cancer has well-defined symptoms, such as rectal bleeding with or without changes in bowel habits, while colon cancer-related symptoms are very vague at the onset, and when the seriousness of symptoms require investigation, the disease is more advanced[27].

Factors related to patient delay

As previously mentioned, lower abdominal symptoms are very common and mostly due to benign, self-limited conditions. Moreover, most are very vague and patients do not relate them to a serious illness. In the complexity of the process of cancer diagnosis, Andersen's Model of Total Patient Delay[28,29], a theoretical framework, defines five time intervals in the decision-making process: Appraisal delay (time between the detection of symptoms and inferring illness); illness delay (period when the patient contemplates between consulting a medical practitioner or self-treating the illness); behavioural delay [delay in making an appointment with the general practitioner (GP)], scheduling delay (time elapsed between making an appointment and the first medical consultation) and treatment delay (until the initiation of treatment). Factors related to the patient are encompassed in the first four time intervals.

Many studies have focused on determining the causes that lead to a delay in seeking medical help once the patient notices the first symptoms, including factors that would increase the delay and others that would reduce it. These factors are listed in Table 2. Most of the studies show that the main factors associated with patient delay are the lack of knowledge and concern about potential risks associated with the symptoms as well as non-recognition of the seriousness of the symptoms[30,31], suggesting that appraisal delay is the main contributor to the global delay related to the patient[28]. This situation entails a misinterpretation of symptoms, attributing them to a benign disease or assuming that they are part of the ageing process. In this way, non-recognition of the seriousness of symptoms will also lead to self-diagnosis, "wait and see" strategies and self-treatment.

Other important factors described in studies are those related to denial and fear of symptoms[32]. They include fear and denial of cancer, fear of poor prognosis, or fear of embarrassing and unpleasant investigations, which are all related to a lack of adequate information. With respect to the symptoms, patients who suffer from persistent or more serious symptoms affecting the person's daily life, such as pain, vomiting or obstruction, delayed seeking treatment less often. In contrast, more common symptoms, such as changes in bowel habits, rectal bleeding, or nonspecific symptoms were associated with more prolonged delays. A recent study that examined medical-advice-seeking behaviours showed that one in five persons experiencing rectal bleeding or changes in bowel habits did not seek medical advice. Moreover, among those seeking help for rectal bleeding or changes in bowel habits, up to 18% and 37%, respectively, delayed seeking treatment by more than 1 mo[33]. There is no clear evidence of the way in which factors such as age, gender, marital

Table 2 Main factors associated with patient delay

Increases delay	Reduces delay
Appraisal delay	
Symptoms attributed to minor illness	Specific symptoms (rectal bleeding, abdominal pain)
Lack of knowledge or failed to recognize symptom severity	Symptoms frequent, severe or affect the person's daily life
Assumed to be part of the ageing process	Pain, vomiting and intestinal obstruction as initial symptoms
Non-specific symptoms (altered bowel habits, unexplained weight loss)	
Self-treatment	
Illness delay	
Younger patients	Comorbidity
Low socioeconomic status	High educational level
Lower educational level	Retirement
Rural areas	
Lack of additive private health insurance	
Family history of cancer	
Behavioural delay	
Fear of pain	Social support
Fear of cancer	Disclosure of symptoms to someone close
Fear of unpleasant or embarrassing investigations	Knowing a person with CRC
Denial of symptoms	
Scheduling delay	
Too busy to visit	Trust in GP
Unpleasant or embarrassing visit	

CRC: Colorectal cancer; GP: General practitioner.

Table 3 Main factors associated with practitioner delay

Increases delay	Reduces delay
Lack of continuity of care	Site (rectum)
Frequent attendance	Experience
Patient's socioeconomic status (lower)	Use of referral guidelines
Initial misdiagnosis	Suspected CRC diagnosis in the referral
Failure to examine or investigate	Urgent referral to hospital
Inaccurate or inadequate tests	
Co-morbidities	
Elderly patients	
Psychiatric co-morbidities	

CRC: Colorectal cancer.

status, socioeconomic status and education level impact delay. Some studies have shown that males and younger people tend to delay more often. Additionally, low socioeconomic status and low educational level seem to be associated, but results are not consistent. In contrast, social support and a trustful relationship with the general practitioner are strongly associated with less delay[25,34]. Finally, increased knowledge about CRC improves timely help-seeking for symptoms, reducing negative perceptions[35].

In sum, the main factors related to patient delay are caused by the lack of knowledge about symptoms, the importance and implications of CRC diagnosis at an early stage, and the diagnostic tools available. Therefore, an effort to educate the general population about CRC is warranted and may help to reduce delay.

Factors related to practitioner delay

One of the steps in the complex process of CRC diagnosis involves the physician that first sees the patient, usually the general practitioner. He must suspect that the symptoms are due to CRC and refer the patient for further investigations. Many factors are associated with practitioner delay (Table 3). Mitchell *et al*[30] performed a systematic review including twenty-nine papers that considered factors that influenced practitioner delay. He described two main factors associated with an increase in delay, as both were considered to be factors in most of the studies included ($\geqslant$ 75%)[30]. The first was initial misdiagnosis, either through prescribing symptomatic treatment or attributing symptoms to other benign conditions. In fact, missed opportunities to diagnose CRC before endoscopic referral occur in 31%-34% of patients presenting with symptoms, entailing an average delay from the first visit > 200 d. Among those patients, there was a mean of 2.41-4.2 missed opportunities. Those patients tended to be older and with more co-morbidities, including congestive heart failure or coronary artery disease. The main diagnostic key was iron- deficiency anaemia, which was associated with the longer delay to referral (> 300 d)[36,37].

The second main factor was failure to examine or investigate. Studies showed a frequent lack of physical examination among patients with lower abdominal symptoms, especially digital rectal examination[30]. In two recent studies, only 25% of patients with rectal carcinoma had a digital rectal examination at their first visit[11], and GPs only performed a physical examination of one in three patients[25]. These results are in accordance with previous studies that showed no improvement over time[38,39].

The available results on the effect of age and comorbidities on delay are conflicting. Although previous studies have noted that elderly patients and those with co-morbidities are referred earlier[30], recent studies suggest the opposite, with more missed opportunities and more delay[36,37,40]. Moreover, psychiatric diseases are also associated with referral delay by the GP[40,41]. Regarding the consultation pattern, a greater interval to diagnosis was observed for patients with an increasing number of visits to the GP due to symptoms related to CRC and those lacking continuity of care[25,42]. Inaccurate or inadequate tests and a negative or a false negative test result increased the delay time[30].

Another important aspect is how the physician performs the request or referral. When the referral is urgent, includes three diagnostic clues, mentions the suspicion of CRC or contains documentation of verbal contact, the delay decreases[25,43]. Furthermore, the use of referral guidelines and the appropriate use of urgent referrals seems to reduce delay[44,45]. Studies have shown that strategies based on training primary

Table 4 National Institute for Health and care excellence referral criteria[7]

High risk referral criteria (any)
Patients ⩾ 40 yr with rectal bleeding and a change of bowel habits persisting ⩾ 6 wk
Patients ⩾ 60 yr with rectal bleeding persisting ⩾ 6 wk without a change in bowel habits and without anal symptoms
Patients ⩾ 60 yr with a change in bowel habits persisting ⩾ 6 wk without rectal bleeding
Patients presenting with a right lower abdominal mass consistent with involvement of the large bowel
Patients presenting with a palpable rectal mass
Patients with unexplained iron deficiency anaemia (< 11 g/100 mL in men, < 10 g/100 mL in non-menstruating women)

care physicians to evaluate patients with digestive symptoms and allowing them open access to endoscopy units reduces waiting time and increases the diagnostic yield[19,46]. Educational programs also increase the appropriateness rate of referrals[47]. Furthermore, a Cochrane review concluded that active local training involving secondary health care specialists and structured referral applications are the only interventions that have an impact on outpatient referral rates[48].

Therefore, it seems important to improve educational programs to reduce initial misdiagnosis. CRC is usually first detected at primary healthcare settings, but each GP diagnoses very few CRC patients each year[49]. Additionally, it is mandatory to generalize digital rectal examinations in patients with lower abdominal symptoms as well as to use referral guidelines and open access endoscopy units to increase the appropriateness of referrals and thus reduce delays.

Factors related to hospital delay: Waiting lists and prioritization

The evaluation of symptoms is one of the most important reasons to perform a colonoscopy, ranging between 38.8% and 57.3% of all referrals for colonoscopy[50-53]. However, most of the colonoscopies performed in symptomatic patients are normal or do not yield changes in the therapeutic approach, so the benefit to most of the patients is scarce[18,23,24,54,55]. This, added to the growing demand of colonoscopy requests related to screening programs, establish the need for prioritization criteria and objective tools with the aim of reducing delay in patients with a high suspicion of CRC, preventing them from being affected by waiting lists. The appropriateness criteria for colonoscopy indications proposed by the American Society for Gastrointestinal Endoscopy[56] and the European Panel on the Appropriateness of Gastrointestinal Endoscopy (EPAGE)[57-63] have shown its limited value as a diagnostic tool in symptomatic patients. Although appropriateness is associated with a high sensitivity for CRC and a fair sensitivity for relevant findings, its specificity and positive predictive values are poor. This is related to their high positivity rate (70%-81.4%). In fact, the rate of appropriateness in colonoscopies due to symptom evaluation ranged between 73% and 95.1%, limiting its use in this scenario. In the next section, we will focus on two of the most promising strategies to reduce diagnostic delay due to waiting time for colonoscopy: Prioritization criteria or predictive indexes and diagnostic biomarkers.

PRIORITIZATION CRITERIA AND CRC PREDICTIVE INDEXES

Strategies for the early diagnosis of CRC in symptomatic patients may improve prognosis[64,65]. In this regard, several risk classification scores based on symptoms have been developed. These classification criteria are intended to determine which patients are most at risk of having CRC, and thus to reduce the delay between the initial consultation in primary care settings and the colonoscopy[6,7,14,15]. The two-week wait (TWW) referral guideline was introduced by the National Health System (NHS)[66] and updated to its most recent version in 2011 (Table 4) by the National Institute of Clinical Excellence (NICE)[7]. It has been the most widely used and evaluated diagnostic criteria. Some other referral guidelines have recently been proposed and validated[67,68]. Moreover, several CRC predictive indexes based on clinical symptoms have been proposed[14,15,69-71].

The TWW emerged in 2000 in response to the low survival rate at 5 years for CRC in Britain compared to other European countries with similar economic resources. The NHS established a prioritization system based on signs and symptoms associated with a high probability of detecting a CRC. Those patients who met any of these criteria should be assessed within 14 d of their referral. The NHS expected that up to 90% of incident CRC would be diagnosed through the TWW. It has been widely implemented across the NHS. Several articles have been published evaluating the efficacy, efficiency and diagnostic accuracy of the TWW[24,54,72-77]. The TWW was implemented in most NHS centres; however, compliance with the guidelines has been poor. This, coupled with the poor specificity of the system, has resulted in a poor cancer detection rate and a steadily growing volume of hospital referrals. The system has been shown to have an adverse impact on the waiting times for routine colorectal referrals[73]. In fact, only 24% of incident CRC cases were diagnosed through the TWW, and no evidence was found that CRC was diagnosed at an earlier stage[75]. Jellema *et al*[24] evaluated the diagnostic accuracy of the TWW. The sensitivity and specificity for CRC detection was

92% and 42% with a 14% PPV and a 3% NPV[24]. In sum, it is a diagnostic tool with low specificity and variable sensitivity and its use is subjected to local circumstances[24,54,72-77].

Two additional sets of prioritization criteria have been recently evaluated. The Scottish Intercollegiate Guidelines Network (SIGN) referral criteria are based on modifications of the TWW[6]. In a recently published article that aimed to compare the faecal immunochemical test (FIT) with the TWW and the SIGN referral criteria, the SIGN referral criteria produced a greater number of referrals (60.1% *vs* 38.1%) and increased the sensitivity for CRC detection (82.5% *vs* 61.9%), but the specificity was inferior when compared with the TWW (42.7% *vs* 65.2%)[54].

Additionally, the Galician Health Service in Spain established indications and priority levels (Ⅰ = fast track, Ⅱ = preferential, Ⅲ = normal) for colonoscopy according to the risk of CRC and significant colonic lesion detection in primary health care settings. These criteria consisted of symptoms, imaging abnormalities and analytical data. Therefore, patients with any of the following situations were stratified to priority level Ⅰ: Palpable right lower abdominal mass; palpable rectal mass; or unexplained iron deficiency anaemia (< 11 g/100 mL in men, < 10 g/100 mL in non-menstruating women). Patients with the following criteria were excluded: NSAID consumption; suspected CRC in imaging studies; rectal bleeding and a change in bowel habits (> 6 wk); patients ⩾ 50 years with a change in bowel habits (preferably more frequent stools), persisting ⩾ 6 wk without rectal bleeding and patients ⩾ 50 years with rectal bleeding persisting ⩾ 3 wk without anal symptoms. If the patient met any of the following conditions, they were stratified to priority level Ⅱ: Faecal haemoglobin concentration > 20 mg/mL or equivalent in the absence of rectal bleeding; high suspicion of inflammatory bowel disease in imaging studies (ultrasound or abdominal CT scan); chronic diarrhoea (> 4 wk evolution), with clinical and laboratory evidence of an inflammatory process after ruling out infectious causes; unexplained iron deficiency anaemia (> 11 g/100 mL in men, > 10 g/100 mL in non-menstruating women); patients < 50 years with persistent rectal bleeding with a negative digital rectal examination < 50 years, with anuscopy/rectoscopy that does not justify the symptoms and, finally, persistent rectal bleeding after medical treatment (2-4 wk) of a benign anal lesion. Finally, priority level Ⅲ consisted of referrals to colonoscopy that did not meet any of the previous conditions but were adequate according to EPAGE Ⅱ criteria. These indications and priority levels were evaluated in symptomatic patients after the implementation of the criteria. They were significantly associated with CRC (Ⅰ = 20.1%, Ⅱ = 19.1%, Ⅲ = 4.8%; $P < 0.001$) and significant colonic lesion (Ⅰ = 35.3%, Ⅱ = 34%, Ⅲ = 19%; $P = 0.002$) detection rates. Additionally, the diagnostic yield for CRC (OR = 2.41; 95%CI: 1.31-4.42) and detection of significant colonic lesions (OR = 1.88; 95%CI: 1.13-3.15) increased when colonoscopies were referred directly from primary care providers[68].

Several studies have been performed to develop predictive indexes for CRC detection in recent years. The aim was to establish objective criteria that are more accurate for CRC and to detect relevant findings, thus reducing the number of referrals to colonoscopy. Selvachandran *et al*[14] developed one of the first predictive systems: The Weighted Numerical Score (WNS). The WNS is derived from the weighting of primary symptoms and symptom complexes and is automatically derived from a patient consultation questionnaire linked to a computerized record[14]. In the validation study, the sensitivity of the WNS for CRC at a 40-point threshold reached 99%. In addition to having similar cancer detection rates as the TWW system, the specificity of the WNS cut-off of 70 was significantly better than that of the TWW system (82.7% *vs* 66.1%; $P < 0.001$)[78]. Thus, the WNS was subsequently validated, both internally and externally, showing similar detection rates with greater specificity. Unfortunately, it has only been validated for the detection of distal tumours and requires licensed software.

Adelstein *et al*[15] published a predictive model based on symptoms collected using a validated questionnaire, demographic variables and medical history. On the basis of a range of symptoms (anaemia, rectal bleeding, abdominal pain and mucus passage to the rectum), age, sex, colonoscopy in the past 10 years, use of nonsteroidal anti-inflammatory drugs or aspirin, and history of irritable bowel syndrome, they obtained a predictive model with an area under the curve (AUC) of 0.83 for CRC detection[15]. The Cancer Prediction in Exeter (CAPER) and the Bristol-Birmingham (BB) equation are two additional CRC scoring systems[70,71]. The CAPER score is derived from a primary care case-control study and the BB equation from a large primary care dataset. Their discrimination characteristics were investigated in two datasets (BB and CAPER dataset) and its diagnostic accuracy for CRC detection was compared with the TWW guideline. Both multivariable symptom scoring systems performed significantly better than NICE referral guidelines: AUC of the BB equation: 0.83 (95%CI: 0.82-0.84) and 0.92 (95%CI: 0.91-0.94), respectively; AUC of the CAPER score: 0.79 (95%CI: 0.79-0.80) and 0.91 (95%CI: 0.89-0.93), respectively; and AUC of the TWW rule: 0.65 (95%CI: 0.64-0.66) and 0.75 (95%CI: 0.72-0.79), respectively[70].

Therefore, prioritization criteria based on symptoms and signs seem to have poor diagnostic accuracy for CRC, while predictive indexes that add demographic variables and/or analytical data worked better. This highlights the need to develop more objective tools to reduce CRC delay due to waiting lists.

BIOMARKERS

Currently, there are several biomarkers available for the

evaluation of symptomatic patients. They include blood and faecal tests, such as serum and faecal haemoglobin (FOBT), serum carcinoembryonic antigen and faecal calprotectin.

Although serum haemoglobin is not a biomarker, its association with the risk of CRC detection and other colorectal diseases is clearly described. As shown previously (Table 1 and Figure 1), iron deficiency anaemia is highly specific for CRC detection (92%), although it lacks sensitivity[22-24]. Other available serum biomarkers, such as carcinoembryonic antigen (CEA), have been evaluated. However, lack of specificity and sensitivity preclude the use of all existing serum markers for the early detection of CRC. CEA determination is limited to surveillance after CRC resection with a curative intent[79].

Faecal calprotectin has recently emerged as a candidate biomarker for intestinal inflammation with a potential clinical application as a diagnostic adjunct in IBD and other pathologies of the gastrointestinal tract[55,80-82]. Calprotectin levels have been found to be significantly elevated in patients with inflammatory and neoplastic conditions[80]. Despite this, the meta-analysis performed by von Roon *et al*[80], which included 7 studies with 2661 patients to evaluate CRC detection, did not show significant differences among patients with CRC and controls. Patients with colorectal neoplasia had non-significantly higher calprotectin levels (132.2 μg/g higher) compared with non-cancer controls (P = 0.18). The sensitivity and specificity of calprotectin for the diagnosis of CRC were 36% and 71%, respectively, with an AUC of 0.66.

Multiple studies have demonstrated that CRC screening with chemical FOBT in average-risk populations significantly reduces CRC mortality[83]. To date, no data are available regarding the effect of FIT on CRC mortality or incidence. However, several studies on diagnostic tests have compared chemical FOBT and FIT for the detection of CRC and advanced adenomas. These studies have shown that FIT is more sensitive and specific for the detection of CRC and advanced adenomas and is a cost-effective screening test[84]. Current CRC screening programs are based mainly on FIT. In contrast, the information available on the evaluation of symptomatic patients is scarce. In the meta-analysis published by Jellema *et al*[24], FIT had a 95% sensitivity and a 84% specificity for CRC detection with a 21% PPV and a 100% NPV (Table 1). However, the studies included in this meta-analysis mixed asymptomatic and symptomatic patients and were performed in secondary care settings. However, the authors concluded that FIT showed good diagnostic performance for CRC.

Four additional studies have recently evaluated the diagnostic accuracy of FIT for CRC detection in symptomatic patients[54,85-87]. In these studies, FIT at different thresholds (10 ng/mL and 20 ng/mL) had an adequate diagnostic accuracy for CRC detection. The ranges of sensitivity, specificity, PPV and NPV were 74.7%-100%, 77.4%-93.9%, 7.6%-53% and 97.8%-100%, respectively. Moreover, in our recently published article, we compared FIT (20 ng/mL cut-off point) with the NICE criteria[7]. Among 787 patients referred for colonoscopy, we detected 97 cases of CRC. FIT had a higher sensitivity (87.6%, 61.9%; P < 0.001) and specificity (77.4%, 42.7%; P < 0.001) for CRC detection than the NICE criteria. Moreover, while the NICE referral criteria was modified according to the CRC location (rectum 76.7%, distal colon 61.4%, proximal colon 43.5%; P = 0.01), FIT sensitivity was not modified by its location (rectum 90%, distal 75%, proximal 87%; P = 0.2)[54]. Finally, McDonald *et al*[85] also evaluated the diagnostic accuracy of FIT for the detection of significant colonic lesions (CRC, advanced adenoma, IBD) in symptomatic patients. They also exhibited good results (sensitivity, 57%; specificity, 99%; PPV, 62% and NPV, 81.6%). These results are concordant with the results obtained in our series (not published). We found that the sensitivity and specificity of FIT for the detection of significant colonic lesions were 60.2% and 82.4%, respectively, and PPV and NPV were 60.2% and 82.4%, respectively.

In summary, biomarkers appear to be a promising tool for the prioritization of CRC in symptomatic patients. Currently, FIT has demonstrated its accuracy as a prioritization tool alone, and its use should be increased. In the coming years, we should see the emergence of new biomarkers.

CONCLUSION

In conclusion, the value of symptoms as predictors of CRC or relevant colonic findings is poor. In the complexity of the cancer diagnosis, delays can occur in the different phases from the appearance of symptoms until final diagnosis (patient-related, physician-related and hospital-related factors). Understanding the factors that produce the delay is the first step to improving the diagnostic process and reducing the time interval from the first symptoms until diagnosis, improving CRC prognosis. The appropriateness criteria for colonoscopy can be a basis to control the quality of referrals, identifying unnecessary tests, but its value as a diagnostic tool is limited, especially in symptomatic patients. Several prioritization criteria and predictive indexes have been developed. All of them have insufficient sensitivity for CRC detection, so CRC cannot be ruled out in those patients who do not meet these criteria. Moreover, these criteria and indexes are nonspecific and are based mainly on the subjective evaluation of symptoms, thus yielding unnecessary colonoscopies. Finally, the use of biomarkers in symptomatic patients is promising. Adding available biomarkers, especially FIT, to risk classification scores and predictive indexes may increase both the sensitivity and specificity of CRC detection, thus reducing the number of patients referred for colonoscopy to evaluate symptoms and increasing the diagnostic yield of colonoscopy in this setting.

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REVIEW

Multiple primary colorectal cancer: Individual or familial predisposition?

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Abstract

Colorectal carcinoma (CRC) is one of the most frequent cancers. Along the surface of the large bowel, several foci of CRC may appear simultaneously or over the time. The development of at least two different tumours has been defined as multiple primary CRC (MPCRC): When more than one tumour is diagnosed at the same time, it is known as synchronous CRC (SCRC), while when a second neoplasm is diagnosed some time after the resection and/or diagnosis of the first lesion, it is called metachronous CRC (MCRC). Multiple issues can promote the development of MPCRC, ranging from different personal factors, such as environmental exposure, to familial predisposition due to hereditary factors. However, most studies do not distinguish this dichotomy. High- and low-pentrance genetic variants are involved in MPCRC. An increased risk for MPCRC has been described in Lynch syndrome, familial adenomatous polyposis, and serrated polyposis. Non-syndromic familial CRCs should also be considered as risk factors for MPCRC. Environmental factors can promote damage to colon mucosae that enable the concurrence of MPCRC. Epigenetics are thought to play a major role in the carcinogenesis of sporadic MPCRC. The methylation state of the DNA depends on multiple environmental factors (*e.g.*, smoking and eating foods cooked at high temperatures), and this can contribute to increasing the MPCRC rate. Certain clinical features may also suggest individual predisposition for MPCRC. Different etiopathogenic factors are suspected to be involved in SCRC and MCRC, and different familial *vs* individual factors may be implicated. MCRC seems to follow a familial pattern, whereas individual factors are more important in SCRC. Further studies must be carried out to know the molecular basis of risks for MPCRC in order to modify, if necessary, its clinical management, especially from a preventive point of view.

Key words: Multiple primary colorectal cancer; Synchronous colorectal cancer; Metachronous colorectal cancer; Chromosomal instability; Microsatellite instabillity; CpG island methylator phenotype

Core tip: Multiple primary colorectal cancer (MCRC),

both Synchronous and Metachronous tumours, is not deeply studied yet, and also has a great clinical impact. Both genetic and environmental factors may affect in the development of MCRC, collaborating in promoting different foci of dysplasia. In general terms, Metachronous forms are mainly related to family factors whereas Synchronous tumours are linked with individual factors. With the exception of cases of hereditary forms of colorectal carcinoma (CRC), the others appears without a well-known molecular basis, and maybe different from sporadic colorectal cancer. For all these reasons, we present a review focused on the state of the art of these particular forms of CRC.

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INTRODUCTION

The colon is one of the localizations where carcinomas most frequently occur. The large bowel mucosa has a great extension. Thus, high- and low-penetrance genetic variants as well as environmental exposure all affect a large field, where several foci of colorectal cancer (CRC) may appear along the surface simultaneously or over time. The development of at least two different tumours has been defined as multiple primary CRC (MPCRC); when more than one tumour is diagnosed at the same time, this is known as synchronous CRC (SCRC), while when a second neoplasm is diagnosed some time after the resection and/or diagnosis of the first lesion, it is called metachronous CRC (MCRC). Initial studies did not distinguished between both concepts, and Moertel *et al*[1] were the first to describe in 1958 the currently most used criteria. MCRC was defined as a pathologically proven adenocarcinoma, separated from the line of anastomosis, different from recurrence, and diagnosed at a minimal interval of 6 mo after the initial CRC; CRCs diagnosed within 6 mo after the initial diagnosis were considered as SCRC[1].

MPCRCs make up 5%-10% of all CRCs. Estimations of the risk of developing MCRC vary widely in the literature, and range from 1.5% to 9%[2,3], depending on the time interval of the series. Recent series describe a risk of MCRC of 3.4%, 10 years after the first diagnosis[4]. On the other hand, large series of CRC estimate a prevalence of SCRC between 3.1% and 3.9%[5,6].

Multiple primary tumours usually arise on a common etiologic substrate, either genetic or environmental. Recurrence after endoscopic polypectomy is considered a risk factor for the development of multicentric CRC. Different adenoma features such as size, villous component, and number and location of polyps, may predict a high risk of metachronous lesions[7]. Nonetheless, recent findings in molecular colorectal carcinogenetics have provided evidence that chromosomal instability, microsatellite instability, and gene methylation are involved in various predisposing lesions or factors for SCRC and MCRC.

As mentioned before, multiple factors can promote the development of MPCRC ranging from different personal factors such as environmental exposure to familial predisposition due to inheritance. However, most studies do not distinguish this dichotomy. There are different entities that increase the risk of MPCRC. First, there are hereditary CRC syndromes (Lynch syndrome, Familial Adenomatous Polyposis), which present germline mutations and promote the development of several lesions overtime[8]. On the other hand, there are diseases and conditions that affect a large area of the colonic mucosa during specific periods of time and promote the formation of several foci of dysplasia, such as inflammatory bowel disease[9]. However, the origin of most of the cases of MPCRCs is still unclear; nowadays, well-defined factors only explain about 10% of SCRCs[8]. Perhaps the basis of most MPCRCs should be described as a situation in which one of the two main factors (genetic predisposition or environmental influence) predominates, or in which these factors are balanced.

There are two main points in which MPCRC stands out. Firstly, tumour multiplicity provides a good model to examine common molecular alterations and, more specifically, a potential field effect[10]. Secondly, and possibly more importantly, there is the possibility of prevention within this subset of CRC, *i.e.*, the existence of different prophylactic actions such as extensive surgery or chemopreventive treatment[11,12]. As is well known, the extension of surgical resection can be influenced by the presence, or at least the risk, of SCRC or MCRC. Moreover surveillance programs can be tailored if risk factors of MCRC are identified in order to reduce morbidity and even mortality[4,13].

Below we give an overview of the current of knowledge of both hereditary and environmental factors that influence SCRC and MCRC, and the importance of gaining more specific knowledge of these factors is adressed. In Table 1 publications are summarized that show the prevalence of and risk factors for MPCRC. In Table 2 publications are summarized that address the main molecular features analysed for MPCRC.

Familial predisposition

There is much evidence that some MPCCRs are linked with a hereditary pattern. Lynch syndrome (LS), also named hereditary nonpolyposis colon cancer (HNPCC), is the most common hereditary CRC syndrome. It has an autosomal dominant hereditary pattern, and it is defined by the presence of a germ-line mutation in one of the four DNA mismatch repair (*MMR*) genes (*MLH1*, *MSH2*, *MSH6* and *PMS2*)[14]. MPCRC tends to appear

Table 1 Different studies about the prevalence of multiple primary colorectal carcinomaand the main clinical risk factors

Ref.	Study design	Prevalence of MPCRC No. of cases (% of global)	Risk factors for MPCRC
[4]	10283 CRC patients. Study of MCRC *vs* solitary CRC	135 (1.3)	Previous SCRC. OR = 3.4, 95%CI (1.9-5.9) Less frequent in rectum. OR = 0.3, 95%CI (0.1-0.6) Not associated with the development of MCRC: Sex, age, TNM stage, or grade of differentiation of the initial CRC
[2]	1298 CRC patients. Study of MPCRC features	53 (4) MPCRC 33 (2.5) SCRC 20 (1.5) MCRC	Lynch > sporadic ($P < 0.001$) MCRC (5.8% *vs* 1.3%) SCRC (5.8% *vs* 2.4%)
[8]	1793 CRC patients. Study of SCRC features	102 (3.6) SCRC	Frequencies of predisposing disease in SCRC patients: 5% FAP (5) 6% SP (6) 2% UC (2)
[9]	1537 CRC patients 69 FAP 780 UC 685 *de novo* CRC		Prevalence of SCRC in special populations: 21% of CRC in FAP 18% of CRC in UC 2.5% of sporadic CRC
[37]	382 CRC patients Study of SCRC *vs* solitary CRC	28 (7.3%) SCRC 208 (54.5%) synchronous adenomas	Male gender: OR = 1.97, 95%CI (1.13-3.45) Age ⩾ 59 yr: OR = 2.57, 95%CI (1.54-4.29) History of adenomas: OR= 3.04, 95%CI: 1.04-8.85 Obstructive tumours: OR = 0.48, 95%CI: 0.27-0.85
[32]	15562 CRC cases. SCRC *vs* solitary CRC	596 (3.8%) SCRC	Male gender: OR = 1.41, 95%CI (1.19 -1.68) Adenomas present: OR = 2.02, 95%CI (1.69-2.41) Aged over 75: OR = 1.31, 95%CI (1.08 -1.59)
[12]	1522 CRC patients. Study of SCRC *vs* solitary CRC	27 (1.8%) SCRC	Male gender SCRC (70%); solitary CRC (56%), $P = 0.001$. Personal history of adenoma SCRC (4%); solitary CRC (1%), $P = 0.001$ Right sided tumour location SCRC (32%); solitary CRC (25%), $P = 0.003$
[57]	382 patients with CRC. Study of MCRC features	28 (7.3%)	Statistical differences: Older than 59 years OR = 2.57, 95%CI (1.54-4.29) History of adenoma OR = 3.04, 95%CI (1.04-8.85) Obstructive CRC OR = 0.48, 95%CI (0.27-0.85) Alcohol univariate analysis $P = 0.006$, no significance in multivariate analysis No statistical significance: Personal history of other tumours History of cancer in first-degree family members Revised Bethesda criteria (at least one criterion) BMI Predominant symptom Predominant localitation
[13]	18782 CRC cases MPCRC features	134 (0.71%) SCRC 300 (1.60%) MCRC	SCRC Men: OR = 1.45; 95%CI: 1.02-2.06 Age older than 65: OR = 1.50, 95%CI (1.02-2.21) Located in proximal colon: OR = 1.70, 95%CI (1.20-2.41) Risk of CRC of first-degree relatives of SCRC patients (OR= 1.86; 95%CI: 1.37-2.53) MCRC: (OR = 2.34; 95%CI: 1.62–3.36) Solitary CRC (OR = 1.75; 95%CI: 1.63-1.88)

CRC: Colorectal cancer; MCRC: Metachronous CRC; SCRC: Synchronous CRC; SSAs: Sessile serrated adenomas; UC: Ulcerative colitis; SP: Serrated polyposis; BMI: Body mass index; HR: Hazard ratio; OR: Odds ratio.

more frequently among patients with LS compared with patients with sporadic CRC.

Win *et al*[15] investigated the MCRC risk in a retrospective cohort of 79 patients with previous rectal adenocarcinoma and with germline *MMR* gene mutations. Twenty-seven per cent of MMR mutation carriers were diagnosed with MCRC. Cumulative risk of MCRC was 19% (95%CI: 9%-31%) at 10 years, 47% (95%CI: 31%-68%) at 20 years, and 69% (95%CI: 45%-89%) at 30 years after surgical resection. In spite of colonoscopy surveillance, 22% of MCRC cases were diagnosed at stage Ⅱ, and 6% at stage Ⅲ. Fante *et al*[2] investigated a subset of 1298 registered patients with CRC, and 53 patients (4.1%) were identified with MPCRC. The frequency in LS patients rose to 11.5% (5.8% SCRC, 5.8% MCRC), whereas the frequency in sporadic CRC was 3.6%, the most commonly reported frequency in most studies[4]. Differences were greater when only metachronous lesions were compared; these were four times more frequent in LS cases (5.8%

Table 2 Summary of studies about the prevalence of multiple primary colorectal carcinoma and the main molecular features of synchronous and metachronous colorectal carcinoma

Ref.	Study design	Prevalence of MPCRC (% of global)	Risk factors for MPCRC	Carcinogenetic pathways
[10]	Solitary (29) MPCRC (12) Study of MPCRC features		No differences: Age Gender Body mass index Tumour location History of CRC of MSI	CIMP-high 17.2% solitary *vs* 66.7% MPCRC *P* = 0.004
[36]	57 MPCRC Comparison of methylation status of solitary CRC *vs* MPCRC 57			Higher methylation for p14 MGMT in MPCRC *P* < 0.05 Correlations: MINT1 (r = 0.8) p16 (r = 0.8), MLH1 (r = 0.9) MGMT (r = 0.6) at the same site
[16]	4760 CRC patients Study of SCRC *vs* solitary CRC	58 (1.2%) SCRC: 42 (72%) sporadic 4 (7%) UC 8(14%)Lynch 1 (2%) FAP 3 (5%) SP	Older patients (*P* = 0.001) Right colon (*P* = 0.0003) Synchronous polyps (*P* = 0.0001) Classical adenoma 47% *vs* 12% SSAs 16% *vs* 0%	(MSI-H) 36% *vs* 12%; (*P* = 0.0005) 92% if SSA precursor
[17]	2884 patients SCRC *vs* solitary CRC	77 (2.7%) SCRC	21 (27%) had a family history of Lynch	54 (32%) MSI-H (> in women and elderly) congruence (MSS/MSI) Yes: 67 patients (87%) No: 10 patients (13%)
[30]	2884 CRC Study of MPCRC methylation state in SCRC *vs* MCRC	33 (1.1%) MCRC 77 (2.6%) SCRC	MSI-H MCRC were younger (64 *vs* 76 years, *P* =0.01)	MSI-H tumors in 12 (36%) MCRC 29 (38%) SCRCP Promoter methylation 50% MCRC 83% SCRCP *P* = 0.03
[35]	2,068 CRC patients SCRC *vs* solitary CRC	47 (2.3%) SCRC	Mean age 68.9 *vs* 65.5 (*P* =0.016) No difference: Family history of CRC BMI	MSI-high (*P* = 0.037). > BRAF (*P* = 0.0041) > CIMP-high (*P* = 0.013) Correlation pairs LINE-1 (r = 0.82; *P* = 0.0072) CpG islands (*P* < 0.0001)

CRC: Colorectal cancer; MCRC: Metachronous CRC; SCRC: Synchronous CRC; SSAs: Sessile serrated adenomas; UC: Ulcerative colitis; SP: Serrated polyposis; BMI: Body mass index; HR: Hazard ratio; OR: Odds ratio; MSI: Microsatellite instability; FAP: Familial adenomatous polyposis.

vs 1.3%; *P* < 0.001)[2]. Hu *et al*[16] reported similar findings: in a study that included 54 SCRC cases, more than 14% were in the context of LS. Moreover, 27.6% of patients with SCRC had first-degree relatives with different LS-related cancers[17].

Being affected by multiple primary neoplasms is a criterion for screening for LS according to the revised Bethesda guidelines[18]. MPCRC is a very important parameter in PREMM126, a computer model to estimate the overall cumulative probability of having a mismatch repair gene mutation. Males under 78 years old with MPCRC have at least a 5% cumulative risk of an MMR gene mutation, calculated by the PREMM126 model, independent of information regarding their relatives. Five per cent is the cut-off recommended for referral for genetic evaluation and/or for considering molecular testing of tumour samples for microsatellite instability (MSI) or immunohistochemistry[19]. As is well known, identifying LS is essential in order to intensify screening colonoscopy.

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder showing mutations in the *adenomatous polyposis coli* (*APC*) gene. It is characterized by the presence of hundreds to thousands of adenomas that can become malignant. Hu *et al*[16] reported that 2% of patients with SCRC suffer from FAP. However, the frequency of SCRC identified in that series seems to be greater than the current findings in global series of CRC, where FAP represents less than 1% of all CRCs. The common surgical management of FAP includes prophylactic resection of the entire colon. When a more conservative approach is taken (for example when the rectum is preserved, or in attenuated forms of FAP), strict surveillance should be done in order to avoid the development of MCRC[20].

Different studies have suggested an association of MPCRC with the serrated pathway, as a consequence of a field defect arising in the mucosa of patients with large

sessile serrated adenoma (SSA), resulting in increased risk for SCRC[16]. Serrated polyposis syndrome refers to a condition characterized by multiple, large and proximal hyperplastic polyps[21]. Although there are still many doubts about the significance and the pathogenesis of serrated polyposis, the main consensus criteria for the diagnosis of serrated polyposis include the possibility of a familial pattern. According to the World Health Organization[22], serrated polyposis is diagnosed if any of the following criteria is met: (1) at least five serrated polyps proximal to the sigmoid colon, two or more of which have a diameter of greater than 10 mm; (2) any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis; and (3) more than 20 serrated polyps of any size but distributed throughout the colon.

A study of 58 patients with SCRC identified 13 patients whose tumours were derived from SSA (SSA-associated SCRC): Three of them (23%) (SSA-associated SCRC) met criteria for serrated polyposis[16]. Moreover, a family history of CRC in patients with serrated polyposis syndrome has been reported in different studies, ranging from 33% to 59% of patients[21,23,24]. Nevertheless, no germline mutation associated with serrated polyposis syndrome has yet been identified. The main carcinogenetic pathway related with serrated adenocarcinoma is the DNA methylation pathway CpG Island Methylation Phenotype (CIMP). CIMP is often related with environmental exposure, and therefore some authors hypothesize about the possibility of an inherited abnormality of epigenetic regulation[25]. Such an abnormality would lead into the accumulation of somatic methylation events in tumour suppressor genes and would synergize with somatic oncogenic activation of *BRAF*, resulting in the development of premalignant serrated lesions. Other investigations found a weak association with mutations in *MUTYH* or *MBD4* genes, especially when adenomas and serrated polyps are simultaneously present[23]. The presence of conventional adenomas in serrated polyposis is also associated with an increased risk of CRC[26].

Excluding the high-penetrance inherited CRC syndromes, around 10% of CRC patients have a family history of the disease. These non-syndromic familial CRCs have been defined as "apparently sporadic forms of the disease that occur in families more often than expected by chance"[27,28]. Samadder *et al*[13] found an increased risk of CRC in first-degree relatives of patients with MPCRC, whereas a relative of a sporadic CRC patient has an increased risk of 1.75 (OR = 1.75, 95%CI: 1.63-1.88) compared with no affected relatives: first-degree relatives of SCRC patients had an about 1.9-fold increased risk of CRC (OR = 1.86, 95%CI: 1.37-2.53), and first-degree relatives of MCRC patients had a 2.5-fold increased risk (OR = 2.34, 95%CI: 1.62-3.36). The differences in risk between solitary CRC and MCRC were statistically significant (OR = 1.41, 95%CI: 1.05-1.91)[13].

When talking about familial predisposition in MPCRC, differences between SCRC and MCRC should be taken into account. Whereas MCRC can be facilitated by some inherited predisposition, with a continuous possibility during the entire lifespan of a person to develop a carcinoma, SCRC appears more likely to be the result of damage through some environmental factor during a specific period of time that enables the concurrence of two tumours at the same time. SCRC tends to be diagnosed at an advanced age compared with sporadic CRC, whereas MCRC tends to appear at an earlier age. This also supports the predominantly familial pattern of MCRC[13]. We have explained above how the risk of having an MCRC in LS is higher than that of having an SCRC[2]. The pathological features of MCRC also suggest a hereditary pattern. It is known that mucinous adenocarcinoma is a typical histologic feature of LS-associated CRC: In a large series of 102 patients with SCRC and 56 patients with MCRC, no differences in the incidence of mucinous carcinomas between SCRC and solitary CRC were found; nevertheless they are more common in metachronous forms[29].

Another factor supporting the hypothesis of the weaker relationship of SCRC with hereditary forms is the higher relation with sporadic MSI forms. Compared with solitary CRC, where about 10%-20% of patients show high MSI, the two types of MPCRC show more than 30% of MSI tumours. In some series, MSI was present in 36% of MCRC cases and in 38% of SCRC cases[30]. Up to 81% per cent of those SCRC cases lose MLH1 protein expression because of promoter hypermethylation; thus, the frequency in SCRC is twice as high as in MCRC (81% *vs* 41%). Both multiple forms associated with MLH1 promoter hypermethylation were more likely to be diagnosed at an older age and showed less frequently CRC in a first-degree relatives[30]. On the other hand, the high prevalence of MSI tumours also explains the most frequent distribution of MPCRC: both SCRC and MCRC used to be located in the proximal colon[31].

Individual predisposition

The screening guidelines for LS list the presence of SCRC as a risk factor. However, LS-associated SCRC accounts for a minority of all SCRC cases. Hu *et al*[16] described LS only in the 38% of SCRC cases. Thus, apart from the already known hereditary forms of CRC, there is an important proportion of MPCRC without a clear basis of inheritance. Some clinical features may suggest individual predisposition for MPCRC. The risk of sporadic CRC increases with age, and therefore an increased prevalence of multiple tumours would be expected in the elderly population. As mentioned before, SCRCs are identified in older patients compared with solitary CRC (median age 70 years *vs* 60 years; $P = 0.001$)[16], and other studies support these findings[5,13]. This association between SCRC and age can be explained by cumulative environmental damage,

because hereditary patterns usually lead to an early onset of the disease. Nevertheless, other studies did not find any differences regarding to the age at presentation[8,32].

As we described above, MSI is associated with SCRC. Although it is frequent in LS, it is not exclusive of it, and MSI is identified in about 10%-15% of sporadic CCR cases[33]. Several small tumour-based studies have found that MSI and abnormal methylation (CIMP-High and *BRAF* mutations) were more frequent in sporadic SCRC and MCRC[13]. Overall chromosomal instability, MSI, and CIMP are implicated in developing various predisposing lesions for MPCRC. In a molecular study of SCRC, Lam *et al*[8] found a 60% positive status for chromosomal instability; 10% was MSI and CIMP-0, whereas 30% was MSI and CIMP positive. Hu *et al*[16] also reported similar findings: They described a signinficantly increased rate of MSI, up to 36% (21/58) *vs* 12% (13/109) in solitary tumours (P = 0.0005), and they also found a difference of 20% in the prevalence of precursor SSA: 22% (13/58) in SCRCs *vs* 2% in solitary CRCs (2/109)) (P = 0.0001). Along these same lines, some authors point out that the pathway by which MPCRC shows MSI differs from the classical mutations of LS[34]. Epigenetics are thought to play a major role in the carcinogenesis of sporadic MPCRC. Between 31% and 62% of SCRC tumours have lost of MLH1 protein expression because of the hypermethylation of the promotor[16,30]. SSAs are the most common lesions of the methylation pathway, as Hu *et al*[16] showed that most (13/21, 62%) of the MSI SCRCs were associated with precursor SSA lesions and were apparently sporadic, with concurrent loss of MLH1 and PMS2 expression, and positive for the *BRAF* V600E mutation. They also found that 22% of SCRCs developed from an SSA, as opposed to only the 2% of solitary CRCs (P = 0.0001). In agreement with these findings, Gonzalo *et al*[10] found a close association between MPCRC and CIMP, identifying 102 CpG sites that showed significant hypermethylation in multiple tumours compared with solitary CRCs: 66.6% of MPCRCs were CIMP-high, whereas 5 of 29 (17.2%) solitary tumours showed CIMP (P = 0.004). Nosho *et al*[35] also described differences in methylation-related features in MPCRC: They found alterations in six of the eight CIMP methylation panel markers, with higher methylation levels of the long interspersed nuclear element 1 (LINE-1) and a higher frequency of *BRAF* mutations. Konishi *et al*[36] analysed the methylation status of a limited number of makers in 57 MPCRCs and 69 solitary CRCs, and found that the methylation status of *p14* and *MGMT* was significantly higher in multiple tumours; in addition, they described concordant methylation within tumour pairs at the same colonic site. Nosho *et al*[35] also found CIMP correlation between tumours at the same colonic site, and no correlation was found for pairs of tumours located in discordant locations. In summary, as the methylation state depends on multiple environmental factors (smoking, eating foods cooked at high temperatures), it may contribute to increasing the rate of MPCRC.

There are several studies that correlate environmental exposure with increased risk of MPCRC. Borda *et al*[37] studied possible risk factors for developing this entity, and they proposed alcohol intake as a risk factor for MCRC and SCRC. The relation between CRC and alcohol intake has been described before, even with moderate intake (about 20-40 g/d)[38]. Moreover, a cumulative alcohol intake of more than 9800 g, calculated as weekly average alcohol intake multiplied by years of drinking, has been described as a risk factor for SCRC[39]. Another study showed that risk of MCRC was not associated with gender, age at diagnosis, country of recruitment, cigarette smoking status, maximum dimension of primary tumour, and histological grade of the rectal cancer[15]. This study associated the risk of MCRC exclusively with the higher stage at diagnosis of the tumour (HR = 6.14, 95%CI: 1.21–13.14; P = 0.03) and a prior diagnosis of SCRC (HR = 11.54, 95%CI: 10.6-12.5; P = 0.04). Tobacco smoke contains a variety of genotoxic substances, including polycyclic aromatic hydrocarbons, nitrosamines, and heterocyclic and aromatic amines. Compounds in cigarette smoke activate the aromatic hydrocarbon receptor which can lead to DNA methylation[40]. Samowitz *et al*[41] described how heavy smoking could be related with CIMP and *BRAF* mutation, but no differences were found in MSI rate, although other authors did find an association[42].

SCRC is more frequently associated with adenomas than solitary CRC. Nowadays, precursor entities such as multiple serrated sessile polyposis are more commonly related with SCRC[43]. Tobacco can lead to an MPCRC because an increased number of polyps, either adenomas or serrated polyps, has been found in patients with direct exposure to tobacco[44]. However, a special pathogenic role has been described for serrated polyposis[21]. The tendency for these lesions to be multiple, when there is an association with smoking, and the frequency of *BRAF* mutation and CIMP, point to a defect that may result from interactions between environment with a low penetrance genetic predisposition[25].

As we have seen, tobacco and alcohol consumption have been adressed in several studies, and both are considered risk factors for CRC. When Borda *et al*[37] investigated both habits in relation with SCRC they found that both SCRC and tobacco were associated with male gender, but they did not find a statistically significant difference using multivariate analysis[37]. A study by Piñol *et al*[12] supports the observation that SCRC is independently associated with gender (P < 0.001). Another risk factor studied by Borda *et al*[37] was body mass index (BMI), and they found that a BMI of less than 21 was a protective factor for MPCRC.

Inflammatory bowel disease (IBD) can increase the risk of CRC. The prevalence of CRC in ulcerative colitis (UC) is 3.7%, increasing to 5.4% in those cases

with pancolitis[45]. Continuous inflammation induces molecular damage and causes a hypermethylation state of the colon mucosa that can promote carcinogenesis. Colonoscopy surveillance may be carried out in order to diagnose the precancerous state of dysplasia. Chromosomal instability and MSI pathways also have a major role in UC-associated CRC. However, there are differences in the instance and frequency of these alterations. *APC* loss of function is less frequent and usually occurs later in the UC-associated dysplasia-carcinoma sequence than in the classical adenoma-carcinoma sequence. By contrast in patients with UC, *p53* mutations occur earlier and are often detected even in mucosa without dysplasia or still indefinite for it. Issa et al studied the CpG methylation status of four genes (*ER*, *MYOD*, *p16* exon 1, and *CSPG2*) in patients with UC[46]. The methylation status was higher in high-grade dysplasia patients both in apparent normal mucosa and in high-grade dysplasia areas, suggesting that methylation precedes dysplasia; increased methylation is also widespread in the mucosa of high-risk patients. Several polymorphisms of genes related with the inflammatory response may increase this CpG methylation state[47]. SCRCs have been frequently found in the colons of patients who had total colectomy for low-grade dysplasia[9]. Therefore it is not coincidental that MPCRC often appears in the context of IBD: SCRCs are present in 18% of UC-related CRCs[9]. In a retrospective study of 64 patients with Crohn´s disease and CRC, Maser *et al*[48] found MCRC in 39% of patients 7 years after segmental of subtotal resection, which increased and at a rate of 0.5% per year after 8 to 10 years. Duration and extension of colitis are the two major features associated with increased risk of CRC[49]. From a review of the literature, Eaden *et al*[45] derived incidence rates of CRC in UC patients which corresponded to cumulative probabilities of 2% by 10 years, 8% by 20 years, and 18% by 30 years. These data suggest an accumulated methylation over time, provoking a field defect that reflects acquired predisposition to CRC and that might promote MPCRC as well[46].

Simultaneous individual and familial predisposition

An important proportion of MPCRC should be described as a whole, in which hereditary component cannot be distinguished from acquired alterations. Although SCRC and MCRC are different entities, they are linked. Patients with MCRC received a diagnosis of SCRC at the time of the initial CRC more often than patients with a solitary CRC (11.1% *vs* 3.1%; $P = 0.001$)[4]. Other studies described the increased risk of developing a MCRC after a SCRC with a hazard ratio 11.54 (HR = 11.54, 95 %CI: 10.6-12.5; $P = 0.04$)[15]. Synchronous adenomas represent a multifocal disease affecting the colonic mucosa. In spite of resection of all the synchronous adenomas, another lesion may appear from a damaged mucosa and develop into a MCRC[50]. The susceptibility to develop two primary neoplasms, simultaneously or consecutively, in the large bowel can be explained in different but not exclusive ways. Firstly, inheritance can mediate cumulative molecular defects that lead to dysplasia at different sites of the colon. On the other hand, one defined enviromental exposure may be implicated in the genesis of synchronous tumours, and continued exposure could result in another consecutive neoplasm. Nowadays, apart from hereditary forms such as LS or FAP, in most cases we do not know what the genetic inherited predisposition for MPCRC is. Whereas MCRC could be mediated by a constitutional factor that generates different polyps over time with a continuous possibility of developing a carcinoma, the carcinogenetic process begins at the same time in two different places of the mucosa for SCRC. This situation arises because of the exposure of a large area of mucosa to the same ethiology factors for a time sufficient to initiate carcinogenesis. Regardless of individual or hereditary causes, once the carcinogenic process has begun it can continue generating new lesions in the remaining colon. The risk for MCRC after diagnosis of the first CRC is higher than the prevalence of single CRC in a sex- and age-matched population, and the risk is higher in the first years following diagnosis, up to 61% in the first three years[4]. The risk is even higher when SCRC was previously identified, so that a close follow up must be carried out.

As we mentioned above, different etiopathogenic factors are suspected to be involved for synchronous and metachronous CRC, and different familial *vs* individual factors may be implicated (Figure 1). MCRC seems to follow a familial pattern, whereas individual factors are more important in SCRC[43].

Since SCRC and MCRC can appear in the same individual, in some occasions both familial and individual conditions concur. A study performed by Greenstein *et al*[9] reported that SCRC accounted for 2.5% of "*de novo*" CRC, and SCRC occurred in 18% of UC patients, and in 21% of patients with FAP[9]. Familial predisposition seems to be important in this development. The methylation state of LINE-1 appears concordant between SCRCs in the same individual, indistinctly of congruent or incongruent localization of the tumours[35]. Moreover, the methylation state of *LINE-1* was also high in normal mucosa of these cases[35]. These findings suggest a similar substrate of the mucosa in relation with environmental exposure, where probably heritable factors modify the phenotype, by mutation of *BRAF*, hypermethylation of promoters of *MMR* genes (*MLH1*), or chromosome instability. Both genetic and environmental factors may influence serrated neoplasia as well to develop into CRC, which in these cases acquires MSI status. A polymorphism in the *MLH1* promoter (-93 G>A) gives an increased risk for loss of *MLH1* function in serrated polyps. This polymorphism does not increased the number of serrated polyps, but appears to promote their malignancy[40]. Moreover, smoking promotes the development of MSI in serrated polyps[25].

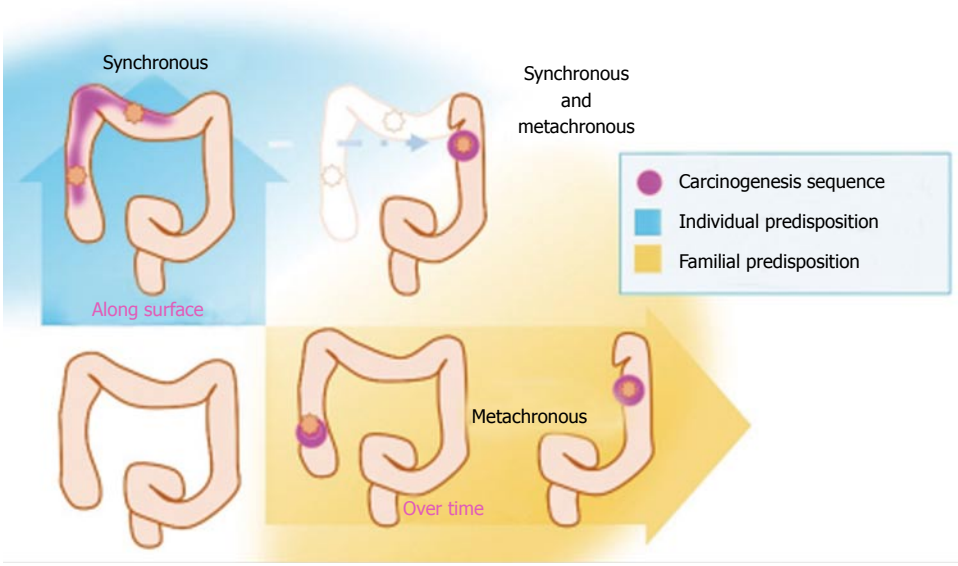

Figure 1 Both familial and individual factors may influence the genesis of multiple primary colorectal cancer. Genetic predisposition can promote the carcinogenesis sequence over time, whereas enviromental factors promote formation of different tumours along the colon surface at the same time.

HETEROGENEITY IN CRC

Finally, according to this feature of CRC, SCRC and MCRC may be defined as clinical entities and sometimes may not reflect the condition of the tumor at the molecular level. Genetic heterogeneity plays a fundamental role in this case. Sometimes MPCRCs show similar clinicopathological features, as shown by Huan *et al*[51], who explored a large cohort with 5346 patients with synchronous, metachronous and solitary CRC, finding similar clinicopathological features between SCRC and MCRC. Moreover, analysing primary CRCs and their metastatic sites[52], checking for mutations KRAS, NRAS, BRAF, PIK3CA, and TP53 genes in 615 patients found for most cases the same mutations between both link.

But in most cases, we have to take on account the proposed role of genetic heterogeneity in individual predisposition to CRC described by Galvan *et al*[53]. Either a single genetic defects or the polygenic conditions produce a cancer-prone condition in the human normal tissue; individual risk of cancer might be further modulated by environmental factors, leading to somatic mutations and ultimately to cancer. This is very striking when talking about the demethylation grade from normal mucosa of healthy people, single cancer patient and multiple CCR, emerging an increased rate of demethylation among normal mucosa of older patients, and in normal mucosa of patients with multiple CRC risk from younger patients, suggesting an inherited predisposition for the apparent field cancerization effect of somatic demethylation[54]. This influence of environmental conditions among hereditary predisposition is clearly shown by Rosty *et al*[55]. They found that the majority of CRCs arising in individuals with serrated polyposis (SP) do not harbor molecular hallmarks of serrated pathway CRCs but show a diverse range of molecular profiles. This suggests that CRC in SP patients may develop from non-serrated polyps through either a derivative of the traditional adenoma pathway. SP could therefore, potentially, be considered a disorder associated with a hypermature or inappropriately aged colonic mucosa, possibly secondary to an alteration in DNA methylation, with a resultant propensity to the development of early onset multiple serrated polyps and conventional adenomas.

Finally, another aspect thar supports the heterogeous condition of CRC is described by Zauber *et al*[56] who evaluated by a set of 6 different markers (LOH for APC, DCC, and mutations of KRAS, BRAF, MSI and methylation of *MMR* genes) 50 patients with SCRC and 5 MCRCs. They found that genetic changes may vary considerably, particularly when the tumors are found in different colon segments. Frequent differences in the molecular findings are also seen between SCRCs sharing the identical microenvironment of the same colon segment. These findings support the hypothesis that MPCRC may follow different pathways of carcinogenesis in the same patient.

CONCLUSION

MPCRC is a rare event but its prevalence is not negligible, and it has a great clinical impact. Both genetic and environmental factors may affect in the development of MCRC, collaborating in promoting different foci of dysplasia. If lesions progress at the same time, SCRCs arise, whereas MCRCs appear if they develop at different time. In general terms, MCRCs are mainly related to family factors whereas SCRC are linked with individual factors. Nevertheless, in some cases both entities arise in the same individual. Due to the heterogeneity of current studies, conclusive information on the molecular basis of MPCRC is scant, with the exception of cases of

hereditary forms of CRC. For this reason it is difficult to draw definitive conclusions at this time. New studies focusing on the carcinogenic mechanism must be done in order to better understand the molecular basis of MPCRC.

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REVIEW

Outcome following incomplete surgical cytoreduction combined with intraperitoneal chemotherapy for colorectal peritoneal metastases

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Abstract

Cytoreductive surgery combined with intraperitoneal chemotherapy can improve survival in appropriately selected patients with colorectal peritoneal metastases. Outcomes are best in those patients in whom a complete cytoreduction can be achieved. Unresectable disease is however encountered in approximately one-quarter of patients at laparotomy. The merits, or otherwise, of proceeding with an incomplete cytoreduction in this setting are unclear. We performed a review of published outcomes following incomplete cytoreduction for colorectal peritoneal metastases. Using the electronic databases, PubMed and MEDLINE, a systematic search of available literature published during the period January 1997 to September 2014 was conducted. Following application of exclusion criteria, 19 papers were identified and included in this review. These comprised fifteen case series, 3 case control studies and one randomised control trial. In the nineteen studies included in this review, 2790 patients underwent cytoreductive surgery with or without intraperitoneal chemotherapy for peritoneal metastases of colorectal origin. Of these, 1732 (62%) underwent a complete cytoreduction while 986 (35%) patients underwent an incomplete cytoreduction. Median survival in the complete cytoreduction group ranged from 11 to 62 mo while survival in the latter group ranged from 2.4 to 32 mo. Of the 986 patients with an incomplete cytoreduction, 331 patients received intraperitoneal chemotherapy and survival in this cohort ranged from 4.5 to 32 mo. An incomplete cytoreduction, with or without intraperitoneal chemotherapy, does not appear to confer a survival benefit. The limited available data points to a palliative benefit in a subset of patients. In the absence of high quality data, the decision as to whether or not to proceed with surgery should be made on an individual patient basis.

Key words: Colorectal carcinoma; Peritoneal metastases; Carcinomatosis; Incomplete cytoreduction; Intraperitoneal chemotherapy

Core tip: Cytoreductive surgery combined with intraperi-

toneal chemotherapy for colorectal peritoneal metastases improves survival in appropriately selected patients following complete cytoreduction. The merits of an incomplete cytoreduction, with or without intraperitoneal chemotherapy, are unclear. The available evidence is heterogenous and of poor quality. The current review has not shown a benefit to surgery in the setting of unresectable disease. Certain patients, particularly those with ascites may however gain from a quality of life point of view.

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INTRODUCTION

Peritoneal metastases (PM) are found in approximately 10% of individuals undergoing resection for colorectal cancer[1] and ultimately occur in up to 35% patients[2-4]. After hepatic metastases, the most common site of cancer recurrence after curative primary resection is the peritoneum[5,6]. Peritoneal metastases have traditionally been associated with a poor prognosis, with patients frequently referred for palliative care. In this setting, median survival in the order of five to seven months was typical[1,7,8]. Recent advances have allowed the introduction of new, more targeted approaches combining systemic chemotherapy with biological agents such as bevacizumab. However, best survival rates achieved with these combinations rarely exceed twenty months[9-11].

Over the past 20 years, a number of studies have shown a survival benefit following combined cytoreductive surgery (CRS) and intraperitoneal chemotherapy for patients with colorectal peritoneal metastases. The primary aim of cytoreductive surgery is to eliminate all macroscopic disease through peritonectomy procedures, and multi-visceral resections if necessary[12,13]. Cytoreductive surgery is combined with intraperitoneal chemotherapy with the aim of irradiating residual microscopic disease[14,15]. A 2003 randomized control trial by Verwaal *et al*[16], showed that patients treated with cytoreduction and heated intraperitoneal chemotherapy (HIPEC) had a median survival of 22.4 mo compared with 12.6 mo for those assigned to systemic chemotherapy alone. Favourable outcomes using this approach have since been demonstrated in a number of case series, including a 2010 multicentre study of 523 patients in which patients undergoing cytoreduction and HIPEC had a median survival of 30.1 mo[17]. The results of a recent meta-analysis by Mirnezami *et al*[18] further supported these encouraging outcomes, with patients undergoing CRS and HIPEC having superior two and five year survival rates when compared to those receiving systemic chemotherapy alone.

In patients undergoing surgery for colorectal peritoneal metastases, a correlation between the completeness of cytoreduction and survival has been shown in a number of studies and confirmed in a recent meta-analysis[18]. Verwaal *et al*[19] found that patients with a complete cytoreduction had a median survival of fifty-two months compared with an eight month median survival in patients with an incomplete cytoreduction. A complete cytoreduction is more likely to be possible and beneficial in the absence of biliary, ureteric, or multilevel bowel obstruction and in patients with lower volume disease [peritoneal carcinomatosis index (PCI) less than twenty][20].

Unfortunately, the pre-operative prediction of those patients in whom a complete cytoreduction will be achievable is difficult. It is well accepted that CT scanning and conventional imaging techniques have a poor sensitivity for identifying peritoneal metastases[21,22]. This can lead to underdiagnosis and understaging, with the result that unresectable disease is first discovered at laparotomy. In an attempt to overcome these limitations, many centres now utilise staging laparoscopy to pre-operatively assess operability and calculate the PCI. A 2012 cohort study by Iversen *et al*[23] found that while pre-operative laparoscopy reduced the rates of open and closed laparotomy, it understated peritoneal tumour in 56% of patients. Furthermore, in patients with metachronous disease, post-operative adhesions may reduce the reliability of the approach in accurately determining the extent and site of recurrent tumour[23]. Ultimately, the tumour burden, PCI, and resectability can only be reliably calculated at laparotomy[24].

While it is evident that a complete cytoreduction combined with intraperitoneal chemotherapy confers a survival benefit, it is not clear what impact, if any, an incomplete cytoreduction has on overall survival and quality of life. In this paper we aim to review the current literature to address the question of whether surgery should be abandoned if a complete cytoreduction cannot be achieved or, would the patient benefit in terms of symptomatic relief or prolongation of life, from an incomplete cytoreduction combined with intraperitoneal chemotherapy.

LITERATURE SEARCH STRATEGIES

A systematic search of available literature using the PubMed and MEDLINE databases provided by the National Library of Medicine was conducted. Search terms included the following keywords, or combinations thereof: "colorectal", "peritoneal", "metastases", "cytoreductive surgery", "intraperitoneal", "HIPEC" and "chemotherapy". The "related citations" function was utilised to broaden the search. Additional relevant publications were obtained by reviewing the reference

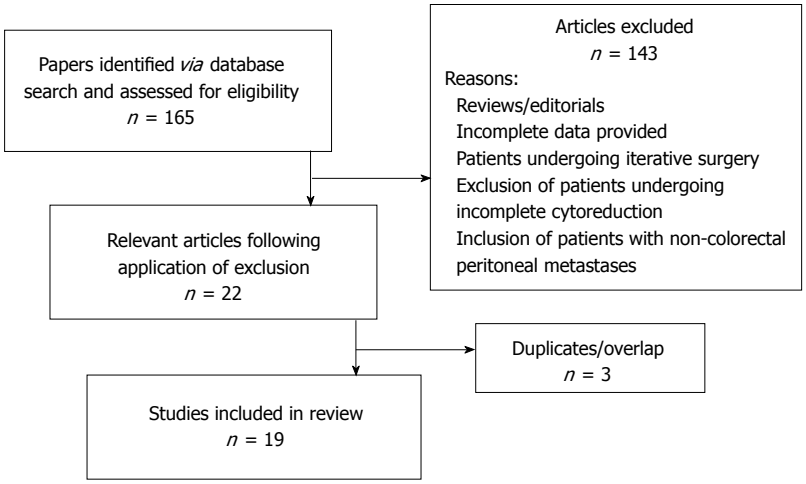

Figure 1 Study flow chart.

sections of all selected articles.

Inclusion/exclusion criteria

Only papers published in the English language during the period January 1997 to September 2014 were included. Only original research articles that studied peritoneal metastases of colorectal origin were included. Papers that omitted the breakdown of survival data according to the primary cancer and a completeness of cytoreduction score (CC-score) were excluded. Studies reporting iterative cytoreductive procedures were also eliminated.

The full text articles of 165 publications were obtained and their relevance assessed. 143 papers were excluded based on the aforementioned criteria. A total of 22 eligible publications were identified. Further review of these papers identified likely overlap between study centres and patient cohorts. Best efforts were made to eliminate any duplication however this was not possible with regard to one paper, in which one-quarter of patients were included in a separate retrospective multicentre study[6,17]. Following this further analysis, 19 papers were ultimately deemed appropriate for inclusion in the current study. This comprised 15 case series[6,17,25-37], 3 case control studies[38-40] and one randomised control trial (RCT)[16]. Figure 1 summarises the selection process.

Completeness of Cytoreduction Score

Eleven of the nineteen studies utilised the completeness of cytoreduction score (CC-Score) as described by Jacquet *et al*[41] in which a CC-0 score indicates that no macroscopic peritoneal tumour remains after cytoreduction, a CC-1 score indicates that persisting tumour nodules are < 2.5 mm, a CC-2 score indicates residual tumour nodules between 2.5 mm and 2.5 cm and finally a CC-3 score indicates tumour nodules > 2.5 cm or a confluence of unresected tumour. The remaining eight studies used four different scoring systems, which for the purpose of this review, were analysed and assigned the most appropriate CC-Score based on the size of the remaining tumour nodules[6,29,30,33,35-37,40]. Three of the papers utilised the R-score, where R0 indicates complete cytoreduction, R1 indicates the persistence of microscopic disease, and R2a, R2b and R2c indicate residual tumour nodules measuring < 5 mm, 5 mm-2 cm and > 2 cm respectively[16,29,37]. Five studies failed to identify specific tumour measurements. Two of these classified resections as: no evidence of macroscopic disease, persisting microscopic disease and persisting macroscopic disease and for comparison purposes, these were assigned scores of CC-0, CC-1 and CC-2/3 respectively[30,33]. The other three studies used the categories; macroscopically complete or macroscopically incomplete, and were assigned appropriate CC scores[35,36,40]. Complete cytoreduction refers to a CC-0 or 1 score whereas a CC-2 or 3 score is classified as incomplete.

LITERATURE SEARCH RESULTS

Patients

In the nineteen studies analysed, a total of 2790 patients underwent cytoreductive surgery and intraperitoneal chemotherapy for peritoneal metastases of colorectal origin during the period 1997 to 2014. Median age provided in seventeen of the studies ranged from 47-67 years. Of the 2790 patients, a CC-0/1 cytoreduction was achieved in 1732 (62%) while a CC-2/3 resection was achieved in 986 (35%). The 986 patients who had final resection scores of CC-2/3 form the basis of our review. The remaining 72 patients are not included in our analysis as a result of unknown or unassigned CC-scores and patients who were lost to follow up or not included in the original analyses.

Synchronous vs metachronous disease

Five studies included patients with synchronous peritoneal metastases only[27,30,35-37]. The study by Pestieau *et al*[26] included patients undergoing resection for both synchronous and metachronous disease, with all but one of the patients in the incomplete cytoreduction group having metachronous peritoneal metastases. The remaining studies included patients with both

synchronous and metachronous disease.

Use of neo-adjuvant chemotherapy

No data was provided regarding the delivery of neo-adjuvant therapy in eighteen of the included studies. The remaining study only included patients who had not received systemic treatment prior to surgery[31].

FACTORS ASSOCIATED WITH AN INCOMPLETE CYTOREDUCTION

Extent of disease

Twelve studies included patients with intra-abdominal disease only[6,16,17, 25,26,28,29,32,37-40] and five of these included patients with peritoneal metastases alone[16,28,32,38,40]. In the seven studies that included patients with non-peritoneal intra-abdominal metastases (hepatic metastases), only one paper included the resection of hepatic metastases in the overall CC-score[29]. In three of these seven studies[6,17,37], the CC-score referred to resection of peritoneal disease only and the remaining three studies did not specify whether the resection of hepatic metastases affected the final CC-score[25,26,39]. Cavaliere *et al*[25] found that the presence of extensive disease at the porta hepatis increased the likelihood of an incomplete cytoreduction. Four of the other studies included patients with extra-abdominal distant metastases (lung and supraclavicular nodes[27], lung only[31], and the other two studies did not specify the site of distant disease[30,36]). The remaining three studies did not specify whether patients also had non peritoneal distant metastases[33-35].

Only two of the studies provided information regarding the actual PCI, or equivalent, in patients in whom a complete cytoreduction was not possible[26,34]. The 55 patients in the study by Pestieau *et al*[26] in whom a complete cytoreduction was not possible, had a median PCI of 20.7 ± 7.6 compared to a PCI of 15.4 ± 7.6 in patients in whom a CC-0/1 resection was possible.

Chua *et al*[34] reported a series of three patients with colorectal peritoneal metastases who underwent incomplete cytoreduction. Two of the patients had a PCI of 11 while the third had a score of 39.The reasons for failure to clear all macroscopic disease in this study were extensive small bowel involvement, liver metastases, and extensive involvement of the lesser sac and diaphragm[34]. Similarly, Winer *et al*[32] found that extensive small bowel involvement or small bowel mesenteric deposits resulted in an aborted or incomplete resection in their 4 patients. Finally, Park *et al*[31] identified the presence of metastases covering a substantial amount of the peritoneal surface as the reason for failure of cytoreduction in the 5 patients in the incomplete cytoreduction group.

Tumour histology

One study specifically reported outcomes in patients with signet ring histology only[32]. Winer *et al*[32] found that tumours with signet ring histology responded poorly to CRS and HIPEC and their five CC-2/3 patients had a median survival of 2.4 mo. In the remaining studies no correlation between completeness of cytoreduction and tumour histological subtype or differentiation was reported.

TREATMENT OF PATIENTS WITH UNRESECTABLE PERITONEAL METASTASES

Extent of surgical resection

In the nineteen studies analysed, 986 patients underwent a CC-2/3 or incomplete resection. No study reported the specific resections that were undertaken in the incomplete cytoreduction group. Two studies reported that in the presence of distant metastases or extensive disease not amenable to a complete cytoreduction, a radical resection of peritoneal disease was not pursued and palliative surgery was performed[30,31]. This, in one study involved the removal of gross tumour deposits or disease that was likely to cause gastrointestinal obstruction, without the administration of intraperitoneal chemotherapy[30] and in the other entailed omentectomy with EPIC[31]. Four studies included patients with extra-abdominal distant metastases. In one study, 10/27 patients had extra-abdominal distant metastases, of whom 5 patients underwent resection of extra-abdominal disease[27]. In another study no patient had resection of their distant disease[30] and the other two studies did not document whether distant metastases were resected[31,36].

Systemic chemotherapy

In three studies[28,38,39], all patients received some form of systemic therapy (neoadjuvant, adjuvant or both). Three series did not document whether their patient cohort received chemotherapy or not and in the remaining thirteen studies the number of the CC-2/3 patients who received systemic therapy was not documented.

Intraperitoneal chemotherapy

In twelve studies[6,16,17,26,28,29,31,33,34,37,39,40] all patients received intraperitoneal chemotherapy (EPIC, IPHC or both) following incomplete cytoreduction (331). In one study the number of patients receiving intraperitoneal chemotherapy was not specified[27]. In the series reported by Huang *et al*[38], a comparison of intraperitoneal chemotherapy *vs* no intraperitoneal therapy following incomplete cytoreduction was performed. Five studies did not utilise this treatment modality. Mitomycin C was the most frequently used chemotherapeutic agent[16,26,27,31,34,37,38] followed by fluorouracil[6,28,40].

OUTCOMES

Survival

A breakdown of survival by completeness of cytro-

Table 1 Survival according to completeness of cytoreduction in patients undergoing surgery for colorectal peritoneal metastases

Ref.	Year	Study size, *n*	CC-0, *n*	Median survival (mo)	CC-1, *n*	Median survival, (mo)	CC-0/1, *n*	Median survival (mo)	CC-2/3, *n*	Median survival (mo)
Pestieau *et al*[26]	2000	104	-	-	-	-	44	24	55	12
Verwaal *et al*[16]	2003	49	18	-	21	20	-	-	10	5
Glehen *et al*[6]	2004	506	271	32.4	106	24	-	-	129	8.4
Carmignani *et al*[27]	2004	27	-	-	-	-	15	20.6	12	9
Mahteme *et al*[40]	2004	18	-	-	-	-	11	32	7	10
Füzün *et al*[28]	2006	29	8	62	18	21	26	37	3	7
Shen *et al*[29]	2008	77	13	NR	35	15.2	-	-	29	4.5
Varban *et al*[37]	2009	14	-	-	-	-	9	23	5	15.4
Chua *et al*[34]	2010	58	-	-	-	-			3	19
Elias *et al*[17]	2010	523	439	33	53	20	-	-	22	7
Cavaliere *et al*[25]	2011	146	124	25	11	11	-	-	11	8
Mulsow *et al*[30]	2011	125	-	-	-	-	31	25	94	8
Chua *et al*[39]	2011	110	72	46	27	35	-	-	11	32
Matsuda *et al*[35]	2012	153	-	-	-	-	31	42	122	10
Park *et al*[31]	2013	29	24	-	0	-	-	-	5	12
Winer *et al*[32]	2013	30	14	-	9	-	23	15.8	4	2.4
Huang *et al*[38] with HIPEC	2013	33	-	-	-	-	14	21.7	19	11
Huang *et al*[38] without HIPEC	2013	29	-	-	-	-	9	18.3	20	5
Kobayashi *et al*[36]	2014	564	-	-	-	-	160	30.5	404	12
Ceelen *et al*[33]	2014	166	79	49	66	22	-	-	21	12

NR: Not reached.

reduction is outlined in Table 1. All studies reported survival using the Kaplan Meier method. Patients in whom a CC-0 resection was achieved had a median survival of 25 to 62 mo, whereas following CC-1 median survival ranged from 11 to 35 mo. In studies where the CC-0 and CC-1 groups were analysed together, a median survival of 15.8 to 42 mo was reported. The 986 patients in whom a CC-2/3 cytoreduction was achieved had a median survival ranging from 2.4 to 32 mo. From the data reported, it is possible to identify only 63 patients in the CC-2/3 group who definitively had peritoneal metastases only and median survival in this cohort ranged from 2.4 to 11 mo. Further analysis of survival data for the CC-2/3 group is outlined in Table 2. Ten of the nineteen studies calculated 5 year survival rates. Two studies documented 5 year survival rates of 3%[30] and 4.7%[36] while the others reported no 5 year survival following incomplete cytoreduction.

Morbidity/mortality

The randomised control trial by Verwaal *et al*[16] was the only study to report perioperative mortality for the incomplete cytoreduction group. Seven out of ten patients in the CC-2/3 group died in comparison to 1/18 (5.5%) patients in the CC-0 group. Furthermore, 80% of the grade 4 toxicities and complications occurred in the CC-2/3 group. The treatment related mortality for the experimental arm (complete and incomplete cytoreduction) was 8%.

DISCUSSION

It is now broadly accepted that the treatment modality of complete cytoreductive surgery with intraperitoneal chemotherapy confers a survival benefit for appropriately selected patients with peritoneal metastases of colorectal origin. It is also accepted that a complete cytoreduction is associated with better outcomes than an incomplete resection. It is less clear however, whether an incomplete cytoreduction is of benefit with respect to survival or quality of life when compared to non-operative approaches. This question is particularly relevant in the setting of unresectable disease first encountered at laparotomy for planned CRS and HIPEC. Despite advances in staging, this situation arises in up to one quarter of patients[23,42,43] and poses a dilemma for the surgeon; should they proceed and remove resectable disease, combining it with HIPEC, or should the procedure be abandoned?

From the current literature review it is not possible to make firm conclusions as to the merits or otherwise of persisting with CRS and HIPEC when it is apparent that it will not be possible to resect all disease. This difficulty arises mainly due to the heterogeneity of patients and reported approaches, most of which has been taken from uncontrolled studies. In the only RCT in the series, the 10 patients in the incomplete group had a median survival of 5 mo compared with 12.6 mo for those undergoing systemic chemotherapy[16]. Perioperative mortality in the incomplete group was high however, impacting significantly on overall survival for this group. Similar results were obtained in the case control series by Mahteme *et al*[40] who reported median survival of 10 mo in those undergoing CRS *vs* 14 mo for those in the standard chemotherapy group. It is however noteworthy that sixteen of the nineteen studies in the

Table 2 Survival following incomplete cytoreduction

Ref.	Year	Study size, *n*	Control[1] median survival (mo)	CC-2/3, *n*	Median survival (mo)	1 yr	2 yr	3 yr	4 yr	5 yr
Pestieau *et al*[26]	2000	104	-	55	12	-	-	-	-	0.0%
Verwaal *et al*[16]	2003	49	12.6	10	5	-	-	-	-	-
Glehen *et al*[6]	2004	506	-	129	8.4	38.0%	-	6.0%	-	0.0%
Carmignani *et al*[27]	2004	27	-	12	9	-	-	-	-	-
Mahteme *et al*[40]	2004	18	14	7	10	-	-	-	-	-
Füzün *et al*[28]	2006	29	-	3	7	-	-	-	-	-
Shen *et al*[29]	2008	77	-	29	4.5	-	-	6.0%	-	0.0%
Varban *et al*[37]	2009	14	-	5	15.4	-	40.0%	-	20.8%	-
Chua *et al*[34]	2010	58	-	3	19	-	-	-	-	0.0%
Elias *et al*[17]	2010	523	-	22	7	-	-	8.5%	-	0.0%
Cavaliere *et al*[25]	2011	146	-	11	8	-	0.0%	-	-	0.0%
Mulsow *et al*[30]	2011	125	-	94	8	39.0%	17.0%	-	-	3.0%
Chua *et al*[39]	2011	110	-	11	32	-	-	-	-	-
Matsuda *et al*[35]	2012	153	-	122	10	-	-	-	-	0.0%
Park *et al*[31]	2013	29	-	5	12	-	-	0.0%	-	0.0%
Winer *et al*[32]	2013	30	-	4	2.4	-	-	-	-	-
Huang *et al*[38] with HIPEC	2013	33	-	19	11	-	-	-	-	-
Huang *et al*[38] without HIPEC	2013	29	-	20	5	-	-	-	-	-
Kobayashi *et al*[36]	2014	564	-	404	12	-	-	-	-	4.7%
Ceelen *et al*[33]	2014	166	-	21	12	-	-	-	-	-

[1]Control: No surgery group/patients treated with systemic chemotherapy alone.

current review reported median survival of 12 mo or less following incomplete cytoreduction. While most of the studies were non-comparative, it is clear that these outcomes are no better than historical controls, or those reported with best systemic treatment. These outcomes suggest no survival advantage to an incomplete cytoreduction. There was however great heterogeneity of patients (many of whom had non-peritoneal distant disease), the extent of surgery, use of HIPEC, and the delivery of systemic treatment. From the literature it is noted that peritoneal metastases of appendiceal adenocarcinoma origin tend to have a better overall survival when compared to true colorectal peritoneal metastases[13,44].

The value of intraperitoneal chemotherapy after CC-2/3 is also unknown. For the twelve studies in the current review in which all patients received intraperitoneal chemotherapy, median survival for the patients with incomplete cytoreduction ranged from 4.1 to 32 mo and, for the five without intraperitoneal chemotherapy, from 2.4 to 15 mo[25,30,32,35,36] (Table 3). In a case-control study, Huang *et al*[38] compared outcomes in CRS/HIPEC with CRS alone. Those in the HIPEC group had a median survival of 11 mo *vs* 5 mo in those who underwent CRS alone. It is important to note that the cut off point, with respect to the size of residual tumour, for use of intraperitoneal chemotherapy varied across the studies in this review, varying from > 1 mm[17] to > 5 mm[6].

While its impact on survival remains debatable, an incomplete cytoreduction with HIPEC may provide symptom relief. Malignant ascites results in abdominal distension and dyspnoea and symptomatic relief with paracentesis is transient at best as failure to treat the cause of the ascites results in rapid reaccumulation of the fluid[45]. Chua *et al*[34] specifically assessed a small number of patients who underwent CC-2/3 resection for colorectal PM. Two out of the three patients reported resolution of their symptoms (abdominal pain, anorexia, distension) postoperatively. Randle *et al*[46] found that partial cytoreduction and HIPEC was successful in treating malignant ascites (no radiological evidence of ascites three months post-operatively) in 84% (243/288) of patients with peritoneal metastases from a variety of primary tumours. This suggests a potential role for intraperitoneal chemotherapy in controlling ascites and improving symptoms. Garofalo *et al*[47] found that laparoscopic HIPEC successfully treated debilitating ascites in 3 patients with PM of colorectal origin. Valle *et al*[48] showed a benefit (at least in the short term) to laparoscopic HIPEC with mitomycin C in patients with PM of colorectal origin. At one month post-operatively, forty-nine out of fifty-two patients (94%) were free of ascites. Eleven of these patients had PM of colorectal origin. These findings suggest a potential benefit, in the presence of unresectable disease at laparotomy, from intraperitoneal chemotherapy. Patients may also benefit, albeit temporarily, from formation of an ostomy, intestinal bypass or adhesiolysis[49].

Factors associated with unresectability have been documented in the literature. The two main causative factors are 5-7 abdominal regions affected by PM and extensive small bowel or mesenteric involvement[50,51]. Only three of the studies in the current review documented the reasons for unresectability. These included extensive small bowel involvement, hepatic metastases

Table 3 Survival following incomplete cytoreduction, with or without intraperitoneal chemotherapy

Ref.	CC-2/3, *n*	Median survival (mo)
With intraperitoneal chemotherapy		
Shen *et al*[29]	29	4.5
Verwaal *et al*[16]	10	5
Elias *et al*[17]	22	7
Füzün *et al*[28]	3	7
Glehen *et al*[6]	129	8.4
Carmignani *et al*[27]	12	9
Mahteme *et al*[40]	7	10
Huang *et al*[38] with HIPEC	19	11
Park *et al*[31]	5	12
Ceelen *et al*[33]	21	12
Pestieau *et al*[26]	55	12
Varban *et al*[37]	5	15.4
Chua *et al*[34]	3	19
Chua *et al*[39]	11	32
Without intraperitoneal chemotherapy		
Winer *et al*[32]	4	2.4
Huang *et al*[38] without HIPEC	20	5
Cavaliere *et al*[25]	11	8
Mulsow *et al*[30]	94	8
Matsuda *et al*[35]	122	10
Kobayashi *et al*[36]	404	12

An unspecified number of patients received HIPEC.

and extensive disease involving the diaphragm and lesser sac[31,32,34]. Similar causative factors were identified in a series reported by van Oudheusden and colleagues[43], however, the main factor was found to be a high PCI with 50% (41/82) of patients undergoing an open and closed laparotomy upon the discovery of a PCI exceeding 20. The literature suggests that a complete cytoreduction confers little survival benefit in patients with a PCI > 17[52] and the association between a high PCI and poor outcomes is documented in eight of the studies[6,16,17,25-28,38] in this review. However, while there are reports showing favourable outcomes in a subset of these patients[53,54], selecting which patients with a high PCI to operate on remains a challenge.

It appears that tumour biology can impact on resectability and outcomes in peritoneal carcinomatosis. Winer *et al*[32] reported outcomes in patients with poorly differentiated tumours. They found that PM was a common finding in patients with signet ring cell subtype in the primary tumour and accounted for 14% of the cytoreductive surgeries performed in their institute. Tumours with signet ring histology are more likely to have metastasised at initial presentation and have an extremely poor prognosis despite advances in systemic chemotherapy[55,56] .The median survival for patients with an incomplete cytoreduction in Winer's series was 2.4 mo. Winer *et al*[32] and Ceelen *et al*[33] concluded that aggressive histological subtypes, including signet ring, are particularly resistant to cytoreductive surgery and this finding is also reported by Chua *et al*[57]. PM with signet ring histology is also associated with an increased risk of death in those undergoing open and closed laparotomy[43] as well as an increased risk of recurrence[58]. These results suggest that patients with signet ring subtype and peritoneal metastases should be approached with caution, particularly when there is a question regarding resectability.

From the published literature it would appear that there is a survival benefit to the delivery of adjuvant systemic treatment following incomplete cytoreduction. Chua *et al*[39] compared outcomes of patients with peritoneal metastases who received systemic chemotherapy *vs* patients who underwent an incomplete cytoreduction, intraperitoneal chemotherapy and systemic chemotherapy. Those in the systemic chemotherapy group alone had median survivals of 11 to 23 mo (depending on the chemotherapy regimen delivered - standard, modern and modern chemotherapy with biological agents) while the median survival of patients who underwent an incomplete cytoreduction was 32 mo. Overall, they found that the administration of a modern chemotherapy regimen improved survival in patients who underwent an incomplete CRS/HIPEC when compared with standard chemotherapy and that an incomplete cytoreduction conferred a survival benefit over systemic chemotherapy alone. Similarly, Glehen *et al*[6] found that adjuvant chemotherapy (irinotecan or oxaliplatin) following incomplete cytoreduction significantly improved survival, when compared with no adjuvant chemotherapy.

Klaver *et al*[59] and Hompes *et al*[50] specifically assessed the impact of palliative chemotherapy (without cytoreductive surgery or HIPEC) on survival in patients with colorectal PM. In a population based study, Klaver *et al*[59] found that patients who received systemic chemotherapy survived for up to 66 wk *vs* 11 wk for those who didn't. They also found that peritoneal metastases are somewhat resistant to fluorouracil monotherapy but may be sensitive to modern, multiagent chemotherapy regimens[59]. Hompes *et al*[50] reported a series of 43 patients with unresectable PM in whom no resection was performed at laparotomy. The overall median survival for these patients was 6.3 mo with those who received palliative chemotherapy having a slightly improved survival of 9.3 mo *vs* 3.1 for those without.

Verwaal *et al*[16] showed particularly adverse perioperative outcomes associated with an incomplete cytoreduction. More recent studies do not provide sufficient data to support or refute this finding. It is however clear that the morbidity associated with CRS and HIPEC has improved significantly and is now comparable with other major gastrointestinal procedures[60]. This factor must be taken into account if a decision is to be made to proceed on an individual patient basis, even when a complete cytoreduction will not be possible.

CONCLUSION

It is generally accepted that the preoperative diagnosis of unresectable peritoneal carcinomatosis precludes an

attempt at cytoreductive surgery and patients should not routinely be exposed to an unnecessary laparotomy. In the setting of unresectable peritoneal disease discovered at laparotomy, there is currently no evidence that an incomplete cytoreduction, with or without HIPEC, will improve survival. However, the available data is of poor quality and the decision to proceed must be made on an individual patient basis, taking into account the site and extent of disease, tumour biology and any palliative benefit that may result, and balancing this with the risk of morbidity. Certain patients, particularly those with ascites may receive a quality of life benefit following incomplete cytoreduction and intraperitoneal chemotherapy.

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REVIEW

Helicobacter pylori in gastric carcinogenesis

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Abstract

Gastric cancer still is a major concern as the third most common cancer worldwide, despite declining rates of incidence in many Western countries. *Helicobacter pylori* (*H. pylori*) is the major cause of gastric carcinogenesis, and its infection insults gastric mucosa leading to the occurrence of atrophic gastritis which progress to intestinal metaplasia, dysplasia, early gastric cancer, and advanced gastric cancer consequently. This review focuses on multiple factors including microbial virulence factors, host genetic factors, and environmental factors, which can heighten the chance of occurrence of gastric adenocarcinoma due to *H. pylori* infection. Bacterial virulence factors are key components in controlling the immune response associated with the induction of carcinogenesis, and *cag*A and *vac*A are the most well-known pathogenic factors. Host genetic polymorphisms contribute to regulating the inflammatory response to *H. pylori* and will become increasingly important with advancing techniques. Environmental factors such as high salt and smoking may also play a role in gastric carcinogenesis. It is important to understand the virulence factors, host genetic factors, and environmental factors interacting in the multistep process of gastric carcinogenesis. To conclude, prevention *via H. pylori* eradication and controlling environmental factors such as diet, smoking, and alcohol is an important strategy to avoid *H. pylori*-associated gastric carcinogenesis.

Key words: *Helicobacter pylori*; Gastric cancer

Core tip: *Helicobacter pylori* (*H. pylori*) is an important etiologic agent in gastric carcinogenesis. Here, we summarize not only recently investigated mechanisms of virulence factors, host genetic factors, and environmental factors, but also potential prevention. The best preventive methods in *H. pylori*-induced carcinogenesis may be achieved through *H. pylori* eradication, dietary, or lifestyle modifications, as well as a better understanding of molecular pathogenesis.

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INTRODUCTION

Gastric cancer remains the third leading cause of cancer death worldwide[1,2]. Although the incidence rates in the United States and many Western countries have declined significantly, the prevalence remains high in Eastern Europe, Central, and South America, and especially in East Asia, where up to 24.18 cases of gastric cancer per 100000 adults were estimated in 2012[3].

Because nearly 40% of patients never report tumor-related symptoms before diagnosis, most gastric cancer cases are advanced-type upon initial presentation, for which prognosis remains poor[4]. Thus, prevention may be the most promising strategy for cancer control.

Despite the fact that the molecular pathways of gastric carcinogenesis remain unclear[5], there are numerous factors that have been associated with gastric carcinogenesis, such as genetic background[6,7], behavioral factors (*e.g*., alcohol, smoking, diet)[8,9], and *Helicobacter pylori* (*H. pylori*). Most importantly, *H. pylori* is the most crucial etiologic agent for gastric adenocarcinoma[10,11], which is involved in 90% of all gastric malignancies[12].

Here, we review the recently investigated mechanisms of *H. pylori*-induced gastric carcinogenesis, focusing not only on epidemiological factors, bacterial virulence factors, host factors, or other environmental factors, but also on preventive management and future directions.

H. pylori as a major risk factor for gastric cancer

H. pylori is a gram-negative microaerophilic bacterium that infects nearly 50% of the world's population. It has been found in every population premeditated, although the incidence varies with age, childhood socio-economic status, education level, living environment, occupation, and geographic regions, in that the incidence is higher in developing countries and much of East Asia[13-15].

In 1994, *H. pylori* was categorized as a class I (definite) carcinogen by the International Agency for Research on Cancer (IARC), a division of the World Health Organization (WHO)[15,16]. Subsequently, it is believed that *H. pylori* is the major risk factor of gastric cancer based on animal studies[17,18], as well as clinical observational and human interventional studies[10,19-21].

The clinical manifestations of *H. pylori* infection are as follows: (1) chronic gastritis, which almost all patients develop and most remain asymptomatic; (2) duodenal ulcer (DU) phenotype, which occurs in 10%-15% of infected individuals; (3) gastric ulcer/adenocarcinoma phenotype, which develops into gastric cancer in 1%-3% of infected individuals; and (4) gastric mucosa–associated lymphoid tissue lymphoma (MALToma), which develops in 0.1% of infected subjects[12,15,22,23]. The DU phenotype with antral colonization is associated with high gastrin and high output of gastric acid, and also related to a lowered risk for gastric cancer occurrence[15,20,24]. However, the gastric adenocarcinoma phenotype, which occurs more frequently when there is proximal colonization of the stomach (pangastritis), brings about damage to gastric glands, causing atrophic gastritis and associated hydrochlorhydria or achlorhydria, and it is characterized by low pepsinogen I and high gastrin levels and a low pepsinogen I/II ratio. This phenotype eventually progresses to a multistep process including intestinal metaplasia, dysplasia, and adenocarcinoma[20,23-25]. This series of histological changes may take as long as 7 or 8 decades[26] and is a well-known characteristic of intestinal-type adenocarcinoma, which is one of two distinct histological variants. It is also believed that *H. pylori* is associated with diffuse-type adenocarcinoma[11], which shows the paucity of glandular structure and comprises poorly cohesive cells that infiltrate the gastric wall[15]. However, pathological sequences of the diffuse-type are less characterized[26].

Gastric adenocarcinoma is also categorized into proximal tumors (esophagogastric junction and gastric cardia) and distal tumors (gastric antrum, body, and fundus)[15]. Proximal gastric cancers have different epidemiological and pathophysiological characteristics compared with distal cancer, and many studies support that this type of cancer is inversely associated with *H. pylori* infection[21,27,28] despite some debates[29,30]. Although the incidence of cancer of the proximal stomach has been increasing, the majority of gastric cancers worldwide arise from the distal stomach, and the significance of *H. pylori* in gastric carcinogenesis remains overwhelming.

PATHOGENESIS OF *H. PYLORI* IN GASTRIC CARCINOGENESIS

As mentioned above, *H. pylori*-induced gastric carcinogenesis in humans rarely occurs among infected individuals. Many studies over the past three decades suggest that the combination of a bacterial virulent strain, a genetically susceptible host, and a predisposed gastric environment may be required for cancer to develop.

Bacterial virulence factors

H. pylori yields various virulence factors that may dysregulate host intracellular signaling pathways and decrease the threshold for neoplastic transformation. Of all virulence factors, *cag*A (cytotoxin-associated gene A) and its pathogenicity island (*cag* PAI) and *vac*A (vacuolating cytotoxin A) are the major pathogenic factors.

***Cag* PAI and *cag*A:** The most well-featured *H. pylori* virulence factor is the *cag* PAI, which is about 40 kb and contains 27-31 genes. The terminal gene of this island, *cag*A, is a highly immunogenic protein often used as a indicator for the entire *cag* PAI locus[26]. It is believed that *cag*A-positive (*i.e.*, *cag* PAI-positive) strains are

linked to more harsh inflammation, higher steps of atrophy, and a larger possibility of advancement to adenocarcinoma of stomach compared with *cag*A-negative (*i.e., cag* PAI-negative) strains[15,31-34]. The estimated relative risk (RR) ranges from 2 to as high as 28.4[23]. However, the same clinical diseases of these are also originated by infections with *cag*A-negative strains, compatible with the assumption that any other bacterial or host factor may contribute to increased risk of a significant clinical outcome[35].

The prevalence of *cag*A differs widely according to region. It varies dramatically, with the prevalence reaching almost 100% in East Asia, and less than 50% in some countries in the West[36]. It has been observed that people with *cag*A-positive strains of *H. pylori* are more susceptible to peptic ulcer disease or gastric adenocarcinoma than are those with *cag*A-negative strains in Western countries[37,38]. In East Asia, most *H. pylori* strains possess the *cag*A gene without regard to the disease; therefore, the pathogenic difference in East Asia is hard to explain concerning the existence of the *cag*A gene alone[39]. Thus, the combined circumstances that permit *cag*A to initiate carcinogenesis remain unclear.

The *cag* PAI encodes a type Ⅳ secretion system (T4SS; *i.e.*, a molecular motors) that injects at least 18 proteins including *cag*A into host cells[14,40].

***Cag*A and glutamate-proline-isoleucine-tyrosine-alanine motifs:** The *H. pylori cag*A protein is a 120- to 140-kDa protein translocated into host cells by the T4SS after bacterial attachment. When the cagA enters the host cell, it can bind to the cell membrane inner surface and undergo tyrosine phosphorylation. This in turn results in morphological changes of the cell, and influences various intracellular signal transduction pathways. In addition, *cag*A exerts pathogenic effects without phosphorylation. Both the phosphorylation-dependent and -independent *cag*A signals interact with many host proteins to trigger downstream pathways, such as the ras/mitogen-activated protein kinase/extracellular signal-regulated kinase pathway[41,42], nuclear factor κB (NF-κB) pathway and B-catenin pathway[43].

Glutamate-proline-isoleucine-tyrosine-alanine (EPIYA) motifs are the sites of *cag*A phosphorylation. According to variations in the encompassing amino acid sequence, four distinct EPIYA-motifs are reported (-A, -B, -C, -D)[44,45]. The first repeat region comprises EPIYA-A/EPIYA-B segments and is present in strains throughout the world. However, the prevalence of the second repeat region varies by geographic area. The respective names of the second repeat region segments of the Western and East Asian strains are EPIYA-C and EPIYA-D[35,46]. In Western strains, an increased number of *cag*A EPIYA-C sites is an significant barometer of the risk of progressing to gastric adenocarcinoma[47]. East Asian strains are nearly the only strains to carry the EPIYA-D motif. These are strains from South Korea, Japan, and China. Many studies have concluded that when infections that occur in the same area are compared, infection with EPIYA-D strains have a higher risk of gastric cancer or peptic ulcer compared to infection with EPIYA-C strains[35,48-50]. However, the role of *cag*A remains unclear. In some reports, *cag*A can micro-evolve within an individual[51] or the EPIYA-B motif may be polymorphic[52]. Thus, further studies are required to explore the association between *cag*A EPIYA motifs and gastric carcinogenesis.

***Vac*A:** All strains of *H. pylori* possess and more than half express the *vac*A gene, which encodes a pore-forming protein that binds to epithelium *via* interaction with protein-tyrosine phosphatases[53]. *vac*A protein is a very potent inhibitor of T cell activation *in vitro*[54], and it has multiple activities, such as pore-formation in membranes, cytochrome C release from mitochondria progressing to apoptosis, and attaching to cell membrane receptors resulting in pro-inflammatory responses[36].

There are many studies showing that differences in *vacA* gene structure are associated with severities of clinical disease. According to variations in *in vitro* vacuolating capacity, studies have reported differences in the signal region (s1 and s2), the middle regions (m1 and m2), and recently the intermediate regions (i1 and i2)[47,55,56]. Investigators have suggested that when compared to s2 or m2 strains, individuals who have been infected with *vac*A s1 or m1 strains may have a heightened risk of gastric cancer and/or peptic ulcers in Africa, Latin America, and the Middle East[57,58]. More recently, i1 strains have been suggested to be correlated not only with inflammatory and dysplastic, but also malignant neoplastic tissue formation in Portugal, Belgium, and Iran[59-61]. However, unlike the above reports, in a reports of subjects from East and Southeast Asia, there was no correlation between the i-region and clinical disease[36]. In a recent long-term study (mean 12.8-year follow-up) based on Spanish populations, there was no correlation between the i-region and clinical outcome either[62]. In addition, there are relatively few studies that have controlled for variables associated with inflammation severity, such as the presence of *cag*A. In summary, despite numerous reports that the *vac*A s1/i1 genotypes are highly pathogenic, no clear association has been observed yet.

Host genetic factors

There is increasing evidence that the nature of the inflammatory response to *H. pylori* is in large part determined by polymorphisms in several host genes encoding cytokines and cytokine receptors.

***IL-1* gene cluster polymorphism:** El-Omar *et al*[63] first reported that pro-inflammatory *IL-1* gene cluster polymorphisms (*IL-1B* gene encoding cytokine *IL-1β* and *IL-IRN* gene encoding its naturally occurring receptor antagonist, IL-1RA) were clearly related

to an intense inflammatory response resulting in hypochlorhydria and high risk of cancer. Subjects with the IL-1B-31*C or -511*T and IL-1RN*2/*2 genotypes have a higher risk of gastric atrophy, gastric cancer, or hypochlorhydria as a result of *H.pylori* infection[23,63,64]. The heightened risk of cancer development with these genotypes was 2- to 3-fold compared with non-inflammatory genotypes[63-65]. These findings have been confirmed in other groups such as Caucasian, Hispanic, and Asian populations[65-71].

In addition, Figueiredo *et al*[66] analyzed the mutual effects of bacterial virulence factors of *H. pylori* (*cag*A-positive, *vac*A s1, and *vac*A m1) and proinflammatory IL-1 genotypes. They showed that pro-inflammatory polymorphisms of IL-1, together with carriage of *H. pylori* with the *vac*A s1 form, heightened the possibility of developing gastric cancer 87-fold compared with individuals who had neither of these risk factors yet were still colonized by *H. pylori*[66]. Crucial evidence, provided by a transgenic study, has confirmed the exclusive role of IL-1β in *H. pylori*-associated gastric carcinogenesis[72]. According to this study, in transgenic mice, human IL-1β stomach-specific expression resulted in gastric cancer and spontaneous gastric inflammation which were associated with early recruitment of myeloid-derived suppressor cells to the stomach.

Despite some conflicting results among Caucasian, Asian, and Hispanic populations, there is a consensus that IL-1B and IL-IRN are crucial cytokine receptors in the pathogenesis of *H. pylori*-induced gastric carcinogenesis[73-76].

Other cytokine gene polymorphism: Additional relations with gastric cancer risk for genetic polymorphisms in TNF-α and IL-10 have been reported[64]. Pro-inflammatory genotypes of TNF-α and IL-10 were each related to an approximately two-fold greater possibility of nocardia gastric cancer[64,65]. Additional reports have suggested that polymorphisms of the Toll-like receptor-4 (*TLR-4*) gene also heightens gastric cancer risk. An 11-fold increase in the odds ratio (OR) for hypochlorhydria was found in the TLR4 + 896G polymorphism carriers. Also, in Caucasian populations, these carriers had significantly more severe atrophic gastritis and inflammation[77].

Host genetics and gastric cancer in the era of Genome Wide Association Studies and future perspectives: In 2008, Sakamoto *et al*[78] first reported that an intronic single nucleotide polymorphism (SNP; rs2976392) in the prostate stem cell antigen (PSCA) was significantly associated with diffuse-type gastric cancer in Japan. Recent two meta-analyses also suggested that PSCA -rs2294008C>T and -rs2976392G>A were potential factors of gastric cancer development in East Asians[79,80]. In addition, it has been thought that the PSCA-rs2294008 polymorphism heightened risk of non-cardiac gastric cancer but protects against proximal cancer in Caucasian populations[81,82]. With recent advances in technology, we can increase our understanding of the genetic mechanisms of gastric carcinogenesis through SNP and next generation sequencing, which could be useful for screening and a necessary step for more effective treatment.

Environmental factors

Environmental factors may also play a role in *H. pylori*-induced gastric carcinogenesis. Salt is a well-known dietary factor. In a Japanese prospective study in 2006[83], a significant correlation between salt consumption and gastric adenocarcinoma was reported in individuals who had both *H. pylori* infection and atrophic gastritis [age- and sex-adjusted hazard ratio, 2.87 (1.14-7.24)]. In addition, according to a recent animal study, high dietary salt intake potentiates the carcinogenic effects of *cag*A-positive *H. pylori* strains[84]. There are some suggestions on the mechanisms by which salt potentiates *H. pylori*-induced gastric carcinogenesis; however, they are not entirely understood. First, salt may destroy the gastric mucosa, thereby leading to inflammation and damage or permitting entry of carcinogens into stomach[14,85]. Second, upregulated production of proinflammatory enzymes and cytokines such as nitric oxide synthase and cyclooxygenase-2 (COX-2) in response to a high-salt consumption may be contributing[86]. Finally, recent reports suggest that high salt concentrations modulate virulence factors, including *cag*A, in *H. pylori*[87,88].

Smoking may be the most significant lifestyle-related risk factor. In a recent systemic review and meta-analysis of cohort studies, it was shown that smoking is correlated with an high relative risk for both gastric cardia [1.87 (1.31-2.67)] and non-cardia cancers [1.60 (1.41-1.80)] significantly[89].

Other factor: Ancestral origin

H. pylori can be divided into seven global populations and subpopulations with distinct geographic distributions, genetically derived from ancestral populations such as those in Africa (Ancestral Africa 1 or 2; AA1 or 2), Europe (Ancestral Europe 1 or 2; AE1 or 2), and East Asia (Ancestral East Asia; AEA). While stomach cancer rates correlate with *H. pylori* prevalence in some areas, in other regions there is no correlation with *H. pylori* prevalence, such as some regions in Africa or South America[90]. In Columbia, the reported gastric cancer rate in the Andes Mountains (approximately 150 per 100000) is 25-fold higher than that in coastal regions (approximately 6 per 100000), in spite of similarly high (approximately 90%) prevalences of *H. pylori* in the two regions[2]. As recently reported[91] in those populations, the authors extracted both human ancestry, from the participants' DNA, and *H. pylori* ancestry, from antral biopsies of the participants, and assessed how coevolution may have had on effect on gastric disease. Remarkably, they found that the interaction between Amerindian host ancestry and *H. pylori* ancestry AA1, which affects the severity of premalignant histopa-

thology, was approximately five-fold larger than the effect of *cag*A (RR = 5.08 *vs* 0.98). This result suggested that ancestral coevolutionary relationships can be significant determinants of gastric cancer.

PREVENTION

The most important primary prevention strategies for gastric cancer potentially include behavioral (dietary or lifestyle) modifications and a decline in the prevalence of *H. pylori*, the major causal factor of gastric cancer[92]. Although the pathogenesis remains unclear, prevention through dietary intervention would include increased fruit, allium, and non-starchy vegetable intake and reduced ingestion of salt or salt-preserved foods and N-nitroso compounds[93-98]. Lifestyle modifications such as maintaining normal weight, limiting alcohol consumption, and smoking cessation may also lower the risk of the disease[99].

H. pylori eradication as a preventative measure for gastric carcinogenesis

H. pylori eradication may be the most efficient method to prevent gastric cancer, in that *H. pylori* infection can persist for decades and slowly progress from preneoplastic lesions to gastric cancer. It is believed that *H. pylori* eradication can suppress the recurrence of peptic ulcers, induce remission of MALToma of the stomach, and lower the rate of recurrence after endoscopic resection of early gastric cancer. However, demonstrating that *H. pylori* eradication directly decreases gastric cancer risk remains challenging.

Currently, a novel meta-analysis of six randomized trials performed in asymptomatic adults estimated a benefit from *H. pylori* eradication (RR = 0.66; 95%CI: 0.46-0.95)[100]. A Chinese randomized controlled trial performed in 2012 in the general adult population concluded that there was a significant decline of gastric cancer risk after 15 years of follow-up (4.6% in the control group, 3.0% in the treated group; OR = 0.61; 95%CI: 0.38-0.96)[101]. Despite some limitations of the study, such as examination of middle-aged groups only and a relative paucity of endpoint data, these well-designed studies have presented good results in terms of eradication therapy for prevention of gastric cancer. In addition, a recently published report from the WHO's IARC, conducted in a working group population, concluded that *H. pylori* eradication can be efficient in gastric cancer prevention, and *H. pylori* screening and treatment strategies would be cost-effective. However, uncertainties regarding the generalizability of the results, cost-effectiveness, and possible adverse outcomes of programs applied in community settings need to be explored[92].

Recent changes in H. pylori eradication subjects

Peptic ulcer, MALToma, and endoscopic treatment of early gastric cancer are well-known indications for *H. pylori* eradication. Recently, it is generally acknowledged that iron deficiency anemia, idiopathic thrombocytopenic purpura, functional dyspepsia, and long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) or proton pump inhibitors (PPIs) are considered highly evident indications[102-106]. In the context described above, Japanese guidelines revised in 2009 strongly recommend (Recommendation grade A) that all *H. pylori* infections should be eradicated regardless of the associated disease[106]. In addition, in the Kyoto Global Consensus Meeting on *H. pylori* Gastritis (From January 30, 2014 to February 1, 2014), it was suggested by 46 authorities that all *H. pylori*-infected individuals, including asymptomatic individuals, should be considered eradication subjects, especially in those with functional dyspepsia[107,108]. Thus, in other East Asian countries such as South Korea and China, where the prevalence of *H. pylori* infection and gastric cancer remains high, careful consideration is required for eradication therapy.

Recent trends in the H. pylori eradication regimens and antibiotics resistances

Although standard triple therapy (PPI + clarithromycin + amoxicillin or PPI + clarithromycin + metronidazole) is still recommended as a first-line regimen in recent Korean and Japanese guidelines[104-106], the increasing rate of eradication failure due to primary resistance to clarithromycin and metronidazole is a global concern[109-111]. Recently, high (≥ 20%) resistance rates of clarithromycin have been reported in the United States and developed countries in Europe and Asia, while relatively low (< 10%) rates have been reported in North Europe[112,113]. Especially, one Japanese multicenter study reported that clarithromycin-resistance rates have increased rapidly from 18.9% in 2002 to 27.2% in 2006[114]. In addition, over 80% of metronidazole-resistance rates have been observed in Africa, Iran, and South America, and 20%-40% of metronidazole-resistance rates have also been reported in United States, Europe, and East Asia[112,115]. Primary quinolone-resistance rates have also been increasing (> 10%) in developed countries in Europe and Asia[112,115,116]. Besides, amoxicillin resistance in Europe has been very low (0% to < 2%) but higher (6%-59%) in Asia, South America, and Africa, and tetracycline resistance has been low or absent (< 5%) in most countries while higher (9%-27%) in South America and Asia[112,115].

With regard to the high resistance to clarithromycin, recent European guidelines[102] recommend that first-line regimens should be tailored according to clarithromycin resistance. In low-resistance (< 20%) regions, standard triple therapy is recommended as a first-line regimen, while in high-resistance (> 20%) regions, bismuth quadruple therapy or sequential/concomitant therapy is recommended first. However, in East Asia, we could not evaluate the superiority of sequential/concomitant therapy over standard therapy[103,104,106,117].

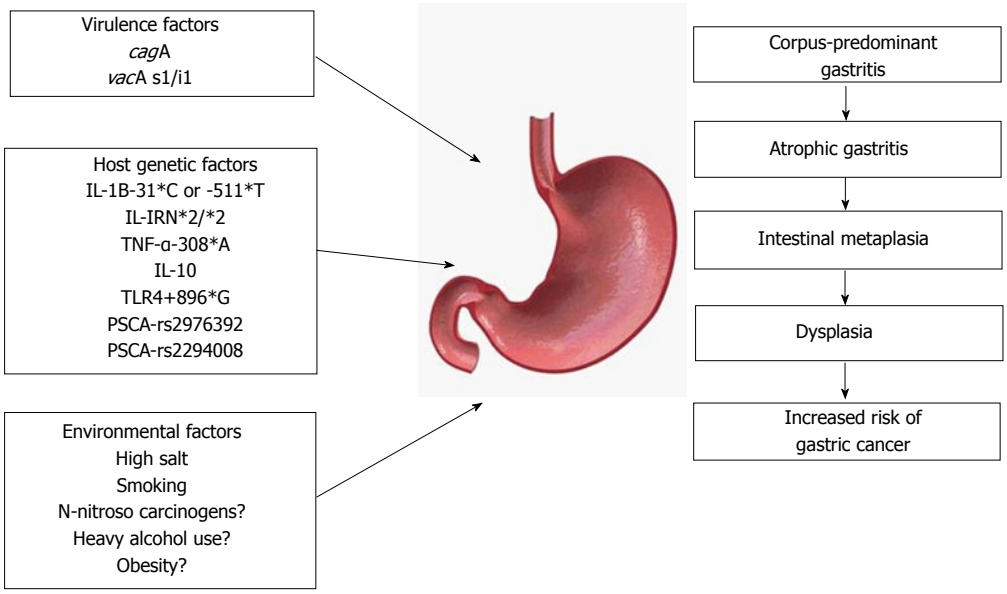

Figure 1 Multiple factors related to *helicobacter pylori*-induced gastric carcinogenesis. IL: Interleukin; TNF: Tumor necrosis factor; TLR-4: Toll-like receptor-4; PSCA: Prostate stem cell antigen.

Thus, to maximize the *H. pylori* eradication treatment effect, individually tailored treatment with consideration of a variety of demographic factors including genetic polymorphisms, antibiotic resistance, and age will be important in the future.

Other protective agents against H. pylori-associated gastric carcinogenesis

The role of aspirin, NSAIDs, and COX-2 inhibitors in gastric carcinogenesis should be considered, because *H. pylori* infection is thought to induce COX-2 overexpression[118,119], and higher levels of COX-2 expression have been observed in gastric carcinoma and premalignant lesions[120,121]. Therefore, it is believed that intervention with aspirin, NSAIDs, and COX-2 inhibitors inhibits or reverses the process of *H. pylori*-related carcinogenesis and prevents the development of gastric cancer[122].

Vitamin C and antioxidants are also considered protective against *H. pylori*-induced gastric carcinogenesis by strengthening the mucosal immune response, neutralizing free radicals, reducing the creation of gastric N-nitroso compounds, inhibiting cell proliferation, and directly influencing *H. pylori* growth[92]. According to a recent meta-analysis of randomized trials conducted in asymptomatic adults, *H. pylori* eradication in combination with antioxidants or vitamins showed a beneficial impact (RR = 0.52; 95%CI: 0.31-0.87)[100]. However, to date, there have been conflicting data in association with gastric cancer and NSAIDs or vitamin C; thus, further studies are required to support the roles of these agents in *H. pylori*-associated gastric carcinogenesis.

In addition, in a recent meta-analysis based on 45 randomized controlled trials, the additional use of probiotics with standard triple therapy was associated with an increased *H. pylori* eradication rate in the per-protocol set (OR = 1.13; 95%CI: 1.10-1.16), a reduction in adverse events (RR = 0.59; 95%CI: 0.48-0.71), and economic burden and a poor compliance rate[123].

FUTURE DIRECTIONS AND CONCLUSION

H. pylori infection is major factor for gastric carcinogenesis. During *H. pylori* infection and subsequent inflammation and carcinogenesis over a time span of decades, numerous factors including bacterial virulence, host genetic, and environmental factors interact and elicit variable clinical outcomes (Figure 1). Thus, understanding the complex mechanisms of a variety of factors is important and may provide future directions for novel therapy.

To date, prevention throughout behavioral management and *H. pylori* eradication may be an important strategy to reduce the occurrence of gastric cancer. A unique contrivance on potential dietary or other chemopreventive agents and related well-designed studies are required. In addition, it is important to take into account whom to eradicate, when to eradicate, and what regimen to use to eradicate *H. pylori* in the general population.

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Endoscopic ultrasound-fine needle injection for oncological therapy

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Abstract

The minimal invasiveness and precision of endoscopic ultrasound (EUS) has lead to both its widespread use as a diagnostic and staging modality for gastrointestinal and pancreaticobiliary malignancies, and to its expanding role as a therapeutic modality. EUS-guided celiac plexus neurolysis is now a well-accepted modality for palliation of pain in patients with pancreatic cancer. EUS-guided ablation, brachytherapy, fiducial marker placement, and antitumor agent injection have been described as methods of performing minimally invasive oncological therapy. EUS-fine needle injection may be performed as adjunctive, alternative, or palliative treatment. This review summarizes the studies to date that have described these methods. A literature search using the PubMed/MEDLINE databases was performed. While most published studies to date are limited with disappointing outcomes, the concept of a role of EUS in oncological therapy seems promising.

Key words: Endoscopic ultrasound-fine needle injection; Endoscopic ultrasound-guided ablation; Photodynamic therapy; Radiofrequency ablation; Cryothermal ablation; Endoscopic ultrasound-guided brachytherapy; Fiducial markers; Endoscopic ultrasound-guided antitumor agent injection

Core tip: In the present review, the novel use of endoscopic ultrasound-fine needle injection (EUS-FNI) in oncological therapy is described. EUS-FNI is a promising method to optimize treatment to a targeted area while minimizing procedure invasiveness and systemic toxicity. EUS-guided ablation, brachytherapy, fiducial marker placement, and antitumor agent injection have been described to date. While these procedures appear to be safe and reasonably well tolerated, their effectiveness and exact role in oncological treatment have yet to be established.

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INTRODUCTION

Since the introduction of endoscopic ultrasound (EUS) for the diagnosis and staging of gastrointestinal and pancreaticobiliary malignancies[1], EUS has increasingly been described as a therapeutic modality. The same minimal invasiveness and precision that favor EUS as a diagnostic modality have generated interest in its therapeutic potential. EUS-guided celiac plexus neurolysis is now a well-accepted modality for palliation of pain in patients with pancreatic cancer, and EUS is now often used to facilitate bile duct identification and access during difficult endoscopic retrograde cholangiopancreatography (ERCP)[2]. More recently, the role of EUS fine needle injection (EUS-FNI) has expanded to include ablation of malignant or pre-malignant tissue, placement of brachytherapy and fiducial markers, and direct delivery of antitumor agents into a targeted lesion under ultrasonographic visualization, theoretically minimizing systemic exposure and increasing local concentration. Injectable agents that have been described for this purpose include lymphocytic cultures, immature dendritic cells, and viral vectors, although most of these studies are limited by their small size, lack of control, and include patients with pancreatic cancer only[2].

EUS-GUIDED ABLATION

Ethanol ablation

Ethanol causes cell-membrane lysis and protein degeneration and has been proposed as a method of ablating the cyst-wall epithelium of premalignant lesions or malignant lesions in poor surgical candidates[3]. After initial cyst needle puncture, cyst fluid is partially aspirated and the cyst is lavaged for several minutes by alternating cyst aspiration and ethanol injection[4]. Ethanol ablation of pancreatic cystic lesions was first described by Gan *et al*[3] in 2005 in a study that included 13 patients with benign mucinous cystic neoplasms and 4 patients with intraductal papillary neoplasms. Complete cyst resolution was noted for 35% patients and cyst size decreased in 9% patients with no reported complications. Cyst resolution was maintained in most patients at long term follow-up[5]. A more recent prospective study of 23 patients with pancreatic cystic neoplasms reported a higher treatment success rate of 52%, however only 2 patients had complete cyst resolution at long-term follow-up[6]. Performing EUS-guided ethanol lavage followed by injection with paclitaxel increased pancreatic cystic tumor resolution to 79% at 6-mo in an abstract of 14 patients[7]. A prospective randomized double-blind single-center study of EUS-FNI of non-malignant mucinous pancreatic cysts with Paclitaxel and Gemcitabine following either ethanol or saline lavage is currently underway, with preliminary results showing 75% complete resolution at 1 year follow-up[8]. To the best of our knowledge, EUS-FNI of Paclitaxel has yet to be performed in malignant lesions.

The use of EUS-FNI for ethanol lavage and ablation of malignant lesions has been described in a small study of adrenal metastatic lesions[9] and in case reports of gastrointestinal stromal tumors, liver metastases, and insulinomas[10-12]. A small study of 19 patients with unresectable pancreatic carcinoma who underwent repeated transgastroenteric injections with dehydrated absolute alcohol reported decreased cancer mass in all patients, with tumor mass decreased over 70% in 12/19 patients[13].

Local ablation

Photodynamic therapy (PDT) utilizes a photosensitizer coupled with light omitted *via* small optic fibers to ablate a targeted area. The photosensitizer is infused systemically but preferentially accumulates in malignant tissue[14,15]. By activating the optic fibers over an area of interest, the omitted light activates the photosensitizer, resulting in the formation of reactive oxygen species that cause tumor necrosis, vascular damage, and local inflammation[16]. PDT has been used for inoperable esophageal, gastric, and biliary malignancies[17]. The percutaneous application of PDT to pancreatic malignancy *via* a hollow metal needle has been shown to be safe and well tolerated, however no survival benefit was seen in one small retrospective study[18]. Chan *et al*[19] demonstrated the feasibility and safety of performing EUS guided PDT in porcine liver, pancreas, kidneys, and spleen, however the degree of necrosis was complete (100%) only in the pancreas. Yusuf *et al*[20] similarly demonstrated successful porcine pancreatic tail necrosis with no observed complications. While, to our knowledge, EUS-guided PDT has yet to be performed in humans, this procedure may be a safe, effective, and less invasive method of locally ablating lesions that cannot be directly accessed endoscopically.

Radiofrequency ablation (RFA) is an ablation technique that uses high-frequency alternating current to create thermal energy and induce coagulative necrosis and may be applied percutaneously, intra-operatively, or endoscopically[21]. RFA has proven successful in the treatment of both hepatocellular carcinoma and liver metastasis[22]. The endoscopic application of RFA for malignant biliary obstruction has been shown to be feasible and safe[23]. The open application of a cool-tip RFA for pancreatic cancer resulted in improved back pain and analgesia, but was associated with significantly high complication rates of up to 50% in patients with pancreatic head cancer, notably due to massive gastrointestinal bleeding[24,25]. A recent small prospective

multi-center pilot study of EUS-RFA of pancreatic head neoplasms and neuroendocrine tumors was reported using a novel monopolar RF probe (1.2 mm Habib EUS-RFA catheter) placed through a 19 or 22 gauge FNA needle. The study reported successful procedure completion in 8/8 (100%) patients with complete cyst resolution noted in 2/6 patients with cystic neoplasms. No reported major post procedure complications were noted[26].

Cryothermal ablation is performed using a cryotherm probe (ERBE Elektromedizin GmbH, Tubingen, Germany), which is an internally carbon-dioxide-cooled bipolar RFA probe. Cryothermal ablation performed under EUS guidance has been shown to reduce tumor size in a small cohort study of 22 patients with locally advanced pancreatic adenocarcinoma, with technical success achieved in 72% patients and no severe early complications noted[27]. The potential application towards EUS ablation of other malignancies is unknown.

The neodymium-doped yttrium aluminum garnet (Nd:YAG) laser is a technology that aims to achieve ablation of a target tissue by the direct application of low-power laser light energy. It has been reported to offer palliative or potentially curative treatment options for hepatocellular carcinoma, colorectal cancer liver metastasis, and malignant thyroid nodules[28]. The EUS-guided application of the Nd:YAG laser ablation of the pancreas has been described in a pig model. Tissue necrosis was observed for all 8 cases with no severe complications[29]. A single case report of EUS-guided Nd:YAG laser ablation through a 22-G needle for a patient with hepatocellular carcinoma of the caudate lobe has been reported[28].

High-intensity focused ultrasound (HIFU) is a non-invasive technique for achieving extracorporeal ablation that induces thermal denaturation of a targeted tissue with minimal to no damage to surrounding tissue. HIFU has been shown to result in tumor ablation and symptom palliation in several studies of patients with liver malignancies[29]. Targeting intra-abdominal tumors with an extracorporeal source may be limited by overlying bowel gas. To overcome this limitation, a new EUS-guided HIFU transducer was developed by Hwang *et al*[30]. This transducer has been shown to ablate liver and pancreatic tissue in a swine model[31].

BRACHYTHERAPY AND FIDUCIAL MARKERS

Interstitial brachytherapy is used for malignancies of the prostate, breast, pancreatic, gynecologic, and brain cancer[32,33]. Radioactive seeds are placed directly into malignant tissue, generating local gamma rays and damaging surrounding tissue. While these seeds are usually placed operatively, EUS-guided brachytherapy has been described in pancreatic[34], esophageal[35], and head and neck tumors[36]. In Sun *et al*[34], 15 patients with unresectable pancreatic cancer underwent placement of 22 seeds of iodine-125 under EUS guidance. Clinical benefit measured by improvement of pain was seen in 30% of patients with partial tumor response noted for 27% patients. Complications included pancreatitis and grade 3 hematological toxicity[34]. A similar study by Jin *et al*[37] used EUS-guided brachytherapy with gemcitabine and 5-flurouracil (5-FU) therapy in 22 patients for up to 24 wk. While no significant increase in survival was seen, tumor growth was effectively controlled in the majority of patients with improvement in pain scores[37].

EUS-guided placement of fiducial markers provides another example of a minimally invasive technique that enables more precise targeting of neoplastic tissue. Typically placed either surgically or percutaneously, fiducial markers are radiographic markers that are placed around a tumor to serve a reference points for radiotherapy. Accurate fiducial placement is important to ensure the correct dose delivery to a target and minimize radiation applied to surrounding normal tissue. A prospective study of 13 patients with mediastinal and abdominal primary or secondary malignancies (with tumors located at the diaphragm dome, porta hepatis, gastroesophageal junction, mediastinum, retrocardiac, paraspinal area adjacent to the thoracic esophagus, and pancreas) demonstrated the feasibility of EUS-guided placement of fiducial markers. Real-time sonographic and fluoroscopic visualization was used to implant the fiducials into the target tissue with a success rate of 85% and post-procedure infection occurring in a single patient[38]. Successful EUS-guided fiducial placement has been described in pancreatic cancer[39-41], esophageal cancer[42], prostate cancer[43], and rectal cancer[44] with varied migration rates depending on the type and length of marker used[45].

EUS-GUIDED ANTITUMOR AGENT INJECTION

A multitude of injectable agents administered by EUS have been used in clinical trials for the treatment of malignancy. These agents include lymphocytic cultures, immature dendritic cells, and viral vectors. Current literature is limited by small sample size, lack of control, and primarily includes patients with pancreatic cancer only. Overall, studies have demonstrated the safety and feasibility of these injectables but have had disappointing clinical outcomes. However, the concept is promising. Local delivery of antitumor agents may optimize therapeutic drug concentration while minimizing systemic toxicity. Additionally, local treatment may allow for tumor downstaging prior to resection or for mass reduction in poor surgical candidates with obstructive symptoms. Larger studies are needed to establish a definitive role and safety profile, and to identify the optimal injectable agents and tumor types for application of this treatment. A new multiple injectable needle has been described that may improve drug distribution and potentially improve

outcomes[46].

Allogenic mixed lymphocytic culture

Inducing cytokine production directly within a tumor has been proposed as a method to increase host antitumor defense and promote tumor regression. Chang *et al*[47] published the first human anti-tumor injection study in a phase I trial of unresectable pancreatic cancer in 2000. The study utilized intratumoral injections of activated allogenic mixed lymphocyte culture (cytoimplant) designed to increase cytokine release. The cytoimplant was formed by coculturing peripheral mononuclear cells from a healthy allogenic donor and the patient. Escalating doses of 3, 6, and 9 billion cytoimplant cells were injected into the tumor bulk under EUS guidance in eight subjects. Two partial responses and one minor response were noted. Side effects were mild and included low-grade fever and nausea[47]. A subsequent randomized multi-center study of conventional chemotherapy *vs* EUS-guided cytoimplant injection was terminated early after survival and tumor response was noted to be inferior in the EUS-FNI arm[48], however this treatment may still have a role as supplemental therapy.

Immature dendritic cells

Dendritic cells are potent antigen-presenting cells that can generate T-cell immune responses and induce antigen-specific aquired immunity[49]. Prior studies have shown that dendritic cells exposed to tumor cells, when introduced to human subjects, generate a strong tumor specific T-cell response upon migration to regional lymph nodes[50]. Hirooka *et al*[4] conducted a trial where five patients with inoperable locally advanced pancreatic cancer were treated with intravenous gemcitabine and biweekly EUS guided immature dendritic cell injections, followed by intravenous infusion of lymphokine- activated killer cells stimulated with anti-CD3 monoclonal antibody (CD3-LAKs). One patient showed partial tumor response leading to tumor resection while two patients had stable disease[51]. In a study by Irisawa *et al*[52], 7 patients had EUS-guided dendritic cells injection, 5 of whom underwent radiation prior to dendritic cell installation to theoretically induce apoptosis and necrosis and increase tumor-associated antigens for dendritic cell cross-presentation. The injections were well tolerated without notable complications and decreased CA 19-9 levels seen in two patients[52]. A phase I clinical trial of patients with resectable pancreatic cancer compared 15 control patients who received standard care to 9 patients who received preoperative EUS-FNI injection of immature dendritic cells and Picibanil (OK-432), a lyophilized mixture of group A Streptococcus pyogenes with anti-neoplastic activity. While there were no significant differences in overall survival times between the two treatment groups, the procedure was well tolerated with mild side effects. A trend towards higher incidence of pancreatic fistulas was seen in the FNI group, however this was not statistically significant (22% *vs* 7%)[53]. A case report of two patients who received EUS-FNA of dendritic cells for advanced gastric cancer has been published[54].

Tumor necrosis factor alpha

Tumor necrosis factor alpha (TNF-α) is an inflammatory cytokine with innate anticancer activity[55]. TNFerade (GenVec, Inc.) is a second generation replication-deficient adenovector carrying the transgene encoding human TNF-α, which is regulated by a chemoradiation-inducible promoter. By injecting TNFerade into tumor cells, TNF-α may be delivered into tumor cells *via* gene transfer[56]. In a phase I/II trial conducted by Hecht *et al*[57], 50 patients with locally advanced pancreatic cancer received five weekly injections of TNFerde at escalating doses, 27 of whom received TNFerade *via* EUS-guidance, along with a combination of 5-FU and radiation. Overall, the procedure was well tolerated, however complete or partial response was noted in only a small percentage of patients[57]. A larger randomized study of 304 patients showed that TNFerade combined with standard treatment was safe but not effective in prolonging survival in patients with locally advanced pancreatic cancer. Receiving EUS-guided *vs* percutaneous application was actually risk factor for inferior progression free survival on multivariate analysis (HR = 2.08; 95%CI: 1.06 to 4.06)[55]. A phase I study of neoadjuvant TNFerade biologic in combination with cisplatin, intravenous 5-FU, and concurrent radiation therapy in patients with locally advanced resectable esophageal cancer has been reported, with fatigue, fever, and nausea the most frequently reported adverse events[58].

ONYX-015

ONYX-015 is a gene-deleted replication-selective adenovirus that targets malignant cells and replicates inside them, ultimately leading to their death[59]. In a phase I/II trail conducted by Hecht *et al*[60], EUS-FNI of ONYX-015 was performed in combination with gemcitabine in 21 patients with advanced pancreatic cancer. Eight treatment sessions were conducted over a period of 8 wk with up to 10 injections received per session. Only 2 patients had partial tumor regression, while 8 patients had stable disease and 11 patients either had progressive disease or had to end the study prematurely. Duodenal perforations occurred in two patients, which were attributed to a stiff endoscope tip; no additional perforations were noted after the protocol was modified to mandate a transgastric approach[60].

CONCLUSION

The role of EUS-FNI for treatment for gastrointestinal malignancies seems promising in theory, but studies are limited and outcomes have been disappointing to date. Larger multi-center randomized trials will be needed

before widespread application may be pursued.

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Role of histone deacetylases in pancreas: Implications for pathogenesis and therapy

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Abstract

In the last years, our knowledge of the pathogenesis in acute and chronic pancreatitis (AP/CP) as well as in pancreatic cancerogenesis has significantly diversified. Nevertheless, the medicinal therapeutic options are still limited and therapeutic success and patient outcome are poor. Epigenetic deregulation of gene expression is known to contribute to development and progression of AP and CP as well as of pancreatic cancer. Therefore, the selective inhibition of aberrantly active epigenetic regulators can be an effective option for future therapies. Histone deacetylases (HDACs) are enzymes that remove an acetyl group from histone tails, thereby causing chromatin compaction and repression of transcription. In this review we present an overview of the currently available literature addressing the role of HDACs in the pancreas and in pancreatic diseases. In pancreatic cancerogenesis, HDACs play a role in the important process of epithelial-mesenchymal-transition, ubiquitin-proteasome pathway and, hypoxia-inducible-factor-1-angiogenesis. Finally, we focus on HDACs as potential therapeutic targets by summarizing currently available histone deacetylase inhibitors.

Key words: Pancreatitis; Pancreatic cancer; Epigenetics; Histone deacetylase; Histone deacetylase inhibitor

Core tip: Histone deacetylases (HDACs) are epigenetic regulators that play an essential role in organ develop-

ment and tissue homeostasis. Aberrant HDAC activity contributes to the development of several diseases, including acute and chronic pancreatitis as well as pancreatic cancer. In acute and chronic pancreatitis the inhibition of HDACs exerts significant positive effects of cytokine- and nuclear factor-κB transmitted inflammation and tissue damage paralleled by reduced oxidative stress. HDACs are expressed in pancreatic cancer and were functionally linked to key processes of tumor progression (epithelial-mesenchymal-transition, the ubiquitin-proteasome pathway and angiogenesis), indicating a pleiotropic effect of HDACs in pancreatic cancerogenesis. Treatment of pancreatic cancer cells *in vitro* with HDAC inhibitors alone and/or in combination with conventional cancer agents resulted in diverse beneficial effects, including inhibition of proliferation and cell cycle as well as apoptosis. Therefore, inhibition of HDACs might be a promising strategy for treatment of pancreatic cancer.

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INTRODUCTION

The pancreas plays a key role in human physiology by its essential functions in gastrointestinal enzymatic digestion and endocrine glucose-dependent regulation of systemic energy metabolism *via* two main functions located in the histo-anatomical endocrine (islets of Langerhans) and exocrine (acinar - ductal) compartment of the pancreas (Figure 1)[1]. The endocrine compartment releases hormones into the blood stream, thereby controlling blood glucose concentration, whereas the exocrine part produces and secrets digestive hydrolytic enzymes into the duodenum. These important physiological tasks of the pancreas become clinically evident, when an acute or chronic inflammatory process like pancreatitis as well as progressive carcinogenesis and subsequently necessary intensive surgery of pancreatic cancer lead to organ destruction and disturbance of the functional integrity of the pancreas (Figure 1)[2-4]. As medicinal therapeutic options of pancreatitis and pancreas cancer are limited and mostly not associated with enhanced therapeutic success until now, the need for new approaches (such as epigenetic interactions) is still urgent in order to improve the quality of live and the outcome of patients with pancreatitis and pancreas cancer[5,6]. In this review we give an overview of the role of epigenetic regulation by histone (de-)acetylation in pancreatic inflammation as well as in development of pancreatic tumors. We will further discuss the potential of histone deacetylase inhibitors (HDACis) as therapeutic approaches for treatment of these pancreatic diseases.

Epigenetic regulation of gene expression is a fundamental mechanism of eukaryotic organisms to ensure that only a subset of genes is actively expressed, thereby enabling the development of organs, specific tissues and their specialized physiologic functions. The term epigenetics describes all heritable changes in gene expression which act independently of the primary structure of the DNA, *i.e.,* the DNA sequence. The two major mechanisms of epigenetics are methylation of DNA and post-translational modification of histone tails[7]. Histones are proteins that package the DNA in structural units called nucleosomes. There are five major classes of histones: H1, H2A, H2B, H3 and H4. H1 are linker histones, whereas two of each of the other four histone classes build the octameric core of the nucleosome[8]. In general, DNA methylation is associated with gene silencing, whereas the effect of histone modifications is dependent on the modification itself, the position of the modification and other surrounding histone modifications[7,9].

The two currently best known histone modifications are histone methylation and acetylation, of which methylation can lead to both, transcriptional activation and repression. Acetylation of histone tails, on the other hand mostly enhances gene expression[8]. This can be explained by the fact that the addition of an acetyl group causes a neutralization of the positive charge of the histone, thereby loosening the contact between DNA and histones and facilitating accessibility of the DNA to transcription-promoting proteins[8]. In contrast, the reverse process, called deacetylation, causes compaction of chromatin and repression of transcription[10]. Deacetylation is performed by a group of enzymes, the histone deacetylases (HDACs), which can be further classified into four groups HDACs I-IV (for details of the different HDAC groups see[10,11]). HDACs play a crucial role in proper development of organs by epigenetic repression of certain genes. However, aberrant activity of HDACs also contributes to development of various human malignancies[10].

HDAC EXPRESSION IN PANCREATITIS

In the last years, intense efforts have been undertaken to gain more detailed insights into the role of HDACs in inflammation and their possible pathogenic involvement in chronic and destructive diseases. As reviewed in detail by others[12-14], HDACs are centrally involved in inflammatory processes in numerous chronic and organ-destructive diseases such as inflammatory bowel disease, chronic respiratory conditions, rheumatoid arthritis and juvenile idiopathic arthritis, allergic diseases and atherosclerosis[12-14]. Here, HDACs influence the antigen presentation, expression of inflammatory mediators and anti-viral responses either directly or indirectly, for instance *via* class II, major histocom-

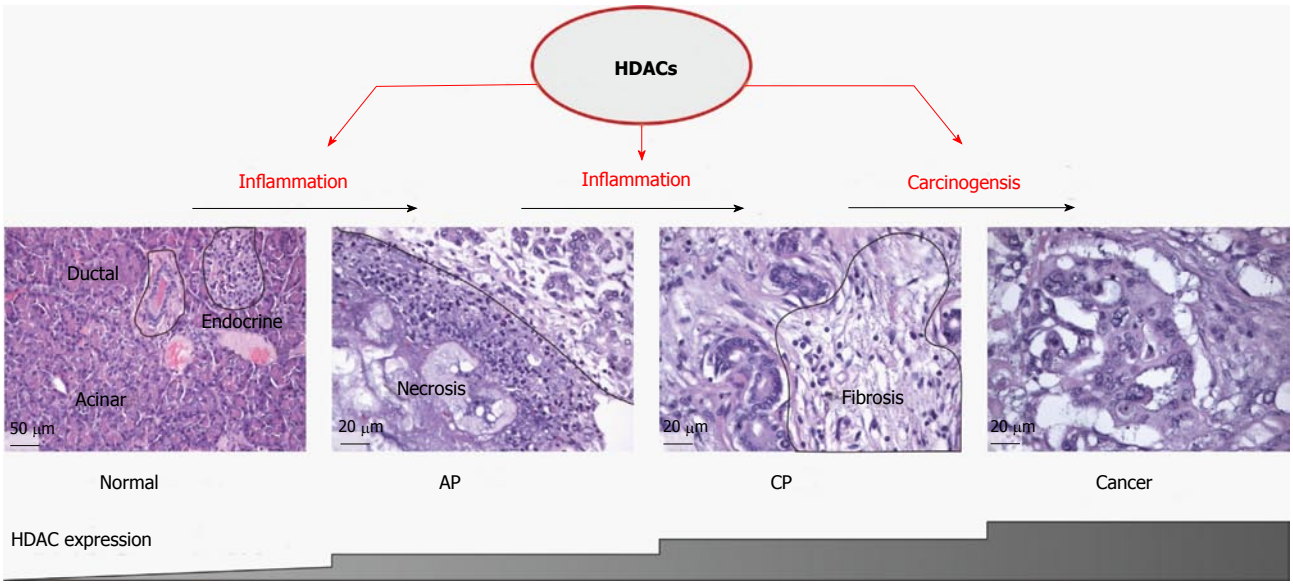

Figure 1 Histone deacetylases and histopathological correlates in the transition from normal to acute/chronic pancreatitis and pancreatic cancer. The trend to increased expression (based on pharmacological inhibition studies) of HDACs from normal to pancreatic cancer tissue is shown in the lower part of the figure. In pancreatic cancer, up-regulation of HDAC-1,-2,-3,-7,-8 could be demonstrated - see text for details - probably offering approaches for personalized therapies based on specific HDAC inhibition. HDAC: Histone deacetylases; AP: Acute pancreatitis; CP: Chronic pancreatitis.

patibility complex, transactivator (CIITT), Interleukin (IL)-10, nuclear factor (NF)-κB, metastatic tumor antigen (MTA)1 or signal transducer and activator of transcription[14,15]. To summarize the functional role of HDACs during pancreatic inflammation and pancreatitis, a recapitulation of relevant inflammatory pathways on cellular and molecular levels involved in acute or chronic pancreatitis (AP, CP) is given in short (reviewed in detail in[16-18]) - in Figures 1 and 2: In the acute phase (AP), neutrophils, followed by monocytes and macrophages, represent the key inflammatory cells secreting the major cytokines and inflammatory mediators. These include, amongst others, tumor necrosis factor (TNF)-α, IL-1β, IL-6, monocyte chemotactic protein (MCP)-1 and platelet activating factor (PAF; being also produced in part by acinar cells)[19]. For development of CP, activation of pancreatic stellate cells as well as infiltrating myeloid cells and particularly macrophages are important on cellular level, whereby NF-κB plays a relevant role on molecular level initiating and promoting fibrosis and scarring of the pancreatic tissue, which results ultimately in loss of exocrine and endocrine functions of the pancreas[20,21]. Additionally, detailed investigations of immune cells revealed that T-cell-subsets play a central role in the pathogenesis of CP by increased counts of $CD4^+$ and $CD8^+$ central memory T-cell subsets (especially $CCR7^+$) which additionally show enhanced IL-10-based response activity towards pancreatitis-associated antigens (mediated *via* $CD4^+CD25^{high}FoxP3^+CD127^-$)[22,23].

The following selected experimental approaches showed an association between inflammatory members and HDACs during pathogenesis of AP and CP, using *in vitro* and *in vivo*-analysis with HDAC inhibitors.

In 2007, the group of Larsen *et al*[24] investigated the possibly protective effect of HDAC inhibition on beta cells after cytokine-induced toxicity. They cultivated the INS-1 beta cell line and intact rat islets treated with the HDACis suberoylanilide hydroxamic acid (SAHA) or trichostatin A (TSA) in the absence or presence of IL-1β and interferon (IFN)-γ. Based on insulin secretion, nitric oxide (NO) formation, inducible NO synthase (iNOS) levels and NF-κB activity as well as viability and apoptosis, the authors could show that HDAC inhibition leads to cytokine-mediated decrease in insulin secretion, paralleled by reduced iNOS levels, NO formation and apoptosis. Furthermore, the IL-1β-induced phosphorylation of the inhibitor protein kappa Bα (IκBα) was inhibited by HDACis. The authors concluded that application of HDACis had a preventive effect on cytokine-induced beta cell apoptosis and impaired beta cell function associated with a down-regulation of NF-κB trans-activating activity.

In 2014, the group of Hartman *et al*[25] analyzed the role of HDAC in trypsin activation, inflammation, and tissue damage in severe acute pancreatitis. After induction of pancreatitis with taurocholic acid in C57Bl/6 mice, the effect of pretreatment with the HDAC inhibitor TSA on serum levels of amylase and IL-6 was determined as well as the pancreatic levels of macrophage inflammatory protein-2 (MIP-2), tissue morphology and myeloperoxidase activity, pro-inflammatory mediators, and trypsin activation in the pancreas and lungs. Using this experimental setting, the authors could demonstrate that pretreatment with TSA results in a significant decrease in amylase levels and a reduction of systemic IL-6 and pulmonary myeloperoxidase activity, as well as the taurocholate-induced gene expression of cyclooxygenase-2, MIP-2, MCP-1, IL-6, and IL-1β in the pancreas. These findings

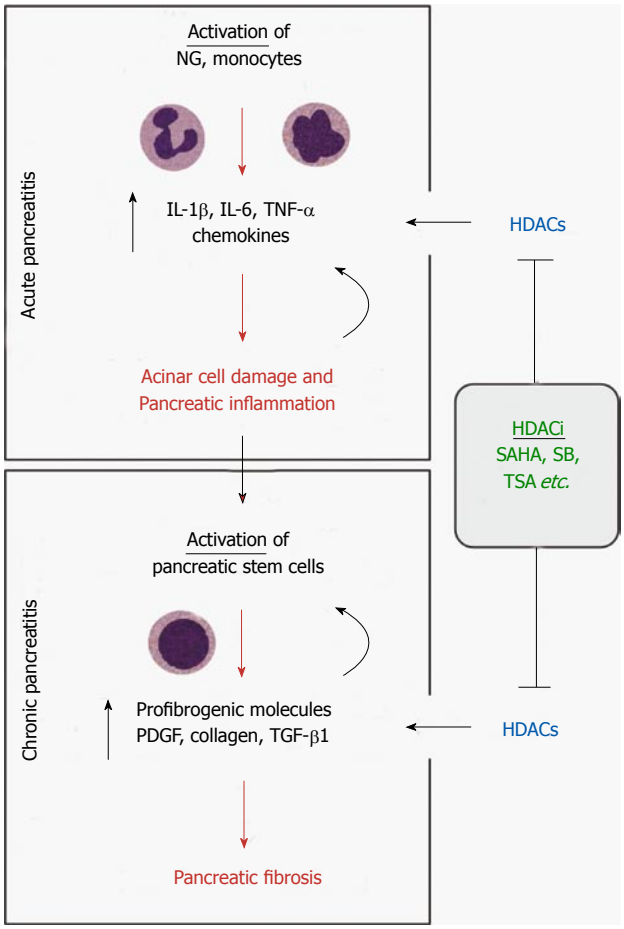

Figure 2 Histone deacetylases in acute and chronic pancreatitis. HDACs induce key pro-inflammatory mediators in AP and CP leading to destruction of pancreatic tissue with necrosis in case of AP and fibrosis/atrophy in case of CP. Inhibition of HDACs by HDACis was shown to significantly antagonist these effects *in vitro* and *in vivo*. HDAC: Histone deacetylases; HDACis: Histone deacetylases inhibitors; NG: Neutrophil granulocyte; IL: Interleukin; TNF: Tumor necrosis factor; PDGF: Platelet-derived growth factor; TGF: Transforming growth factor; SAHA: Suberoylanilide hydroxamic acid; SB: Sodium butyrate; TSA: Trichostatin A.

suggest that HDACs are involved in the pathogenetic process of AP such as inflammation and tissue damage.

Recently, the group of Kanika *et al*[26] studied the effect of HDAC inhibition on inflammation and fibrogenesis in L-Arginine(Arg)-induced pancreatitis and -associated fibrosis in Wistar rats. Looking at biochemical estimations, histological alterations, DNA damage, and the expression of various proteins, post-treatment with sodium butyrate (SB) decreased L-Arg-induced oxidative and nitrosative stress, DNA damage, histological alterations, and fibrosis. Interestingly, post-treatment with SB significantly decreased the expression of α-smooth muscle actin, IL-1β iNOS, and 3-nitrotyrosine. Overall, the authors concluded that post-treatment with SB could alleviate L-Arg-induced pancreatic damage and fibrosis in rats[26].

These findings are summarized in Figure 2: Taken together, the pre- or post-treatment of AP and CP with the three different HDAC inhibitory substances SAHA, TSA and SB resulted in a significant decrease of inflammatory mediators in AP and CP with reduced disease progression compared to untreated controls. Interestingly, none of the mentioned experimental trials have carried out a sub-analysis of the HDAC classes and their members which could selectively be involved in this specific disease model. This approach could lead to the development of high selective HDAC-inhibitors to reduce systemic effects of pan-HDACis, because individual members of HDAC classes are specifically involved in the modulation of immune response in acute and chronic inflammatory diseases (reviewed in detail in[14]).

HDAC EXPRESSION IN PANCREATIC TUMORS

The development from normal to cancerous cells is driven by complex modifications. Alternative pathways like epigenetic alterations become more and more interesting than progression models for mutations of different proto-oncogenes or tumor suppressor genes. One alternative way is the modification of histones by histone deacetylation. By removing acetyl groups from nucleosomes, histones, and non-histone proteins, HDACs do restrict the availability to access transcription factors or repressors[27], implicating that over-expression of HDACs can lead to aberrant gene expression and carcinogenesis[28].

Ductal adenocarcinoma of the pancreas, or simply called pancreatic cancer (PC), ranks among the most lethal of all malignancies in humans. In general, little is known about the role of HDACs in neoplasms derived from pancreatic endocrine and acinar cells; therefore the following paragraphs focus mainly on PC.

Recent studies revealed that under conditions of pancreatitis, adult exocrine acinar cells can differentiate and gain metaplastic ductal characteristics. This differentiation is also known as acinar-to-ductal metaplasia (ADM) and in mouse models, ADM is a precursor lesion of PC[29,30]. Wauters *et al*[31] investigated the role of Sirtuine 1 (SIRT1) and its inhibition by Leptomycin B and nicotinamide in a mouse model and human pancreatic exocrine cell culture experiments. Localized in the nucleus of normal exocrine acinar cells, SIRT1 is inhibited by the protein deleted in breast cancer 1 (DBC1). In ADM, the co-localization of SIRT1 and DBC1 is disrupted and SIRT1 translocates into the cytoplasm, ending up in SIRT1-driven effects like cell differentiation and certain roles during multistage carcinogenesis[32-34]. The Wnt/β-catenin pathway plays an important role in embryonic acinar cell differentiation and Prolferation. Wauters *et al*[31] discovered that in pancreatic acini, SIRT1 is a regulator of the Wnt/β-catenin signialing pathway and SIRT1 inhibition resulted in maintenance of Wnt/β-Catenin signaling. In conlusion, Murtaugh *et al*[35] propose that in normal pancreas, DBC1 balances SIRT1 activity and acinar cells remain differentiated. In 2007, Nakagawa *et al*[36] investigated the expression profile of class I HDACs in human cancer tissues. Amongst others, they stained 20 PC samples with class

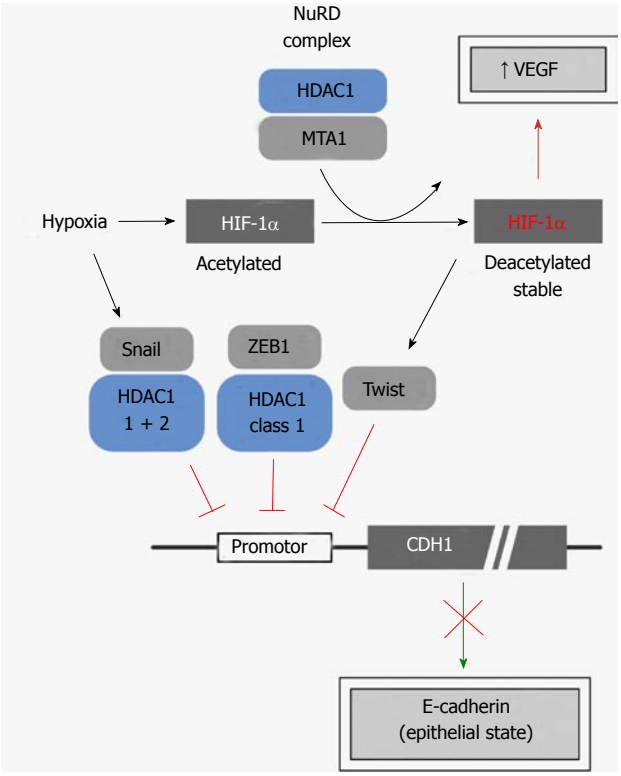

Figure 3 Histone deacetylase involvements in hypoxia inducible factor-1-mediated response to hypoxia. As suggested by Miyake *et al*[51], HIF-1α is possibly regulated and stabilized by two subunits of the NuRD complex: HDAC1/MTA1. Stabilized HIF-1α induces neo-angiogenesis by up-regulation of VEGF and, furthermore, contributes to EMT *via* Twist and subsequent inhibition of E-Cadherin expression (CDH1). Expression of E-Cadherin can be additionally repressed by complexes of either HDAC class I with ZEB1 or HDAC1 and 2 with Snail at the CDH1 promoter[56]. HDAC: Histone deacetylases; HIF1: Hypoxia inducible factor-1; MTA1: Metastasis-associated protein 1; VEGF: Vascular endothelial growth factor; EMT: Epithelial-mesenchymal transition; ZEB1: Zinc finger E-box-binding homeobox 1.

I HDAC antibodies. Immunoreactivity was observable for HDAC1 in 17 PC (85%), for HDAC2 in 18 PC (90%), for HDAC3 in 20 PC (100%) and for HDAC 8 in 18 PC (90%) samples.

Lehmann *et al*[37] discovered a significant correlation between class I HDAC expression and an increased nuclear translocation of RelA/p65. RelA/p65 is a member of the NFκB family transcription factors and a key regulator in pancreatic carcinogenesis. The NFκB family is involved in the regulation of many genes which participate in functions like cell survival, proliferation, differentiation, and inflammation[38]. In addition, Weichert *et al*[39] pointed out that high expression rates of RelA/p65 are correlated with the activation of the NFκB pathway in PC. Furthermore, they linked their results on class I HDAC expression to higher tumor grades and poor prognosis.

It has been also reported that HDAC2 plays a role in therapeutic resistance in PC, since inhibition of HDAC2 leads to up-regulation of the BH3-only protein NOXA. This in turn makes PC cells vulnerable to etoposide-induced (topoisomerase II inhibitor) apoptosis as well as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis[40,41].

In addition, a relation between oncoproteins of the Myc family and HDAC2 up-regulation has been demonstrated: In PC, the c-Myc oncogene is highly expressed, whereas CCNG2 is under-expressed. CCNG2 is known to stop cell cycle progression by inducing G1/S phase cell cycle arrest[42]. Marshall *et al*[43] showed that trichostatin A, a pan-HDACI, is able to improve CCNG2 expression and significantly elevates CCNG2 protein expression. On the contrary, they showed that transcriptional repression of CCNG2 contributes to N-Myc and HDAC2-induced cell proliferation. This suggests a potential benefit by using HDACIs in the treatment of PC as well.

In growing solid tumors, like PC, tumor cells experience specific microenvironmental conditions - in particular, a decreased oxygen level, called hypoxia[44]. Hypoxia in the microenvironment of tumors can lead to radio/chemo-resistance and metastasis[45-47]. Cellular response to hypoxia is controlled by many intracellular accumulating transcription factors, of which Hypoxia-inducible-factor-1 (HIF-1) plays an important role in the events induced by hypoxia[44]. HIF-1 is composed by the HIF-1α and HIF-1β subunits[48]. Denslow *et al*[49], Liu *et al*[50] and Miyake *et al*[51] showed that the expression of HDAC1 positively correlates with the expression of HIF-1α and metastasis-associated protein 1 (MTA1) in PC and that the expression of HIF-1α is possibly regulated through HDAC1/MTA1 subunits of the Nucleosome Remodeling Deacetylase (NuRD) complex, a HDAC containing repressor complex of proteins with the capability of ATP-dependent chromatin remodeling (Figure 3). As these changes are associated with poor prognosis, the inhibition of HDAC1 seems to be a promising therapeutic target[51].

In recent studies, HDACs have been connected with epithelial-mesenchymal transition (EMT), a process that contributes to PC progression[52]. EMT is described as a turning of tumor cells from an epithelial into a mesenchymal phenotype, thereby becoming more invasive - a process which can lead to the development of metastases[53]. E-cadherin regulates metastasis of PC and is suppressed by a Snail/HDAC1/HDAC2 repressor complex. Similar to HIF-1, gene expression of *Snail* is upregulated by hypoxia[54]. Von Burstin *et al*[55] showed that down-regulation of E-cadherin is associated with disorganization and loss of cell-cell adhesion in EMT and that inhibition of histone deacetylation seems to be one possibility to intervene E-cadherin down-regulation in PC. In cancer cells, E-cadherin is repressed by transcription repressors like Snail and Zinc finger E-box-binding homeobox 1 (ZEB1) which regulate the recruitment of HDAC1 and HDAC2 to the E-Cadherin promotor (Figure 3)[56].

The ubiquitin-proteasome pathway regulates the degradation of intracellular proteins, including proteins which are involved in cell cycle regulation and differentiation. In order to survive, tumor cells are more dependent on the ubiquitin-proteasome pathway

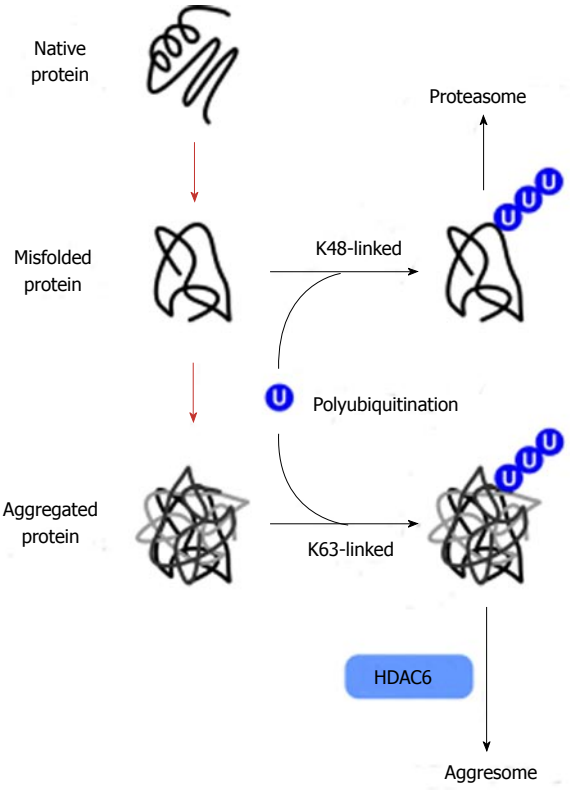

Figure 4 Role of histone deacetylase 6 in protein turnover. HDAC6 facilitates un-/misfolded protein degradation by recruiting ubiquinated proteins to the aggresome or proteasome thus protecting tumor cells from apoptosis - see text for details[58]. HDAC6: Histone deacetylase 6.

than healthy cells, because tumor cells show more accumulation of mis- or unfolded proteins than other cells[57] - see also Figure 4. Aldana-Masangkay *et al*[58] detected that HDAC6 is able to bind ubiquinated proteins and to activate the proteasome pathway. In consequence, HDAC6 protects tumor cells from apoptosis by helping to reduce the intercellular amount of mis- or unfolded proteins. As shown by Rodriguez-Gonzalez *et al*[57] HDAC6 inhibitors break up aggressomes, an aggregation of misfolded proteins, in PC. Furthermore, combination of HDAC6 and proteasome inhibitors increases proteasome-induced apoptosis in cancer cells (Figure 4)[59]. Frankland-Searby *et al*[60] found that patients with a solid tumor like PC benefit from a combination of bortezomib (proteasome inhibitor) and a specific HDAC6 inhibitor.

The nerve growth factor IB, also known as Nur77, affects proliferation as well as apoptosis. *Nur77* gene encodes an orphan nuclear receptor that positively regulates antigen-induced apoptosis of thymocytes[59]. HDAC7 was shown to be a key regulator in the negative selection of thymocytes and ensures down-regulation of the *Nur77* gene[61]. Recently, Ouaïssi *et al*[62] determined the expression pattern of *Nur77* gene simultaneously with the expression pattern of genes encoding for HDACs and SIRTs in PC. They recognized an overexpression of *HDAC7* and *HDAC2* as well as *Nur77* in a significantly high percentage of PC compared to benign tumors and chronic pancreatitis. Although the function of *Nur77* seems to be divergent and therefore further studies are needed to clarify the involvement of the *HDAC7/HDAC2/Nur77* axis in the pathogenesis of PC, those findings suggest new approaches in the design of anti-PC therapy[62].

In summary, especially class Ⅰ and Ⅱ HDACs influence events involved in pancreatic cancerogenesis. Significant correlations of the NFκB-family member RelA/p65 and class Ⅰ HDACs imply possible effects on functions like cell survival, proliferation, differentiation, and inflammation, which all play a role in cancerogenesis[37,38]. Primarily class Ⅰ HDACs show importance in the regulation of apoptosis and cell cycle in mainly three different ways: (1) inhibition of HDACs (HDAC2 and 7) induces up-regulation of BH-3 only protein *NOXA*, *CCNG2* gene expression and *Nur77*[41,43,61]; (2) moreover, HDACs are involved in EMT of PC tumor cells *via* the Snail/HDAC1/HDAC2 complex that suppresses E-Cadherin expression; and (3) in the oxygenation of PC microenvironment by regulating the expression *HIF-1α* through *HDAC1/MTA1*[51,55]. All these findings suggest that HDAC inhibitors (HDACi) would interfere with cancerogenesis in PC on different points and are therefore a highly promising tool in anti-PC therapy.

HDAC-INHIBITORS: FROM THE BENCH TO THE BED

The development of HDACis as therapeutics for chronic diseases and cancer arose from the functional understanding of the underlying dys-regulation of HDACs. The acetylation status of histones is controlled by the opposing actions of two enzyme classes, the histone acetyltransferases (HATs), which transfer acetyl groups to lysine residues within the N-terminal tails of core histones, and the HDACs which remove the acetyl groups[63]. Histone hyperacetylation is associated with transcriptional activity. The rate of regulation and affection through HDACis lies by 20% of all known genes, whereof almost one half is down-regulated and the other half is up-regulated[64].

The family of HDACis includes naturally occurring and synthetically generated compounds which target the HDAC enzyme family. These compounds vary in their chemical structure, their biological activity, and their specificity. There are two HDACis - vorinostat (Zolinza®) and romidepsin (Istodax®) - which have received approval from the United States Food and Drug Administration (FDA) for treatment of cutaneous T-cell lymphoma (CTCL). Romidepsin also got approved for the treatment of peripheral T-cell lymphoma[65,66].

The HDACis can be grouped by their structure into hydroxamic acid, cyclic peptide, bibenzimide, and short-chain fatty acid group (Table 1). The group of hydroxamates (vorinostat, givinostat, abexinostat, panobinostat, belinostat, and trachostatinA) exerts nonspecific HDAC inhibition by affecting all classes of

Table 1 Overview of histone deacetylase inhibitors based on their structure, class specificity; current clinical trials and suggested therapeutic effects[63,77,84-88]

Structure class	HDAC class specificity	HDAC inhibitor	Clinical trials	Effects
Hydroxamic acid	I, II, IV	Trichostatin A	-	-
		Quisinostat	Phase I	-
		Vorinostat	FDA approved (2006), phase II, III	Vorinostat significantly sensitized pancreatic cancer cells for radiotherapy
		Panobinostat	Phase II, III	Panobinostat induced the death of pancreatic tumor cell by apoptosis
		Resminostat	Phase I, II	-
		Abexinostat		-
		Belinostat		-
	I, II	Givinostat		The orally active HDAC inhibitor ITF2357 (givinostat) favors β-cell survival during inflammatory conditions
Cyclic peptide	I	Depsipeptide	FDA approved (2009), phase I, II	-
Benzamides	I HDAC1	Entinostat	Phase II	-
		Mocetinostat	Phase I, II	Mocetinostat + gemcitabine might be an effective treatment for gemcitabine-refractory pancreatic cancer
Fatty acid	I, II	Valporic acid	Phase I, II, III	Valporic acid may protect β-cells from palmitate-induced apoptosis and ER stress *via* GSK-3β inhibition, independent of ATF4/CHOP pathway
	I, II	Butyrate	Phase II	Butyrate regulates both the survival and replication of human β-cells

HDAC: Histone deacetlyase; FDA: Food and Drug Administration.

HDACs[67,68].

The group of cyclic peptides includes compounds like depsipeptide (romidespin) and trapoxin. The benziamides include entinostat and mocetinostat. The hydroxamates, cyclic peptides and benzamides have potent inhibition properties in the nanomolar range. HDAC isotype-selective inhibitors like tubacin, mocetinostat and PC-34501 inhibit HDAC6; in addition HDAC1 and 8 are also becoming available[69,70].

It is a current topic of discussion whether to choose a broad-spectrum HDACi or a class specific HDACi. Furthermore, there are emerging hypotheses about the combination of HDACis with other signaling compounds like miRNA inhibition, in order to obtain better inhibition outputs[6].

The response to HDACis is complex and involves transcriptional effects as well as non-transcriptional effects in the cell: Lee *et al*[71] summarized the multimodal effects through HDACis including apoptosis, cell-cycle arrest, necrosis, autophagy, differentiation, and migration. Normal cells are up to ten times more resistant to HDACi-induced cell death than transformed cells. As an example, they described that vorinostat induced DNA double strand breaks (DSB) in normal and transformed cells in the cell culture, but normal cells were able to repair the DSB without almost any loss in viability[71].

In pancreatic cell lines, HDACis were shown to be potent anticancer drugs as single compounds but also as adjuvant drugs when combined with DNA-damaging agents, ionizing radiation or other approaches such as silencing through small interfering RNA[72,73]. Vincent *et al*[74] showed that Drosophila Eyes Absent Homologue-2 (*EYA2*) is silenced in the majority of PC and investigated the role of epigenetic mechanisms of *EYA2 gene* silencing in pancreatic cancers. Knockdown of *EYA2* increased cell proliferation in pancreatic cancer cell lines. Silencing of *EYA2* expression in pancreatic cancer cell lines correlated with histone deacetylation and was reversible with HDACis.

Peulen *et al*[75] described that HDAC inhibition in human pancreas cell lines with chemical inhibitors (SAHA, MS-275 and celecoxib) significantly impaired proliferation of a human pancreatic cell line (BxPC-3 cells) *in vitro*.

Yee *et al*[76] showed in human pancreatic adenocarcinoma cells that the combination of the HDACi suberoylanilide hydroxamic acid (SAHA) and ML-60218 (inhibitor of RNA polymerase Ⅲ) led to suppression of colony formation and proliferation, cell cycle arrest, and apoptotic cell death. The enhanced cytotoxicity was accompanied by up-regulation of the pro-apoptotic regulator BAX and the cyclin-dependent kinase inhibitor p21 (CDKN1A).

Mhedi *et al*[77] examined human pancreatic cancer cell lines (Panc-1, BxPC-3, SOJ-6) and an immortalized epithelial cell line of a normal human pancreatic duct (HPDE/E6E7): A significant variation in HDACs and SIRTs protein expression levels was seen among individual cell samples. The *in vivo* results showed that panobinostat

(LBH589) exhibited a tumor reduction efficacy similar to the chemotherapeutic drug gemcitabine. In line with its *in vitro* activity, panobinostat also achieved a significant reduction of tumor growth in a BxPC-3 pancreatic tumor cell line subcutaneous xenograft mouse model[77].

In a xenograft model of pancreatic cancer, Lee *et al*[78] tested the effects of combined (vorinostat) SAHA and bortezomib treatment with or without gemcitabine on cell growth, apoptosis and expression of related proteins. The triple combination of vorinostat, bortezomib, and gemcitabine resulted in the strongest antitumor effects *in vitro*.

Currently, there are 7 clinical trials concerning HDACis in PC[79]. In general, there are more than 80 clinical trials investigating more than eleven different HDACis in solid and hematological malignancies, either as mono-therapies or in combination with other antitumor agents[63].

Vorinostat

The FDA approval for vorinostat was given after two phase II clinical trials in CTCL patients. Vorinostat showed similar effects as standard therapy in CTCL patients, but with a higher relief from pruritus. It was well tolerated with some adverse effects like diarrhea, fatigue and nausea. The response rates in solid cancer like breast, colorectal or lung cancer were poor. The use as a single agent has been unsuccessful, but the combination with conventional cancer agents seemed to be highly beneficial[78,80,81].

Depsipeptide

The bicyclic peptide is connected with potent cytotoxic effects *in vitro* and *in vivo*. Depsipetide was tested in a range of clinical trials (phase I/II/III) in colorectal, renal, breast neoplasms as well as hematological malignancies; and showed limited activity as monotherapy in acute myeloid leukemia and myelodysplastic syndrome[82,83].

CONCLUSION

The pancreas plays a key role in the exocrine and endocrine functional integrity of the organism which is severely affected by processes like acute or chronic inflammation as well as cancerogenesis. It is clear today that epigenetic regulators, such as HDACs are involved in development and progression of pancreatic diseases as shown during the last years in diverse *in vitro* and *in vivo* models. In this review, we investigated current literature to comprehensively summarize the role of HDACs in AP and CP as well as in PC. HDACs are overly expressed in PC and are associated with EMT, angiogenesis, and consequently with poor prognosis. HDACis were shown to have multifariously anti-tumor effects in PC, especially in combination with standard chemotherapeutics. Based on the data presented in this review, targeting HDACs can be a promising therapeutic option for treatment of PC and should be prospectively assessed in future clinical trials.

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How to improve colon cancer screening rates

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Abstract

Colorectal carcinoma is a common cause of death throughout the world and may be prevented by routine control, which can detect precancerous neoplasms and early cancers before they undergo malignant transformation or metastasis. Three strategies may improve colon cancer screening rates: convince the population about the importance of undergoing a screening test; achieve higher efficacy in standard screening tests and make them more available to the community and develop new more sensitive and efficacious screening methods and make them available as routine tests. In this light, the present study seeks to review these three means through which to increase colon cancer screening rates.

Key words: Colon cancer screening; Colon cancer; Screening tests; Colonoscopy; New technology

Core tip: Colorectal carcinoma is a common cause of death and may be prevented by convincing the population about the importance of undergoing a screening test; achieve higher efficacy in standard screening tests and develop new more sensitive and efficacious screening methods.

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INTRODUCTION

Colorectal carcinoma (CRC) is a common cause of death throughout the world in both men and women. Many forms of CRC may be prevented by routine control, which can detect precancerous neoplasms

and early cancers before they undergo malignant transformation or metastasis[1]. Consensus evidence-based recommendations call for screening of all people, beginning at age 50. Some screening tests have proven to be effective and are recommended at varying intervals, depending on each patient's risk of developing CRC[2]. Unfortunately, colorectal screening is underused, and at least 40% of age-eligible adults do not adhere to up-to-date screening guidelines[3]. So what can be done to improve screening rates in colon cancer?

Three strategies may increase compliance on colon cancer screening rates: (1) convince the population about the importance of undergoing a screening test; (2) achieve higher efficacy in standard screening tests and make them more available to the community; (3) develop new more sensitive and efficacious screening methods and make them available as routine tests. In this light, the present study seeks to review these three means through which to increase colon cancer screening rates.

CONVINCING PROFESSIONALS AND EDUCATION FOR POPULATION

Multiple strategies have improved CRC screening rates, including media education and medical publications[4,5]. Electronic medical records (EMR), for example, provide specific information necessary to improve screening rates.

Increasingly, investigators are recognising that enhancements of primary care practices require changes in physician and staff roles in order to produce effective medical teams, with medical assistants (MAs) playing a key role[6].

The National Colorectal Cancer Roundtable has disclosed guidelines that recommend the colon cancer screening, using reminder systems that are mainly controlled by physician. Screening guidelines and personal experience are useful to detect early tumors, primarily in patients with a high risk of CRC, using only local resources[7]. EMR-based reminders have improved the CRC screening rates and should be disseminated to the population and medical practioners by physicians and medical societies by making a recommendation, developing a screening policy, using the reminder systems and measurements, and even improving one's own performance. All of these tools are important in the struggle to increase colon cancer screening rates.

Making a recommendation

A recommendation from a physician is the most influential factor in determining whether a patient is screened for CRC. The evidence supporting the vital role of a physician's recommendation derives from many types of research-based and population sources, and is geographically constant[8].

Physicians are increasingly aware of the importance of screening to reduce mortality caused by CRC[9]. In fact, 98% of primary care physicians responding to a national survey reported that they screen for CRC. While this is encouraging, many patients do not receive this needed recommendation when they are visiting their doctor. Assessing the patient's risk status, discussing their needs, and offering several test options can all serve to increase the likelihood of a patient receiving the proper screening. At minimum, a physician should offer the patient a choice between a high-sensitivity, multiple sample stool blood test (FOBT or FIT) and a colonoscopy[10].

Developing a screening policy

Office policy is the foundation of a systematic approach to cancer screening. Only a systematic approach will achieve the goal of a recommendation for every appropriate patient[9].

Consider the following when developing your screening policy: (1) national screening guidelines; (2) realities of your practice; (3) patient history and risk level; (4) patient preferences and insurance coverage; and (5) local medical resources[10].

As part of a high-quality screening program for your practice, develop a policy for an annual stool blood test (FOBT/FIT). There is no evidence from randomized controlled trials that one specific screening method is the "best". However, based on modeling studies that assume 100% patient adherence for stool testing and colonoscopy, years of life saved through annual high-quality stool-blood screening programs are comparable to high-quality colonoscopy-based screening programs when positive stool tests are followed by colonoscopy[11,12].

Reminders

Implementation of EMR-based reminders or prompts has been shown to improve CRC screening rates, although provider compliance with prompts is variable. Reminder systems can be directed towards physicians or patients or both. Reminders directed at patients are further endorsed by strong evidence in that they have proven to be effective in screening for both breast cancer and CRC. Chart prompts, ticklers and logs, and electronic medical records can all provide cues for physicians and their teams to take action. Postcards, letters, prescriptions, in-person conversations, and phone calls can encourage patients to follow through with screening[13,14]. To achieve high screening rates with take-home stool blood tests, reminder and tracking systems are therefore essential[9-12].

Measuring and improving performance

During staff meetings, allow time for your team to report what is working well, what can be done differently, whether or not documentation procedures need improvement, and if there are additional ways to support the members of the team. Elicit feedback from your team and your patients to learn valuable

information about any and all opportunities to improve your system[4,9,10].

It is essential to complete one review that will serve as a baseline comparison for all future investigations. An initial audit can be completed simultaneously with the baseline review. Audits are not complicated, and the simplest audit involves reviewing a specified number of patient records and documenting key elements[9].

SCREENING TESTS

The perfect knowledge of each exam limits and periodicity is necessary to make the CRC screening test more efficacious. People with a high risk for CRC should not be included in a routine screening used for the general population. Their screening must be started early in a shorter period, and using various tests. Those with previous CRC are not included in screenings, but rather in either follow-up or surveillance.

Recently published guidelines grouped CRC screening tests into cancer prevention and detection tests. Prevention methods have the potential to detect both câncer and polips, whereas detection methods generally show a low sensitivity for polyps and an even lower sensitivity for cancer. However, these are easier to execute and are more cost efficient. The United States Preventive Task Force recommends CRC screening for the average at-risk population, using an anual fecal occult blood test (FOBT), a periodic flexible sigmoidoscopy (FS), or a colonoscopy[15,16].

CRC detection methods

The FOBT can be easily executed. Guidelines recommend the collection of two to three stool samples. Dietary restrictions and suspension of medication, such as aspirin, is controversial. FOBT must be done annually. Patients with positive results should receive a medical referral to undergo a colonoscopy[17].

Fecal immunochemical test (FIT) is one of the favorite detection tests. FIT has proven to produce a more effective performance than the guaiac-based FOBT[18]. This test must be done annually, and patients with positive results should receive a medical referral to undergo a colonoscopy[17].

A stool DNA test detects exfoliated-DNA from neoplastic cells in stool samples. This test is more costly than other stool tests. Moreover, intervals and periodicity of the exam is still uncertain[17].

Methods of CRC prevention

Flexible sigmoidoscopy requires partial or total bowel preparation, which may cause some discomfort when executed without anaesthesia. FS evaluates only the distal colon, where most of the lesions are located. However, some patients with only right colon lesions may receive a misdiagnosis. Patients with positive results in FS must undergo a colonoscopy to fully examine the colon to identify synchronic lesions. Negative results do not guarantee the abscence of polyps or cancer on the proximal colon. The recomended interval is every 5 years.

Colonoscopy requires total bowel preparation and usually intravenous sedation (conscious or deep). At least one working-day is missed and a companion is necessary. These tests present low risks (perforation, bleeding), usually associated with polypectomy. Nevertheless, this procedure still offers a great advantage over other methods, as it is able to detect both early and advanced lesions and allows for imediate treatement in early cases. The interval recomended for the average risk population is every 10 years, which may vary, depending on the findings and personal risk[17].

Double-contrast barium enema is an alternative method for a full colon examination. This procedure requires complete bowel prepation. Patients with positive results receive a medical referral to undergo a colonoscopy. The sensitivity of this exam is lower than that of a conventional colonoscopy, and the impact on mortality reduction is uncertain. The recommended interval is every 5 years[17].

Computed tomography colonography (virtual colonoscopy) requires a complete bowel preparation. Patients with positive results receive a medical referral to undergo a colonoscopy. Despite radiation exposure, the risks are still quite low. The recommended interval is every 5 years[17].

If any of the above tests have positive results the patient receive a medical referral to undergo a colonoscopy[19].

Diferent guidelines recommend CRC screening for the average risk population, using anual FOBT, periodic FS, or colonoscopy, beginning at 50 years of age and continuing with follow-up exams until 75 years of age if negative results are found[15,16,20]. The high risk population may begin colonoscopy examinations at an earlier age, with shorter intervals than average at-risk patients.

COLONOSCOPY

Colonoscopy is largely used to evaluate the colon. In 2010, in the United States alone, it is estimated that over 3.3 million colonoscopy were performed[21]. As a major advantage, this test frequently allows for the treatment of some affections immediately upon diagnosis (*e.g.,* polypectomy, dilatation, hemostasis), behaving as a propedeutic and therapeutic method.

When properly executed by a well-trained professional, under adequate bowel preparation, a colonoscopy can be considered safe, precise, and easily tolerated by patients. As an operator-dependent method, where results may vary largely from one professional to other, quality indicators (QI) should be observed. Theses indicators were established by the American Society for Gastrointestinal Endoscopy (ASGE) and the American College of Gastroenterology Task Force on Quality in Endoscopy in 2006 and updated in 2012[21]. The aim is to improve the quality of the exam

and reduce complications, especially the number of missed lesions.

QI in colonoscopy were organized in three moments: before, during, and after the procedure. Every endoscopist must understand and target each item. Knowing the technique is not enough; it must also be well-executed. The main value of a colonoscopy as a screening method depends on the quality of the exam, as the findings (particularly polyps) are definitive to determining the interval of future colonoscopies.

QUALITY INDICATORS

Before the exam

Recommendation: Colonoscopies should be properly recommended and respect adequate intervals. Target: ⩾ 80%[19].

Informed consent: Patients or their guardian must sign an informed consent form. Risks, benefits, and alternative methods must be discussed and well understood before the exam. Target: ⩾ 98%[19].

Follow-up: Colonoscopy intervals must be respected, based on the findings (normal exam, polyps, cancer). Target: ⩾ 90%[19].

Inflamatory bowel disease surveillance - Chronic disease and ulcerative colitis: Adequate colonoscopy intervals must be respected. ASGE recommends that patients undergo an annual or bi-annual colonoscopy 8-10 years after the disease has been diagnosed due to a higher CRC risk[22]. Target: > 90%[19].

During exam

Quality of bowel preparation: Endoscopists must register the quality of bowel preparation (QBP) on the exam report. Terms used can be "excellent, good, fair, poor", "adequate ou inadequate" or the Boston Bowel Preparation Scale or Ottawa Bowel Preparation Scale can be used. QBP directly influences the interval between future colonoscopies. Exams with inadequate bowel preparations should be repeated at one-year intervals. Target: > 98%[19].

Adequate bowel preparation: Colon must be properly cleansed to perform a colonoscopy. The index of patients recomended to repeat the colonoscopy in one year should not exceed 15%. Target: > 85% patients with adequate bowel preparation[19].

Cecum intubation documented with photography: Exams must reach the cecum with the proper identification of anatomical masks and photographic documentation. Target: > 90% all exams and > 95% screening exams[19].

Adenoma detection rate in asymptomatic average risk patients: Adenoma detection rate (ADR) must be over 25% in screened populations with gender differentiation (> 30% for males and > 20% for females)[19].

Withdrawal time: Withdrawal time should be routinelly measured. Target > 98%[19].

Screening exams with normal results: The withdrawal time must be above 6 min. This indicator attempts to guarantee that the colon is appropriately examined, given that there is a clear association between the withdrawal time and the ADR. Target: > 6 min[19].

Polypectomy: Pedunculated polyps and sessile polyps of up to 2 cm should undergo endoscopic resection. Only in cases of failure or the impossibility of resection should these patients receive a referral for surgery. Target: > 98%[19].

After exam

Complications: Perforation incidence in all colonosocpies should be < 1:500 exams. In the case of screening colonoscopy, this value should be < 1:1000. The bleeding incidence for post-polypectomy should be < 1%, considering both imediate and late bleeding.

Management of post-polypectomy: > 90% of post-polypectomy bleeding cases should be resolved regardless of the type of intervention.

Surveillance recommendation: Interval before subsequent colonoscopy should be logged in the patient's medical records and sent to the patient after histological evaluation. Target: > 90%[19].

Ideally, all endoscopists should measure, register, and interpret their own quality indicators in colonoscopy. If an indicator target is not reached, the entire exam process should be analyzed in order to identify the failure and optimize the quality of the test. Colonoscopy, to be cost-effective as a CRC screening method, must be executed according to quality indicator parameters[19].

Bowel preparation for colonoscopy

In addition to playing an important role in the quality of the exam, bowel preparation is a common reason for low adherence to screening programs. During the pre-colonoscopy evaluation, the endoscopist should explain, in detail and as many times as necessary, the importance of colon cleansing, clarify the proper procedures to go about it, and elucidate the questions surrounding the subject in an attempt to demystify this step in the exam process.

The drugs used for bowel preparation may be different from one medical service to another. The choice must be based on the patient's profile, understanding capacity and comorbidities. Some principles, however, are applicable to all cases: (1) dietary restrictions: One to four days before exams, associated with the use of

Table 1 Recommendations for colonoscopy intervals according to previous exam findings[22]

Post-polypectomy follow-up	
No polyps	10 yr
Hyperplastic polyps in rectum/sigmoid	10 yr
Low risk adenoma	
1-2 tubular adenomas, < 10 mm	5-10 yr
High risk adenoma	
3-10 adenomas	3 yr
> 10 adenomas	< 3 yr
Villous adenoma(s) or tubular adenoma (s) ⩾ 10 mm	3 yr
Adenoma with high gradedysplasia	3 yr
Serrated polyps/lesions	
Serrated poliposis	1 yr
⩾ 10 mm or with dysplasia or traditional serrated adenoma	3 yr
< 10 mm in proximal colon and without dysplasia	5 yr

laxative drugs[23]; (2) anti-hemetic: Metoclopramide and ondansetron are commonly administered before laxatives to improve one's tolerance to bowel preparation. Evidence of the benefits of these drugs in tolerance and quality of bowel preparation are controversial[23]; (3) oral hydratation: Clear liquids should be ingested to prevent dehydration and optimize colon cleansing; (4) walking: Patients restricted to bed may have poor bowel preparation; (5) split dosis: In addition to the patient's better tolerance, split dose bowel preparation usually promotes better colon cleansing than does a single dose[23].

Post-colonoscopy follow-up

Colonoscopy intervals are a key-points in CRC screening. This interval is often a decision made by the physician who requested the first exam. However, not all non-endoscopists know how to correctly interpret the results of colonoscopy exams and hystological findings to determine the best interval. In these situations there is a tendency to shorten intervals. Unnecessary and early request of colonoscopy commits its cost-effectiveness, exposes patients to unnecessary risks, and operates the health care system.

The most recent recommendation regarding post-polypectomy surveillance was published in 2012[24] and adapted as a clinical decision tool from AGA in 2014. They recommend follow-up based on endoscopic and histological findings (Table 1).

Only recently, however, were serrated lesions surveillance recommendations actually published. Many endoscopists and even pathologists do not know this entity. However, it is known that this group of lesions passes through a different neoplasm transformation sequence at the proximal colon and, therefore, should undergo strict surveillance.

To follow the recommendations above, a complete exam (up to the ceccum) must be performed, with excellent quality of bowel preparation and the complete removal of all polyps. If any of these criteria are not attained, future examintervals must be reduced[22].

NEW SCREENING METHODS AND TECHNOLOGY

The need to detect colorectal adenomas and cancer has led to the implementation of new methods and in upon current colonoscopy technology.

Stool DNA testing (Fecal DNA testing - COLOGUARD)

Cologuard is the first stool-based test intended for the qualitative detection of colorectal neoplasia associated with DNA markers and with the presence of occult hemoglobin in human stool samples. A positive result may indicate the presence of CRC or advanced adenoma (AA), and should be followed by diagnostic colonoscopy. Cologuard is recommended in the screening of adults of either sex, 50 years of age or older, who are at a typical average risk for CRC. Cologuard is not intended to replace diagnostic colonoscopies or surveillance colonoscopies in high-risk individuals. The test is an automated assay aimed at detecting tumor-specific DNA changes, including aberrant methylated *BMP3* and *NDRG4*, a mutant form of *Kras*, beta-actin, and hemoglobin[23].

The safety and effectiveness of Cologuard was established in a clinical trial that screened 10023 subjects in a cross-sectional study at 90 sites throughout the United States and Canada. The trial compared the performance of Cologuard to the FIT, a commonly used non-invasive screening test that detects blood in stool samples. Of the 9989 participants evaluated in this study, 65 (0.7%) presented colorectal cancer and 757 (7.6%) presented advanced precancerous lesions (AA or sessile serrated polyps measuring ⩾ 1 cm in their largest dimension) in their colonoscopy exams. The sensitivity for detecting colorectal cancer was 92.3% with DNA testing and 73.8% with FIT (P = 0.002). The sensitivity for detecting advanced precancerous lesions was 42.4% with DNA testing and 23.8% with FIT (P < 0.001). The rate of detection of polyps with high-grade dysplasia was 69.2% with DNA testing and 46.2% with FIT (P = 0.004); the rates of detection of serrated sessile polyps measuring 1 cm or more were 42.4% and 5.1%, respectively (P < 0.001). Specificities with DNA testing and FIT were 86.6% and 94.9%, respectively, among participants with nonadvanced or negative findings (P < 0.001); these values were 89.8% and 96.4%, respectively, among those with negative results in colonoscopy exams (P < 0.001). The numbers of people who needed to be screened to detect one type of cancer were 154 with colonoscopy, 166 with DNA testing, and 208 with FIT. This method was not investigated in patients with a history of colorectal cancer, adenomas, or other related cancers, nor in patients who have been diagnosed with a relevant family (hereditary) cancer syndrome, such as hereditary non-polyposis colorectal cancer syndrome (HNPCCC or Lynch Syndrome) and in Inflammatory Bowel Disease[24]. The United States FDA approved Cologuard in 2014. It is important to stress

custs and availability of this method. Nevertheless, no data is available regarding changes in guidelines for CRC screening.

Check cap

Prepping for a colonoscopy requires people to swallow foul-tasting liquids designed to cleanse the colon. The preparatory process is burdensome and uncomfortable, and the colonoscopy procedure requires sedation. Currently, all available screening technologies require patients to compromise accuracy (as with fecal occult blood testing) or safety and comfort (colonoscopies and virtual CT scans). The newest method in colon cancer screening - capsule endoscopies - requires patients to go through bowel preparation that is even more intense than what they would normally go through for a colonoscopy. As a result, far too many people forgo screening or postpone it for years, thus diminishing their chances to be among those who survive colon cancer. It is clear that high-accuracy, non-intrusive screening methods are needed[25,26].

Check-Cap[27] is a new technology that is currently in development for CRC imaging. Check-Cap is a capsule device that produces images of the colon using low dose radiation (total dose equivalent to a single plain abdominal radiograph) and creates a 3-dimensional reconstructed image of the colon surface. The imaging capsule is swallowed by the patient and moves passively through the gastrointestinal tract. The capsule employs x-rays and patients drink an oral contrast solution to label fecal material, but they are not required to take any laxative preparation. Data from the Check-Cap is captured on a hand-worn data receiver, which is reviewed later by a gastroenterologist. Patients continue their daily routines after Check-Cap ingestion. The clinical performance of the Check-Cap device is currently under investigation (see also the Check-cap website).

According to Chatrath *et al*[28], more data are needed to establish the safety and efficacy of the Check-Cap System before its implementation as a CRC screening modality. However, their survey suggests that Check-Cap, or a device with similar characteristics and performance, could contribute significantly to screening adherence among patients who refuse to undergo a colonoscopy exam.

Colon capsule endoscopy

Colon capsule endoscopy was introduced in 2006 as a wireless, minimally invasive technique for the imaging of the large bowel that does not require sedation or gas insufflation. Its high procedural costs, the need for extensive bowel cleansing in order to gain reasonable polyp detection rates, and the inability to take biopsies, thus requiring additional conventional colonoscopy to confirm finding and remove polyps, has limited its use[29].

Colon capsule endoscopy has proven to be a feasible and exceptionally safe procedure to view the entire colon. Diagnostic accuracy of colon capsule endoscopy for the detection of significant colon polyps (> 6 mm) can be compared to conventional colonoscopy reported sensitivities and specificities for the detection of significant polyps in the range of 39.0%-87.5% and 54.0%-88.0%, respectively[30].

Current indications target patients on whom conventional colonoscopy cannot be or has been incompletely performed[29]. Other potential applications, such as colorectal cancer screening or the diagnostic surveillance of inflammatory bowel disease still require further clarification[31].

Technological advances in colonoscopy

Inadequate colon preparation, inability to reach the cecum (*e.g.,* incomplete colonoscopy), quick withdrawal times (< 6 min), and patient-related factors are some of the important causes of overlooked lesions. Despite the quality indicator in colonoscopy exams, the primary reason for missing colorectal adenomas and early cancers is poor visualization of the proximal aspect of colonic folds, anatomical flexures, and the area around the ileocecal valve[32]. These anatomical sites tend to be hidden from the standard forward-viewing colonoscope (140°-170° angle of view) and can often only be seen through endoscopist manipulation of the colonoscope, including efforts to flatten folds and straighten flexures, as well as the prolonged retroflexion of the colonoscope itself[32,33].

Third Eye® technology (retroscope and panoramic)

The Third Eye retroscope is an auxillary, through-the-scope device able to retroflex 180° while extended from the working channel of any standard colonoscope and is intended to detect polyps located on the proximal folds and at the anatomical flexures of the colon. A miniaturized video camera is located in the tip of the device as well as a light-emitting diode (LED) illumination that provides a continuous retrograde image during the examination process.

In two prospective, multicenter studies, including n = 249 and n = 298 human subjects, respectively, incremental polyp detection rates with the third eye were 14.8% for all polyps and 16.0% for adenomas, as compared to 13.2% for all polyps and 11.0% for adenomas in the second study[34,35].

Leufkens *et al*[36], in a prospective, randomized, international multicenter trial including n = 349 subjects, demonstrated an additional detection rate of 29.8% for all polyps and 23.2% for adenomas.

The third eye panoramic is a novel prototype, single-use video cap containing two side viewing lenses fitted onto a standard colonoscope. Only one feasibility study performed with 17 patients is available in the literature, but no data regarding polyp detection is available[37].

Fuse® full spectrum endoscopy® colonoscopy platform

The Fuse® Full Spectrum Endoscopy® colonoscopy platform is a standard flexible, reusable, reprocessable colonoscope that provides a high resolution, 330° field

of view achieved by the use of three imagers and LED groups positioned at the front and on the sides of the colonoscope tip, from which images are displayed on three contiguous video monitors[38]. Gralnek *et al*[38] compared the adenoma miss rates restuling from Fuse colonoscopies using a standard forward-viewing (SFV) colonoscopy, concluded that Fuse colonoscopy has the potential to improve the efficacy of CRC screening and surveillance (7.5%) as well as SFV (40.8%) adenoma miss rates, $P < 0.0001$. Dispite this, there is an important point to consider: The difficulty to resect polyps in very difficult positions.

Extra-wide-angle-view colonoscope

A prototype extra-wide-angle-view colonoscope (144°-232° lateral-backward-viewing lens that works in tandem with a standard 140° forward-viewing lens), when compared to standard forward-viewing colonoscopy (140° angle of view), is able to identify significantly more polyps (68% *vs* 51%; $P < 0.0001$)[39]. However, the same authors reported in an abstract manner that no significant difference was observed in adenoma detection rates (ADR mean 1.1 *vs* 1.0, per patient, $P = 0.36$) between the prototype and standard colonoscopes when compared to another study[40,41].

NaviAidTM G-EYETM balloon colonoscope

The NaviAid™ G-EYE™ Systemis is a colonoscope with a balloon, which can be inflated, attached to the flexible tip of a standard colonoscope[41]. The mechanical flattening and straightening of haustral folds with the inflated balloon allows one to view hidden anatomical areas, thus increasing adenoma detection.

Gralnek *et al*[41] assessed safety and feasibility of this device in a prospective cohort study performed with patients ho had received a referral for CRC screening and concluded that the NaviAid™ G-EYE™ balloon colonoscope appeared to be safe and feasible for usein colonoscopies.

Shpak *et al*[42] reported that the NaviAid™ G-EYE™ balloon colonoscopy detected 81% more adenomas ($P < 0.001$) than did the standard colonoscope. Moreover, there was only a 7.5% adenoma miss rate reported with balloon colonoscopy. In addition, the authors reported that the "first pass" adenoma detection rate using standard colonoscopy was 25.9% as compared to 40.4% using NaviAid™ G-EYE™ balloon colonoscopy (P = NS). Time to the cecum and cecal intubation rates were similar between groups. There were no adverse events reported.

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Targeting cancer testis antigens for biomarkers and immunotherapy in colorectal cancer: Current status and challenges

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Abstract

Colorectal cancer ranks third among the estimated cancer cases and cancer related mortalities in United States in 2014. Early detection and efficient therapy remains a significant clinical challenge for this disease. Therefore, there is a need to identify novel tumor associated molecules to target for biomarker development and immunotherapy. In this regard, cancer testis antigens have emerged as a potential targets for developing novel clinical biomarkers and immunotherapy for various malignancies. These germ cell specific proteins exhibit aberrant expression in cancer cells and contribute in tumorigenesis. Owing to their unique expression profile and immunogenicity in cancer patients, cancer testis antigens are clinically referred as the most promising tumor associated antigens. Several cancer testis antigens have been studied in colorectal cancer but none of them could be used in clinical practice. This review is an attempt to address the promising cancer testis antigens in colorectal cancer and their possible clinical implications as biomarkers and immunotherapeutic targets with particular focus on challenges and future interventions.

Key words: Cancer testis antigens; Colorectal cancer; Testis specific genes; Biomarkers; Immunotherapy

Core tip: Despite of the availability of enormous tumor antigens, there is a dearth of targets for biomarkers and immunotherapy for clinical cancer management. Cost-effectiveness and invasiveness associated with colonoscopy hinders its implications in less developed and developing countries. Colorectal cancer treatment including surgery and radiation has significant side effects on normal tissues. Recently a new category of antigens has been discovered which are expressed in tumor cells but not in normal tissues except the immuno-privileged testis. Targeting such antigens would be specific to the cancer cells with no deleterious

effects on normal cells. Scope of these magic bullets in colorectal cancer is discussed in this review.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in both men and women causing the global incidence of more than 1.2 million cases and 600000 deaths every year[1]. Histologically, Adenocarcinoma represents the most common type of CRC (about 95%) and other histotypes include neuroendocrine neoplasms, gastrointestinal stromal tumors, primary colorectal lymphoma, leiomyosarcoma, melanoma and squamous cell carcinoma. Clinically CRC can be classified as genetic/hereditary and non-hereditary/sporadic[2,3]. Hereditary CRCs can be further categorized as hereditary non-polyposis colorectal cancer and multiple polyps CRC which can be further sub-divided in to several subgroups depending upon the genetic basis[4]. Considering the slow development of CRC in comparison to other cancers, early detection of precancerous lesions may significantly improve the efficacy of therapeutic modalities and consequently, reducing the CRC-related deaths. Detection of CRC and its precursors (polyps) mainly relies upon colonoscopy but due to its invasive nature and the cost involved, it has limited applications in developing countries like India[5]. Less invasive detection test such as fecal occult blood test and stool analysis have low sensitivities which highlights the need to explore novel, sensitive, non-invasive biomarkers that can facilitate early detection, staging, disease progression and prediction of therapeutic outcome to determine optimized treatment for CRC. CRC treatment encompasses surgical or endoscopic resection, followed by second line of therapeutic interventions including chemotherapy, radiation and targeted therapy which often causes systemic toxicity and side effects. The toxicity incurred results in compromised quality of life of CRC patients and emphasizes the need to explore other therapeutic modalities such as immunotherapy. Immunotherapy is not commonly used as a treatment option but recent advances in tumor immunology and identification of tumor specific antigens reignited the interest in immunotherapy. Molecular identification of tumor antigens for immunotherapy may pave the way for novel therapeutics and their integration with conventional therapies can have substantial impact towards improving the outcomes of patients with CRC. In this context, cancer testis (CT) antigens are regarded as the promising targets for biomarker development and immunotherapy. The aberrant expression of CT antigens in cancer cells but not in other somatic tissues except testis forms the basis for their clinical implications as biomarkers and immunotherapy[6-8]. Over the past two decades, there is a huge influx of promising clinical studies which revealed significant future prospects to study these Cancer testis antigens for clinical translation.

DISCOVERY OF CANCER TESTIS ANTIGENS

The search for novel tumor associated antigens (TAA) for biomarker development and immunotherapeutic targets led to the identification of distinct categories of TAAs. Broadly, TAAs can be divided as tumor shared antigens [antigens present in both differentiated and cancer cells such as overexpressed antigens (MUC1)] and tumor specific antigens [antigens expressed specifically in cancer cells such as mutated antigens (p53, Ras)]. However, both tumor shared antigens and tumor specific antigens have their respective limitations which hamper their clinical implications. Tumor shared antigens cannot serve as targets for biomarker and targeted therapy because of their non-specific expression in other somatic tissues whereas tumor specific antigens are not abundantly expressed in cancers. Interestingly, in the early 1990s, a unique class of TAAs designated as CT antigens was identified which are primarily expressed in germinal cells of immuno-privileged testis and placenta and yet exhibits aberrant expression in multiple malignancies. The term cancer testis antigen (CTA) was coined by Old *et al*[9]. Melanoma associated antigen-1 (MAGE-1) was the first identified CT antigen which exhibited autologous T cell response in melanoma patients[10]. Later, it was shown to be expressed in several other malignancies as well[11,12]. The method (T cell epitope cloning) used for the identification of MAGE-1 was based on *in vitro* stimulation of peripheral blood cells with autologous tumor cells and subsequent gene identification by re-stimulation with cells transfected with cDNA libraries of tumor cells. Employing the same strategy, some other members of MAGE family (MAGE-A2, MAGE-A3), BAGE and GAGE-1 were identified[13-16]. Later, serological analysis of cDNA expression libraries (SEREX) was developed and led to the discovery of several CT antigens including synovial sarcoma/X breakpoint 2 (SSX-2) and New York oesophageal squamous cell carcinoma 1 (NY-ESO-1)[17-19]. Shortly after this, differential gene expression libraries were employed to compare total mRNA of normal tissues *vs* testis and resulted in the discovery of Sperm associated antigen 9 (SPAG9) and A-kinase anchor protein 4 (AKAP4)[20,21]. To compile the growing list of CT antigens, a database was developed by Ludwing Institute for Cancer Research (http://www.cta.Incc.br/)[22]. So far, more

than 180 members of CT antigens have been identified. Because of their exceptionally restricted expression in cancer cells, CT antigens are considered as excellent targets for diagnostic and prognostic biomarkers and immunotherapy. Theoretically, targeting these CT antigens will not cause any deleterious side-effects on normal cells[23]. However, there are many challenges to be addressed before translating their implications from benchside to bedside.

CANCER TESTIS ANTIGENS IN COLORECTAL CANCER

There is growing line of evidences indicating the expression of several cancer testis (CT) antigens in CRC. However, only few of the CT antigens exhibited high frequency of expression that could provide clinical applications. Chronologically, the first CT antigen characterized in CRC was MAGE family[10]. MAGE family of genes comprises of over 65 genes that are encoded from X chromosome[24]. Their function in germ cells of testis is poorly defined but they are highly immunogenic in cancer patients, generating both humoral as well as cytotoxic T cell responses. That's why there are several ongoing clinical studies to analyze the antitumor immunotherapeutic potential of MAGE antigens and/or their epitopes. It has been demonstrated that CRC tissues expressed some of MAGE antigen with low frequency, particularly, MAGE-A1 and MAGE-A3. Importantly, there are few contradictory reports regarding other members of MAGE family such as MAGE-A12, MAGE-B1 and MAGE-B2[24,25]. These antigens were demonstrated to have no expression in 34 CRC specimens tested by employing RT-PCR[24]. In an independent study by Burgdorf *et al*[26], 47% liver biopsy specimens with metastatic CRC were shown to express six distinct members of MAGE family (MAGE A-1, A-3, A-4, A-6, A-10 and A-12). Furthermore, MAGE-A12 expression was also shown in disseminated tumor cells (found in blood) of CRC patients[27]. These findings are corroborated with the earlier studies of MAGE antigen in melanoma where, MAGE-A1 expression was demonstrated in 48% of metastatic melanoma *vs* 16% of primary melanoma indicating the possible correlation of MAGE expression in late metastatic cancers[28]. Such inter-tumor variations in the expression of CT antigens are common. Yet, another study by Chen *et al*[29] in 250 CRC tissue specimens revealed that 36% of CRC specimens expressed at least one member of MAGE-A family. So far, MAGE-8 is the member of MAGE family that displayed highest frequency of expression (44%) in CRC tumor specimens[30]. Apart from MAGE family, in a cohort of 121 CRC patients, it was demonstrated that several CT-X antigens are expressed in CRC tissue specimens in contrast to matched adjacent non-cancerous tissues including *SCP-1* (1.7%), *SSX-2* (2.5%), *SSX-4* (2.5%), *SSX-1* (5.0%), *CT10* (6.6%), *NY-ESO-1* (9.9%), *MAGE-1*, (11.6%) *LAGE-1* (15.7%), *MAGE-4* (22.3%) and *MAGE-3* (27.3%)[31]. While most of the CT antigens are testis-restricted, some of them also show weak expression in normal tissues and are termed as testis specific genes. Some examples of testis specific CT antigens expressed in CRC includes *HSP105*[32], *GPA34*[33], *RAP80/UIMC1*[34], *TRAG-3*[35], *cTAGE* variants[36], *NY-CO-58/KNSL6*[37], *NW-BR-3*[38], *RBP1L1*[39], *KU-MEL-1*[40], *HSP60*[41], *RNF43*[42], *KIF18A* (*SW#108*)[43] and *TOMM34*[44]. Some of the CT antigens such as ADAM-1, FTHL17, GAGE-1 to 8, MORC, MMA-1A, MMA-1B, PAGE-1, RAGE-4, SCAGE-ac, SGY-1, SPO11, TAF2Q, TDRD, TEX15 and TPX-1are reported to be not expressed in CRC tissue sections[24,45-47]. However, these studies were conducted with small sample size hence confirmation in a large cohort is required to validate the results.

FUNCTIONS OF CT ANTIGENS

A lot of clinical research and trials have been conducted to explore the clinical potential of Cancer testis antigens but their role in carcinogenesis is still poorly-understood. CT antigens are proposed to be activated due to global demethylation associated with carcinogenesis[48,49]. In a different speculative proposal, CT antigen expression is considered as a part of gametogenesis gene activation program that imparts the oncogenic potential and malignant properties to a neoplastic cell[50,51]. At the same time, these CT antigens being highly immunogenic also render the cancer cells prone to the immuno-surveillance thereby raising a concern about their positive role in cancer progression. To understand the role of CT antigens in metastasis, Alves *et al*[52] compared the expression of CT antigens in primary and metastatic lesions and found no significant difference between the two sets indicating no correlation of CT antigen expression with metastasis. However, there are some CT antigens including prostate-associated gene 4, SCP-1, and SPANX, expression of which is directly correlated with liver metastasis of CRC[29]. Likewise, a well-characterized CT antigen, SPAG9 was also shown to be associated with early stages of CRC suggestive of its potential implications as an early diagnostic biomarker. In addition, the role of SPAG9 is also proposed in cellular migration and invasion as depicted by reduced migratory and immiratory potential of CRC cells post-siRNA mediated downregulation of SPAG9[53]. Another testis-selective cancer testis gene, *TSP50*, was demonstrated to be associated with poor prognosis in CRC[54].

Few of the CT antigens were shown to have functional relevance as well. For example, MAGE family members are proposed to be involved in modulation of p53[55]. Outside the *MAGE* gene families, antiapoptotic properties of GAGE-7 have been reported, as GAGE-7C was shown to render a human tumor-derived cell line resistant to apoptosis induced by interferon-γ (INF-γ) or Fas and also prevented killing induced by taxol

and ionizing radiation[56]. Yet another CT-X antigen, AKAP4 interacts with cyclic adenosine monophosphate dependent protein kinase A and is involved in sperm motility[57]. In contrast to our very limited knowledge of CT-X function, most of the non-X CT antigens have well-defined roles in spermatogenesis and fertilization. For instance, SCP-1, is a part of the synaptonemal complex and is involved in chromosome pairing during meiosis[58], OY-TES-1 acts in acrosin packaging in the acrosome of sperm heads[59], SPO11 is a meiosis-specific endonuclease[60] and the brother of the regulator of imprinted sites is a recently described paralog of the epigenetic modulatory protein CCCTC-binding factor (CTCF), and is involved in the epigenetic reprogramming occurring during spermatogenesis[61]. SPAG9 is a sperm-associated JNK-binding protein that has a role in spermatozoa-egg interaction[62]. Although, some of the CT antigens such as MAGE family members and NY-ESO-1 have been well characterized even in clinical studies and trials, we still have limited knowledge about how these CT antigens contribute in cancer cell development and evolution. In this context, there are reports suggesting that CT antigens are intrinsically disordered proteins which play important roles in transcriptional regulation and signaling *via* regulatory protein networks in cancer cells[63]. Considering the wide ranged expression of more than 170 CT antigens in tumors of different histological origins at different stages of disease progression, it can be speculated that these testis specific genes are activated as a part of dedifferentiation program during carcinogenesis and are crucial for tumor development. During tumorigenesis, neoplastic cells undergo tremendous metabolic stress and bypass several anti-tumor processes including apoptotic signals and immune attack[64]. Under such stressful conditions, it would be quite inappropriate for a cancer cell to channelize its energy towards gene activation and formation of proteins which are irrelevant to the cancer cell. In fact, cancer cells would selectively use the part of the cellular energy to activate a set of gene expression program involved in gametogenesis and embryogenesis, in order to counteract stress signals and attain the malignant characteristics such as motility and invasion which eventually helps the cancer cells to thrive under stressful conditions. As for now, the functional relevance of CT antigens might not be well characterized but their role in carcinogenesis seems to be very vital.

CURRENT CHALLENGES

Although CT antigens are undoubtedly the sure-shot promising targets for various clinical interventions based on their unique expression patterns, there is a marked variation in the expression frequencies observed by different studies. *CT* genes are classified into three major groups based on their expression pattern which include testis restricted, testis/brain restricted and testis selective. This classification is based on genome wide analysis of gene expression data that showed out of 153 *CT* genes, 39 genes are present only in adult testis and placenta classified as testis-restricted, 14 genes are expressed in brain termed as testis/brain-restricted, and 85 genes, ranked in testis selective based on the ratio of testis/placenta expression relative to normal adult tissue[65]. An example of such discrepancy is clearly represented by CAGE-1. Shi *et al*[66] reported the expression of *CAGE* mRNA in 30.8% colorectal tumors whereas, an independent study revealed *CAGE* expression in 90% tissue specimens by RT-PCR. Importantly, both groups found weak expression of CAGE in a portion of normal matched tissue specimens. Such discrepancies might be due to the differences in the experimental procedures, epidemiological variations, and inter and intra-tumor heterogeneity.

There are few CT antigens which are reported in CRC cell lines but not in tissue specimens. Some studies have reported the expression of *CTA* genes in CRC cell lines but not within CRC tissue. Such examples and their expression frequency in CRC cell lines are *MCAK* (5/6), *TAG-1* (4/4), *TAG-2A* (2/4), *TAG-2B* (1/4), *TAG-2C* (2/4)[67,68]. Some of these genes are quite promising however, further studies in tissue specimens are needed to establish their clinical utility. To validate the expression of above mentioned CT antigens, recently, MCAK expression was examined in paired colorectal tumor tissue samples and the corresponding normal tissues of 120 patients. Results showed the expression of MCAK in normal tissues and significant increased expression in CRC tissue specimens which correlated with poor prognosis and lymph node metastasis[69]. The expression of MCAK in normal tissues puts a significant challenge for its clinical implications as a diagnostic biomarker. Yet, another CT antigen POTE was shown to be differentially expressed in 6 of 6 prostate, 12 of 13 breast, 5 of 5 colon, 5 of 6 lung, and 4 of 5 ovarian cancers[70]. However, the expression of *POTE* gene was also confirmed by *in situ* RNA hybridization in normal tissues including prostate, ovary, testis, and placenta[71]. Thus, an important control that should be taken in to account while determining the expression of CT antigens is matched adjacent non-cancerous tissues (ANCT) especially for clinically relevant data. It is also noteworthy to point out the fact that ANCT may not be considered as "normal" because these tissues might have underlying, undiagnosed disease. In this regard, Chen *et al*[72] reported variations in the expression of CTA genes in sets of disease free normal tissues suggesting that CT antigens might be expressed before clinical manifestation or histopathopathological changes in the tissues. Also, depending on the sampling method, ANCT can be a section of the tumor with no morphological signs of hyperplasia but may have underlying genomic lesions that cause CTA expression. This inherent heterogeneity in a clinical challenge while exploring the clinical potential of CT antigens.

In the earlier years of CT antigens identification by SEREX, most of the studies in CRC were focused at gene expression analysis which restricted their clinical

translation because of the variability observed in gene and protein expression of certain CT antigens. In this context, the gene expression of *NY-ESO-1* in CRC was established by several studies ranging from 6% (34/567[49] to 9.9% (12/121)[31] with few exceptions. In particular, Chen *et al*[19] reported no expression of *NY-ESO-1* in CRC (0/16) by employing RT-PCR. In terms of protein expression also, NY-ESO-1 expression was detected in 8.3% (1/12) by immunohistochemical analysis[31] which is again contradicted by another study showing no NY-ESO-1 protein expression[73]. MAGE is another CT antigen which is well-studied in CRC. Immnohistochemical analysis of MAGE family members revealed by Jungbluth *et al*[74] revealed no expression of MAGE family in 15 CRC tissue specimens tested. Later, serological analysis of CRC patients demonstrated anti-MAGE-A3 antibodies in 8% of CRC patients indicating the MAGE antigen expression at least, in a fraction of CRC patients[37]. Such variations in the analysis of expression of CT antigens might also stem from the demographic variations but it is quite important to validate the protein expression in clinical samples to minimize the genomic instability driven discordances. Confirmed antigen expression also paves the way for future immunotherapeutic studies towards designing the better vaccines to improve the mounted immune response.

SPAG9: A CT ANTIGEN THAT STANDS OUT AS A BIOMARKER

Over the past two decades, there is an emergence of innumerable biomarkers and therapeutic targets for various malignancies but it is rare to find a tumor antigen that is expressed in almost all cancers. Interestingly, there is only one CT antigen that appears to be most promising biomarker and therapeutic target among all other antigens. This testis specific gene called as Sperm associated antigen 9 was first identified by Shankar *et al*[20], in 1998 as a testis specific gene having unique palindromic sequences and encoding a leucine zipper dimerization. It is a single copy gene encoded from chromosome 17q21. Further characterization of SPAG9 revealed it as a c-Jun N-terminal kinase-interacting protein involved in MAPK pathway[62,75]. The first report showing the expression of SPAG9 in cancer cells demonstrated its mRNA and protein expression in 90% epithelial ovarian cancer (EOC) tissue specimens but not in matched ANCT specimens. In addition 67% EOC patients exhibited circulating antibodies against SPAG9 suggesting its implications as an immunotherapeutic target[76]. Later, same group demonstrated SPAG9 expression (both mRNA and protein) in renal cell carcinoma[77], cervical cancer[78,79], breast cancer[80,81], thyroid cancer[82], chronic myeloid leukemia[83], colorectal cancer[53] and bladder transitional cell carcinoma[84] establishing its clinical utility as a biomarker. In CRC *per se*, SPAG9 expression was detected in 74% of CRC tissue specimens with no discrepancy in gene and protein expression[53]. Further, humoral response was generated in 70% CRC patients. In addition, depletion of SPAG9 in colorectal cancer cells resulted in inhibition of cellular proliferation, migration and invasion *in vitro*[53]. Recently, SPAG9 serum levels were determined in endometrial cancer patients and the cut off levels of 15 ng/mL could provide the sensitivity of 74% and specificity of 83% to detect endometrial cancers[85,86]. SPAG9 expression was also found in brain cancer/astrocytoma[87,88], prostate cancer[89,90], hepatocellular carcinoma[91], lung cancer[92], vulva cancer and non skin melanoma[93]. Till date, SPAG9 is the most versatile and promising CT antigen that can be clinically translated for biomarker development and immunotherapeutic use. To the best of our knowledge, none of the other CT antigens studied so far have showed such a consistency in clinical data among different studies.

Mechanistically, SPAG9 is involved in cellular proliferation, probably by regulating cyclin proteins as reported in hepatocellular carcinoma and prostate cancer[89-91]. In prostate cancer, the role of SPAG9 is not only restricted to cellular growth/proliferation but also in angiogenesis[90]. In astrocytoma and prostate cancer, SPAG9 is associated with cellular migration and invasion by modulating MMPs[88,90]. Table 1 summarizes the gene and protein expression of SPAG9, serological analyses of SPAG9 antigen levels and antibody responses in different cancers studied so far. There is a growing line of evidences that SPAG9 is indeed important for oncogenic properties of cancer cells and contributes towards tumor progression. Hence, future studies to establish its clinical implications in a large cohort of patients are warranted.

IMMUNOTHERAPEUTIC IMPLICATIONS OF CT ANTIGENS IN COLORECTAL CANCER

The search for tumor-specific and tumor-abundant antigens is still going on to facilitate the rational design of cancer immunotherapy strategies. In CRC, while conventional therapy such as chemotherapy and radiation are useful for the majority of patients, it is not good enough for patients with relapsed cancer and for those of advanced CRC stages. Chemoresistance is another problem that develops with increased exposure of conventional chemotherapy. At that time, immunotherapy can be a good choice to integrate with the conventional interventions to kill the residual tumor cells, strengthen the immune system and further improve the survival rate. There have been limited studies exploring the relevance of CT antigens for immunotherapeutic purposes in CRC patients. MAGE

Table 1 Expression and humoral response of SPAG9 in various cancers demonstrating its clinical relevance as a biomarker and mmunotherapeutic target

Cancer	SPAG9 mRNA expression *n* (%)	SPAG9 Protein expression *n* (%)	SPAG9 expression in matched adjacent non-cancerous tissues	Serological detection of SPAG9 antibodies *n* (%)	Expression in cell lines	Clinical relevance and concluding remarks	Ref.
Epithelial ovarian cancer	18 (90)	18 (90)	No	20 (67)	A-10, SKOV-6, Caov-2	No correlation between SPAG9 expression and tumor stages	[76]
Cervical cancer	54 (82)	54 (82)	No	53 (80)	SiHa, HeLa, CaSki, C-33A	SPAG9 expression in cervical tissue specimens was associated with early stages of cervical cancer Ablation of SPAG9 in cervical cancer cells resulted in inhibition of cellular proliferation, migration an invasion *in vitro* and *in vivo*	[78,79]
Breast cancer	88 (88)	88 (88)	No	80 (80)	MCF-7, BT-474, SK-BR-3, MDA-MB-231	SPAG9 expression was not correlated with tumor stages but showed significant association with early grades. In addition, High SPAG9 immunoreactivity score correlated with lymphovascular invasion and high risk of recurrence SPAG9 ablation in triple negative breast cancer cells resulted in inhibited cellular proliferation, colony formation , migration and invasion and reduced tumor growth *in vivo*	[80,81]
Renal cell carcinoma	46 (88)	46 (88)	No	40 (77)	A704, ACHN, Caki-1, Caki-2 NII-AKS395 NII-AKS413 NII-AKS414	SPAG9 expression was significantly associated with lymph node invasion and metastasis in clinical specimens siRNA mediated SPAG9 downregulation inhibited cellular proliferation, migration and invasion *in vitro* and *in vivo*	[77]
Thyroid cancer	108 (78)	108 (78)	No (not in multinodal goitres and follicular adenoma samples tested)	92 (78)	WRO, FTC-133, BC-PAP, 8305C	Both SPAG9 expression and humoral response were associated with early stages of thyroid cancer Depletion of SPAG9 resulted in inhibition of cellular growth and colony forming ability of thyroid cancer cells	[82]
Fine needle aspirates of PTC	6 (38) PTC	-	8 (40) benign nodules	-	-	No clinical relevance	[94]
Endometrial cancer	-	Serum SPAG9 antigen (with cut off 17 ng/mL) was used to determine endometrial malignancy (sensitivity = 74%, specificity = 83%)	No SPAG9 levels found in women benign diseases	36 (72)		No significant association of serum SPAG9 antigen levels with histological type, FIGO stage, tumor grade, size, myometrial invasion, lymphovascular space invasion, cervical involvement, adnexal involvement, peritoneal cytology or lymph node status of endometrial tumors Serum SPAG9 levels were found to be negatively correlated with tumor grades	[85,86]
Colorectal cancer	58 (74)	58 (74)	No	38 (70)	COLO 205, HCT 116	SPAG9 expression was correlated with early stages but not with grades, lymph nodes positivity or metastasis SPAG9 expression depletion resulted in decreased tumor growth *in vivo* and reduced migration and invasion *in vitro*	[53]
Bladder transitional cell carcinoma	101 (81)	101 (81)	No	96 (77)	HTB-2, HTB-9, HTB-1, UM-UC-3	High SPAG9 expression (> 60% SPAG9 positive cells) was found to be significantly associated with superficial non-muscle invasive stage and low grade tumors *In vitro* downregulation of SPAG9 caused G_0-G_1 arrest, inhibition of cellular proliferation, migration and invasion	[84]

Chronic myeloid leukemia	106 (88)	106 (88)	No	106 (88)	K562, KCL-22	No correlation with stages	[83]
Prostate cancer	-	54 (36.5)	No	-	REPW-1, PC-3, DU-145	SPAG9 expression in clinical specimens is associated with advanced tumor stages and gleason score SPAG9 could supercharge prostate cancer proliferation with cyclin D1 and cyclin E upregulation SPAG9 depletion caused reduction in angiogenesis and migration	[89,90]
Brain cancer (Astrocytoma)	-	63 (60)	No	-	SW1783, SF295, TG905, U251 and U87 (SPAG9 not expressed in A172)	SPAG9 expression was found to positively correlated with tumor grades. SPAG9 depletion was accompanied by downregulation of MMP9 suggesting the possible role of SPAG9 in cellular invasion. PODXL is a critical mediator of the promoting effect of SPAG9 on astrocytoma cell invasion, possibly through upregulation of MMP9 expression	[89,90]
Hepatocellul-ar carcinoma	-	47 (48.5)	No	-		High SPAG9 expression is strongly correlated with multiple tumors, advanced TNM stage, tumor size, serum AFP levels and tumor relapse SPAG9 modulates cell proliferation through cyclin regulation	[91]
Non small cell Lung cancer		63 (52.5)	No	-	A549, H1299	Overexpression of SPAG9 correlated with poor tumor differentiation, advanced p-TNM stage, nodal metastasis and poor overall survival SPAG9 might act as an important promoter in lung cancer progression and invasion *via* MMP9 regulation and JNK activation	[92]
Non melanoma Skin cancer	-	18 (90) basal cell carcinoma and 18 (82) squamous cell carcinoma	weak SPAG9 expression in 25% normal skin cases	-	-	Significant negative correlation between SPAG9 expression and tumor grade and significantly higher H score values in grade I SCC cases	[93]

EOC: Epithelial ovarian cancer; SPAG9: Sperm associated antigen 9; FIGO staging: Federation of Gynecology and Obstetrics staging; G_0: G_0 phase of cell cycle; G_1: G_1 phase of cell cycle; REPW-1: Human normal prostate epithelial cells; cyclin D1: Cyclin D1protein; cyclin E: Cyclin E protein; SCC: Squamous cell carcinoma; PTC: Papillary thyroid cancer.

antigens have also been tested as immunotherapy targets in phase II clinical trials in metastatic CRC patients and results were promising with low toxicity. This vaccine was artificially synthesized by using helper/killer-hybrid epitope long peptide of MAGE-A4 cancer antigen and was used in combination with OK432 and Montanide ISA-51[95]. In CRC, HSP105 also showed promising results in mouse model system in preclinical investigation[32]. In addition, in a recent phase I clinical trial for advanced CRC using combination of chemotherapy and immunotherapy illustrated some limited positive responses[96]. This limited success rate of immunotherapy might be attributed to the low frequency of CT antigen expression in CRC tissues. However, we have several other new CT antigens which are not yet characterized in CRC patients. Recently emerged promising CT antigens such as SPAG9 antigens can be targeted in combination with other CT antigen to improve the efficacy of immunotherapeutic vaccines. Conceptually, these testis specific genes might provide new clinical tools as we move even closer to an era of more personalized therapeutics.

CONCLUSION

Our current understanding of CT antigen expression and immune response in CRC is still in early stages of translational clinical research. Compiling together, there is a scope for improvement despite the low frequency of expression of several CT antigens in CRC. It might be related to the fact that only a sub-population of CRC patients can derive the benefit from CT antigens based therapies or multi-biomarker approach is the answer to improve the clinical management through detection, prediction and prognosis. The combination of CT antigens that can be employed for this purpose needs to be explored. With the advent of personalized therapy, CT antigens can provide an option to the clinicians to design the targeted and tailored medicine for the patients to obtain maximum benefit from their therapeutics. Analysis of antigen specific humoral and cellular response will shed more light towards designing the optimal therapeutic regimen for the patients. In conclusion, CT antigens are promising targets and might provide a new avenue for improved biomarkers

and therapeutics.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Outcome of curative resection for perihilar cholangiocarcinoma in Northeast Thailand

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Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at nkhuntikeo@gmail.com. Participants gave informed consent for data sharing.

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Abstract

AIM: To examine survival outcomes of perihilar cholangiocarcinoma (PCCA) resection including mortality, morbidity and prognostic factors.

METHODS: Multivariate analyses were carried out based on the survival data of all patients with histologically confirmed PCCA who underwent curative resection at Srinagarind Hospital from January 2006 to December 2011.

RESULTS: There were 29 (19%) cases of intrahepatic CCA that involved hilar and 124 (81%) with hilar bile-duct cancer. R0 resection was carried out on 66 (43.1%) patients of whom 50 (32.7%) also had lymph node metastasis. The other patients underwent R1 resection. The overall 5-year survival rate was 20.6% (95%CI: 13.8-28.4) and median survival time was 19.9 mo. Postoperative mortality was 2%, and 30% of patients had complications. Patients without lymph node metastasis were 60% less likely to die than those with metastasis. Achieving R0 led to a 58% reduction in the chance of mortality as compared to R1.

CONCLUSION: To achieve a better survival outcome, focus should center on performing radical surgery and detection of patients with early stage cancer.

Key words: Perihilar cholangiocarcinoma; Thailand; Curative resection; Five-year survival; Prognostic factors

Core tip: Cholangiocarcinoma is usually fatal because detection most commonly occurs during late stage disease. Early detection leads to a substantially better survival outcome Thus, priority should be placed on early stage detection allowing curative radical surgery.

Titapun A, Pugkhem A, Luvira V, Srisuk T, Somintara O, Saeseow O, Sripanuskul A, Nimboriboonporn A, Thinkhamrop B, Khuntikeo N. Outcome of curative resection for perihilar cholangiocarcinoma in Northeast Thailand. *World J Gastrointest Oncol* 2015; 7(12): 503-512 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i12/503.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i12.503

INTRODUCTION

Cholangiocarcinoma (CCA) is the most common primary liver cancer in the northeast of Thailand where it has its highest incidence worldwide[1,2] and where it is one of the major causes of death. CCA is divided into three types according to the location of the primary tumor. Perihilar cholangiocarcinoma (PCCA) is the most common type. It occurs when the tumor originates or includes the confluence of the hepatic duct and accounts for 67% of all cases. It is followed by distal cholangiocarcinoma and intrahepatic cholangiocarcinoma which account for 27% and 6% of cases, respectively[3].

PCCA requires a major hepatectomy with bile duct resection, caudate lobectomy and regional lymphadenectomy to achieve a free margin resection and the best survival outcome[4-6]. Due to the anatomical complexity of the hepatic hilum, early lymphatic metastasis and vascular invasion, various techniques have been used to achieve margin negative resection (R0) to improve survival outcome and to reduce operative mortality[7].

Because caudate bile ducts are open to hepatic duct confluence, most PCCA have microscopic tumor invasion to the caudate lobe[8]. Caudate lobectomy is necessary to achieve margin negative resection and better survival without increasing post-operative mortality[9,10]. Preoperative biliary drainage (PBD) is performed to relieve suffering from jaundice or cholangitis as it reduces postoperative morbidity and mortality in patients who undergo right hepatectomy, who have a future liver remnant volume of $\leqslant$ 30%[11,12] and a preoperative total bilirubin of $\geqslant$ 170 μmol/L (10 mg/dL)[13]. It is not recommended that PBD be performed systemically due to potential complications such as sepsis, vascular injuries, and tumor seeding. Preoperative portal vein embolization (PVE) is widely used for pre-operative preparation to induce hypertrophy of remnant liver. It has resulted in minimized post-operative complications especially in cirrhotic patients[14] or future liver remnant volume (FLR) < 25% in normal liver[15]. Right or left trisectionectomy is performed for Bismuth IV PCCA and patients show an increasing number of margin negative resections and also improved long-term survival[16-18]. Combined major hepatectomy with portal vein and hepatic artery resection have high R0 resection with acceptable mortality[19,20].

Although outcomes of curative resection for PCCA have continuously been improving over the last decade, the 5-year survival rate is unsatisfactorily low. This has also been the case for Thailand where there are few studies on the outcome of curative resection for PCCA and 5-year survival, even though advances have been made in early stage diagnosis and surgical procedures. This study addresses this limitation in Thailand. It is based on a large cohort size and examines survival outcomes of PCCA liver resection including mortality, morbidity and prognostic factors.

MATERIALS AND METHODS

Between January 2006 and December 2011, 153 patients from northeast Thailand received curative hepatic resection for PCCA at Srinagarind Hospital, Khon Kaen, the tertiary referral center for northeast Thailand. Data relating to patient survival are examined and compared. We excluded patients who had gross residual tumor and required palliative procedures. Patient

Table 1 Characteristics of the 153 of patients with perihilar cholangiocarcinoma presented as number and percentage unless specified otherwise

Characteristics	*n* (%)
Gender	
Male	113 (73.9)
Female	40 (26.1)
Mean ± SD of age (yr)	56.8 ± 8.22
Symptoms	
Median (min-max) duration (d)	60 (3-300)
Abdominal discomfort	92 (60.1)
Jaundice	90 (58.8)
Weight loss	37 (24.2)
Fever	18 (11.8)
Co-morbidity	32 (20.9)
Hypertension	14 (9.1)
Diabetes mellitus	15 (9.8)
Renal failure	2 (1.3)
Chronic obstructive pulmonary disease	4 (2.6)
Hepatitis	3 (1.9)
Others	6 (3.9)

survival was measured from the day of operation until death or the end of the study on 19 September 2014 so that there was a minimum follow-up of 33 mo. This study was approved by Institutional Ethics Committee, Khon Kaen University, No. 571283.

We report continuous data as mean ± SD, and range. Categorical variables were reported as percentages. Median survival time and the 5-year survival were estimated using Kaplan-Meier methods. Log-rank test was used to compare survival experience between selected groups of patients. Cox proportional hazard model was used to determine factors affecting the overall survival. Initially we explored the effect of each factor, independently, on the survival. Based on the results from these bivariate models, all variables with a p-value of less than 0.2 were included into the full model as the starting of the model fitting using backward elimination processes. The least significant factor was removed from the full model, one at a time, where its effect on the model was assessed using a likelihood ratio test. In the final model that contained all factors that were statistically significant survival was used as the basis to estimate the hazard ratios and their 95%CI. Tests for proportional hazard model assumptions and model's goodness-of-fit were also implemented. A *P* value of £ 0.05 was considered statistically significant. All statistical analyses were done using Stata version 13 (Stata Corp, College Station, TX, United States).

RESULTS

Of the 153 patients included in the analysis, 113 (73.9%) were male and 40 (26.1%) were female with a combined mean age of 56.8 ± 8.2 years (Table 1). Most of the patients presented with abdominal discomfort 92 (60.1%) and jaundice 90 (58.8%).Thirty seven patients showed weight loss (24.3%) and 18 had fever (11.8%).The median duration of symptoms was 60 d (range 3-300 d). Thirty two patients had co-morbidity that included hypertension, diabetes, renal failure, pulmonary disease and hepatitis.

Table 2 Type and staging of perihilar cholangiocarcinoma

Characteristics	*n* (%)
Type	
Bismuth I	5 (3.3)
Bismuth II	9 (5.9)
Bismuth IIIa	65 (42.5)
Bismuth IIIb	41 (26.8)
Bismuth IV	4 (2.6)
Right intrahepatic mass involving hilar	22 (14.4)
Left intrahepatic mass involving hilar	7 (4.6)
Staging	
Stage 0	9 (5.9)
Stage I	9 (5.9)
Stage II	65 (42.5)
Stage IIIa	17 (11.1)
Stage IIIb	47 (30.7)
Stage IVa	4 (2.6)
Stage IVb	2 (1.3)

Classification of resected tumors

Most tumors originated from the hepatic hilum, namely, 5 patients (3.3%) with Bismuth type I, 9 (5.9%) Bismuth type II, 65 (42.5%) Bismuth type IIIa, 41 (26.8%) Bismuth type IIIb, and 4 (2.6%) Bismuth type IV. There were 29 patients who had intrahepatic mass with hilar invasion (Table 2).

According to the American Joint Cancer Committee (AJCC) staging for perihilar bile duct tumors 7th edition patients were group as carcinoma in situ (*n* = 9, 5.9%), stage I (*n* = 9, 5.9%), stage II (*n* = 65, 42.5%), stage IIIa (*n* = 17, 11.1%), stage IIIb (*n* = 47, 30.7%), stage IVa (*n* = 4, 2.6%), and stage IVb (*n* = 2, 1.3%), patients who had intrahepatic tumor were also included in this staging system. Four patients with stage IVa, Bismuth type IV were classified as T4, and all underwent curative trisectionectomy. Two patients with stage IVb had celiac lymph node metastasis all of whom underwent curative resection with extensive lymphadenectomy.

Patients who had co-morbidity were group as carcinoma in situ (*n* = 3, 9.4%), stage I (*n* = 1, 3.1%), stage II (*n* = 18, 56.2%), stage IIIa (*n* = 3, 9.4%), stage IIIb (*n* = 7, 21.8%) and none were in stage IV.

Histological findings showed 91 papillary adenocarcinoma (59.4%), 58 tubular adenocarcinoma (37.9%), 1 adenocarcinoma with squamous metaplasia (0.6%), 2 poorly differentiated adenocarcinoma (1.3%), and 1 undifferentiated carcinoma (0.6%).

Preoperative preparation

The 90 patients who presented with jaundice had a mean total bilirubin level of 17.6 mg/dL. A total of 37 patients (41%) underwent preoperative percutaneous biliary drainage with a decrease in total serum bilirubin to 3.9 mg/dL on the day before surgery. Fifty-three

Table 3 Preoperative biliary drainage in jaundice patients (*n* = 90)

	Biliary drainage 37 (41%)	Non-biliary drainage 53 (59%)	*P* value
TB at presentation (mean ± SD)	19.4 ± 10.5	12.1 ± 15.8	0.06
TB at day before surgery (mean ± SD)	3.9 ± 4.1	17.1 ± 14.3	0.04
Post-operative complication	35%	37.70%	0.80
Length of hospital stay (mean ± SD)	15.6 ± 9.7	16.3 ± 7.6	0.56
Mortality (%)	0	2 (3.7)	-
Median survival time, mo (95%CI)	30.4 (16.3-58.7)	17 (12.2-26.6)	0.02
5-year survival rate, %(95%CI)	29.5 (14.1-46.7)	7.3 (0.9-22. 4)	0.02

TB: Serum total bilirubin.

patients (59%) underwent surgery without preoperative drainage. These had a total serum bilirubin of 17.1 on the day before surgery (Table 3).

Combined preoperative portal vein embolization (PVE) was performed on 10 patients (6.5%) who had an estimated small FLR, right hepatectomy on 4 patients, extended right hepatectomy on 5 patients and one patient underwent left hepatectomy.

Operative procedure

Major hepatectomy was carried out on 145 patients comprising 63 patients who underwent right hepatectomy, 35 patients who underwent extended right hepatectomy including trisectionectomy and 47 patients who had a left hepatectomy. Extrahepatic bile duct resection alone was performed in 8 patients due to limited tumor involvement. Mean operative time was 326 ± 125 min, blood loss was 1274.2 ± 1312.5 mL and length hospital stay was 15 ± 8.2 d.

Post-operative complications occurred in 70.3%, 37.1%, 29.8% of right hepatectomy, right extended hepatectomy and left hepatectomy patients, respectively. There were no significant differences in operative time, blood loss or length of hospital stay between the major hepatectomy procedures.

Combined portal vein resection was performed in 12 patients (7.8%). Mean operative time was 463.3 ± 101.1 min, blood loss was 1804.2 ± 1664.6 mL and length hospital stay was 17.7 ± 8.7 d.

R0 resection was achieved 31.7%, 37.5%, 51.4% and 53.2% of right hepatectomy, bile duct resection, right extended hepatectomy and left hepatectomy patients, respectively. Overall R0 resection was achieved in 66 (43.1%) of patients and R1 (indicated by microscopic residual tumor) in 87 (56.6%) of patients (Table 4).

Operative morbidity and mortality

Mortality at 30 d was 2% with 1 patient dying of postoperative bleeding, renal failure and myocardial infarction, and 2 patients dying after being discharged from hospital. Post-operative complications occurred in 46 patients (30%). Four patients had post-operative bleeding (2.6%) and all underwent re-operation. There were 13 patients who had bile leakage (8.5%) all of whom received conservative treatment. Eleven patients had intra-abdominal collection, 7 of whom underwent percutaneous drainage. There were pleural effusions in 12 patients (7.8%), 9 (5.9%) had wound infection, 7 (1.2%) developed pneumonia, and 2 (1.3%) had urinary tract infection. Jaundice patients who underwent preoperative biliary drainage had comparable post-operative complications, mortality and length of hospital stay to non-biliary drainage patients (Table 3).

Survival analysis

Median survival time after curative resection was 19.9 mo (95%CI: 14.2-23.8; Figures 1 and 2A). Patients with R0 had the longest median survival time of 40.2 (95%CI: 22.4-57.9) mo. The overall survival rate was 68% (95%CI: 60%-74.7%) after 1 year, 33.7% (95%CI: 26.3%-41.2%) after 3 years, and 20.6% (95%CI: 13.8%-28.4%) after 5 years (Figure 3).

Survival according to stage

Following are survival rates according to the AJCC staging for perihilar bile duct tumor 7th edition 2010, including all 153 patients. Stage 0, 5-year survival rate was 71.1% (95%CI: 23.3%-92.3%); Stage Ⅰ, 5-year survival rate was 51.8% (95%CI: 16.4%-78.8%); Stage Ⅱ, 5-year survival rate was 25.3% (95%CI: 15% -37%); Stage ⅢIa, 5-year survival rate was 17.6% (95%CI: 4.3%-38.3%); Stage ⅢIb, 5-year survival rate was 2.68% (95%CI: 0.2%-11.8%); Stage IVa and stage Ⅳb had no 5-year survival rate.The patient groups with an early stage showed significantly better long-term survival ($P < 0.001$).

Twenty-nine patients who had intrahepatic tumor all had tumor with periductal invasion or nodal metastasis, according to AJCC staging for intrahepatic bile duct tumor 7th edition 2010, they were in stage IVa. The 5-year survival rate was 20.1%, median survival time was 14.9 mo comparable to overall survival ($P = 0.39$)

Survival according to residual tumor status

R0 resection resulted in a significantly better survival with 5-year survival rate of 35.6% (95%CI: 23.1%-48.3%) compared to R1 resection which resulted in a 5-year survival rate of 6.4% (95%CI: 0.9%-20.3%; $P < 0.001$) (FigureS 2B and 3).

Survival according to histopathology

Lymph node metastasis had a major influence on the patient's survival. The 5-year survival rate of lymph node negative (N0) patients was 29.7% (95%CI: 20%-40%), while for lymph node metastasis (N1) patients it was 2.5% (95%CI: 0.2%-11.1%; $P < 0.001$) (Figures 2C and 3).

There was very good long-term survival in the lymph node negative patients within the R0 group. The 5-year survival rate was significantly higher in the R0N0

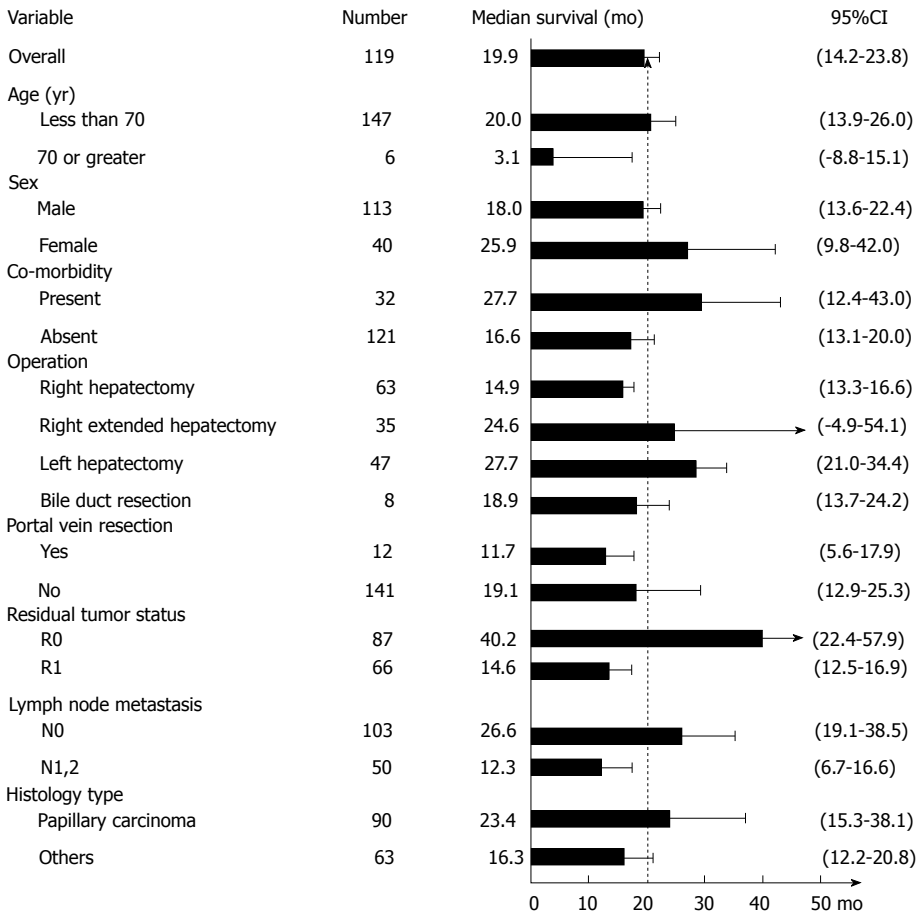

Figure 1 Median survival (mo) of clinico-pathological parameters.

group 47% (95%CI: 31.2%-61.3%) compared with the R1 or N1groups 6.9% (95%CI: 2%-16.3%; $P < 0.001$) (Figure 2D).

Bivariate and multivariable survival analyses

The results of our bivariate analysis are shown in Table 5. There are 4 significant parameters associated with long-term survival: age < 70 ($P = 0.003$), residual tumor status ($P < 0.001$), lymph node metastasis ($P < 0.001$), and papillary histology type ($P = 0.012$). In the multivariable model, where the effect of age was taken into account, however, only the 3 factors excluding age were significantly associated with overall survival (Table 6). Achieving R0 resulted in a 58% (HR = 0.42; 95%CI: 0.28-0.62; $P < 0.001$) reduction in the chance of mortality as compared to R1. Likewise, patients without lymph node metastasis were 60% (HR = 0.40; 95%CI: 0.27-0.59; $P < 0.001$) less likely to die than those who had metastasis.

Patients with other types of invasive cholangiocarcinoma had a 58% (HR = 1.58; 95%CI: 1.08-2.30; $P = 0.018$) higher chance of mortality as compare to papillary carcinoma.

DISCUSSION

Results from this study showed that curative resection of PCCA in Srinagarind hospital, Khon Kaen, Thailand had low perioperative morbidity, mortality and a 5-year survival rate comparable to recent studies. Five-year survival after curative resection for PCCA in Asia ranges from 0% to 64%[7,10,17-19,21-26] and in North America and Europe from 10% to 38%[4,27-29], post-operative morbidity was 26.3%-75% and mortality rates were 0%-11% (Table 7). There are only two previous studies from Thailand[23,26], and one review article by Khuntikeo *et al*[30], which found a 5-year survival of 0%-10.8%. The substantial improvements shown in our northeast Thailand cohort are most likely to be due to better, more radical surgical procedures, patient selection, and preoperative care.

This study did not show that preoperative biliary drainage in jaundice patients reduced post-operative complications, mortality or length of hospital stay. Preoperative biliary drainage patients showed a better rate of survival, but this was not significant in multivariate analysis.

A small number of patients underwent preoperative PVE because CT was not used to estimate remnant liver volume. The benefit of PVE could not be evaluated due to lack of clear indications.

We found that patients who had co-morbidity had a better survival outcome because these patients regularly had medical attention that increased the

Table 4 Operative procedure

Procedures	*n* (%)	Operative time; min (mean ± SD)	Blood loss; mL (mean ± SD)	Hospital stay; d (mean ± SD)	Morbidity	R0
Overall	153	326 ± 125	1274.2 ± 1312.5	15 ± 8.2	30%	43.1%
Right hepatectomy	63 (41.2)	334.3 ± 118.6	1480.2 ± 1644.9	15.4 ± 9	70.3%	31.7%
Right extended hepatectomy[1]	35 (22.9)	365.4 ± 133.7	1200.6 ± 881.5	15.6 ± 8.2	37.1%	51.4%
Left hepatectomy	47 (30.7)	319.4 ± 112	1180.5 ± 1082.9	15.2 ± 7.2	29.8%	53.2%
Extra hepatic duct resection	8 (5.2)	150.6 ± 55.4	481.2 ± 380.7	8.6 ± 5	0	37.5%
Portal vein resection	12 (7.8)	463.3 ± 101.1	1804.2 ± 1664.6	17.7 ± 8.7	50%	50%

[1]Included right trisectionectomy.

Table 5 Bivariate analysis of clinico-pathological parameters

Variable	Number (person-months)	IRR/100	HR	95%CI	*P* value
Age (10 yr added)	153 (4026.8)	2.96	0.99	(0.97-1.01)	0. 743
Age (yr)					0.003
Less than 70	147 (3984.4)	2.84	1		
70 or greater	6 (42.4)	14.15	4.71	(2.02-10.97)	
Sex					0.066
Male	113 (2729.4)	3.33	1		
Female	40 (1297.4)	2.16	0.68	(0.44-1.04)	
Co-morbidity					0.281
Present	32 (1009.3)	2.48	0.79	(0.51-1.23)	
Absent	121 (3017.5)	3.12	1		
Operation					0.061
Right hepatectomy	63 (1389.1)	3.89	1		
Right extended hepatectomy	35 (1059.3)	2.36	0.63	(0.39-1.03)	
Left hepatectomy	47 (1412.6)	2.27	0.6	(0.39-0.93)	
Bile duct resection	8 (165.8)	4.83	1.12	(0.53-2.36)	
Portal vein resection					0.259
Yes	12 (3909.8)	2.92	1.49	(0.77-2.86)	
No	141 (117.0)	4.27	1		
Residual tumor status					< 0.001
R1	87 (2386.8)	4.63	1		
R0	66 (1640.0)	1.8	0.40	(0.27-0.59)	
Lymph node metastasis					< 0.001
N1, 2	103 (3248.6)	6.17	1		
N0	50 (778.2)	2.19	0.38	(0.26-0.55)	
Histology type					
Papillary carcinoma	90 (2687.4)	2.38	1		0.012
Others	63 (1339.4)	4.11	1.61	(1.11-2.33)	

IRR: Incidence rate per 100 person-months.

Table 6 Multivariable analysis of clinico-pathological parameters

Variable	IRR/100	Unadjusted HR	Adjusted HR[1]	95%CI	*P* value
Residual tumor status					< 0.001
R1	4.63	1	1		
R0	1.8	0.40	0.42	(0.28-0.62)	
Lymph node metastasis					< 0.001
N1, 2	6.17	1	1		
N0	2.19	0.38	0.4	(0.27-0.59)	
Histology type					
Papillary carcinoma	2.38	1	1		0.018
Others	4.11	1.61	1.58	(1.08-2.30)	

[1]Each hazard ratio was adjusted for age and other factors listed in the table. IRR: Incidence rate per 100 person-months.

chance of detecting an early stage tumor. Early stages (stages 0, Ⅰ and Ⅱ) were found in 68.7% of patients who had co-morbidity compare to 50.4% of patients who did not.

This study validates John Hopkin's definition of perihilar cholangiocarcinoma[3]. *i.e.,* that any tumor involving hepatic duct bifurcation should be treated and staged like perihilar tumor, because there are no differences in the prognosis and treatment strategy. Lymph node metastasis has been identified as a strong factor indicating a very poor prognosis[4,7,9,10,29]. This is also the case in our study since of the 32.7% patients who had lymph node metastasis only 2.5% had a 5-year survival.

Papillary carcinoma associated with intraductal tumor and less aggressive behavior resulted in a significantly longer median survival after resection of 55.7 mo compared to the nodular sclerosing type of 33.5 mo[28,31]. This study shows that papillary carcinoma is an independent prognostic factor with a 5-year survival rate of 27.7%, whereas other CCA types resulted in only a 10.2% survival rate.

Recent studies showed that R0 resection was the factor indicating a good prognosis resulting in a 40.7%-52% 5-year survival compared to R1 resection with 5-year survival of 7.9%-32%[7,17,19,25]. More radical surgery had to be considered in order to achieve a more negative margin, such as combined vascular resection where studies have reported a 5-year survival of 47.6%-58% with mortality of 2%-8.8%[19,20], and left or right trisectionectomy resulting in a 5-year survival of 36.8%-64.2% with an acceptable mortality of 0%-1.2%[16-18,32,33].

Our study, which was based on current surgical techniques, showed a 5-year survival in the R0 group

Table 7 Five-year survival after curative resection for perihilar cholangiocarcinoma in previous studies

Ref.	Place	Year	Resections	Morbidity	Mortality	R0	5-year survival rate
Present study	Thailand	2014	153	30%	2.00%	43.1%	20.6%
Nimura *et al*[21]	Japan	2000	100	49%	9.00%	61.0%	26.0%
Kondo *et al*[22]	Japan	2004	40	48%	0	95.0%	NA
Dinant *et al*[27]	Netherlands	2005	54	59%	11.00%	46.0%	38.00%
Jarnagin *et al*[28]	United States	2005	106	62.3%	7.50%	77.0%	NA
DeOliveira *et al*[29]	United States	2007	173	61%	5.00%	19.0%	10.00%
Ito *et al*[4]	United States	2008	38	32%	0	63.0%	33.00%
Paik *et al*[18]	Korea	2008	16	75%	0	81.2%	64.00%
Khuntikeo *et al*[23]	Thailand	2008	30	76.70%	6.70%	NA	0%
Hirano *et al*[24]	Japan	2010	146	44%	3.40%	87.0%	35.5%
Igami *et al*[25]	Japan	2010	298	43%	2.00%	74.0%	42.0%
Nagino *et al*[19]	Japan	2010	50	54%	2.00%	66.0%	30.3%
Cheng *et al*[10]	China	2012	176	26.3%	2.90%	78.4%	13.5%
Natsume *et al*[17]	Japan	2012	201	44.2%	1.00%	84.9%	35.2%
de Jong *et al*[6]	United States	2012	224	NA	6.70%	66.5%	20.2%
Nagino *et al*[7]	Japan	2013	574	57.30%	4.70%	76.5%	32.5%
Pattanathien *et al*[26]	Thailand	2013	58	NA	NA	46.6%	10.8%

NA: Not available.

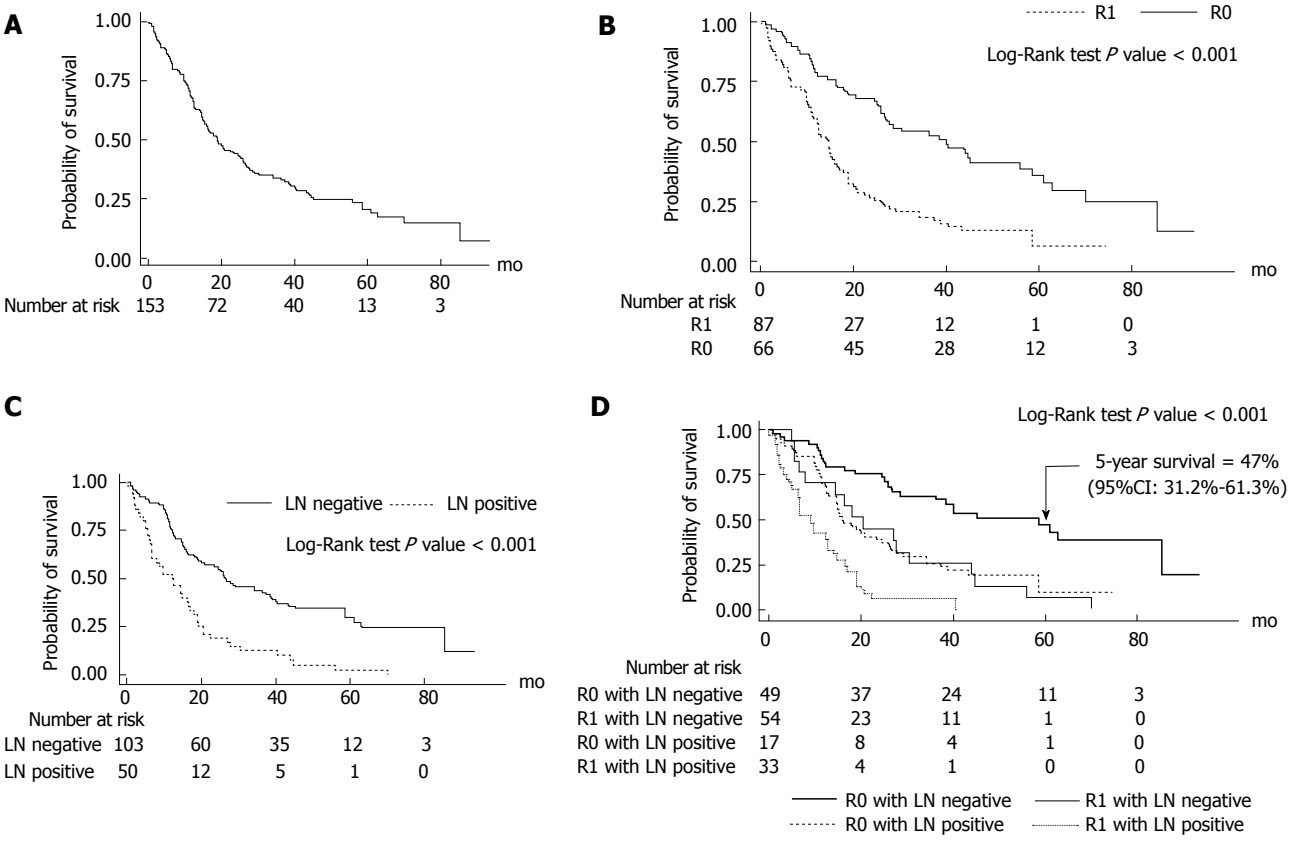

Figure 2 Kaplan-Meier survival curve. A: Kaplan-Meier survival curve of perihilar cholangiocarcinoma; B: Kaplan-Meier survival curve of perihilar cholangiocarcinoma according to residual tumor status; C: Kaplan-Meier survival curve of perihilar cholangiocarcinoma according to lymph node metastasis; D: Kaplan-Meier survival curve of perihilar cholangiocarcinoma according to residual tumor status and lymph node metastasis.

of 35.6%, which was much greater than in the R1 group with a survival of 6.4%. Right hepatectomy was the procedure that achieved the lowest rate of R0 resection, probably due to the preservation of segment 4 liver parenchyma which increased the chance of a histologically positive margin. Appropriately designed studies are needed to investigate this finding (Table 4).

R0 resection was carried out in 43.1% of all patients which compares favorably with previous studies where the R0 resection ranged from 19% to 95% (Table 6).

There is one randomized control trial showing the benefit of chemotherapy in advanced biliary tract cancer[34], but there are no randomized trials to prove the benefit of chemotherapy in an adjuvant setting.

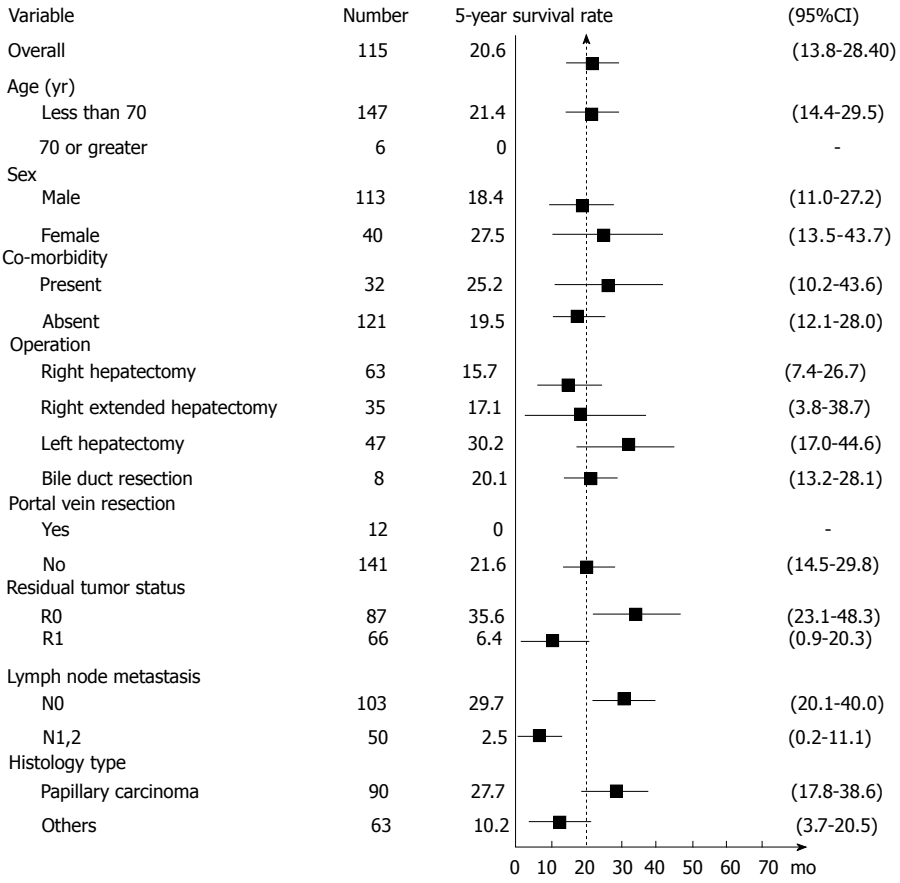

Figure 3 Five-year survival rate of clinico-pathological parameters.

This study was not designed to prove the benefit of chemotherapy but there is one retrospective study from our institute from the years 2009-2011 that may imply benefit of adjuvant chemotherapy. The study included 263 patients who underwent curative resection for all types of cholangiocarcinoma. Patients who received adjuvant chemotherapy had a significantly longer median survival time of 21.6 mo compared to those with no adjuvant chemotherapy of 13.4 mo. Benefit was also found in lymph node metastasis, R1 resection, higher carbohydrate antigen 19-9 and higher stage[35].

Based on the information from this study, we can suggest measures to improve long-term survival outcome. Focus should center on: (1) screening tools and screening policy to detect early lymph node negative cases; and (2) radical surgical techniques and perioperative care to improve the R0 resection rate and to minimize post-operative morbidity and mortality.

In conclusion, curative resection in PCCA is possible with current surgical procedures resulting in a two-fold greater survival outcome compared to previous studies from Thailand. Independent factors that were associated with good survival outcome were R0 resection, no lymph node metastasis, and papillary histology.

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COMMENTS

Background

The highest incidence worldwide of perihilar cholangiocarcinoma (PCCA) occurs in the northeast of Thailand. Major hepatectomy with bile duct resection is the standard curative procedure; however, to date the 5-year survival rate in Thailand of 0%-10.8%, has been unsatisfactorily low.

Research frontiers

Curative resection in PCCA is safe with improved surgical procedures resulting in a two-fold greater survival outcome compared to previous studies from Thailand. Independent factors that were associated with good survival outcome were R0 resection, no lymph node metastasis, and papillary histology.

Innovation and breakthroughs

Advances in surgical technique combined with early stage diagnosis can lead to a substantially improved prognosis and 5-year survival in PCCA patients.

Applications

To improve long-term survival outcome focus should center on (1) radical surgical techniques and perioperative care to improve the R0 resection rate and to minimize post-operative morbidity and mortality; and (2) screening tools and screening policy to detect early lymph node negative cases.

Terminology

Perihilar cholangiocarcinoma is defined as “tumors that are located in the extrahepatic biliary tree proximal to the origin of the cystic duct”.

Peer-review

The present study has been performed in the place that the highest incidence of cholangiocarcinoma is reported. The study was well designed and conducted with great invention.

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Management of asymptomatic primary tumours in stage IV colorectal cancer: Review of outcomes

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Author contributions: Wilkinson KJ and Roohullah A designed the research; Wilkinson KJ performed the research; Wilkinson KJ, Roohullah A, Chua W and Ng W analyzed the data and wrote the paper.

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Data sharing statement: All data referred to in this review is taken directly from the outcome data from the original cited papers. No additional data are available.

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Abstract

AIM: To compare outcomes for patients presenting with stage IV colorectal cancer and an asymptomatic primary tumour, undergoing primary tumour resection (PTR) plus palliative chemotherapy *vs* primary chemotherapy up-front.

METHODS: A literature search was conducted using MEDLINE and EMBASE. The primary outcome was overall survival. Secondary outcomes included perioperative mortality, morbidity and delayed surgical intervention rates in patients undergoing PTR and subsequent complication rates in patients with an un-resected primary tumour. Tertiary outcomes included impact on systemic treatment and identification of prognostic factors relevant for survival in this cohort.

RESULTS: Twenty non-randomised studies met the inclusion criteria. Eleven studies included comparative overall survival data. Three studies showed an overall survival advantage for PTR, 7 studies showed no statistically significant advantage, and 1 study showed a significant worsening in survival in the surgical group. The perioperative mortality rate ranged from 0% to 8.5%, and post-operative morbidity rate from 10% to 35%, mainly minor complications that did not preclude subsequent chemotherapy. The rate of delayed primary-tumour related symptoms, most commonly obstruction, in patients with an un-resected primary tumour ranged from 3% to 46%. The strongest independent poor prognostic factor was extensive hepatic metastases, in addition to poor performance status, M1b stage and non-use of modern chemotherapy agents.

CONCLUSION: Based on the current literature, both PTR and up front chemotherapy appear appropriate initial management strategies, with a trend towards an overall survival advantage with PTR. The procedure has a low post-operative mortality, and most complications are transient and minor. The results of recruiting randomised trials are eagerly anticipated.

Key words: Colorectal cancer; Resection; Primary tumour; Asymptomatic; Unresectable metastases; Chemotherapy; Complications

Core tip: The management of asymptomatic primary tumours in stage IV colorectal cancer is under debate. A literature review was performed focusing on this cohort, with patients undergoing primary tumour resection (PTR) *vs* up front chemotherapy. Survival appears equivalent with both management strategies, with a trend to an advantage in PTR. Surgical mortality is low and most morbidity transient. Most studies are retrospective, small and non-randomised. Larger randomised controlled trials are awaited.

Wilkinson KJ, Chua W, Ng W, Roohullah A. Management of asymptomatic primary tumours in stage IV colorectal cancer: Review of outcomes. *World J Gastrointest Oncol* 2015; 7(12): 513-523 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i12/513.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i12.513

INTRODUCTION

Colorectal cancer is the third most common cancer in men and the second in women worldwide[1]. Approximately 20% of patients present with stage IV disease, and the vast majority (70%-80%) of these patients are incurable. There is no consensus regarding the appropriate management of an asymptomatic or minimally symptomatic primary lesion in these patients. While patients presenting with symptoms suggestive of obstruction, bleeding or perforation are often surgically managed to palliate these acute symptoms, the majority of patients present with systemic symptoms (*e.g.*, weight loss, fatigue, anorexia) and an asymptomatic primary lesion. There are no published randomised controlled trials addressing this clinical question. The CAIRO4[2] and SYNCHRONOUS[3] trials (colon cancer) and GRECCAR-8 trial[4] (rectal cancer) are currently recruiting with results not expected to be available for a number of years.

There is an increasing body of evidence suggesting a survival advantage in patients undergoing primary tumour resection (PTR). This includes post-hoc analyses of randomised trial data[5,6], meta-analyses[7] and population-wide registry data[8]. Improved survival outcomes in advanced disease associated with surgical debulking have a well-established evidence base in epithelial ovarian[9] and renal[10] malignancies. However, most of the currently published evidence relating to colorectal cancer encompasses patients with both symptomatic and asymptomatic primary tumours. More pertinently, most studies include a heterogeneous population, including a significant proportion of patients with poor performance status at diagnosis, who are unfit for PTR. Selection bias may thus skew survival outcomes in favour of the PTR cohort who are likely to be of superior performance status, have fewer co-morbidities, and possibly less burden of disease at diagnosis. Many of the current reviews use data collected in the era prior to routine use of modern chemotherapy regimes and biological agents, including the vascular endothelial growth factor-A monoclonal antibody bevacizumab, and the epidermal growth factor receptor (EGFR) inhibitors cetuximab and panitumumab. These have all had a major impact on survival and therefore it is essential to review patients in this current clinical context.

PTR reduces the risk of subsequent local tumour related complications, primarily obstruction, but also perforation, bleeding and fistulae formation. These complications often warrant emergency surgery, which has a higher rate of peri-operative mortality and morbidity than elective surgery. This may be more problematic when the patient has myelosuppression due to systemic chemotherapy. Any subsequent emergency surgery may also interrupt the use of systemic chemotherapy. This may be a more critical delay later in the course of the patient's illness as their burden of disease increases. Intact primary tumours may cause systemic complications including weight loss, anorexia, nutritional depletion and pain. They can also cause local complications (diarrhoea, faecal incontinence, *etc*.) that can impact significantly on quality of life.

Arguments supportive of non-resection strategies up front [primary chemotherapy (PC)] include the risks of post-operative morbidity and mortality. Surgery can delay the use of systemic chemotherapy. Furthermore, the risks of complications from an un-resected primary lesion have been quoted by some to be relatively low[11]. Modern chemotherapy regimes are associated with high response rates, suggesting that chemotherapy may be sufficient to control the primary[12]. A recent Cochrane Collaboration Systematic Review[13] did not find consistently improved outcomes after PTR (although it identified a paucity of sound clinical trials), and current NCCN guidelines support primary resection only in the setting of symptomatic disease[14].

This review was designed to summarise the current literature available, focusing primarily on the outcomes of overall survival, and additional outcomes of perioperative morbidity and mortality, delayed complication rates in both groups, and impact on subsequent chemotherapy. Identification of prognostic markers was also reviewed.

MATERIALS AND METHODS

An extensive literature search was conducted using MEDLINE and EMBASE. Results were limited to 1980-2015 and restricted to English language articles. Search subject headings and MeSH terms included Colorectal Neoplasms, Colon Neoplasms and Rectal Neoplasms, Stage IV, General Surgery, Drug Therapy and the keywords asymp* and symp*. The search strategy was designed to be broad and relevant articles

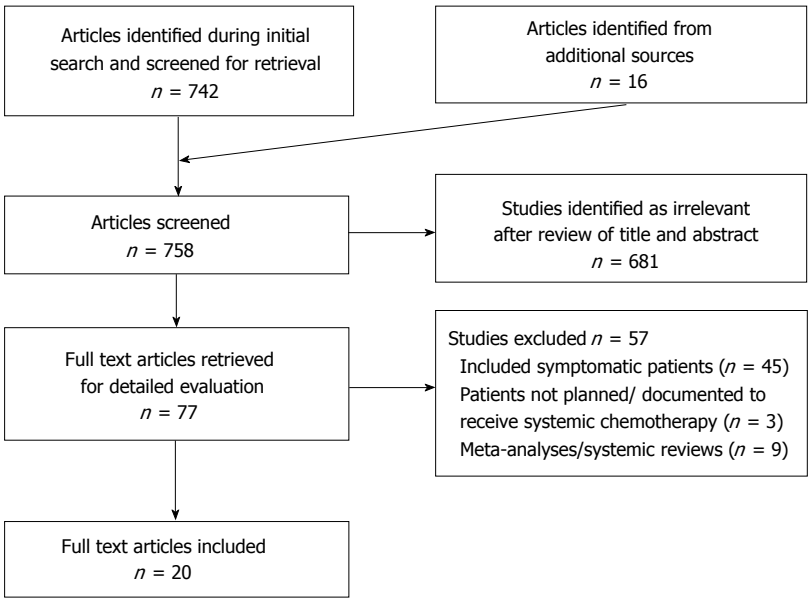

Figure 1 Flow diagram of literature search.

were manually searched to include articles with relevant asymptomatic groups or subgroups. The citations of relevant studies were examined to identify additional articles (Figure 1).

Only studies in which the patients were planned for systemic chemotherapy (after PTR or upfront) were included. Inclusion criteria specified patients with confirmed adenocarcinoma of the colon or rectum, excluding other histological diagnoses. Exclusion criteria included patients undergoing upfront "curative" resection of the primary tumour with staged/simultaneous resection of metastases. Patients undergoing non-resection surgery upfront (including diverting stoma, internal bypass, *etc*.) were also excluded. For the primary outcome overall survival, all articles were two arm studies in which PTR and PC were compared. For the remaining outcomes, single arm studies involving patients undergoing either PTR or PC were also included.

The primary outcome of interest was overall survival (defined as date of diagnosis to date of death). Survival was determined by the Kaplan-Meier method and distributions compared by the log rank test in all cited articles. The overall significance level was set at 0.05. Secondary outcomes were peri-operative (30 d) mortality, post-operative morbidity (any recorded complication), and delayed surgical intervention for complications in patients undergoing PTR. Other secondary outcomes included the development of delayed primary tumour related symptoms warranting intervention in patients undergoing PC. Tertiary outcomes included the impact of treatment choice on subsequent systemic chemotherapy (timing from diagnosis to chemotherapy, and development of grade 3 or 4 chemotherapy-related toxicities), and prognostic variables influencing overall survival in the asymptomatic cohort, which was determined by multivariate analysis, using the Cox proportional hazards model.

RESULTS

Study characteristics

Twenty studies met the inclusion and exclusion criteria (Table 1). Eleven studies, all retrospective in nature, compared the outcomes of patients undergoing PTR followed by systemic chemotherapy, *vs* PC upfront. Of these, 1 study included an asymptomatic subgroup within a larger cohort. All of these studies provided overall survival data. A further 7 studies were single-arm studies looking at patients undergoing PC, 4 retrospective and 3 prospective. An additional 2 studies, both retrospective, were single-arm studies following the outcomes of patients undergoing PTR.

All studies included patients with both colon or rectal cancers, except Boselli *et al*[15] and McCahill *et al*[16] who excluded patients with rectal malignancies. All patients in the Matsuda *et al*[17] study had peritoneal metastases from a colorectal primary at diagnosis.

The vast majority of studies were single institution, retrospective reviews. The median age of patients ranged from 52-73. The proportion of males ranged from 50%-65%. The majority used modern 1st line chemotherapy regimes (fluoropyrimidine based doublet with oxaliplatin or irinotecan), though 5 studies conducted prior to the routine use of these agents used single agent fluoropyrimidine (5-fluorouracil) only, and in 1 study this data was missing. Five studies documented use of bevacizumab, though in many this data was missing, and only one study quoted specific use of EGFR monoclonal antibodies.

Outcomes

Overall survival: Median overall survival (Table 2) was compared in 11 studies. In the majority of studies, in acknowledgement of the risk of selection bias and confounding in retrospective studies, an attempt was made to provide adjusted survival data. This was

Table 1 Study characteristics

Ref.	Years data collected	Country	Type of study	Total *n*	% of group receiving chemo	Predominant chemotherapy regime	Targeted agent use
2 arms: PTR *vs* PC				(PTR *n*/PC *n*)	(PTR/PC)		
Yun *et al*[18] (2014)	2000-2008	South Korea	Retrospective, propensity-score matched cohort, single centre	416 (218/198)	66/100	Doublet	ND
Matsumoto *et al*[19] (2014)	2005-2011	Japan	Retrospective, single centre	88 (41/47)	85/100	Doublet	Approx 50% received targeted agent
Ahmed *et al*[42] (2014) Subgroup	1992-2005	Canada	Retrospective, multicentre	834	100/100	ND	< 2%
Cetin *et al*[22] (2013)	2006-2010	Turkey	Retrospective, multi centre	99 (53/46)	100/100	Doublet	100% received bevacizumab
Boselli *et al*[15] (2013)	2010-2011	Italy	Retrospective, single centre	48 (17/31)	65/100	Doublet	> 50% received bevacizumab 1st line
Seo *et al*[20] (2010)	2001-2008	South Korea	Retrospective, single centre	227 (144/83)	100/100	Doublet	5%-10% received bevacizumab; 5%-10% received EGFR monoclonal antibody
Galizia *et al*[25] (2008)	1995-2005	Italy	Retrospective, single centre	65 (42/23)	100/100	Singlet	Nil
Benoist *et al*[26] (2005)	1997-2002	France	Retrospective, case matched, single centre	59 (32/27)	94/100	Singlet	Nil
Michel *et al*[21] (2004)	1996-1999	France	Retrospective, single centre	54 (31/23)	97/100	Doublet	Nil
Ruo *et al*[43] (2003)	1996-1999	United States	Retrospective, single centre	230 (127/103)	ND/83	Singlet	Nil
Scoggins *et al*[44] (1999)	1985-1997	United States	Retrospective, single centre	89 (66/23)	ND/100	Singlet	Nil
Single arm: Primary chemotherapy				*n*	% group receiving chemo		
Yun *et al*[23] (2014)	2000-2011	South Korea	Retrospective, single centre	259	100	Doublet	ND
McCahill *et al*[16] (2012)	2006-2009	United States	Prospective Phase 2	86	100	Doublet	100% received bevacizumab
Clements *et al*[45] (2009)	2003-2006	United Kingdom	Retrospective, single centre	37	92	Doublet	ND
Bajwa *et al*[27] (2009)	1999-2005	United Kingdom	Retrospective, single centre	67	100	Doublet	ND
Poultsides *et al*[24] (2009)	2000-2006	United States	Retrospective, single centre	233	100	Doublet	48% received bevacizumab 1st line
Muratore *et al*[46] (2007)	2000-2004	Italy	Prospective, single centre	35	100	Doublet	Nil
Sarela *et al*[47] (2001)	1997-2000	United Kingdom	Retrospective and prospective, single centre	24	87	Singlet	Nil
Single arm: Primary tumour resection				*n*	% group receiving chemo		
Maeda *et al*[28] (2013)	2001-2009	Japan	Retrospective, single centre	94	85	Doublet	33% received targeted agent
Matsuda *et al*[17] (2012)	1998-2007	Japan	Retrospective, single centre	40	74	Doublet	ND

PTR: Primary tumour resection; PC: Primary chemotherapy; ND: Not documented.

presented as adjusted hazard ratios, or using matched patient cohorts.

In 3 studies, there was a statistically significant improvement in median overall survival in the PTR group. In 2 of these studies, this difference remained significant after adjustments, and in the third no attempt was made to calculate such adjustments. The magnitude of the unadjusted median overall survival benefit in these studies ranged from 3-7 mo.

In 7 studies, there was no statistically significant improvement in overall survival in the PTR group (and in 4 of these studies adjusted outcomes measures were used). However, in 3 of these studies (Yun *et al*[18]; Matsumoto *et al*[19]; and Seo *et al*[20]), there was a definite trend to an overall survival advantage with PTR that didn't quite meet statistical significance. In 2 further studies[21,22], an unadjusted improvement in median overall survival in the PTR group of 7 and 6 mo respectively did not meet statistical significance, likely due to small sample sizes.

Only 1 study (Boselli *et al*[15]) suggested a survival disadvantage with PTR, but this study was an outlier (see Discussion below).

Primary tumour related complications: Sixteen studies looked at the rate of development of primary

Table 2 Overall survival

Ref.	Unadjusted median OS (mo)			Adjusted survival outcomes: Is PTR superior?
	PTR	PC	*P* value	
Galizia *et al*[25] (2008)	15	12	$P = 0.03$	Yes (HR for death PC = 3.91, 95%CI: 2.83-4.99, $P = 0.01$)
Ahmed *et al*[42] (2014) Subgroup	15	8	$P < 0.01$	Yes (analysis not shown)
Ruo *et al*[43] (2003)	16	9	$P < 0.001$	No adjusted survival data
Yun *et al*[18] (2014) Matched cohort	17	14	P = NS	No (HR for death PC = 1.16, 95%CI: 0.89-1.52, $P = 0.27$)
Matsumoto *et al*[19] (2014)	24	23	P = NS	No (HR for death PTR = 0.72, 95%CI: 0.42-1.25, P = NS)
Seo *et al*[20] (2010)	22	14	P = NS	No (HR for death PC = 1.73, 95%CI: 0.94-3.16, $P = 0.07$)
Benoist *et al*[26] (2005) Matched cohort	23	22	P = NS	No (HR not reported, $P = 0.753$)
Cetin *et al*[22] (2013)	23	17	P = NS	No adjusted survival data
Michel *et al*[21] (2004)	21	14	P = NS	No adjusted survival data
Scoggins *et al*[44] (1999)	14	17	P = NS	No adjusted survival data
Boselli *et al*[15] (2013)	4	5	P = NS	No (HR for death PTR = 2.1, 95%CI: 1.06-4.5, $P = 0.03$)

NS: Not significant ($P > 0.05$); PTR: Primary tumour resection; PC: Primary chemotherapy; HR: Hazard ratio.

tumour related complications requiring intervention in patients undergoing PC (Table 3). This varied from 3.5% to 40%. The mean time to onset of complications ranged from 3-11 mo. The predominant complication was obstruction, with very low reported rates of bleeding, perforation and pain. Interventions to manage obstruction included both surgical (resection, de-functioning stoma or bypass procedures) and non-surgical (*e.g.*, endoscopic stenting, radiotherapy, *etc.*). For the majority of these procedures, the authors commented that they were well tolerated and the patient was able to proceed with ongoing systemic treatment subsequently.

Three studies reviewed predictive variables for the development of complications requiring intervention. Matsumoto *et al*[19] identified inability to fully traverse the tumour at diagnostic colonoscopy as the only positive factor. For patients who subsequently developed obstruction, the mean time from diagnosis to onset was 2 mo in those with a non-traversable lesions *vs* 16 mo in those with a traversable lesion ($P = 0.01$). Yun *et al*[23] identified rectal tumours and tumours > 5 cm as positive predictive factors on multivariate analysis. Poultsides *et al*[24] did not find any positive correlation with reference to patient age, site of tumour, bevacizumab use, extent of metastatic disease, baseline CEA, Albumin, LDH or Alkaline phosphatase level.

Perioperative mortality and morbidity in PTR group: Peri-operative (30 d) mortality rates were reported in 12 studies (Table 4). In the vast majority, the rate was less than 2%. In the review by Boselli *et al*[15], there was a very high perioperative mortality rate (29%), but of note the PC group also had a high rate (19%), and the difference between the groups was not statistically significant.

With respect to morbidity, the most common post-operative complications were minor-wound infections, prolonged post-operative ileus, urinary infections/retention, and respiratory tract infections. Anastomotic leaks and intra-abdominal collections/sepsis were the most commonly reported major complications, and occurred in 0%-4% of patients in which specific complication rates were documented.

Impact on subsequent systemic therapy: Three studies looked at the median delay from diagnosis to commencement of chemotherapy, and predictably this was prolonged in the PTR group. In the Galizia *et al*[25] study, the interval was 35 d in the PTR group *vs* 8 d in the PC group ($P < 0.01$), in the Benoist *et al*[26] study 44 d *vs* 15 d respectively, and in the Seo *et al*[20] review 37 d *vs* 7 d respectively ($P < 0.01$).

The rates of significant (grade 3 or 4) chemotherapy related toxicities were also considered by the above authors, and no differences were identified between the groups in any study. Galizia *et al*[25] reported rates of 45% in PTR group *vs* 43% in PC group ($P = 0.89$), and Benoist *et al*[26] recorded 50% *vs* 37% respectively ($P = 0.46$). Seo *et al*[20] looked specifically at grade 3 or 4 gastro-intestinal toxicities, and the rates were similar between groups (10% *vs* 12 % respectively, $P = 0.7$).

Prognostic variables affecting overall survival: Eight studies looked at prognostic factors influencing overall survival in the whole cohort (PTR and PC groups combined). Table 5 summarises the variables found to be independently prognostic on multivariate analyses, and the hazard ratio for death (presence *vs* absence of factor) is documented where statistically significant.

Age and sex were reviewed in most studies, and were not independent factors in any study. Performance status was examined in 4 studies, and was an independent factor in 2 of these, with hazard ratios for death of 2.7 and 3.2 for patients with an ECOG performance status $\geqslant 2$ *vs* < 2. Bajwa *et al*[27] noted the presence of more than one primary tumour was a predictor for poorer overall survival in this cohort (OR for death 3.37, 95%CI: 1.21-9.3, $P = 0.02$).

The extent of hepatic parenchymal involvement by metastatic disease was a strong poor prognostic marker in 2 out of 4 reviews, with a hazard ratio for death of up to 5.8 for extensive disease *vs* limited disease. Metastatic dissemination to at least two distant sites

Table 3 Primary tumour related complications in patients undergoing primary chemotherapy

Ref.	% of patients requiring intervention for primary tumour related complications	Most common complication	Comment
Yun *et al*[18] (2014)	3%	Obstruction > perforation	Mean onset of complications = 8 mo
Cetin *et al*[22] (2013)	4%	Obstruction > rectovesical fistula	-
Muratore *et al*[46] (2007)	6%	Obstruction > haemorrhage	-
Clements *et al*[45] (2009)	8%	All obstruction	-
Scoggins *et al*[44] (1999)	9%	All obstruction	Mean onset of complications = 3 mo
Poultsides *et al*[24] (2009)	11%	Obstruction > perforation > pain	-
Seo *et al*[20] (2010)	14%	Obstruction > bleeding	-
Benoist *et al*[26] (2005)	15%	All obstruction	-
McCahill *et al*[16] (2012)	16%	Obstruction > perforation, pain	Majority onset of complications < 12 mo
Michel *et al*[21] (2004)	22%	All obstruction	Mean onset of complications = 4 mo
Yun *et al*[23] (2014)	22%	Obstruction > perforation	Mean onset of complications = 7 mo
Matsumoto *et al*[19] (2014)	26%	Majority obstruction	-
Ruo *et al*[43] (2003)	29%	All obstruction	Majority onset of complications < 6 mo
Galizia *et al*[25] (2008)	30%	Obstruction> perforation > haemorrhage	Mean onset of complication = 11 mo
Sarela *et al*[47] (2001)	33%	Obstruction > pain > tenesmus	Mean onset of complication = 9 mo
Bajwa *et al*[27] (2009)	40%	Obstruction > bleeding	

Table 4 Complications in patients undergoing primary tumour resection

Ref.	Post-operative (30 d) mortality %	Post-operative morbidity		Requiring subsequent surgical intervention (%)
		%	Most common complication	
Cetin *et al*[22] (2013)	0	ND	ND	6% (all rectovesical fistula)
Benoist *et al*[26] (2005)	0	19	Wound infection, cardio-respiratory, intra-abdominal abscess, UTI	ND
Galizia *et al*[25] (2008)	0	21	All minor	0%
Maeda *et al*[28] (2013)	0	21	Wound infection, ileus, anastomotic leak	ND
Michel *et al*[21] (2004)	0	ND	ND	ND
Seo *et al*[20] (2010)	0	35	Urine retention, wound complication, ileus.	2%
Yun *et al*[18] (2014)	1	10	Ileus, wound infection, anastomotic leak	ND
Matsuda *et al*[17] (2012)	2	15	Wound infection, ileus	11%
Ruo *et al*[43] (2003)	2	21	Wound infection, ileus, intra-abdominal infection	3%
Matsumoto *et al*[19] (2014)	2	20	ND	ND
Scoggins *et al*[44] (1999)	5	30	Wound infection, UTI, sepsis	ND
Boselli *et al*[15] (2013)	29	35	Wound infection, UTI, pneumonia	ND

ND: Not documented; UTI: Urinary tract infection;

(M1b stage) *vs* disease confined to one organ (M1a stage) conferred a worse prognoses in 2 out of 3 studies in which it was assessed, though the magnitude of the effect (hazard ratio) was low. In a review by Matsuda *et al*[17], for which the whole patient cohort had peritoneal carcinomatosis, the degree of peritoneal involvement (limited *vs* extensive) and the presence *vs* absence of ascites were not found to be significant prognostic factors.

In general, the location of the primary tumour (right colon *vs* left colon *vs* rectum) was not prognostic in this cohort. Only one review, by Bajwa *et al*[27] found tumours proximal to the splenic flexure conferred a worse prognosis than distal tumours (OR for death 2.61, $P = 0.007$). Tumour differentiation was again only prognostic in one study (Seo *et al*[20]), with "high grade" tumours (poorly differentiated, mucinous or signet ring histology) conferring a worse prognosis. T stage and N stage were not prognostic in this group with metastatic disease at diagnosis. Maeda *et al*[28] looked at two inflammation-based prognostic indices- the neutrophil to lymphocyte ratio and the Glasgow prognostic score (GPS), which scores patients based on their baseline level of C-reactive protein and Albumin at diagnosis (with points allocated for high C-reactive protein and hypoalbuminaemia). A neutrophil:lymphocyte ratio $\geqslant 3$ (*vs* < 3) was associated with poorer survival on multivariate analysis (OR = 1.97, 95%CI: 1.74-3.39; $P = 0.01$), as was a GPS of 2 (*vs* 0-1) (OR = 1.95, 95%CI: 1.05-2.72; $P = 0.03$).

The use of doublet chemotherapy, with a 5-fluorouracil doublet (oxaliplatin or irinotecan) also improved survival in the 2 papers in which it was reviewed[17,20].

Only one study reviewed prognostic factors in sub-groups (site of metastases) specific to the primary treatment modality (PTR *vs* PC). Yun *et al*[18] reported that, in their unmatched cohort, patients with liver, lung and peritoneal metastases all had improved survival in the PTR arm in comparison to the PC arm.

DISCUSSION

The decision regarding resection of an asymptomatic primary tumour, in a patient with a good performance

Table 5 Independent prognostic factors influencing overall survival on multivariate analysis, with hazard ratios or odds ratios for death

Ref.	Age	Sex	ECOG PS ⩾ 2	Tumour location: Right colon	Tumour differentiation	T stage	N stage	M1b (*vs* M1a)	Presence of liver mets	Extent of hepatic involvement	Pre treatment CEA	Chemotherapy regime: Non use of Oxaliplatin/ Irinotecan
Cetin *et al*[22] (2013)	a	a									a	
Yun *et al*[18] (2014)[1]	a	a		a	a	a	a	HR 1.39	HR 1.31		a	
Galizia *et al*[25] (2008)	a	a	HR 3.18	a	a	a	a			HR 5.79[2]	a	
Matsuda *et al*[17] (2013)	a	a		a	a		a		a		a	HR 2.57
Bajwa *et al*[27] (2009)	a		a	OR 2.61	a					a	a	
Maeda *et al*[28] (2013)	a	a	OR 2.7[3]	a	a	a	a	OR 1.66			a	
Seo *et al*[20] (2010)	a	a	a	a	HR 2.82[4]			a		HR 2.41[5]	a	HR 1.89[6]
Michel *et al*[21] (2004)										a		

a: Factor investigated by authors and found to be non-significant on multivariate analysis; [1]Unmatched cohort; [2]> 50% hepatic replacement (*vs* < 50% hepatic replacement); [3]ECOG PS ⩾ 1 (*vs* 0); [4]High grade (*vs* low grade); [5]> 5 liver metastases (*vs* < 5); [6]Oxaliplatin use only. ECOG: Eastern Co-operative.

status, is complex. For many, the key question is that of a survival advantage. The above summary suggests that both PTR and PC survival outcomes are equivalent, with a trend towards an overall survival advantage with PTR. In this cohort, PTR is relatively safe with most morbidity being minor and transient, and the vast majority of patients being able to proceed with systemic chemotherapy, with a mean delay of 5-7 wk post surgery. For the PC group, the most common complication is obstruction, with a median rate of occurrence of approximately 20%.

This review is novel because it looks specifically at asymptomatic patients receiving systemic chemotherapy in both arms, by default excluding those with poorer performance status. It is this cohort in whom the decision regarding PTR *vs* PC is the most complex for the multidisciplinary team. This review provides a current overview, including many recently published studies, with data collection in the modern chemotherapy era. Many previously published reviews of asymptomatic patients have included some studies with symptomatic primary tumours[29,30], or included trials with data mainly collected prior to 2005[31,32], when the therapeutic landscape was very different.

The trend to a survival advantage complements and parallels several studies looking at the general population (combined symptomatic and asymptomatic primary tumours at diagnosis). In a recent large meta-analysis of 15 studies involving 12416 patients by Ahmed *et al*[33], the median overall survival was 4 mo longer in patients undergoing PTR *vs* PC, and 6 mo longer in a subgroup receiving second and third generation chemotherapy. In a large cancer registry review by Tsang *et al*[8] of 11706 patients, all receiving chemotherapy, there was a 4 mo improvement in median overall survival in those undergoing PTR *vs* those declining it. Similarly, in a recent SEER database cohort review[34], using stratified propensity-score methods, there was a significantly improved overall and cancer-specific survival in patients undergoing PTR (adjusted HR of death = 0.40, 95%CI: 0.39-0.42; $P < 0.001$). However, the power of the model is limited by the prognostic variables available in the SEER database, which don't include details regarding tumour burden and patient performance status, and thus selection bias is still a major limitation, though the magnitude of the benefit is hypothesis-generating.

However, the four papers with the most recent data in this review did not show an overall survival advantage with PTR. In an era where median overall survival in stage Ⅳ disease in recent trials is approaching 30 mo, it is possible that improved response rates may be enough to control asymptomatic primary lesions. In a small prospective trial of 16 patients by Karoui *et al*[12], 69% of primary colonic tumours achieved major histological tumour regression after neoadjuvant chemotherapy with FOLFOX or FOLFIRI chemotherapy. However, when comparing the histological response of the primary tumour compared with liver metastases, other small series have suggested that this may be poorer in the former[35], and this requires further investigation.

The main criticism of the current literature is the poor quality of evidence, with the vast majority of studies being retrospective, non-randomised single institution reviews, with their inherent risk of selection bias between the groups, and confounding. However, most reviews did attempt to control for these. Small sample sizes were another common limitation, and it is likely that many studies were underpowered to translate clinically significant improvements in overall survival into statistically significant results. Older meta-analyses including some of the trials in this review have suggested a survival advantage for PTR. Many included studies can also be criticized for their missing data in respect to accurate documentation of specific chemotherapy regimes or targeted agent used, which is a critical factor in the equation.

One review with anomalous results was Boselli *et al*[15], with very high surgical mortality and morbidity rates, and very low overall survival in both arms (4-5 mo). This was a small ($n = 48$), single institution review, with only 17 patients in the PTR group. The mean age of patients was older than other reviews (72), and significantly this cohort had a high proportion of patients with extensive hepatic metastatic disease

(47% of PTR group and 58% of PC group has > 50% of liver parenchyma replaced by tumour) and a high proportion with documented hepatic failure (Childs Pugh B score 35% *vs* 55% respectively). All post-operative deaths were attributed to hepato-renal failure and heart failure. Given the significantly disproportionate results, reflecting a patient group skewed towards very extensive metastatic disease, the validity of this study is in question.

No quality of life data exists in the literature for this patient population. In the palliative setting, patient reported outcomes, both global quality of life (including functional outcomes) plus symptomatic scores are essential. Treatment options need to be evaluated with respect to their impact on symptoms which can significantly impair patients' quality of life, such as pain, diarrhoea, tenesmus, faecal incontinence, *etc*. It is very likely that symptomatic local complications, particularly in rectal cancer patients, were under-reported in the included studies, given their retrospective nature. Pain from locally advanced rectal cancer can be an extremely debilitating complication, and other local complications can significantly impede social, emotional and physical functioning. Future studies should focus on global and symptomatic quality of life outcomes, and indeed most currently recruiting RCTs do have these as a secondary endpoint.

Most studies in this review failed to differentiate between colonic and rectal tumours. Anatomical restriction due to complex invasion patterns (*e.g.*, to pelvic bones, genitourinary organs, major blood vessels and nerves) can make PTR more complicated or infeasible in rectal cancers. The use of up-front radiotherapy for "local control" was also poorly reported in the studies, and thus it is impossible to tease out the potential benefits of this as an alternative definitive primary therapy for rectal cancers. In addition, the cancers have different clinical trajectories. Given the above arguments, future trials should separate colon and rectal tumours as different entities, and look specifically at adjuvant or high dose palliative radiotherapy upfront, and the subsequent outcomes.

"Obstruction" was by far the most common delayed complication in the PC group. It must be remembered that in a proportion of patients presenting with obstructive symptoms, this may be due to peritoneal disease or adhesions secondary to surgeries, and therefore PTR is not a guarantee for prevention of such complications, and may contribute to such. Studies should compare complication rates in both arms, and only a minority of the papers did. The clinically relevant questions regarding the efficacy and morbidity of interventions to manage obstruction have not been covered in this review. Surgical therapeutic options include diverting stoma, internal bypass and palliative resection, and local therapies include laser coagulation and radiotherapy in rectal cancers. Many patients are now managed endoscopically with self-expanding metal stents (SEMS), and their rate of use has been increasing since their introduction in the 1990s. A recent meta-analysis of 88 trials involving patients using SEMS[36] reported a clinical success rate of 92%, with a median rate of re-intervention (required for stent blockage, migration, failure or perforation) of 20%. The efficacy and risks of each intervention are essential to relay to the patient if obstructive or other complications develop.

Many currently accepted prognostic variables in colorectal cancer reflect predictive markers for the development of metastatic disease (*e.g.*, T stage, N stage, *etc*.). However, once patients have metastatic, incurable disease, the most relevant prognostic markers reflect the burden of disease and the patient's overall performance status, and this is reflected by the findings above, with hepatic tumour burden, multiple sites of metastatic disease and poor ECOG performance status the most relevant indices. A criticism of most of the included papers is that they looked at prognostic factors for the whole cohort of patients, and did not differentiate between the treatment arms to assess for interaction. This data has been reported in the literature for the combined asymptomatic and symptomatic cohort. For example, in their pooled retrospective analysis of 4 first line chemotherapy trials, Faron *et al*[6] identified a significant interaction between PTR and the location of the primary tumour - the OS benefit of PTR being greater in rectal tumours than colon tumours. Tsang *et al*[8] used subgroup analyses based on tumour location and found that PTR conferred a significant survival advantage in both colon (OS HR = 0.39; 95%CI: 0.37-0.42; $P < 0.0001$) and rectal primaries (OS HR = 0.46; 95%CI: 0.43-0.50; $P < 0.0001$). The same authors analysed subgroups based on age, and in patients aged greater than 70 years, the survival benefit of PTR also persisted. Gresham *et al*[37] performed subgroup analyses based on extent of metastases, and found that the effect of PTR on OS was not modified by this. Most recently, Ishihara *et al*[38] used propensity score analysis to confirm a cancer-specific mortality benefit of PTR irrespective of number of organs involved in metastatic disease, and for locally advanced disease.

In tandem with all spheres of oncology, the decision regarding PTR *vs* PC needs to be individualised. More specific prognostic and predictive markers, to identify who may benefit from each strategy, are required. It is being increasingly appreciated that somatic mutation status is not only predictive of response to therapy, but also probably prognostic. In a retrospective review of 188 patients with colorectal cancer[39], those with KRAS mutations were found to have poorer outcomes, with a disease-specific survival of 2.6 years in KRAS mutant patients *vs* 4.8 years in wild type patients ($P = 0.0003$). Further work in this field is greatly anticipated.

There is no currently published randomised trial data because previously designed trials (including the ISAAC trial, ClinicalTrials.gov number NCT01086618) failed to recruit sufficiently. One reason for this may be the entrenched beliefs of clinicians, with a disparity between oncologists and surgeons. A recent survey of attitudes

of clinicians showed that medical oncologists were more likely to prefer PC if a patient had an asymptomatic sigmoid or caecal lesion, whereas surgeons (colorectal and general) preferred a primary surgical approach[40]. Indeed, over the past two decades there has been a trend towards non operative management in Stage IV colorectal cancer, and the annual rate of PTR has decreased from 74.5% in 1988 to 57.4% in 2010 ($P < 0.001$)[41]. As the surgeon is usually the initial specialist for these patients, these beliefs may hinder recruitment to such trials. However, it is imperative that the currently recruiting RCTs do accrue enough patients to further clarify this grey area, and provide clarification on the suggestion of a survival advantage.

The above review advocates that both PTR followed by systemic chemotherapy and PC are appropriate up front treatment options in patients with asymptomatic primary lesions. There is a trend for a survival advantage in PTR, though the results of currently recruiting randomised trials and meta-analyses including recent trials are paramount to clarify this in the modern era. For those undergoing PTR, multiple studies confirm this is relatively safe and most patients can proceed to systemic treatment uneventfully. Patients with a higher burden of disease, particularly liver metastases, have poorer prognosis overall, though it remains to be clarified whether their primary mode of treatment modulates this, and the relevance of subgroups based on site, extent of disease and patient characteristics. Better validated prognostic tools are required to individualize patient management in this grey area.

COMMENTS

Background

The optimal management of the asymptomatic primary tumour in patients presenting with stage IV colorectal cancer is contentious, with no published randomised control trial data currently available.

Research frontiers

Previously published registry data and meta-analyses of retrospective small trials have suggested a possible survival advantage of primary tumour resection (PTR) in the general stage IV patient population. However, these have included patients with poor performance status unsuitable for aggressive therapy, and selection bias is a major limitation. In contrast, there has been a trend towards a reduction in the rates of primary tumour resection (PTR) in patients presenting with incurable disease. This discrepancy has peaked interest in the debate, and multiple randomised controlled trials are currently recruiting. In parallel with the trend for personalized oncology, identification of prognostic markers in this cohort and predictive markers for benefit or detriment of both management strategies are essential and currently lacking.

Innovations and breakthroughs

This review article is novel in its focus specifically on patients with good performance status presenting with asymptomatic lesions, who comprise the most complex clinical management challenge. Although most included papers suggest equivalent survival, the review identifies a trend to a survival advantage with PTR which is hampered by the methodological limitations of the included small studies. The review also highlights the relative safety of surgery in this cohort, and the minimal impact on subsequent systemic therapy. It also identifies the rate and nature of complications arising in patients undergoing non-surgical management and reports on what factors predict the development of such complications.

Applications

This review further informs members of the multidisciplinary team managing patients with incurable stage IV colorectal cancer, aiding their decision making based on the best available evidence to date.

Terminology

PTR: Primary tumour resection; PC: Primary chemotherapy.

Peer-review

In this review paper, Wilkinson *et al* evaluated management of asmptomatic primary tumours in stage IV colorectal cancer, referring previous studies. This is a carefully done study and the findings are of considerable interest.

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