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WJGO covers topics concerning carcinogenesis, tumorigenesis, metastasis, diagnosis, prevention, prognosis, clinical manifestations, nutritional support, molecular mechanisms, and therapy of benign and malignant tumors of the digestive tract. The current columns of *WJGO* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of gastrointestinal oncology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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Inflammation-associated microsatellite alterations: Mechanisms and significance in the prognosis of patients with colorectal cancer

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Abstract

Microsatellite alterations within genomic DNA frameshift as a result of defective DNA mismatch repair (MMR). About 15% of sporadic colorectal cancers (CRCs) manifest hypermethylation of the DNA MMR gene *MLH1*, resulting in mono- and di-nucleotide frameshifts to classify it as microsatellite instability-high (MSI-H) and hypermutated, and due to frameshifts at coding microsatellites generating neo-antigens, produce a robust protective immune response that can be enhanced with immune checkpoint blockade. More commonly, approximately 50% of sporadic non-MSI-H CRCs demonstrate frameshifts at di- and tetra-nucleotide microsatellites to classify it as MSI-low/elevated microsatellite alterations at selected tetranucleotide repeats (EMAST) as a result of functional somatic inactivation of the DNA MMR protein MSH3 via a nuclear-to-cytosolic displacement. The trigger for MSH3 displacement appears to be inflammation and/or oxidative stress, and unlike MSI-H CRC patients, patients with MSI-L/EMAST CRCs show poor prognosis. These inflammatory-associated microsatellite alterations are a consequence of the local tumor microenvironment, and in theory, if the microenvironment is manipulated to lower inflammation, the microsatellite alterations and MSH3 dysfunction should be corrected. Here we describe the mechanisms and significance of inflammatory-associated microsatellite alterations, and

propose three areas to deeply explore the consequences and prevention of inflammation's effect upon the DNA MMR system.

Key words: Microsatellite instability; Microsatellite stable; Elevated microsatellite alterations at selected tetranucleotide repeats; Colorectal cancer; Mismatch repair; Inflammation; *MSH3*

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Core tip: Inflammation can trigger microsatellite stable colorectal cancers (CRCs) to acquire a nuclear-to-cytoplasm displacement of the DNA mismatch repair protein MSH3, rendering the CRC with di- and tetra-nucleotide microsatellite instability (MSI-low/elevated microsatellite alterations at selected tetranucleotide repeats) and modifying the biological behavior of the CRC towards metastasis and poor patient survival. We herein discuss the mechanisms and significance of these induced inflammatory-associated microsatellite alterations, and suggest three content areas to further examine interventions that may modify the observed behavior of these CRCs.

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INTRODUCTION

The Cancer Genome Atlas (TCGA) for colorectal cancers (CRCs) clarified that there are two types of sporadic CRCs - hypermutated and non-hypermutated. Most hypermutated CRCs have a defect in their mismatch repair (MMR) system due to the loss of MLH1 function by promoter silencing of the *MLH1* locus, resulting in high levels of insertion/deletion (I/D) mutations at microsatellite loci (microsatellite instability high: MSI-H)^[1]. Most MSI-H CRCs exhibit proximal location, mucinous, undifferentiated histology, abundant CD8⁺/Th1 T cell infiltrations, and less aggressive clinical behavior, and are susceptible for immune checkpoints blockade^[2,3]. Among non-hypermutated CRCs, I/D mutations in microsatellite loci with larger repeat units (di- and tetra-nucleotide repeats) are frequent and have been shown to be caused by tumor cells' exposure to inflammatory tumor-microenvironments^[4,5]. In this review, we describe and discuss the penetrance and causes of inflammation-associated microsatellite alterations (IAMAs), and their significance to patients' prognoses in CRC. We also raise "Provocative Questions" whose answers could contribute not only to

understand the biology of IAMAs but also to treatment of CRC with IAMAs.

MSI-H, MSI-L AND EMAST IN CRC

Microsatellites or simple sequence repeats are composed of 1-6 nucleotide repeats, occupy 3% of the total human genome, and are located in both coding and non-coding regions^[6]. MSI is defined as continuous length changes in simple DNA repeat sequences within microsatellite loci^[7]. MSI in CRC was first reported by Aaltonen *et al*^[8] and Thibodeau *et al*^[9] followed by Ionov *et al*^[10] in 1993. It was then shown that a subset of sporadic CRC tumors and tumors from hereditary nonpolyposis colon cancer (HNPCC) exhibit MSI and MMR-defects^[11]. Subsequently, germline mutations in *MSH2*, *MLH1*, *PMS2* and *MSH6* were found in different HNPCC families^[12-18] and tumors from these families exhibited MSI^[19,20]. A causal relationship between MMR-defect, MSI and cancer susceptibility was shown by knockout mouse studies^[21-24]. Genetic complementation studies using tissue cultured MSI-positive CRC cells also confirmed that MSI is caused by MMR-deficiency in human cells^[25-27]. It was also shown that MSI exhibited in 10%-15% of sporadic CRC cases was due to transcriptional down-regulation of *MLH1* expression through promoter hyper-methylation^[28].

MSI in CRCs was defined at an international workshop meeting sponsored by the National Cancer Institute in 1998^[2]. A panel of five microsatellite markers - two markers with mononucleotide repeats and three markers with dinucleotide repeats - were validated to be classified as follows: High-frequency MSI (MSI-H: 2 or more of 5 markers show instability), low-frequency MSI (MSI-L: 1 of 5 markers shows instability), and microsatellite stable (MSS: none of 5 shows instability) CRCs. It was also confirmed that MSI-H in CRC is caused by defective MMR, mainly *MSH2* and *MLH1*, and manifests as sporadic and hereditary forms of CRCs. Both sporadic and inherited MSI-H CRCs have unique clinical and pathological features compared to MSI-L/MSS sporadic CRCs^[2]. At this NIH meeting, the presence of CRCs with MSI-L was appreciated and discussed. However, the etiology of MSI-L and the distinction between MSI-L and MSS CRC remained unclear. Another type of microsatellite alteration, called elevated microsatellite alterations in selected tetra-nucleotide repeats (EMAST), where insertion/deletion mutations in the loci with tri- and/or tetra-nucleotide but not with mono- and/or dinucleotide repeats was recognized as a component of CRC but its etiology and clinic-pathological significance was not determined^[2].

Although a consensus on the definition of MSI-L CRC was reached at the NCI meeting, two subsequent studies showed that approximately 80% of non-MSI-H CRC exhibited mutation at < 1 microsatellite locus when a large number of the loci with di-nucleotide repeats were tested for frame-shift mutations, indicating

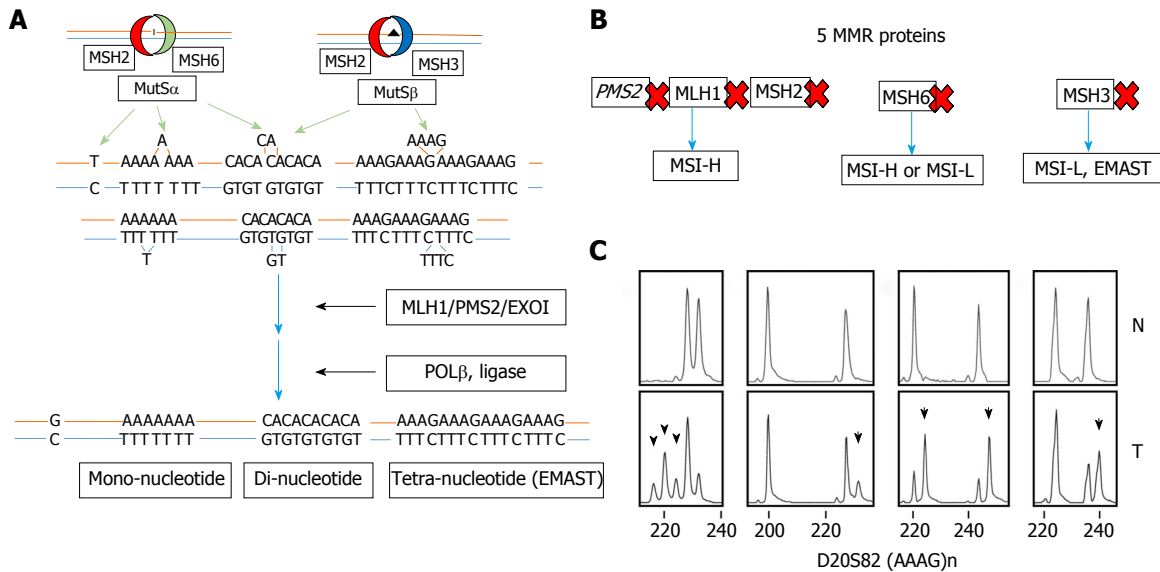


Figure 1 Human DNA mismatch repair. A: Two DNA recognition complexes MutS α , which recognizes insertion-deletion (I/D) loops of 1-2 repeated nucleotides for repair, and MutS β which recognizes I/D loops of 2 or greater nucleotides for repair, are the key protein complexes of MMR. The MLH1 and PMS2 complex, also known as MutL α , then helps execute the repair with the exonuclease Exo1, polymerase β and DNA ligase to fully effect repair; B: Specific efficiency in one of the five DNA MMR proteins yields differing microsatellite instability (MSI) results. Loss of MLH1, MSH2 or PMS2 will yield frameshifts at mono-, di- and tetra-nucleotide microsatellite markers. Loss of MSH6, inactivating MutS α only, will yield mononucleotide mostly but some dinucleotide microsatellite frameshifts, whereas loss of MSH3, inactivating MutS β , will yield di- and tetranucleotide microsatellite frameshifts, but no mononucleotide microsatellite frameshifts; C: Examples of fragment analysis comparing normal colon tissue (upper panels) with tissue (lower panels) demonstrating frameshifts in the tetranucleotide marker D20S82. MMR: Mismatch repair; MSI: Microsatellite instability; CRC: Colorectal cancer.

that most of CRC is MSI-L, and that the NCI reference panel was inadequate for detection of MSI-L CRC^[29,30]. These studies also showed that there were no genetic or clinic-pathological characteristics of tumors to separate MSI-L from MSS CRC. However, both studies observed that the incidence of MSI was non-randomly distributed among non-MSI-H CRC, suggesting that some tumors were more susceptible than others to slippage mutations at microsatellite loci, especially loci with dinucleotide repeats^[29,30]. The reason for the observed variation in instability and its pathological significance in patients' prognoses remained unclear.

I/D mutations in loci with selected tetra-nucleotide repeats (EMAST), such as (AAAG) $_n$ or (ATAG) $_n$, have been reported in non-CRCs including non-small cell lung, bladder, ovary, head and neck, skin and kidney cancers^[31]. Haugen *et al.*^[32] first described the frequency of EMAST in CRC, its relationship to MSI-L and its possible cause. They used the five NCI-endorsed MSI markers plus 2 additional markers with dinucleotide repeats to identify MSI-H, MSI-L and MSS CRC. They also used 7 EMAST markers and defined EMAST-positive if one or more of the 7 markers showed ID mutations^[32]. They found that EMAST is common in sporadic cases of non-MSI-H CRC (approximately 50%) and is associated with decreased nucleus MSH3 expression in tumor cells. Using MSH3-proficient and -deficient colon cancer cell lines, they also showed evidence that EMAST and low levels of instability at dinucleotide loci repeats - but not with mononucleotide repeats - in non-MSI-H CRC cells are caused by loss of MSH3^[32]. Frequent incidence of EMAST in CRCs was confirmed by 2 other studies^[33,34].

The genetic cause of EMAST due to the loss of MSH3 was also proven by other studies using tissue cultured human cells^[35,36].

BIOCHEMICAL BASIS OF MICROSATELLITE ALTERATIONS

Accumulated evidence supports that MSI-H, MSI-L and EMAST are caused by defects in some components of MMR^[37]. When DNA polymerase copies template DNA containing microsatellite loci, it mistakenly adds or deletes a repeat unit in the newly synthesized DNA strand (Figure 1A). The DNA polymerase slippage errors create loops between the two strands, which are recognized and repaired by MMR. *In vitro* experiments using cell extracts and/or purified proteins demonstrate that there are 5 MMR proteins involved in MMR reactions in human cells (Figure 1)^[38]. MSH2 plays a major role in recognition of mismatched DNA. MLH1 and PMS2 are the main proteins responsible for down-stream MMR reactions. If MSH2, MLH1 or PMS2 lose their function, slippage errors at microsatellite loci with mono-, di- and tetra-nucleotide repeats are not fixed at all, resulting in MSI-H (Figure 1B). There are 2 pathways for mismatch recognition: (1) MSH2 and MSH6 form a dimer called MutS α that preferentially recognizes mismatched nucleotides and loops containing 1-2 nucleotides; (2) MSH2 and MSH3 form a dimer called MutS β that recognizes loops containing 2 or more nucleotides generated at di- and tetra-nucleotide repeats, including the EMAST loci (Figure 1A)^[39]. Defects in MSH6 result in increased

Table 1 Expression of MSH3 protein within the epithelium of normal colonic mucosa and adenoma of patients with mono- or bi-allelic germline mutation in *MSH3*

Tissue (epithelium)	Monoallelic <i>MSH3</i> germline mutation	Bi-allelic <i>MSH3</i> germline mutation
Normal colonic mucosa	MSH3 expressed	MSH3 absent
Colon adenoma	Not obtained	MSH3 absent

Extracted from Adam *et al.*^[42].

missense mutations and in instability at mononucleotide repeats (Figure 1A)^[40]. When only MSH3 is disabled, increases in instability at di-, tri- and tetra-nucleotide repeats (EMAST) but not at mononucleotide repeats are observed (Figure 1)^[32]. Biochemical data indicates that loops containing 2 nucleotides are preferentially recognized by MutS β over MutS α ^[41]. Thus, when loss of MSH3 leaves many loops containing 2 or more nucleotides unrepaired, MutS α may repair some but not all such loops, resulting in low levels of mutation in di-nucleotide repeat loci and high levels of mutation in loci with tetra-nucleotide repeats (EMAST) loci (Figure 1B and C).

MSI-L AND EMAST ARE CAUSED BY MSH3 FUNCTIONAL LOSS IN CRC

The first evidence that loss of MSH3 may result in MSI-L and/or EMAST in CRC was reported by Haugen *et al.*^[32] in 2008. They used the colon cancer cell line HCT116 that is deficient in MLH1 due to a hemizygous inactivating mutation in exon 9, and is also deficient in MSH3 due to a homozygous frameshift inactivating mutation in exon 7. Thus, this cell line showed the MSI-H phenotype. Introduction of a normal human chromosome 3 carrying a wild-type *MLH1* to HCT116 complemented MLH1-deficiency^[25]. The resulting HCT116 with chromosome 3 exhibited stability in loci with mononucleotide repeats but showed low levels of instability at loci with dinucleotide repeats: MSI-L, and high degree of instability at EMAST loci. They further introduced a normal human chromosome 5 carrying wild-type MSH3 into HCT116 + 3 cells. The resulting HCT116 + 3 + 5 cells exhibited complete stability at loci with mono-, dinucleotide repeats and EMAST loci. Finally, they introduced MSH3-shRNA to HCT116 + 3 + 5 cells to knock-down MSH3 and showed that specific knock-down of MSH3 resulted in an MSI-L/EMAST phenotype.

The second evidence that loss of MSH3 results in MSI in loci with di- and tetra-but not mono-nucleotide repeats is from a discovery of two families with bi-allelic MSH3 germ-line mutations, reported by Adam *et al.*^[42]. Patients with bi-allelic inactivation mutations of the MSH3 locus suffered from a colorectal adenoma polyposis syndrome and early occurrence of multiple adenoma polyps and tumors in other organs. As expected, the expression of MSH3 was null in normal colon and adenoma polyps from these patients (Table 1). MSI assays showed that instability at di-nucleotide repeat loci and EMAST loci, but not loci with

mononucleotide repeats, was detected in adenoma polyps but not in normal colon cells from the same patient. This is because adenoma is monoclonal while the normal colon of these patients consists of mixture of cells with MSI at different loci, masking each alteration that occurred in individual colon cells with the exception of germline alleles. However, there is likely dinucleotide and tetranucleotide instability within normal tissues if they were compared to heterozygous *MSH3* germline relatives, or relatives that are homozygous normal for *MSH3* mutation. These results support that MSI-L/EMAST in sporadic CRC is caused by loss of MSH3 function.

EVIDENCE THAT MSI-L/EMAST IN SPORADIC CRC IS INDUCED BY INFLAMMATION THROUGH DISPLACEMENT OF MSH3 FROM NUCLEUS TO CYTOPLASM

While homogeneous loss of nuclear MSH3 can be detected in adenoma polyp with bi-allelic germline *MSH3* mutations, heterogeneous loss of nuclear MSH3 is frequently detected in sporadic CRC exhibiting MSI-L/EMAST (Figure 2A). These results suggest that local loss of MSH3 expression in sporadic MSI-L/EMAST CRC may be not due to genetic loss of MSH3. TCGA data shows that the frequency of *MSH3* somatic mutations in CRC is about 6.6%. This does not explain the high incidence of MSI-L/EMAST (approximately 50%) in CRC. Furthermore, most *MSH3* mutations are frameshift mutations in exon 7 that are a resulting target from *MLH1* inactivation in sporadic CRC (Table 2)^[1].

Lee *et al.*^[34] found that EMAST CRC is enriched in in the tumor microenvironment of CD8⁺ T cells compared to non-EMAST CRC, suggesting that some immunological and inflammatory responses are active in EMAST CRC. They also found that EMAST is significantly high in ulcerated tumors. Devaraj *et al.*^[43] further showed that EMAST-positive rectal tumors are associated with the presence of chronic inflammation. These observations led them to hypothesize that inflammation may somehow affect MSH3 function that induces MSI-L/EMAST.

Tseng-Rogenski *et al.*^[4,36] demonstrated that several main inflammatory factors, including oxidative stress (hydrogen peroxide), interleukin 6 (IL6) and prostaglandin E2 (PGE2) induce displacement of MSH3 from the nucleus to the cytoplasm in several

Table 2 Comparison of type of mismatch repair gene mutations between sporadic hypermethylated *MLH1* colorectal cancers and *POLE* mutation colorectal cancers from TCGA

<i>MLH1</i> promoter hypermethylation	22/35 (63%) of hypermutated CRCs	8/22 (36%) with MSH3 frameshift mutation 1/22 (4.5%) with MSH3 missense/nonsense mutation 0/22 (0%) with MSH2 mutation 5/22 (23%) with MSH6 frameshift mutation 4/22 (18%) with MSH6 missense/nonsense mutation
<i>POLE</i> mutation	13/35 (37%) of hypermutated CRCs	3/13 (23%) with MSH3 frameshift mutation 2/13 (15%) with MSH3 missense/nonsense mutation 5/13 (38%) with MSH2 missense/nonsense mutation 0/13 (0%) with MSH6 frameshift mutation 7/13 (54%) with MSH6 missense/nonsense mutation

Both types of CRCs are hypermutated, containing hundreds of somatic mutations in genomic DNA. Note that the *MLH1* hypermethylated CRCs demonstrate higher frequency and consistent frameshift mutations in *MSH3* and *MSH6* as compared to *POLE* mutated CRCs, which contain some frameshifts but higher frequency of missense/nonsense mutations in *MSH3*, *MSH2* and *MSH6*. Extracted from: Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 333-337. CRCs: Colorectal cancers.

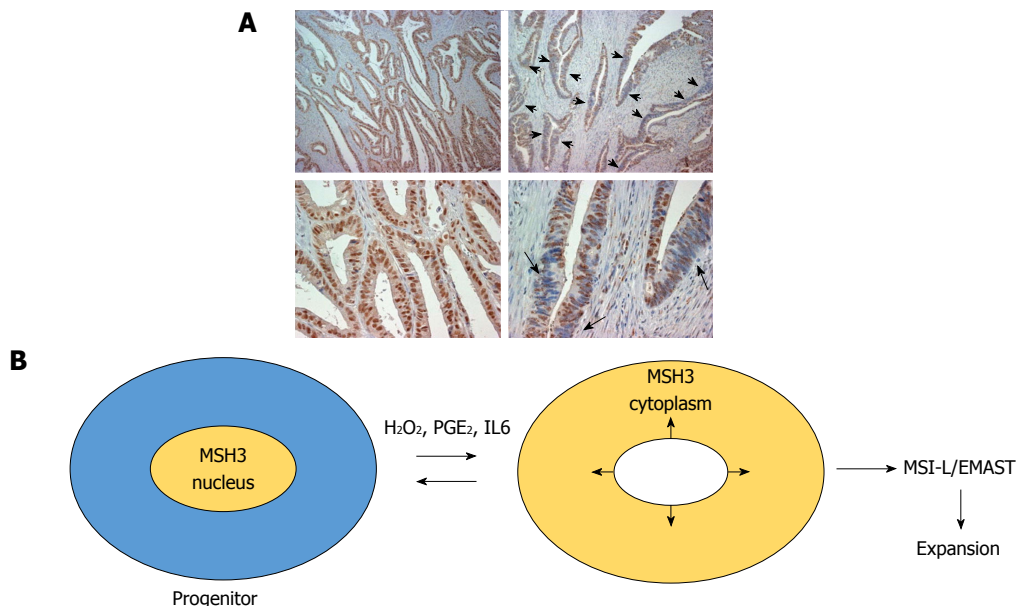


Figure 2 MSH3 expression in sporadic colorectal cancer. A: Immunohistochemistry for MSH3 in sporadic CRC. Arrows show heterogeneous expression of MSH3 in cells and within nuclei in the epithelium; B: Model of MSH3 displacement from the nucleus to the cytosol with inflammatory stimuli to allow accumulation of tetranucleotide frameshift mutations. Progenitor cells could be affected earlier such that subsequent daughter cells amplify the accumulated frameshift mutations. MSI: Microsatellite instability; CRC: Colorectal cancer; EMAS: Elevated microsatellite alterations at selected tetranucleotide repeats.

cancer cell lines. Importantly, other MMR proteins including *MLH1*, *MSH2* and *MSH6* do not move from the nucleus to the cytoplasm in response to these stimuli. Repeated treatment of several microsatellite stable colon cancer cell lines with IL6 induced microsatellite instability at EMAS loci. However, other inflammatory cytokines including $\text{TNF}\alpha$, $\text{IFN}\alpha$, $\text{IFN}\beta$, and $\text{IL1}\beta$ did not have such an effect. Tseng-Rogenski *et al.*^[41] also showed that phosphorylation of STAT3 may be required for displacement of MSH3 when induced by IL6. These studies convincingly show that not all, but some, inflammatory factors induce EMAS through loss of MSH3 from the nucleus (Figure 2B).

Evidence that an inflammatory micro-environment induces MSI-L (low levels of MSI at the loci with dinucleotide repeats) has been shown in regenerated colon tissues from ulcerated colitis (UC) patients. The

first study, reported by Brentnall *et al.*^[44], showed for the first time the presence of MSI-L but not MSI-H in colon tissues from UC patients. The second study, by Ozaki *et al.*^[45], isolated crypts from UC-derived CRC, UC-derived hyperplasia and UC-regenerated colons through laser micro-capture and tested for the presence of microsatellite instability in DNA. Ozaki *et al.*^[45] detected MSI-L but not MSI-H in some crypts but not in others, regardless of whether they were from cancer or non-cancer tissues. They also showed that MSI was not detected from stroma cells from these UC patients. Each crypt showed a different MSI-profile, indicating that MSI-L occurs independently at the crypt level. Our recent study showed that regenerated colon cells and CRCs from UC patients have a high frequency of MSH3 displacement from the nucleus to the cytoplasm, and demonstrate MSI-L/EMAS^[46].

These results further support the role of inflammation in displacement of MSH3-induced MSI-L/EMAST in human tissues including cancers.

PROGNOSTIC VALUE OF MSI-L/EMAST IN CRC

Several studies have examined the impact of MSI-L and/or EFAST genotypes on patient prognoses in CRC. There have been 4 studies evaluating the prognosis values of MSI-L^[47-50]. Kohonen-Corish *et al.*^[47] showed that patients with stage C colon cancers defined as MSI-L by the NCI panel plus one tetra-nucleotide marker (*MYCL1*) showed poor overall survival (OS) compared to patients with MSI-H and/or MSS colon cancers. Similar results were obtained by Wright *et al.*^[48]. They showed that stage C CRC patients that are positive for MSI-L as defined by the NCI panel, plus an additional 2 markers with mono-nucleotide repeats, 3 with di-nucleotide repeats and the tetra-nucleotide *MYCL1* marker, exhibited poor cancer-specific survival compared to MSS CRC patients^[48]. They also observed that most MSI-L CRC exhibited MSI at one di- or tetra-nucleotide but not at mono-nucleotide repeat markers^[48]. Lee *et al.*^[49] examined 3019 CRC cases for MSI using an NCI microsatellite marker panel and evaluated prognoses of those patients. Similar to other studies, they showed that most MSI-L CRC exhibited MSI at dinucleotide repeats, and patients with MSI-L CRCs was associated with poor OS by Cox regression analysis^[49]. Although the previous 2 studies suggested that MSI-L may have a significant prognostic value for stage C CRC patients, Lee *et al.*^[49] did not examine the prognostic significance of MSI-L for cancer-specific survival in their large cohort. In contrast to the above three studies, Azzoni *et al.*^[50] reported that MSI-L is associated with improved patient survival as compared to MSS CRC. However, the percentage of MSI-H cases in their cohort was unusually high (37%: 68 of 184 cases) compared to other studies (10%-15%), suggesting the presence of some bias in the studied cohort. Lastly, a study reported by Garcia *et al.*^[51] did not find any association between MSI-L and disease-free survival (DFS) or OS in stage II and III CRC cohorts.

There are 2 studies examining the relationship between EFAST and OS in CRC; they found no association between the two^[33,51]. However, when both MSI-L and EFAST cases were combined, Garcia *et al.*^[51] found that MSI-L/EFAST was associated with shorter DFS but not OS compared with non-MSI-L/EFAST CRC. In their cohort, MSI-H CRC patients exhibited the highest survival. Thus, the MSI-L/EFAST genotype in CRC may be associated with recurrence and/or metastasis after surgery. There appears to be heterogeneity even among MSI-L/EFAST CRC patients^[52,53]. One group of MSI-L/EFAST CRC exhibited loss of heterozygosity (LOH) at chromosome 9p24.2. and the other did not exhibit 9p24.2 LOH. When the prognoses of these two groups were compared, the one with 9p24.2 LOH at stage

III showed improved survival after surgery and OS in Kaplan-Meier analysis and in multi variate analysis over the one without 9p24.2. LOH at stage III^[53]. The results also showed that MSI-L/EFAST/9p24.2 LOH is an independent factor that predicts improved OS in stage II/III CRC. Thus, MSI-L/EFAST may be associated with recurrence, but additional genetic or epigenetic changes may modify the behavior of recurrent tumors^[53]. Overall, the data presented so far suggest that MSI-L and/or EFAST could be a biomarker for DFS and/or OS of stage II and/or III CRC. However, additional studies using a population-based large cohort are needed to confirm the prognostic value of MSI-L and EFAST.

One concern regarding EFAST is that various studies have not reached a full consensus on the definition of EFAST. As described above, current evidence supports the idea that MSI-L and EFAST in sporadic CRC share the same etiology: both are induced by the absence of nuclear MSH3 in response to exogenous inflammatory factors such as IL6, and oxidative stress^[4]. Based on these observations, we propose that EFAST cancer is a non-MSI-H cancer, and MSI at EFAST markers is not caused by loss of other MMR proteins including MLH1, MSH2, PMS2^[51,53]. The next question should be whether or not non-EFAST CRC really exists. Similar to MSI-L in CRC^[29,30], almost all CRC could be EFAST-positive if a large number of EFAST markers are used. A recent study by Cortes-Ciriano *et al.*^[54] showed that all non-MSI-H cancers contain various levels of frame-shift mutations in microsatellite loci with mono-, di-, tri- and tetra-nucleotide repeats. Considering that all tumor tissues contain some degree of inflammatory elements, many of those mutations could be induced by the loss of MSH3 triggered by inflammation in the tumor-microenvironment. Furthermore, a study for UC suggested that frequent exposure to inflammation increased the incidence of MSI-L and EFAST^[46]. Thus, while the purpose of the MSI assay is primarily to detect MMR-deficient CRC, the purpose of an EFAST assay could be to distinguish CRCs whose precursors were exposed to high levels of inflammation to CRCs whose precursors were exposed to lower levels of inflammation. Therefore, the results of the studies by Kohonen-Corish, Wright, Lee and Garcia could be re-interpreted according to the idea that high levels of inflammatory tumor-microenvironments not only induce MSI-L/EFAST in cancer cells at the primary site but also include some property that promotes recurrence and/or metastasis when they disseminate. Additional studies will be required to determine whether the numbers and kinds of EFAST markers and cut-off levels for determining EFAST-positive/negative used so far are adequate to distinguish CRCs with different prognoses^[31].

PROVOCATIVE QUESTIONS

Here, we have raised three questions whose answers can be important for not only clinical but also basic

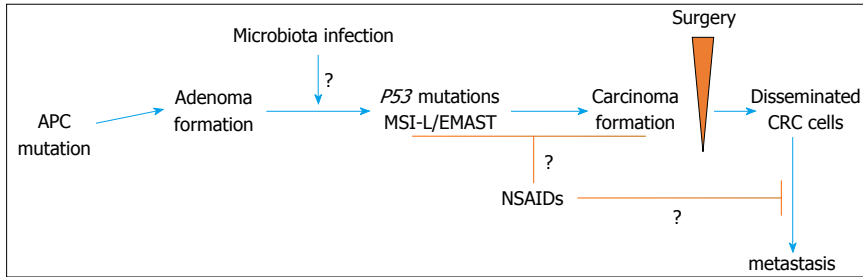


Figure 3 Model of adenoma-to-carcinoma formation in the human colon, with actual and potential sites of interventions to improve survival. MSI: Microsatellite instability; CRC: Colorectal cancer; EMAS: Elevated microsatellite alterations at selected tetranucleotide repeats.

aspects of MSI-L/EMAST in CRC (Figure 3).

Question 1: Does treatment of CRC with non-steroidal anti-inflammatory drugs reduce not only recurrence/metastasis but also the incidence of MSI-L/EMAST?

The idea that inflammation is associated with recurrence and/or metastasis is indirectly supported by observations that an intake of the anti-inflammatory drug, aspirin, may not only prevent adenomas^[55] and CRC formations^[56], but also prevent recurrence and metastasis of CRC following surgery^[57]. Other non-steroidal anti-inflammatory drugs (NSAID) including celecoxib and rofecoxib, specific inhibitors of cyclooxygenase 2 (COX-2), have been shown to reduce the incidence of adenomas^[58-60]. But it was also found that COX-2 inhibitors suppressed colorectal tumor growth and metastasis in mouse models^[61,62]. Furthermore, Chan *et al.*^[63] reported that the regular intake of aspirin after curative surgery reduced cancer-specific mortality in a sub-group of CRC cells expressing a high level of COX-2 protein. In addition, CRCs expressing HLA class I compared to those not expressing HLA class I are susceptible for aspirin treatment after diagnosis^[64]. Also, CRCs with *PIK3CA* mutations responded better to aspirin treatment after diagnosis than did CRCs with wild-type *PIK3CA*^[65]. However, a recent study by Gray *et al.*^[66] showed that the efficacy of aspirin on cancer-specific survival, and OS was associated with levels of COX-2 expression but not with mutational status of *PIK3CA* in CRCs. Ng *et al.*^[67] showed that aspirin and COX-2 inhibitors improved recurrence-free survival, DFS and OS of stage III CRC patients who either received fluorouracil (FU) plus leucovorin (LV) or FU plus LV with irinotecan. These studies support the idea that NSAIDs can be used as part of adjuvant therapy for stage I - III CRC, however, the efficacy of NSAIDs on the recurrence/metastasis of CRC are still under investigation through several randomized controlled trials^[57].

Ma *et al.*^[68] showed that PGE2 and its receptor, the prostaglandin E receptor 2 (EP2), are necessary for colon cancer formation in inflammatory tissue environments. Compared to wild-type mice treated with azoxymethan (AOM) followed by dextran sodium sulfate (DSS), AOM/DSS-treated EP2-knockout and prostaglandin E synthase (*Ptges*)-knockout mice bore a significantly reduced number of colon tumors.

They identified neutrophil, probably myeloid-derived suppressor cells (MDSC), and cancer-associated fibroblast (CAF) as the main cell components recruited in tumor-microenvironments, expressing EP2, responding to PEG2, and contributing to tumor formation. These cells form a positive-feedback loop of COX-2-PGE2-EP2-NF- κ B-COX-2 cycles, and produce TNF- α and IL6^[68]. The presence of MDSC and CAF in the tumor-microenvironment are also significantly associated with stage progression and a poor prognosis for CRC, while activation of Th1 helper and cytotoxic memory T cells play a key role in anti-tumor activities preventing recurrence and/or metastasis in CRC^[69,70]. Interestingly, Zelenay *et al.*^[71] showed that, depending on the level of COX-activity in cancer, the immunological landscape of tumor-microenvironments can be switched between anti-tumor and inflammatory pro-tumor. Therefore, the level of PEG2 and of COX-2 may be major factors in controlling immunological responses to cancer cells, and thereby a patient's prognosis. Regarding the relationship between MSI-L/EMAST and PEG2, we have observed that the exposure of colon cancer cells in tissue cultures to PGE2 triggers movement of MSH3 from the nucleus to the cytoplasm, which may induce MSI-L/EMAST. Therefore, it is reasonable to speculate that MSI-L/EMAST in CRC may be associated with high levels of COX-2 expression in cancer cells and/or in tumor-microenvironments. This could be the reason why patients with MSI-L/EMAST CRCs exhibit a shorter RFS^[51,53]. Thus, reduction of PGE2 by NSAIDs may reduce the incidence and recurrence/metastasis of MSI-EMAST. If this is the case, MSI-L/EMAST could be a biomarker for susceptibility to the NSAIDs treatment.

Question 2: Do microbiota play a role in MSI-L/EMAST formation, adenoma/carcinoma transition and recurrence/metastasis?

Lee *et al.*^[34] discovered that EMAS is less frequent in colorectal adenomas and well-differentiated adenocarcinomas than in moderately differentiated and poorly differentiated adenocarcinomas, suggesting that EMAS is progressively acquired during the histological adenoma-carcinoma sequence, from adenoma to well-differentiated carcinomas to moderately and poorly differentiated carcinomas. Because a key gene alteration responsible for adenoma-carcinoma sequence

in CRC is *p53* mutation^[72], MSI-L/EMAST formation may be associated with *p53* mutation. In fact, Ahrendt *et al.*^[73] reported that EMAST is associated with *p53* mutations in non-small cell lung cancer. Li *et al.*^[74] observed an association between LOH at *TP53* and EMAST in CRC. Interestingly, *p53* mutations are the most frequently found in inflammatory bowel disease (IBD)-associated CRC among other gene mutations (60%-90%)^[75,76]. One half of the *p53* mutations are C:G>T:G transitions, thought to be caused by nitric oxide exposure due to increased inducible nitric oxide synthase expression in IBD^[75]. Our preliminary data showed that IBD-associated CRC exhibit a higher frequency of MSI-L/EMAST than do sporadic CRC (unpublished data). Taken together, these results suggest that the inflammatory tissue environment may enhance *p53* mutations and MSI-L/EMAST formation in sporadic adenomas, leading to carcinoma transition. As mentioned earlier, MSI-L/EMAST in stage II CRC patients is associated with shorter RFS, suggesting that the inflammatory tumor-environment in primary tumor tissues somehow promotes recurrence or metastasis. These observations lead to the next question: What establishes an inflammatory environment in colorectal adenoma and carcinoma?

Microbiota in the colon and rectum create an inflammatory microenvironment and promote CRC formation^[77]. Several bacterial organisms including *Fusobacterium nucleatum* (*F. nucleatum*), Enterotoxigenic *Bacteroides fragilis* (*ETBF*), and colibactin-producing *Escherichia coli* (*E. coli*) are epidemiologically associated with CRC, and have been found to be enriched in CRC^[77,78]. The enrichment of *F. nucleatum* was also found in colorectal adenoma relative to non-adenoma or surrounding tissues^[79-81]. McCoy *et al.*^[79] showed that *F. nucleatum* abundance in colorectal adenoma is associated with local inflammatory cytokine gene expression including IL-10 and TNF- α . Kostic *et al.*^[80] investigated the effect of *F. nucleatum* infection on the development of intestinal tumors in *APC*^{Min/+}, IL10^{-/-} and T-bet^{-/-} X Rag2^{-/-} mice. There was an increase in the number of tumors in *APC*^{Min/+} mice. Importantly, infection with *F. nucleatum* accelerated adenocarcinoma formation in the small intestines of *APC*^{Min/+} mice compared to sham-treated control mice. In contrast, infection with *F. nucleatum* did not induce any tumor formation in IL10^{-/-} and T-bet^{-/-} X Rag2^{-/-} mice. These results suggest that the effects of *F. nucleatum* may manifest on existing adenomas, and may stimulate adenoma-carcinoma transition by creating an oxidative stress-rich, carcinogenic environment^[80]. It would be interesting to determine whether *F. nucleatum* -induced adenocarcinomas in *APC*^{Min/+} mice gain *p53* mutations. Kostic *et al.*^[80] further showed that infection of tumor tissues with *F. nucleatum* results in recruitment of MDSCs, tumor-associated macrophages, and dendritic cells in tumor tissues, and modulate the tumor immune micro-environment that promote tumor progression. In

addition, they found the up-regulation of genes that are down-stream of NF- κ B including *PTGS2* (*COX-2*), *IL6*, *IL1 β* , and *TNF* in both human and mouse CRC infected with *F. nucleatum*^[80]. It is tempting to speculate that *F. nucleatum*-induced adenocarcinoma may gain MSI-L/EMAST in response to oxidative stress, PEG2 and/or IL6 that cause displacement of MSH3 from the nucleus to the cytoplasm. Recently, a heavy load of *F. nucleatum* has been associated with MSI-H CRC, proximal colon cancer and a poor prognosis^[81-84]. Yu *et al.*^[85] showed that *F. nucleatum* infection in primary CRC is associated with recurrence after surgery followed by adjuvant chemotherapy. They showed that *F. nucleatum* induces chemoresistance in infected cells through autophagy^[85]. One of the reasons why 5-FU-based adjuvant therapy does not have benefit for a sub-group of MSI-H CRC^[86,87] could be partly explained by the infection of *F. nucleatum*^[85]. It is also possible that the CpG Island Methylator Phenotype (CIMP) including promoter methylation of the *MLH1* locus could be induced by chronic inflammation due to a heavy load of *F. nucleatum* infection^[82]. Considering that infection of *F. nucleatum* is associated with recurrence of CRC after surgery, a group of such CRCs may exhibit MSI-L/EMAST CRC^[51,53].

Another bacterium, *ETBF*, is also associated with CRC^[88-90] and can target colorectal cells to promote an adenoma and/or adenoma-carcinoma transition in *APC*^{Min/+} mice^[91]. *ETBF* produces a metalloprotease toxin called BFT. BFT binds to the surface of colorectal epithelial cells and induces E-cadherin cleavage, resulting in an increase in barrier permeability and inducing an inflammatory micro-environment with Th-17/IL-17 predominance^[91,92]. Th-17/IL-17 plays a major role in *ETBF* tumorigenesis because the depletion of CD4⁺ T cells and blockade of IL-17 inhibited it. IL-17 attracts neutrophils, MDSCs and macrophages, and induces carcinogenic and immunosuppressive factors including nitric oxide, ROS, and Arg1 in mouse models^[92]. Colibactin-producing *E. coli* is also associated with CRC^[93,94] and initiates inflammation and promotes adenoma formation in *APC*^{Min/+} mice^[94] and in *APC*^{Min/+}, IL10^{-/-} mice^[95]. Taken together, infection with all three bacterial organisms, that are found to be associated with CRC, induces an inflammatory environment in adenoma tissue and promotes adenoma and/or a transition from adenoma to carcinoma in mouse models. It would be interesting to determine whether MSI-L/EMAST and *p53* mutations coincide with bacterial-induced transitions to adenoma/carcinoma. Recently, Scott *et al.*^[96] showed that the efficacy of 5-FU treatment maybe largely influenced by microbiota in the gut.

Question 3: Is MSH3 a component of DNA damage signaling?

The MutS β hetero-duplex between MSH3 and MSH2 not only functions in MMR but may also play a role

in double strand break (DSB) repair *via* homologous recombination (HR)^[97-100]. DNA double strand breaks (DSB) induce cell death if not repaired. Cells have evolved two pathways to re-connect the broken DNA ends: Non-homologous end joining (NHEJ) and homologous recombination (HR). If one of these pathways is disabled when DSB is created, cells use the other pathway for survival. The HR reaction starts with a nuclease-mediated resection of broken DNA ends to be coated by the single stranded (ss) DNA-binding protein, replication protein A (RPA). Then, Ataxia telangiectasia and Rad3-related (ATR) kinase is recruited to the RPA-coated ssDNA *via* an ATR-interacting partner (ATRIP). The topoisomerase II β -binding protein 1 (TOPBP1), which is recruited to the DSB site, interacts with ATRIP and activates ATR. Activated ATR phosphorylates CHEK2 that regulate cell cycle progression. TOPBP1 also interacts with polo-like kinase (PLK) that phosphorylate RAD51 for its loading on resected ssDNA^[101]. Burdova *et al.*^[99] showed that recruitment of ATR/ATRIP to RPA-coated ssDNA is mediated by MutS β which binds to the loop structure formed within the ssDNA. Therefore, MutS β is required in the early stage of HR-DSB repair and its loss due to an MSH2 or MSH3 defect forces a cell to use NHEJ for survival under the presence of DSBs^[98,100]. Thus, when oxidative stress causes DSBs, it may induce elimination of MSH3 from the nucleus, resulting in activation of Ataxia-telangiectasia mutated (ATM)^[102] but not ATR, and dependence of NHEJ for survival.

An intriguing question is why and how nuclear MSH3 proteins translocate in response to oxidative stress or exposure to IL6 or PGE2 (Figure 2B)^[4,36]. H₂O₂ and oxidative stress causes DSBs, resulting in activation of NF- κ B^[103,104]. IL6 and PGE2 are mediators that possibly form a loop associated with activation of NF- κ B through STAT3^[105-107]. IL6 activates STAT3, which directly interacts with the NF- κ B family member RELA, contributing to constitutive NF- κ B activation^[105], and COX2/PGE2 also activates STAT3, leading to NF- κ B activation^[106]. We found that MSH3 itself is a shuttling protein. It contains a bona fide bipartite nuclear localization signal (NLS) that directs its nuclear import to perform DNA repair (unpublished data). It also contains two functional nuclear export signals (NESs) that allow it to exit the nucleus upon the treatment of a pro-inflammatory cytokine, IL-6 (unpublished data). Among the other main MMR proteins including MSH2, MSH3 is the only MMR protein that shifts into the cytoplasm upon oxidative stress or IL6 treatment, suggesting that MSH3 moves alone or does so with other unknown partner proteins. Recent data indicate that the NF- κ B Essential Modulator (NEMO), when used as a bait, can pull down MSH3, suggesting physical interaction between these two proteins^[108]. As one of the three components of the IKK complex, NEMO's role in regulating the NF- κ B pathway is well documented^[104]. It is possible that simultaneous or sequential movement of MSH3, NEMO and ATM in

the cell may transmit a DNA damage signal to NF- κ B, depending on the degree of DNA damage. Further studies are necessary for clarify these possibilities.

MSI-L/EMAST IS COMMON IN HUMAN CANCERS

Since the discovery of MSI in CRC, MSI-L and EMAST have been examined in cancers from other organs and tissues. MSI-L has been found in stomach^[109], cervical^[110], pancreatic^[111], ovarian^[112], skin^[113], nerve^[114], breast, endometrial^[115], liver^[116], esophageal^[117], eye^[118], soft tissue^[119], gallbladder^[120], head and neck^[121], prostate^[122], lung^[123] and cancers of the urinary tract^[124]. EMAST has also been widely detected in other various human cancers^[31]. A recent study by Cortes-Ciriano *et al.*^[54] showed that there are MSI-H prone cancers including colorectal, esophageal, stomach and endometrial cancers, and non-MSI-H prone cancers that include ovarian, kidney, liver, breast, head and neck, cervical, lung, pancreatic, bladder, prostate, skin, adrenal, cortical and thyroid cancers. They also showed that most non-MSI-H cancers exhibit different degrees of MSI at not only loci with mono- but also loci with di-, tri- and tetra-nucleotide repeats, suggesting that inflammation-induced MSH3 replacement from the nucleus to the cytoplasm is probably common in human cancers^[54,125,126]. Thus, the answers to the provocative questions raised above may also apply to many human cancers.

CONCLUSION

MSI-L/EMAST is common in human cancers. MSI-L/EMAST is caused by displacement of MSH3 from the nucleus to the cytoplasm in replicating cells triggered by inflammatory stimuli, and can be termed Inflammatory-Associated Microsatellite Alterations (IAMAs). MSI-L/EMAST is associated with recurrence and/or metastasis in CRC patients. MSI-L/EMAST CRC is a heterogeneous group and consists of sub-groups with different genetic changes and prognoses.

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Advance in plasma *SEPT9* gene methylation assay for colorectal cancer early detection

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Abstract

This review article summarizes the research advances of the plasma-based *SEPT9* gene methylation assay for the clinical detection of colorectal cancer and its limitations. Colorectal cancer is a common malignancy with a poor prognosis and a high mortality, for which early detection and diagnosis are particularly crucial for the high-risk groups. Increasing evidence supported that *SEPT9* gene methylation is associated with the pathogenesis of colorectal cancer and that detecting the level of methylation of *SEPT9* in the peripheral blood can be used for screening of colorectal cancer in susceptible populations. In recent years, the data obtained in clinical studies demonstrated that the *SEPT9* gene methylation assay has a good diagnostic performance with regard to both sensitivity and specificity with the advantage of better acceptability, convenience and compliance with serological testing compared with fecal occult blood tests and carcinoembryonic antigen for colorectal cancer (CRC). Furthermore, the combination of multiple methods or markers has become a growing trend for CRC detection and screening. Nevertheless, the clinical availability of the methylated *SEPT9* assay is still limited because of the large degree of sample heterogeneity caused by demographic characteristics, pathological features, comorbidities and/or technique selection. Another factor is the cost-effectiveness of colorectal cancer screening strategies that hinders its large-scale application. In addition, improvements in its accuracy in detecting adenomas and premalignant polyps are required.

Key words: Plasma; *SEPT9*; Methylation; Colorectal cancer; Early detection

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Core tip: The methylated *SEPT9* gene has been implicated as a biomarker for colorectal cancer associated with the pathogenesis of colorectal cancer (CRC). In this article, we reviewed the literature on the correlation of *SEPT9* gene and colorectal cancer and the theoretical basis of the *SEPT9* gene methylation assay. Then, we focused on the diagnostic performance of the *SEPT9* gene methylation assay for CRC by analyzing the clinical trial studies and compared that assay with other methods. Finally, we discussed the limitations of the *SEPT9* gene methylation assay in clinical application. We hope that this article can provide a comprehensive overview of the progress achieved in the *SEPT9* methylation assay for both the basic and clinical sciences.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common malignant tumors of the digestive system and results in significant morbidity and mortality. As it is estimated, there were approximately 135430 new cases of colorectal cancer, including men and women, in 2017^[1]. The incidence is higher in men than women and markedly increases with age^[2]. CRC kills almost 700000 people every year, making it the world's fourth deadliest cancer (after lung, liver and stomach cancers)^[3]. As research has shown, the incidence and mortality rates of CRC vary up to 10-fold worldwide, with distinct gradients across human development, pointing towards widening disparities and an increasing burden in countries in transition^[4]. In general, its incidence and mortality rates are still rising rapidly in many low-income and middle-income countries.

The initial symptoms of colorectal cancer, however, are atypical, leading to a poor prognosis and high fatality rate. Therefore, screening of CRC in the population is of great significance for its early diagnosis and treatment. Currently, CRC screening approaches are divided into two categories: Invasive and noninvasive methods. The invasive methods, such as colonoscopy, remain the main screening tools due to their very good diagnostic performance, enabling the detection and removal of precancerous lesions^[5]. However, it requires thorough bowel preparations. Additionally, discomfort and privacy infringement contribute to poor compliance among patients. Non-invasive screening approaches, which include fecal occult blood tests (FOBT), fecal immunochemical tests (FITs) and

carcinoembryonic antigen (CEA), are more easily acceptable. However, their effectiveness may not be guaranteed. Although various guideline-recommended methods are available for CRC detection, patient compliance remains low. The data in 2013 showed that only approximately 57% of eligible adults adhered to the screening recommendations provided by the United States Preventive Services Task Force^[6]. Thus, it is very important to develop an efficient approach to enhance patient compliance that can be applied to screening the general population.

Studies^[7-9] have shown that the DNA methylation of certain genes is closely related to the development of colorectal cancer. Beggs *et al.*^[10] verified that methylation changes contribute substantially to the progression from normal mucosa to adenoma and to carcinoma; for instance, GRASP, which encodes the general receptor for phosphoinositide 1-associated scaffold protein, was differentially methylated in colorectal cancer. Aberrant DNA methylation in the genome may contribute to malignant transformation by silencing multiple tumor-suppressor genes. This type of epigenetic alteration is believed to occur early in tumor development and may precede genetic changes^[11]. In recent years, *SEPT9* gene methylation has been recognized as a hotspot and is considered to be a specific biomarker of the early stages of colorectal cancer. It may be a reliable indicator for screening CRC among high-risk individuals. This paper reviews the progress in the plasma-based *SEPT9* gene methylation assay for the detection of colorectal cancer.

SEPT9

As we know, there are 14 members (SEPT1-SEPT14) in the SEPT gene family, whose protein products Septins are a series of highly conserved GTP binding protein family. In humans, there are 13 genes, respectively named SEPT1 to SEPT13; the *SEPT9* gene is located on the human chromosome 17q25. 3^[12], contains 17 exons, and spans 240×10^3 bp. The 5'-end regulatory regions of the *SEPT9* gene have a -C- phosphor -G- site (CpG island), which is the main site of DNA methylation. In mammals, 60%-90% of CpG sites are methylated, and most of the remaining unmethylated residues are clustered in CpG islands within functional gene promoters^[13]. It has been shown^[12,14,15] that SEPT 9 has 18 distinct transcripts encoding 15 polypeptides, with two transcripts (SEPT9_v4 and v4*) encoding the same polypeptide.

SEPT9 GENE AND COLORECTAL CANCER

In recent years, growing evidence has shown that the *SEPT9* gene is associated with malignant tumors. Peterson *et al.*^[16] used immunoprecipitation and immunofluorescence studies to analyze SEPT9_i1 and found that it interacts with both α and γ tubulin.

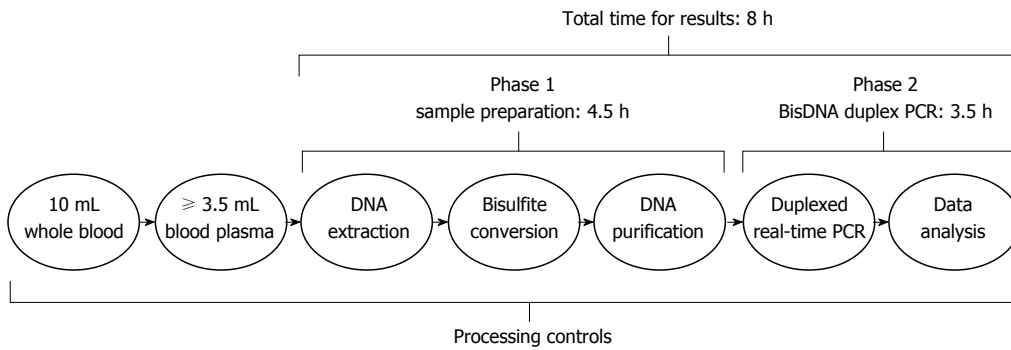


Figure 1 The outline of the Epi proColon work flow. The test consists of the Epi proColon Plasma Quick kit, PCR kit, and Control kit. The total assay time is approximately 8 h. For the Plasma Quick kit, 3.5 mL of plasma was mixed with an equal volume of lysis buffer; after incubating for 10 min, magnetic beads and absolute ethanol were added. After 45 min, impurities were removed from the magnetic beads by centrifugation; the purified DNA was then released from the beads in the elution buffer and treated at 80 °C with a solution of ammonium bisulfite for deamination of cytosine^[34]. After a series of washing steps, the converted DNA (bisulfite-modified DNA, bisDNA) was captured by magnetic beads. The bisDNA was assayed with the PCR kit on a Duplexed Real-Time PCR device. Finally, methylated SEPT9 and PCR results were recorded by the instrument software. In the whole working flow, the processing controls were included to monitor the execution of the procedure and ensure the validity of the test result and model^[34].

SEPT9_i1-expressing cells demonstrated dramatic chromosome segregation defects, centrosome amplification and cytokinesis defects, which indicates that SEPT9_i1 increases genomic instability in the process of tumorigenesis through two potential molecular mechanisms: defective chromosome segregation and cytokinesis failure. Additionally, expression of HIF^[17], JNK^[18] and Rho signaling pathways^[19] may also be potential mechanisms of colorectal cancer development in which the *SEPT9* gene is involved.

SEPT9 gene encodes a protein called septin-9, which is part of a group of proteins called septins. Septins are involved in various biological processes such as division of cytoplasm, cell polarization, vesicle transport and membrane reconstruction. The septin-9 protein also appears to act as a tumor suppressor, which means that it regulates cell growth and prevents cells from dividing too fast or in an uncontrolled way^[20]. When the methylation occurs at a CpG island, genes with high levels of 5-methylcytosine in their promoter region are transcriptionally silent^[21]; DNA methylation gradually accumulates on long-term silenced gene and may result in the inactivation of cancer suppressor genes. Tóth *et al.*^[22] have found that *SEPT9* mRNA expression decreased from adenoma to dysplasia to carcinoma in the progression of colon neoplastic disease, which presents a strong significant correlation of *SEPT9* methylation with the mRNA's low expression in CRC. Thus, downregulation of *SEPT9* mRNA and the decrease in *SEPT9* expression may account for the pathological progression from benign to malignant lesions in colon tissues.

PLASMA SEPT9 GENE METHYLATION ASSAY

Methods of the SEPT9 gene methylation assay

Due to epigenetic silencing of the *SEPT9* gene by promoter methylation in plasma, the company

Epigenomics AG first studied SEPT9 methylation based on the SEPT9 biomarker available in Europe in 2008^[23]. After one year, a commercial kit was finalized and the first generation of the CE-marked Epi proColon real-time PCR kit was launched. This CE-marked IVD (*In vitro* Diagnostic) kit became publicly available in Europe in 2010. Currently, the second generation of the assay is commercially available as the Epi proColon 2.0 assay^[24].

In general, The Epi proColon test is an *in vitro* diagnostic PCR method for the qualitative detection of SEPT9 DNA methylation levels in plasma derived from patients' whole blood specimens (Figure 1). To perform the test, approximately 10 mL of whole blood is a source of sufficient plasma for the analysis. The testing cycle performed with the current manual workflow takes approximately 8 h. As a first step, a minimum of 3.5 mL of blood plasma is isolated from the blood sample. Then, the Epi proColon 2.0 test consists of two phases. In Phase I, DNA is extracted from the plasma fraction and treated with bisulfite-conversion reagents and purified to obtain highly purified DNA^[25]. In Phase II, the test detects the hyper-methylated v2 region of the SEPT 9 gene and a region of the ACTB (β -actin) gene as an internal control by duplex real-time PCR^[26]. Finally, the Epi proColon 2.0 test only reports qualitative positive and negative results. A positive test is indicative of an increased likelihood for having CRC and a colonoscopy is recommended as a follow-up for diagnostic evaluation.

Diagnostic performance of plasma SEPT9 gene methylation

Increasingly, studies^[27-30] are suggesting that the methylation status of SEPT9 is a reliable index for screening CRC. For evaluating its diagnostic performance, we have collected several research results in which the sensitivity and specificity are key indicators. Table 1

Table 1 Sensitivity and specificity of the *SEPT9* gene methylation assay for colorectal cancer detection

Publications	Number of cases	Sensitivity	Specificity	Algorithm	Assay used	Ref.
Tóth <i>et al</i> (2012)	184 (92 CRC, 92 no evidence of disease)	95.6% (95%CI: 89.2%-98.8%)	84.8% (95%CI: 75.8%-91.4%)	1/3	Epi proColon 2.0	[27]
		79.3% (95%CI: 69.6%-87.1%)	98.9% (95%CI: 94.1%-100%)	2/3		
Church <i>et al</i> (2014)	1516 (53 CRC, 1457 without CRC)	48.2% (95%CI: 32.2%-63.6%)	91.5% (95%CI: 89.7%-93.1%)	1/3	Epi proColon 1.0	[31]
Potter <i>et al</i> (2014)	1544 (44 CRC, 1500 non-CRC)	68.0% (95%CI: 53%-80%)	80.0% (95%CI: 78%-82%)	-	Epi proColon 1.0	[34]
Su <i>et al</i> (2014)	234 (172 CRC, 62 controls)	88.4%	93.5%	-	MSP-DHPLC	[28]
Johnson <i>et al</i> (2014)	301 (101 CRC, 200 non-CRC)	73.3% (95%CI: 63.9%-80.9%)	81.5% (95%CI: 75.5%-86.3%)	-	Epi proColon 1.0	[32]
Jin <i>et al</i> (2014)	476 (135 CRC, 341 non-CRC)	74.8% (95%CI: 67.0%-81.6%)	87.4% (95%CI: 83.5%-90.6%)	2/3	Epi proColon 2.0	[29]
Ørntoft <i>et al</i> (2015)	300 (150 CRC, 150 controls)	73.0% (95%CI: 64%-80%)	82.0% (95%CI: 75%-88%)	1/3	Epi proColon 1.0	[33]
Sharif <i>et al</i> (2016)	90 (45 CRC, 45 controls)	84.4%	99.0%	-	MS-HRM assay	[52]
Wu <i>et al</i> (2016)	1031 (291 CRC, 740 non-CRC)	73.0% 76.6% (95%CI: 71.3%-81.4%)	97.5% 95.9%	-	Epi proColon 2.0 New SEPT9 assay	[30]
Nian <i>et al</i> (2016)	25 studies, 9927 samples (2975 CRC, 6952 non-CRC)	71.0% (95%CI: 67%-75%)	92.0% (95%CI: 89%-94%)	2/3	Epi proColon 2.0	[35]

CRC: Colorectal cancer.

shows the data from clinical trials using the *SEPT9* gene methylation assay published since 2012.

From the table, it can be seen that the plasma *SEPT9* gene methylation assay exhibited a high overall sensitivity and specificity for CRC detection. Moreover, with the improved method used in the subsequent studies, especially after the application of the second-generation *SEPT9* methylation assay (Epi proColon 2.0, Epigenomics AG, Germany), the detection sensitivity increased from approximately 48.2%-73.3%^[31-34] to approximately 71.0%-95.6%^[27,29,30,35], while the specificity improved from 80.0%-91.5% to 84.8%-98.9%. Meanwhile, Wu *et al*^[30] reported that the new *SEPT9* assay, with enhanced technical simplicity and a lower cost, presented a sensitivity of 76.6% and a specificity of 95.9%, which not only did not differ in performance compared with Epi proColon 2.0 but also reduced the complexity of the testing process and appeared to be a simpler, cheaper, more efficient, convenient, and user-friendly alternative for CRC screening. Additionally, methylation of *SEPT9* detected by MSP-DHPLC (methylation-specific polymerase chain reaction (PCR)-denaturing high-performance liquid chromatography)^[28] shows that the sensitivity and specificity are as high as 88.4% and 93.5%, respectively, which also appears to be a useful biomarker in a clinical laboratory setting. Tóth *et al*^[36] measured the positive predictive value and negative predictive value, which reached up to 93.8% (30/32) and 84.6% (22/26), respectively, supporting the reliability of this assay for CRC detection. Nian *et al*^[35] also estimated an area under the curve (AUC) of 0.88 and diagnostic odds ratio of 27 (95%CI: 18-42) using a bivariate mixed effect model. Furthermore, Ørntoft *et al*^[33] found that the clinical sensitivity for

CRC stages I-IV was 37%, 91%, 77%, and 89%, respectively. In comparison, Jin *et al*^[29] described that methylated *SEPT9* was positive in 66.7% of stage I (12/18), 82.6% of stage II (19/23), 84.1% of stage III (37/44), and 100% of stage IV (5/5) cases in 90 cases of CRC whose stages were identified based on the surgically resected specimens. The results indicate that advanced stage CRCs are more easily detected by *SEPT9* methylation than the early stage. Although the sensitivity and specificity reported in Table 1 come from different studies, leading to the variation in the ability to detect CRC, these results are still comparable because the majority of studies used Epi proColon products as the commercialized tests, and multiple PCR reactions are performed in all of these studies, which determine the final test result.

As for the test performance of other non-invasive CRC detection approaches, according to retrospective case control studies^[27,31,37,38], the FOBT identifies individuals with CRC with a sensitivity between 33% and 79% and a specificity between 87% and 98%. Another recent case control study by Tóth *et al*^[27] showed that the FOBT was positive in 29.4% (5/17) of NED (no evidence of disease) and 68.2% (15/22) of CRC and that elevated CEA levels were detected in 14.8% (4/27) of NED and 51.8% (14/27) of CRC. Both the FOBT and CEA showed a lower sensitivity and specificity than *SEPT9* (95.6% and 84.8%). In addition, Lee *et al*^[39] reported that the sensitivity was as high as 79% (95%CI: 69%-86%) for FIT for CRC with a specificity of 94% (95%CI: 92%-95%) by meta-analysis, which is at the same level as *SEPT9*^[27]. Johnson *et al*^[32] obtained estimates of 68.0% (95%CI: 58.2-76.5%) for the sensitivity and 97.4% (95%CI: 94.1%-98.9%) for the specificity of FIT, and drew the

conclusion that the sensitivity of the Epi proColon test was statistically comparable to FIT by analyzing the paired samples. A study by Song *et al.*^[40] also showed that the SEPT9 assay exhibited significantly higher sensitivity than the FIT test (75.6% vs 67.1%, $P < 0.05$) in pooled data of the symptomatic population. In general, compared with these other CRC detection tests, the SEPT9 gene methylation assay shows a good diagnostic performance in both sensitivity and specificity with the advantage of better acceptability and compliance of serological testing.

Hence, the promoter hyper-methylation analysis of plasma SEPT9 DNA has the potential to serve as a non-invasive screening method for the identification of specific biomarkers, enabling early detection of CRC in a large population. This approach holds promise for increased accuracy, safety, affordability, and patient compliance^[41].

Combined detection of the SEPT9 assay with other colorectal cancer detection tests

The combination of multiple methods or markers has become an increasing trend in CRC detection and screening. A recent study conducted by Wu *et al.*^[30] demonstrated that the combination of SEPT9 + FIT had a high sensitivity for CRC detection (94.4%), and the sensitivity of combined examination of SEPT9 + FIT + CEA was 97.2% (76.6%, SEPT9 alone). Another study^[42] found that the sensitivity of joint examination of SEPT9 and FIT in CRC diagnosis was 97.8% (80.0%, SEPT9 alone) and that the specificity was 52.9%, whereas the advanced adenoma diagnosis was 67.6% (10.8%, SEPT9 alone) and 47.4%, respectively, which suggested that the combination of the SEPT9 and FIT assays not only significantly enhanced the sensitivity for CRC detection but also increased the positive detection rate for advanced adenoma. In the study of Yu *et al.*^[43], it was seen that the under-ROC curve area of SEPT9 with CEA and FOBT for CRC detection reached 0.935. Furthermore, other than the tests mentioned above, SEPT9 may be combined with other existing biomarkers for CRC detection, such as glycoprotein markers or other methylation markers^[12]. A study published by Tänzer *et al.*^[44] demonstrated the combined analysis of methylation status of SEPT9 and ALX4 to be highly significant in the detection of colorectal polyps with a sensitivity and specificity reaching 71% and 95%, respectively, indicating the potential use of the combined methods in detecting advanced precancerous colorectal lesions. However, further studies are still required to evaluate the effect of combined biomarker assays on CRC detection and screening.

Limitations of the SEPT9 methylation assay

Although the plasma-based SEPT9 methylation assay performs well with regard to both sensitivity and specificity, its clinical availability is still limited. As we can see in Table 1, there is a large degree of

heterogeneity among studies, which may be due to many causes, especially the impacts of non-tumor-related factors on DNA methylation, such as aging, sex, race, hormone levels, dietary factors^[45], lifestyle factors (smoking and alcohol consumption)^[46], and other environmental exposure factors. Song *et al.*^[47] found a high PDR (positive detection rate) of SEPT9 methylation in normal subjects and cancer patients over 60 years, which may reflect increased SEPT9 gene methylation levels with age. Additionally, the increased false negative rate of the SEPT9 assay is associated with diabetes, arthritis and arteriosclerosis ($P < 0.05$)^[33], which can explain why the diagnostic performance of the SEPT9 assay varies compared to previous retrospective case-control studies. Nevertheless, not enough is known to approximate the effect of demographic characteristics, pathological features and/or comorbidities on the results of the SEPT9 methylation assay. Moreover, using a 2/3 algorithm test has a high true negative rate, although its sensitivity was higher with a 1/3 algorithm test^[35]. On account of the capability of excluding non-cancer samples and avoiding the rate of misdiagnosis, the 2/3 algorithm is recommended for CRC detection. Therefore, the technique and method selection could also affect the laboratory results and lead to heterogeneity. Further studies should pay more attention to examining the variation in diagnostic accuracy and validating potential confounding factors affecting DNA methylation status, in the design of future experimental studies. These non-neoplastic factors should be taken into consideration when evaluating DNA methylation to avoid the influence those caused on the testing results.

The cost-effectiveness is another limitation that limited large-scale application of the SEPT9 methylation assay. It was reported^[48] that the methylated SEPT9-based strategies were not a cost-saving with the costs of \$8400 to \$11500 per quality-adjusted life-year gained in comparison with established screening strategies including FOBT, FIT, and colonoscopy. The current cost of the methylated SEPT9 test in Europe is approximately 150 Euros, considerably more than fecal tests^[31]. In brief, FIT dominated methylated SEPT9 and was preferred among all of the alternatives^[49,50]. Even so, the biomarker for colorectal cancer screening still offers potential benefits over current methods, but in order to realize its full potential, the plasma-based assay will need to be acceptable to clinicians and patients compared to current technologies and the medical environment. As the emerging SEPT9 methylation assay becomes available clinically, the decision over whether to adopt it will require weighing its costs, utilization and longitudinal adherence against the alternative of putting efforts into improving current screening strategies. At the population level, methylated SEPT9 yielded incremental benefit at acceptable costs when it increased the fraction of the population screened more than it was substituted for other strategies^[48]. Thus, screening costs, utilization,

adherence, and follow-up are the influential determinants of the cost-effectiveness of colorectal cancer screening strategies.

Moreover, the capability of the *SEPT9* gene methylation assay for detecting adenomas, which is the most common precancerous lesion of CRC, is limited. For early stage CRC (Stage I), polyps or adenomas, methylated *SEPT9* alone presented quite low sensitivity with approximately 35%^[25], 20%^[51] and 11.2%^[31], respectively, indicating that this biomarker may be far from sufficient and effective at screening asymptomatic CRC patients, despite the diagnostic value of detecting advanced stage CRCs (III-IV). With the transformation of the medical pattern, the focus of hygiene work is switching to prevention rather than curing. Thus, the detection of precancerous or early stage colorectal cancer is very crucial for the health workers to identify high-risk groups and to provide an accurate early diagnosis. Still, this assay faces significant challenges nowadays when introduced for detecting early pre-invasive pathological changes, such as adenomas and premalignant polyps. On the one hand, there is plenty of room for improvement in the method of the methylated *SEPT9* assay itself, such as amelioration of DNA isolation or enhancement of PCR efficiency. On the other hand, the combination of the *SEPT9* assay with other markers in CRC detection is at its initial stage, in spite of the detection rate increasing to 37%^[44] by applying an additional methylation marker like *ALX4*, but further research is still needed to evaluate the effect of joint detection and to explore its possibility, for the sake of improving the sensitivity for detection of early cancers and advanced adenomas. More studies on early-stage CRC are expected in the future.

FUTURE PERSPECTIVES

Taken together, the use of the plasma-based methylated biomarker *SEPT9* gene should be the alternative approach for CRC screening due to greater diagnostic performance, convenience, and compliance in comparison with non-serological methods. The methylated *SEPT9* assay showed relatively high pooled sensitivity, whereas it was also affected by many factors, leading to the high level of heterogeneity. Future clinical diagnostic studies of methylation in blood should consider the impacts of these factors, especially non-neoplastic factors (e.g., aging, sex, lifestyle, coexistent disease, methodology) on diagnostic accuracy. Moreover, the cost of the *SEPT9* methylation assay is still much higher than the FOBT and FIT. And further investigation of early CRC is still required, as a result of its sensitivity for the asymptomatic population in the screening setting still not being satisfactory, but improvements in accuracy can be expected as the diagnostic technology evolves.

In the future, deciphering epigenetic information including DNA methylation and applying it to the

selection of appropriate detection methods and the development of relevant therapy is likely to transform the diagnosis and treatment of colorectal cancer, consequently decreasing mortality.

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Vitamin D in esophageal cancer: Is there a role for chemoprevention?

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Abstract

Vitamin D has emerged as a promising anti-cancer agent due to its diverse biological effects on tumor differentiation, apoptosis and suppression of cellular proliferation. Current evidence suggests a protective role of vitamin D in colon cancer. The effect of vitamin D on esophageal cancer remains controversial. Multiple studies investigated the association between vitamin D and esophageal cancer, employing different modes of assessment of vitamin D status such as serum 25-hydroxyvitamin D levels, vitamin D dietary intake or exposure to ultraviolet B (UVB) radiation. Genetic variations of the vitamin D receptor (VDR) gene and VDR expression in esophageal specimens have also been investigated. Ecological studies evaluating exposure to UVB radiation yielded an inverse correlation with esophageal cancer. When vitamin D dietary intake was assessed, direct association with esophageal cancer was observed. However, circulating 25-hydroxyvitamin D concentrations showed inconsistent results. In this review article, we present a detailed summary of the current data on the effects of vitamin D on various histological subtypes of esophageal cancer and their precursor lesions. Well-powered prospective studies with accurate measurement of vitamin D status are needed before chemoprevention with vitamin D is recommended, as current evidence does not support a chemopreventive role of vitamin D against esophageal cancer. Future studies looking at the incidence of esophageal cancer in patients with pre-cancerous lesions (Barrett's esophagus and squamous cell dysplasia) receiving vitamin D supplementation are needed.

Key words: Vitamin D; Vitamin D receptor; Esophageal adenocarcinoma; Esophageal squamous cell carcinoma; Genetic polymorphism

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Core tip: Vitamin D has emerged as a promising anti-cancer agent due to its diverse biological effects on tumor differentiation, apoptosis and suppression of cellular proliferation. Ecological studies evaluating exposure to ultraviolet B radiation yielded an inverse correlation with esophageal cancer. When vitamin D dietary intake was assessed, direct association with esophageal cancer was observed. However, circulating 25-hydroxyvitamin D concentrations showed inconsistent results.

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INTRODUCTION

« Sol est remediorum maximum » (The sun is the best remedy)--Pliny, the Elder.

This remark, attributed to Pliny, exemplifies the healing properties of sunlight known since ancient times^[1]. The fact that most of the beneficial effects of sunlight are mediated by vitamin D came to light by experimental studies on Rickets in the 1930s^[1,2]. Epidemiologic research in the 1980s showed that incidence and death rates for certain cancers were lower among individuals with higher exposure to sunlight^[3]. Researchers hypothesized that variation in vitamin D levels might account for this association. Since then, laboratory studies elucidated several antineoplastic properties of vitamin D such as its role in promoting cellular differentiation, decreasing cancer cell growth, stimulating apoptosis, and inhibiting angiogenesis^[4,5].

Vitamin D appears to have a protective role in colorectal and breast cancers but confirmatory data for cancers of other organs such as prostate or esophagus remains lacking^[6-9]. Esophageal cancer is a major public health concern due to increasing incidence and poor survival rates after diagnosis. Numerous studies investigated the association between vitamin D status and esophageal cancer with inconsistent results.

The aim of this review is to present the available scientific evidence for the role of vitamin D in esophageal squamous cell cancer (ESCC), esophageal adenocarcinoma (EAC) and their precursor lesions-squamous cell dysplasia and Barrett's esophagus (BE) respectively.

LITERATURE SEARCH

A PubMed search of all studies published in English from 2006 to 2016 was performed. Medical subject headings (MeSH terms) used were "vitamin D", "calcitriol", "vitamin D receptor", "sun", "sunlight",

"esophageal neoplasm", "esophageal adenocarcinoma", "Barrett's esophagus", and "esophageal squamous cell carcinoma". References of relevant articles were also reviewed and selected.

VITAMIN D METABOLISM AND ANTI-CANCER PROPERTIES

The two main sources of vitamin D are diet and solar radiation. Provitamin D in the skin is converted to previtamin D by ultraviolet B (UVB) radiation, which is then converted to vitamin D₃ (cholecalciferol) through isomerization. Vitamin D₃ is hydroxylated in the liver to form 25-hydroxycholecalciferol [25(OH)D₃]. Another hydroxylation reaction occurs in the kidneys, where 25(OH)D₃ is converted to the biologically active form 1 α ,25(OH)₂D₃ (calcitriol), involved in bone and calcium metabolism^[4]. Calcitriol also regulates its own catabolic cascade: it induces the expression of the CYP24A1 gene, which encodes the 24-hydroxylase enzyme. The latter converts 25(OH)D₃ and 1 α ,25(OH)₂D₃ to the less active metabolites 24,25(OH)₂D₃ and 1 α ,24,25(OH)₃D₃ respectively. This is the rate-limiting step of vitamin D catabolism^[4].

Calcitriol, thought to be the metabolite involved in the anticancer properties of vitamin D, binds to the vitamin D receptor (VDR). The calcitriol-VDR complex binds to the retinoid X receptor (RXR), forming the heterodimer VDR-RXR, which translocates to the nucleus and binds to the vitamin D response element (VDRE) on a particular gene, with subsequent transcription and translation of various proteins, including the ones involved in the vitamin D anti-carcinogenic properties, *i.e.*, anti-proliferation, apoptosis, differentiation, and angiogenesis inhibition^[4,5] (Figure 1). Calcitriol inhibits proliferation by inducing cell cycle arrest at the G0/G1 phase. Cyclins and cyclin-dependent kinase inhibitors regulate cell cycle progression and induce G1 cell-cycle arrest. Interestingly, cyclin-dependent kinase inhibitor 1A contains a VDRE, which accounts for the anti-proliferative effects of vitamin D^[4,10]. Apoptosis is another key mechanism in inhibiting carcinogenesis. Calcitriol induces the expression of pro-apoptotic proteins and activates caspase, a cysteine protease that mediates apoptosis. In addition to its apoptotic and anti-proliferative effects, vitamin D inhibits angiogenesis. In prostate cancer, vitamin D interrupts signaling of an angiogenic factor, interleukin 8, leading to decreased endothelial cell migration and possibly metastasis^[4].

Osteopontin and E-cadherin are two proteins induced by vitamin D with antagonistic growth regulatory activity. While osteopontin promotes cellular invasion^[11], E-cadherin suppresses cell growth by inhibiting the transcriptional activity of β -catenin, a protein that induces genes involved in promoting cell growth and proliferation^[12]. In colon adenocarcinoma, for instance, E-cadherin is preserved as opposed to low osteopontin levels^[13]. Subsequently, high levels of calcitriol would

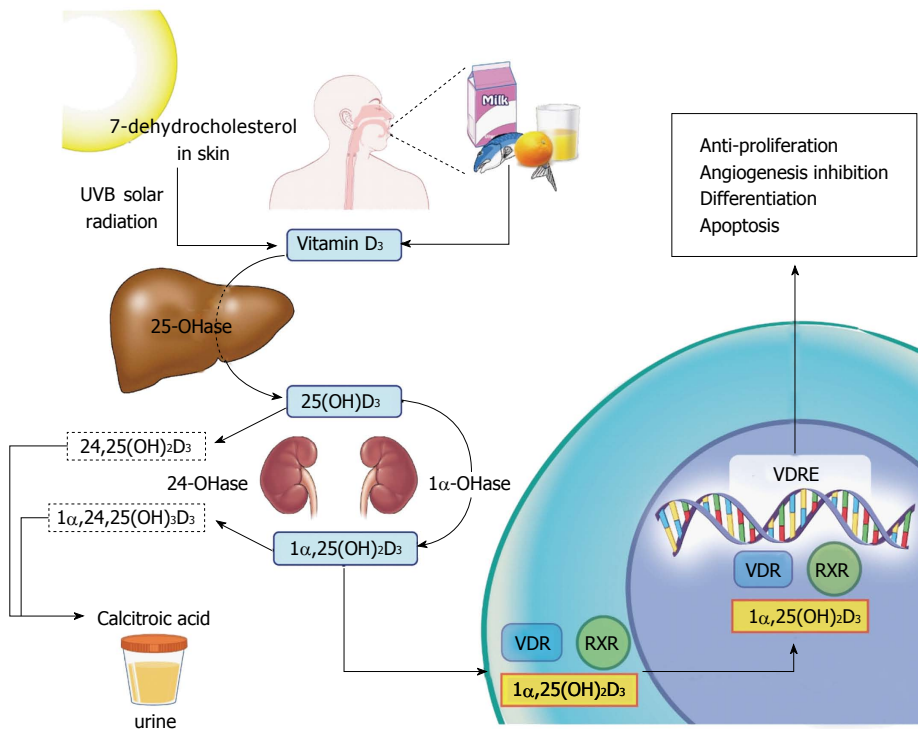


Figure 1 Vitamin D metabolism and anti-cancer properties. UVB: Ultraviolet B radiation; VDR: Vitamin D receptor; RXR: Retinoid X receptor; VDRE: Vitamin D response element; 25(OH)ase: 25-hydroxylase; 24(OH)ase: 24-hydroxylase; 1α (OH)ase: 1α -hydroxylase; 25(OH)D₃: 25-hydroxyvitamin D; $1\alpha,25$ (OH)₂D₃: $1\alpha,25$ -dihydroxyvitamin D; $1\alpha,24,25$ (OH)₃D₃: $1\alpha,24,25$ -trihydroxyvitamin D; 24,25(OH)₂D₃: 24,25- dihydroxyvitamin D.

lead to further E-cadherin-induced tumor suppression with low osteopontin levels, and subsequent cell growth inhibition^[14].

From an immunologic perspective, multiple cells are known to be involved in EAC and its precursor lesions, BE and reflux esophagitis: In addition to the dendritic cells and CD4 T cells, signaling pathways involved include NF- κ B, Wnt and Hedgehog pathways. Immunologically, the role of vitamin D in esophageal cancer remains inconclusive and unclear. For instance, vitamin D was shown to inhibit the Hedgehog signaling cascade which is overexpressed in BE. Similarly dendritic cells, increased in BE and EAC, are maintained in an immature form by vitamin D. On the other hand, BE is characterized by a Th2-predominant response and data suggests that $1\alpha,25$ -hydroxyvitamin D promotes the Th2 response. In addition, vitamin D was shown to increase interleukin-4 cytokine production, which has been implicated in BE. In view of these multiple contradictory effects on neoplastic progression, the role of vitamin D in esophageal cancer needs to be evaluated^[15].

MODES OF ASSESSMENT OF VITAMIN D STATUS

Serum concentration of vitamin D seems to be the most accurate indicator of a patient's vitamin D status and is usually monitored to treat vitamin D deficiencies. More than 50 vitamin D metabolites have

been identified over the past years but only two gained particular attention: $1\alpha,25$ (OH)₂D₃ and 25(OH)D₃. While $1\alpha,25$ (OH)₂D₃'s half-life is around 4 h and levels are widely dependent on an individual's calcium needs, 25(OH)D₃ has a half-life of around 3 wk, reflecting more accurately a patient's vitamin D stores, and therefore widely accepted as an indicator of an individual's vitamin D status^[16]. The normal levels are considered to be 10-68 ng/mL (24.9-169.5 nmol/L) with different cut-offs in various assays and laboratories^[17]. Sunlight is a major contributor to vitamin D status. Many studies attempted to validate different UVB exposure questionnaires and found correlations ranging between 0.16 and 0.4 for vitamin D serum concentration and reported UVB exposure^[18-21]. Correlations noted were not strong however, raising the hypothesis that sun exposure alone does not explain serum vitamin D levels^[19]. Multiple studies also used dietary vitamin D intake as a surrogate of vitamin D status and showed a good correlation between dietary vitamin D intake and serum vitamin D levels. This correlation could be stronger for instance, in wintertime, when exposure to UVB radiation is reduced^[19].

Taking vitamin D dietary intake, lifetime UVB exposure and vitamin D serum concentrations into account seems to be the most accurate method to assess an individual's vitamin D status^[19]. As a matter of fact, Giovannucci *et al.*^[22] built a predictor score to assess long-term vitamin D status using multiple determining factors of vitamin D exposure including dietary and

Table 1 Studies investigating correlations between vitamin D and esophageal squamous cell dysplasia and carcinoma

Ref.	Study design/location	Vitamin D exposure/status/genetics studies	Statistical correlation
Abnet <i>et al</i> ^[27]	Cross-sectional study China	25-hydroxyvitamin D serum level	RR = 1.86, 95%CI: 1.35-2.62
Chen <i>et al</i> ^[28]	Prospective study China	25-hydroxyvitamin D serum level	ESCC in men: HR = 1.77, 95%CI: 1.16-2.70
Lipworth <i>et al</i> ^[29]	Case-control study Italy	Vitamin D dietary intake	ESCC: OR = 0.58, 95%CI: 0.39-0.86
Tran <i>et al</i> ^[30]	Case-control study Australia	Ultraviolet B radiation	ESCC: No association
Wang <i>et al</i> ^[24]	Case-control study China	Genetic polymorphisms	ESCC: No association

ESCC: Esophageal squamous cell carcinoma; OR: Odds ratio; RR: Relative risk; HR: Hazard ratio; CI: Confidence interval.

supplementary vitamin D intake, geographic residence, race, physical activity and body mass index^[22].

On another note, two genome wide association studies of vitamin D levels have been conducted and common genetic variants of genes involved in vitamin D metabolism pathways were identified^[23,24]. Subsequently, multiple single nucleotide polymorphisms (SNPs) were investigated in an attempt to find correlations with esophageal cancer^[24,25].

ESOPHAGEAL CANCER AND VITAMIN D

Esophageal cancer encompasses two histological subtypes: ESCC and EAC, which differ epidemiologically, by risk factors and outcomes. ESCC is the most common esophageal cancer worldwide with an increased incidence in developing countries. Esophageal squamous dysplasia is the histologic precursor of ESCC. Developed countries witness a higher prevalence of EAC^[26], which is commonly related to chronic acid reflux exposure, with BE being the main risk factor for EAC. Potential associations of vitamin D have been investigated in both histological subtypes of esophageal cancer as presented below.

Esophageal squamous cell dysplasia

Only one study based on the Linxian population in China investigated the role of vitamin D in esophageal squamous cell dysplasia and found a linear association between vitamin D levels and development of squamous dysplasia: 230 out of 724 patients had esophageal squamous dysplasia. Patients diagnosed with esophageal squamous dysplasia had higher median levels of 25(OH)D₃ levels compared to controls (36.5 nmol/L vs 31.5 nmol/L, $P = 0.0004$)^[27].

Esophageal squamous cell carcinoma

Three studies evaluated vitamin D status and ESCC with diverging results depending on mode of assessment of Vitamin D status^[28-30] (Table 1). While one study in China concluded a direct correlation between ESCC and measured serum 25(OH)D₃ concentrations^[28], another study conducted in Italy noted an inverse association between increased dietary vitamin D intake and

ESCC^[29]. The third one, done in Australia, found no association between ESCC and lifetime UVB radiation exposure^[30].

The study from China was population-based and included 2018 participants, out of which 545 developed ESCC, with an overall trend towards higher concentrations in serum 25(OH)D₃ in those who developed cancers. Multivariate analysis demonstrated increased risk with higher 25(OH)D₃ values (4th quartile hazard ratio (HR): 1.30, 95%CI: 0.97-1.73, $P = 0.013$). When stratified by gender, ESCC risk remained increased in men with higher vitamin D levels (4th quartile HR = 1.77, 95%CI: 1.16-2.70, $P = 0.003$) but not in women. These conclusions could not be extrapolated to other populations due to overall low vitamin D levels and high rate of exposure to polycyclic aromatic hydrocarbons in this study population, with the latter factor placing them at higher risk for neoplasia^[28]. It is worthwhile noting however, that pre-neoplastic lesions with squamous cell dysplasia were also found to have an E-cadherin/osteopontin disequilibrium, with E-cadherin suppression and osteopontin up-regulation leading to increased risk of cell growth, proliferation and subsequently malignant transformation with higher calcitriol levels^[14].

The study from Italy was a case-control study with 304 patients and investigated the association between dietary vitamin D intake over the prior two years and ESCC^[29]. In ESCC patients, an inverse relationship was noted between vitamin D intake and esophageal neoplasia. The highest tertile corresponded to > 3.5 µg/d with a risk reduction of around 40% compared to lowest tertile (< 2.51 µg/d).

The last case-control study from Australia assessed UVB exposure and prevalence of ESCC. No relationship was observed between lifetime UVB radiation and ESCC (OR = 0.94, 95%CI: 0.82-1.09) in contrast to EAC and esophago-gastric junction adenocarcinoma^[30].

An association between SNPs in the genes involved in vitamin D pathway and ESCC was also evaluated: Wang *et al*^[24] investigated 12 SNPs in four genes known to be part of the vitamin D pathway: vitamin D binding protein, 7-dehydrocholesterol reductase, 25-hydroxylase and 24-hydroxylase or CYP24A1.

SNPs related to vitamin D levels were not found to be associated with ESCC risk.

The rate-limiting step of vitamin D synthesis was also investigated in regards to ESCC. In one study of 42 patients with esophageal cancer of which 39 had ESCC, CYP24 gene expression was assessed by semi-quantitative RT-PCR assay. Cases with lower CYP24 expression ($n = 25$) had significantly higher survival rate compared to patients with increased CYP24 expression ($n = 17$, $P < 0.05$), making of CYP24 a "candidate oncogene" that might serve as a biomarker of increased ESCC risk^[31].

Barrett's esophagus

Vitamin D dietary intake and supplementation have been studied with regards to BE. An Irish study evaluated the association between vitamin D intake assessed *via* food questionnaires, among patients with BE ($n = 224$), reflux esophagitis ($n = 230$) and EAC ($n = 227$), compared to 260 healthy controls^[32]. Vitamin D intake was not found to be associated with reflux esophagitis or BE. After adjusting for reflux symptoms however, a positive correlation emerged between patients with BE and the highest tertile of dairy products intake (≥ 493.2 g/d) (OR = 1.94, 95%CI: 1.01-3.71). This could imply that patients are consuming dairy products to treat their symptoms, rather than an actual association with BE, as proposed by the authors^[32]. In a clinical trial studying the effect of vitamin D supplementation on BE, 3 of the first 10 evaluable patients had BE with high-grade dysplasia. After 2 wk of vitamin D supplementation (50000 units weekly), 2 out of 3 patients with BE had regression to low-grade dysplasia on pathology, suggesting a potential benefit of vitamin D in BE^[33].

Three studies assessed VDR expression in BE^[25,34,35]. Trowbridge *et al.*^[34] compared VDR expression in normal esophagus, BE and normal gastric tissue, by immunofluorescent staining. No VDR expression was detected in normal squamous mucosa in contrast to normal gastric mucosa and BE mucosa. This suggests a restriction of VDR expression to columnar epithelium and glandular structures, as well as potential chemopreventive effects of vitamin D in patients with BE. Those findings were reproducible in a Dutch study where VDR mRNA had a 2-fold higher expression in BE epithelium compared to squamous epithelium^[25]. In another study comprising 37 patients with BE and 107 with EAC, VDR expression was found to be increased in both BE (95%) and EAC (79%), but significantly higher in BE^[35]. This implies that VDR might be involved early on in EAC development.

Esophageal adenocarcinoma

To date, the studies that examined the association between vitamin D status and EAC showed inconsistent results^[30,32,36-38]. Several of these studies were either population-based or ecologic studies with lack

of information on 25(OH)D₃ levels either before or after EAC diagnosis, and therefore relied on various other measures of vitamin D status such as sunlight exposure or dietary vitamin D intake.

The studies that examined the association between vitamin D and EAC are summarized in Table 2. Only 2 studies evaluated the association of serum 25(OH)D₃ concentrations and EAC. Abnet *et al.*^[36], in a nested case-control study, examined the relationship between upper gastrointestinal cancers and circulating serum 25(OH)D₃ levels. No significant association was noted with EAC when comparing patients with highest and lowest categories of 25(OH)D₃ levels (50-75 nmol/L vs < 25 nmol/L, OR = 1.63, 95%CI: 0.25-2.12)^[36]. Another US-based study also did not show any association between 25(OH)D₃ levels and incidence or prevalence of EAC among patients with BE^[38]. Giovannucci *et al.*^[22] used a predicted 25(OH)D₃ level derived by modeling various factors that can affect vitamin D status such as UVB, dietary vitamin d intake, supplementation, skin pigmentation and body mass index. A 25nmol/L increment in predicted vitamin D resulted in 17% reduction in total cancer incidence and 29% reduction in cancer mortality. However, the study did not mention the rates of EAC in particular, although there was an inverse association with esophageal cancer incidence (RR = 0.37, 95%CI: 0.17-0.80)^[22].

Data from animal models have shown that dietary vitamin D is associated with tumor inhibition and reduction of tumor growth, especially in colorectal cancer and breast cancer^[39-41]. However, the epidemiologic studies for EAC have been contradictory^[32,37,42]. In fact, in an Irish study, patients with the highest tertile of vitamin D intake had increased risk of EAC compared to the lowest tertile (OR = 1.99, 95%CI: 1.03-3.86)^[42]. In another population-based study in the US, no association was found between vitamin D intake and EAC (OR = 1.10, 95%CI: 0.86-1.40)^[37]. Similar results were found in a meta-analysis that concluded that higher intake of vitamin D results in a non-significant increase in the risk of EAC (OR = 1.45, 95%CI: 0.65-2.24)^[5]. The current evidence hence fails to establish a relationship between vitamin D intake and EAC.

The other significant contributor of vitamin D status is sunlight exposure. To date only one study examined UVB exposure as a risk factor for EAC^[30]. Patients with EAC were 41% less likely to have high levels of lifetime ambient UVB radiation compared to population controls (OR = 0.59, 95%CI: 0.35-0.99). Although the study did not check serum vitamin D levels to establish the diagnosis of vitamin D deficiency, the study results were adjusted for several potential confounders such as body mass index, reflux symptoms, education, smoking, alcohol and *Helicobacter pylori* infection, following which the inverse association remained between UVB and EAC. The same inverse association was seen between number of nevi, which is a

Table 2 Studies investigating correlations between vitamin D and Barrett's esophagus or esophageal adenocarcinoma

Ref.	Study design/location	Vitamin D exposure/status/ genetics studies	Statistical correlation	Other
Tran <i>et al</i> ^[30]	Case-control study Australia	Cumulative ambient ultraviolet B radiation	EAC risk: OR = 0.59, 95%CI: 0.35-0.99 EAC risk for every 107 J/m ² increase in radiation: OR = 0.82, 95%CI: 0.72-0.93	
Mulholland <i>et al</i> ^[32]	Case-control study Ireland	Vitamin D dietary intake <i>via</i> food questionnaire	EAC risk: OR = 1.99, 95%CI: 1.03-3.86 BE risk: no association	
Mayne <i>et al</i> ^[37]	Case-control study United States	Vitamin D dietary intake	EAC: no association	
Thota <i>et al</i> ^[38]	Retrospective study of a prospectively collected database	25-hydroxyvitamin D serum levels	EAC: no association	
Abnet <i>et al</i> ^[36]	Nested case-control study United States, Finland, China	25-hydroxyvitamin D serum levels	BE with HGD: no association EAC: no association	
Trowbridge <i>et al</i> ^[43]	Retrospective study United States	Vitamin D receptor expression	Not assessed	VDR expression decreased with tumor dedifferentiation VDR expression lower in neoadjuvant therapy responders
Trowbridge <i>et al</i> ^[34]	Retrospective study United States	Vitamin D receptor expression	Not assessed	VDR expression increased in Barrett's esophagus
Zhou <i>et al</i> ^[35]	Descriptive United States	Vitamin D receptor expression	Not assessed	VDR expressed in 95% of BE (35/37) VDR expressed in 78% of EAC (86/109)
Janmaat <i>et al</i> ^[25]	Cohort study Netherlands	Vitamin D receptor polymorphisms	EAC: 2 GT copies: OR = 0.50, 95%CI: 0.27-0.96 BE: 2 GT copies: OR = 0.46, 95%CI: 0.26-0.80	VDR expression is 2 fold higher in BE as compared to normal esophagus
Chang <i>et al</i> ^[45]	Case-control study Ireland	Vitamin D receptor polymorphisms	EAC: rs2238139 TT: OR 0.26, 95% CI: 0.07-0.93 EAC: rs2107301 TT: OR = 0.19, 95%CI: 0.06-0.67	
Zgaga <i>et al</i> ^[5]	Meta-analysis United States	Ultraviolet B radiation Vitamin D intake Vitamin D serum levels	Vitamin D level and overall esophageal cancer: OR = 1.39, 95%CI: 1.03-1.74 Vitamin D intake and EAC: OR = 1.45, 95%CI: 0.65-2.24	

EAC: Esophageal adenocarcinoma; BE: Barrett's esophagus; HGD: High-grade dysplasia; VDR: Vitamin D receptor; OR: Odds ratio; CI: Confidence interval.

surrogate marker of sun exposure, and EAC, further supporting the hypothesis of sun exposure and tumor inhibition^[30].

In an attempt to find biomarkers predicting the malignant potential of an esophageal lesion, response to treatment and prognosis, investigators have evaluated the genetics involved in the vitamin D pathway in regards to EAC. The focus has mainly been on the VDR expression in different tissues as well as SNPs of some of the genes in the vitamin D signaling pathway.

Trowbridge *et al*^[43] looked at VDR expression using immunofluorescence in 15 biopsy specimens of patients with EAC. Greater average mean fluorescence, a reflection of higher VDR expression, was observed for moderately and well-differentiated tumors (111.7) compared to poorly differentiated tumors (98.7), which highlights the anti-carcinogenic properties of vitamin D through VDR, particularly differentiation. This was also established in colon adenocarcinoma where decreased

VDR expression was noted with progressive de-differentiation^[44].

Apart from assessing VDR expression level, VDR polymorphisms in EAC have also been investigated. Vitamin D exerts many of its biological effects by binding to VDR and VDR gene polymorphisms may alter mRNA stability and transcriptional activity.

In an Irish population-based case-control study, 224 cases of EAC were identified and 256 controls were selected^[45]. Variants in the VDR gene were explored and TT homozygotes at rs2238139 and rs2107301 SNPs seemed to have a reduced risk of EAC compared to individual with CC alleles at those sites (OR = 0.26, 95%CI: 0.007, 0.93 and OR = 0.19, 95%CI: 0.06-0.67, respectively). However when permutation analyses were done, there was no significant association between EAC and VDR polymorphisms^[45]. A later study identified two SNPs of the VDR gene associated with reduced risk of reflux esophagitis, BE and EAC^[25]. Patients with the rs1989969 T/rs2238135

G haplotype had a lower risk for reflux esophagitis (OR = 0.48, 95%CI: 0.28-0.81), BE (OR 0.46, 95%CI: 0.26-0.80) as well as EAC (OR = 0.50, 95%CI: 0.27-0.96). Both of these haplotypes appear to be associated with reduced VDR expression. The authors studied the mechanism by which those SNPs work and discovered that the rs1989969 T allele lead to the appearance of a GATA-1 transcription factor binding site, which is known to be a negative transcriptional regulator. This haplotype could be exerting its direct biological effects on the rate of reflux esophagitis with a subsequent decreased rates of BE and EAC^[25]. Those findings could have significant clinical implications in terms of identifying patients who would benefit from vitamin D chemoprevention.

CONCLUSION

In summary, data continues to be inconsistent and firm conclusions regarding the chemopreventive role of vitamin D in esophageal cancer cannot be made. While vitamin D studies struggle with measuring the combined influences of dietary vitamin D intake and sunlight, vitamin D serum levels are a single point measure in time, and levels are known to change throughout the year. As a matter of fact, while an inverse association exists between UVB radiation and EAC, this was not observed with vitamin D intake. Serum 25(OH) D₃ levels appear to be associated with higher risk of ESCC especially in Chinese population. No association was noted however between vitamin D serum levels and EAC. Studies have been population-specific making it difficult to apply findings to other populations. Multiple genetic studies provided new grounds for future investigations such as SNPs leading to the appearance of transcription sites with known negative regulatory roles. VDR expression is increased in BE as compared to EAC or normal squamous epithelium, making of VDR a potential biomarker in selecting those who could benefit from vitamin D as a chemopreventive agent. Well-powered prospective studies with accurate measurement of vitamin D status are needed before chemoprevention with vitamin D is recommended, as current evidence does not support a chemopreventive role of vitamin D against esophageal cancer. Future studies looking at the incidence of esophageal cancer in patients with pre-cancerous lesions (BE and squamous cell dysplasia) receiving vitamin D supplementation are needed.

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Retrospective Study

Impact of duration of adjuvant chemotherapy in radically resected patients with T4bN1-3M0/TxN3bM0 gastric cancer

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Abstract**AIM**

To provide evidence regarding the postoperative treatment of patients with T4bN1-3M0/TxN3bM0 gastric cancer, for which guidelines have not been established.

METHODS

Patients who had undergone curative resection between 1996 and 2014 with a pathological stage of T4bN1-3M0/TxN3bM0 for gastric cancer were retrospectively analyzed; staging was based on the 7th edition of the American Joint Committee on Cancer staging system. The clinicopathological characteristics, administration of adjuvant chemotherapy, and patterns of recurrence were studied. Univariate and multivariate analyses of prognostic factors were conducted. The chemotherapeutic agents mainly included fluorouracil, platinum and taxanes, used as monotherapy, doublet, or triplet regimens. Patterns of first recurrence were categorized as locoregional

recurrence, peritoneal dissemination, or distant metastasis.

RESULTS

The 5-year overall survival (OS) of the whole group ($n = 176$) was 16.8%, and the median OS was 25.7 mo (95%CI: 20.9-30.5). Lymphovascular invasion and a node positive rate (NPR) ≥ 0.8 were associated with a poor prognosis ($P = 0.01$ and $P = 0.048$, respectively). One hundred forty-seven (83.5%) of the 176 patients eventually experienced recurrence; the most common pattern of the first recurrence was distant metastasis. The prognosis was best for patients with locoregional recurrence and worst for those with peritoneal dissemination. Twelve (6.8%) of the 176 patients did not receive adjuvant chemotherapy, while 164 (93.2%) patients received adjuvant chemotherapy. Combined chemotherapy, including doublet and triplet regimens, was associated with a better prognosis than monotherapy, with no significant difference in 5-year OS (17.5% *vs* 0%, $P = 0.613$). The triplet regimen showed no significant survival benefit compared with the doublet regimen for 5-year OS (18.5% *vs* 17.4%, $P = 0.661$). Thirty-nine (22.1%) patients received adjuvant chemotherapy for longer than six months; the median OS in patients who received adjuvant chemotherapy for longer than six months was 40.2 mo (95%CI: 30.6-48.2), significantly longer than the 21.6 mo (95%CI: 19.1-24.0) in patients who received adjuvant chemotherapy for less than six months ($P = 0.001$).

CONCLUSION

Patients with T4bN1-3M0/TxN3bM0 gastric cancer showed a poor prognosis and a high risk of distant metastasis. Adjuvant chemotherapy for longer than six months improved outcomes for them.

Key words: Gastric cancer; T4bN1-3M0/TxN3bM0; Recurrence; Distant metastasis; Adjuvant chemotherapy

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Core tip: Patients with T4bN1-3M0/TxN3bM0 gastric cancer have a poor prognosis after curative resection. Due to limited evidence and a lack of guidelines for clinical practice, T4bN1-3M0/TxN3bM0 gastric cancer remains a challenging clinical problem. Our retrospective study is complementary to large-scale phase III prospective trials and showed that the most common pattern of first recurrence for this population is distant metastasis and that prolonged adjuvant chemotherapy may improve patient outcomes. This finding will need to be confirmed by future prospective randomized controlled studies to improve the outcomes for patients with T4bN1-3M0/TxN3bM0 gastric cancer.

Wang QW, Zhang XT, Lu M, Shen L. Impact of duration of adjuvant chemotherapy in radically resected patients with

T4bN1-3M0/TxN3bM0 gastric cancer. *World J Gastrointest Oncol* 2018; 10(1): 31-39 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i1/31.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i1.31>

INTRODUCTION

Nearly one million new cases of gastric cancer (GC) were diagnosed in 2012, making it the fifth most common malignancy worldwide^[1]. Geographically, GC is most common in East Asian countries including China, Japan and Korea (45% in China). In contrast to the situation in Japan and Korea, GC in China is often detected at a locally advanced or advanced stage. Complete resection with a D2 lymphadenectomy remains the cornerstone of curative treatment; however, more than half of resectable GC patients develop recurrence despite achieving an R0 resection^[2].

Efforts to reduce the risk of recurrence and improve survival have focused on perioperative treatment. Postoperative adjuvant chemotherapy in GC is primarily supported by two large randomized phase III studies: The Japanese ACTS-GC^[3] (Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer) and the Asian CLASSIC^[4] (Capecitabine and Oxaliplatin Adjuvant Study in Stomach Cancer) trials. Both of these trials showed a survival benefit after D2 gastrectomy compared with surgery alone. A recent study, SAMIT^[5] (Japanese Stomach Cancer Adjuvant Multi-Institutional Trial), compared additional chemotherapy with single-agent fluoropyrimidine but failed to show a survival benefit. However, GC patients who were resectable at the most advanced stage (T4bN1-3M0/TxN3bM0, mostly III C) were not included in the CLASSIC trial; moreover, this patient population made up only 5% of the sample in the ACTS-GC study and 10% in the SAMIT study. Considering that R0 resection of the primary cancer had barely been achieved due to the locally advanced stage, these patients were at the highest risk for disease recurrence and were more likely to benefit from adjuvant chemotherapy. Due to the limited evidence as well as the difficulties in therapeutic management, T4bN1-3M0/TxN3bM0 gastric cancer remains a challenging problem in clinical practice.

A Korean retrospective study^[6] that focused on stage IV [T4N1-3M0/T1-4N3M0, American Joint Committee on Cancer (AJCC) 6th edition^[7]] GC patients, who were equivalent to the T4bN1-3M0/TxN3bM0 (AJCC 7th edition^[8]) patients in the current study, showed that patients who received adjuvant chemotherapy exhibited a survival benefit compared with patients who received surgery alone. However, the Korean study did not discuss the appropriate adjuvant therapy modality, which remains undefined for T4bN1-3M0/TxN3bM0 GC patients.

In view of the limited evidence regarding T4bN1-

3M0/TxN3bM0 GC, the difficulty of R0 resection, and the high risk of disease recurrence in this population, the aim of this retrospective study was to discuss the appropriate adjuvant therapy modality for patients with the most locally advanced GC.

MATERIALS AND METHODS

Patients

A total of 326 consecutive patients with primary GC with a pathological stage of T4bN1-3M0/TxN3bM0 based on the AJCC (7th edition) staging system who underwent potentially curative resection (R0) between October 1996 and December 2014 were identified in the database of Peking University Cancer Hospital. Of these patients, 18 had a distant metastasis that was detected before surgery, 48 had distant metastasis or peritoneal seeding (including positive peritoneal cytology) identified during the operation, 26 were given preoperative chemotherapy, 21 had a positive resection margin, 37 had recurrence within one month after surgery, and 176 with T4bN1-3M0/TxN3bM0 disease were available for analysis (Figure 1). All patients had histologically confirmed gastric or gastroesophageal junction adenocarcinoma.

Treatment and recurrence

A total of 145 (82.4%) patients had metastasis in sixteen or more regional lymph nodes with a median number of 20 metastatic lymph nodes (range: 0-70) and a median node positive rate (NPR) of 0.60 (range: 0.0-1.0). D2 lymph node dissection, according to the NCCN Clinical Practice Guidelines in Oncology-Gastric Cancer (Version 1.2017), was performed in 136 (77.3%) patients, and the median number of dissected lymph nodes was 33 (range: 2-108); 49 (27.8%) patients showed invasion of the adjacent structures and underwent a gastrectomy with bloc resection of the involved structures. A total of 132 (75%) patients underwent resection at a single institution in the Peking University Cancer Hospital.

Adjuvant chemotherapy was administered to 164 (93.2%) patients after curative resection. The chemotherapy regimens included monotherapy (capecitabine/S1/5-FU, $n = 10$), doublet chemotherapy (FOLFOX, $n = 33$; XELOX, $n = 34$; SOX, $n = 39$; capecitabine/S1+cisplatin, $n = 9$; paclitaxel+capecitabine, $n = 15$; paclitaxel+ cisplatin/oxaliplatin, $n = 4$) and triplet chemotherapy (based on 5-FU including cisplatin, oxaliplatin, epirubicin, paclitaxel, docetaxel, etoposide, and mitomycin, $n = 20$); 12 patients did not receive adjuvant chemotherapy. Fourteen patients received intra- or postoperative intraperitoneal perfusion of cisplatin/paclitaxel/5-FU, and four patients received postoperative chemoradiotherapy. All adverse events were assessed using the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 2.0. Dose modifications were made for patients who experienced hematologic or non-hematologic toxicity.

Disease recurrence was determined by radiologic or histological examination; the sites of recurrence were documented separately and included anastomotic sites, regional lymph nodes, peritoneum, ovary, adrenal gland, liver, lung, bone, extra-abdominal lymph nodes, and Virchow's lymph nodes. Based on these sites, the patterns of the first recurrence were categorized as locoregional recurrence (anastomotic sites and regional lymph nodes), peritoneal dissemination (ovary and the peritoneum), or distant metastasis (the liver, lung, bone, Virchow's lymph nodes, extra-abdominal lymph nodes, and adrenal gland).

Follow-up evaluation

Patients were followed every 3 mo for the first 2 years and then at 6-mo intervals until the fifth year. Regular follow-up evaluations consisted of a physical examination, routine laboratory tests, abdominal computed tomography (CT) scan, endoscopy, and chest X-ray.

Statistical analysis

The statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) software, version 21.0. Disease-free survival (DFS) was defined as the time from surgery until the recurrence of GC or death from any cause. Overall survival (OS) was defined as the time from surgery until death from any cause. Continuous variables were transformed to dichotomous variables in the survival analysis. χ^2 tests were used to compare clinicopathological characteristics between groups. Variables known to have prognostic value were selected in the final multivariable Cox proportional hazards model. Kaplan-Meier curves for disease-free survival and OS were compared using a log-rank test. A P -value of < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Our study included a group of 176 patients with metastasis in sixteen or more regional lymph nodes (TxN3bM0) or invasion of adjacent structures (T4bN1-3M0) in whom achieving R0 resection was difficult and who were assumed to be at high risk for recurrence. All patients, including 131 females and 45 males aged 25-81 years (56.4 ± 11.1 years), had histologically confirmed gastric or gastroesophageal junction adenocarcinoma; most had poorly differentiated adenocarcinoma. Of the 176 patients, 156 (88.6%) were classified as stage III C based on the AJCC TNM Staging Classification for Carcinoma of the Stomach (7th ed, 2010). The clinicopathological characteristics of the patients are listed in Table 1.

Survival and prognostic factors

Based on the follow-up data updated on July 31, 2015, the median follow-up time for the 176 patients was

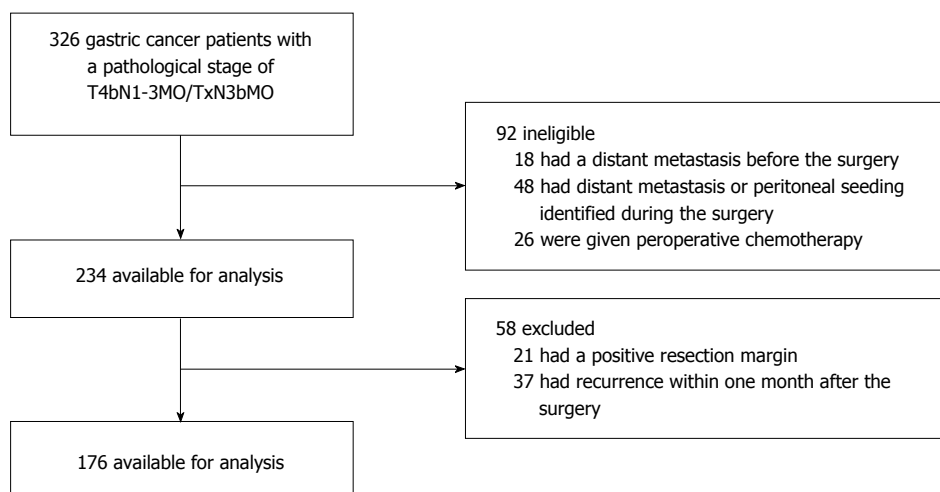


Figure 1 Study flow diagram.

Table 1 Relationship between clinicopathological characteristics and prognosis of T4bN1-3M0/TxN3bM0 gastric cancer patients

Clinicopathological characteristics	All patients (n = 176)		5-yr OS (%)	P value
	n	%		
Sex				
Male	131	74.4%	17.4%	0.702
Female	45	25.6%	15.8%	
Age (yr)				
≥ 60	68	38.6%	21.8%	0.799
< 60	108	61.4%	13.5%	
Tumor location				
Upper third	43	24.4%	17.7%	0.614
Middle third	56	31.8%	19.6%	
Lower third	62	35.2%	19.6%	
Total	15	8.5%	0.0%	
Tumor grade (differentiation)				
Moderate	15	8.5%	19.3%	0.241
Poor	161	91.5%	16.5%	
Lymphovascular invasion				
Yes	139	79.0%	10.3%	0.010
No	37	21.0%	30.6%	
No. of positive LNs				
0	4	2.3%	37.5%	0.174
1-6	17	9.7%	31.2%	
7-15	10	5.7%	0.0%	
≥ 16	145	82.4%	15.8%	
No. of dissected LNs				
≥ 30	106	60.2%	20.6%	0.326
< 30	70	39.8%	11.6%	
Positive LN ratio				
≥ 0.8	34	19.3%	6.2%	0.048
< 0.8	142	80.7%	20.5%	
Pathologic T stage ¹				
T2	5	2.8%	40.0%	0.420
T3	20	11.4%	30.6%	
T4a	102	58.0%	12.6%	
T4b	49	27.8%	21.2%	
Stage ¹				
IIIA	5	2.8%	40.0%	0.237
IIIB	15	8.5%	35.9%	
IIIC	156	88.6%	14.0%	

¹Recorded based on the American Joint Committee on Cancer (AJCC) TNM Staging Classification for Carcinoma of the Stomach (7th edition, 2010). LN: Lymph node; OS: Overall survival.

Table 2 Multivariate analysis of the prognostic factors for overall survival of T4bN1-3M0/TxN3bM0 gastric cancer patients

Clinicopathological characteristics	P value	Odds ratio	95%CI	
			Lower	Upper
Lymphovascular invasion	0.01	1.80	1.15	2.8
Node positive rate	0.14	1.36	0.90	2.1
Stage	0.49	0.71	0.34	1.5

LN: Lymph node.

47.4 mo (range: 2-202 mo). By the end of the follow-up period, 123 patients had died, 37 patients were alive, and 16 patients (9.1%) had been lost to follow-up.

The 5-year OS of the group was 16.8%; the median OS was 25.7 mo (95%CI: 20.9-30.5). The 3-year DFS of the whole group was 9.8%, while the median DFS was 11.7 mo (95%CI: 10.0-13.4). The univariate analysis showed that lymphovascular invasion and NPR ≥ 0.8 were associated with a poor prognosis ($P = 0.01$ and $P = 0.048$, respectively), while stage IIIC was not significantly associated with a poor prognosis according to the Kaplan-Meier method ($P = 0.237$, Table 1).

In the multivariate analysis, lymphovascular invasion was an independent prognostic factor ($P = 0.01$, HR: 1.8, 95%CI: 1.15-2.8) for OS in T4bN1-3M0/TxN3bM0 GC patients (Table 2).

Patterns of recurrence

During the follow-up period, 147 (83.5%) of the 176 patients with T4bN1-3M0/TxN3bM0 GC experienced recurrence; the first recurrence was localized to a single site in 78.9% of patients, two sites in 13.6% of patients, and three or more sites in 6.8% of patients. As shown in Table 3, the most common pattern of first recurrence was distant metastasis (45.6%), followed by peritoneal dissemination (25.9%) and locoregional recurrence (22.5%). Nine patients (6.1%) who

Table 3 Overall survival according to patterns of recurrence in T4bN1-3M0/TxN3bM0 gastric cancer patients after curative resection

Recurrent sites	Recurrent patients (<i>n</i> = 147)		Median OS (mo)	5-yr OS (%)	<i>P</i> value
	<i>n</i>	%			
Locoregional	33	22.5%	33.9	28.0%	0.001
Peritoneal	38	25.9%	16.0	0.0%	
Distant	67	45.6%	21.3	14.7%	

Table 4 Overall survival of patients with T4bN1-3M0/TxN3bM0 gastric cancer according to distant site of metastasis

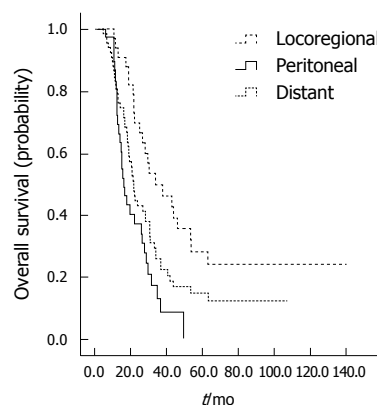
Distant metastasis site	Recurrent patients (<i>n</i> = 147)		Median OS (mo)	5-yr OS (%)
	<i>n</i>	%		
Liver	26	17.7%	18.3	15.5%
Lung and pleura	12	8.2%	16.8	0.0%
Bone	10	6.8%	30.7	29.2%

experienced combined patterns of recurrence were excluded from the survival analysis. The prognosis was best for patients with locoregional recurrence and worst for those who had peritoneal dissemination. Figure 2 presents the OS for each group. The 5-year OS rates were 28.0%, 0% and 14.7% for locoregional recurrence, peritoneal dissemination and distant metastasis, respectively, which showed statistically significant differences ($P = 0.001$).

We further analyzed OS according to the most distant metastatic sites; the most frequent site of distant metastasis was the liver, followed by the lung (including malignant pleural effusion), bone, and other distant sites. Eight of ten patients had bone metastases as the first recurrence site without liver or lung metastases. The median OS for patients with bone metastasis from GC was 30.7 mo, while that for patients with other metastatic sites was 21.9 mo ($P = 0.35$). The median OS for patients with lung metastasis was significantly shorter than that for patients with other metastatic sites (16.8 mo vs 22.4 mo, $P = 0.04$) (Table 4). The results showed that patients with bone metastasis had a better prognosis, whereas patients with lung and pleura metastasis had a worse prognosis than those with other metastatic sites.

Adjuvant chemotherapy

During the follow-up period after curative resection, 12 patients did not receive adjuvant chemotherapy because of their poor condition or rejection of chemotherapy; 164 (93.2%) of the 176 patients received at least one cycle of adjuvant chemotherapy. Combined chemotherapy, including doublet and triple regimens, was associated with a better prognosis than monotherapy but with no significant difference in 5-year OS (0% in the monotherapy group and 17.5% in the combined chemotherapy group, $P =$

**Figure 2 Overall survival of patients with T4bN1-3M0/TxN3bM0 gastric cancer after curative resection according to the patterns of recurrence.**

0.613). Triple adjuvant chemotherapy showed no significant survival benefit over the doublet regimen ($P = 0.449$). The 5-year OS rates were 0%, 17.4%, and 18.5% for the monotherapy, doublet chemotherapy and triple chemotherapy groups, respectively ($P = 0.661$); the 3-year DFS rates were 0%, 5.3%, and 5.3%, respectively ($P = 0.583$, Table 5). The patient characteristics, except for age, were similar in the three groups; approximately 60.0% of patients in the monotherapy group, 40.7% in the doublet group, and 28.0% in the triplet group were older than 60 years ($P = 0.202$).

In our study, various chemotherapeutic agents, including platinum-, taxane-, epirubicin-based regimens, did not show any significant differences in survival benefit (data not shown).

The median number of cycles of adjuvant chemotherapy was six, and the median time of adjuvant chemotherapy was 4.2 mo. Thirty-nine (22.1%) of the 176 patients received adjuvant chemotherapy for longer than six mo, as shown in Table 5. A longer duration of adjuvant chemotherapy was significantly associated with a better prognosis; the median OS was prolonged to 40.2 mo (95%CI: 30.6-48.2) in patients given adjuvant chemotherapy for longer than six months, compared with 21.6 mo (95%CI: 19.1-24.0) in patients given adjuvant chemotherapy for less than six months ($P = 0.001$). The median DFS was 23.2 mo (95%CI: 21.5-24.9) in patients given adjuvant chemotherapy for longer than six months, compared with 9.9 mo (95%CI: 7.6-12.3) in patients receiving adjuvant chemotherapy for less than six months ($P = 0.0001$) (Table 5, Figure 3). The patient characteristics were similar between the two groups.

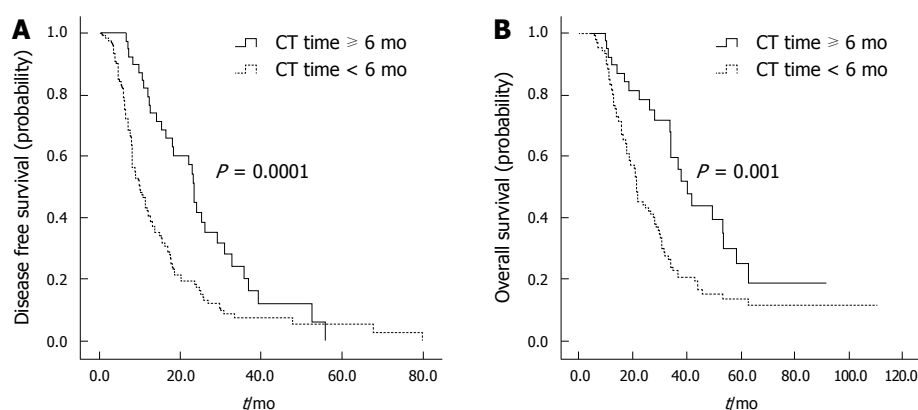
Treatment compliance, modifications and adverse events

Of the 164 patients who received adjuvant chemotherapy, only 39 patients continued the treatment for over six months. The most common reasons for withdrawal of treatment included the refusal of the patients to continue treatment due to inadequate

Table 5 Relationship between adjuvant treatment and the prognosis of T4bN1-3M0/TxN3bM0 gastric cancer patients

Treatment		<i>n</i>	Median DFS (mo)	3-yr DFS (%)	<i>P</i> value	Median OS (mo)	5-yr OS (%)	<i>P</i> value
Adjuvant chemotherapy	Yes	164	12.3	10.4%	0.000	25.7	16.1%	0.532
	No	12	2.8	0.0%		18.7	22.2%	
Chemotherapy	Mono-therapy	10	6.7	0.0%	0.583	20.3	0.0%	0.661
	Doublet	134	12.0	5.3%		26.3	17.4%	
Regimen	Triple	20	13.0	5.3%	0.000	29.7	18.5%	0.001
	≥ 6 mo	39	23.2	20.2%		40.2	25.0%	
Adjuvant chemotherapy time	< 6 mo	125	9.9	7.3%		21.6	13.4%	

DFS: Disease-free survival.

**Figure 3** Kaplan-Meier curves of disease-free survival (A) and overall survival (B) for T4bN1-3M0/TxN3bM0 gastric cancer patients after curative gastrectomy according to the duration of adjuvant chemotherapy. *P* value by log-rank test. A: Disease-free survival (DFS): 23.2 mo vs 9.9 mo, *P* = 0.0001; B: Overall survival: 40.2 mo vs 21.6 mo, *P* = 0.001. CT: Chemotherapy.

social support (32%), adverse events (28%), the detection of relapse or metastasis (14.6%), or other factors (25.4%). A total of 114 patients (69.5%) required dose modifications or chemotherapy delays, including 24/39 (61.5%) in the chemotherapy ≥ 6 mo group and 90/125 (72.0%) in the chemotherapy < 6 mo group. Of the 154 patients who received doublet or triplet regimens, 20 patients (13.0%) switched to monotherapy because of adverse events or upon their request.

Adverse events, including hematologic and non-hematologic toxic effects, were analyzed. The most frequent grade 3 or 4 adverse events were neutropenia (20.3%), nausea and vomiting (7.3%), anorexia (6.7%), and diarrhea (3.7%). Overall, 44 patients (26.8%) developed grade 3 or 4 toxicities (data not shown).

DISCUSSION

The aim of this retrospective study was to provide evidence for clinical treatment of T4bN1-3M0/TxN3bM0 GC patients after curative resection. This population is at the most advanced stage of GC at which resection is possible; therefore, R0 resection is difficult, and the risk of recurrence is high. Currently, controversy exists regarding whether prolonging the duration of adjuvant

chemotherapy, intensifying adjuvant chemotherapy, or undergoing preoperative chemotherapy will improve the prognosis for these patients. More efforts to explore appropriate adjuvant therapy modalities are necessary for clinical practice.

Despite undergoing standardized adjuvant chemotherapy followed by curative resection performed by experienced surgeons in our high-volume GC centers, patients with T4bN1-3M0/TxN3bM0 GC had a high risk of recurrence and a poor prognosis. The 5-year OS of the entire group was 16.8%, which is significantly lower than that of patients with stage III disease, ranging between 40%-70% in most phase 3 trials^[3,9]. Patients at stage III C accounted for 88.6% of our study population; the 5-year OS for these patients was far lower than that of patients with stage III C GC reported in another study (14.0% vs 30.2%)^[10]. Moreover, a Korean study^[6] showed that the 5-year OS rate of the patients who received adjuvant chemotherapy with T4bN1-3M0/TxN3bM0 GC was 39.6%; only 61.7% of these patients experienced recurrence^[11]. However, the 5-year OS of patients in our study who received adjuvant chemotherapy for longer than 6 mo was only 25%, and 147 (83.5%) of the 176 patients experienced recurrence.

Several factors may be responsible for the poor prognosis of patients in our study. First, new diagnostic

modalities such as endoscopic ultrasound (EUS), positron emission tomography/computed tomography (PET/CT), magnetic resonance imaging (MRI), and laparoscopic staging, were not used for preoperative staging of patients treated during the early part of the study, which may have reduced the accuracy of staging and led to the advanced gastric cancer be treated as resectable gastric cancer improperly^[12-14]. Therefore, patients included in this study may be mixed with advanced patients actually, and these errors can be avoided using new staging approach. Second, the risk of non-regional lymph node metastases is increased in patients with N3b, although all tumors with T4bN1-3M0/TxN3bM0 are staged regardless of the M1 category; additionally, without appropriate clinical information, surgical pathologists may be unaware that particular lymph node metastases are already distant metastases and they may be classified as N3b instead of M1. Third, Korean and Japanese surgeons have performed more D2+ lymphadenectomies, total gastrectomies, multivisceral resections, and Billroth II digestive tract reconstructions than their Chinese counterparts; indeed, the OS of Korean patients was longer than that of Chinese patients, especially for those with stage III disease^[15]. Fourth, 39 patients in our study underwent limited lymph node dissections, whereas only 4 patients received postoperative chemoradiotherapy, as the INT 0116 study established postoperative chemoradiotherapy as a standard of care for patients who undergo < D2 dissections^[16]. These facts reflect the medical status in China and contribute to a new understanding of T4bN1-3M0/TxN3bM0 patients, who mostly belong to stage III C, while they are distinct from conventional stage III C GC patients with regard to the biological behavior and prognosis of the disease.

In our study, the most common pattern of first recurrence was distant metastasis; sites of distant metastasis and locoregional recurrence accounted for 45.6% and 22.5%, respectively, of patients with T4bN1-3M0/TxN3bM0 recurrent GC. Patients with locoregional recurrence showed a better prognosis than patients with distant metastasis, suggesting that systemic therapy, rather than local therapy, was more likely to benefit patients with T4bN1-3M0/TxN3bM0 GC. According to the results of the ACTS-GC and CLASSIC trials^[3,9], adjuvant chemotherapy with one year of S1 or 6 mo of the XELOX regimen after a D2 gastrectomy was confirmed to be the standard adjuvant treatment for locally advanced gastric cancer. Without definitive data favoring combined therapy over monotherapy, especially in GC patients with the most advanced stage of T4bN1-3M0/TxN3bM0, it remains unclear whether an intensified or longer duration of adjuvant chemotherapy provides an additional benefit.

In our study, triple adjuvant chemotherapy showed no significant survival benefit compared with a doublet regimen. Recently, the SAMIT study and the ITACA-S study, both of which compared poly-chemotherapy vs

monotherapy, failed to show any benefit for patients in an adjuvant setting^[5,17]. Intensifying adjuvant chemotherapy is almost considered too difficult to provide additional benefit. It is of note that patients who received adjuvant chemotherapy for longer than six months in our study benefited significantly from the treatment, with the median OS prolonged to 40.2 mo. In contrast, the median OS was 21.6 mo for patients who received chemotherapy for less than six months. It is therefore suggested that prolonged adjuvant chemotherapy may improve the outcomes for patients at a high risk of distant recurrence. However, only 22.1% of the patients completed all six months of chemotherapy, which may be explained by the frailty of GC patients after surgery, along with the toxicity of adjuvant poly-chemotherapy. In this case, active dose modification based on the adverse events of chemotherapy should to be performed to ensure adequate chemotherapy time and additional benefit from the treatment.

While preoperative chemotherapy may theoretically be superior to postoperative chemotherapy for several reasons^[18-20], preoperative chemotherapy has been widely used for patients with T4bN1-3M0/TxN3bM0 GC in clinical practice. However, whether perioperative or postoperative chemotherapy is more beneficial for T4bN1-3M0/TxN3bM0 patients lacks data supported by prospective studies; the ongoing RESOLVE study (NCT01534546) to compare perioperative chemotherapy of SOX vs SOX/XELOX as postoperative chemotherapy in locally advanced gastric cancer with D2 dissection may provide additional evidence. Moreover, patients in arm C of the RESOLVE study will receive 8 cycles of perioperative SOX followed by 3 cycles of S-1 monotherapy, which may provide evidence for prolonged adjuvant chemotherapy.

Based on the classification and statistical analysis, 26 patients with T4b disease were excluded from our study because they had a positive resection margin, which indicates that at least one-third of T4b patients according to preoperative staging failed to eventually undergo R0 resection. Preoperative chemoradiotherapy (CRT) may increase resectability and improve the outcomes of T4b patients. The role of CRT continues to be evaluated in many ongoing clinical trials worldwide, such as the Trial of Preoperative Therapy for Gastric and Esophagogastric Junction Adenocarcinoma (TOPGEAR, NCT01924819) and the ARTIST-II trial in patients with lymph node-positive GC after D2 gastrectomy.

Due to the small sample sizes and the heterogeneity of therapy administered over a long period, the results in this study have been mixed and biased. Although this study was conducted based on retrospective data, we think that the bias may be reduced by the fact that the surgeries were performed in our high-volume GC centers and patients had access to good medical care. Indeed, this study is the largest retrospective analysis of the effect of adjuvant therapy

on patients with T4bN1-3M0/TxN3bM0 GC; the results reflect the current medical situation for the treatment of gastric cancer in China and are complementary to those of large-scale phase III prospective trials.

Undoubtedly, along with an in-depth understanding of molecular and gene profiling, personalized precision medicine as well as adjuvant and perioperative multimodal therapies^[21] will be crucial for improving the outcomes of conventional adjuvant chemotherapeutic treatments in the future.

In conclusion, patients with T4bN1-3M0/TxN3bM0 gastric cancer showed a poor prognosis, with the most common pattern of first recurrence being distant metastasis rather than locoregional recurrence. Adjuvant chemotherapy for longer than six months may improve the outcomes of this patient group. However, a prospective randomized controlled study will be required to confirm these findings and to improve the outcomes for patients with T4bN1-3M0/TxN3bM0 gastric cancer.

ARTICLE HIGHLIGHTS

Research background

In view of the limited evidence regarding T4bN1-3M0/TxN3bM0 GC, as well as the difficulty of achieving R0 resection and the high risk of disease recurrence, this retrospective study is complementary to large-scale phase III prospective trials and may provide implications for clinical practice.

Research motivation

The population targeted in our study is difficult to treat with no accepted standard of care. This study is the largest retrospective analysis of the effect of adjuvant therapy on patients with T4bN1-3M0/TxN3bM0 GC. Furthermore, our study explored the patterns of recurrence and their relationships to the prognosis of these patients.

Research objectives

To provide evidence regarding the postoperative treatment of patients with T4bN1-3M0/TxN3bM0 gastric cancer, for which guidelines have not been established.

Research methods

Patients who had undergone curative resection between 1996 and 2014 with a pathological stage of T4bN1-3M0/TxN3bM0 for gastric cancer were retrospectively analyzed; staging was based on the 7th edition of the American Joint Committee on Cancer staging system. The clinicopathological characteristics, administration of adjuvant chemotherapy, and patterns of recurrence were studied. Univariate and multivariate analyses of prognostic factors were conducted. The chemotherapeutic agents mainly included fluorouracil, platinum and taxanes, used as monotherapy, doublet, or triplet regimens. Patterns of first recurrence were categorized as locoregional recurrence, peritoneal dissemination, or distant metastasis.

Research results

The 5-year overall survival (OS) of the whole group ($n = 176$) was 16.8%, and the median OS was 25.7 mo (95%CI: 20.9-30.5). Lymphovascular invasion and a node positive rate (NPR) ≥ 0.8 were associated with a poor prognosis ($P = 0.01$ and $P = 0.048$, respectively). One hundred forty-seven (83.5%) of the 176 patients eventually experienced recurrence; the most common pattern of the first recurrence was distant metastasis. The prognosis was best for patients with locoregional recurrence and worst for those with peritoneal dissemination. Twelve (6.8%) of the 176 patients did not receive adjuvant chemotherapy, while 164 (93.2%) patients received adjuvant chemotherapy. Combined

chemotherapy, including doublet and triplet regimens, was associated with a better prognosis than monotherapy, with no significant difference in 5-year OS (17.5% vs 0%, $P = 0.613$). The triplet regimen showed no significant survival benefit compared with the doublet regimen for 5-year OS (18.5% vs 17.4%, $P = 0.661$). Thirty-nine (22.1%) patients received adjuvant chemotherapy for longer than six months; the median OS in patients who received adjuvant chemotherapy for longer than six months was 40.2 mo (95%CI: 30.6-48.2), significantly longer than the 21.6 mo (95%CI: 19.1-24.0) in patients who received adjuvant chemotherapy for less than six months ($P = 0.001$).

Research conclusions

Patients with T4bN1-3M0/TxN3bM0 gastric cancer showed a poor prognosis, with the most common pattern of first recurrence being distant metastasis rather than locoregional recurrence. Adjuvant chemotherapy for longer than six months may improve the outcomes of this patient group.

Research perspectives

To date, few retrospective studies have analyzed the survival and prognosis factors for T4bN1-3M0/TxN3bM0 GC patients; however, due to the small sample sizes and different treatment regimens, the results have been mixed. No meta-analyses have been conducted on this topic. However, a prospective randomized controlled study will be required to confirm these findings and to improve the outcomes for patients with T4bN1-3M0/TxN3bM0 gastric cancer.

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Clinical Practice Study

Neoadjuvant hyperfractionated accelerated radiotherapy plus concomitant 5-fluorouracil infusion in locally advanced rectal cancer: A phase II study

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Abstract

AIM

To evaluate the efficacy and tolerability of neoadjuvant hyperfractionated accelerated radiotherapy (HART)

and concurrent chemotherapy in patients with locally advanced infraperitoneal rectal cancer.

METHODS

A total of 30 patients with histopathologically confirmed T2-3/N0+ infraperitoneal adenocarcinoma of rectum cancer patients received preoperative 42 Gy/1.5 Gy/18 days/bid radiotherapy and continuous infusion of 5-fluorouracil (325 mg/m²). All patients were operated 4-8 wk after neoadjuvant concomitant therapy.

RESULTS

In the early phase of treatment, 6 patients had grade III-IV gastrointestinal toxicity, 2 patients had grade III-IV hematologic toxicity, and 1 patient had grade V toxicity due to postoperative sepsis during chemotherapy. Only 1 patient had radiotherapy-related late side effects, *i.e.*, grade IV tenesmus. Complete pathological response was achieved in 6 patients (21%), while near-complete pathological response was obtained in 9 (31%). After a median follow-up period of 60 mo, the local tumor control rate was 96.6%. In 13 patients, distant metastasis occurred. Disease-free survival rates at 2 and 5 years were 63.3% and 53%, and corresponding overall survival rates were 70% and 53.1%, respectively.

CONCLUSION

Although it has excellent local control and complete pathological response rates, neoadjuvant HART concurrent chemotherapy appears to not be a feasible treatment regimen in locally advanced rectal cancer, having high perioperative complication and intolerable side effects. Effects of reduced 5-fluorouracil dose or omission of chemotherapy with the aim of reducing toxicity may be examined in further studies.

Key words: Hyperfractionated accelerated radiotherapy; Rectal cancer; Neoadjuvant chemoradiotherapy

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Core tip: This study includes a first phase II study evaluating neoadjuvant hyperfractionated accelerated radiotherapy plus concomitant infusional 5-fluorouracil (5-FU) chemotherapy in locally advanced rectal cancer (not resectable cancer). This regimen may allow clinicians to design other neoadjuvant hyperfractionated accelerated radiotherapies. This study showed excellent local control but high rate of perioperative complications. Decreasing or modifying the 5-FU dose could provide better local control.

Gural Z, Saglam S, Yucel S, Kaytan-Saglam E, Asoglu O, Ordu C, Acun H, Sharifov R, Onder S, Kizir A, Oral EN. Neoadjuvant hyperfractionated accelerated radiotherapy plus concomitant 5-fluorouracil infusion in locally advanced rectal cancer: A phase II study. *World J Gastrointest Oncol* 2018; 10(1): 40-47 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i1/40.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i1.40>

INTRODUCTION

Rectal cancer is associated with a high incidence of local recurrence and distant metastasis^[1,2]. In randomized studies, local-regional recurrence despite mesorectal resection has been reported to occur in 15% to 30% of the patients undergoing surgery alone^[3-8]. In this regard, addition of preoperative and postoperative treatments to surgery have been shown to significantly improve local recurrence and survival rates^[9-13], leading to standard administration of such treatments. Currently, preoperative chemoradiation (CRT) is the preferred treatment regimen in these patients, owing to low local recurrence rates and higher chance of sphincter-sparing surgery; although, studies comparing preoperative and postoperative CRT are relatively limited.

Besides conventional radiotherapy (RT) consisting of 45-50 Gy/1.8-2 Gy/5-6 wk, hypofractionated and hyperfractionated accelerated RT (HART; 42 Gy/1.5 Gy/18 d) are also used. HART reduces the risk of repopulation in tumor cells by shortening the treatment time and increases the repair capacity of normal tissues after sublethal damage through the reduction of the fraction dose. Thus, a survival advantage is provided in favor of normal cells, since tumor cells exhibit a poor repair mechanism^[14]. In this background, a fractionated HART scheme was examined in this study.

Therefore, this study was carried out to observe the early and late effects of HART regimen in combination with neoadjuvant chemotherapy in patients diagnosed with locally advanced rectal cancer.

MATERIALS AND METHODS

Patient selection

Previously untreated patients with histologically confirmed adenocarcinoma of the rectum (mid and distal \leq 12 cm from the anal verge) were included in the study at Istanbul University Oncology Institute. Patient inclusion criteria were as follows: presence of resectable tumor; Karnofsky performance score \geq 80; adequate bone marrow reserve (hemoglobin $>$ 11 g/dL, white blood cell $>$ 3500 mL, platelet count $>$ 100000 mL), normal kidney and liver function tests (creatinine $<$ 1.3 mg/dL, alanine aminotransferase and aspartate aminotransferase $<$ 80 U/L), and \leq 70 years of age. Patients who had received pelvic RT previously and patients with clinically detected distant metastases were excluded from the study. Clinical staging prior to treatment was accomplished based on physical examination, tumor markers (carcinoembryonic antigen, CA19-9), complete blood count and biochemistry tests, positron emission-computed tomography, pelvic-diffusion magnetic resonance imaging (MRI), and endorectal ultrasound. This prospective study was approved by the local ethics committee. A written informed consent was obtained from all patients prior to treatment.

Table 1 Patient characteristics

Characteristic	n = 30
Sex, M/F	19/11
Age, median (range)	53 (30-70)
Tumor location, distance from anal verge	
≤ 5 cm	19 (63)
> 5 cm	11 (37)
Clinical TN stage	
T2N2	1 (3)
T3N0	2 (7)
T3N1	15 (50)
T3N2	12 (40)
Tumor differentiation	
Well	10 (33)
Moderate	10 (33)
Poor	4 (14)
Mucinous	3 (10)
Signet ring cell	3 (10)

Unless otherwise stated, data are presented as n (%). M: Male; F: Female.

Preoperative CRT

All patients received preoperative HART (42 Gy/1.5 Gy/18 d/bid) and concurrent continuous infusion of 5-fluorouracil (5-FU; 325 mg/m²) and were hospitalized during treatments to observe the possible acute side effects.

Prior to RT planning, computed tomography was performed in prone position with belly board, with a 0.5 cm slice thickness for all patients. Gross tumor volume and clinical target volume were estimated by the radiologist and radiation oncologist. Patients were treated with a 3-D conformal RT technique, through posterior and lateral fields using a linear accelerator (18 MV) and with an isodose of 95% of planned target volume. RT regimen was defined by a fraction dose of 150 cGy/fr given 2 times/d, 5 d/wk, with a minimum 8 h between fractions. Total dose was 4200 cGy and total treatment duration was 18 d.

Port or subclavian catheter was used to give 5-FU in the form of a continuous infusion during the entire treatment. The daily dose of 5-FU that was given to patients was 325 mg/m²[15]. Surgery was performed 4-8 wk after the completion of CRT.

Low anterior or abdominoperineal resection (total mesorectal excision) was performed depending on the location of the tumor and response rate. Four cycles of 5-FU (400 mg/m², D1-5, q 28 d) plus folinic acid (20 mg/m², D1-5, q 28 d) were administered postoperatively.

Assessment of efficacy and side effects

The primary endpoint was pathological response rate after CRT, and secondary endpoints included the local control rate, surgical margin positivity, survival and toxicity. Patients were assessed for toxicity during CRT on a daily basis. During the period between the end of CRT and surgery, patient assessments for side effects were performed weekly. Acute radiation toxicity criteria of the Radiation Therapy Oncology Group

and the European Organization for Research and Treatment of Cancer (EORTC) were used for side effect assessments^[16]. Pathologic response and staging were defined according to the Dworak regression scoring system^[17] and TNM staging system^[18], as described by the American Joint Committee on Cancer.

Statistical analysis

Statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, United States) statistical software. Survival was calculated using the Kaplan-Meier method.

RESULTS

Thirty patients (19 males and 11 females) who were diagnosed with locally advanced rectum cancer between October 2007 and March 2009 were included. The median age was 53 years (range: 30-70 years). Patient characteristics are summarized in Table 1. There were only 2 patients with T3N0 disease, and one of them had positive circumferential margins in staging MRI.

Pathological findings

Surgery was performed in all subjects except for one, who was found to have metastases during the early period after the CRT. Surgery was performed at week 4 in 15 patients and between weeks 6 and 8 in 13 patients. Twelve patients (41%) underwent sphincter-sparing surgery. According to the Dworak total regression scoring system, 6 of 29 (21%) patients who underwent surgery had grade IV (total) regression, and 9 patients (31%) had grade III (near total) regression. Corresponding figures for grade II, I and 0 regression were 11 patients (38%), 2 patients (7%) and 1 patient (3%), respectively.

Positive margins were found in 2 patients (6.6%). In 14 patients, mesorectal fascia invasion was detected in staging MRI and only 2 of those patients had positive radial surgical margin. Comparison of ypT and cT yielded a down-staging rate of 59%. Clinical and pathological tumor stages are shown in Table 2. The median number of lymph nodes that were excised was 25 (2-58), respectively. No pathologic lymph nodes were present in 19 (63%) patients. With regard to N stage, 20 (69%) patients were found to have down-staging.

Local control and survival

One (3.3%) patient had local recurrence while distant metastases were found in 13 (43.3%) patients during a median follow up of 60 mo (5-78 mo). None of the patients with T3N0 disease had local recurrence. Overall, 14 patients (46.6%) died during the study period. The causes of death were systemic metastasis (13 patients) and chemotherapy-related toxicity (1 patient). Median time to progression was 59 mo (2-78

Table 2 Clinical (cT2) and pathological (ypT) tumor stages

	cT2	cT3	Total
ypT0	-	6 (20.6)	6 (20.6)
ypT1	-	3 (10.3)	3 (10.3)
ypT2	-	8 (27.5)	6 (20.6)
ypT3	1	11 (37.9)	12 (41.3)
Total	1	28	29

Data are presented as *n* (%).

Table 3 Surgical complications

Timing of the complication	
Perioperative	6 (20.6) ¹
Early postoperative	4 (13.7) ²
Late postoperative	2 (6.8) ³

¹Bladder-urethra injury (*n* = 4), rectum perforation (*n* = 1), necrosis due to proctectomy (*n* = 1); ²Acute renal failure (*n* = 3), perirectal abscess (*n* = 1); ³Colovaginal fistula (*n* = 1), perirectal abscess (*n* = 1). Data are presented as *n* (%).

mo). The 2- and 5-year disease-free survival (DFS) rates were 63% and 53%, while the 2- and 5-year overall survival (OS) rates were 70% and 53.1%, respectively. The patients with complete or near-complete pathological response were compared to patients with less favorable group for survival. We found no significant difference in either group for DFS (*P* = 0.63) and OS (*P* = 0.32).

Toxicity and complications

Early side effects of preoperative CRT: The highest frequency of side effects occurred at weeks 3-4. During the acute phase 6 (20%) patients developed grade III-IV gastrointestinal system toxicity (3 grade III tenesmus/diarrhea and 3 grade IV tenesmus and diarrhea), and 2 (6.7%) patients developed grade III-IV hematopoietic system toxicity (1 grade III leucopenia and 1 grade IV neutropenia). There were no interruptions in RT due to toxicity, while in 4 patients chemotherapy was interrupted for 1 wk. Perianal abscess formation was observed in 3 patients before the planned date of surgery. One patient experienced spontaneous perforation at the tumor zone prior to surgery.

Perioperative complications: One patient had spontaneous perforation of the colon before surgery. Surgery was complicated in 4 patients with urethra-bladder injury, and in 1 patient with rectal perforation. Temporary nephrostomy tube was inserted in 3 patients. One patient developed incontinence and impotence due to nerve damage caused by bladder injury. Total proctectomy procedure was performed in 1 patient due to sudden onset of ischemia during mesorectal resection. Perirectal abscesses developed in 2 patients. Surgical complications are shown in Table 3.

Postoperative chemotherapy: Sixteen (53%) patients underwent adjuvant chemotherapy. Chemotherapy was not given to 13 patients with pathologic complete response after surgery or who had preoperative grade IV toxicity due to CRT. Grade V toxicity (sepsis) was seen in only 1 patient after three cycles of chemotherapy. Adjuvant treatment was terminated prematurely in 2 patients due to grade IV hematologic toxicity.

DISCUSSION

Despite the continuous search for effective multidisciplinary treatment protocols, patients diagnosed with rectum cancer remain a high-risk population for local and distant recurrence. This study provided encouraging results with neoadjuvant HART plus chemotherapy.

A variety of preoperative RT regimens is used in patients with rectum cancer, and conventional RT (45-50 Gy/5 wk) represents the standard regimen for preoperative concurrent CRT. While a statistically significant advantage in terms of local recurrence rates was reported in 14 previous studies examining this regimen, a survival advantage could be shown in only 2 studies for preoperative RT^[9,19]. In these studies, patients with early stage disease (I) and no requirement for preoperative CRT represented the majority of the participants. In a Polish study comparing short-term preoperative RT and conventional CRT, a statistically significant superiority of CRT was observed in terms of complete response rates (*P* < 0.0001); however, no difference was found in local control and survival^[20]. In a randomized study from France comparing preoperative RT and CRT, better pathologic complete response rate (11.4% vs 3.6%, *P* < 0.0001) and reduced local recurrence (8% vs 16.5%, *P* < 0.051) were observed in the CRT arm^[10]. In the similarly designed EORTC 22921 study, lower local recurrence was demonstrated in the CRT arm (*P* < 0.001)^[21].

Several phase II studies administering HART alone or with concurrent chemotherapy have also been performed^[22-28]. In the HART study by Bouzourene *et al.*^[29] none of the patients had complete response and 8% of the patients had local remission. In another study by Voelter *et al.*^[23] examining HART and CT, the reported positive circumferential resection margin was 21% and local control was 100%. In our study, radial surgical margin positivity was 7%, and after a median follow-up of 60-mo the local control rate was 97%. Local recurrence was seen in only 1 patient preoperatively staged as T3N1 and the radial surgical margin was pathologically positive in this patient. In contrast with a phase II study by Marsh *et al.*^[26], where 17 patients receiving preoperative capecitabine and HART had a complete response of 18%, the complete response rate was 21% (grade IV) and the

Table 4 Studies investigating hyperfractionated accelerated radiotherapy regimen for locally advanced rectal cancer

Study	Number of patients	Design	Follow-up (mo)	Total RT dose	Intervals (wk)	Concomitant chemotherapy	pCR ¹	Local control	Down-staging
Coucke <i>et al</i> ^[24] 2006	250	Prospective	39 mo	41.6 Gy/1.6 Gy	1 wk	None	1.20%	91.70%	38%
Ceelen <i>et al</i> ^[22] 2007	50 vs 91	Prospective	67 mo vs 28 mo	41.6 Gy/1.6 Gy vs 45 Gy/1.8 Gy	13 d vs 6 wk	None vs 5-FU bolus chemotherapy	4% vs 18%	94% vs 95.6%	30% vs 51%
Voelter <i>et al</i> ^[23] 2006	33	Prospective	104 mo	41.6 Gy/1.6 Gy	1wk	CPT-11	NA	100%	33%
Brooks <i>et al</i> ^[42] 2006	20	Prospective	31 mo	25 Gy/1.67 Gy (CHART)	1 wk	None	NA	95%	NA
Widder <i>et al</i> ^[43] 2005	184	Prospective	43 mo	25 Gy/2.5 Gy	1 wk	None	NA	97.90%	NA
Bouzourene <i>et al</i> ^[29] 2003	104	Prospective	40 mo	41.6 Gy/1.6 Gy	1 wk	None	0%	92.30%	43%
Marsh <i>et al</i> ^[26] 2010	17	Prospective	NA	50.4-55.2 Gy/1.2 Gy	4-6 wk	Capecitabine 825 mg/m ² -twice per day	18.80%	NA	81.25%
The present study	30	Prospective	60 mo	42 Gy/1.5 Gy	6-8 wk	5-FU (325 mg/m ²) continuous infusion	21%	96.70%	59%

¹Pathological complete response; NA: Not available; RT: Radiotherapy; pCR: Pathological complete response.

Table 5 Biological equivalent doses^[44]

Regimen	Tumor control/acute normal tissue complication probability		Late normal tissue complication probability
	Bed (Gy) ($\alpha/\beta = 10$ Gy)		Bed (Gy) ($\alpha/\beta = 3$ Gy)
	No time correction	With time correction	
25 Gy/5 fr/5 d (d = 5 Gy)	37.5	37.5	66.7
50 Gy/25 fr/33 d (d = 2 Gy)	60.0	44.4	83.4
42 Gy/28 fr/18 d (d = 1.5 Gy)	48.3	41.7	63.0

Equation 1: Linear quadratic based isoeffect, basic formula without time correction, BED = $nd(1+d/\alpha/\beta)$, where n = number of fractions, d = dose (Gy) per fraction, α/β = the LQ quotient, Equation 2: Time-corrected LQ- formula, BED = $nd(1+d/\alpha/\beta-\gamma/\alpha(T-T_k))$, where γ/α = repair rate (set to 0.6 Gy/d), T = overall treatment time and T_k = proliferation delay (set to 7 d, or maximally T).

near-complete response rate was 31% (grade III) among our participants. Studies with HART regimen are shown in Table 4.

The primary aim of this study was to search for possible therapeutic strategies that may help increase the rate of pathological tumor response and to decrease late side effects. In the regimen examined herein, decreased fraction size and shortened total treatment duration were hypothesized to result in decreased late and early side effects, respectively. Treatment duration and doses were different from those administered in conventional RT schemes. Therefore, a biological effective dose formula was used for dose calculations instead of the given dose, according to a time-corrected linear quadratic model^[30,31]. Biological equivalent doses are shown in Table 5.

In this study combining HART and concurrent chemotherapy, 8 patients developed (26.6%) CRT-related grade III-IV toxicity. Although there was an increase in acute reactions, these effects were generally tolerable and RT was completed without interruption in all patients. In 4 patients, chemotherapy was interrupted shortly due to chemotherapy-related acute side effects.

Toxicity was increased as a result of combined use of chemotherapy and RT regimen together with a higher chemotherapy dose as compared to conventional chemotherapy. The highest incidence of side effects was observed at weeks 3 and 4, which correspond to the development of acute mucosal side effects.

In addition, there is some literature data available on early side effects in rectum cancer patients treated with neoadjuvant conventional CRT. For example, in the EORTC 22921 study, grade III-IV toxicity occurred in 14% of the patients^[21]. In that study, the probable cause of increased side effects was the total treatment duration and impaired tissue repair as a consequence of shorter intervals between fractions of the chosen HART regimen. In a retrospective study where neoadjuvant CRT and HART alone were compared, no grade III-IV toxicity was reported in the HART arm of the study^[22]. In the Phase II 93-01 study, patients were treated with neoadjuvant HART with no significant increase in acute side effects^[32]. In another phase II study with preoperative HART and concurrent irinotecan (CPT-11), addition of chemotherapy was associated with an increase in grade III-IV toxicity^[23],

while the most common grade III-IV side effects observed in this study included diarrhea (24%) and infection (9%). In that phase II study, early side effects were more frequent than in our study. Probably, reduced incidence of diarrhea in this study could be explained on the basis of sparing the bowel volume out of the RT field.

Bowel perforation occurring in 2 of our patients raises the question of whether a period of 4 wk allows adequate time with normal tissue recovery following an intensive therapy regimen with neoadjuvant HART and concurrent chemotherapy. 5-FU is known to affect the repair mechanism in intestinal cells^[33] and the 5-FU dose used in this study might have played a role in the development of perforation in 2 of our patients.

The ideal duration between neoadjuvant therapy and surgery remains a source of debate. The objective of early surgery following short-term RT is to reduce or prevent long-term side effects. However, delayed surgery has been reported to result in increased rates of tumor regression and pathological complete response. In randomized studies utilizing short-term preoperative RT, the time between RT and surgery is relatively short^[19,34], posing some challenges in the interpretation of the effects of the timing of surgery following RT. Early and delayed surgery were compared in the Stockholm III study where local control, DFS and OS were found to be similar in between three arms^[35]. In the randomized Istanbul R-01 study examining the ideal timing for surgery after preoperative CRT, no significant associations were observed between the time-to-surgery and regression rates or local control rates. Surgical margin seems to be the most important factor for local recurrence^[36].

In our study, no surgery-related deaths occurred (0/29). In a phase II study utilizing HART and concurrent CPT-11, the postoperative complication rate was 27%, similar to other neoadjuvant CRT studies^[23]. Operative complications were recorded in 7% of the cases in this study. Occurrence of late toxicity only in 1 patient suggests that the strategy of utilizing HART to reduce late toxicity may prove to be successful. While no late side effects were observed in the 91-10 study with preoperative HART^[37], in another study comparing conventional CRT with HART alone, late side effects were more frequently observed in the HART arm^[22].

In this study, the ability of the HART regimen to achieve a higher tumor regression rate due to decreasing tumor repopulation was examined. In this regard, complete and near-complete response was achieved in 21% and 31% of the participants, respectively. In a previous study comparing HART alone vs conventional CRT regimens, lower complete response rates observed in the HART arm underscores the additive effect of chemotherapy^[22]. Similarly, in the French and EORTC studies comparing conventional RT and CRT, the reported pathological complete response rates in the CRT arm were 11.4% and 14%, respectively^[38,39]. In our study, HART with concurrent

chemotherapy was found to achieve complete or near-complete tumor regression in 52% of the patients. Preoperative HART scheme appeared to be capable of increasing tumor response and local control rates, but no difference was found for OS in phase II studies^[22]. This study showed no survival benefit despite a high pathological response rate. A study by Petrelli *et al.*^[36,40] and randomized Istanbul R-01 study did not find any correlation between pathological complete response rate and survival.

Circumferential (lateral) margin positivity was found in 2 patients, whereas only 1 patient showed local recurrence during a median follow-up period of 60 mo. Thirteen patients had distant metastases. Extensive hepatic metastases were found in early phase in 3 patients who died due to systemic disease.

In conclusion, earlier studies have proven the feasibility of HART treatment in terms of early and late side effects in this patient population. As in our study, improved local control rates and tumor regression may be achieved with HART but with higher toxicity. Toxicity could be reduced by giving chronomodulated concomitant capecitabine in Brunch Study^[41]. A plausible option would be to reduce the dose of 5-FU to reduce toxicity.

ARTICLE HIGHLIGHTS

Research background

Currently, preoperative chemoradiation (CRT) is the preferred treatment regimen in locally advanced rectal cancer patients, owing to low local recurrence rates and higher chance of sphincter-sparing surgery. Besides conventional radiotherapy consisting of 45-50 Gy/1.8-2 Gy/5-6 wk, other radiotherapy schemes are also used. The hyperfractionated accelerated radiotherapy (HART) scheme reduces the risk of repopulation in tumor cells by shortening the treatment time and increases the repair capacity of normal tissues. In this background, a HART scheme and the combination of infusional 5-fluorouracil (5-FU) was examined in this study to augment the pathological complete response.

Research motivation

Local recurrence is still a substantial problem for locally advanced rectal cancers. Investigating tolerability and the effect of different radiotherapy schemes on local control other than conventional and hypofractionated radiotherapy can be a solution.

Research objectives

This study was mainly designed to observe the early and late effects of HART regimen in combination with neoadjuvant chemotherapy in patients diagnosed with locally advanced rectal cancer. The primary aim of this study was to search for possible therapeutic strategies that may help increase the rate of pathological tumor response and to decrease late side effects.

Research methods

Previously untreated locally advanced rectal cancer patients with histological confirmation were included in the study. The patients were clinically staged according to positron emission-computed tomography and pelvic-diffusion magnetic resonance imaging. All patients received preoperative HART (42 Gy/1.5 Gy/18 d/bid) and concurrent continuous infusion of 5-FU (325 mg/m²) and were hospitalized during treatments to observe the possible acute side effects. Total mesorectal excision was performed 4-8 wk after the completion of chemoradiotherapy. Four cycles of 5-FU (400 mg/m², D1-5, q 28 d) plus folinic acid (20 mg/m², D1-5, q 28 d) were administered postoperatively. The primary

endpoint was pathological response rate after CRT, and secondary endpoints included the local control rate, surgical margin positivity, survival and toxicity.

Research results

Thirty patients were included between October 2007 and March 2009. The median age was 53 years. Most of the patients clinically staged as T3N+ disease (90%). Surgery was performed at week 4 in half of the patients. Twelve patients (41%) underwent sphincter-sparing surgery. The Dworak total regression scoring system was used to evaluate pathological response, and grade IV (total) regression was found in 6 of 29 (21%) patients; nine patients (31%) had grade III (near total) regression. Positive margins were found in 2 patients (6.6%). One (3.3%) patient had local recurrence during a median follow-up of 60 mo. The 5-year disease-free survival rate was 53%, while the 5-year overall survival rate was 53.1%. There were no interruptions in RT due to toxicity, while in 4 patients chemotherapy was interrupted for 1 wk. Sixteen (53%) patients underwent adjuvant chemotherapy.

Research conclusions

Improved local control rates and tumor regression may be achieved with HART but with higher acute toxicity. Toxicity could be reduced by giving chronomodulated concomitant chemotherapy or reducing the dose of 5-FU. Surgery timing has no effect on survival but still should be considered because of increased acute side effects due to HART fractionation. Besides an increased pathological response rate, this study showed no survival benefit.

Research perspectives

Different HART schemes can be examined with concomitant chemotherapy in the future studies. Because of the high incidence of acute toxicity, fraction dose and chemotherapy doses should be designed properly for new studies.

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Comparison between laparoscopic and open surgery for large gastrointestinal stromal tumors: A meta-analysis

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Abstract

AIM

To investigate whether laparoscopic surgery is as safe and feasible as open resection for patients with larger gastrointestinal stromal tumors (GISTs) (≥ 5 cm).

METHODS

A systematic search of PubMed, EMBASE, Web of Science and the Cochrane Library database was performed. Relevant studies of laparoscopic and open surgery for GISTs of > 5 cm published before December 2016 were identified from these databases. The quality of the studies was assessed by the Newcastle-Ottawa Quality Assessment Scale. The tumor size, operation time, blood loss, postoperative hospital stay, complication rate, and disease-free survival rate were assessed. The software Stata (version 12.0) was used for the meta-analysis.

RESULTS

Five clinical trials comprising 209 patients with GISTs of similar larger sizes were evaluated. The pooled analysis of 100 patients in the laparoscopic resection group and 109 patients in the open resection group demonstrated that laparoscopic surgery was significantly associated with a shorter postoperative hospital stay ($P < 0.001$).

and less blood loss ($P = 0.002$). Moreover, there were no statistically significant differences in the operation time ($P = 0.38$), postoperative complication rate ($P = 0.88$), or disease-free survival rate ($P = 0.20$) between two groups.

CONCLUSION

Our findings revealed that for patients with large GISTs of comparable sizes, laparoscopic surgery did not significantly influence the operation factors or clinical outcomes compared with open surgery. This suggests that laparoscopic resection is as acceptable as open surgery for treatment of large gastric GISTs.

Key words: Laparoscopic resection; Open resection; Gastrointestinal stromal tumor; Meta-analysis; Clinical outcome

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Core tip: Whether laparoscopic resection is also effective and feasible for treatment of larger gastric gastrointestinal stromal tumors (GISTs) (> 5 cm) remains unknown. This meta-analysis collected up-to-date clinical data of comparison of laparoscopic and open resection for larger gastric GISTs (> 5 cm). Our results showed that laparoscopic resection is an upgraded minimal invasive technique with a shorter postoperative hospital stay and less intraoperative blood loss compared with open surgery in treating patients with larger GISTs.

Cui JX, Gao YH, Xi HQ, Cai AZ, Zhang KC, Li JY, Wei B, Chen L. Comparison between laparoscopic and open surgery for large gastrointestinal stromal tumors: A meta-analysis. *World J Gastrointest Oncol* 2018; 10(1): 48-55 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i1/48.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i1.48>

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common gastrointestinal sarcomas. They usually arise from the interstitial cells of Cajal and regulate gastrointestinal motility^[1,2]. GISTs are often characterized by cellular markers such as CD117 (a receptor tyrosine kinase protein also known as tyrosine-protein kinase Kit). The stomach is the most prevalent location of GISTs, and the proximal stomach is involved in about two-thirds of suffering patients^[3]. It is well accepted that the malignant potential of GISTs depends on the tumor size, cell mitotic rate, and tumor location^[4].

Although substantial advances have been made in the targeted therapies for these tumors, surgical resection is still the most important component in the treatment of primary GISTs with no evidence of

metastasis. Because wide margins (> 5 cm) and lymph node dissection are not necessary in the surgical management of GISTs^[5], laparoscopic surgery seems to be more suitable for resection of these tumors. Various types of laparoscopic procedures for GISTs have been performed in a few specialized centers, including wedge resection of the stomach, intragastric tumor resection, and combined endoscopic-laparoscopic resection, etc. However, during laparoscopic surgery, these tumors must be handled with great care because rupture of their capsule confers a near 100% risk of recurrence.

Several studies and meta-analyses have shown that laparoscopic resection for gastric GISTs is as safe and efficacious as open surgery; additionally, laparoscopy is associated with less blood loss, less morbidity, and quicker recovery^[6-8]. The long-term survival of patients with GISTs mainly depends on the tumor progression, and laparoscopic surgery does not increase the risk of tumor relapse and metastasis. The clinical practice guidelines for the management of GISTs released by the National Comprehensive Cancer Network and the Japanese Study Group on GIST note that laparoscopic surgical resection is the preferred therapy for relatively small GISTs with a diameter of < 5 cm^[9].

However, most cohort studies have focused on laparoscopic surgery for relatively smaller tumors; few have been designed for evaluation of larger GISTs (> 5 cm)^[10-14]. Although the size limit was not clearly stated, the practice guideline of the European Society for Medical Oncology recommends application of laparoscopic procedures in patients with large GISTs^[15]. However, the complex surgical skills and long learning curve associated with laparoscopic surgery might prevent its application to larger GISTs to some extent^[16]. Therefore, the feasibility and safety of laparoscopic surgery for GISTs of > 5 cm remains unclear. Additionally, whether 5 cm is the most appropriate cutoff for performance of minimally invasive procedures in patients with larger GISTs remains controversial. This meta-analysis was performed to assess the short- and long-term results of patients with larger gastric GISTs (> 5 cm) undergoing laparoscopic surgery.

MATERIALS AND METHODS

Literature search

Systematic electronic searches of PubMed, EMBASE, the Cochrane Library, the Clinical Trials Database, Web of Science, and Google Scholar were performed to identify relevant articles published up to 30 December 2016, utilizing the following search terms: "gastrointestinal stromal tumor," "GIST," "laparoscopic," "laparoscopy," "open resection," "gastrectomy," and "stomach". Citations and references of identified studies were also reviewed for additional literature and trials. The language of the publications was limited to English.

Table 1 Main characteristics of enrolled trials

Ref.	Region	Year	Study design	Study period	Sample size		Tumor size (cm)		CS	Follow-up (mo)
					LAP	Open	LAP	Open		
Kim <i>et al</i> ^[10]	South Korea	2012	OCS (R)	1998-2011	24	14	6.1 ± 1.3	7.2 ± 1.7	0	49.3 (8.4-164.4)
Lin <i>et al</i> ^[11]	China	2014	OCS (R)	2007-2012	23	23	7.2 ± 1.6	7.3 ± 1.5	1	34.0 (6-78)
Hsiao <i>et al</i> ^[12]	Taiwan	2015	OCS (P)	2002-2012	18	37	6.1 ± 1.0	6.0 ± 0.9	0	43.2 (16.8-133.2)
Takahashi <i>et al</i> ^[13]	Japan	2015	OCS (R)	1995-2011	12	15	7.5 ± 1.9	5.5 ± 0.73	3	63 (7-154)
Khoo <i>et al</i> ^[14]	Japan	2016	OCS (R)	2002-2015	23	36	NA	NA	1	45

OCS: Observational clinical study; R: Retrospective study; P: Prospective study; NA: Not available; CS: Convention surgery.

Study selection

The inclusion criteria were as follows: (1) The studies involved patients with gastric GISTs larger than 5 cm; (2) The specific interventions were laparoscopic and open surgical resection; (3) The clinical outcomes were the operation time, intraoperative blood loss, conversion rate, length of hospital stay, adverse events, and long-term outcomes (overall survival, disease-specific survival, or recurrence rate); (4) Controlled studies (randomized controlled trials, cohort studies, and case-control studies) were included for the pooled analysis. However, case reports and case series were included for the systematic review; and (5) The informative data and full text of the articles were available.

The exclusion criteria were as follows: (1) The patients had GISTs that were located outside of the stomach or complicated with mixed disease; (2) Duplicate publications; (3) the size of the GIST was not specifically stated; (4) The article was a case report or review; and (5) The publication was in a language other than English.

Data extraction and management

Two reviewers independently screened the titles and abstracts of the publications. Once deemed acceptable, the whole manuscripts were obtained and screened. Controversial issues were resolved by discussion or referred to a third reviewer. Another two reviewers independently extracted the data using a unified form and resolved any discrepancies through discussion. The variables of interest included the author, study period, number of patients, tumor size, operation time, blood loss, length of postoperative hospital stay, complication rate, and long-term outcome (namely disease-free survival). In addition, if the original studies included the median, range, and size of a sample, we estimated the mean and variance using the methods described by Hozo *et al*^[12].

The quality of the included papers was assessed using the Newcastle-Ottawa Quality Assessment Scale^[17]. This scale ranges from 0 to 9 points; studies with a score of ≥ 6 were considered methodologically sound.

Statistical analysis

The meta-analysis was performed using weighted

mean differences (WMDs) for continuous variables, odds ratios for dichotomous variables, and hazard ratios for time-to-event variables. Statistical heterogeneity was assessed by performing χ^2 tests and calculating the Higgins I^2 statistic, and a value of $P < 0.10$ or $I^2 > 50\%$, indicated statistical significance. A fixed-effects model was generally employed. If the heterogeneity was statistically significant, a random-effects model was adopted. Publication bias was evaluated by Begg's test. A P value of < 0.05 was considered significant. Statistical analyses were performed using Stata software (version 12.0; StataCorp, College Station, TX, United States).

RESULTS

Enrolled studies and quality assessment

No eligible randomized controlled trials were identified, but 5 nonrandomized trials were analyzed (209 patients with GISTs of similar size). Overall, 100 patients underwent laparoscopic resection and 100 underwent open resection. A flow chart of the search strategy is illustrated in Figure 1. The main characteristics and quality assessment results of the included studies are shown in Tables 1 and 2, respectively.

Tumor size

Four studies reported no statistically significant differences in tumor size between the laparoscopy and open group, while Kim *et al*^[10] reported that the tumor size in the open group was significantly larger than that in the laparoscopy group. Additionally, in the pooled data from a fixed-effects model with no significant heterogeneity ($I^2 = 53.3\%$, $P = 0.073$) (Table 3), no significant difference was identified in the total analysis [WMD = -0.038 cm, 95% confidence interval (95%CI): -0.699 to 0.362, $P = 0.632$] (Figure 2).

Operative factors

All enrolled studies provided data for analysis of the operation time. The results showed no significant difference between the two groups (WMD = 7.17 min, 95%CI: -56.02 to 70.36, $P = 0.824$) (Figure 3A). Because obvious heterogeneity was detected ($I^2 = 92.9\%$, $P = 0.000$) (Table 3), a random-effects model was employed.

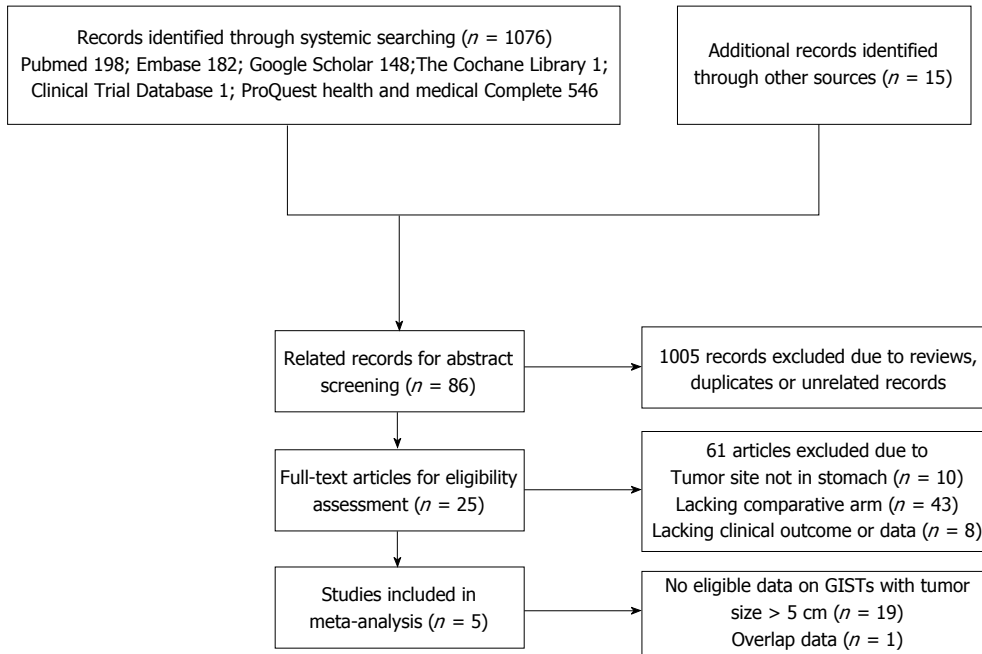


Figure 1 Flow chart of study selection process. GISTs: Gastrointestinal stromal tumors.

Table 2 Newcastle-Ottawa Scale Assessment of enrolled studies

Ref.	Selection (0-4)				Comparability		Outcome (0-3)			Total
	REC	SNEC	AE	OINP	SCB	SCA	AO	FU	AFC	
Kim <i>et al</i> ^[10]	1	1	1	1	1	0	1	1	1	8
Lin <i>et al</i> ^[11]	1	1	1	1	1	1	1	1	0	8
Hsiao <i>et al</i> ^[12]	1	1	1	1	1	0	1	1	1	8
Takahashi <i>et al</i> ^[13]	1	1	1	0	1	0	1	1	1	7
Khoo <i>et al</i> ^[14]	1	1	1	1	1	1	1	1	1	9

REC: Representativeness of the exposed cohort; SNEC: Selection of the no exposed cohort; AE: Ascertainment of exposure; OINP: Outcome of interest not presented in the start of study; SCB: Study controls for basic characteristics; SCA: Study controls for additional factor; AO: Assessment of outcome; FU: Follow-up; AFC: Adequacy of follow up.

Table 3 Summary results of meta-analysis of clinical outcomes

Outcomes	No. of studies	Effect value	95%CI of effect	Heterogeneity	
				I^2 (%)	P value
Tumor size	4	WMD = -0.0.38	-0.699 to 0.362	53.3	0.073
Operation time	5	WMD = 7.17 min	-56.02 to 70.36	92.9	0.000
Blood loss	4	WMD = -47.47 mL	-93.20 to -1.73	63.2	0.043
Postoperative complications	5	OR = 0.93	0.34 to 2.50	0.0	0.858
Postoperative stay	5	WMD = -2.81 d	-3.68 to -1.94	38.7	0.163
Progression-free survival	5	HR = 0.64	0.35 to 1.19	0.0	0.553

WMD: Weighted mean differences.

Four studies reported data regarding intraoperative blood loss; Lin *et al*^[11] reported that laparoscopic surgery was associated with less blood loss. The heterogeneity between the studies was significant ($I^2 = 63.2\%$, $P = 0.043$); therefore, the analysis was performed with a random-effects model. In the pooled data, a significant difference was found among these three groups (WMD = -47.47 mL, 95%CI: -93.20 to -1.73 mL, $P = 0.042$) (Figure 3B).

Among all enrolled studies, five patients in the

laparoscopy group reportedly underwent conversion to open surgery. One conversion resulted from the surgeons' initial learning curve for laparoscopy, one was due to dense adhesion to liver, and the other three occurred because of failure to secure the tumor in the visual field of the laparoscope.

Short-term outcomes

All five studies reported postoperative complications. The pooled data revealed no significant difference

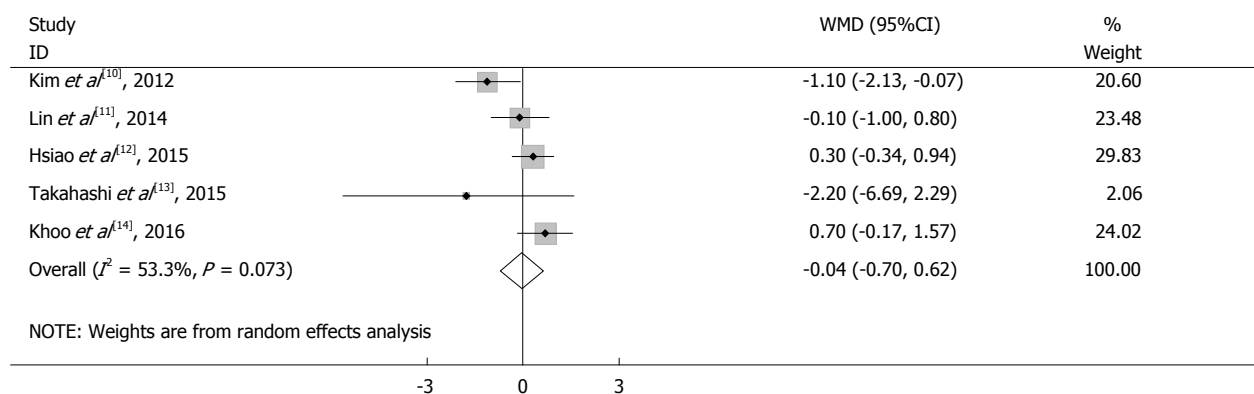


Figure 2 Meta-analysis of tumor size in laparoscopic surgery and open surgery groups.

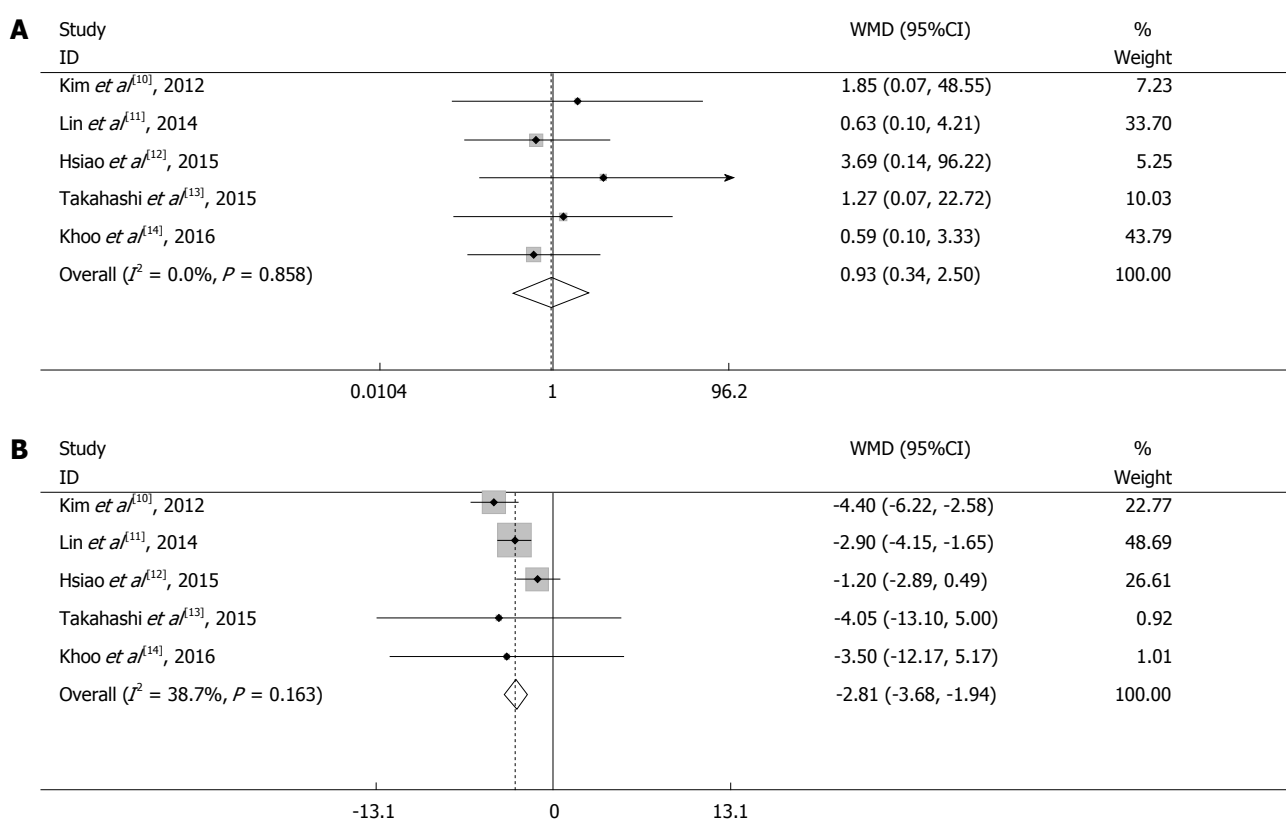


Figure 3 Meta-analysis of operative factors in laparoscopic surgery and open surgery group. A: Pooled analysis of operation time; B: Pooled analysis of blood loss.

between the two groups (odds ratio = 0.93, 95%CI: 0.34 to 2.50, $P = 0.88$) (Figure 4A). A fixed-effects model was used because of the lack of significant heterogeneity ($I^2 = 0.0\%$, $P = 0.858$).

Five studies reported data regarding the postoperative hospital stay. A fixed-effects model was employed because of insignificant heterogeneity ($I^2 = 38.7\%$, $P = 0.163$). The postoperative hospital stay was significantly shorter in the laparoscopy than open group (WMD = -2.81 d, 95%CI: -3.68 to -1.94, $P < 0.001$) (Figure 4B).

Long-term outcomes

All eligible studies reported the progression-free survival of patients. Figure 5 shows a forest plot of

disease-free survival and the results of the meta-analysis. No significant difference was observed in patients with larger GISTs who underwent laparoscopic vs open surgery (hazard ratio = 0.64, 95%CI: 0.35 to 1.19, $P = 0.157$). No obvious heterogeneity was observed in this study; therefore, a fixed-effects model was applied in the survival meta-analysis ($I^2 = 0.0\%$, $P = 0.553$) (Figure 5).

Publication bias

Publication bias was evaluated based on the postoperative hospital stay using Begg's and Egger's tests. No publication bias was identified in the five studies (Begg's test, $P = 0.773$; Egger's test, $P = 0.825$) (Figure 6).

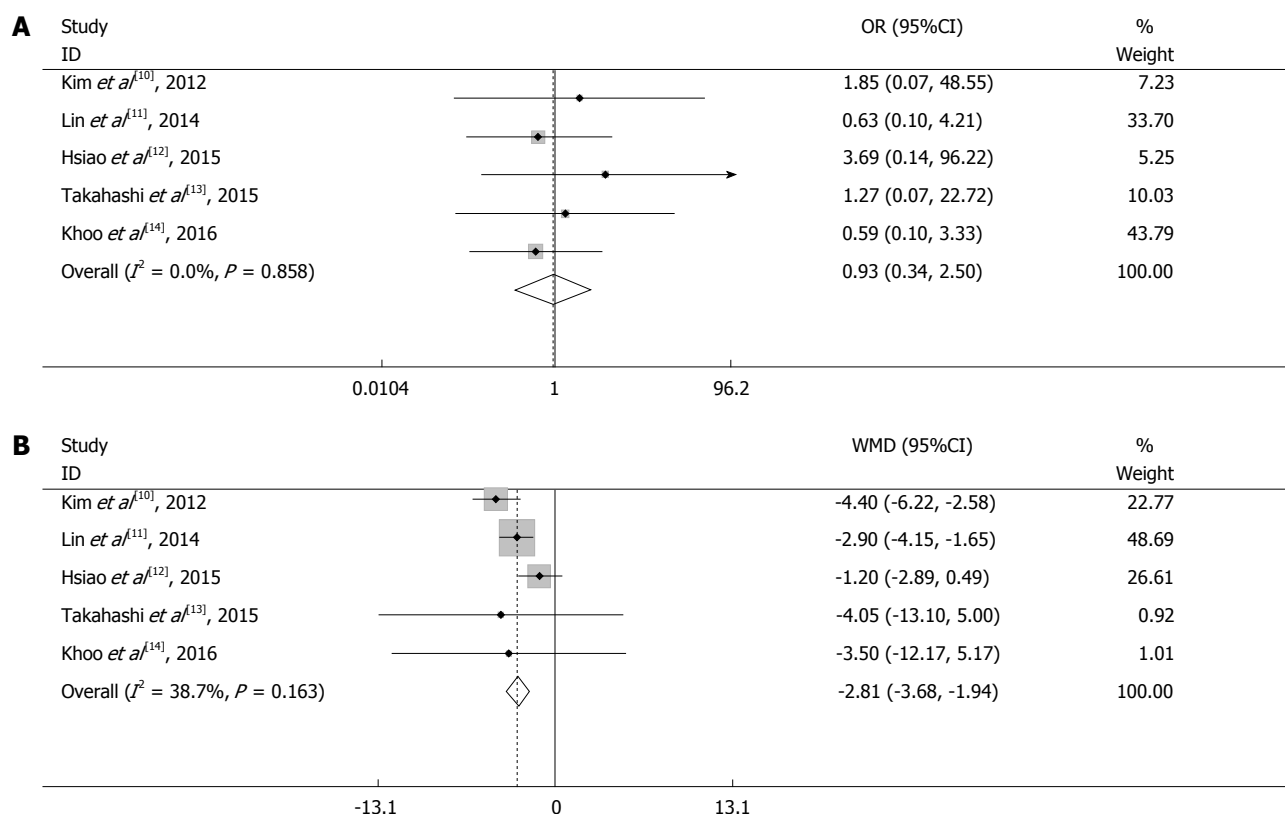


Figure 4 Meta-analysis of short-term outcomes in laparoscopic surgery and open surgery groups. A: Pooled analysis of postoperative complications; B: Pooled analysis of postoperative hospital stay.

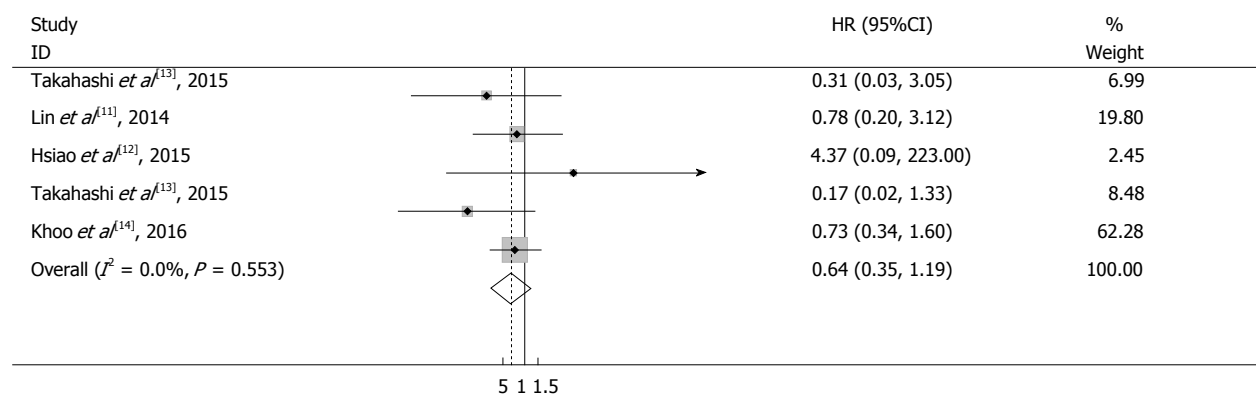


Figure 5 Meta-analysis of progression-free survival in laparoscopic surgery and open surgery groups.

DISCUSSION

Recent studies have suggested that the prognosis of GISTs is mainly based on the tumor size and histological features rather than achievement of wide resection margins^[18]. Therefore, laparoscopic resection is more frequently performed for treatment of patients with GISTs using the advances currently being made in surgical techniques.

Although randomized controlled trials are the first choice for high-quality meta-analyses, we failed to enroll any randomized controlled trials in this study. There are several obstacles to design and perform randomized controlled trials, such as ethical issues and organization

difficulty^[19]. Finally, five nonrandomized controlled studies (one prospective and four retrospective) were enrolled; all were assessed according to the Newcastle-Ottawa Quality Assessment Scale and scored > 6, ensuring their high quality.

Our pooled analysis demonstrated faster recovery and less blood loss in the laparoscopy than open surgery group. Less trauma caused by laparoscopic surgical intervention, only a mild acute inflammatory response, and earlier postoperative activities are considered to contribute to the shorter postoperative hospital stay. Although the blood loss volume might have varied according to the different methods used among the studies, the results of our work indicate

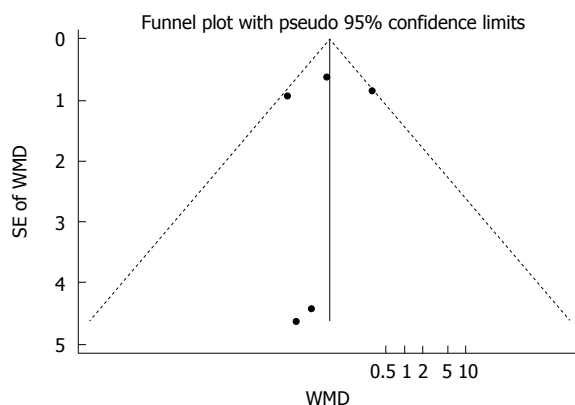


Figure 6 Funnel plot of postoperative hospital stay in laparoscopic surgery and open surgery groups. WMD: Weighted mean difference.

that laparoscopic surgery might reduce patients' surgical trauma to some extent. Furthermore, there was no difference in the postoperative complications between the two groups, adding to the safety of laparoscopic surgery in patients with larger GISTs.

Our review also indicated that laparoscopic resection for larger GISTs is feasible with a conversion rate of 5%, which is similar to other laparoscopic procedures such as laparoscopic gastrectomy^[20,21]. The oncological outcome is one of the most concerning problems that prevents application of laparoscopy to the surgical treatment of larger GISTs^[22]. Our results showed no difference in the disease-free survival of patients with larger GISTs who underwent laparoscopy vs open surgery (hazard ratio = 0.643, 95%CI: 0.349 to 1.185, $P = 0.157$), suggesting that the performance of a laparoscopic procedure does not profoundly influence the oncological outcome compared with open surgery.

Several limitations in our study should be addressed. First, the limited number of patients might affect the reliability of the results (209 patients across 5 studies). Second, most of the patients' tumor sizes ranged from 5 to 10 cm; therefore, the results might not be suitable for patients with GISTs of > 10 cm. Third, treatment of larger GISTs in laparoscopic surgery requires greater surgical skill to prevent tumor rupture and gain adequate resection margins. Therefore, the inclusion of single-center studies with various levels of surgical techniques might have contributed to the bias of our meta-analysis. Finally, the use of different risk classifications and drug therapies within the groups might have also contributed to the bias of recurrence or progression-free survival^[23].

In conclusion, this meta-analysis has demonstrated that laparoscopic surgery is as safe and feasible as open surgery for resection of larger GISTs (> 5 cm, mainly 5-10 cm). Moreover, laparoscopic surgery might offer the advantage of faster recovery and less trauma over open surgery in patients with GISTs. More multicenter randomized controlled clinical trials are needed to clarify and confirm the role of laparoscopic

surgery in patients with larger GISTs.

ARTICLE HIGHLIGHTS

Research background

Laparoscopic resection of relatively small gastric gastrointestinal stromal tumors (GISTs) is currently well-accepted and has been proven as safe and feasible as traditional open surgery. However, whether laparoscopic resection is also effective and feasible for treatment of larger gastric GISTs (> 5 cm) remains unknown.

Research motivation

The authors aimed to explore whether laparoscopic resection is also effective and feasible for treatment of larger gastric GISTs (> 5 cm), just as the same situation in smaller GISTs.

Research objectives

Laparoscopic resection for small GISTs is now well-accepted. However, whether laparoscopic surgery is as safe and feasible as open resection for patients with larger GISTs (≥ 5 cm) remains controversial.

Research methods

A systematic search of PubMed, EMBASE, Web of Science and the Cochrane Library database was performed. Relevant studies of laparoscopic and open surgery for GISTs of > 5 cm published before December 2016 were identified from these databases. The meta-analysis was performed using Stata (version 12.0) applying weighted mean differences for continuous variables, odds ratios for dichotomous variables, and hazard ratios for time-to-event variables.

Research results

In terms of operative and oncological factors, our research demonstrated that laparoscopic surgery was significantly associated with a shorter postoperative hospital stay ($P < 0.001$) and less blood loss ($P = 0.002$) in resecting larger GISTs. Moreover, there were no statistically significant differences in the operation time ($P = 0.38$), postoperative complication rate ($P = 0.88$), or disease-free survival rate ($P = 0.20$) between two groups.

Research conclusion

This research stands as the first meta-analysis focusing on this specific type of GISTs. The meta-analysis has demonstrated that laparoscopic surgery is as safe and feasible as open surgery for resection of larger GISTs (> 5 cm, mainly 5-10 cm). Moreover, laparoscopic surgery might offer the advantage of faster recovery and less trauma over open surgery in patients with GISTs.

Research perspectives

Laparoscopic resection is as acceptable as open surgery for treatment of large gastric GISTs.

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Leptomeningeal metastases originated from esophagogastric junction/gastric cancer: A brief report of two cases

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Abstract

Leptomeningeal carcinomatosis is a very rare manifestation in patients diagnosed with esophagogastric junction and gastric cancer. Its prognosis is ominous and therapy outcomes are disappointing. Herein, we present two patients; one initially diagnosed with gastric cancer and leptomeningeal carcinomatosis but no other evidence of metastatic disease and the other one initially diagnosed with esophagogastric junction cancer, who recurred solitary with leptomeningeal seedings several years after the initial diagnosis and treatment. Furthermore, a thorough and short review of the literature is carried out.

Key words: Esophagogastric junction cancer; Gastric cancer; Leptomeningeal carcinomatosis; Prognosis; Investigation; Therapy

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Core tip: Leptomeningeal carcinomatosis (LMC) is related with ominous prognosis and the median survival varies between a few weeks to months. Even LMC is extremely rare in patients diagnosed with esophagogastric junction and gastric cancer, physicians should be alerted when neurological symptoms occurred, are persistent and could not be explained. A single diagnosis test procedure itself is not absolutely sensitive and the investigation algorithm may comprise a gadolinium enhanced brain magnetic resonance imaging and cerebrospinal fluid cytology tests.

Kountourakis P, Papamichael D, Haralambous H, Michael M, Nakos G, Lazaridou S, Fotiou E, Vassiliou V, Andreopoulos D. Leptomeningeal metastases originated from esophagogastric junction/gastric cancer: A brief report of two cases. *World J Gastrointest Oncol* 2018; 10(1): 56-61 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i1/56.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i1.56>

INTRODUCTION

Esophagogastric junction (EGJ) and gastric cancer (GC) constitute a major health issue worldwide and are often diagnosed in advanced stage with dismal prognosis^[1]. Leptomeningeal carcinomatosis (LMC) is defined as cancerous infiltration of the arachnoid membrane and the pia mater with devastating prognosis. Several routes of spread to meninges have been suggested; direct infiltration from bone metastases, *via* arteries and lymphatics, perineural and perivascular spaces, retrograde flow of Batson's venous plexus^[2]. Among cancer patients, it is most often related to breast and lung tumors, melanoma, leukemia and lymphoma^[3]. Only a few cases of EGJC and GC patients have been reported with LMC as an upfront disease manifestation. Most of the cases are presented several months or years after the initial diagnosis with synchronous diffuse disease spread. It is reported a 0.16%-0.69% of GC cases with LMC diagnosis, meanwhile it is clinically diagnosed in 2%-4% of all cancer patients^[4,5]. Herein, we present two patients with LMC and primary site diagnosis of EGJ and GC, respectively (Table 1).

CASE REPORT

Case 1

A 64-year-old female was referred to our Centre in September 2015. Her disease symptoms started almost 3 wk before with severe episodes of headache, dizziness visual and hearing loss (mainly right). From her past medical and family history nothing important to be mentioned. Initially, she was investigated by a computed tomography (CT) brain scan (Figure 1A) with no evidence of suspicious findings and pain

Table 1 Characteristics of patients diagnosed with leptomeningeal carcinomatosis

Characteristics	Case 1	Case 2
Age (yr)	64	57
Sex	Female	Male
Primary neoplasm	Stomach	EGJ
Histology	Adenocarcinoma gr III, signet ring cells	Adenocarcinoma gr III, signet ring cells
Disease status	Initial diagnosis	Recurrence
Systemic disease	None other than LMC	None other than LMC
Main neurological symptoms	Headache, dizziness, visual and hearing loss	Headache, dysphasia, temporary left side paresis
CSF cytology	Positive	Positive
Brain imaging studies	CT: No findings MRI: Positive	CT: No findings MRI: Positive

EGJ: Esophagogastric junction; LMC: Leptomeningeal carcinomatosis; CSF: Cerebrospinal fluid; CT: Computed tomography; MRI: Magnetic resonance imaging.

killers were administrated with no benefit. Afterwards, steroids were administrated empirically with initial improvement of symptoms for a few days. Due to deterioration, further investigation by brain magnetic resonance imaging (MRI) revealed diffuse meningeal enhancement (Figure 1B). It was extended also into the internal auditory canal and optic nerves sheaths more into the right side. CT chest, abdomen, pelvis scans revealed only thickness in the area of gastric cardia and further investigation by an upper GI endoscopy confirmed the diagnosis of a poorly differentiated gastric adenocarcinoma with signet ring features (Figure 2). Cerebrospinal fluid cytology (CSF) confirmed the diagnosis of LMC (Figure 3). Full blood count and biochemistry tests were within normal values and tumor markers' evaluation revealed CEA = 33.3 ng/mL. Her clinical status deteriorated rapidly, was in coma, and the other day of IT with Methotrexate (MTX, 12.5 mg) the patient died, approximately within 4 wk after the initial onset of disease symptoms.

Case 2

A 51-year-old man was diagnosed with an EGJ poorly differentiated adenocarcinoma (cT3N+, M0) in July 2009. He was therefore commenced on peri-operative chemotherapy with epirubicin, cisplatin and capecitabine regimen and on 02/11/2009 he was operated (Ivor-Lewis gastrectomy, ypT2N1, R0, gr III adenocarcinoma with signet ring features, Figure 4). He remained disease-free until September 2015 when he experienced neurological symptoms such as dysphasia, headaches and temporary left side paresis. Initially, investigations by CT brain (Figure 5A), chest, abdomen, pelvis scans revealed no evidence of disease. Subsequently, a brain MRI scan revealed findings of LMC (Figure 5B). A CSF cytology investigation confirmed the diagnosis consistent with GC origin (Figure 6), meanwhile a

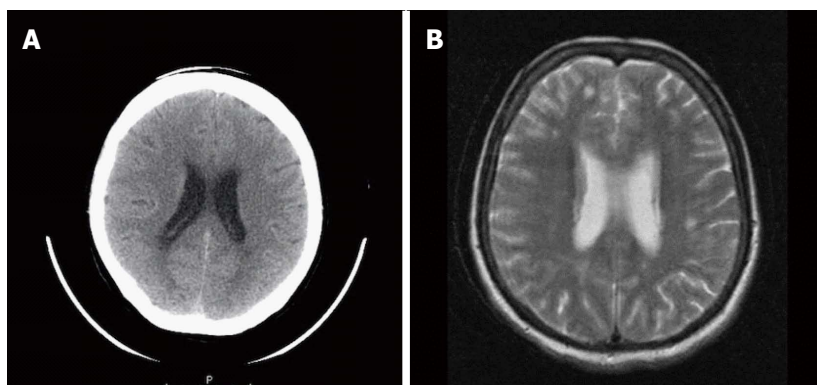


Figure 1 Patient No. 1. A: Computed tomography brain did not reveal brain lesions; B: Magnetic resonance imaging brain showed findings consistent with leptomeningeal carcinomatosis.

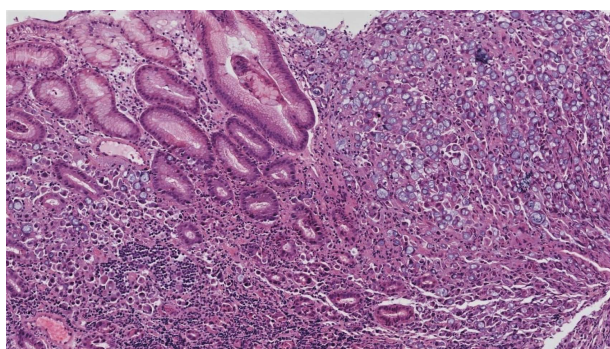


Figure 2 Patient No. 1. Diffuse infiltration of gastric mucosa from a poorly differentiated poorly cohesive gastric adenocarcinoma (including mixed adenocarcinoma with > 50% signet ring cells features (HE 100 x).

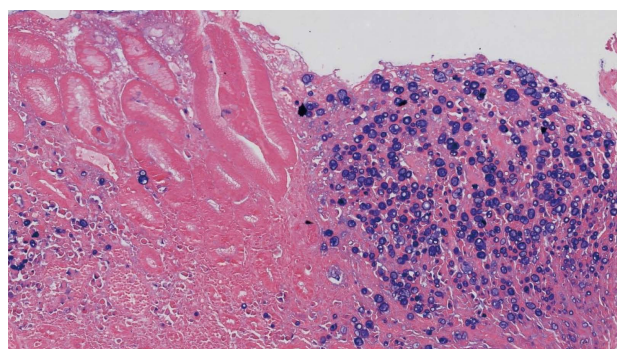


Figure 4 Patient No. 2. Alcian blue highlights difference in mucin production between cancer cells (blue) and normal gastric tissue (no presence), helping us also determine about the extent of the infiltration (Alcian blue 200 x).

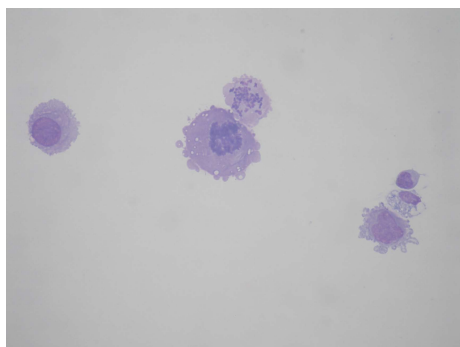


Figure 3 Patient No. 1. Four atypical cells, one lymphocyte and one macrophages next to the lymphocyte. Atypical cells are isolated, two of those show mitotic activity. The size of atypical cells and lymphocyte could be compared (Hemacolor 40 x).

flow cytometric test confirmed the presence of a non hematopoietic cell population. Full blood count and biochemistry tests were within normal values and tumor markers' evaluation revealed CEA = 6.3 ng/mL, CA 19-9 = 98.1 µg/mL. He was treated with intrathecal MTX (12.5 mg) twice-weekly with relatively good clinical response initially. After three weeks of treatment he was put on "maintenance" application once weekly, but unfortunately his performance status was rapidly deteriorated after a couple of

weeks. Therefore, his treatment was changed to MTX, Cytosine Arabinoside (40 mg) and Dexamethasone (4 mg). He received 3 and 5 applications with good clinical response, in October and in December 2009, respectively. Afterwards, the CT chest-abdomen-pelvis re-staging scans revealed no clear evidence of local recurrence or metastatic disease, meanwhile the MRI brain performed revealed slight improvement of meningeal enhancement. In July 2016, his performance status deteriorated with severe episodes of headaches, dizziness, dysphasia, visual and hearing loss and consciousness deduction. He was re-challenged with IT but with modest improvement and died in September 2016.

DISCUSSION

Leptomeningeal carcinomatosis is often presented with non specific clinical symptoms like headache, nausea and vomiting. Supratentorial involvement could cause altered mental and personality status, dysphasia and seizures. When infratentorial lesions occurred, they are mainly presented with cranial nerve palsies and related symptoms^[6,7]. The gadolinium enhanced MRI and CSF are the main investigation procedures. The sensitivity and specificity of the brain MRI are

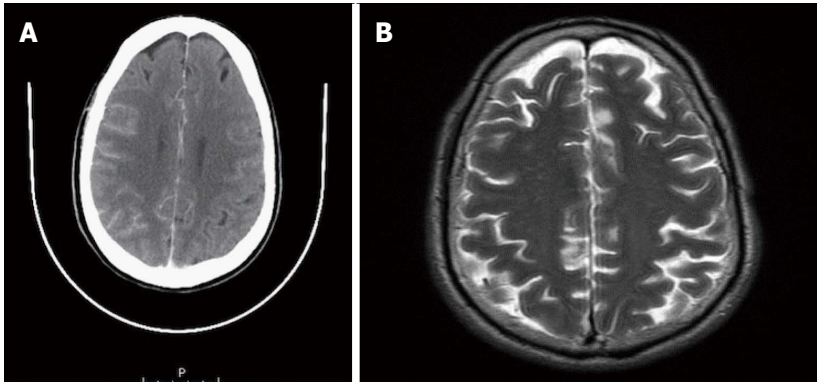


Figure 5 Patient No. 2. A: Computed tomography brain did not reveal brain lesions; B: Magnetic resonance imaging brain showed findings consistent with leptomeningeal carcinomatosis.

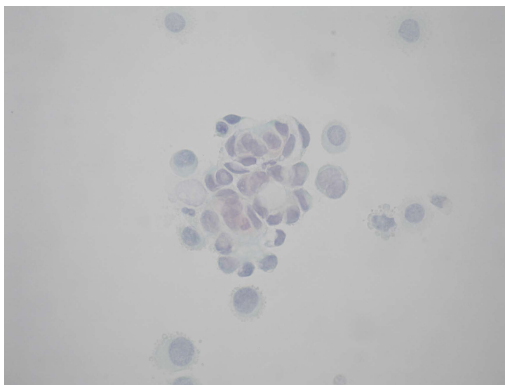


Figure 6 Patient No. 2. An irregular cluster of atypical cells. The cells show intermediate size, degeneration changes, indistinct cytoplasmic borders and moderate size of nuclei. Nuclear/cytoplasmic ratio is increased (Papanicolaou 40 ×).

66%-76% and 75%-77%, respectively, but could be almost two times higher than those of the CT^[8-10]. Approximately in 67% of cases imaging studies reveal findings such as focal or diffuse abnormal meningeal enhancement and nodules detection^[11]. Moreover, meningeal enhancement is suggestive but does not confirm the diagnosis. Infection or inflammatory causes, intracranial hypertension but even a lumbar puncture procedure before MRI scan could induce diffuse meningeal enhancement for a period of weeks to months and give false positive results^[12]. After first sampling of CSF examination the possibility of a positive result is approximately 54%. This raises to 91% after multiple cytology investigations^[6]. On the contrary, a negative cytology investigation after three lumbar punctures does not rule out the diagnosis of LMC in the context of other positive tests, like an MRI with characteristic findings.

Reviewing the literature, it is obvious that this is a rare manifestation. In a retrospective analysis of medical records from Memorial Sloan-Kettering Cancer Center of consecutive 90 patients with LMC treated from 1975 to 1981, none was diagnosed with EGJ/GC^[6]. From MD Anderson cancer Center, between 1985

to 2001 in more than 1500 EGJ/GC patients, eight cases with leptomeningeal seedings were identified^[13]. Furthermore, in a Korean retrospective multicenter analysis conducted between 1995 to 2007, 54 patients were identified with LMC from a total 22154 GC patients^[14].

There is no doubt that LMC is related with ominous prognosis. Treatment goals initially focus to improvement of neurological symptoms and quality of life and secondarily to prolongation of survival. In the meantime, median survival varies between a few weeks to months. Current treatment approaches may be IT, systemic therapy and or cranio-spinal radiation therapy (RT) but the results of these approaches are disappointing and a lot of times confusing and conflicting. There are no robust data from multicenter prospective studies to support the superiority of IT vs best supportive care. A study compared MTX/ Ara-C/hydrocortisone combination vs single agent MTX. Primary sites were the lung ($n = 33$), breast ($n = 13$) and stomach ($n = 5$). Superiority was revealed for the combination arm for median overall survival (18.6 vs 10.4 wk, $P = 0.02$) and cytology negative conversion (38.5% vs 13 %, $P = 0.03$), respectively^[15].

Moreover, a prospective study provided no benefit of IT added to systemic treatment and RT. The first group consisted of 54 patients treated with RT, IT and systemic therapy vs 50 patients treated with RT and systemic chemotherapy. There was no differences in median survival (4 mo) and long term survivors^[16]. It should be underlined, that in both groups approximately 60% of patients were diagnosed with breast cancer, 15% with lung cancer and the other 25% with various other types of cancer (the percentage of EGJ/GC cases is not clarified, if any). It should be also stated that the aforementioned studies reviewed, reflect various solid tumors with highly variable prognosis, including breast cancer which has a more indolent history, and the regimens of IT chemotherapy and RT administered are not distinguished based on the various types of solid tumors.

In addition, a multicenter retrospective analysis of patients with GC and LMC revealed no evidence of an additional effect of cranio-spinal RT to IT^[14]. Due to the fact that in most cases blood brain barrier is destroyed and LMC is related with highly permeable blood vessels in vascularized tumors, it could be also speculated that systemic therapy may be effective. An experimental model supports this hypothesis^[17].

In conclusion, even LMC is rare in patients diagnosed with EGJC and GC, physicians should be alerted when neurological symptoms occurred, are persistent and could not be explained. Furthermore, a single diagnosis test procedure itself is not absolutely sensitive and the investigation algorithm should comprise a gadolinium enhanced brain MRI and repeated CSF cytology tests. Unfortunately, disease prognosis is dismal and newly developed targeted drugs with improved CNS penetration and better outcomes remains a priority.

ARTICLE HIGHLIGHTS

Case characteristics

Two patients are presented. Both of them were presented with severe neurological symptoms and their further investigation revealed the initial diagnosis and the recurrence of gastric cancer (GC)/esophagogastric junction cancer (EGJC), respectively.

Clinical diagnosis

Two cases are presented. One initially diagnosed with GC and leptomeningeal carcinomatosis (LMC) but no other evidence of metastatic disease and the other one initially diagnosed with EGJC, who recurred solitary with leptomeningeal seedings several years after the initial diagnosis and treatment.

Differential diagnosis

Meningeal enhancement is suggestive but does not confirm the diagnosis. Infection or inflammatory causes, intracranial hypertension but even a lumbar puncture procedure before magnetic resonance imaging (MRI) scan could induce diffuse meningeal enhancement give false positive results.

Laboratory diagnosis

Elevation of CEA levels were reported in both patients and mild elevation of CA19-9 was reported in patient with EGJC recurrence.

Imaging diagnosis

Brain MRI images revealed diffuse meningeal enhancement consistent with LMC.

Pathological diagnosis

CSF cytology confirmed the diagnosis of LMC in both patients.

Treatment

Chemotherapy, IT therapy.

Related reports

Only a few cases of EGJC and GC patients have been reported with LMC as an upfront disease manifestation or as solitary disease recurrence.

Term explanation

EGJC/GC are diseases with high malignant potential.

Experiences and lessons

Even LMC is extremely rare in patients diagnosed with EGJC and GC,

physicians should be alerted when neurological symptoms occurred, are persistent and could not be explained.

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ORIGINAL ARTICLE

Retrospective Study

- 62** Preliminary study of automatic gastric cancer risk classification from photofluorography
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Retrospective Study

Preliminary study of automatic gastric cancer risk classification from photofluorography

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Abstract**AIM**

To perform automatic gastric cancer risk classification

using photofluorography for realizing effective mass screening as a preliminary study.

METHODS

We used data for 2100 subjects including X-ray images, pepsinogen I and II levels, PG I /PG II ratio, *Helicobacter pylori* (*H. pylori*) antibody, *H. pylori* eradication history and interview sheets. We performed two-stage classification with our system. In the first stage, *H. pylori* infection status classification was performed, and *H. pylori*-infected subjects were automatically detected. In the second stage, we performed atrophic level classification to validate the effectiveness of our system.

RESULTS

Sensitivity, specificity and Youden index (YI) of *H. pylori* infection status classification were 0.884, 0.895 and 0.779, respectively, in the first stage. In the second stage, sensitivity, specificity and YI of atrophic level classification for *H. pylori*-infected subjects were 0.777, 0.824 and 0.601, respectively.

CONCLUSION

Although further improvements of the system are needed, experimental results indicated the effectiveness of machine learning techniques for estimation of gastric cancer risk.

Key words: Gastric cancer; *Helicobacter pylori*; Mass screening; Photofluorography; Automatic data processing

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Core tip: We developed an automatic gastric cancer risk classification system that analyzes X-ray images as a preliminary study. To evaluate the effectiveness of our system, we performed a retrospective analysis of patients who underwent photofluorography and ABC (D) stratification by blood inspection. From the experimental results, we found that machine learning techniques might have a potential for extracting additional gastric cancer risk information. The collaborative use of image-based risk information and ABC (D) stratification will provide more reliable gastric cancer risk information.

Togo R, Ishihara K, Mabe K, Oizumi H, Ogawa T, Kato M, Sakamoto N, Nakajima S, Asaka M, Haseyama M. Preliminary study of automatic gastric cancer risk classification from photofluorography. *World J Gastrointest Oncol* 2018; 10(2): 62-70 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i2/62.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i2.62>

INTRODUCTION

Gastric cancer remains the third leading cause of cancer mortality in the world, and East Asian countries, including China, South Korea and Japan, have the

highest mortality rates^[1,2]. In Japan, the number of gastric cancer-related deaths each year is approximately 50000, and there has been no change over the past several decades.

Many studies on gastric cancer have been carried out, and epidemiological studies have revealed that *Helicobacter pylori* (*H. pylori*) infection is a main cause of gastric cancer^[3,4]. Consequently, in 1994, the International Agency for Research on Cancer (IARC) at the World Health Organization (WHO) declared that *H. pylori* infection can be classified as a group I carcinogen^[5]. An animal experiment using Mongolian gerbils^[6] and a prospective cohort study by Uemura *et al.*^[7] indicated that the main cause of gastric cancer is *H. pylori* infection. It has also been reported that about half of the world's population is infected with *H. pylori* and that its prevalence is highly variable depending on age, geography and economic factors^[8]. Although auto-immunization, drug-induced suffering and infectious diseases can cause gastritis and/or gastric cancer, most cases are due to *H. pylori* infection^[9,10]. In Japan, the incidence of *H. pylori*-negative gastric cancer was reported to be 0.3%-0.6%^[11,12], and almost all cases of gastric cancer are derived from *H. pylori*-induced gastritis. Moreover, *H. pylori* infection rates in Japan differ according to the year of birth, and generations born in the 1970s or later have extremely low infection rates^[13]. Meanwhile, recent studies have shown that *H. pylori* eradication therapy reduces the risk for development of gastric cancer^[14,15]. *H. pylori* eradication therapy for *H. pylori*-infected patients with gastritis has been covered by national health insurance since February 2013 in Japan, the first country in the world to do so. Hence, mass screening methods with consideration of gastric cancer risk are required^[16,17].

ABC (D) stratification combining serum pepsinogen (PG) and *H. pylori* antibody has gradually been introduced for evaluation of gastric cancer risk^[18]. It has been reported that the combination of these serum markers is effective for evaluating pre-malignant conditions of the gastric mucosa^[19]. Since pre-malignant stages of atrophic gastritis, intestinal metaplasia and dysplasia, which can be detected from serum markers, lead to gastric adenocarcinoma, ABC (D) stratification is expected to become a new standard non-invasive inspection method for evaluation of gastric cancer risk^[20]. On the other hand, the effectiveness of photofluorography and endoscopy for gastric cancer mass screening has also been evaluated. Hence, evaluation of gastric cancer risk from clinical image data is a crucial issue for the mass screening.

Recently, it has been reported that ABC (D) stratification and radiological findings of photofluorography have a good correlation with gastric cancer risk^[21]. Since the main cause of gastric cancer and its risk factors have been clarified, a diagnostic technique for gastric cancer risk and/or *H. pylori* infection from photofluorography would play an important role in risk-based mass

screening^[22,23].

In this study, we performed a preliminary investigation of automatic gastric cancer risk classification using photofluorography for realizing effective risk-based mass screening.

MATERIALS AND METHODS

We performed a preliminary study for classification of gastric cancer risk from photofluorography. Then we developed an automatic risk classification system utilizing machine learning techniques for achieving our objective.

Study subjects

Data for X-ray images (8-bit gray scale, 1024 × 1024 pixels), *H. pylori* antibody, pepsinogen I (PG I) level, pepsinogen II (PG II) level, PG I/PG II ratio, *H. pylori* eradication history and interview sheets were used in this study. These data were acquired at the Medical Examination Center of Yamagata City Medical Association that specializes in gastric cancer mass screening from April 2012 to March 2013. We used X-ray images of eight positions for each subject. *H. pylori* antibody titers were measured by enzyme-linked immunosorbent assay kits (E Plate Eiken *H. pylori*, Eiken Chemical Co., Ltd., Tokyo, Japan). PG I level and PG II level were measured by Auto pepsinogen I BML-2G and Auto pepsinogen II BML-2 (BML, Inc., Ltd., Saitama, Japan), respectively. The cut-off value of *H. pylori* antibody titers was 10 U/mL, and the cut-off values of PG levels were PG I < 70 ng/mL and PG I/PG II ratio < 3. Subjects in whom these serum markers were measured were categorized into three or four groups corresponding to their gastric cancer risk as shown in Table 1. In ABC (D) stratification, group A is defined as a very low gastric cancer risk group, group B is defined as a middle-risk group, and groups C and D are defined as high-risk groups, with group D generally being included in group C^[21].

Automatic gastric cancer risk classification system

We developed an automatic gastric cancer risk classification system for identification of *H. pylori* infection status and atrophic level from photofluorography. In the first stage, *H. pylori* infection status classification was performed. In the second stage, atrophic level classification was applied to *H. pylori*-infected subjects. First, for gastric cancer risk classification, we derived image features from X-ray images for representing changes inside the stomach caused by *H. pylori* infection. In training procedures, we calculated more efficient image features that had high correlations with values of *H. pylori* antibody and serum markers. Specifically, we obtained new image features by projecting the original image features to a space that provided high correlations with values of PG levels and *H. pylori* antibody titers via Kernel Canonical Correlation

Table 1 ABC (D) stratification

	A	B	C (D)
<i>H. pylori</i> antibody level	-	+	+ (-)
PG levels	-	-	+

Patients with *H. pylori* antibody level ≥ 10 U/mL were classified as (+) and patients with PG I ≤ 70 ng/mL and PG I/PG II ratio ≤ 3 were classified as (+). *H. pylori*: *Helicobacter pylori*.

Analysis (KCCA)^[24]. Next, we classified these image features by a Support Vector Machine (SVM)^[25]. An SVM technique is a machine learning technique that is often used for classification problems. Since multiple X-ray images were taken for each subject, the classification results of all X-ray images were integrated by an accuracy-based voting method. The values of *H. pylori* antibody and serum markers were used only in training procedures, and our system enabled classification of the risk of gastric cancer from only X-ray image information. Namely, if we want to estimate gastric cancer risk via our system, input data are only X-ray images, and calculated image features are automatically converted to new features considering PG levels and *H. pylori* antibody titers for the gastric cancer risk classification. A more detailed mathematical explanation of our system is given in^[26].

Statistical analysis

The verification method was 15-fold cross-validation. The gold standard for evaluating our system was the result of ABC (D) stratification by blood inspection. Sensitivity, specificity and Youden index (YI) were used as evaluation criteria for each stage's classification. A receiver operating characteristic (ROC) curve was generated based on each stage's classification result. ROC curves were obtained by changing the threshold that determines gastric cancer risk. Accuracy, precision, false positive rate and false negative rate were calculated. We also utilized a confusion matrix for evaluation of our system. A confusion matrix is often used in the field of machine learning, and it represents information about actual and predicted classification results obtained by a classification system. In this study, Togo R, Ishihara K, Ogawa T and Haseyama M from the Graduate School of Information Science and Technology, Hokkaido University took charge of the statistical analysis since they have an advanced knowledge of statistical analysis.

RESULTS

The total number of subjects was 2535, and subjects who had undergone *H. pylori* eradication therapy and had suspected false negative results in ABC (D) stratification were excluded as shown in Figure 1. Specifically, we excluded 175 subjects who had undergone *H. pylori* eradication therapy, and we excluded 260 subjects in

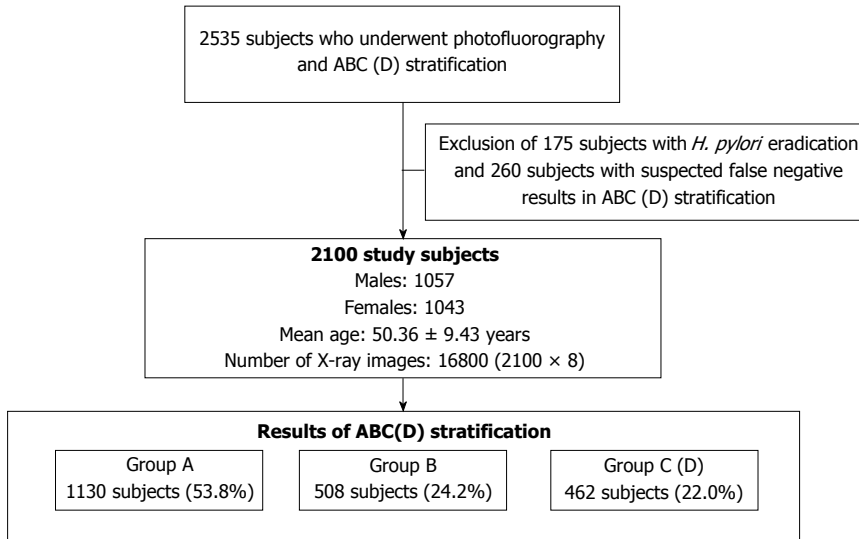


Figure 1 Target selection flowchart. *H. pylori*: *Helicobacter pylori*.

Table 2 Confusion matrix for the first stage

		Predicted class	
		<i>H. pylori</i> non-infection	<i>H. pylori</i> infection
True class	Group A	979	151
	Group B or C (D)	102	868

H. pylori: *Helicobacter pylori*.

Table 3 Confusion matrix for the second stage

		Predicted class	
		Non-severe	Severe
True class	Group B	331	177
	Group C (D)	98	364

group A with PG I levels ≤ 30 ng/mL, PG II levels ≥ 15 ng/mL or PG I/PG II ratio < 4 . If the training data included data for such subjects, it would have caused classification performance degradation since the correlation between radiological findings and ABC (D) stratification results for them might be eliminated. Consequently, data for 2100 subjects (1057 males and 1043 females; mean age, 50.36 ± 9.43 years) were used for analysis. There were 1130 subjects (53.8%) in group A, 508 subjects (24.2%) in group B and 462 subjects (22.0%) in group C (D).

Our system was evaluated with 16800 X-ray images for 2100 subjects. In the first stage, we performed *H. pylori* infection status classification. The number of subjects classified into each class is shown as a confusion matrix in Table 2. Of the 970 subjects who belonged to groups B and C (D) in ABC (D) stratification, 868 were correctly classified into the high gastric cancer risk group (*H. pylori* infection) using only X-ray image information. Also, of the 1130 subjects who belonged to group A in ABC (D) stratification, 999 were correctly classified into

the low gastric cancer group (*H. pylori* non-infection). On the other hand, 102 of the 2100 subjects (4.8%) were incorrectly classified into the *H. pylori* non-infection group in our system. Specifically, sensitivity (*H. pylori* infection), specificity (*H. pylori* non-infection) and YI were 0.884, 0.895 and 0.779, respectively. Other evaluation criteria were as follows: accuracy was 0.889, precision was 0.907, false positive rate was 0.105 and false negative rate was 0.116. Figure 2 shows examples of X-ray images correctly or incorrectly classified in the first stage. The ROC curve of the first stage that was obtained by changing the threshold determining *H. pylori* infection is shown in Figure 3.

Next, we examined whether our system can be applied to more specific atrophic level classification. In the supplementary experiment of the second stage, we focused on *H. pylori*-infected subjects and applied atrophic level classification to them. The number of subjects classified into each class is shown as a confusion matrix in Table 3. The experimental results showed that 364 of the 462 subjects who belonged to group C (D) in ABC (D) stratification were correctly classified into the severe atrophic level group based on the condition of the stomach shown in X-ray images. Sensitivity (severe), specificity (non-severe) and YI in the second stage were 0.777, 0.824 and 0.601, respectively. Other evaluation criteria were as follows: accuracy was 0.800, precision was 0.809, false positive rate was 0.176 and false negative rate was 0.223. Figure 4 shows examples of X-ray images correctly or incorrectly classified in the second stage. The ROC curve of the second stage that was obtained by changing the threshold determining the severity of atrophic level is shown in Figure 5.

DISCUSSION

It is a critical issue to evaluate gastric cancer risk for realizing effective gastric cancer mass screening^[27].

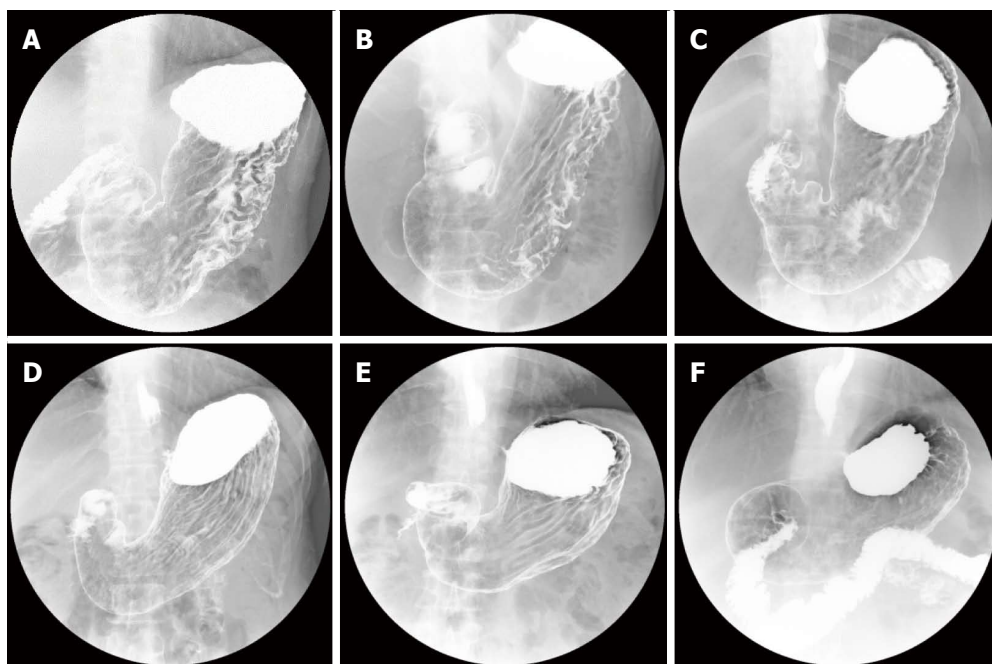


Figure 2 Examples of X-ray images correctly or incorrectly classified in the first stage. A: True class: Group B or C (D). Predicted class: *H. pylori* infection (Correct classification); B: True class: Group B or C (D). Predicted class: *H. pylori* infection (Correct classification); C: True class: Group B or C (D). Predicted class: *H. pylori* non-infection (Incorrect classification); D: True class: Group A. Predicted class: *H. pylori* non-infection (Correct classification); E: True class: Group A. Predicted class: *H. pylori* non-infection (Correct classification); F: True class: Group A. Predicted class: *H. pylori* infection (Incorrect classification). *H. pylori*: *Helicobacter pylori*.

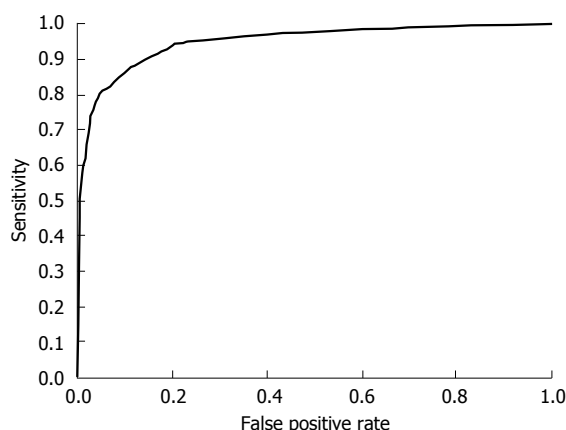


Figure 3 Receiver operating characteristic curve of the first stage generated by changing the threshold.

H. pylori eradication therapy as primary prevention and early detection of gastric cancer as secondary prevention should be implemented more effectively. Concretely, it is necessary to identify individuals with a high gastric cancer risk for more detailed examination and continuous gastric cancer screening based on their *H. pylori* infection status and atrophic level.

ABC (D) stratification has already been introduced in some areas for gastric cancer risk screening. However, ABC (D) stratification may have a disadvantage for detecting individuals with high gastric cancer risk. Since individuals in whom *H. pylori* has been eradicated and individuals with a high atrophic level who have a high gastric cancer risk are often classified into group A in

ABC (D) stratification^[28-30], the false-negative rate is a problem. Thus, since even if individuals in group A in ABC (D) stratification can develop gastric cancer^[31], the combined use of image-based inspection is mandatory for evaluation of gastric cancer risk^[32]. Photofluorography or endoscopy remains the gold standard of gastric cancer mass screening in Japan since clinicians can examine conditions of the stomach through the images. Hence, supporting image-based inspections will lead to more efficient gastric cancer mass screening.

Endoscopy is superior to photofluorography for detection of cancerous lesions in image-based inspections^[33]. In Japan, endoscopy has been recommended for gastric cancer mass screening programs in addition to photofluorography since 2016. Results of studies in South Korea have provided useful suggestions. In South Korea, a selective (*i.e.*, photofluorography or endoscopy) gastric cancer mass screening program was started in 2002^[34,35]. Lee *et al.*^[33] reported that the proportion of individuals who underwent endoscopic examination in the National Cancer Screening Program (NCSP) in South Korea increased greatly from 31.15% in 2002 to 72.55% in 2011. The NCSP provides biennial gastric cancer mass screening with either photofluorography or endoscopy for men and women over 40 years of age. On the other hand, the proportion of individuals who underwent photofluorography in the NCSP decreased from 68.85% in 2002 to 32.8% in 2011. Lee *et al.*^[33] also reported that the rate of participation in the NCSP increased from 7.40% in 2002 to 45.40% in 2011, and the number of individuals examined by

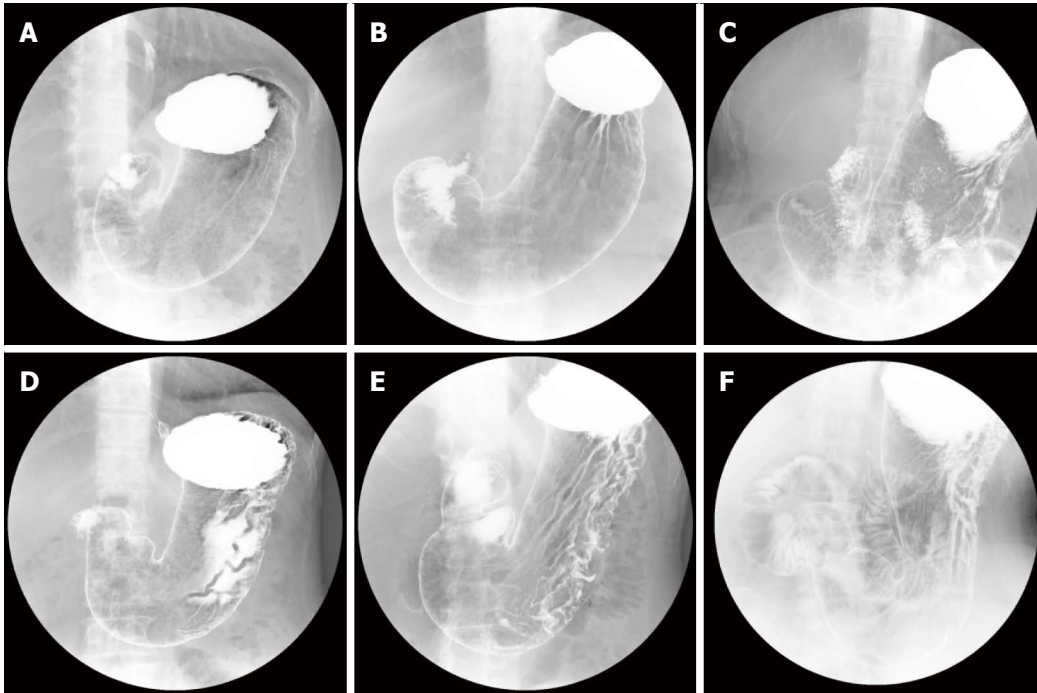


Figure 4 Examples of X-ray images correctly or incorrectly classified in the second stage. A: True class: Group C (D). Predicted class: Severe (Correct classification); B: True class: Group C (D). Predicted class: Severe (Correct classification); C: True class: Group C (D). Predicted class: Non-severe (Incorrect classification); D: True class: Group B. Predicted class: Non-severe (Correct classification); E: True class: Group B. Predicted class: Non-severe (Correct classification); F: True class: Group B. Predicted class: Severe (Incorrect classification).

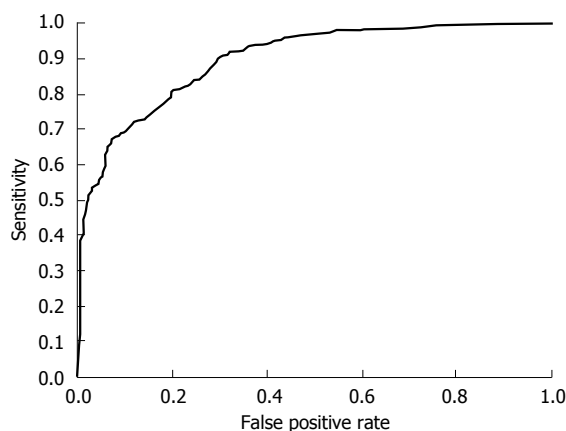


Figure 5 Receiver operating characteristic curve of the second stage generated by changing the threshold.

photofluorography increased in accordance with an overall increase in the percentage of participants in the NCSP in South Korea. This indicates the importance of automatic gastric cancer risk classification systems for photofluorography even under the condition of selective gastric cancer mass screening. In Japan, it will take a long time to establish endoscopic examinations due to an insufficient number of medical specialists and regional disparities of clinicians. The uneven distribution of clinicians who have experience in endoscopy is a bottleneck for endoscopic mass screening. Furthermore, the number of individuals who can be examined in one day by endoscopy is much smaller than the number of

individuals who can be examined by photofluorography. Although photofluorography involves radiation exposure, facilities have been constructed and inspection methods have been established. Each type of inspection has advantages and disadvantages, the above-described situation should be considered for establishing a gastric cancer risk classification system^[36].

In this study, we developed an automatic gastric cancer risk classification system as a preliminary study. Our system analyzes X-ray images and provides *H. pylori* infection status or atrophic severity level. It should be noted that the most important classification is the first stage, and the second stage is a supplementary experiment to verify whether our system can perform more detailed atrophic level classification. Experimental results indicated that risk-based information can be provided by our system. In the first stage, the most important risk classification, 88.9% of the subjects were correctly classified into the low gastric cancer risk group (*H. pylori* non-infection) and the high risk group (*H. pylori* infection). The purpose of our system is to improve the final accuracy of clinicians' diagnosis by providing risk-based information from image data. Gastric cancer risk information based on X-ray images is useful for identification of high-risk individuals and for reducing the burden on clinicians. Results of studies on identification of risk information for gastric cancer from photofluorography and examination of its application should be helpful for the future of gastric cancer mass screening. Moreover, the threshold determining each risk group in our system can be continuously changed

depending on the demands of clinicians. Namely, it is possible to decrease false negative cases by enhancing sensitivity based on the threshold for gastric cancer mass screening. Therefore, the combination of the results of ABC (D) stratification and our system will provide more reliable information for clinicians.

As an example of gastric cancer mass screening using our system, more specific examinations can be performed for individuals who have positive results in the first stage and had not received *H. pylori* eradication therapy are led to more specific examinations. The Japanese national health insurance now covers *H. pylori* eradication therapy for *H. pylori*-infected patients with gastritis detected by endoscopic examination. If those patients have positive results in the examination, *H. pylori* eradication therapy will be conducted and they will be followed up by gastric cancer screening.

Our study has some limitations. First, although the gold standard of our system was ABC (D) stratification and *H. pylori* eradication history, there are often contain false negative or false positive cases. Ideally, *H. pylori* infection status and atrophic level should be evaluated by radiological findings of photofluorography or endoscopy, and these results should be used for the gold standard. However, since this preliminary study focused on mass screening data, we utilized ABC (D) stratification as the simplest inspection with a high objectivity. Secondly, the exclusion rule of this study is our limitation. The advantage of image-based risk information is that gastric cancer risk information can be estimated from individuals who have undergone *H. pylori* eradication therapy since the presence or absence of atrophy of the stomach remains a key factor for them. However, *H. pylori*-eradicated individuals and individuals with suspected false negative results of ABC (D) stratification were excluded from our study due to the lack of a gold standard of ABC (D) stratification. Instead, we performed a supplemental experiment for evaluation of stomach atrophy in this study. We will target *H. pylori*-eradicated individuals and suspected false negative individuals as a future work.

We presented a gastric cancer risk classification system using photofluorography as a preliminary study. The first step of our experimental results indicates that gastric cancer risk information can be provided by machine learning techniques. Although further investigation and improvements of the system are needed, it is expected that collaborative use of image-based risk information derived by our system and ABC (D) stratification will enable more accurate evaluation of gastric cancer risk.

ARTICLE HIGHLIGHTS

Research background

Gastric cancer is one of the most common malignancies, and has the highest mortality rates in East Asian countries. Although ABC (D) stratification is effective method for evaluating gastric cancer risk, photofluorography still

plays an important role in gastric cancer mass screening since image-based evaluation is mandatory.

Research motivation

If gastric cancer risk information can be provided automatically by analyzing X-ray images, it would be helpful for the future of gastric cancer mass screening.

Research objectives

The aim of this study was investigation of potential of machine learning techniques using photofluorography.

Research methods

We developed an automatic gastric cancer risk classification system for identification of *Helicobacter pylori* infection status and atrophic level from photofluorography. All of 2100 patients' data were acquired at the Medical Examination Center of Yamagata City Medical Association in Japan, from April 2012 to March 2013. From DICOM data, we extracted the image data while securing anonymity.

Research results

Experimental results suggested that image-based risk information can be calculated by our system.

Research conclusions

Although further investigation and improvement of the system are needed, this retrospective study indicated that machine learning techniques analyzing X-ray images can provide effective gastric cancer risk information. Also, we discussed the potential of machine learning techniques and the future of gastric cancer mass screening.

Research perspectives

In the field of breast cancer, computer-aided supporting systems have already become a part of routine clinical work for detection of breast cancer or abnormalities. Gastric cancer as well as breast cancer requires effective and highly accurate mass screening. We believe that this preliminary study will contribute the next step of the future of gastric cancer mass screening.

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***Fusobacterium nucleatum* and colorectal cancer: A review**

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Abstract

Fusobacterium nucleatum (*F. nucleatum*) is a Gram-negative obligate anaerobe bacterium in the oral cavity and plays a role in several oral diseases, including periodontitis and gingivitis. Recently, several studies have reported that the level of *F. nucleatum* is significantly elevated in human colorectal adenomas and carcinomas compared to that in adjacent normal tissue. Several researchers have also demonstrated that *F. nucleatum* is obviously associated with colorectal cancer and promotes the development of colorectal neoplasms. In this review, we have summarized the recent reports on *F. nucleatum* and its role in colorectal cancer and have highlighted the methods of detecting *F. nucleatum* in colorectal cancer, the underlying mechanisms of pathogenesis, immunity status, and colorectal cancer prevention strategies that target *F. nucleatum*.

Key words: *Fusobacterium nucleatum*; Carcinoma; Colon and rectal carcinoma; Host immunity; Gut microbiome

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Core tip: *Fusobacterium nucleatum* (*F. nucleatum*) promotes the progress of colorectal adenomas involving in multiple potential mechanisms. *F. nucleatum* positivity in colorectal cancer (CRC) is different in different research groups. Some potential biomarkers may be regarded as a criterion for judging CRC prognosis. Some chemoprevention and immunotherapy strategies on *F. nucleatum*-positive colorectal cancer need to be further explored in the future.

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INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent malignant neoplasm and the fourth most frequent cause of cancer death in the world, and the five-year survival rate is nearly 65%^[1]. For a long time, the mortality rate of CRC has declined in areas where medical resources are abundant, while the mortality rate has risen in areas with poor medical conditions^[2]. CRC is a complex disease that is influenced by both genetic and environmental factors such as dietary habits and lifestyle. Recently, increasing evidence has indicated an association between the intestinal microbiota and CRC^[3-5].

More than 100 trillion (10^{14}) microorganisms reside in the intestinal tract and play an extremely important role in human health. These microbes maintain intestinal homeostasis by regulating various biological activities such as mucosal barrier, immune and metabolic functions^[6,7]. Once the intestinal balance is damaged, it may cause numerous intestinal diseases including inflammatory bowel diseases (IBD) and colorectal neoplasms^[8-10]. There is accumulating evidence to suggest that the gut microbiota is associated with colorectal neoplasms^[11-18]. Several studies have validated that the levels of *Bacteroides*, *Prevotella*, *Escherichia coli*, *Bacteroides fragilis* (ETBF), *Streptococcus gallolyticus*, *Enterococcus faecalis*, and *Streptococcus bovis* are significantly higher in CRC tissue compared to those in adjacent normal tissue^[4,11-16,18]. ETBF has been confirmed to selectively stimulate *STAT3* in the colon, induce inflammation infiltrates of T helper type 17 and promote the development of CRC^[19]. *Enterococcus faecalis* has been reported to facilitate tumorigenesis through activating the DNA damage pathways^[20]. Furthermore, the abundance of both *Fusobacterium nucleatum* (*F. nucleatum*) and *C. difficile* was found to be significantly higher in CRCs compared to the healthy control group^[21]. Additional studies have also confirmed that *F. nucleatum* associates with some Gram-negative bacteria, including *Streptococcus*, *Campylobacter spp.* and *Leptotrichia*, and synergistically promotes the occurrence of CRC^[22,23].

F. nucleatum, a common Gram-negative anaerobic bacterium, is one of the most prevalent species in the oral cavity, and several studies have demonstrated that *F. nucleatum* is associated with oral inflammation diseases, such as periodontitis and gingivitis^[24-26]. It has also been associated with pancreatic cancer, oral cancer, and premature and term stillbirths^[27-30]. In addition, *F. nucleatum* is closely connected with liver abscess^[9,31], appendicitis and infections of the head and neck, including mastoiditis, tonsillitis and maxillary sinusitis^[32-35]. Increasing evidence has indicated that the levels of *F. nucleatum* are significantly elevated in tumor tissues and stool specimens of CRC patients relative to those in normal controls^[36-42]. Researchers have reported that *F. nucleatum* may contribute to the development of CRC and that it is considered to be a

potential risk factor for CRC progression^[17,43]. Investigators have demonstrated that a higher abundance of *F. nucleatum* in CRC is associated with a shorter survival time^[44]. Several researchers have also shown that a high-abundance of *F. nucleatum* induces a series of specific tumor molecular events, including CpG island methylator phenotype (CIMP), microsatellite instability (MSI), and genetic mutations in *BRAF*, *CHD7*, *CHD8* and *TP53*^[44,45]. However, *F. nucleatum* was previously regarded as a passenger bacterium in human intestinal tract^[46,47]. Recently, it has been considered to be a potential initiator of CRC susceptibility^[37,45]. Kostic *et al.*^[48] have confirmed that *F. nucleatum* promotes colorectal tumorigenesis in *Apc^{min/+}* mice. Rubinstein *et al.*^[43] have reported that *F. nucleatum* stimulates tumor cell growth in CRC by activating β -catenin signaling and inducing oncogenic gene expression *via* the FadA adhesion virulence factor. Together, these studies show that *F. nucleatum* plays an important role in the initiation of CRC and promoting tumor cell growth in CRC, supporting that *F. nucleatum* is a cause of CRC rather than a consequence. In this review, we have summarized the recent reports on *F. nucleatum* and its role in CRC and have highlighted the methods of detecting *F. nucleatum* in CRC, the underlying mechanisms of pathogenesis, immunity status, and colorectal prevention strategies that target *F. nucleatum*.

F. nucleatum invades human epithelial cells, activates β -catenin signaling, induces oncogenic gene expression and promotes growth of CRC cells through the FadA adhesion virulence factor.

METHODS FOR DETECTING *F. NUCLEATUM* IN CRC

To detect *F. nucleatum* in CRC, investigators have used several different methods, including fluorescent quantitative polymerase chain reaction (FQ-PCR), fluorescence in situ hybridization (FISH), quantitative real-time polymerase chain reaction (qPCR), and droplet digital polymerase chain reaction (ddPCR). Furthermore, sample collection methods also vary among studies, some of which are derived from formalin-fixed paraffin-embedded (FFPE) CRC tissues, CRC frozen tissues, genomic DNA, and feces collected from CRC patients.

As shown in Table 1, the detection method and the detection rate of *F. nucleatum* in CRC differ among studies. In one Chinese study, the *F. nucleatum* abundance was measured in frozen tissues from 101 CRC patients by FQ-PCR, and FISH analysis was conducted on 22 CRC FFPE tissues with the highest abundance of *F. nucleatum* to confirm the FQ-PCR results, and the positive rate of *F. nucleatum* was detected to be 87.13% (88/101)^[40]. Analyzing 598 CRC patients in 2 American nationwide prospective cohort studies, researchers detected the abundance of *F. nucleatum* in FFPE tissue samples obtained from CRC patients by qPCR and found that the positive percentage of *F. nucleatum*

Table 1 Positive detection rates of *Fusobacterium nucleatum* in colorectal cancer reported by different research groups

Author (publish date)	Total cases (n)	Positive cases (n)	Positive percentage	Detection method	Detection samples
Li <i>et al</i> ^[40] (3/2016)	101	88	87.13%	FISH and FQ-PCR	Frozen tissue and FFPE tissue
Mima <i>et al</i> ^[38] (8/2015)	598	76	13%	qPCR	FFPE tissue
Nosho <i>et al</i> ^[49] (1/2016)	511	44	8.6%	qPCR	FFPE tissue
Tahara <i>et al</i> ^[45] (1/2014)	149	111	74%	qPCR	Genomic DNA
Ito <i>et al</i> ^[39] (2/2015)	511	286	56%	qPCR	FFPE tissue
Suehiro <i>et al</i> ^[50] (3/2017)	158	85	54%	ddPCR	Feces

qPCR: Quantitative real-time polymerase chain reaction; FQ-PCR: Fluorescent quantitative polymerase chain reaction; ddPCR: Droplet digital polymerase chain reaction; FISH: Fluorescence in situ hybridization; FFPE: Formalin-fixed paraffin-embedded.

accounted for 13% (76/598) of the CRC samples. This detection rate was significantly lower than that reported in the Chinese study (87.13%)^[38]. In one Japanese study, the experimental specimens were obtained from CRC FFPE tissues from 511 Japanese patients, and the abundance of *F. nucleatum* was detected by qPCR. *F. nucleatum* was detected in 8.6% (44/511) of the CRC tissue samples, which was similar, albeit slightly lower, to that reported in the USA (13%)^[49]. In another study, the richness of *F. nucleatum* was evaluated by qPCR, and the samples were prepared from genomic DNA extracted from 149 primary CRC tissue samples; *F. nucleatum* was detected in 74% (111/149) of the CRC tissue samples^[45]. In a recent study, the samples consisted of FFPE tissues from 511 CRC patients, and *F. nucleatum* was detected in 56% (286/511) of the CRC patients by qPCR^[39]. In another study, *F. nucleatum* was detected in the stool samples collected from CRC patients, and the sensitivity and specificity were found to be 72.1% (75/104) and 91.0%, respectively, while the high-abundance of *F. nucleatum* in patients exhibited a false positive rate of 7.0%^[42]. In another study, the levels of *F. nucleatum* were measured in fecal specimens from Japanese CRC patients by ddPCR, and *F. nucleatum* was found to be present in 54% (85/158) of the specimens^[50]. Furthermore, some researchers used a qPCR assay to detect *F. nucleatum* in FFPE tissue from CRC patients and revealed that *F. nucleatum* was present in 2.5% (4/157) of rectal cancers and 11% (19/178) of cecum cancers, with a significant linear trend along all subsites^[51]. The percentage of *F. nucleatum*-enriched CRC gradually increases from rectum to cecum^[51], suggesting that the rate at which *F. nucleatum* is present may also differ among intestinal sites.

Common specimens for detecting *F. nucleatum* in CRC include frozen tissues, FFPE tissues, genomic DNA and feces. The use of both frozen tissue and FFPE tissue specimens are limited by surgery or colonoscopy. Specimens derived from the feces of CRC patients are easy to obtain, but they often result in high false positive detection rates. As mentioned above, qPCR, ddPCR, FQ-PCR and FISH are applied to detect the levels of *F. nucleatum*. While the qPCR assay is the most popular technique to measure the abundance of *F. nucleatum* in CRC tissues, it is difficult to detect *F. nucleatum* in the feces^[52]; in addition, a higher false

positive rate is seen in the high abundance group of *F. nucleatum*^[42]. It has been reported that ddPCR improved the sensitivity of *F. nucleatum* detection in the feces compared to qPCR, and ddPCR was demonstrated to be 1000 times more sensitive than qPCR^[53]. In addition, ddPCR resulted in a higher detection rate of low concentrations of microorganisms compared with qPCR^[54]. FQ-PCR is a convenient and rapid method for detecting pathogens and displays a higher sensitivity and specificity than qPCR^[55]. In addition, it is difficult to contaminate FQ-PCR during experimental operation compared with qPCR^[55].

UNDERLYING MECHANISMS OF

F. NUCLEATUM PATHOGENESIS IN CRC

A previous study has shown that lymph node metastases are present in 52 out of 88 (59.1%) cases with a high-abundance of *F. nucleatum* and in 0 out of 13 (0%) subjects with a low-abundance of *F. nucleatum*, which indicates that a high abundance of *F. nucleatum* is associated with CRC progression and metastasis^[40]. It has been suggested that high levels of *F. nucleatum* may be associated with poor outcomes of CRC. Some researchers have also reported that the load of *F. nucleatum* DNA in CRC tissue is correlated with higher colorectal cancer-specific mortality^[44] and that *F. nucleatum* DNA may serve as a potential poor prognostic biomarker^[44]. *Fusobacterium* was shown to be enriched in the mucosa-adherent microbiota and have the ability to adhere to and invade human epithelial and endothelial cells^[27,52,56]. Recently, several researchers have suggested that *F. nucleatum* is a pathogenic bacterium rather than a bacterium that promotes colorectal carcinogenesis^[43,57]. Several studies have shown that its virulence factors are closely linked with colorectal lesions. It has been demonstrated that *F. nucleatum* invades human epithelial cells, activates β -catenin signaling, induces oncogenic gene expression and promotes growth of CRC cells *via* the FadA adhesion virulence factor^[43]. A second virulence factor, an autotransporter protein, Fap2, has been shown to potentiate the progress of CRC *via* inhibiting immune cell activity^[58].

As shown in Figure 1, *F. nucleatum* attaches and

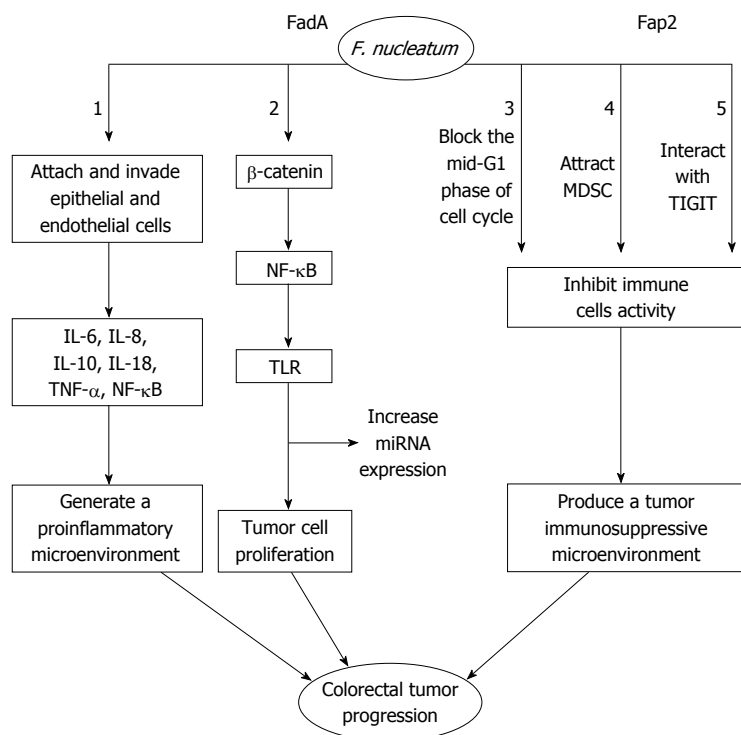


Figure 1 Underlying mechanism of *Fusobacterium nucleatum* pathogenesis in colorectal cancer. (1) In pathway 1, the FadA in *Fusobacterium nucleatum* (*F. nucleatum*) adheres to and invades human epithelial cells and endothelial cells, and inflammatory cytokine (IL-6, IL-8, IL-10, IL-18, TNF- α and NF- κ B) levels increase in a proinflammatory microenvironment that accelerates the progression of colorectal tumors; (2) In pathway 2, FadA interacts with E-cadherin on the epithelial cell, activates β -catenin signaling, increases NF- κ B inflammatory gene expression and promotes tumor cells proliferation. However, *F. nucleatum*-infected cells increase the expression of miRNA by activating Toll-like receptor and further promote the release of miRNA; (3) In pathway 3 and 4, *F. nucleatum* dampens human T-cell activation in a tumor immunosuppressive microenvironment that supports tumor cell growth by blocking the mid-G1 phase of cell cycle and attracting myeloid-derived suppressor cells; and (4) In pathway 5, the interaction between Fap2 of *F. nucleatum* and the human inhibitory receptor TIGIT induce human lymphocytes cell death and generate a tumor immunosuppressive microenvironment that promotes colorectal tumor progression. MDSC: Myeloid-derived suppressor cell; TLR: Toll-like receptor.

invades human epithelial and endothelial cells^[27,56]. This attachment and invasion depends on the *F. nucleatum* FadA adhesion protein^[59,60]. The FadA protein exists in two main forms. The first form is the intact pre-FadA consisting of 129 amino that is anchored to the membrane, and the second form is the secreted mature FadA (mFadA) consisting of 111 amino acids that are secreted outside of *F. nucleatum*^[61]. When mFadA combines with pre-FadA, the pre-FadA-mFadA is internalized, and FadAc is activated^[61]. The internalization of the pre-FadA and mFadA complex ensures that *F. nucleatum* binds to and invades host epithelial cells^[61]. The host endothelial receptor for FadA is the vascular endothelial cadherin (CDH5), which is a member of the cadherin family^[59]. The CDH5 receptor is required for *F. nucleatum* to adhere to and invade endothelial cells^[59]. *F. nucleatum* invasion induces the production of cytokines such as interleukin-8 (IL-8), which is regulated by the p38 MAPK signaling pathway but independent of Toll-like receptor (TLR), NOD-1, NOD-2 and Nuclear Factor-kappaB (NF- κ B) signaling^[62]. *F. nucleatum* promotes the expression of several inflammatory genes such as NF- κ B and cytokines, including IL-6, IL-8 and IL-18^[43]. *F. nucleatum* also promotes the release of

inflammatory cytokines particularly IL-8, IL-10 and tumor necrosis factor- α (TNF- α) in a proinflammatory microenvironment that accelerates colorectal tumor progression^[37,62,63]. Another receptor of FadA is the cell-adhesion molecule E-cadherin expressed on non-CRC and CRC cells^[43]. E-cadherin is a strong tumor suppressor that inhibits tumor growth and development^[64].

FadA binding to wnt7b E-cadherin on CRC cells promotes *F. nucleatum* adhesion and invasion of host epithelial cells, activates β -catenin signaling that leads to increased expression of *Wnt* genes, oncogenes, transcription factors, and inflammatory genes, and promotes tumor cells proliferation^[43]. FadAc, but not mFadA, binds specifically to the E-cadherin-5, the cytoplasmic or the transmembrane domains of E-cadherin, and results in E-cadherin phosphorylation and internalization^[43,65]. As a result, a series of events, which include a decrease in β -catenin phosphorylation, an accumulation of β -catenin in the cytoplasm, and translocation toward the nucleus, leads to the activation of β -catenin-regulated transcription (CRT)^[43]. CRT increases the expression of *wnt* signaling genes such as *wnt7a*, *wnt7b* and *wnt9a*, the oncogenes *myc* and cyclin D1, transcription factors such as the lymphoid enhancer factor (LEF-1), NF- κ B such as NF- κ B2, T cell factor

such as TCF1, TCF3 and TCF4, and proinflammatory cytokines including IL-6, IL-8 and IL-18^[43]. On the other hand, *F. nucleatum* infected cells increase the expression of microRNA-21 (miR21) by activating TLR4 signaling to MYD88, which leads to the activation of NF- κ B^[41]. Subsequently, hyperactive NF- κ B attaches to the promoter of miR21 and induces the oncogenic cascade in CRC^[41]. Moreover, *F. nucleatum* reduces CD3⁺ T-cell density in CRC tissue^[38]. A previous study has shown that FDC364, sonic extract of *F. nucleatum*, inhibits human T-cell responses to antigens and mitogens^[66]. By blocking the mid-G1 phase of cell cycle, the *F. nucleatum* inhibitory protein suppresses human T-cell activity^[67]. This effect may promote an immunosuppressive microenvironment that allows tumor cell growth^[67]. By releasing short-chain fatty acids (acetate, propionate, and butyrate) and short-peptides (formylmethionyl-leucyl-phenylalanine), *F. nucleatum* also selectively attracts myeloid-derived suppressor cells (MDSCs)^[48,68]. MDSCs, a group of heterogeneous cells, show strong T-cell suppressive activity in the immune response^[69]. MDSCs and their effectors are key components of the neoplasm and promote tumor progression^[48,70]. *F. nucleatum*-associated tumors increase the myeloid-lineage infiltrating cells, including CD11b⁺, tumor-associated macrophages (TAMs), M2-like TAMs, tumor-associated neutrophils, conventional myeloid dendritic cells (DCs) and CD103⁺ regulatory DCs^[48]. These cells play an important role in dampening antitumor immunity and promoting tumor progression^[69,71-73]. Collectively, these studies have shown that *F. nucleatum* produces a tumor immunosuppressive microenvironment and promotes CRC progression. Fap2, a galactose-sensitive adhesion protein, plays an important role in coaggregation and cell adhesion^[74]. In *F. nucleatum*, the virulence factor Fap2 protein suppresses immune cell activities through interacting with TIGIT^[58]. The interaction between Fap2 and TIGIT protects tumors containing *F. nucleatum* from host immune cell attack^[58]. TIGIT is an inhibitory receptor in humans that is expressed on T cells and natural killer (NK) cells^[75]. The Fap2 has also been reported to induce human lymphocyte cell death^[57]. In addition, Fap2 mediates *F. nucleatum* enrichment by interacting with Gal-GalNAc overexpressed in colorectal tumors^[76]. Gal-GalNAc is a host polysaccharide overexpressed in CRC^[76]. In summary, *F. nucleatum* produces a tumor immunosuppressive microenvironment that promotes CRC progression.

F. NUCLEATUM AND IMMUNITY STATUS IN CRC

Some researchers have demonstrated that *F. nucleatum* modulates the tumor immune microenvironment while promoting CRC development^[48]. Recently, it has been confirmed that biomarkers such as immune antibodies, miRNA, TAMs, and tumor-infiltrating T-cell subsets play a significant role in *F. nucleatum*-associated

CRC^[44,48,77,78].

Several studies have shown that *F. nucleatum* infection causes high levels of serum *F. nucleatum*-IgA antibodies in CRC patients^[77]. Researchers have confirmed that serum anti-*F. nucleatum*-IgA combined with CA19-9 and CEA has a higher sensitivity than CA19-9 and CEA alone in screening early CRC^[77]. This study suggests that serum *F. nucleatum*-IgA antibodies may be regarded as a potential diagnosing biomarker for early CRC^[77]. In addition, some researchers have found that the levels of the *fadA* gene in colon tissue from CRC patients are > 10-100 times higher in comparison with normal subjects^[43]. This study also reveals a gradual increase in *fadA* gene copies in normal individuals compared to CRC patients^[43]. The *fadA* gene has become a potential ideal diagnostic marker to identify individuals with CRC risk^[43]. The *miR-21* gene has been demonstrated to promote tumor cell growth and migration *via* inhibiting sec23a protein expression^[79]. The data also indicated that *F. nucleatum* induces a high level of miR-21 expression in advanced CRC^[41]. The amount of miR-21 in CRC tissues has been shown to be associated with poor clinical outcomes^[41]. Studies have reported that non-coding RNAs (lncRNAs) play a crucial role in the diagnosis and prognosis of CRC^[80]. One study has found that low levels of NR_034119 and NR_029373 are associated with a short survival rate of CRC^[80]. These researchers suggested that several lncRNAs (NR_034119, NR_029373, NR_026817, and BANCR) are potential diagnostic biomarkers for CRC and that NR_034119 and NR_029373 are potential prognostic indicators for CRC^[80]. Another study reported that the level of lncRNA PANDAR was higher in CRC cells and tissues relative to adjacent normal tissues^[81], and high levels of PANDAR expression were associated with short overall survival^[81]. The authors suggested that the amount of PANDAR expression may be a prognostic indicator for CRC.

A previous study reported that *F. nucleatum*-positive tumors increased TAM infiltration^[48]. TAMs play an important role in innate immunity, and subpopulations of regulatory T-lymphocytes (Tregs) are a component of the acquired immunity. A recent study has found that intense infiltration of TAMs in colorectal tumor tissue is associated with shorter disease-free survival and overall survival of CRC^[78]. Infiltration of TAMs CD68⁺/iNOS⁻ in colorectal tumor stroma is confirmed to be related to the poor prognosis of CRC^[78]. Some researchers have reported that tumor-infiltrating T-cell subpopulations distinctly regulate the prognosis of CRC^[82]. For instance, in tumor-infiltrating T-cell subsets, CD45RO⁺-cell density, but not that of FOXP3⁺-cell, is significantly associated with a long survival of CRC patients^[82]. CD45RO⁺-cell is considered to be a potential good prognostic biomarker for CRC^[82]. The FOXP3⁺ transcription factor, which plays an important role in regulating the immune system, is regarded as an immunosuppressive factor. Some scholars have reported that infiltration of FOXP3⁺ in colorectal tumor

stroma is associated with a poor prognosis in CRC^[78]. However, several researchers also suggest that FOXP3⁺-cells are generally associated with a good prognosis of CRC^[83]. An article recently published in Nature Medicine has shown that distinct tumor-infiltrating FOXP3⁺-T cell subpopulations have an opposite approach to determining CRC prognosis. The development of inflammatory FOXP3⁺ (lo) non-T_{reg} cells was shown to be associated with tumor invasion by intestinal bacteria, particularly *F. nucleatum*^[84]. In this study, CRC patients with a high infiltration of FOXP3⁺ (lo) T cells exhibit a significantly better prognosis, compared to those with a FOXP3⁺ (hi) T_{reg} cell infiltration^[84]. When FOXP3⁺ (hi) T_{reg} cells are depleted from CRC tissues, antitumor immunity is augmented^[84]. The elimination of FOXP3⁺ (hi) T_{reg} cells has been suggested to play a crucial role in suppressing CRC formation^[84]. Recent research has also found that prudent diets such as whole grain and dietary fiber reduce the risk of *F. nucleatum*-positive CRC^[85].

In conclusion, anti-*F. nucleatum*-IgA, the *fadA* gene, and lncRNAs may be considered as potential diagnostic biomarkers during the early stage of *F. nucleatum*-positive CRC. The CD45RO⁺-cell and FOXP3⁺ (lo) T cell biomarkers are associated with a favorable prognosis in *F. nucleatum*-positive CRC, while the miR-21, lncRNA PANDAR, and TAMs CD68⁺/iNOS⁻ biomarkers are associated with a poor clinical prognosis of *F. nucleatum*-positive CRC.

PREVENTION STRATEGIES THAT TARGET *F. NUCLEATUM* IN CRC

Currently, cancer prevention strategies have been mainly focused on chemoprevention and immunotherapy. Chemoprevention, which involves the use of aspirin, cyclo-oxygenase-2 (COX-2) inhibitors, and selective EP2 antagonists, plays an important role in *F. nucleatum*-associated CRC. Immunotherapies, such as antibody treatment, immune-checkpoint blockade therapy and adoptive cell transfer therapies, may aid in the prevention of *F. nucleatum*-positive CRC.

Chemoprevention, including the use of aspirin, COX-2 inhibitors, and selective EP2 antagonists, plays a significant role in the mechanisms of *F. nucleatum*-positive CRC. For instance, some researchers have reported that regular aspirin use lowers CRC incidence and mortality and reduces the risk of distant metastasis of CRC^[85,86]. Regular doses of aspirin were also associated with a lower risk of CRC and low levels of CD45RO (PTPRC)⁺T cells, CD3⁺T cells or CD8⁺ T cells^[87]. Aspirin induces neutrophils apoptosis^[88] and triggers a lipoxin-driven immune-regulatory effect^[89]. Aspirin directly inhibits T-cell activation and proliferation and suppresses cytokine production involved in the T cell-mediated adaptive immune response^[90]. Tumor-infiltrating immune cells have been associated with a good prognosis in CRC^[91,92]. The amount of *F. nucleatum* is inversely proportional to CD3⁺ T-cell density in colorectal carcinoma

tissue^[38]. These data indicate that aspirin may support the host immune system and prevent the development of *F. nucleatum*-associated CRC.

In addition, FadA in *F. nucleatum* specifically binds to E-cadherin and activates *Wnt* signaling^[43]. *F. nucleatum* increases expression of inflammatory genes and *Wnt* genes^[43]. A recent study has reported that EP2 enhances the expression of NF- κ B-targeted proinflammatory genes induced by TNF- α in neutrophils^[93]. The levels of cytokines such as TNF- α and IL-6, COX-2, chemokine CXCL1, and *Wnt* are significantly higher in tumor lesions of EP2-abundant mice than those in EP2- deficient mice^[93]. This study revealed that EP2 promotes colon tumorigenesis by means of expanding inflammation and shaping a tumor microenvironment^[93]. PF-04418948, a selective EP2 antagonist, significantly inhibits the formation of colon tumors^[93]. This suggests that selective EP2 antagonists may be promising drugs for the chemoprevention of *F. nucleatum*-associated CRC.

Furthermore, COX expression in BrA^{AV600E} cells may prevent CD103⁺ DC activation and accumulation in tumors^[94]. By suppressing local T-cell effector, COX-2 also promotes immune evasion and resistance to antigen-specific cancer immunity^[95]. COX-2 is also considered an inhibitor of antigen-specific tumor immunotherapy^[95]. This is powerful evidence that supports that COX inhibitors reduce the risk of CRC by inhibiting inflammatory pathways, and COX inhibitors may be important for immune-based therapy in CRC patients. In conclusion, aspirin, EP2 antagonists, and COX-2 inhibitors may be important tools for preventing *F. nucleatum*-associated CRC.

Immunotherapies, including antibody treatment, immune-checkpoint blockade therapy and adoptive cell transfer therapies, may be effective strategies for preventing *F. nucleatum*-positive CRC. For example, the interaction between Fap2 and TIGIT receptor protects tumors against immune cell attack and, accordingly, inhibits antitumor immunity and supports tumor cells growth^[58]. Fap2 also induces lymphocyte cell death^[57]. Fap2 mediates *F. nucleatum* enrichment via its interaction with Gal-GalNAc that is overexpressed in CRC, which may exacerbate the inhibition of antitumor immunity^[76]. Therefore, anti-Fap2 antibody development may favor antitumor immune response and be a potential immunotherapy in *F. nucleatum*-positive CRC. *F. nucleatum* inhibits T-cell activity and stimulates lymphocyte cell death, which protects tumors from immune cell attack. *F. nucleatum* may have immunosuppressive function in the tumor immune microenvironment.

Recently, the approach to cancer immunotherapy involves immune-checkpoint blockade, such as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed death protein 1 (PD-1). CTLA-4 and PD-1 have been reported to be involved in T cell-mediated antitumor immunity^[96,97]. It was speculated that blockade of CTLA-4 and PD-1 may shape the antitumor immunity response and be an effective immunotherapy

for *F. nucleatum*-associated CRC. Other CRC treatment strategies involving *F. nucleatum*, such as *miR-21* blockade may play a significant role in *F. nucleatum*-positive CRC, as *F. nucleatum* increases expression of *miR-21* by activating TLR4 signaling to NF- κ B^[41]. It has been demonstrated that *miR-21* promotes tumor cells proliferation and migration by down-regulating the expression of the sec23a protein^[79]. The inhibition of *miR-21* suppresses the metastasis of colorectal tumor cells by regulating programmed cell death 4^[98]. In a *miR-21* knockout mouse model, expression of proinflammatory and procarcinogenic cytokines was decreased, suggesting that *miR-21* deficiency promotes the apoptosis of tumor cells by suppressing STATA3 and Bcl-2 activation^[99]. It has been suggested that the *miR-21* blockade may be a potential treatment strategy for *F. nucleatum*-associated CRC. Some adoptive cell transfer therapies, such as NK cells^[100], cytokine-induced killer cells^[101], and tumor-infiltrating lymphocytes^[102], are also being used to strengthen antitumor immunity in clinical practice. These adoptive cell transfer therapies may also be considered as an immunotherapy approach in CRC associated with *F. nucleatum*.

In sum, CRC prevention strategies that target *F. nucleatum* are mainly focused on chemoprevention, which includes the use of aspirin, COX-2 inhibitors and selective EP2 antagonists, and immunotherapy, which includes anti-Fap2 antibody treatment, CTLA-4, PD-1, *miR-21* blockade therapies and adoptive cell transfer therapies.

CONCLUSION

In summary, the gut microbiota, especially *F. nucleatum*, has been extensively associated with CRC. *F. nucleatum* promotes the progression of CRC via multiple potential mechanisms. The positive detection rate of *F. nucleatum* in CRC samples varies among different studies. FadA combined with anti-*F. nucleatum*-IgA may improve the diagnosis of CRC. Several potential biomarkers, such as *miR-21*, LncRNA PANDAR, TAMs CD68⁺/iNOS⁻, FDXP3⁺ (lo) T cells and CD45RO⁺ cells, may be considered as criteria for determining CRC prognosis. Furthermore, chemoprevention and immunotherapy strategies should be further explored in the future.

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Clinical Practice Study

Sessile serrated adenoma detection rate is correlated with adenoma detection rate

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Kinoshita H, Niimi K, Ono S, Yoshida S, Yamada A, Kodashima S, Yamamichi N and Hirata Y performed endoscopic procedures, checked the manuscript and suggested improvement; Hayakawa Y and Minatsuki M checked the manuscript and suggested improvement; Ushiku T and Fukayama M provided advice on the histopathological diagnoses, checked the manuscript and suggested improvement; Koike K gave the final approval of the manuscript.

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Abstract

AIM

To investigate the association between adenoma detection rate (ADR) and sessile serrated adenoma detection rate (SSADR) and significant predictors for sessile serrated adenomas (SSA) detection.

METHODS

This study is a retrospective, single-center analysis. Total colonoscopies performed by the gastroenterologists at the University of Tokyo Hospital between January and December 2014 were retrospectively identified. Polyps were classified as low-grade or high-grade adenoma, cancer, SSA, or SSA with cytological dysplasia, and the prevalence of each type of polyp was investigated. Predictors of adenoma and SSA detection were examined using logistic generalized estimating equation models. The association between ADR and SSADR for each gastroenterologist was investigated by calculating a correlation coefficient weighted by the number of each gastroenterologist's examination.

RESULTS

A total of 3691 colonoscopies performed by 35 gastroenterologists were assessed. Overall, 978 (26.5%) low- and 84 (2.2%) high-grade adenomas, 81 (2.2%) cancers, 66 (1.8%) SSAs, and 2 (0.1%) SSAs with cytological dysplasia were detected. Overall ADR was 29.5% (men 33.2%, women 23.8%) and overall SSADR was 1.8% (men 1.7%, women 2.1%). In addition, 672 low-grade adenomas (68.8% of all the detected low-grade adenomas), 58 (69.9%) high-grade adenomas, 29 (34.5%) cancers, 52 (78.8%) SSAs, and 2 (100%) SSAs with cytological dysplasia were found in the proximal colon. Adenoma detection was the only significant predictor of SSA detection (adjusted OR: 2.53, 95%CI: 1.53-4.20; $P < 0.001$). The correlation coefficient between ADR and SSADR weighted by the number of each gastroenterologist's examinations was 0.606 ($P < 0.001$).

CONCLUSION

Our results demonstrated that ADR is correlated to SSADR. In addition, patients with adenomas had a higher prevalence of SSAs than those without adenomas.

Key words: Sessile serrated adenoma; Sessile serrated adenoma detection rate; Adenoma detection rate; Colonoscopy; Interval colorectal cancer

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Core tip: Sessile serrated adenomas (SSAs) are difficult to detect and are associated with interval colorectal cancer (CRC). To reduce interval CRC and CRC death, SSA detection is important, and evaluation of the sessile serrated adenoma detection rate (SSADR) is crucial. In Western countries, there have been some reports showing the correlation of adenoma detection rate (ADR) and SSADR. However, in Asian countries, little is known about the correlation between ADR and SSADR. We investigated the association between ADR and SSADR and significant predictors for SSA detection in Japanese population. We found that ADR is correlated with SSADR, and patients with adenomas have a higher prevalence of SSAs than those without adenomas.

Ohki D, Tsuji Y, Shinozaki T, Sakaguchi Y, Minatsuki C, Kinoshita H, Niimi K, Ono S, Hayakawa Y, Yoshida S, Yamada A, Kodashima S, Yamamichi N, Hirata Y, Ushiku T, Fujishiro M, Fukayama M, Koike K. Sessile serrated adenoma detection rate is correlated with adenoma detection rate. *World J Gastrointest Oncol* 2018; 10(3): 82-90 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i3/82.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i3.82>

INTRODUCTION

Colorectal cancer (CRC) is one of the major causes of cancer mortality in the world^[1]. Incidence of CRC has been increasing in Japan, and it is now the second leading cause of cancer-related death^[2]. Colonoscopy currently plays a central role in CRC screening^[3-5]. Total colonoscopy has been shown to reduce the risk of death from CRC by removing precancerous adenomas^[5]. Total colonoscopy and detection of adenomas are imperative for preventing CRC. The adenoma detection rate (ADR) has been reported to be an excellent quality indicator of total colonoscopy^[6,7]. ADR is also associated with the risk of interval CRC and death^[8,9].

However, there have been some reports indicating that total colonoscopy is less effective in reducing the risk of cancer in the proximal colon^[10,11]. The presence of sessile serrated adenomas (SSAs) in the right colon, which would progress *via* the serrated pathway to CRC, is thought to be a potential reason. A serrated pathway is an alternative pathway in which serrated polyps replace the traditional adenoma as precursor lesions to CRC^[12]. CRCs derived from serrated pathways account for 20%-30% of all CRCs^[13,14]. SSAs are usually flat or sessile, and are occasionally covered by a mucous cap^[13]. They are difficult to detect because of their subtle morphology, and even when detected, are often incompletely resected. In addition, some SSAs are reported to progress to invasive cancer in a short period of time^[15,16]. Therefore, SSAs are thought

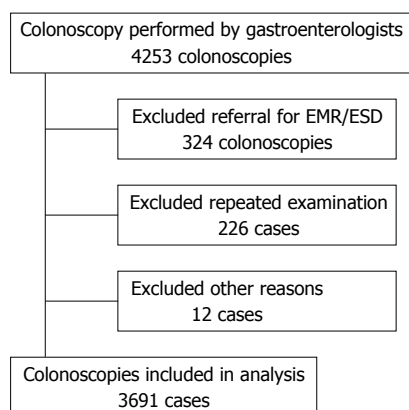


Figure 1 Study flow chart. EMR: Endoscopic mucosal resection; ESD: Endoscopic submucosal dissection.

to be strongly associated with interval CRC^[16,17]. To reduce interval CRC and CRC-related death, detection of SSAs is important, and evaluation of the SSA detection rate (SSADR) is crucial. Recently, there have been few reports suggesting that SSADR is associated with ADR^[17,18]. However, to the best of our knowledge, there has been no report in Asian countries showing a correlation between ADR and SSADR. In this context, we investigated the association between ADR and SSADR with significant predictors for SSA detection in total colonoscopy screening or surveillance in the Japanese population.

MATERIALS AND METHODS

Patients

This study is a retrospective, single-center analysis. We extracted data on total colonoscopies performed at the University of Tokyo Hospital between January and December 2014 by reviewing electronic medical records. All total colonoscopies performed by gastroenterologists were included in this analysis. Indications for total colonoscopy were classified as surveillance total colonoscopy, positive fecal occult blood test, screening for other symptoms (e.g., abdominal pain, anemia, and chronic diarrhea), and others. The following colonoscopies were excluded: repeated examinations during the study period and referral colonoscopies for endoscopic mucosal resection/endoscopic submucosal dissection (Figure 1). All gastroenterologists involved in this study had more than 5 years of experience in total colonoscopy.

In this study, we classified the pathology of each resected polyp into the following categories: low- or high-grade adenoma, cancer (including intramucosal cancer), SSA, or SSA with cytological dysplasia (Figures 2 and 3). Polyps that were resected but not histologically evaluated, and endoscopically detected polyps that were not resected, were determined to be non-neoplastic. The histological definition for SSAs was in accordance with the definition of the Japanese Society for Cancer of the Colon and Rectum^[19]. SSAs had two or more of

the following features in more than 10% of the serrated area: (1) Dilated crypt; (2) irregularly branching crypt; and/or (3) dilation of the base of the crypt which often has a boot, L, or inverted T shape. SSA with cytological dysplasia was defined as a dysplastic area, similar to conventional adenoma^[19,20]. In our institution, the comprehensive retrospective analysis of each patient's medical record was approved by our ethics committee (No. 2058); this study is included in that category. The present study was performed in accordance with the Declaration of Helsinki.

Procedure

The bowel preparation method in our institution was as follows: (1) 10 mL of 0.75% sodium picosulfate the day before endoscopy; and (2) 2–4 L of polyethylene glycol (Niflec: EA Pharma, Tokyo, Japan) on the morning of the endoscopy.

Video processor unit EVIS LUCERA SPECTRUM or EVIS LUCERA ELITE (Olympus Corporation, Tokyo, Japan) and single-channel lower gastrointestinal endoscope (PCF-Q260AZI, PCF-Q260AI, PCF-PQL, CF-240AI; Olympus Co.) were used. The choice of the endoscope was left to the discretion of each endoscopist.

Almost all colonoscopies were performed without sedation, but in some special cases where patients could not tolerate the colonoscopy procedure, conscious sedation using diazepam with or without pentazocine was administered.

Examination items

The polyp detection rate and location of each polyp were investigated. The proximal colon was defined as the area proximal to the splenic flexure (transverse colon, ascending colon, and cecum), while the distal colon was defined as the area distal to the splenic flexure (descending colon, sigmoid colon, and rectum). ADR was calculated as described in previous literature^[6,21]: the proportion of colonoscopies where at least one colorectal low- or high-grade adenoma or cancer was detected. SSADR was calculated in the same way: the proportion of colonoscopies where at least one SSA or SSA with cytological dysplasia was detected.

Factors possibly related to adenoma detection and SSA detection was assessed: (1) Patients' age; (2) patients' sex; (3) years of colonoscopy experience of the endoscopist; (4) withdrawal time; (5) cecal intubation rate; and (6) bowel cleansing level. Withdrawal time was defined as the time from identification of cecum to identification of anus in colonoscopy cases where no polyps were removed. The bowel cleansing level was classified as "adequate" or "non-adequate" according to the ASGE/ACG task force recommendations. "Adequate" was defined as the examination allowed for the detection of polyps > 5 mm in size^[6,22].

Statistical analysis

Characteristics of patients were summarized and compared between the presence (+) or absence (-)

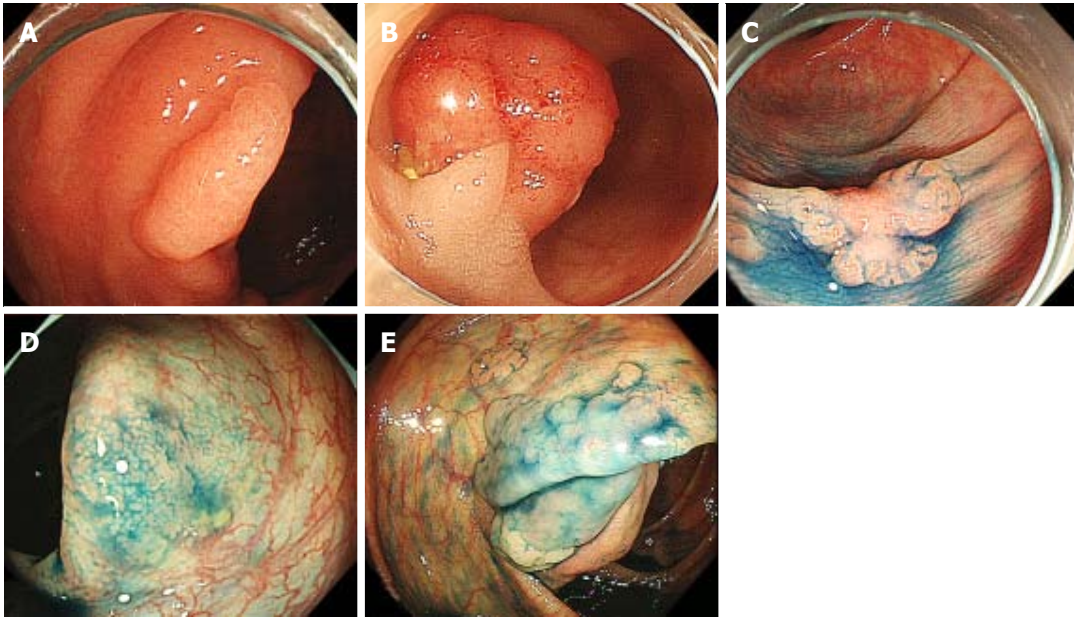


Figure 2 Typical endoscopic pictures of each polyp. A: Low grade adenoma; B: High grade adenoma; C: Cancer; D: Sessile serrated adenoma; E: Sessile serrated adenoma with cytological dysplasia.

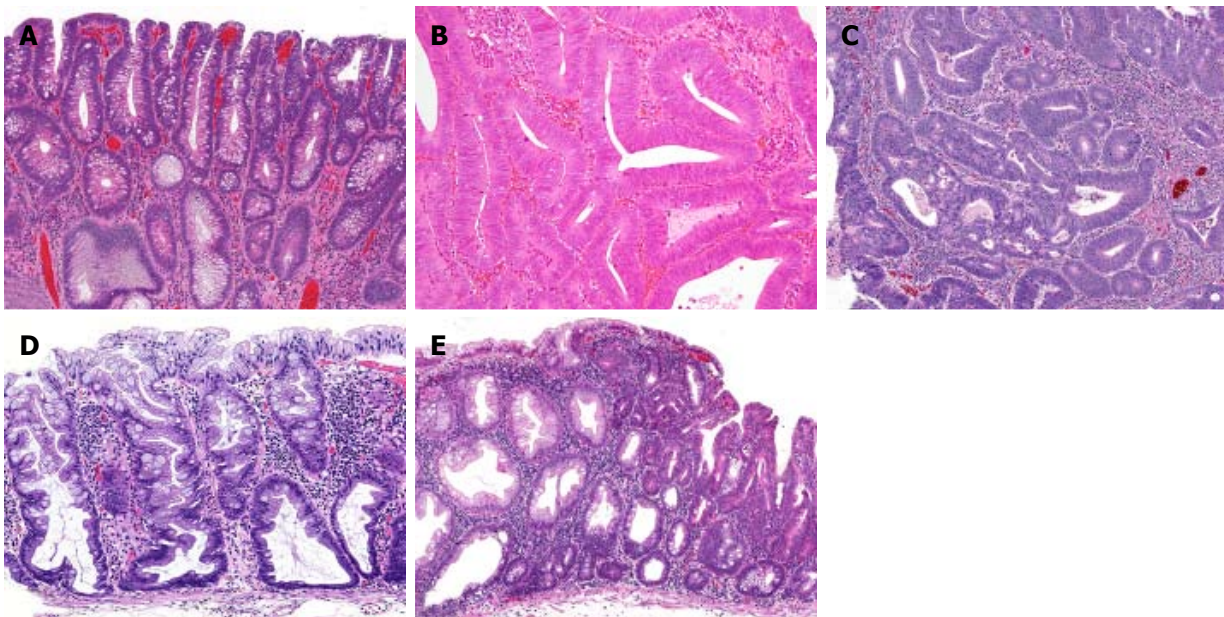


Figure 3 Histopathological pictures of each polyp. A: Low grade adenoma; B: High grade adenoma; C: Cancer; D: Sessile serrated adenoma; E: Sessile serrated adenoma with cytological dysplasia.

of adenoma detection using *t*-test or chi-squared test. Gastroenterologists' experience and their average withdrawal time that was calculated after excluding polypectomy were also summarized. Predictors of ADR were examined using logistic generalized estimating equation models, which explain the adenoma detection probability of each total colonoscopy by patient- and gastroenterologist-level variables. We used robust sandwich variance estimators that specified each gastroenterologist as a cluster to compute 95% confidence intervals (CI) and *P*-values. Predictors of SSADR were similarly examined, but adenoma detection

of corresponding total colonoscopy was added as a predictor. The bivariate association of SSADR and ADR of each gastroenterologist were illustrated by a scatter plot and correlation coefficient that were weighted by the number of performed total colonoscopies. All analyses were conducted using SAS version 9.4 (Cary, NC, United States).

RESULTS

Study group and characteristics of colonoscopies

A total of 4253 colonoscopies were performed by

Table 1 Patient characteristics

	Total (<i>n</i> = 3691)
Age, mean ± SD (yr)	63.5 ± 13.3
Sex: Male (%)	2224 (60.3)
Adequate bowel cleansing (%)	3585 (97.1)
Cecal intubation rate (%)	3636 (98.5)
Indications for colonoscopy (%)	
Surveillance	1314 (35.6)
Fecal occult blood test	538 (14.6)
Screening for other symptoms	544 (14.7)
Others	1295 (35.1)

Others include screening before surgery or chemotherapy, patients' desire, and so on.

Table 2 Gastroenterologist characteristics

	<i>n</i> = 35
Sex: Male (%)	25/35 (71.4)
Years of experience in colonoscopy (%)	
5-9	24/35 (68.6)
10-14	6/35 (17.1)
≥ 15	5/35 (14.3)
Number of colonoscopies performed (%)	
≤ 100	19/35 (54.3)
100-200	10/35 (28.6)
≥ 200	6/35 (17.1)
Withdrawal time: Mean (SD), min	10.1 ± 6.9

gastroenterologists during the study period. Overall, 562 colonoscopies were excluded based on the predetermined criteria, and 3691 colonoscopies were included in the analysis (Figure 1). Baseline characteristics of colonoscopies are shown in Table 1. Adequate bowel cleansing and cecal intubation rate were observed in 3585 (97.1%) cases and 3636 (98.5%) cases, respectively.

Characteristics of gastroenterologist

Baseline characteristics of gastroenterologists are shown in Table 2. All gastroenterologists had at least 5 years of colonoscopy experience; 16 (45.7%) gastroenterologists performed more than 100 cases a year.

Detection of each polyp

Low- and high-grade adenomas, and cancers were found in 978 (26.5%) cases, 84 (2.2%) cases and 81 (2.2%) cases, respectively. Overall ADR was 29.5% (men 33.2%, women 23.8%). SSAs and SSAs with cytological dysplasia were found in 66 (1.8%) cases and 2 (0.1%) cases, respectively. Overall SSADR was 1.8% (men 1.7%, women 2.1%).

The location of each polyp was also investigated. Altogether, 672 low-grade adenomas (68.8% of all the detected low-grade adenomas), 58 (69.9%) high-grade adenomas, 29 (34.5%) cancers, 52 (78.8%) SSAs, and 2 (100%) SSAs with cytological dysplasia were found in the proximal colon.

Predictors for adenoma detection

Univariable and multivariable analyses were performed

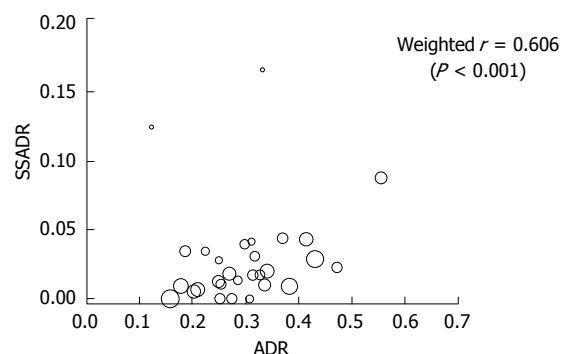


Figure 4 Weighted scatter plot and correlation coefficient for detection rates of sessile serrated adenomas and adenomas of each gastroenterologist. The area of the circle is proportional to the number of colonoscopies performed. SSADR: Sessile serrated adenomas; ADR: Adenomas.

to evaluate factors associated with adenoma detection (Table 3). In our institution, the cecal intubation rate was almost 100%, but could not be used in the analyses. Mean withdrawal time was 10 min, and there were only 2 gastroenterologists whose withdrawal time was less than 6 min. According to the scatter diagram plotting each endoscopist's ADR against their mean withdrawal time, as previously reported^[7], the recommended ADR level of 25%^[6] corresponded to a withdrawal time of 8 min. All factors, except for years of colonoscopy experience, were significantly associated with adenoma detection in both analyses with a 5% significance level. Being a woman (adjusted OR: 0.61, 95%CI: 0.54-0.70; *P* < 0.001) and those with non-adequate bowel cleansing (adjusted OR: 0.32, 95%CI: 0.19-0.52; *P* < 0.001) had a statistically inverse relationship with adenoma detection. Mean withdrawal time ≥ 8 min had statistically significant correlation with adenoma detection (adjusted OR: 1.77, 95%CI: 1.28-2.46; *P* < 0.001).

Predictors for sessile serrated adenoma detection

Univariable and multivariable analyses were performed to evaluate factors associated with SSA detection (Table 4). Both analyses revealed that adenoma detection was the only significant predictor for SSA detection (adjusted OR: 2.53, 95%CI: 1.53-4.20; *P* < 0.001). Mean withdrawal time ≥ 8 min tended to be associated with SSA detection, but was not statistically significant (adjusted OR 1.53; 95%CI: 0.62-3.75; *P* = 0.35).

Correlation between ADR and SSADR

As for the correlation between ADR and SSADR, a scatter diagram of ADR and SSADR is shown in Figure 4. The correlation coefficient between ADR and SSADR weighted by the number of each gastroenterologist's examinations was 0.606 (*P* < 0.001).

DISCUSSION

In the present study, a relatively strong association between ADR and SSADR was observed. Some reports

Table 3 Odds ratio estimates from logistic generalized estimating equations for adenoma detection

Variable	Univariable model		Multivariable model	
	OR (95%CI)	P	OR (95%CI)	P
Patient-level variable				
Age (yr)	1.02 (1.02, 1.03)	< 0.001	1.02 (1.02, 1.03)	< 0.001
Female	0.63 (0.55, 0.71)	< 0.001	0.61 (0.54, 0.70)	< 0.001
Non-adequate bowel cleansing	0.36 (0.22, 0.57)	< 0.001	0.32 (0.19, 0.52)	< 0.001
Endoscopist-level variable				
Endoscopist's experiment (yr)	0.98 (0.94, 1.02)	0.36	0.99 (0.96, 1.02)	0.55
Mean withdrawal time \geq 8 min (<i>vs</i> < 8 min)	1.72 (1.23, 2.41)	0.0015	1.77 (1.28, 2.46)	< 0.001

Multivariable model simultaneously adjusted for listed variables. Confidence intervals and *P*-values were calculated by robust variance specifying a gastroenterologist as a cluster.

Table 4 Odds ratio estimates from logistic generalized estimating equations for sessile serrated adenoma detection

Variable	Univariable model		Multivariable model	
	OR (95%CI)	P	OR (95%CI)	P
Patient-level variable				
Adenoma detection (<i>vs</i> none)	2.44 (1.45, 4.09)	< 0.001	2.53 (1.53, 4.20)	< 0.001
Age (yr)	0.99 (0.98, 1.01)	0.27	0.99 (0.98, 1.00)	0.07
Female	1.28 (0.77, 2.11)	0.34	1.40 (0.85, 2.29)	0.19
Non-adequate bowel cleansing	0.50 (0.07, 3.47)	0.48	0.60 (0.08, 4.28)	0.61
Endoscopist-level variable				
Endoscopist's experiment (yr)	0.99 (0.89, 1.10)	0.86	1.00 (0.91, 1.09)	0.96
Mean withdrawal time \geq 8 min (<i>vs</i> < 8 min)	1.74 (0.70, 4.29)	0.23	1.53 (0.62, 3.75)	0.35

Multivariable model simultaneously adjusted for listed variables.

have described the correlation of ADR and SSADR in Western countries patients^[17,18]; however, to our knowledge, the prevalence of SSAs or SSADR in Asian populations has not yet been fully investigated and appropriate SSADR has not been determined. Therefore, our study holds importance, as it is the first report to demonstrate the correlation between SSADR and ADR in Asian populations.

There is controversy regarding the prevalence of SSAs, which differs among previously published studies, varying from 2%-10%^[13,17,18,23,24]. In our institution, the prevalence of SSAs was approximately 2%, which is lower than previously reported results in Western populations. Each endoscopist's cognitive capability to detect SSAs may differ in degree. Payne *et al*^[25] reported that the prevalence of SSAs varied among endoscopy centers. In addition, Abdeljawad K *et al*^[26] reported that a review of pathology slides by an experienced gastrointestinal pathologist increased the prevalence of SSAs, and the prevalence of SSAs increased over the study period, suggesting that each endoscopist improved his detection skills over time. However, the gastroenterologist's ADR in this study was approximately 30%, which is within the standard of quality indicators for colonoscopy specified by ASGE^[6]. Therefore, the quality of the present study is assured. The quality of the pathological evaluation was also high, because the experienced gastrointestinal pathologist (U.T.), who was acquainted with the definition of the Japanese Society for Cancer of the Colon and Rectum, reassessed the pathology slides. As previously mentioned, the prevalence of SSAs

in Asian populations has not been determined, as there may be a difference between races. It is mandatory to investigate the true prevalence of SSAs in Asian populations in the future.

The factors associated with SSA detection were investigated, and our study demonstrated that adenoma detection at the patient level was the only independent significant factor associated with SSA detection. Previous reports have shown that when a patient presented with serrated lesions, especially SSAs, he/she was also more likely to have advanced neoplasia^[23,27-29]. These results were compatible with previous reports and suggested that ADR is correlated with SSADR.

A withdrawal time of \geq 8 min was not a statistically significant factor for SSA detection, although it was significantly related to adenoma detection. However, considering that ADR and SSADR are correlated, a longer duration of inspection seems to improve ADR and SSADR. In this study, the total number of SSAs was quite small. This may be a reason why a significant association between withdrawal time and SSA detection was not found.

We acknowledge that there were several limitations in our study. First, this study was a retrospective single center study, and the number of SSA cases was small. Second, there were many cases of total colonoscopy surveillance in the present study in addition to total colonoscopy screening. As previously stated, the target ADR should be changed according to patient risk^[30]. However, factors associated with adenoma detection in this study were similar to those in previous reports.

Moreover, Anderson JC reported that the serrated polyp detection rate was similar for screening or surveillance indications, suggesting that both indications could be used to derive the serrated polyp detection rate in practice^[31].

Rex *et al.*^[32] has also recently reported that using overall ADR to calculate ADR from screening, surveillance, and diagnostic colonoscopies would be just as effective as a screening-only ADR. Taking this into account, the current findings can be applied to clinical practice to some extent. Finally, the ratio of adequate bowel cleansing in this study was much higher than in previous studies. The ASGE guidelines recommend that the quality of bowel cleansing should be evaluated after retained fluid or stool has been suctioned^[6]. In our institution, if fluid and stool were retained, gastroenterologists suctioned as much as possible to identify polyps ≥ 5 mm in size. Such cases were considered adequate in our study, and therefore, the ratio of the "adequate" level was high.

In conclusion, our study suggests that ADR is correlated with SSADR. In addition, patients with adenomas may have a higher prevalence of SSAs than those without adenomas. A large-scale prospective study will be needed to validate these findings.

ARTICLE HIGHLIGHTS

Research background

Sessile serrated adenomas (SSAs) are difficult to detect and strongly associated with interval colorectal cancer (CRC). It is necessary to investigate the factors which influence SSA detection and to evaluate the SSA detection rate (SSADR).

Research motivation

In Western countries, some reports have described the correlation of ADR and SSADR. However, to the best of our knowledge, there has been no report in Asian countries showing a correlation between ADR and SSADR. In this context, we investigated the association between ADR and SSADR with significant predictors for SSA detection in total colonoscopy screening or surveillance in the Japanese population.

Research objectives

The main objectives were as follows; the prevalence of each polyp (low-grade or high-grade adenoma, cancer, SSA, or SSA with cytological dysplasia), each gastroenterologist's ADR and SSADR, the association between ADR and SSADR for each gastroenterologist and predictors of adenoma and SSA detection.

Research methods

Total colonoscopies performed by the gastroenterologists at the University of Tokyo Hospital between January and December 2014 were retrospectively identified. The prevalence of each type of polyp was investigated. Predictors of adenoma and SSA detection were examined using logistic generalized estimating equation models. The association between ADR and SSADR for each gastroenterologist was investigated by calculating a correlation coefficient weighted by the number of each gastroenterologist's examination.

Research results

A total of 3691 colonoscopies by 35 gastroenterologists were assessed. 978 low grade adenomas (26.5%), 84 high grade adenomas (2.2%), 81 cancers (2.2%), 66 SSAs (1.8%) and 2 SSAs with cytological dysplasia (0.1%) were

detected. Adenoma detection was the only significant predictor of SSA detection (adjusted OR: 2.53, 95%CI: 1.53-4.20; $P < 0.001$). The correlation coefficient between ADR and SSADR weighted by the number of each gastroenterologist's examinations was 0.606 ($P < 0.001$).

Research conclusions

Our study suggests that ADR is correlated with SSADR. Some reports have described the correlation of ADR and SSADR in Western countries patients; however, to our knowledge, the prevalence of SSAs or SSADR in Asian populations has not yet been fully investigated and appropriate SSADR has not been determined. Therefore, our study holds importance, as it is the first report to demonstrate the correlation between SSADR and ADR in Asian populations. In addition, patients with adenomas may have a higher prevalence of SSAs than those without adenomas.

Research perspectives

This study was a retrospective single center study, and the number of SSA cases was small. Therefore, a large-scale prospective study will be needed to validate these findings.

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Case of pancreatic metastasis from colon cancer in which cell block using the Trefle® endoscopic scraper enables differential diagnosis from pancreatic cancer

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Abstract

Endoscopic transpapillary brush cytology and forceps biopsy during endoscopic retrograde cholangiopancreatography are generally used to obtain pathological evidence of biliary strictures. Recently, the new endoscopic scraper Trefle® has been reported and demonstrated high cancer detectability in malignant biliary strictures. This device is used to scrape the stricture over the guidewire, and, in the original method, the tissue and/or cell samples obtained are subjected

to histological and/or cytological analysis separately. However, discrimination of chunks of tissue is hampered by the opacity of the surrounding fluid. We have developed a cell block technique for the Trefle® device without dividing obtained specimens into tissue and cellular components, which is the simplest method and enables immunohistochemical analysis. We present a case of obstructive jaundice diagnosed immunohistochemically as pancreatic metastasis from colon cancer using cell block sections obtained with the Trefle® device, which procedure is as easy as conventional brush cytology.

Key words: Trefle®; Cell block; Endoscopic scraper; Pancreatic metastasis; Biliary strictures

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Core tip: We described a case of pancreatic metastasis from colon cancer in which cell block technique with the specimens obtained by the new endoscopic device Trefle® was useful in the differential diagnosis from pancreatic cancer. The combination of cell block technique and Trefle® might be a promising method in the diagnosis of biliary strictures because this procedure is as easy as conventional brush cytology.

Kato A, Naitoh I, Kato H, Hayashi K, Miyabe K, Yoshida M, Hori Y, Natsume M, Jinno N, Yanagita T, Takiguchi S, Takahashi S, Joh T. Case of pancreatic metastasis from colon cancer in which cell block using the Trefle® endoscopic scraper enables differential diagnosis from pancreatic cancer. *World J Gastrointest Oncol* 2018; 10(3): 91-95 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i3/91.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i3.91>

INTRODUCTION

Accurate diagnosis of biliary strictures is challenging, despite development of various imaging modalities. It is essential to diagnose the cause of biliary strictures using pathological evidence prior to selection of the appropriate therapy. Endoscopic transpapillary brush cytology during endoscopic retrograde cholangiopancreatography (ERCP) is conventionally used to obtain specimens for pathological diagnosis of biliary strictures, because it is technically easy and rapid. To provide larger tissue samples and improve sensitivity, endoscopic transpapillary forceps biopsy is also frequently performed^[1,2]. However, forceps biopsy is technically more difficult and time-consuming than brush cytology^[3,4]. Benign and malignant lesions can be diagnosed using cytology specimens, but these cannot be subjected to immunohistochemical analysis, despite its utility for differential diagnosis. The Trefle® endoscopic scraper (Piolax Medical Devices, Yokohama, Japan) enables detection of cancer in malignant biliary strictures^[5]. This device has three scraping loops and was designed to access biliary strictures over the

guidewire and obtain tissues and/or cell samples for histology or cytology. The procedure using the Trefle® device is almost identical to that for conventional brush cytology; scraped tissues and/or cell samples, together with bile juice, are aspirated from the side port of the outer sheath into a syringe. In the original method, after allowing the aspirate to settle in a sterile tube, specimens were divided into tissue and fluid components for histological and cytological analyses, respectively. However, discrimination of chunks of tissue is hampered by the opacity of the surrounding fluid. Therefore, a simpler method of processing specimens obtained using the Trefle® device is required.

The cell block technique improves diagnostic yield and facilitates immunohistochemical analysis^[6-9]. We typically use the Trefle® device to obtain specimens from biliary strictures, which, together with aspirated bile juice and affixed tissues, are poured into a sterile tube. The tube is sent to the Pathology Department for evaluation by the cell block method, which enables differentiation of benign from malignant lesions, as well as immunohistochemical analysis during any time of need. We report here a case of obstructive jaundice with pancreatic metastasis from colon cancer, differential diagnosis of which from pancreatic cancer was performed by immunohistochemical examination of cell block sections obtained using the Trefle® device.

CASE REPORT

A 69-year-old male underwent laparoscopic low anterior resection for rectal adenocarcinoma (stage IV; pT4N2M1 according to the American Joint Committee on Cancer 7th Edition Cancer Staging Manual) 18 mo prior and received adjuvant chemotherapy [FOLFOX (folinic acid, 5-fluorouracil, and oxaliplatin) plus panitumumab as the first line and FOLFIRI (folinic acid, 5-fluorouracil, and irinotecan) plus bevacizumab as the second line]. Metastases to the liver and lung occurred despite administration of second-line chemotherapy, and the patient presented with epigastric pain and jaundice. Laboratory evaluation revealed high aspartate/alanine transaminase levels (777/394 IU/L) and bilirubin/direct bilirubin levels (11.4/7.3 mg/dL) (Table 1).

Contrast-enhanced computed tomography (CT) revealed a defined 1.5 cm × 1.5 cm mass, which was poorly enhanced in both the early and late phases, at the pancreatic head, dilated common bile duct and upstream main pancreatic duct, as well as masses in both lobes of the liver and both lungs (Figure 1A and B). The patient was diagnosed with obstructive jaundice due to primary pancreatic ductal adenocarcinoma or pancreatic metastasis from colon cancer. Therefore, we planned to perform endoscopic biliary drainage to treat the obstructive jaundice and obtain histopathological evidence.

An ERCP demonstrated a biliary stricture of the lower common bile duct approximately 2 cm in length, as well as dilatation of the proximal bile duct (Figure

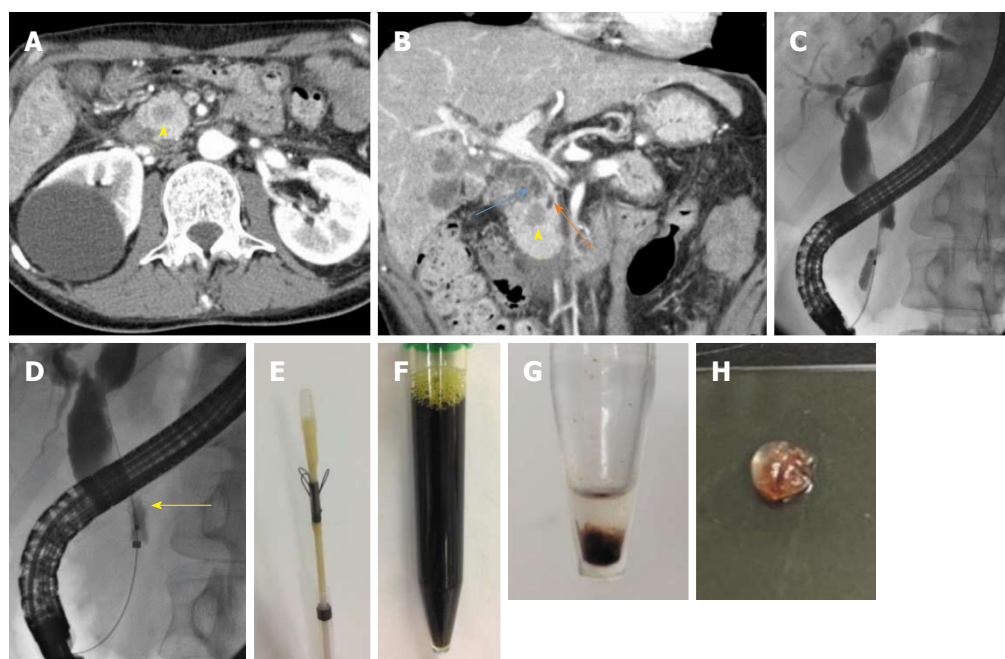


Figure 1 Imaging findings and samples obtained using the Trefle® device. A and B: Abdominal computed tomography indicated a poorly enhanced region (yellow arrowhead), dilated common bile duct (blue arrow), and upstream main pancreatic duct (orange arrow); C: Endoscopic retrograde cholangiopancreatography demonstrated a biliary stricture; D: The Trefle® device was inserted and opened, and the scraping loops were identified under fluoroscopic guidance (yellow arrow); E: Appearance of the Trefle® device; F: Appearance of samples obtained; G and H: Appearance of the centrifuged deposit.

Table 1 Laboratory data

Variable	Value	Reference range
White blood cell	10.6	$3.6-9.6 \times 10^3/\mu\text{L}$
Hemoglobin	12.1	13.2-17.2 g/dL
Platelet	541	$148-339 \times 10^3/\mu\text{L}$
C-reactive protein	5.55	$\leq 0.30 \text{ mg/dL}$
Aspartate transaminase	777	13-33 IU/L
Alanine transaminase	394	6-30 IU/L
Lactate dehydrogenase	405	119-229 IU/L
Alkaline phosphatase	4861	115-359 IU/L
γ -glutamyl transpeptidase	1347	10-47 IU/L
Amylase	404	37-125 IU/L
Total bilirubin	6.3	0.3-1.2 mg/dL
Direct bilirubin	4.3	0.0-0.3 mg/dL
Carcinoembryonic antigen	12.5	$< 5.0 \text{ ng/mL}$
Carbohydrate antigen 19-9	13280.0	$< 37.0 \text{ U/mL}$

1C). After performing endoscopic sphincterotomy (EST), the Trefle® device was inserted into the bile duct over the guidewire. Next, the scraping loops of the device were opened and passed through the stricture in the proximal-to-distal direction under fluoroscopic guidance (Figure 1D). All specimens including aspirated bile juice and tissues were transferred to a sterile tube; the scraping loops were cut using scissors (Figure 1E and F). The centrifuged deposit was fixed in formalin overnight. Next, the deposit was washed in saline, mixed with 1% sodium aspartate, and centrifuged again. Finally, the deposit was put a few drop of 1 M calcium chloride and embedded in paraffin, yielding a cell block (Figure 1G and H). The cell block was sectioned for hematoxylin-and-eosin (HE) and immunohistochemical staining. The lesion was confirmed to be moderately differentiated

adenocarcinoma, which by immunohistochemical staining was focally positive for cytokeratin 7 (CK 7) and positive for CK 20 and caudal type homeobox 2 (CDX 2). These findings were consistent with those of previous resected specimens, confirming the final diagnosis of pancreatic metastasis from colon cancer (Figure 2).

A covered self-expanding metal stent (SEMS) was inserted to resolve the symptoms and establish biliary drainage. The third-line chemotherapy regimen, FOLFIRI plus ramucirumab, was administered based on the results of immunohistochemical examination, and the patient is alive at the time of writing. The combination of the cell-block technique and the Trefle® device was useful for making decisions regarding management of this patient.

DISCUSSION

This case demonstrated contrast-enhanced CT findings compatible with typical pancreatic ductal adenocarcinoma with hypovascular tumor and a dilated upstream main pancreatic duct. In this case, differential diagnosis of pancreatic metastasis of colon cancer was necessary, because the patient had a medical history of colon cancer with distant metastasis. However, pancreatic metastasis from colon cancer is rare in clinical practice. Pancreatic metastases from non-pancreatic primary tumors are rare, accounting for approximately 2% of all pancreatic neoplasms^[10], and arise most commonly from primary tumors of the kidney, lung, breast, and colon. Immunohistochemistry is essential for identifying the primary site of metastatic neoplasms using molecular markers. Determination of CK 7, CK 20, and CDX 2

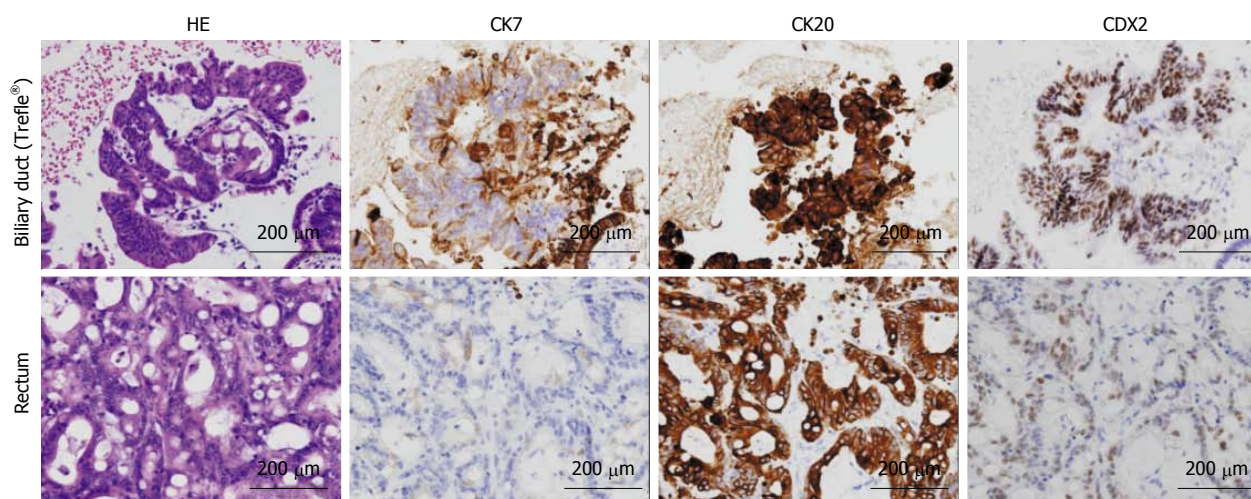


Figure 2 Histological findings. Immunohistochemical staining revealed that cancer cells in cell block specimens obtained using the Trefle® device were focally positive for cytokeratin 7 (CK 7), and positive for CK 20 and caudal type homeobox 2 (CDX 2). These findings were consistent with those of rectal resection specimens.

expression is useful for distinguishing colon cancer. CK 7 is expressed by various cancers, including that of the pancreas, but not the gastrointestinal tract. In contrast, CK 20 is expressed by most gastrointestinal tumors-including primary colonic, pancreatic, and gastric cancers, but is non-specific. CDX 2 is also expressed by colon adenocarcinoma, but at very low levels in most gastric and pancreatic tumors^[11-13]. Biopsy specimens are generally required for immunohistochemical analysis; cytology specimens are unsuitable for this purpose. However, the cell block method is appropriate for immunohistochemical analysis. Use of sodium aspartate as a fixative increases the cellularity, increasing morphological detail and improving the diagnostic sensitivity. The cell block method can also generate multiple sections for staining and immunohistochemistry^[14]. The efficacy of cell block method has been reported in the bile duct cytology and endoscopic ultrasound-guided fine needle aspiration of pancreas and gastrointestinal solid neoplastic lesions^[7-9].

The amount of tissue collected from the biliary tract by brush cytology is insufficient for immunohistochemical analysis, despite the need for immunohistochemical analysis to diagnose various diseases of the biliary tract, such as IgG4-SC or metastasis from cancer in other organs. Although endoscopic transpapillary forceps biopsy can be performed to obtain larger tissue samples, its success is dependent on operator skill because it is technically more difficult than brush cytology. Hence, alternative techniques that yield tissue samples of adequate size are required. In this case, we used the Trefle® endoscopic device, which has been demonstrated to be superior to forceps biopsy in terms of histologic/cytologic sample yield (93.5% vs 83.7%) and cancer detection (64.7% vs 51.3%)^[5]. Specimens obtained using the Trefle® device are divided into tissue and fluid components for histological and cytological analyses, respectively. However, distinguishing chunks of tissue is hampered by the opacity of the surrounding

fluid. In addition, some tissue may remain in the fluid component. Therefore, a simpler and more efficient specimen-processing method is needed. We typically subject specimens obtained using the Trefle® device to the cell block method to enable differentiation of benign and malignant lesions, as well as immunohistochemical examination. In the case presented herein, the cell block method with the Trefle® device facilitated differential diagnosis of a biliary stricture. Further studies involving larger populations are needed to confirm the efficacy of this method.

In conclusion, we describe a case of pancreatic metastasis from colon cancer in which the cell block technique, together with immunohistochemistry, enabled differential diagnosis from pancreatic cancer. The combination of the cell block technique and the Trefle® device shows promise for diagnosis of biliary strictures as it is as easy as conventional brush cytology.

ARTICLE HIGHLIGHTS

Case characteristics

The patient underwent resection for rectal adenocarcinoma presented metastases to the liver and lung with epigastric pain and jaundice.

Clinical diagnosis

The patient was diagnosed with obstructive jaundice.

Differential diagnosis

Primary pancreatobiliary carcinoma or pancreatic metastasis from colon cancer.

Laboratory diagnosis

Laboratory evaluation revealed the findings of obstructive jaundice.

Imaging diagnosis

The patient was diagnosed with obstructive jaundice due to primary pancreatic ductal adenocarcinoma.

Pathological diagnosis

Immunohistochemical findings of the cell block sections obtained using the

Trefle® endoscopic scraper were consistent with those of previous resected specimens, confirming the final diagnosis of pancreatic metastasis from colon cancer.

Treatment

A covered self-expanding metal stent was inserted to resolve the symptoms and establish biliary drainage and the third-line chemotherapy regimen for colon cancer was administered.

Related reports

There have been few reports dealing with the combination of a scraper Trefle® and cell block method for histocytological diagnosis of malignant biliary strictures.

Experiences and lessons

The combination of the cell block technique and the Trefle® device shows promise for diagnosis of biliary strictures as it is as easy as conventional brush cytology.

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World Journal of Gastrointestinal Oncology (*World J Gastrointest Oncol*, *WJGO*, online ISSN 1948-5204, DOI: 10.4251) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJGO covers topics concerning carcinogenesis, tumorigenesis, metastasis, diagnosis, prevention, prognosis, clinical manifestations, nutritional support, molecular mechanisms, and therapy of benign and malignant tumors of the digestive tract. The current columns of *WJGO* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of gastrointestinal oncology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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Prospective Study

Prospective real-time evaluation of diagnostic performance using endocytoscopy in differentiating neoplasia from non-neoplasia for colorectal diminutive polyps (≤ 5 mm)

Takahiro Utsumi, Yasushi Sano, Mineo Iwatate, Hironori Sunakawa, Akira Teramoto, Daizen Hirata, Santa Hattori, Wataru Sano, Noriaki Hasuike, Kazuhito Ichikawa, Takahiro Fujimori

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Author contributions: Utsumi T, Sano Y, Iwatate M, Sunakawa H, Teramoto A, Hirata D, Sano W, Hasuike N, Ichikawa K and Fujimori T designed the study; Utsumi T collected the data; Utsumi T, Sano Y and Iwatate M drafted the study, analyzed the data and wrote the manuscript.

Institutional review board statement: This study was approved by the Institutional Review Board at Sano Hospital.

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Abstract

AIM

To clarify the diagnostic performance of endocytoscopy for differentiation between neoplastic and non-neoplastic colorectal diminutive polyps.

METHODS

Patients who underwent endocytoscopy between October and December 2016 at Sano Hospital were prospectively recruited. When diminutive polyps (≤ 5 mm) were detected, the lesions were evaluated by endocytoscopy after being stained with 0.05% crystal violet and 1% methylene blue. The diminutive

polyps were classified into five categories (EC 1a, 1b, 2, 3a, and 3b). Endoscopists were asked to take a biopsy from any lesion diagnosed as EC1b (indicator of hyperplastic polyp) or EC2 (indicator of adenoma). We have assessed the diagnostic performance of endocytoscopy for EC2 and EC1b lesions by comparison with the histopathology of the biopsy specimen.

RESULTS

A total of 39 patients with 63 diminutive polyps were analyzed. All polyps were evaluated by endocytoscopy. The mean polyp size was 3.3 ± 0.9 mm. Among the 63 diminutive polyps, 60 were flat and 3 were pedunculated. The mean time required for EC observation, including the time for staining with crystal violet and methylene blue, was 3.0 ± 1.9 min. Histopathologic evaluation showed that 13 polyps were hyperplastic and 50 were adenomas. The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of EC2 for adenoma compared with EC1b for hyperplastic polyp were 98.0%, 92.3%, 96.8%, 98.0% and 92.3%, respectively. There were only two cases of disagreement between the endoscopic diagnosis made by endocytoscopy and the corresponding histopathological diagnosis.

CONCLUSION

Endocytoscopy showed a high diagnostic performance for differentiating between neoplastic and non-neoplastic colorectal diminutive polyps, and therefore has the potential to be used for "real-time histopathology".

Key words: Endocytoscopy; Diagnostic performance; Diminutive polyp; Endocytoscopic classification; Real-time histopathology

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Core tip: Single-Charge Coupled Device integrated type endocytoscopy (OLYMPUS, Japan) is going to be newly launched in 2018. Endocytoscopy with approximately 500-fold magnification capability allows us to observe both structural and cellular atypia *in vivo*, and is expected to be used as an optical biopsy. In our prospective study, we aimed to clarify the diagnostic performance of endocytoscopy in differentiating neoplasia from non-neoplasia for colorectal diminutive polyps (≤ 5 mm). The diagnostic performance of endocytoscopy met the threshold of the Preservation and Incorporation of Valuable endoscopic Innovations statement for "resect-and-discard" and "diagnose-and-leave" strategies.

Utsumi T, Sano Y, Iwatate M, Sunakawa H, Teramoto A, Hirata D, Hattori S, Sano W, Hasuike N, Ichikawa K, Fujimori T. Prospective real-time evaluation of diagnostic performance using endocytoscopy in differentiating neoplasia from non-neoplasia for colorectal diminutive polyps (≤ 5 mm). *World J Gastrointest Oncol* 2018; 10(4): 96-102 Available from: URL: <http://www.wjgnet.com>

INTRODUCTION

Colorectal cancer (CRC) is a major cause of morbidity and mortality worldwide^[1]. Resection of adenomas with colonoscopy has been shown to decrease the risk of subsequent CRC and CRC-related death^[2,3].

Technological advances in colonoscopy have now made it possible to determine the histopathology of colorectal polyps *in vivo*, which may potentially reduce the cost of histopathological assessment of adenomas and avoid unnecessary polypectomy for hyperplastic polyps^[4]. The American Society for Gastrointestinal Endoscopy (ASGE) has suggested key thresholds for assessing the histology of diminutive polyps (≤ 5 mm) using endoscopic technology in the Preservation and Incorporation of Valuable endoscopic Innovations (PIVI) document. This specified an agreement rate of $\geq 90\%$ for assignment of post-polypectomy surveillance intervals and a negative predictive value of $\geq 90\%$ for recto-sigmoid polyps with an adenomatous histology. However, it has been difficult for endoscopic real-time histologic assessment of diminutive polyps to meet the PIVI criteria, except for high-confidence assessment made by experts using narrow-band imaging (NBI)^[5,6].

The single-Charge Coupled Device integrated-type endocytoscope, which will be newly launched in early 2018 by Olympus Medical Systems Corporation (Tokyo, Japan), is based on the technology of light contact microscopy (Figure 1). It has ultra-high magnification capability, allowing observation of both structural and cellular atypia *in vivo* and in real time^[7-10]. The diagnostic accuracy of endocytoscopy for differentiating neoplastic from non-neoplastic colorectal polyps has already been reported to be 94.1%-100%^[9-11]. A randomized study of colorectal lesions larger or equal to 5mm in diameter showed that the diagnostic accuracy of endocytoscopy was not inferior to that of biopsy^[11]. Although it is expected that endocytoscopy will eventually be used as real-time histopathologic assessment, few studies have examined its diagnostic performance for diminutive polyps.

Therefore, we conducted the present prospective study to clarify the diagnostic performance of endocytoscopy for differentiating neoplastic from non-neoplastic diminutive colorectal polyps.

MATERIALS AND METHODS

Patients

Consecutive adult patients 20 years of age or older who underwent total colonoscopy with an endocytoscope between October and December 2016 in Sano Hospital were enrolled in this study. The exclusion criteria were as follows: (1) Patients with a history of inflammatory

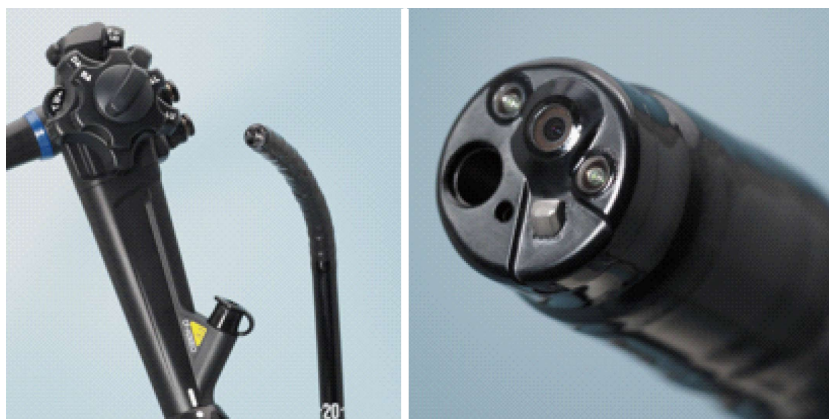


Figure 1 Technology of light contact microscopy, endocytoscope.

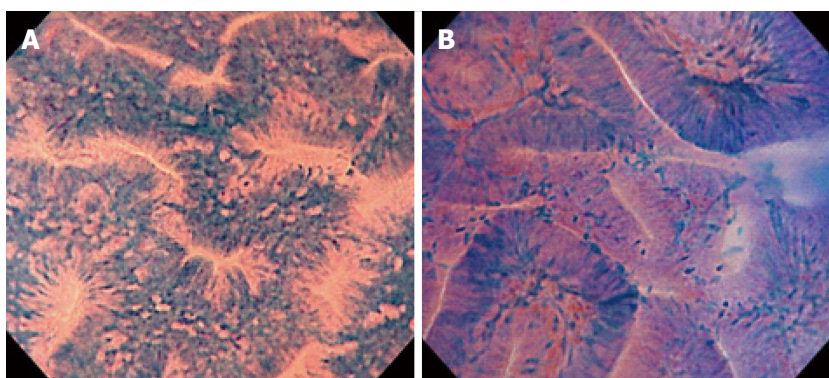


Figure 2 Typical images of endocytoscopic (EC) 1b and EC2. A: EC1b shows narrow serrated lumina and small roundish nuclei; B: EC2 shows slit-like smooth lumina and uniform fusiform or roundish nuclei.

bowel disease, hereditary polyposis syndrome, or Lynch syndrome; (2) patients with poor bowel preparation; and (3) patients without diminutive polyps. Written informed consent was obtained from all patients before the examination. The study protocol was approved by the institutional review board of Sano Hospital, Kobe, Japan. The clinical trial was registered in a clinical trial registry (UMIN 000023738).

Methods

The procedures were performed by 6 endoscopists who had extensive experience in magnifying colonoscopy (> 1000 cases). All had only limited experience of endocytoscopy before the study. We employed a prototype endocytoscope (CF-Y0058-1) with capability of approximately 500-fold magnification, which had the same specifications as the one released by Olympus, with a LUCERA video processor. We used a distal attachment fitted to the tip of the endocytoscope, which facilitated stable attachment to the surface of the polyps in all cases. All diminutive colorectal polyps were detected by white light imaging and evaluated by endocytoscopy as follow. First, the lesions were stained with 0.05% crystal violet and 1% methylene blue for 30 seconds using a non-traumatic tube^[12]. More dye was added if the staining of polyps

was initially inadequate. Endoscopists were asked to assign an endocytoscopic (EC) classification (1a, 1b, 2, 3a, and 3b) for each polyp after staining^[10]. The EC classification was based on the morphology of the lumina and the shape of nuclei in the lesions. EC1a was assigned to indicate normal mucosa, EC1b for hyperplastic polyp, EC2 for adenoma, and EC3a/3b for cancer. When many diminutive polyps were detected, only the first three were studied so as not to prolong the examination unnecessarily. Any inflammatory polyps were omitted from the study. When diminutive polyps were classified as EC1b or EC2, biopsy was performed (Figure 2). We then assessed the diagnostic performance of the endocytoscopy for EC2 and EC1b lesions by comparison with the corresponding histopathology of the biopsy specimen.

The location, size and shape (Paris classification) of all polyps and the examination time including dye staining were recorded^[13]. The biopsy specimens were diagnosed histopathologically by an experienced gastrointestinal histopathologist (T.F) according to the criteria of the World Health Organization without endoscopic diagnosis^[14]. We prospectively evaluated the diagnostic performance of endocytoscopy for differentiating between neoplastic and non-neoplastic polyps.

Table 1 Characteristics of the studied patients and resected polyps

Total patients, <i>n</i>	39
Male/female	23/16
Age (mean ± SD, yr)	65.8 ± 13.1
Polyps, <i>n</i>	63
Size (mean ± SD, mm)	3.3 ± 0.9
Location, right/left	33/30
Shape, protruding/flat/depressed	3/60/0
Histopathology	
Hyperplastic polyp	13
Adenoma	50

Table 2 Diagnostic performance of endocytoscopy for differentiating adenoma from hyperplastic polyp among diminutive colorectal polyps

EC classification	Hyperplastic polyp	Adenoma
	<i>n</i>	<i>n</i>
EC 1b	12	1
EC 2	1	49

Sensitivity 98.0% (95%CI: 89.3%-99.9%); Specificity 92.3% (95%CI: 64.0%-99.8%); Accuracy 96.8% (95%CI: 89.0%-99.6%); Positive predictive value 98.0% (95%CI: 89.3%-99.9%); Negative predictive value 92.3% (95%CI: 64.0%-99.8%). EC: Endocytoscopic.

Statistical analysis

Continuous variables are expressed as mean ± SD. In this study, the 95% confidence interval (95%CI) for diagnostic performance was calculated.

RESULTS

During the study period, 71 patients underwent total colonoscopy using an endocytoscope. Among these patients, 4 with a history of ulcerative colitis, 3 with poor bowel preparation, and 25 without diminutive polyps were excluded, leaving a total of 39 patients with 63 diminutive polyps for analysis. Table 1 shows the characteristics of the eligible patients and resected polyps. All eligible polyps were evaluated by endocytoscopy. The mean polyp size was 3.3 ± 0.9 mm. Among the 63 diminutive polyps, 60 were flat and 3 were pedunculated. The mean duration of EC observation, including the time taken for staining with crystal violet and methylene blue, was 3.0 ± 1.9 min. The relationship between endoscopic diagnosis using the EC classification and pathological diagnosis of colorectal diminutive polyps in terms of the ability to differentiate between adenoma and hyperplastic polyp is shown in Table 2. The sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of EC2 for adenoma compared with EC1b for hyperplastic polyp were 98.0%, 92.3%, 96.8%, 98.0% and 92.3%, respectively. There were two cases of disagreement between the endoscopic and histopathological diagnoses.

DISCUSSION

In this study, endocytoscopy achieved a high diagnostic performance for differentiating neoplastic from non-neoplastic diminutive colorectal polyps. To our knowledge, this is the first prospective study to have evaluated the performance of endocytoscopy for diminutive polyps in real time.

The diagnostic performance of endocytoscopy for diminutive polyps met the PIVI criteria for assessment of histology. A meta-analysis of studies that had evaluated image-enhanced endoscopy techniques such as NBI, i-SCAN, and Fuji Intelligent Chromo Endoscopy concluded that the diagnostic performance of NBI met the threshold only when assessments could be made with high confidence^[6]. The rate of high confidence for NBI-based diagnosis without magnification is reported to range from 75% to 80%^[4,15,16]. Endocytoscopy was able to achieve a high diagnostic performance for all diminutive colorectal polyps regardless of confidence level.

However, the diagnostic performance in our present study tended to be slightly lower than that in previous studies of endocytoscopy^[9,10]. There are several possible reasons for this difference. First, although some previous studies using endocytoscopy demonstrated a diagnostic accuracy of almost 100% in differentiating neoplasia from non-neoplasia, most of the polyps analyzed were small (6–9 mm) or large (≥ 10 mm). It is generally more difficult to diagnose diminutive polyps accurately than in the case for small or large polyps because any neoplastic changes in the former are expected to be minimal. Second, some earlier studies excluded lesions that were not imaged well by endocytoscopy. In order to obtain a fully magnified image with an endocytoscope, the scope should physically be in contact to the lesion. Factors including colonic peristalsis, movement due to breathing and heartbeat often make it difficult to obtain evaluable images using endocytoscopy. Kudo *et al.*^[10] reported that endocytoscopy yielded sufficiently clear images in 95.5% of the cases they studied. These factors also need to be considered when discussing the diagnostic performance of endoscopic instruments.

In the present study, there were two cases of disagreement between the EC diagnosis and the histopathological diagnosis made by biopsy. We investigated the causes of disagreement in these two cases. In one of them, although the endoscopist diagnosed the polyp as EC1b, the pictures taken suggested that it should have been diagnosed as EC2 (Figure 3). This suggests the need for more experience with real-time evaluation of 500-fold magnification images for assessment of cellular and nuclear morphology. In the other case, the polyp had a shallow depressed area on its surface, and the EC diagnosis of EC2 was based on the features in this depressed area. Histopathologic evaluation demonstrated a hyperplastic polyp, but a section of the pathology specimen revealed

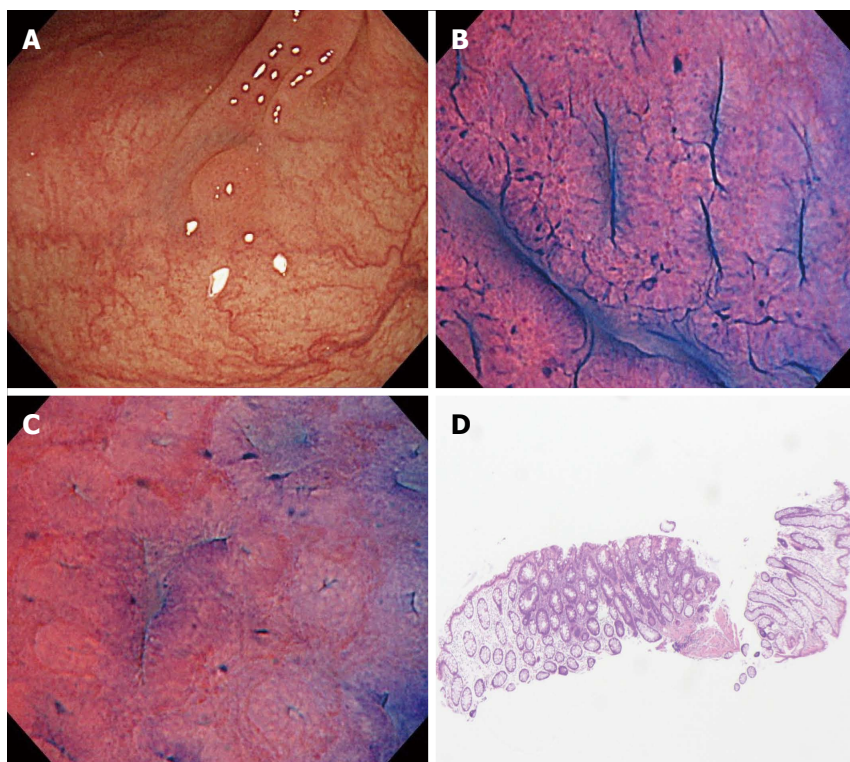


Figure 3 A case of disagreement between endocytoscopic (EC) 1b diagnosis and histopathologic diagnosis (adenoma). A: Flat-type lesion (0-IIa) 3 mm in size; B: The polyp was located in the sigmoid colon. The EC images showed slit-like smooth lumina; C: The EC images showed roundish lumina; D: The nuclei were not clear due to inadequate staining. An endoscopist diagnosed the polyp as EC1b. Histologic examination revealed an adenomatous polyp.

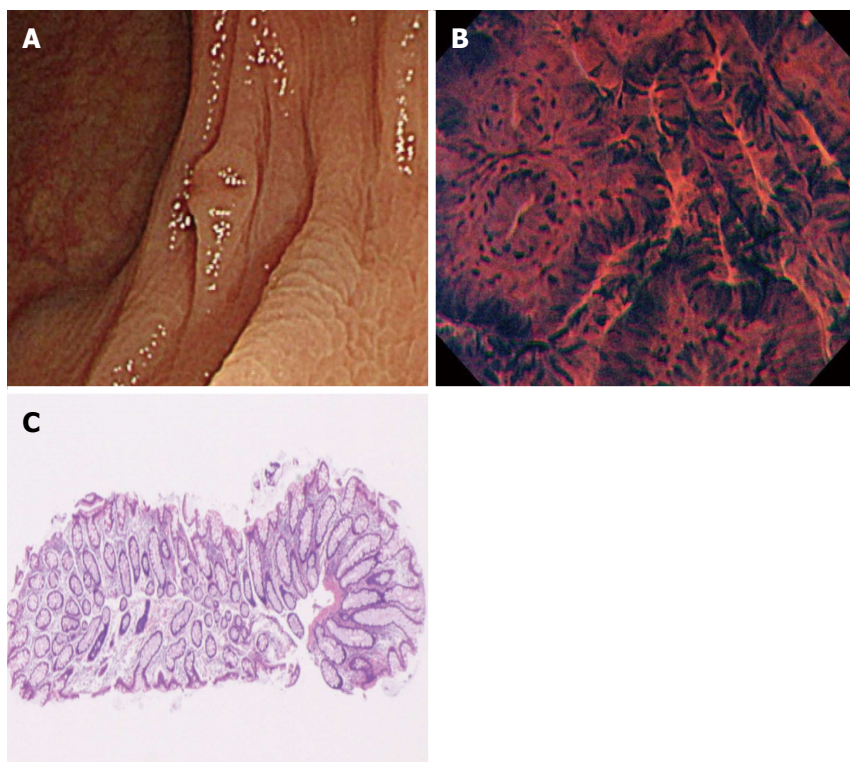


Figure 4 A case of disagreement between endocytoscopic (EC) 2 diagnosis and histopathologic diagnosis (hyperplastic polyp). A: Flat-type lesion (0-IIa) with a shallow depressed area, 3 mm in size; B: The polyp was located in the transverse colon. Endoscopic imaging in the shallow depressed area showed typical EC2 features with slit-like smooth lumina and uniform fusiform and roundish nuclei; C: The polyp was diagnosed as EC2. Histologic examination revealed a hyperplastic polyp without a depressed area.

no depressed area (Figure 4). We speculate that this discrepancy resulted from inadequate resection of the biopsy specimen, which did not provide the part showing adenomatous change.

Because endocytoscopy has a high diagnostic performance for diminutive polyps, it is applicable as a means of assessing real-time histopathology. However, to justify the clinical application of endocytoscopy, clarification of its disadvantages is essential. In the present study, it took approximately 3 min to observe each lesion, including the time required for staining. For this reason, it is not realistic to carry out endocytoscopy for all lesions when many polyps were detected. Solving this problem represents a significant challenge.

This study had some limitations. First, because we were using a prototype endocytoscope that was available to our institute for only a limited period, the number of samples was too small. Second, this study was performed at a single community-based hospital. Additional multicenter studies will be needed to confirm the present results. Third, all colonoscopists who performed endocytoscopy had enough experience of magnifying colonoscopy. The assessment during colonoscopy is a different experience from evaluations of only endoscopic still images. Endoscopists are required to obtain clear evaluable images in a colonic environment that is moving or contains remnant stool material^[17,18]. It remains to be examined whether non-experts in magnifying endoscopy can deliver highly reliable diagnoses using endocytoscopy.

We conclude from this study that endocytoscopy can achieve high diagnostic performance in terms of differentiating neoplastic from non-neoplastic diminutive colorectal polyps. Real-time histopathologic assessment by endocytoscopy *in vivo* has the potential to replace the conventional diagnosis by histopathologists *in vitro*.

ARTICLE HIGHLIGHTS

Research background

Endocytoscopy, which will be newly launched in 2018, allows us to observe both structural and cellular atypia *in vivo*. The Preservation and Incorporation of Valuable endoscopic Innovations (PIVI) document described key thresholds for assessing the histology of diminutive polyps (≤ 5 mm) using endoscopic technology. However, few studies about endocytoscopy have examined its diagnostic performance for diminutive colorectal polyps.

Research motivation

This study before the launch of endocytoscopy can help to understand the usefulness of endocytoscopy.

Research objectives

To clarify the diagnostic performance of endocytoscopy for differentiating neoplastic from non-neoplastic diminutive colorectal polyps.

Research methods

We prospectively recruited patients who underwent endocytoscopy between October and December 2016 at Sano Hospital. Diminutive polyps were evaluated by endocytoscopy after being stained with 0.05% crystal violet and 1% methylene blue. The diminutive polyps were classified according to the endocytoscopic (EC) classification. Endoscopists have assessed the diagnostic

performance of endocytoscopy for EC2 (indicator of adenoma) and EC1b (indicator of hyperplastic polyp) lesions by comparison with the histopathology of the biopsy specimen.

Research results

A total of 39 patients with 63 diminutive polyps were analyzed. The accuracy and negative predictive value of EC2 for adenoma compared with EC1b for hyperplastic polyp were 98.0% and 92.3%. Endocytoscopy showed a high diagnostic performance for differentiating between neoplastic and non-neoplastic colorectal diminutive polyps. However, because endocytoscopy was available to our institute for only a limited period, the number of samples was small.

Research conclusions

The diagnostic performance of endocytoscopy for colorectal diminutive polyps met the PIVI criteria for assessment of histology.

Research perspectives

Real-time histopathologic assessment by endocytoscopy has the potential to save histopathologic diagnosis. Additional multicenter studies are needed to confirm our result.

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EGFR amplification induces sensitivity to antiEGFR therapy in pancreatic acinar cell carcinoma

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Abstract

Pancreatic acinar cell carcinoma (PACC) is a rare cancer. When the tumor is metastatic, few therapeutic options are available. Precision medicine using next-generation sequencing is defined by the administration of drugs based on the tumor genetic mutations. The usage of precision medicine for finding new therapeutic options for rare cancers is an emerging field. We have reported here the case of a patient bearing a multitreated metastatic PACC. This patient underwent somatic and constitutional exome analyses. The analyses revealed in the liver metastasis an amplification of the *EGFR* gene. Accordingly, the patient was treated with off-label usage of panitumumab. We observed rapid response with necrosis of the liver metastasis, while no efficacy was observed in the primary tumor. An exome analysis of the primary tumor revealed amplification of *HER2* and *MET* with *EGFR* amplification. Such amplifications are known as a resistance mechanism to antiEGFR therapy. Our results suggest that exome analysis may be helpful to highlight targets in rare cancers, such as PACC. *EGFR* amplification in this pathology should be determined and could be used as a biomarker to propose antiEGFR therapy.

Key words: Pancreatic cancer; Acinar cell carcinoma; Exome; Genetic mutations; Precision medicine

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Core tip: The role of genetic profiling for therapy of rare cancer for precision medicine is currently under investigation. This case report reports, for the first time, that pancreatic acinar cell carcinoma could benefit from precision medicine and that *EGFR* gene amplification could be targetable by anti*EGFR* monoclonal antibody in this pathology.

Richard C, Niogret J, Boidot R, Ghiringhelli F. *EGFR* amplification induces sensitivity to anti*EGFR* therapy in pancreatic acinar cell carcinoma. *World J Gastrointest Oncol* 2018; 10(4): 103-107 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i4/103.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i4.103>

INTRODUCTION

Pancreatic acinar cell carcinoma is a rare cancer. This cancer accounts for about 1% of all cases of pancreatic cancers. Patients at diagnosis often present a large tumor with distant metastases in the liver or in other organs^[1]. No standard treatments have been proposed in metastatic settings.

Despite the absence of response to chemotherapy, a recent publication suggested that the prognosis of pancreatic acinar cell carcinoma could be better than classical pancreatic ductal adenocarcinoma^[2]. Another report^[3] described 112 cases of pancreatic acinar cell carcinoma. Eighty-eight patients underwent surgical resection. The overall survival rate was 43.9% at 5 years and the median overall survival was 41 mo. However, for unresectable pancreatic acinar cell carcinoma we found several studies describing the usage of chemotherapy for treatment of pancreatic ductal adenocarcinoma^[1,4-7]. However, due to small sample sizes, the level of evidence is limited. Recent advances in genetic testing have revealed that pancreatic acinar cell carcinoma could have genetic mutation that could be targetable in 30% of cases. Here, we provide the first report (to our knowledge) of an *EGFR* amplification in a metastatic pancreatic acinar cell carcinoma, with exceptional and rapid response only in metastases harboring only *EGFR* amplification.

CASE REPORT

A 54-year-old man with past history of nephroblastoma at young age that had been treated by surgery and chemotherapy presented with diarrhea associated with important low weight of 10 kg. On June 2017, a CT scan revealed two liver metastases and voluminous mass in the head of the pancreas, without compression of the biliary tract or duodenum. The patient benefited from pancreatic biopsy upon endoscopic ultrasound. Histology

revealed a tumor with large area of fibrous stroma. Acinar architecture was observed with pyramidal-shaped cells surrounding small lumina. The malignant cells were monomorph, with round nuclei and prominent nucleoli having eosinophil cytoplasm. Immunohistochemically, expression of EMA, cytokeratin 7 and absence of WT1, synaptophysin, and chromogranin A markers were shown. Ki67 was expressed in 30% of the tumor nuclei. The diagnosis of metastatic acinar cell pancreatic cancer was given.

The patient received four cycles of FOLFIRINOX, which resulted in tumor progression, and then two cycles of gemcitabine plus nabpaclitaxel, again with tumor progression. The patient was included in the EXOMA trial (NCT02840604). A biopsy of a liver metastasis was performed with a blood withdraw. Then, the patient benefited from somatic and constitutive exome sequencing. The tumor mutational burden was 410 mutations. We limited our analysis to a set of 324 genes, selected due to their roles in prediction of response or resistance to therapy or their associations with cancer predisposition. Our gene list was inspired from the recently published gene list of the MD Anderson Cancer Center used for clinical trial of precision medicine^[8].

We observed an unknown mutation in the *CUL2* and *PBRM1* genes. *PBRM1* encodes a tumor suppressor and component of the SWI/SNF chromatin protein complex. Inactivating mutations of *PBRM1* are frequently found in renal tissues^[9]. *CUL2* is a cullin protein. Cullins are associated with RING proteins and ubiquitin E3 ligases. This complex regulates various cellular processes, including proliferation, differentiation and apoptosis. Loss of *PBRM1* activity is also associated with chromosomal instability, due to inability to promote cohesion^[10]. Because of the presence of *PBRM1* mutation, we searched for chromosomal instability using TITAN software. Titan is a Python/R package for analyzing subclonal copy number alterations and loss of heterozygosity in whole genome and exome sequencing of tumors^[11].

We observed a ploidy near 3, where 21 chromosomal fragments of more than 10 mB were amplified and 4 chromosomal segments of more than 10 mB were deleted. The number of clones in this tumor was one. Interestingly, we observed a large chromosome 7 amplification containing the *EGFR* gene locus, resulting in the presence of three copies of the *EGFR* gene (Figure 1A). Extensive analysis of the amplifications revealed that only *EGFR* but not *MET* (Figure 1A) nor *ERBB2* (Figure 1B) were amplified. Based on this observation, anti*EGFR* (panitumumab) treatment was given.

At 2 wk after the first injection, we observed clinical improvement for the patients, with 2 kg weight gain and disappearance of liver pain. After two cycles of chemotherapy, we observed a dissociated response with complete necrosis of the liver metastasis, which was tested for exome analysis (Figure 2A and B). In

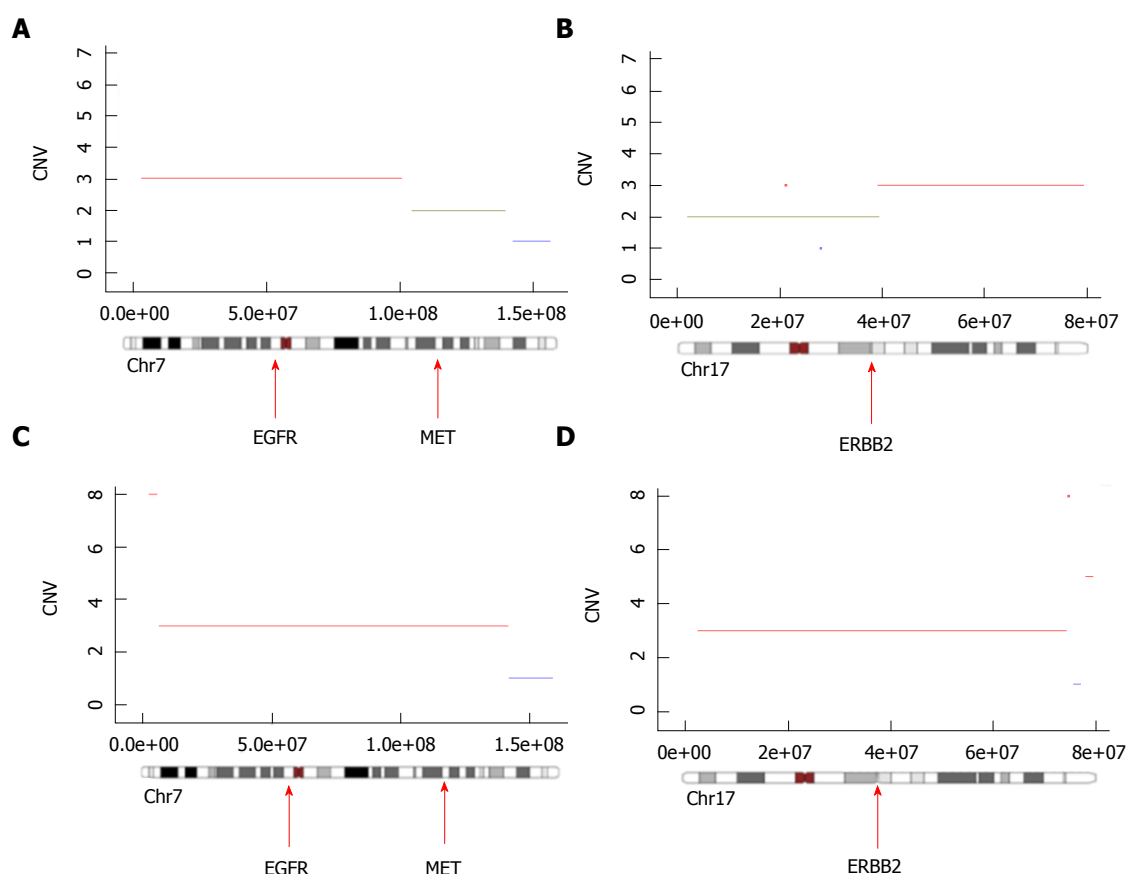


Figure 1 Representation of chromosomal amplification and deletion in chromosomes 7 (A, C) and 17 (B, D) in the primary tumor (C, D) and the liver metastasis (A, B). Portions in red are amplified, portions in blue are deleted and portions in green are diploid. Genes of interest are indicated by a red arrow.

contrast, we did not observe any changes in the tumor characteristics of the primary tumor (Figure 2C and D). A second exome analysis was performed on the primary tumor. Interestingly, we observed in this tumor *EGFR* amplification but concomitant amplification of *ERBB2* and *Met* loci (Figure 1C and D), suggesting the presence of an intrinsic tumor resistance mechanism to anti*EGFR* therapy.

The primary tumor contained two clones, including one with strong similarity to the liver metastatic one, suggesting that only one clone was at the origin of the metastasis process.

DISCUSSION

Pancreatic acinar cell carcinoma is a rare disease of the pancreas. This is a tumor with poor prognosis, like ductal adenocarcinoma. The mean survival is around 2 years and the 3-year survival rate is about 25%^[12,13]. Because of the rarity of the disease, few trials address the therapeutic strategy to treat metastatic pancreatic acinar cell carcinoma. Classically, these tumors are considered as chemoresistant ones. Patients are treated with first-line therapy, like pancreatic ductal adenocarcinoma, and then proposed for palliative care.

In contrast to ductal adenocarcinoma of the pancreas, we have little information on the underlying genetic alterations that dictate the development of

pancreatic acinar cell carcinoma. Only a study of 23 cases of pancreatic acinar cell carcinoma was extensively characterized by exome sequencing fluorescence *in situ* hybridization and microsatellite instability analysis^[14]. This study underlined some mutations that could be targetable, such as those in genes coding for members of the Fanconi anemia pathway and mutations in genes such as *BRCA2*, *PALB2*, *BAP1*, *ATM*, *BRAF* and *JAK1*. Such mutations could be targetable by PARP inhibitors, BRAF inhibitors and JAK1 inhibitors respectively. However, we did not observe any mutations in these genes in our patient.

Pancreatic acinar cell carcinoma presents frequently with a large number of chromosomal alterations and a major intratumoral heterogeneity. These data suggest that these tumors are chromosomally unstable^[15], although the mechanism(s) which explain chromosomal instability is(are) unclear. We could suspect that it may explain its aggressive behavior and resistance to therapy^[16]. In this report, the presence of *PBRM1* mutation, which is classically associated with chromosomal instability, could explain this instability. Such instability should induce some amplification of oncogenes that could be targetable. In this case, the liver metastasis presented amplification of *EGFR*, which could be oncogenic but also a target for anti*EGFR* therapy.

Efficacy of anti*EGFR* therapy is restrained by the

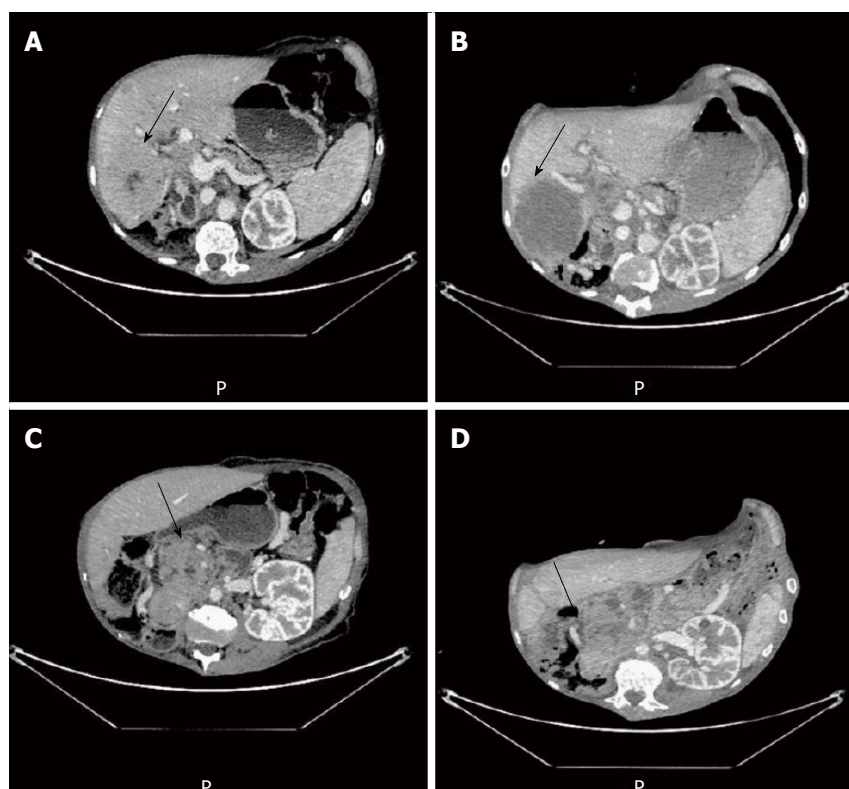


Figure 2 Primary tumor and liver metastasis response to FOLFIRI plus panitumumab. A and B: CT scan axial images of liver metastasis at baseline and 4 wk of therapy; C and D: Axial images of at primary pancreatic tumor at baseline and 4 wk of therapy. Lesions are indicated by a arrow.

presence of *KRAS-NRAS* mutations^[17]. However, none of the pancreatic acinar cell carcinomas had *KRAS* mutation in previous series, in contrast to ductal adenocarcinomas^[18-20].

Accordingly, we did not detect mutation either on *KRAS* or on *NRAS* in our patient. We observed, for the first time, amplification in the *EGFR* locus in a pancreatic acinar cell carcinoma. In colorectal cancer, *EGFR* amplification was previously described as a biomarker associated with anti*EGFR* efficacy^[21,22]. The presence of a dissociation response between liver metastasis and primary tumor suggest the presence of a clonal heterogeneity between the two tumor sites, confirmed by our bioinformatic analysis of copy number alterations. Indeed, this analysis underlined that the primary tumor contained two clones, while the liver metastasis contained only the anti*EGFR*-sensitive clone. The mechanism of resistance to anti*EGFR* therapy is pleiotropic and includes presence of *KRAS* and *NRAS* mutations, *PIK3CA* and *PTEN* alterations, mutation in the extracellular domain of *EGFR*, *HER2* and *MET* amplifications gave strong rationale to explain the resistance of the primary tumor to anti*EGFR* therapy.

Together, this report provides the first description of a major and rapid response of pancreatic acinar cell carcinoma to anti*EGFR* therapy related to *EGFR* amplification. This report also gives rationale to perform multiple biopsies or liquid biopsy to address

tumor heterogeneity, which could explain dissociated response.

ARTICLE HIGHLIGHTS

Case characteristics

A pancreatic cancer with liver metastasis.

Clinical diagnosis

Amplification of the *EGFR* gene is targetable in pancreatic acinar carcinoma.

Differential diagnosis

Histology and molecular biology are required for the diagnosis.

Laboratory diagnosis

Genetic testing provides information on targetable tumor mutation.

Imaging diagnosis

Computed tomography scan underlines liver metastasis necrosis.

Treatment

The patient was treated with off-label usage of panitumumab.

Term explanation

This is the first report of *EGFR* amplification in acinar cell pancreatic cancer, and the first report of panitumumab efficacy in such disease.

Experiences and lessons

Our findings suggest that exome analysis may be a helpful tool to highlight targets in rare cancers, such as pancreatic acinar cell carcinoma. *EGFR*

amplification in this pathology should be determined and could be used as biomarker to propose anti*EGFR* therapy.

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Targeted therapy or immunotherapy? Optimal treatment in hepatocellular carcinoma

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cause of cancer mortality in the United States and the second leading cause of cancer mortality worldwide. Sorafenib is the only food and drug administration (FDA) approved as first line systemic treatment in HCC. Regorafenib and nivolumab are the only FDA approved second line treatment after progression on sorafenib. We will discuss all potential first and second line options in HCC. In addition, we also will explore sequencing treatment options in HCC, and examine biomarkers that can potentially predict benefits from treatments such as immune checkpoint inhibitor. This minireview summarizes potential treatments in HCC based on clinical trials that have been published in manuscript or abstract format from 1994-2018.

Key words: Sequencing treatment; Sorafenib; Hepatocellular carcinoma treatments; Nivolumab; Regorafenib; Lenvatinib; Cabozantinib; Immunotherapy; Biomarker; Pembrolizumab; Ramucirumab; Alpha-fetoprotein; Neoantigen; Tumor mutational burden; Interferon-gamma

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Core tip: Hepatocellular carcinoma (HCC) is the fifth leading cause of cancer mortality in the United States and the second leading cause of cancer mortality worldwide. There are some potential treatment options for first and second line HCC, there are also new biomarkers that can predict benefits from treatments such as immune checkpoint inhibitors.

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Abstract

Hepatocellular carcinoma (HCC) is the fifth leading

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth leading

cause of cancer mortality in the United States and the second leading cause of cancer mortality worldwide^[1]. Sorafenib has been the only food and drug administration (FDA) approved first line treatment in HCC since 2007. Lenvatinib is another promising treatment in first line HCC, demonstrated non-inferiority in median overall survival (mOS) compared to sorafenib^[2]. Nivolumab also might have activity in first line HCC. In the second line treatment of HCC, there are 2 FDA approved medications regorafenib and nivolumab. In addition, other targeted therapies such as cabozantinib or pembrolizumab might be beneficial in second line treatment of HCC.

We will discuss the options of systemic treatment in HCC both for first and second line, the optimal sequencing of treatments, their side effects, and potential biomarkers that may predict benefits of therapy.

FIRST LINE SYSTEMIC TREATMENT IN HCC

Sorafenib is a tyrosine kinase inhibitor that inhibits vascular endothelial growth factor receptor 1 (VEGFR1), VEGFR2, VEGFR3, platelet-derived growth factor receptor-beta, KIT and RAF/ mitogen-activated protein/MEK. In the phase III (SHARP trial) of 602 HCC patients with Child Pugh Class A (preserved liver function), mOS in sorafenib was 10.7 mo^[3]. Although it is the first line and only therapy that improves mOS in first line patients, most of patients could not tolerate at the full dose of sorafenib due to the side effects. In the oncology community, most patients are started on lower dose, for example 200 mg PO BID with potential up titration. The most common adverse events (AEs) were diarrhea (39%), fatigue (22%), hand-foot skin reaction (21%), rash (16%), and alopecia (14%)^[3]. The common grade 3/4 AEs were hypophosphatemia (11%), diarrhea (8%), hand-foot skin reaction (8%), thrombocytopenia (4%), and hypertension (2%)^[3]. Even though there was no difference in survival benefits whether or not patients are started at a full dose (400 mg BID) or reduced dose (200 mg BID), it improved cost-effective in sorafenib treatment^[4,5]. Therefore sorafenib is most beneficial for patients with Child Pugh Class A with preserved liver function. In a retrospective subanalyses of phase III SHARP study, sorafenib has shown mOS of 14 mo in HCV patients^[6]. In the SHARP study, the top 3 risk factors for HCC in the sorafenib group were Hepatitis C (29%), alcohol (26%), and hepatitis B (19%). In the phase III of Asia Pacific study in 226 HCC patients with Child Pugh Class A, up to 73% patients were HBV positive. This study reported the mOS was 6.5 mo in sorafenib vs 4.2 mo in placebo group^[7]. In a retrospective study of 59 unresectable HCC patients who received sorafenib that included Child Pugh Class A (26), B (23), and C (10)^[8]. The mOS were 8.3, 4.3, and 1.5 mo, respectively^[8]. In this study, the top 3 risk factors for HCC were alcohol (38%) and viral hepatitis B/C (26%). This retrospective study suggested that sorafenib may exert the maximum benefit in Child Pugh Class A patient, regardless of etiology for HCC.

Some of the side effects emerged from sorafenib suggested that hypertension (HTN) and diarrhea may be correlated with efficacy. In a retrospective study in 41 HCC patients (Child Pugh Class A/B, 25/16 patients), showed development of HTN led to better response to sorafenib treatment, with mOS of 18.2 mo vs 4.5 mo in patients without HTN^[9]. Another retrospective study in 112 patients with advanced HCC showed that diarrhea can also predict the response to sorafenib treatment as well. Patients with diarrhea demonstrated longer mOS of 14.1 mo vs 7.1 mo when compared to patients without diarrhea^[10].

POTENTIAL FIRST LINE SYSTEMIC TREATMENT OPTIONS IN HCC

Lenvatinib is a multiple kinase inhibitor that inhibits VEGFR 1-3, fibroblast growth factor receptor 1-4, platelet derived growth factor receptor (PDGFR) alpha, c-Kit and RET proto-oncogene. In the randomized phase III (REFLECT) study of lenvatinib vs sorafenib in first line treatment of unresectable HCC in 954 patients (1:1) with Child Pugh Class A, it showed mOS in lenvatinib vs sorafenib was 13.6 mo and 12.3 mo, respectively. It met its primary endpoint of non-inferiority and it achieved the secondary endpoints with the median progression free survival (PFS) of 7.4 mo vs 3.7 mo and the time to progression (TTP) was 8.9 mo vs 3.7 mo^[2]. The most common AEs were hypertension (42%), diarrhea (39%), decreased appetite (34%), decreased weight (31%), and fatigue (30%)^[2]. The common grade 3/4 AEs were hypertension (23%), decreased weight (8%), decreased platelet count (6%), elevated aspartate aminotransferase (5%), and decreased appetite (5%)^[2]. The usage dose is oral 8 mg (weight < 60 kg) or 12 mg (weight ≥ 60 kg) once daily. In the phase 2 study of lenvatinib in 46 HCC patients with Child Pugh Class A, the objective response rate (ORR) was 37%^[11]. The most common causes of HCC in phase 2 study were Hepatitis C (58.7%), Hepatitis B (32.6%), and Alcohol (4.3%).

Nivolumab is an immune checkpoint inhibitor that inhibits PD-1. In a phase I / II study (CHECKMATE 040) of nivolumab in advanced HCC patients in the dose-expansion phase, there were 56 sorafenib naïve patients. All patients were uninfected with viral hepatitis (55 with Child Pugh Class A and only 1 Child Pugh Class B)^[12]. This study showed ORR of 23% and OS rate of 82% at 9 mo^[12]. Nivolumab showed 23% of partial response (PR) in HCC sorafenib naïve patients, it could be considered as a potential first line treatment^[12]. It demonstrated that nivolumab might be beneficial for first line treatment in HCC patients. A phase III study of nivolumab compared to sorafenib as a first line treatment is ongoing.

SECOND LINE TREATMENT OPTIONS IN HCC

Regorafenib, is an oral multikinase inhibitor specifically inhibits VEGFR-1, 2, 3. It was approved by FDA on April

27, 2017 as a second line treatment in HCC patients who have been previously progressed with sorafenib. In this study, the median treatment time on first line sorafenib was 7.8 mo for both patient groups^[13]. This study showed mOS of 10.6 mo in regorafenib groups (379) vs 7.8 mo in placebo groups (194)^[13]. The median PFS was 3.1 mo in regorafenib vs 1.5 mo in placebo group^[13]. The ORR in regorafenib group was 11%^[13]. In the phase III (RESORCE) study of regorafenib in 573 HCC patients with Child Pugh Class A, the most common AEs were hand-foot skin reaction (52%), diarrhea (33%), fatigue (29%), anorexia (24%), and hypertension (23%)^[13]. The common grade 3/4 AEs were hypertension (13%), hand-foot skin reaction (13%), fatigue (6%), increased blood bilirubin (6%), and increased AST (4%)^[13]. The etiologies of HCC in this study were hepatitis B (38%), alcohol use (24%), and hepatitis C (21%)^[13]. In this study (RESORCE) showed that 199 patients out of 374 patients who received regorafenib had experience of hand-foot skin reaction during cycle 1, these patients had better mOS of 14.1 mo vs 6.6 mo in patients who did not experience hand-foot skin reaction. It also showed HR of 0.52^[14]. It suggests that hand-foot skin reaction should be managed properly to get a better response of regorafenib and mOS benefit.

Nivolumab, is an immunotherapy that inhibits PD-1. It was granted approval by FDA on September 22, 2017 as a second line systemic treatment in HCC patients who have been treated with or intolerant to sorafenib. The phase I / II study of nivolumab with dose escalation that included 48 patients with Child Pugh Class A and B7, in addition to dose expansion in 214 patients (Child Pugh Class A)^[12]. In the dose-escalation phase, ORR was 15%, 6 mo and 9 mo OS rates were both 66%, and mOS was 15 mo^[12]. In the dose expansion phase, ORR was 20%, 6 mo and 9 mo OS rates were 83% and 74%, only the group in sorafenib progressor without viral hepatitis reached mOS of 13.2 mo and the rest of the groups did not reach mOS^[12]. In the dose expansion phase, the patients were divided into 113 patients without HBV or HCV (56 untreated/intolerant of sorafenib and 57 progressed post sorafenib)^[12]. In addition, this phase also included 51 patients with HBV and 50 patients with HCV^[12]. The study demonstrated transient decreased HCV RNA in some HCV infected patients and no reactivation in HBV infected patients. The most common AEs were fatigue (25%), pruritus (20%), diarrhea (18%), rash (11%), and increased AST level (11%)^[12]. The grade 3/4 AEs were increased AST (4%), rash (2%), diarrhea (2%), and fatigue (2%)^[12]. The dose is 3 mg/kg (240 mg) every 2 wk.

In a retrospective analysis of this study, PD-L1 was showed as biomarker that predicted response to nivolumab in 174 out of 214 patients. The ORR was 26% vs 19% in patients with PD-L1 \geq 1% compared with PD-L1 < 1%, it suggested that PD-L1 could be a potential biomarker associated with nivolumab treatment^[12].

Cabozantinib is an oral tyrosine kinase inhibitor

including VEGFR, MET, RET, KIT, and FLT3. In the phase III (CELESTIAL) study of cabozantinib vs placebo in 707 HCC patients with Child Pugh Class A who previously received sorafenib^[15]. The characteristics of the patients were the median age of patients was 64 years, 82% male patients, 38% HBV infected, 25% HCV infected, 78% had extrahepatic spread, 30% had macrovascular invasion, and 27% had received two prior systemic therapy^[15]. This study has achieved mOS of 10.2 mo in cabozantinib vs 8 mo in placebo group^[15]. It also achieved median PFS of 5.2 mo in cabozantinib vs 1.9 mo in placebo group, and ORR of 4% in cabozantinib group vs 0.4% in placebo group^[15]. The most common grade 3/4 AEs were hand-foot syndrome (17%), HTN (16%), increased AST (12%), fatigue (10%), and diarrhea (10%)^[15]. It suggested that cabozantinib has the potential to be an effective treatment for second line HCC.

Pembrolizumab is an immunotherapy that inhibits PD-1. In the Phase 2 study (KEYNOTE-224) of Pembrolizumab in 104 HCC patients with Child Pugh Class A who progressed on sorafenib treatment. The primary endpoint of this study was achieved with ORR of 16.3% with 1 CR^[16]. The median PFS was 4.8 mo and the 6 mo PFS and OS rates were 43.1% and 77.9%, respectively^[16]. About 94% of patients who responded, continue to respond at 6 mo^[16]. The most common AEs were fatigue (21.2%) and increased AST (12.5%)^[16]. The etiologies of HCC were HBV (21.2%) and HCV (26%)^[16]. The grade 3-5 AE was reported in 25% of patient with 1 death due to ulcerative esophagitis^[16]. This study showed that pembrolizumab might have a good response in advanced HCC patients who progressed on sorafenib.

Ramucirumab is a fully monoclonal antibody (IgG1) that inhibits VEGFR2. In the phase III study of ramucirumab vs placebo as a second line treatment in 565 HCC patients with Child Pugh Class A (REACH)^[17]. Eventhough there was no significantly improvement in mOS between patients who received ramucirumab vs placebo (9.2 mo vs 7.6 mo), ORR in ramucirumab group was higher than the placebo group (7% vs < 1%)^[17]. The most common AEs were peripheral edema (36%), liver injury (30%), bleeding or haemorrhage (26%), ascites (22%), and fatigue (21%)^[17]. The grade 3/4 AEs were liver injury (14%), hypertension (13%), ascites (5%), bleeding or haemorrhage (5%), and asthenia (5%)^[17]. The etiologies of HCC in this study were Hepatitis B (35%) and Hepatitis C (27%)^[17]. In the prespecified subgroup retrospective analysis of 250 patients with α -fetoprotein (AFP) \geq 400 ng/mL, the mOS was 7.8 mo (ramucirumab group) vs 4.2 mo (placebo group)^[17]. It suggested that ramucirumab could be beneficial in HCC patients with AFP \geq 400 ng/mL. AFP can potentially be used as a biomarker to predict the response of ramucirumab treatment in HCC patients. A phase III study looking for HCC patients with AFP \geq 400 ng/mL not prespecified is ongoing.

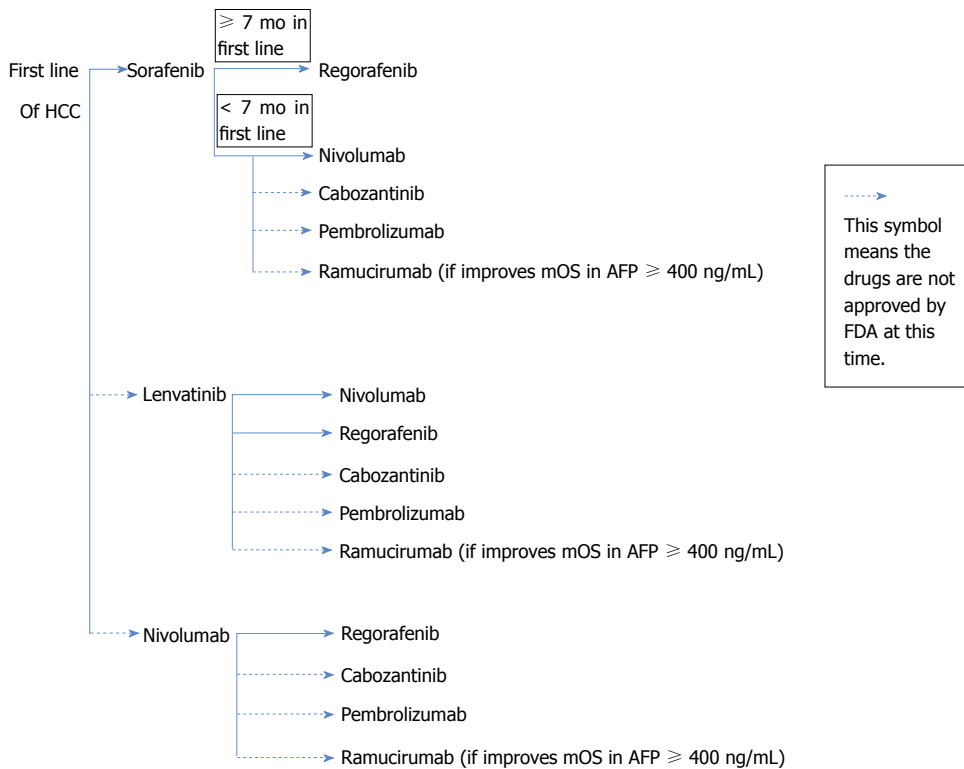


Figure 1 Potential sequencing treatment options in hepatocellular carcinoma. The only food and drug administration (FDA) approved for first line systemic treatment for hepatocellular carcinoma (HCC) is sorafenib. If patients tolerate sorafenib well and could stay on therapy for at least 7 mo, regorafenib (FDA approved) would be a preferred second line option. If patients could not tolerate sorafenib well or received less than 7 mo of treatment with sorafenib, the next second line options will be nivolumab (FDA approved) and could be cabozantinib or pembrolizumab after get approval by FDA. Another potential first line option will be lenvatinib or nivolumab after get approval by FDA. If patients progress on lenvatinib, then second line options will be nivolumab, regorafenib, cabozantinib, pembrolizumab. For patients who progress on nivolumab, then second line options will be regorafenib, cabozantinib, pembrolizumab. Another possible option of second line treatment after patients progress after the above first line treatment could be ramucirumab if the phase III study shows improvement of mOS in HCC patients with AFP ≥ 400 ng/mL. FDA: Food and drug administration; mOS: Median overall survival; RR: Response rate.

SEQUENCING TREATMENTS IN HCC IN THE FUTURE

Sorafenib is the only FDA approved first line treatment in HCC. It is beneficial in HCC patients with Child Pugh Class A and especially in patients with HCV. As demonstrated in a retrospective analysis of HCV patients which comprised 29% of the total patient populations in SHARP study, the mOS was 14 mo, while mOS of the overall population was only 10.9 mo. When patients experience side effects such as HTN or diarrhea, these side effects should be managed aggressively to minimize premature discontinuation of sorafenib. In a two retrospective studies in patients who had HTN or diarrhea were linked to a better mOS compared to patients who did not experience HTN or diarrhea. For instance, the mOS in HTN group was 18.2 mo vs 4.5 mo in group without HTN, the mOS in patients with diarrhea was 14.1 mo vs 7.1 mo in patients without diarrhea (Figure 1).

If patients with Child Pugh Class A tolerate sorafenib well in the first line setting, regorafenib would be a good choice as a second line treatment due to similar toxicities profiles of the two medications. Regorafenib was only studied in patients with Child Pugh Class A. For patients who have difficulty tolerating toxicities of

sorafenib, nivolumab could be a good option as a second line treatment, it achieved ORR of 15%-20%. Nivolumab will be beneficial in patients with Child Pugh Class A/B7. Nivolumab achieved higher RR in PD-L1 $\geq 1\%$ (positive) compared to tumors with PD-L1 $< 1\%$ (negative), 26% and 19% respectively. However nivolumab does not seem to offer differential outcomes regardless of the length of treatment on first line therapy. Even though cabozantinib or pembrolizumab or ramucirumab have not been FDA approved at this time. Once become FDA approved, then cabozantinib or pembrolizumab could be other second line options. If the phase III study in HCC patients with AFP ≥ 400 ng/mL shows improvement mOS with ramucirumab, then the strategy for second line treatment may include testing of AFP. For patients with AFP ≥ 400 ng/mL, ramucirumab could be a second line option.

Lenvatinib has shown non-inferiority to sorafenib in a phase III study, therefore it would be a first line treatment in HCC if granted FDA approval. It could be a good alternate to sorafenib for patients who prefer to have less hand-foot syndrome and/or diarrhea. Once patients progress, the second line treatment options are nivolumab (in patients with Child Pugh Class A or B7 only and PD-L1 +) and regorafenib (in Child Pugh Class

A). Other potential second line options are cabozantinib, pembrolizumab, or ramucirumab.

Nivolumab as first line treatment if granted FDA approval, it will be beneficial for patients who have no contraindication to immunotherapy or who have severe HTN at baseline. If patients could not tolerate or progressed while on nivolumab, the second line options could be regorafenib. Other potential second line options are cabozantinib, pembrolizumab, or ramucirumab.

POTENTIAL BIOMARKERS TO MAXIMIZE THE RESPONSE OF TREATMENT IN HCC

AFP

AFP stands for alpha-feto protein, it is used as a diagnostic and prognosis marker in HCC patients. In a single-institution prospective study, preoperative value of AFP > 400 ng/mL in 108 resectable HCC patients, correlated with higher recurrence rates and lower survival rates at 2 years^[18]. In a prespecified group of 250 HCC patients in a phase III ramucirumab trial (REACH) with a baseline AFP \geq 400 ng/mL, mOS of ramucirumab and placebo was 7.8 mo and 4.2 mo, respectively^[17]. In the group (310 patients) where baseline AFP < 400 ng/mL, there was no difference in mOS between ramucirumab and placebo. Therefore, AFP could be used as a marker to predict response with ramucirumab treatment. Phase III of ramucirumab study is ongoing in HCC patients with AFP \geq 400 ng/mL and the mOS benefit needs to be validated in patients with AFP \geq 400 ng/mL, once the preliminary data is available.

PD-L1

A programmed death ligand-1 could be a potential biomarker to predict the efficacy of immune checkpoint inhibitors. PD-L1 can be detected using several assays, and the definition of PD-L1 positivity and the methodology of measuring PD-L1 are required to understand about the role of PD-L1 in HCC^[19]. In a phase II dose expansion cohort study of nivolumab in HCC patients either progressed or intolerant of sorafenib, RR was 26% vs 19% in patients with PD \geq 1% and PD-L1 < 1%, respectively^[12]. PD-L1 \geq 1% therefore appears to indicate higher RR in HCC and it also predicts response of nivolumab treatment with mOS benefit.

FUTURE DIRECTION BIOMARKERS

Neoantigen

A tumor-specific mutated peptides on the surface of cancer cells initiate neoantigen production. Each tumor cell causes genetic mutations due to alteration of peptides (amino acid sequencing), it produces neoantigen signature that contains four amino acid strings of peptides^[20]. Neoantigen signature is seen in patients with long term clinical benefit of therapy (no evidence of disease for > 6 mo)^[20]. Neoantigen was investigated

using whole exome sequencing in DNA of tumor cell. Neoantigen can be used as a biomarker to predict the response to immune checkpoint inhibitor treatment. The higher number of neoantigen in a tumor that binds to major histocompatibility complex (MHC) class I, it would be recognized easier by T cells to activate T cells. A prospective study of 18 non-small cell lung cancer (NSCLC) samples from patients who received pembrolizumab (anti-PD-1, an immunotherapy), high mutational burden related to high neoantigen (median of 112 candidate neoantigen per tumor) and associated with improvement of PFS for 14.5 mo^[21]. This study showed high mutational burden at least 200 nonsynonymous mutations (mutations that altered protein in cancer cells) per sample, it related to durable clinical benefit (partial or stable response > 6 mo). High mutational burden by itself was not enough to predict durable clinical benefit, because in a few patients without durable clinical benefit also had high mutational burden. In addition to high mutational burden, high number of neoantigen was a better prediction of treatment response. It showed better PFS in patients with high neoantigen compared to low neoantigen group, with PFS of 14.5 mo vs 3.5 mo, respectively^[21]. Another prospective study in 64 stage IV melanoma patients who received ipilimumab or tremelimumab (anti-CTLA-4) demonstrated long term clinical benefit in 11 out of 25 patients with high number of mutational load, in addition 14 patients with high number of mutational load without long term clinical benefit^[20]. In the second set of 39 melanoma patients who received anti-CTLA-4, 25 patients with high neoantigen had long term clinical benefit to anti-CTLA-4^[20].

Tumor mutational burden

Tumor mutational burden (TMB) refers to DNA sample that can be detected in blood, and it is considered one example liquid biopsy. This non-invasive test is helpful and convenience especially if tumor tissue is inadequate. This biomarker might help to predict the response of immune checkpoint inhibitor. In a retrospective analysis of atezolizumab (anti-PD-L1) in NSCLC patients, blood was used to extract TMB to predict benefit in patients who received atezolizumab. It included 211 NSCLC patients in POPLAR and 583 NSCLC patients in OAK trial^[22]. The TMB was minimum 10 single nucleotide variants (SNV) from cell free-DNA in plasma. In the POPLAR study, patients with TMB \geq 10, the atezolizumab group showed better PFS hazard ratio (HR) of 0.68 and OS HR of 0.59 compared to docetaxel group^[22]. In the OAK study, PFS and OS were also better in the atezolizumab group compared to docetaxel group with HR of 0.73 and 0.69, respectively^[22]. From this data, tumor mutational burden could be beneficial as a biomarker for the efficacy of immune checkpoint inhibitor. Prospective studies using TMB in NSCLC patients are ongoing. It needs further investigation for HCC patients in the future.

Interferon gamma

A cytokine that is produced by several cells including CD4+ T helper cell type 1 (Th1 cells), CD8+ cytotoxic T cell, macrophage, mucosal epithelial cell, natural killer cell (NK), and NK T cell^[23-25]. It inhibits cellular proliferation and causes apoptosis^[26]. A study in 48 HCC patients who received curative treatment (surgery/RFA), a higher risk of tumor recurrence was observed in patients with lower levels of interferon gamma (IFN- γ)^[27]. IFN- γ can therefore be a potential marker to predict HCC recurrence. In two prospective studies from 17 NSCLC and 21 melanoma patients who received pembrolizumab (anti-PD-1), these studies analyzed IFN- γ mRNA to predict response treatment of pembrolizumab. It showed longer PFS and OS in NSCLC patients with high level vs low level of IFN- γ (5.12 vs 2 mo; 10.15 vs 4.86 mo). It also showed longer PFS in melanoma patients with high level vs low level of IFN- γ (4.99 mo vs 1.86 mo)^[28].

CONCLUSION

HCC is the second leading cause of cancer mortality worldwide. Sorafenib is the only FDA approved first line treatment in unresectable HCC. Sorafenib has shown median OS response in HCC patients with HCV infection. There are others potential first line treatments in HCC such as lenvatinib and nivolumab, although not FDA approved, hold great promise based on phase III studies. The second line treatments of HCC patients who progressed or intolerant to sorafenib, include regorafenib and nivolumab. Regorafenib demonstrated higher median OS in HCC patients who tolerated sorafenib for at least 7 mo. Nivolumab has been reported to be more beneficial in HCC patients with Child Pugh Class A/B7, and achieved higher RR in patients with PD-L1 \geq 1%. Other potential options for second line treatments are cabozantinib (phase III) or pembrolizumab (phase II).

There are two current biomarkers that used to predict response of treatment such as PD-L1 and AFP. For instance, PD-L1 indicates higher RR in nivolumab study, and AFP \geq 400 ng/mL shows a trend for higher median OS in ramucirumab subgroup analysis phase III study. In addition, other future biomarkers that might be used to predict response of treatment are neoantigens, tumor mutational burden and IFN- γ . These biomarkers need further validation in large randomized clinical trials.

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Risk of gastric cancer development after eradication of *Helicobacter pylori*

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important risk factor for gastric cancer (GC) development through the Correa's gastric carcinogenesis cascade. However, *H. pylori* eradication alone does not eliminate GC, as pre-neoplastic lesions (atrophic gastritis, intestinal metaplasia and dysplasia) may have already developed in some patients. It is therefore necessary to identify patients at high-risk for gastric cancer after *H. pylori* eradication to streamline the management plan. If the patients have not undergone endoscopy with histologic assessment, the identification of certain clinical risk factors and non-invasive testing (serum pepsinogen) can predict the risk of atrophic gastritis. For those with suspected atrophic gastritis, further risk stratification by endoscopy with histologic assessment according to validated histologic staging systems would be advisable. Patients with higher stages may require long-term endoscopic surveillance. Apart from secondary prevention to reduce deaths by diagnosing GC at an early stage, identifying medications that could potentially modify the GC risk would be desirable. The potential roles of a number of medications have been suggested by various studies, including proton pump inhibitors (PPIs), aspirin, statins and metformin. However, there are currently no randomized clinical trials to address the impact of these medications on GC risk after *H. pylori* eradication. In addition, most of these studies failed to adjust for the effect of concurrent medications on GC risk. Recently, large population-based retrospective cohort studies have shown that PPIs were associated with an increased GC risk after *H. pylori* eradication, while aspirin was associated with a lower risk. The roles of other agents in reducing GC risk after *H. pylori* eradication remain to be determined.

Key words: Gastric adenocarcinoma; Stomach cancer; *Helicobacter pylori*; Chemoprevention; Intestinal metaplasia

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Abstract

Helicobacter pylori (*H. pylori*) infection is the most

Core tip: Although *helicobacter pylori* (*H. pylori*)

infection is the most important risk factor for gastric cancer (GC) development, eradication of this bacteria does not guarantee the elimination of GC risk, as pre-neoplastic lesions may have already developed. It is therefore necessary to identify patients at high-risk for GC after *H. pylori* eradication by either endoscopy with histologic assessment or non-invasive testing. Long-term endoscopic surveillance is advisable for high-risk patients. Future studies are necessary to investigate medications that may modify the GC risk after *H. pylori* eradication.

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INTRODUCTION

Gastric cancer (GC) is the fifth most common cancer worldwide, with an estimation of 952000 new cases (6.8% of all incident cancer cases) in 2012^[1]. The disease burden is particularly high in East Asian countries where around half of the new cases are diagnosed. It is the third leading cause of cancer related mortality in the world, with 723000 deaths (8.8% of all cancer deaths) in a year. Around two-thirds of patients are diagnosed with GC at an advanced stage when curative surgery is not possible^[2,3]. Despite the advances in surgery and chemotherapy, the prognosis remains dismal in patients with advanced disease, with a median survival of less than one year.

The global prevalence of *Helicobacter pylori* (*H. pylori*) infection in adults ranges from 19% to 88%^[4]. *H. pylori* infection is one of the major risk factors for GC development (a relative risk of 2.8 as shown in a recent meta-analysis)^[5]. It is estimated that *H. pylori* infection attributes to 89% of non-cardia GC cases, which in turn accounts for 78% of all GC cases^[6]. *H. pylori* is classified by the International Agency for Research on Cancer of the World Health Organization as class I human carcinogen^[7]. It is postulated that *H. pylori* infection triggers and promotes the Correa's cancer cascade^[8]—a multistep process involving sequential changes of the gastric mucosa from chronic gastritis to atrophic gastritis, intestinal metaplasia, dysplasia and finally adenocarcinoma. Atrophic gastritis, intestinal metaplasia and dysplasia are considered to be pre-neoplastic lesions. In a population-based cohort study, the risk of GC was increased in patients with atrophic gastritis, intestinal metaplasia and dysplasia as compared to those with normal gastric mucosa by a hazard ratio (HR) of 4.5, 6.2 and 10.9, respectively^[9].

H. PYLORI ASSOCIATED GC

There are multiple pathways by which *H. pylori* leads

to GC development. *H. pylori* incites acute-on-chronic inflammation, leading to a high turnover rate of gastric epithelium as well as a microenvironment in which high levels of reactive oxygen and nitrogen radicals promote persistent DNA damage^[10-13]. *H. pylori* can also induce epigenetic changes including CpG island methylation of tumor suppressor genes such as E-cadherin^[14,15]. The aberrant expression of activation-induced cytidine deaminase *via* the effect of nuclear factor (NF)- κ B can alter nucleotides in the tumor-related genes^[16,17]. The induction of double-stranded DNA breaks and alteration of microRNAs expression further contribute to the genetic instability^[11,18]. The interplay between *H. pylori*, gastric microbiome and the exogenous factors in producing carcinogens further adds complexity to the *H. pylori*-induced carcinogenesis^[18]. *H. pylori* eradication can reduce or even eliminate gastric mucosal inflammation and reverse the *H. pylori*-associated molecular events^[15,18].

GC AFTER H. PYLORI ERADICATION

Although *H. pylori* is a major risk factor of GC, eradication of *H. pylori* does not completely eliminate the risk of subsequent GC development. It has been shown that *H. pylori* eradication could only reduce GC by 33%-47%^[19,20]. The fact that a significant proportion of *H. pylori*-eradicated subjects progress to develop GC is likely related to the baseline gastric histology at the time of eradication. The development of pre-neoplastic lesions including atrophic gastritis, intestinal metaplasia and dysplasia undermines the effect of *H. pylori* eradication in reducing GC^[21,22]. In a prospective, randomized study involving 1630 *H. pylori*-infected subjects conducted by Wong *et al*^[21], the beneficial effect of *H. pylori* eradication was limited to patients without baseline pre-neoplastic lesions (atrophic gastritis, intestinal metaplasia and dysplasia). No GC was diagnosed among patients who received *H. pylori* eradication therapy without pre-neoplastic lesions during a follow-up of 7.5 years. A meta-analysis of 10 studies involving 7955 patients by Chen *et al*^[22] also showed similar findings.

H. pylori eradication is found to reverse chronic gastritis in the majority of patients and atrophic gastritis in some patients^[23-25], but not for intestinal metaplasia^[24,26]. The presence of intestinal metaplasia is therefore considered to be a "point of no return" in the GC cascade. However, *H. pylori* eradication has been shown to slow the progression of intestinal metaplasia to GC^[25,27]. A study of 2258 patients with a much longer follow-up duration (up to 15 years) showed that *H. pylori* eradication reduced GC risk even in those with intestinal metaplasia and dysplasia^[28]. In concordance with this study, a randomized controlled trial of 544 patients concluded that *H. pylori* eradication after endoscopic resection of early GC could reduce the risk of metachronous GC by 65%^[29]. Since most of these patients with early GC would have concurrent pre-neoplastic lesions in the stomach, the findings would

support the potential benefits of *H. pylori* eradication to prevent GC development even in the presence of advanced gastric histology.

A recent nationwide population-based study from Sweden showed that treatment for *H. pylori* could reduce GC and non-cardia GC development when compared to background population^[30]. Overall, about 0.2% of patients developed GC after *H. pylori* treatment. However, the risk reduction was only apparent 5 years after *H. pylori* eradication treatment (standardized incidence ratio of 0.31), suggesting a long lag time of benefits by chemoprevention.

SURVEILLANCE FOR HIGH-RISK PATIENTS AFTER *H. PYLORI* ERADICATION

Eradication of *H. pylori* before the development of atrophic gastritis can nearly eliminate GC risk^[31]. As discussed, among patients who have already developed atrophic gastritis, eradication of *H. pylori* can only halt and partially reverse the progression of gastric mucosal damage, and therefore this group of patients is still at increased risk for GC development. According to the Kyoto Global Consensus statement^[32], patients with *H. pylori* infection diagnosed non-invasively and at risk for atrophic gastritis should undergo endoscopy for histological assessment. These risk factors include age range in which atrophic gastritis are prevalent in that particular population, a prior history of gastric ulcer, a pretreatment serum pepsinogen I level of less than 70 ng/mL and a pepsinogen I : II ratio of less than 3. The degree and extent of atrophic gastritis and intestinal metaplasia are important in predicting subsequent GC risk. Two validated histologic staging systems, Operative Link for Gastritis Assessment (OLGA)^[33] and Operative Link on Gastric Intestinal Metaplasia Assessment (OLGIM)^[34], have been proposed for further risk stratification. Those with OLGA or OLGIM stages III–IV are considered to be at high risk of GC development, and may be considered for a long-term endoscopic surveillance program^[31]. Surveillance programs are considered to be cost effective only in this group of high-risk patients^[32,35,36]. The aims of secondary prevention programs are to remove intraepithelial lesions and early GC before the lesions become invasive, thereby reducing GC-related deaths^[31,32]. Currently, there are insufficient data to guide the optimal management strategies for patients with lower OLGA and OLGIM stages. Even the optimal surveillance intervals for high risk patients are based on expert opinions rather than data from clinical trials^[37].

ROLES OF MEDICATIONS IN GC DEVELOPMENT AFTER *H. PYLORI* ERADICATION

There are still sparse data on the modifiable risk factors for GC after *H. pylori* eradication. Increasing evidence

has emerged showing that certain medications may increase GC risk, while some are shown to reduce cancer risk. However, the majority of these studies included both *H. pylori*-infected and *H. pylori*-negative subjects. In the following sections, medications that could potentially modify GC risk after *H. pylori* eradication, including proton pump inhibitors (PPIs), aspirin, cyclooxygenase-2 (COX-2) inhibitors, statins, metformin as well as lifestyle factors will be discussed. Their effects are summarized in Table 1.

Proton pump inhibitors

Since its introduction in the 1980s, PPIs have become one of the most commonly prescribed medications worldwide^[38]. PPIs lead to profound acid suppression which could worsen atrophic gastritis^[39], particularly in *H. pylori*-infected subjects^[40]. In addition, the increase in gastrin (a potent growth factor that has trophic effect on gastric mucosa) in response to the hypochlorhydria would stimulate enterochromaffin-like cell hyperplasia^[40]. A meta-analysis of four studies (one cohort and three case-control studies) showed that the risk of GC was increased by 43% among PPI users^[41]. However, the *H. pylori* status was unknown in these studies, and multiple biases including protopathic and indication biases were present.

Recently, we conducted a territory-wide retrospective cohort study recruiting 63397 *H. pylori*-eradicated subjects^[42]. PPIs use (defined as at least weekly use) was shown to be associated with an increased GC risk (HR = 2.44) even after *H. pylori* eradication, while histamine-2 receptor antagonists (H2RA) were not a significant risk factor. Compared with non-PPIs use, the risk increased with increasing frequency (HR 2.43 for weekly to less than daily use, and HR 4.55 for daily use) and duration of PPIs use (HR = 5.04, 6.65 and 8.3 for ≥ 1 year, ≥ 2 years and ≥ 3 years, respectively). The adjusted absolute risk difference for PPIs vs non-PPIs use was 4.29 excess GC cases per 10000 person-years. H2RA was chosen as a negative control exposure in this study to address the issue of indication bias. Prescriptions of PPIs and H2RA within six months prior to GC diagnosis were excluded to reduce protopathic bias. One intriguing observation from this study was that the cohort of PPIs users who had not received *H. pylori* eradication therapy had the lowest incidence rate of GC (0.8 per 10000 person-years) when compared to that of the two *H. pylori*-eradicated cohorts with and without PPIs use (8.1 and 2.9 per 10000 person-years, respectively). It thus appears that prior *H. pylori* infection is still a more important risk factor than PPIs use in the determination of GC risk, and PPIs increase GC risk only in those with baseline pre-neoplastic lesions induced by prior *H. pylori* infection. The study, however, did not investigate whether the increased GC risk existed for all kinds of PPIs.

Aspirin

Recent meta-analyses investigating the potential role of aspirin concluded that aspirin was associated with

Table 1 Pharmacological modalities to reduce risk of gastric preneoplastic lesions and/or cancer

References	Drugs	Study design	Number of subjects	Results
You <i>et al</i> ^[92] , 2006	Vitamin and garlic supplement	Randomized controlled trial	3365	No protective effect
Leung <i>et al</i> ^[56] , 2006	Rofecoxib	Randomized controlled trial	213	Regression of IM: (a) antrum (24.5% <i>vs</i> 26.9% for placebo) (b) corpus (4.3% <i>vs</i> 2.2% for placebo) OR of IM regression: (a) celecoxib alone (OR = 1.72; 95%CI: 1.07-2.76) (b) <i>H. pylori</i> eradication followed by celecoxib (OR = 1.48; 95%CI: 0.91-2.40)
Wong <i>et al</i> ^[57] , 2012	Celecoxib	Randomized controlled trial	1024	(a) celecoxib alone (OR = 1.72; 95%CI: 1.07-2.76) (b) <i>H. pylori</i> eradication followed by celecoxib (OR = 1.48; 95%CI: 0.91-2.40)
Cheung <i>et al</i> ^[53] , 2018	Aspirin	Population-based retrospective cohort study	63605	PS-adjusted HR of GC: 0.30 (95%CI: 0.15-0.61)
Cheung <i>et al</i> ^[42] , 2018	Proton pump inhibitors	Population-based retrospective cohort study	63397	PS-adjusted HR of GC: 2.44 (95%CI: 1.42-4.20)

IM: Intestinal metaplasia; OR: Odds ratio; PS: Propensity score; HR: Hazard ratio; GC: Gastric cancer.

a reduced GC risk in observational studies, while post-hoc analysis of randomized trials showed statistically non-significant trend favoring aspirin use^[43,44]. The chemopreventive effect of aspirin is mediated *via* both cyclooxygenase (COX)-2 and non-COX related pathways, including phosphatidylinositol 3-kinase (PI3K)^[45,46], NF- κ B^[47], Wnt- β -catenin, extracellular signal-regulated kinase (ERK) and activated protein1 (AP-1)^[48].

However, most published data included both *H. pylori*-infected and *H. pylori*-negative subjects. A few studies showed that the chemopreventive effect of aspirin was higher in *H. pylori*-infected subjects on stratified analysis^[49-51]. As shown in a case-control study, the chemopreventive effect of aspirin use was higher in *H. pylori*-infected subjects [odds ratio (OR) = 0.39] than in the whole cohort (OR = 0.60), and no statistically significant difference was noted for *H. pylori*-negative subjects^[50]. Another population-based study from Sweden showed that the ORs were 0.70 for the whole cohort and 0.60 for *H. pylori*-infected subjects, again without statistically significant difference for *H. pylori*-negative subjects^[51]. Similarly, a Taiwanese nationwide retrospective cohort study found that the HR of GC with regular use of non-steroidal anti-inflammatory drugs (NSAIDs) was lower for *H. pylori*-infected (HR = 0.52) than non-infected subjects (HR = 0.80)^[52].

A recent territory-wide retrospective cohort study recruiting 63605 *H. pylori*-eradicated subjects showed that aspirin use (defined as at least weekly use) was associated with a reduced GC risk (HR = 0.30)^[53]. The protective effect increased with increasing frequency, duration and dose of aspirin (all *P*-trend < 0.001), being most prominent in those who used aspirin daily (HR = 0.21), for at least 5 years (HR = 0.07) and at a dose of at least 100 mg (HR = 0.15). The protective effect of aspirin appeared to be larger in *H. pylori*-eradicated subjects (HR = 0.30) than that reported by a meta-analysis including both *H. pylori*-infected and *H. pylori*-negative subjects (pooled OR = 0.78)^[43]. This should be interpreted with caution, however, as it is not a head-to-

head comparison with different patient characteristics.

However, one of the major side effects of aspirin is gastrointestinal bleeding, and the risk-benefit profile of aspirin use on GC prevention in *H. pylori*-infected subject remains to be determined. The adjusted absolute risk difference was only 2.52 fewer GCs per 10000 person-years for aspirin users after *H. pylori* eradication^[53]. Future studies to address the risk-benefit profile are warranted. Nonetheless, the evidence from this territory-wide cohort study may provide further support for aspirin use in the consideration of the risk-benefit profile of aspirin use in preventing cardiovascular events and various cancers. The United States Preventive Services Task Force favors the use of low-dose aspirin for the primary prevention of cardiovascular disease and colorectal cancer in adults aged 50 to 59 years who have a more than 10% 10-year risk of cardiovascular disease and are not at increased risk of bleeding^[54].

COX-2 inhibitors

COX-2 is an enzyme involved in the conversion of arachidonic acid to prostaglandins, and its overexpression is found in gastric intestinal metaplasia and cancer^[55]. Two randomized trials have been performed to evaluate the potential benefit of COX-2 inhibitors^[56,57]. In the study of 213 *H. pylori*-eradicated subjects with intestinal metaplasia, the use of rofecoxib did not significantly regress intestinal metaplasia and its severity over 2 years^[56]. The study by Wong *et al* showed that celecoxib use for 2 years could regress advanced gastric lesions in *H. pylori*-infected subjects, but a synergistic effect was not observed in those who had *H. pylori* eradicated^[57].

Statins

Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is one of the key enzymes for cholesterol synthesis^[58], and are widely used for the primary and secondary prevention of cardiovascular diseases. Besides, it has been proposed to have chemopreventive effects on solid organ tumors in *in-vitro* studies, by halting cell-cycle progression^[59], inducing

apoptosis^[60], inhibiting angiogenesis^[61], and inhibiting the growth of tumor cells^[62].

To date no data from randomized clinical trials are available concerning the role of statins in GC prevention. A meta-analysis^[63] of 11 studies (eight observational, three post-hoc analyses of 26 clinical trials) reported a significant reduction in GC risk with statin use (adjusted OR = 0.68), in a dose-dependent manner. However, conflicting results exist among observational studies, with some showing statins to be protective against GC^[64-67], while no such benefit was observed in other studies^[68-74]. This is likely due to the heterogeneity of different studies, and the recruitment of both *H. pylori*-infected and *H. pylori*-negative subjects. The confounding effect of *H. pylori* would significantly affect the causal relationship and the magnitude of any beneficial effect. Therefore, studies dedicated to investigate the chemopreventive effect of statin on GC after *H. pylori* eradication are warranted.

Metformin

An increased GC risk by around 19% among patients with diabetes mellitus (DM) was reported by a meta-analysis of 17 studies (11 cohort studies, six case-control studies)^[75]. But among diabetic patients who take metformin, the GC risk appears to be lower^[76]. The anti-cancer activity by metformin is proposed to be mediated by two pathways. First, as metformin is an insulin sensitizer, it reduces the production of insulin and insulin-growth factors (IGFs). Proliferation of cancer cells expressing IGF receptors is stimulated by the IGFs signaling pathway^[77]. Second, the activation of AMP-activated protein kinase (AMPK) and the subsequent inhibition of the mammalian target of rapamycin pathway is shown to inhibit the growth of cancer cells^[78].

A recent meta-analysis of seven cohort studies concluded that metformin use was associated with a reduced GC risk (HR = 0.76)^[76]. However, significant heterogeneity was noted among these studies. The chemopreventive role of metformin remains controversial in clinical studies, as data from randomized clinical trials are not available. While a protective effect with varying effect estimate was shown for some studies^[79-83], others failed to demonstrate such association^[84,85]. The failure to adjust for *H. pylori* infection and the severity of DM further complicates the debate over this issue.

A population-based cohort study of 2603 Japanese subjects showed the GC risk was larger with higher hemoglobin A1c (HbA1c) levels^[86]. The age- and sex-adjusted incidence of GC among individuals with HbA1c levels of 5.0%-5.9%, 6.0%-6.9% and $\geq 7.0\%$ were 2.5, 5.1, and 5.5 per 1000 person-years. *H. pylori* infection and a higher HbA1c level ($\geq 6.0\%$) had a synergistic effect on increasing GC risk. The protective effect of metformin on GC may therefore be due to a better DM control instead of the anti-cancer effects found in *in-vitro* studies. This study, however, did not adjust for the effect of various medications and comorbidities, and therefore

the independent role of HbA1c level remains to be determined.

Future studies to include a homogenous group of patients (*i.e.*, only *H. pylori*-eradicated subjects) and factor in the effect of DM control (as reflected by HbA1c level) as well as medications that may modify cancer risk are crucial to investigate (1) the chemopreventive role of metformin in GC in diabetic patients; and (2) whether HbA1c is an independent risk factor for GC. As HbA1c is a time-varying covariate, not only the baseline HbA1c level but also the dynamics throughout the follow-up should be taken into consideration in order to derive a more precise effect estimate.

Lifestyle factors

Lifestyle factors that could potentially affect the risk of preneoplastic lesions and GC include smoking, alcohol use, high salt intake, vitamins and antioxidants. Concentrated salt intake is proposed to cause excessive cell replication (hence increased rate of endogenous mutations), to incur mucosal damage with associated inflammatory changes and to induce atrophic changes in gastric mucosa^[87]. Ascorbic acid, on the other hand, is linked with a protective effect against intestinal metaplasia and GC by reducing the gastric pH^[88]. Atrophic gastritis favors the proliferation of anaerobic bacteria which reduce nitrate (abundant in various kinds of food) to nitrite, in turn reacting with other nitrogen-containing components to generate N-nitroso carcinogens.

However, data on the roles of these factors in *H. pylori*-eradicated subjects are lacking. A randomized control trial on *H. pylori* eradication showed that alcohol consumption (OR = 1.67) was independently associated with intestinal metaplasia progression^[89]. Another study of more than 3000 Chinese subjects with baseline chronic atrophic gastritis showed that the risk of transition to dysplasia nearly doubled among smokers while the risk of intestinal metaplasia was mildly increased^[90]. In another study involving 3433 patients, the risk of progression to dysplasia or GC increased with increasing years of cigarette smoking, but decreased among those with higher levels of ascorbic acid (OR = 0.2, highest vs lowest tertile)^[91].

The roles of vitamin and antioxidant supplements also remain controversial as shown by a three-arm trial which randomized 3365 *H. pylori*-infected subjects to eradication therapy, vitamin supplement (vitamin C, vitamin E and selenium) and garlic supplement (aged garlic extract and steam-distilled garlic oil)^[92]. While *H. pylori* treatment reduced the risk of preneoplastic lesions and GC, similar beneficial effect was not observed for vitamin or garlic supplements.

CONCLUSION

H. pylori infection is the most important risk factor for GC development. However, *H. pylori* eradication does not entirely eliminate the GC risk, as pre-neoplastic

lesions (atrophic gastritis, intestinal metaplasia and dysplasia) may have already developed. It is therefore necessary to identify patients at high risk for GC after *H. pylori* eradication to streamline the management plan. The detection of atrophic gastritis by non-invasive testing (serum pepsinogen) or endoscopy with histologic assessment, followed by further risk stratification with validated histologic staging systems (e.g., OLGA and OLGIM) would be advisable. Patients with higher stages may require regular endoscopic surveillance. Apart from secondary prevention to reduce deaths by diagnosing GC at an early stage, identifying medications that could potentially modify the GC risk would be desirable. The potential roles of a number of medications have been suggested by various studies, including PPIs, aspirin, statins and metformin. However, several drawbacks need to be acknowledged. First, there are currently no randomized clinical trials to address the impact of these medications on GC risk. Second, the majority of these studies recruited both *H. pylori*-infected and *H. pylori*-negative subjects, but not specifically *H. pylori*-eradicated ones. In addition, previous studies failed to adjust for the effect of concurrent medications on GC risk. The failure to take into account the effect of *H. pylori* infection and concurrent medications will undoubtedly bias the causal relationship and the effect estimate. Recently, large population-based retrospective cohort studies have shown that PPIs were associated with an increased GC risk after *H. pylori* eradication, while aspirin was protective. The roles of statin and metformin in reducing GC risk after *H. pylori* eradication remain to be determined.

Owing to the relatively low incidence of GC and the long lag time of cancer development, investigating the potential modifiable factors (including medications) for GC development by randomized clinical trials would require a large sample size and long follow-up duration which are technically difficult and resource-intensive. Future research should focus on high-risk population including those with underlying pre-neoplastic lesions, family history of GC or those who have undergone endoscopic removal of early gastric tumors. Another research direction would be the use of population-based retrospective cohort study design for pharmaco-epidemiological studies on GC. As such, studies can be carried out in a short period of time with large sample size, despite the relatively rare incidence of GC. We have previously examined the effects of PPIs and aspirin among *H. pylori*-eradicated subjects in population-based retrospective cohort studies. There are still other potential chemopreventive agents that remain to be explored, for example, statins and metformin. The concept of “drug repurposing” has recently been advocated in the field of oncology: Currently approved drugs with a non-oncology primary purpose may be used for chemoprevention or as an adjunctive treatment. The use of population-based retrospective cohort studies can help in identifying potential drug candidates and directing the path to future randomized

clinical trials.

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Emerging evidence of the molecular landscape specific for hematogenous metastasis from gastric cancer

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Abstract

Gastric cancer (GC) is one of the most frequently

diagnosed cancers in the world. Most GC patients are diagnosed when the cancer is in an advanced stage, and consequently, some develop metastatic lesions that generally cause cancer-related death. Metastasis establishment is affected by various conditions, such as tumor location, hemodynamics and organotropism. While digestive cancers may share a primary site, certain cases develop hematogenous metastasis with the absence of peritoneal metastasis, and vice versa. Numerous studies have revealed the clinicopathological risk factors for hematogenous metastasis from GC, such as vascular invasion, advanced age, differentiation, Borrmann type 1 or 2 and expansive growth. Recently, molecular mechanisms that contribute to metastatic site determination have been elucidated by advanced molecular biological techniques. Investigating the molecules that specifically participate in metastasis establishment in distinct secondary organs will lead to the development of novel biomarkers for patient stratification according to their metastatic risk and strategies for preventing and treating distinct metastases. We reviewed articles related to the molecular landscape of hematogenous metastasis from GC.

Key words: Gastric cancer; Hematogenous metastasis; Hepatic metastasis; Molecular mechanism; Biomarker; Premetastatic niche

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Core tip: Gastric cancer (GC) has high cancer-related mortality, which is mainly caused by distant metastasis including hematogenous metastasis. Numerous steps are required to establish a metastatic focus, and understanding the molecular mechanisms of each step is necessary to conquer metastasis. Development and dissemination of sequencing technology have elucidated some of the molecular biological mechanisms associated with cancer metastasis. This review aims to summarize the molecules reportedly contributing to hematogenous metastasis from GC and to become

the groundwork for the further development of novel biomarkers and molecular targets.

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INTRODUCTION

Malignant tumor cells have characteristics unlike noncancerous cells, such as autonomous growth, immortalization, invasion and metastasis. Among these characteristics, metastasis greatly affects the quality of life of patients and is the main cause of cancer-related mortality. Understanding the mechanism and management of metastasis is urgently required to improve the prognosis of cancer patients.

The establishment of metastasis is affected by various conditions. Metastatic sites depend on anatomical and hemodynamic structures of the vascular system^[1]. Digestive cancers have a higher incidence of hepatic metastasis than other malignancies, and colon cancer tends to metastasize to the liver more frequently than rectal cancer because of the portal vein reflux. Alternatively, the frequency of hepatic metastasis is lower in gastric cancer (GC) than that in colon cancer despite the similar portal vein reflux in both cancers^[2]. Cancers have respective organotropism that cannot be illustrated by only the anatomical viewpoint. A compatibility between circulating tumor cells and a premetastatic niche is required, which is referred to as the seed and soil hypothesis^[3]. This hypothesis has pioneered the molecular biological understanding of the mechanism of tumor metastasis. Recently, improved sequencing technology has provided new insight into the steps required for tumor metastasis, such as vascular invasion, detachment, survival in hypoxic or non-anchored environments, immune evasion, tissue engraftment, and colonization^[4,5]. In addition to metastatic organotropism due to the primary organ, intratumor and intertumor heterogeneity contributes to metastatic target organ determination^[6,7]. Only an appropriate subclone with suitable attributes for a certain microenvironment can form a metastatic focus in a corresponding organ. In this article, we review the molecules associated with hematogenous metastasis from GC and microenvironment establishment for hepatic metastasis that is representative of hematogenous metastasis and list the molecules in Table 1 and Figure 1.

GC is the third leading cause of cancer-related death in both sexes worldwide^[8]. The prognosis of patients with GC is dismal: The 5-year survival for all patients is approximately 50% and is only 25%-30% for patients with advanced GC due to a lack of curative therapeutic

agents and sensitive biomarkers predicting recurrence^[9]. Concerning peritoneal dissemination that is the most frequent metastasis from GC, development of recent therapeutic strategies might improve the prognosis of GC patients^[10,11]. Surgical resection of hepatic metastasis can improve the outcome of GC patients, though the adaptation of surgical treatment for hematogenous metastasis is limited^[12]. The development of remedies against hematogenous metastasis has stalled. Elucidating the molecular biological mechanisms specific for hematogenous metastasis from GC will be a significant and effectual step for the development of novel biomarkers and therapeutic target molecules, which will lead to the improvement of patients' prognoses.

EPITHELIAL MESENCHYMAL TRANSITION AND INVASION INTO THE CIRCULATION

Epithelial mesenchymal transition and invasion into the circulation are the first steps for distant metastasis from the primary lesion. To spread to other organs through the blood stream, tumor cells must invade the basal lamina, reach and invade vessels, and detach from the primary tumor nodule. Then, single tumor cells or tumorspheres must acquire a mesenchymal phenotype and resist anoikis to arrive at a target organ. We have listed the genes that reportedly contribute to these steps and summarized the studies below.

Vimentin

Vimentin (VIM) is a type III intermediate filament protein that is mainly expressed in mesenchymal cells and an important marker of epithelial mesenchymal transition (EMT)^[13]. Epithelial cancer cells acquire motility and metastatic potential by cellular re-programming to a mesenchymal phenotype. Increased vimentin expression has been reported in various cancers including gastrointestinal cancers^[14,15]. Zhao *et al.*^[16] explored the clinical significance of VIM expression and human epidermal growth factor receptor 2 (HER2) status in GC tissues by immunohistochemistry (IHC). They found that VIM expression was significantly correlated with older age, advanced stage, poorly differentiated type, venous invasion, hepatic metastasis and recurrence and that HER2 status was correlated with advanced cancer, poor differentiation, venous invasion, hepatic metastasis and recurrence. There was a significant correlation between VIM expression and HER2-positivity. VIM expression was detected in 9.8% in GC patients and was not detected in early GC patients. The 3-year survival of the patients with vimentin-positive GC was significantly poorer than that of patients with vimentin-negative GC. VIM positivity was an independent prognostic factor in multivariate analysis with respect to overall survival. VIM plays an important role in metastasis and may have a more requisite role in the establishment of hematogenous metastasis in GC. EMT inhibitors in-

Table 1 Molecules reported to be associated with hematogenous metastasis from gastric cancer

Molecule	Full name	Biological function	Specimens	Detection methods	<i>In vivo</i>	Associating molecules and cells	Ref.
EMT and invasion into the circulation							
VIM	Vimentin	Type III intermediate filament	GC tissue	IHC	-	HER2	[16]
GPR155	G protein-coupled receptor 155	Seven-pass transmembrane receptor	GC tissue, GC cell line	qPCR, IHC	-	TWIST1, WNT5B, p-ERK1/2, p-STAT1	[19]
Survival in the circulation							
HIF-1 α	Hypoxia inducible factor-1 alpha	Transcription factor in response to hypoxia	GC tissue	IHC	-	-	[25]
EGFL7	Epidermal growth factor-like domain-containing protein 7	Epidermal growth factor for vasculogenesis	GC tissue, GC cell line	qPCR, WB, IHC	Yes	AKT, SNAI1	[30]
Premetastatic niche							
CXCL1	C-X-C motif chemokine ligand 1	Inflammatory chemokine binding CXCR2	CRC cell line, liver (M) ¹ , lung (M) ¹ , cecum (M) ¹	ELISA, FCM	Yes	CXCR2, VEGF-A, MDSCs	[36]
TIMP1	Tissue inhibitor of metalloproteinase 1	Inhibitor of MMPs	Plasma, CRC tissue, CRC cell line, liver (M) ¹	qPCR, ELISA	Yes	SDF-1, Neutrophil	[43]
			Plasma, PDAC tissue, PDAC cell line (M), liver (M)	qPCR, ELISA, IHC	Yes	PI3K, CD63, SDF-1, HSC, Neutrophil	[44]
Exosome	-	Cell-derived membrane vesicle	CRC tissue, serum, CRC cell line	qPCR, WB	Yes	miR-203	[51]
			PDAC cell line, liver (M) ¹ , lung (M) ¹ , spleen (M) ¹ , kidney (M) ¹ , brain (M) ¹ , bone marrow (M) ¹	WB, IHC, IF, FCM, Proteomics	Yes	Proteins ²	[52]
Migration, invasion and proliferation at the target organs							
NFKB1/p105	Nuclear factor kappa B subunit 1	Transcription factor	GC tissue	FCM	-	-	[55]
			GC tissue	FCM	-	Ki-67	[57]
MAP1LC3	Microtubule associated protein 1 light chain 3	Subunit of MAP1 and associated with autophagy	GC tissue	IHC	-	-	[63]
BECN1	Beclin1	Autophagy regulator and component of PI3K complex	GC tissue	IHC	-	-	[63]
SQSTM1/p62	sequestosome 1	Activator of NF-kB signaling	GC tissue	IHC	-	-	[63]
MFS4	Major facilitator superfamily domain containing 4	Multi-pass transmembrane protein	GC tissue, GC cell line	qPCR	-	BMP2, NUDT13, OCLN	[64]
PAK1	p21 (RAC1) activated kinase 1	serine/threonine p21-activating kinase	GC tissue, GC cell line	qPCR, WB, IHC, IF	Yes	ATF2, miR-132, CD44, FN1	[66]
Angiogenesis							
VEGF-D	Vascular endothelial growth factor-D	Growth factor for angiogenesis	GC tissue	IHC	-	-	[70]
TYMP	Thymidine phosphorylase	Angiogenic factor	GC tissue	IHC	-	-	[72]
			GC tissue	IHC	-	-	[73]
Biomarkers predicting hematogenous metastasis from GC							
IL-6	Interleukin-6	Inflammatory cytokines	Serum	ELISA, CLEIA	-	HGF	[78]
Glut1	Glucose transporter-1	Glucose transporter	GC tissue	IHC	-	-	[79]
HER2	Human epidermal growth factor receptor 2	Epidermal growth factor receptor	GC tissue	IHC, FISH	-	-	[80]
			GC tissue	IHC, FISH	-	-	[81]
NCPAP3	NTase domain containing non-canonical poly(A) polymerase 3	mRNA stabilizing factor	GC tissue, GC cell line	qPCR	-	-	[82]
NPM1	Nucleophosmin 1	Nucleolar protein	GC tissue	IHC	-	-	[83]
CXCR4	C-X-C motif chemokine receptor 4	Inflammatory chemokine receptor binding CXCL12	GC tissue	IHC	-	CXCL12	[84]
CXCL12	C-X-C motif chemokine ligand 12	Inflammatory chemokine binding CXCR4	GC tissue	IHC	-	CXCR4	[84]

D-Dimer	-	Fibrin degradation product	Plasma	LEIA	-	-	[85]
Fibrinogen	-	Coagulation factor	Plasma	Clauss clotting method	-	-	[86]
CD44v6	CD44 variant 6	Adhesion molecule	GC tissue	qPCR, IHC	-	-	[87]

¹(M): Specimen obtained from mouse; ²70 proteins were listed in the original article. IHC: Immunohistochemistry; qPCR: Quantitative reverse transcription polymerase chain reaction; *TWIST1*: Twist family bHLH transcription factor 1; *WNT5B*: Wingless-type MMTV integration site family, member 5B; p-: Phosphorylated; ERK1/2: Extracellular signal-regulated kinase 1 and 2; *STAT1*: Signal transducer and activator of transcription 1; WB: Western blotting; AKT: AKT serine/threonine kinase; *SNAIL*: Snail family transcriptional repressor 1; CRC: Colorectal cancer; ELISA: Enzyme-linked immunosorbent assay; FCM: Flow cytometry; MDSCs: Myeloid-derived suppressor cells; MMPs: Matrix metalloproteinases; *SDF-1*: Stromal cell-derived factor 1; PDAC: Pancreatic ductal adenocarcinoma; PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; HSC: Hepatic stellate cell; miR: MicroRNA; IF: Immunofluorescence; Ki-67: Marker of proliferation Ki-67; MAP1: Microtubule-associated proteins 1; BMP2: Bone morphogenetic protein 2; *NUDT13*: Nudix hydrolase 13; *OCN*: Occluding; *ATF-2*: Activating transcription factor 2; FN1: Fibronectin 1; CLEIA: Chemiluminescent enzyme immuno assay; HGF: Hepatocyte growth factor; FISH: Fluorescence *in situ* hybridization; GC: Gastric cancer.

cluding TGF- β signaling pathway inhibitor might be a therapeutic agent for hematogenous metastasis from GC^[17].

G protein-coupled receptor 155

G protein-coupled receptors (*GPCRs*) are seven-pass transmembrane receptors that participate in diverse physiological processes including visual sensing, immune response, cell viability, and tumor metastasis^[18]. Ligand binding to *GPCRs* activates the G protein and intracellular signaling. Because there are numerous *GPCRs* and they are the origin of many intracellular signals, *GPCRs* represent 30%-50% of the targets of currently marketed therapeutic drugs^[19]. *GPR155* is a member of the *GPCR* family and little is known about its function. Our recent global expression analysis of primary GC tissues obtained from patients with synchronous hepatic metastasis and without metastasis to the peritoneal cavity or distant lymph nodes uncovered that *GPR155* was a molecule specific for hematogenous metastasis^[20]. *GPR155* was the most downregulated gene in GC tissues with synchronous hepatic metastasis compared with GC tissues without hepatic metastasis. In stage IV GC, the expression level of *GPR155* was significantly lower in patients with synchronous hematogenous metastasis compared with patients without hematogenous metastasis. In stage II/III GC, the patients in the *GPR155* low expression group had significantly higher cumulative incidence of hematogenous recurrence. Multivariate analysis showed that downregulated expression of *GPR155* mRNA was an independent predictor of hematogenous metastasis. Furthermore, we revealed that the expression level of *GPR155* was inversely correlated with the expression of *TWIST1* and *WNT5B*, which have been well known to play pivotal roles in *EMT*. Inhibition of *GPR155* expression using siRNA specific for *GPR155* increased the level of p-ERK1/2 and p-STAT1 and cell proliferation and invasion capacity *in vitro*. We found that *GPR155* may represent a molecule specific for hematogenous metastasis from GC *via* *EMT* and cell viability promotion and may be a putative biomarker for diagnosing and predicting hematogenous metastasis from GC. *GPR155* is a transmembrane receptor, is expected to be a

druggable target.

SURVIVING IN THE CIRCULATION

When tumor cells detach from the primary nodule and enter the circulation, they are exposed to stress from hypoxia in the portal vein and a non-adherent state. Activation of an alternative metabolic pathway under hypoxia and acquisition of anoikis resistance are necessary to endure these environmental selective pressures^[21,22]. A subclone that evolves to adapt itself to this severe environment for epithelial cells can reach the new soil alive. Here, we review the molecules that contribute to environmental adaptation that are reportedly related to hematogenous metastasis from GC.

Hypoxia inducible factor-1 alpha

The hypoxic environment is known to be related to angiogenesis, a malignant tumor phenotype and resistance to therapies^[23]. The adaptation to a hypoxic environment is an important advantage for the development of distant metastases^[24]. Hypoxia inducible factor-1 alpha (*HIF-1 α*) expression is suppressed under normal oxygen partial pressure by the ubiquitin-proteasome pathway. When oxygen supply becomes deficient, the concentration of *HIF-1 α* is elevated, promoting transcription of vascular endothelial growth factor (*VEGF*), glucose transporter 1, platelet derived growth factor subunit B, carbonic anhydrase 9, *etc.*, by forming a heterodimer with *HIF-1 β* ^[25]. Some studies have suggested the utility of *HIF-1 α* inhibitor to suppress cancer cell activity^[26,27]. GC cells that have detached from a primary lesion can survive and engraft in the portal vein, which is hypoxic, to form metastatic loci. Chen *et al*^[28] showed that *HIF-1 α* overexpression in GC tissue was more frequent in patients with hepatic metastases than without hepatic metastasis. They also reported that *HIF-1 α* was higher in patients with peritoneal metastasis than in patients without peritoneal metastasis, but the population of high *HIF-1 α* still tended to be large in patients with hepatic metastasis. *HIF-1 α* must play an important role in distant metastases including hematogenous metastasis from GC.

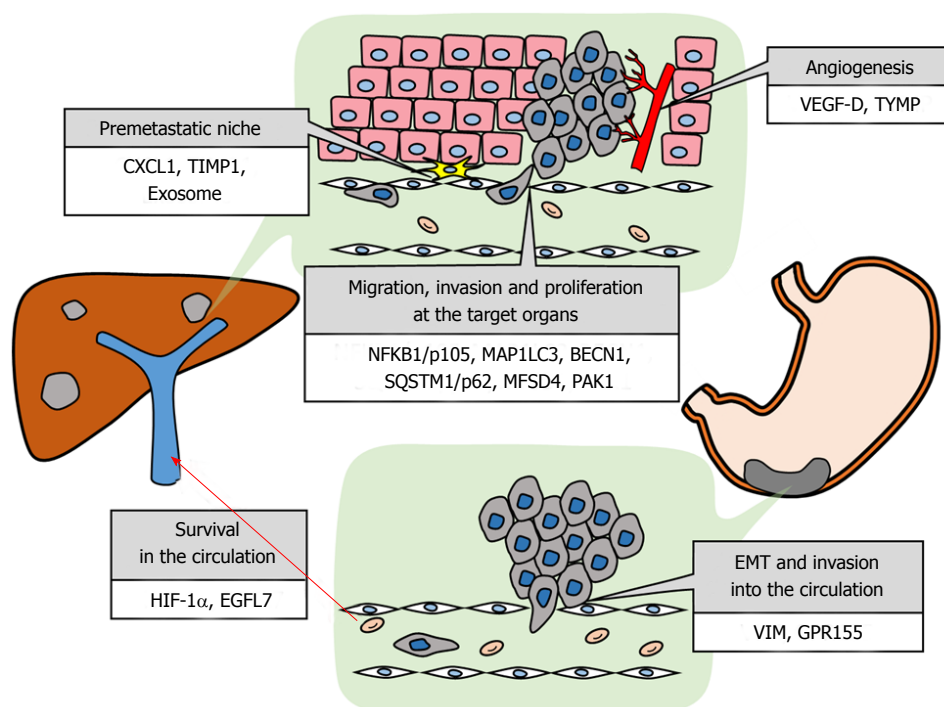


Figure 1 Schema of molecules associated with each step of the establishment of hepatic metastasis from gastric cancer. VIM: Vimentin; GPR155: G protein-coupled receptor 155; HIF-1 α : Hypoxia inducible factor-1 alpha; EGFL7: Epidermal growth factor-like domain-containing protein 7; CXCL1: C-X-C motif chemokine ligand 1; TIMP1: Tissue inhibitor of metalloproteinase 1; NFKB1/p105: Nuclear factor kappa B subunit 1; MAP1LC3: Microtubule associated protein 1 light chain 3; BECN1: Beclin1; SQSTM1/p62: Sequestosome 1; MFSD4: Major facilitator superfamily domain containing 4; PAK1: P21 (RAC1) activated kinase 1; VEGF-D: Vascular endothelial growth factor-D; TYMP: Thymidine phosphorylase.

Epidermal growth factor-like domain-containing protein 7

In a physiological state, epithelial cells, including neoplastic cells, suppress anoikis by adhering to the extracellular matrix (ECM) and adjacent cells *via* integrin or cadherin, and a loss of adhesion induces apoptosis^[29]. Anoikis resistance is an important factor for metastasizing to distant organs in various cancers^[30]. In GC, anoikis resistance has been relatively well investigated in peritoneal metastasis, which is the most frequent metastasis from GC^[31,32]. Luo *et al.*^[33] demonstrated that epidermal growth factor-like domain-containing protein 7 (EGFL7) promoted metastasis by activating EMT through an EGFR-AKT-Snail signaling pathway and by protecting GC cells from anoikis. Overexpression of EGFL7 significantly decreased apoptotic GC cells in suspension culture, and GC cells treated with *EGFL7*-specific shRNA had a significantly higher percentage of apoptotic cells. Moreover, they showed that EGFL7-overexpressing cells grew into larger tumors and were more likely to metastasize to the liver compared to *EGFL7*-underexpressing CG cells *in vivo*. Although the mouse xenografts in their study were ectopic subcutaneous tumors, the results suggested that EGFL7 should play a pivotal role in the establishment of hematogenous metastasis *via* anoikis resistance.

PREMETASTATIC NICHE

In 1978, Schofield^[34] postulated that the microenvironment could maintain hematopoietic stem cells and

advocated the concept of the niche for stemness in the spleen. Recently, the concept has been extended to a metastatic niche as the microenvironment that is conducive to the survival and proliferation of metastatic cancer cells^[35]. A premetastatic niche is the soil in secondary organs that is formed before the arrival of circulating tumor cells by factors from primary tumor cells that adjust the premetastatic niche. Studies on blocking premetastatic niche formation may provide novel treatment strategies to prevent distant metastasis and cure cancers, as cancers cannot be cured when they metastasize to distant organ. To the best of our knowledge, there have been no reports regarding a hematogenous premetastatic niche. Hence, elements reported to be involved in premetastatic niche formation in gastrointestinal cancers are introduced below.

C-X-C motif chemokine ligand 1

C-X-C motif chemokine ligand 1 (*CXCL1*) encodes an 11 kDa chemokine and is a member of the CXC family. *CXCL1* is secreted by macrophages and epithelial cells and acts as a chemoattractant for neutrophils^[36]. *CXCL1* participates in angiogenesis, inflammation, wound healing and development of the spinal cord, and its aberrant expression facilitates tumorigenesis, cell proliferation and metastasis in certain cancers^[37,38]. In colorectal cancer (CRC), *CXCL1* contributes to premetastatic niche formation by recruiting C-X-C motif chemokine receptor (*CXCR2*)-positive myeloid-derived suppressor cells (MDSCs)^[39]. *VEGF-A* secreted by primary CRC cells

stimulates tumor associated macrophages (TAMs) in the primary focus to produce *CXCL1*. The secreted *CXCL1* drives circulating MDSCs to the premetastatic liver. MDSCs isolated from premetastatic livers of xenograft mice bearing human CRC cells in the cecal wall promote CRC cell survival. The cancer cells in the primary focus drive MDSCs to the liver *via* *CXCL1* from TAMs and might form a premetastatic niche to evade innate and adaptive immune responses.

Tissue inhibitor of metalloproteinase 1

The balance of proteases and their inhibitors is essential to maintain homeostasis in the ECM. Matrix metalloproteinases (MMPs) are proteinases that decompose the ECM, and their overexpression is reportedly associated with tumor spread and metastasis^[40]. Several studies have reported the correlation between MMP overexpression and poor prognosis in several malignant tumors^[41]. Thus, it was hypothesized that inhibition of MMPs would result in a therapeutic anticancer effect^[42]. Tissue inhibitor of metalloproteinase (TIMP) inhibits MMP activity and prevents tissue destruction by forming a complex with MMPs^[43]. However, increased expression of TIMP1 is negatively correlated with survival in patients with several cancer types^[44,45]. Additionally, in GC patients, the association between TIMP1 overexpression and poor outcome has been reported^[46]. Seubert *et al.*^[47] described that TIMP1 created a premetastatic niche for hepatic metastasis from CRC, which explains the paradoxical phenomenon where TIMP expression correlated with poor prognosis in cancer patients even though TIMP inhibits MMPs. In their study, high TIMP1 levels in plasma and CRC tissue were associated with hepatic metastasis in CRC patients, and TIMP1-overexpressing tumors transplanted subcutaneously diverted intravenously injected cancer cells to the liver in a mouse model. Additionally, they demonstrated that TIMP1 established a premetastatic niche by recruiting stromal cell-derived factor 1 (SDF-1)-dependent neutrophils to the liver. Grünwald *et al.*^[48] demonstrated that TIMP1 secreted by pancreatic cancer activated hepatic stellate cells (HSCs) *via* CD63 and phosphatidylinositol 3-kinase signaling and increased susceptibility of the liver to pancreatic cancer cells. Activated HSCs expressed SDF-1, attracting neutrophils to the liver, which formed a premetastatic niche. *In vivo*, systemic increases in TIMP1 lead to more hepatic metastases after injections of pancreatic cancer cells, which did not occur in TIMP1 or CD63 knockout mice. HSCs were reported to participate in the formation of a premetastatic niche in other studies^[49]. TIMP1 overexpression was observed in GC tissue, and therefore TIMP1 might contribute to the formation a hepatic premetastatic niche in GC^[46].

Exosomes

Exosomes are small membrane vesicles derived from

most eukaryotic cells *in vivo* and *in vitro*^[50]. Derived exosomes exist not only in the ECM but also in bodily fluids, including blood, urine and cerebrospinal fluid, circulating in the body. Past studies have indicated that exosomes are associated with various biological processes, participating in apoptosis, angiogenesis, inflammation, coagulation and antigen presentation^[51]. Moreover, exosomes function as a cargo transporting proteins and nucleic acids to target cells and act as a communication tool between distant cells^[52]. Recently, exosomes from cancer cells were reported to facilitate cancer progression and metastasis and to suppress anti-tumor immunity^[53,54]. Takano *et al.*^[55] described that circulating exosomal microRNA (miR)-203 was associated with distant metastasis in CRC patients. Exosomal miR-203 that originated from primary CRC was reportedly incorporated into monocytes and promoted the differentiation of monocytes to M2-tumor-associated macrophages (TAMs). In a xenograft mouse model, miR-203-transfected CRC cells developed more liver metastases than control CRC cells. Their result suggested that exosomes bearing miR-203 contribute to the establishment of a premetastatic niche *via* TAM promotion in the liver of CRC patients. Yu *et al.*^[56] demonstrated that exosomes derived from pancreatic cancer induced a liver premetastatic niche. They performed proteomic analysis on exosomal proteins and revealed that these proteins were involved in pancreatic cancer growth, invasion and metastasis. Interestingly, another study showed that exosomes had respective organotropism, which was prescribed by integrin on their membranes^[57]. The organotropism of exosomes depended on the combination of the alpha chain and beta chain of integrin and distinct cells in the target organ took up the circulating exosomes. Exosomes from cancer cells were delivered by integrin to a particular organ and formed a premetastatic niche *via* contained proteins or nucleic acids, leading to metastatic organotropism. Further exploration of exosomes should uncover more insights on organotropism and the mechanisms of metastasis.

MIGRATION, INVASION AND PROLIFERATION AT THE TARGET ORGANS

Cancer cells that arrive at a metastatic organ are trapped in a capillary plexus. Subsequently, the adhesion of cancer cells to epithelial cells is driven by selectin and integrin families^[58]. Then, cancer cells migrate to the interval of epithelial cells and invade target organ tissue *via* adhesion to and decomposition of the basal lamina. Among the cancer cells that arrive at a metastatic target organ, only the cells that have acquired the capacities of adhesion, migration, invasion and proliferation can form a new tumor focus. We summarized the reported molecules associated with these steps.

Nuclear factor kappa B subunit 1

Ohyama *et al.*^[59] examined nuclear factor kappa B subunit 1 (NFKB1/p105) immunofluorescence intensity in GC cells isolated from 43 clinical specimens using flow cytometry. The intensity was higher in patients with hepatic metastasis than in patients without hepatic metastasis and was positively correlated with venous invasion. In contrast, the intensity was lower in patients with peritoneal metastasis than in patients without peritoneal metastasis. NFKB1/p105 intensity was not associated with nodal metastasis, lymphatic invasion, serosal invasion or histological type. Kimura *et al.*^[60] also reported a correlation between the p105-labeling rate detected by flow cytometry and hepatic metastasis from GC. In addition, they reported that NFKB1/p105 positivity by flow cytometry correlated positively with Ki-67 positivity, an index widely used for cell proliferation^[61]. NFKB1/p105 in GC cells is a putative biomarker specific for hematogenous metastasis. It was also reported that 5-FU resistance might be overcome *via* suppression of phosphorylated NFKB in Epstein-Barr virus-positive gastric cancer^[62]. The development of therapeutic agent targeting NFKB might lead to improvement of GC patients' prognosis.

Autophagy-related proteins

Autophagy is an intracellular degradation system that delivers cytoplasmic proteins and organelles to the lysosome, and it is an important system for maintaining intracellular homeostasis against pathogens and nutrient stress^[63]. Additionally, there are contradictory aspects in autophagy regarding neoplasia. In non-cancerous cells, autophagy protects cells from adverse effects leading to malignant transformation, such as reactive oxygen species, aberration of organelles and DNA damage^[64]. In contrast, autophagy acts as an important anti-apoptotic mechanism in established cancer cells resisting hypoxia, malnutrition and therapeutic agents^[65]. Therefore, inhibiting autophagy should be a viable therapeutic strategy for cancers, and effective treatments with an anti-autophagy agent have been reported^[66]. Sharifi *et al.*^[67] showed that autophagy was necessary for metastatic cells to migrate and invade by focal adhesion disassembly *via* proteolysis of paxillin. Their work marked the first anti-metastatic effect *via* autophagy inhibition and was a notable achievement. Masuda *et al.*^[68] indicated the correlation between autophagy-related proteins, microtubule associated protein 1 light chain 3, beclin1 and sequestosome 1/p62, and clinicopathological features. They investigated the expression of these proteins by IHC in 510 GC tissues. Autophagy, as determined by the expression of these proteins, was significantly correlated with poor survival rates and incidence of hepatic metastasis, but not with incidence of peritoneal metastasis. Understanding the role of autophagy in tumor survival and metastasis would help develop specific autophagy inhibitors and might improve

the outcome of patients with GC.

Major facilitator superfamily domain containing 4

Major facilitator superfamily domain containing 4 (MFSD4) is located on chromosome 1q32.1 and encodes a multi-pass transmembrane protein, and its biological function has not yet been determined. We recently detected MFSD4 as a biomarker specific for hepatic metastasis of GC by sequencing RNA from the GC tissue of patients with or without hepatic metastasis^[69]. Patients with low MFSD4 expression in primary GC tissues had significantly higher cumulative incidence of hepatic recurrence, and reduced MFSD4 expression was an independent risk factor of metachronous and synchronous hepatic metastasis. We indicated that DNA methylation in CpG islands of MFSD4 was one of the suppressive mechanisms of transcription. Furthermore, GC cell migration and invasion abilities were significantly increased by inhibition of MFSD4 expression using siRNA. MFSD4 should be a promising biomarker predicting hepatic metastasis in GC patients.

p21 (RAC1) activated kinase 1

p21 (RAC1) activated kinase 1 (PAK1) is a serine/threonine-protein kinase that plays a critical role in cytoskeleton dynamics, cell adhesion, migration, proliferation, apoptosis and mitosis^[70]. Liu *et al.*^[71] delineated the downstream pathway of PAK1 in which PAK1 acted as an oncogenic factor. PAK1 suppressed the expression of miR-132 *via* phosphorylating activating transcription factor 2 (ATF2), which bound to the promoter of miR-132. Phosphorylation of ATF2 inhibited its nuclear translocation and resulted in the diminution of miR-132. Additionally, their bioinformatics analysis revealed direct targets of miR-132, including CD44 and fibronectin 1, whose inhibition induced tumor apoptosis. Furthermore, miR-132 overexpression inhibited cell adhesion and migration *in vitro* and hematogenous metastasis *in vivo*. The patients with lower miR-132 expression in GC tissue had significantly poorer prognoses, and hepatic metastatic tissues expressed significantly lower miR-132 compared with primary GC tissues while there were no differences between primary GC tissues and lymph node metastases or peritoneal metastases. PAK1 and its downstream pathway should be a useful biomarker and therapeutic target for hematogenous metastasis from GC.

ANGIOGENESIS

As in the primary lesion, growth factors and angiogenic factors are required for metastatic focus growth. Tumor angiogenesis is necessary to supply nutrients and oxygen, and to carry out metabolites for tumor growth^[72]. Additionally, an increase in blood vessels leads to further metastatic opportunities. In this section, we introduce studies that investigated the relationship between angiogenic factors and hematogenous

metastasis.

Vascular endothelial growth factor-D

Effectiveness of anti-VEGF and anti-VEGFR monoclonal antibodies were proved in clinical management^[73-75]. Several studies have shown the association between vascular endothelial growth factor-D (VEGF-D) and lymph node metastasis in GC^[76,77]. Deng *et al.*^[78] indicated that VEGF-D is associated with hepatic metastasis from GC. They investigated the correlation between hepatic metastasis and the expression levels of VEGF-A, VEGF-C, VEGF-D, VEGFR-3, and CD34 by IHC. VEGF-D, VEGFR-3, CD34, Lauren classification and lymph node metastasis were associated with hepatic metastasis after radical surgery in univariate analysis, and VEGF-D was the only independent indicator of hepatic metastasis in multivariate analysis. They concluded that VEGF-D is an important factor for predicting hepatic metastasis of GC. The VEGF family plays a key role in angiogenesis and lymphangiogenesis. Their study lacks evidence of the molecular mechanism of hepatic metastasis establishment, though VEGF-D might contribute to hepatic metastasis *via* angiogenesis, which increases intratumor blood flow and nourishes the metastatic tumor.

Thymidine phosphorylase

Thymidine phosphorylase (TYMP) is an enzyme involved in pyrimidine nucleotide metabolism and was recently reported to be identical to platelet-derived endothelial cell growth factor (PD-ECGF). PD-ECGF has angiogenic activity *in vitro* and *in vivo*^[79]. Kimura *et al.*^[80] investigated the association of clinicopathological features with the expression of VEGF and TYMP in IHC analysis. In their study, there was a significant correlation between the positive expression of VEGF and lymphatic invasion. Additionally, the positive expression of TYMP and VEGF was significantly correlated with the frequency of hepatic recurrence. Moreover, patients with positivity of both TYMP and VEGF had significantly unfavorable prognoses. Their results indicated that combination analyses of TYMP and VEGF expression in GC appear to be well-characterized indicators of prognosis and suggested that co-expression of TYMP and VEGF, molecules contributing to angiogenesis, supported hepatic metastasis formation. Maeda *et al.*^[81] also reported that TYMP was associated with angiogenesis and hepatic metastasis from GC. They showed a correlation between TYMP expression and microvessel density in GC tissue by IHC. TYMP-positive patients had higher microvessel density and a significantly higher frequency of hepatic metastasis. Their result suggested that microangiogenesis promotes the establishment of hepatic metastasis.

BIOMARKERS PREDICTING

HEMATOGENOUS METASTASIS FROM GC

Many cancer-related genes that should be putative

biomarkers and therapeutic targets have been reported in the past^[82,83]. In recent decades, the progress and generalization of sequencing technologies have enriched our molecular knowledge regarding cancers and revealed the molecular mechanisms specific for distinct metastatic organs and hematogenous metastasis^[84,85]. While some studies have described mechanisms contributing to the establishment of hematogenous metastasis or downstream pathways, other studies have described biomarkers for predicting hematogenous metastasis. These biomarkers are useful for patient stratification, selection of therapeutic strategy and postoperative surveillance according to individual risk of metastasis and recurrence. Additionally, further investigation of molecular mechanisms might lead to the development of novel therapeutic target molecules. We listed the molecules reported as biomarkers of hematogenous metastasis in Table 1 and outlined some of them below^[86-95].

Interleukin-6

Interleukin-6 (IL-6) is a representative inflammatory cytokine that participates in B cell maturation, T cell differentiation, activation of natural killer cells, suppression of regulatory T cells and cancer cachexia^[96]. Additionally, IL-6 involvement in the biological activity of cancer cells has been previously reported^[97,98]. High IL-6 expressing tumor cells formed more distant metastases in breast cancer, lung cancer and hepatocellular carcinoma^[99,100]. Furthermore, adhesion of tumor cells to targeted organs, which leads to metastatic focus formation, is facilitated in high IL-6 expressing organs such as brain, lung, liver and bone marrow^[101]. In GC, the association between IL-6 and clinicopathological factors has been reported^[102,103]. Ashizawa *et al.*^[86] assessed the correlation of preoperative serum levels of IL-6 with GC patients' characteristics. They found that serum IL-6 level was significantly related to advanced stage, tumor depth, lymphatic invasion, venous invasion and hepatic metastasis. IL-6 expression in GC tissue or serum might be related to distant metastasis, and in particular, hepatic metastasis.

Glucose transporter-1

Glucose intake is increased in malignant tumor cells, which is facilitated by glucose transporters. Glucose transporter-1 (Glut1), a member of the glucose transporter family, is overexpressed in several cancers and is correlated with malignant phenotypes^[104]. The association between Glut1 and GC was first reported in 2000. Kim *et al.*^[105] showed that high Glut1 protein expression was associated with an intestinal type of GC. In 2001, Kawamura *et al.*^[87] demonstrated that Glut1-positive GC by IHC had a significantly higher incidence of hepatic metastasis whereas there was no statistical significance regarding the correlation between Glut1 and peritoneal metastasis. Additionally, they showed that Glut1-positive GC cells were localized mainly in the central part of the tumor. The result was consistent with

an adaptation to a hypoxic environment at the center of the tumor. It is expected that the transcription of Glut1 is activated *via* increased HIF1- α . Glut1 could be a putative biomarker for hepatic metastasis from GC.

Human epidermal growth factor receptor 2

HER is a member of the epidermal growth factor receptor family. HER2 is involved in the pathogenesis and poor prognosis of breast cancer and GC, and monoclonal antibodies to HER2, trastuzumab and pertuzumab have been applied clinically worldwide^[106,107]. The major role of HER2 is to promote cell proliferation, suppress apoptosis, and facilitate tumorigenesis^[108]. A few studies have reported the association between HER2 expression and GC patients' prognoses^[109,110]. Lee *et al.*^[88] analyzed the relationship between HER2 expression and computed tomography (CT) imaging in GC patients. In their cohort of 276 patients, hepatic metastases were more frequently found in HER2-positive GC while peritoneal metastasis was more often found in HER2-negative GC. Hepatic metastases were significant independent factors that predict HER2-positive cancers. Similarly, Matsusaka *et al.*^[89] reported the correlation between HER2 positivity and hepatic metastasis in 1466 GC patients. In their data, the incidence of hepatic metastasis was significantly higher in HER2-positive patients, and moreover, HER2-positive patients had a significantly lower incidence of peritoneal metastasis. These two studies suggested that HER2 positivity was associated with hepatic metastasis from GC specifically and was negatively associated with peritoneal metastasis. As supportive data, a meta-analysis also demonstrated that HER2 positivity was associated with differentiated type and intestinal type, and differentiated type and Borrmann type1/2 are reportedly risk factors for hepatic metastasis from GC^[111]. The mechanism is unknown; however, HER2 positivity may be a predictive biomarker for hepatic metastasis from GC.

NTase domain containing non-canonical poly(A) polymerase 3

We recently focused on genes reflecting the metastatic potential of GC cells and identified a NTase domain containing non-canonical poly(A) polymerase 3 (NCPAP3) as a predictor for hepatic metastasis^[90]. NCPAP3 has been shown to regulate translation by acting as an mRNA stability factor and the gene has a mutation predicting worse prognosis in multiple myeloma^[112]. NCPAP3 expression was decreased in GC tissue compared with adjacent noncancerous mucosae in most patients. Patients with lower NCPAP3 expression have a shorter overall survival and disease-free survival. Furthermore, lower NCPAP3 expression was significantly correlated with the cumulative incidence of hepatic metastasis while there was no significant difference in the cumulative incidence of peritoneal metastasis by NCPAP3 expression. Additionally, we revealed the mechanisms for suppression of NCPAP3 expression. Copy number alterations at the

NCPAP3 locus were observed in the GC tissues of 35% of patients and in 50% of GC cell lines. Additionally, 42% of GC cell lines harbored single nucleotide variants, and all of these cell lines expressed lower NCPAP3 mRNA. Aberrant DNA methylation was not observed in GC cell lines. NCPAP3 not only associates with the malignant phenotype of GC but may also be a predictive biomarker specific for hepatic metastasis.

Nucleophosmin 1

Nucleophosmin 1 (NPM1) is a nucleolar phosphoprotein involved in numerous cellular processes, including centrosome duplication, histone assembly, protein chaperoning and cell proliferation^[113,114]. NPM1 downregulates the tumor suppressor cyclin dependent kinase inhibitor 2A in the nucleolus and has inhibitory effects by activating transcription factor 5 (ATF5) and abrogating ATF5-induced G(2)/M cell cycle blockade^[115,116]. Some studies revealed that positive expression of NPM1 in GC tissue was associated with poor prognosis in postoperative GC patients. Zhou *et al.*^[117] and Li *et al.*^[118] found that NPM1 level was linked to more advanced tumor stages and was an independent indicator for prognosis and recurrence. Ding *et al.*^[91] indicated a correlation between NPM1 expression and clinicopathological features including metastatic site. Patients with NPM1-positive GC had significantly higher rates of hepatic metastasis and recurrence. While the molecular basis remains to be elucidated, NPM1 expression might predict hepatic metastasis from GC.

CONCLUSION

The development of molecular techniques and bioinformatics has led to accumulated knowledge and an understanding of the mechanisms of distant metastasis from cancer. Cancers generate manifold subclones as seeds based on their genomic instability and heterogeneity. Subsequently, subclones that pass through the selection of each step for metastasis and adapt to the secondary organ, the so-called soil, have the opportunity to metastasize. Moreover, cancer cells create a pre-metastatic niche *via* secretion of exosomes. However, knowledge of the mechanism specific for hematogenous metastasis is scarce, and the full picture of organotropism has not yet been elucidated. Hematogenous metastasis is a factor that strongly contributes to poor prognosis in GC. Therefore, understanding and controlling its mechanism are significant issues. Further studies on this theme should improve GC patients' prognoses.

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Retrospective Cohort Study

Trans-anal minimally invasive surgery for rectal neoplasia: Experience from single tertiary institution in China

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Abstract

AIM

To evaluate the feasibility and safety of trans-anal minimally invasive surgery (TAMIS) from single institute in China.

METHODS

A retrospective review was conducted for patients with rectal neoplasia, who underwent TAMIS using single incision laparoscopic surgery-Port from January 2013 till January 2016 by a group of colorectal surgeons from Gastrointestinal Center Unit III, Peking University Cancer Hospital. Patients' demographic data, surgical related information, post-operational pathology, as well as peri-operative follow-up were all collected.

RESULTS

Twenty-five patients with rectal neoplasia were identified consequently. Complete full-thickness excision was achieved in all cases without conversion. 22 (88%) cases had rectal malignancies [6 were adenocarcinomas and 16 were neuroendocrine tumors (NET)], while 3 patients had adenomas. Mean surgical duration was 61.3 min, and mean post-operative stay were 2.7 d. Post-operational examination demonstrated 5 cases had positive resection margin: 2 adenocarcinoma cases and 1 NET case with positive lateral margin, and the other 2 NET cases with positive basal margin. The curve of operation time for TAMIS cases suggested a minimum of 10 cases for a laparoscopic surgeon proficient with

this technique.

CONCLUSION

TAMIS was demonstrated to be reproducible and safe, with a relatively short learning process for laparoscopic surgeons in selected cases for rectal neoplasia. Long-term oncological outcome needs to be determined by further investigation.

Key words: Rectal neoplasia; Resection margin; Trans-anal minimally invasive surgery

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Core tip: Local excision was regarded as the conversational treatment for early stage rectal neoplasia. Recent evidence, however, revealed certain disadvantages. Minimally invasive surgery has been adopted in treating rectal cancer. This study was the first well-documented retrospective trail demonstrating the safety and feasibility of trans-anal minimally invasive surgery. Short-term follow-up showed no serious post-operative complications (over grade IIIa by CD classification), meanwhile, lateral resection margin should be evaluated pathologically and surgeons proficient for laparoscopic surgery would be confident over the learning curve regarding 10 cases.

Chen N, Peng YF, Yao YF, Gu J. Trans-anal minimally invasive surgery for rectal neoplasia: Experience from single tertiary institution in China. *World J Gastrointest Oncol* 2018; 10(6): 137-144 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i6/137.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i6.137>

INTRODUCTION

As a challenging area, low rectum has drawn significant attention and caution due to anatomic features. The rates of sphincter-preserving surgery have largely increased, due to the application of neoadjuvant chemoradiation as well as the laparoscopic approach, or both. It, however, is still the fact that colostomy or temporary ileostomy might be necessary in around 10%-30% of the patients with neoplasia in the mid-low third of the rectum^[1]. For benign neoplasia or early-stage malignancy located in mid-low rectum, a variety of treatments might be available. Tran-anal local excision is commonly recommended. This approach, however, should be limited to well-selected patients since high-quality oncological excision could not be guaranteed due to exposure and visibility^[2,3]. Another meaningful technique might be trans-anal endoscopic microsurgery (TEM), first introduced by Buess *et al*^[4] and considered as an alternative approach, providing acceptable oncological outcome with less postoperative complications and better function, compared with radical excision. Nevertheless, TEM is embedded with

certain disadvantages, such as high cost, complexity of the instruments, rather steep learning curve and limited indications, resulting in failure of widespread adoption^[5]. Marked advances in instrumental innovation (single port) and technical expertise (laparoscopy) led to the creation of trans-anal minimally invasive surgery (TAMIS). At present, two well-designed platforms for TAMIS, the GelPOINT Path and the single incision laparoscopic surgery (SILS) Port have gained the approval of Food and Drug Administration for use^[6,7]. Previous literature has demonstrated that TAMIS might be an alternative choice for rectal lesions, offering several technical advantages compare with TEM: Firstly, with the widespread of laparoscopic surgery, laparoscopic instruments which has been already available could easily be applied in the TAMIS setting, meanwhile the unique apparatus employed by TEM could not be compatible with laparoscopic platform; secondly, the soft platform-SILS Port could provide safer trans-anal access as well as less sphincter traction, compared with rigid channel employed by TEM.

In the present study, SILS Port platform was applied using a standard laparoscopic setting, and the characteristics of patients underwent TAMIS were collected as well as the short-term outcome, aiming to demonstrating the utility of this TAMIS technique with both favorable and unfavorable factors.

MATERIALS AND METHODS

This study population consisted of consecutive patients identified from a single institution retrospectively from January 2013 till January 2016. Data from individual patients were reviewed and analyzed. The indications for TAMIS were as follows: benign neoplasia (adenomas over 2.5 cm in diameter); low grade (G1) neuroendocrine tumors with diameter less than 2 cm, and for curative intent stage I rectal cancer with favorable histological features (mri-lymph node negative cT1, with diameter less than 3 cm, moderate to well differentiation, and no mri-lympho-vascular invasion).

Exclusion criteria were patients with certain conditions: invasive rectal tumor (over mri T2 or lymph node positivity), history of inflammatory bowel disease, severe hemorrhoids or anal stricture, fecal incontinence, or with contraindications to general anesthesia. Patients with diagnosis of malignancy underwent preoperative staging with 3-Tesla pelvic MRI or endorectal ultrasound to determine depth of invasion and status of lymph nodes. Standard preoperative imaging, such as computed tomography of the chest, abdomen, and baseline blood test for carcinoembryonic antigen (CEA) were completed before surgical intervention. All TAMIS surgeries were performed in single high-volume tertiary hospital by trained laparoscopic colorectal surgeons under general anesthesia. Technically, TAMIS was a platform whereby standard laparoscopic instruments and cameras were used combined with disposable multi-channel port positioned trans-anally with gas insufflation of the rectum.

Table 1 Patients' clinical and pathological characteristics

	Benign (<i>n</i> = 3)	Malignant ¹ (<i>n</i> = 22)	All (<i>n</i> = 25)
Mean age, yr (SD)	55.3 (7.5)	51.3 (13.8)	51.8 (13.2)
Gender			
Male	1	8	10 (40%)
Female	2	14	15 (60%)
Mean body mass index, kg/m ² (SD)	23.9 (1.3)	23.9 (3.0)	23.9 (2.9)
Pre-TAMIS excision	0	5	5 (20%)
Mean lesion size, cm (SD)	1.1 (0.7)	1.1 (0.5)	1.1 (0.5)
Mean distance from anal verge, cm (SD)	9.3 (0.6)	8.3 (1.6)	8.4 (1.6)
Final pathology			
Benign	3		3 (12%)
Malignant			22 (88%)
Adenocarcinoma		6	6 (24%)
Mid-high differentiation		6	6 (24%)
Low differentiation		0	0
T0 (no residual tumor)		0	0
T1		5	5 (20%)
T2-3		1	1 (4%)
NET		16	16 (64%)
Lymph-vascular invasion	0	1	1 (4%)
Positive margin	0	5	5 (20%)
Position			
Lloyd-Davies	3	15	18 (72%)
Jackknife	0	7	7 (28%)
Mean duration of surgery, min (SD)	58.0 (37.0)	61.8 (24.7)	61.3 (25.5)
Mean blood loss, mL (SD)	5 (0)	8.6 (4.4)	8.2 (4.3)
Mean length of post-operative stay	2.3 (1.5)	2.8 (1.4)	2.7 (1.4)

¹Includes *in situ* and invasive adenocarcinoma and neuroendocrine tumors. NET: Neuroendocrine tumors.

Full mechanical bowel preparation (polyethylene glycol) was performed, and all patients received preoperative antibiotics which were continued for 1 d in all patients postoperatively.

Surgical procedure

Trans-anal minimally invasive surgeries were performed using the SILS Port (Covidien-Medtronic, Minneapolis, MN). Pneumo-rectum was maintained with CO₂ insufflation with flow set to 40 L/min and pressure set to 15 mmHg (range, 10-18 mmHg). A high-definition 30° 5 mm or 10 mm camera lens was used in combination with standard laparoscopic graspers and electrocautery or Ethicon Endo-Surgery HARMONIC ACE (Figure 1). After marking the area of resection (Figure 2A), the dissection was started around 5 mm from the lesion margins to obtain a full thickness excision (Figure 2B). The defect was closed in all patients using a running suture of Vicryl 3-0 or V-lock 3-0 (Covidien) (Figure 2C). The surgical specimen were pinned on a cork board and sent fresh for histopathological examination (Figure 2D). Liquid diet was prescribed during post-operational day 1-3. Patients were discharged routinely on the following day of surgery with prolonged stay for special patients, depending on case complexity and occurrence of complications. Data was collected retrospectively in a common database. Complications were graded according to the Clavien-Dindo classification. The size of specimen, surgery duration, final pathological diagnosis and other peri-operative factors were recorded. Follow

up consisted of postoperative visits at 2- and 8-wk after TAMIS, with digital rectal examination.

Statistical analysis

Summary data were presented as mean ± SD for continuous variables and percentages for discrete variables. All statistical tests were two-sided and a *P* value less than 0.05 was considered significant. The analysis was performed using SPSS 19.0 (IBM Switzerland Ltd., Zurich, Switzerland).

RESULTS

Demographics

A total of 25 patients were enrolled in this study, baseline patient demographics and tumor characteristics were summarized in Table 1. The mean age of the patients was 51.8, 10 (40%) patients were male, with the mean body mass index 23.9 kg/m². Five (20%) out of 25 patients had pre-TAMIS local excision before admission. Mean size of the lesions was 1.1 cm (range from 0.5 to 2 cm) in diameter, meanwhile, the mean distance from lesions to anal verge was 8.4 cm (range from 5 to 10 cm). The pathological examination revealed that 3 (12%) patients was diagnosed benign lesions (adenomas), on the other hand, 22 (88%) were malignant, among which 16 with neuroendocrine tumors (NETs) and 6 patients with adenocarcinoma (5 patients pT1, and 1 pT3). Positive lymph-vascular invasion was seen in 1 patient, and resection margin

Table 2 Characteristics of patients with positive resection margin

Patients' No	Age	Gender	BMI (kg/m ²)	Distance from anal verge (cm)	Diameter (cm)	Surgery duration (min)	Position	Post-op stay	Type of positive margin	Pathology	Post-op treatment
1	58	2	19.9	7	2	50	Lloyd-Davies	1	Lateral	Adenocarcinoma	Curative surgery
2	75	2	23.2	10	2	60	Lloyd-Davies	1	Basal	NET-G1	Imatinib
3	64	2	26.1	6	1.5	45	Lloyd-Davies	4	Basal	NET-G1	Imatinib
4	63	2	20.0	10	0.5	60	Lloyd-Davies	2	Lateral	NET-G1	Imatinib
5	59	1	24.8	8	1.5	30	Lloyd-Davies	3	Lateral	Adenocarcinoma	Chemo-radiation

NET: Neuroendocrine tumors.



Figure 1 Settings for trans-anal minimally invasive surgery. The SILS-Port® was inserted through the anus. Assisting trocars and routine laparoscopic instruments were placed. A high-definition 30° 5 mm or 10 mm laparoscopic camera lens was chosen. SILS: Single incision laparoscopic surgery.

was interpreted positive (less than 1 mm) in 5 patients. 18 (72%) patients had their surgeries performed in the Lloyd-Davies position, meanwhile, 7 patients in jackknife position in terms of anterior lesions. There was no intra-operative conversion from TAMIS to laparoscopic radical resection, and the mean duration of TAMIS surgeries was 61.3 min (range from 25 to 105 min), with mean blood loss 8.2 mL (range from 5 to 20 mL). There was no operative mortality or serious complication (over grade 3 by Clavien-Dindo grading system), and the mean length of hospital stay was 2.7 d post-operatively. Patients were follow-up more than 3 mo.

Learning curve for TAMIS

TAMIS surgeries were performed by a group of surgeons proficient with laparoscopic skills. Figure 3 demonstrated the correlation between cases and duration of TAMIS surgeries, indicating the learning curve of this technique. A trend line showed a steep decline in the surgery duration from 1 to 10 cases, and after 10 cases, this line stayed relatively steady.

Positive resection margin

In this study, we noted that 5 (20%) patients had

positive resection margin (defined as less than 1 mm from the cutting edge) by post-operative pathological examination: 2 patients were diagnosed with adenocarcinoma, meanwhile the other 3 were low grade NETs (G1), as shown in Table 2. Interestingly, for cases of adenocarcinoma, positivity occurred at the lateral resection margin, while for cases of NETs, results showed 1 case had positive lateral margin and 2 cases had positive basal margin. All 5 patients with positive margin underwent MDT discussion for further treatment strategies. Finally, for the 2 patients with adenocarcinoma, 1 had curative surgery (low anterior resection), and 1 underwent post-operative chemo-radiation; for the 3 NETs patients, adjuvant imatinib were recommended by oncologists.

DISCUSSION

Local excision (LE), as an alternative approach, has been employed under certain circumstances (benign adenomas, early stage adenocarcinomas, and low grade neuroendocrine tumors) for curative intent in rectal neoplasia. However, due to the difficulties in exposure and dissection, LE has been applied only in selected cases^[8,9]. Since introduced by Buess *et al*^[4], TEM has progressively become another recommended surgical procedure in clinical practice. The application of TEM, nevertheless, has been notably slow, due to the instrumental obstacles: Surgeons were compelled to operate through a rigid rectoscope, limiting triangulation and the subsequent instrumental manipulation, compared with the standard experience laparoscopically. With the widespread of laparoscopic approach, abdominal and pelvic operations have undergone magnificent changes. Using single-port system with common laparoscopic instruments, trans-anal laparoscopic resection has recently become more accessible. TAMIS, first reported by Atallah *et al*^[10], was a novel trans-anal platform for full-thickness local excision of rectal benign and malignant tumors. TAMIS was more than a local excision technique, based on the laparoscopic platform with curative intent, by adopting the full-thickness resection and wound-sewing under camera and grasper. Several studies with limited cases have been published, demonstrating the better exposure

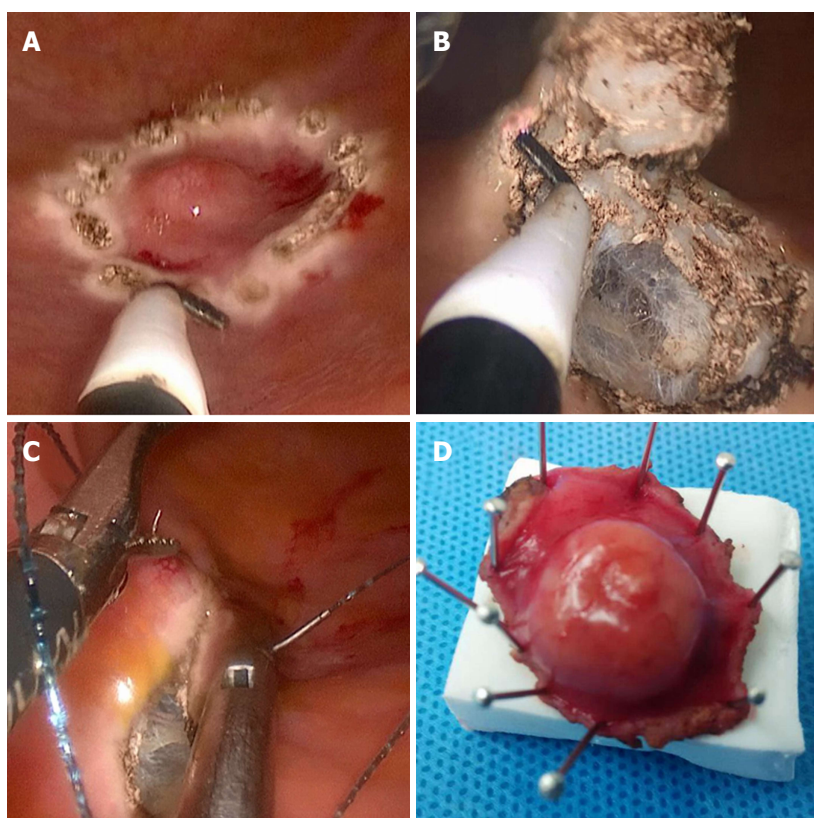


Figure 2 Procedures of trans-anal minimally invasive surgery. A: Intra-operative view of TAMIS showed resection margin was marked by electrocautery; B: An endoscopic grasper and electrocautery were used to facilitate a full-thickness excision; C: The defect of rectal wall was closed using STRATAFIXTM; D: The surgical specimen was pinned on plastic board with indicative orientation. TAMIS: Trans-anal minimally invasive surgery.

of operative field and easier instrumental manipulation, with the help of high-definition flexible camera and constant gas insufflation^[5,7,11,12]. TAMIS settings provided a more precise resection margin and dissection plane, following the full-thickness excision principle.

This paper presented preliminary data from a single-center series of 25 consecutive patients affected by rectal neoplasia, including 22 cases malignancies (6 cases with adenocarcinomas, 16 cases with G1 neuroendocrine tumors) and 3 cases with adenomas, treated by TAMIS. All surgeries were successfully achieved without intra-operative conversion. In the first few cases, TAMIS surgeries were started with considerably better condition, like middle aged, female patients, then all patients meeting the inclusive criteria were suggested afterwards. Therefore, the mean age of enrolled patients was 51.8, and 60% were female due to the safety concerns. Five patients underwent local excision pre-operatively, with positive or unknown margins. In terms of precise orientation of the neoplastic residue or scar, all 5 patients underwent pre-TAMIS endoscopic examination with lesion clipped. Excision was done following the clips. Cases were selected based on the principle of trans-anal local excision by the National Comprehensive Cancer Network (NCCN) guideline version 2017. 1^[13]. Post-operational pathology revealed that 3 cases with benign adenomas, while the other 22 were malignant. Among those, 16 (64%) were G1 NETs,

and 6 were adenocarcinoma, with 5 T1 tumor and 1 case T3 tumor. No lymph-vascular invasion was seen. Interestingly, for the case with pT3 tumor, pre-operative mri demonstrated T1 (mucosa invasion) while trans-anal ultrasonography showed T1-T2 (putative muscularis invasion), fortunately negative resection margin was achieved by full-thickness resection. Higher risk of recurrence and curative surgery was informed, however, this patient refused surgical intervention due to concerns of anal function, with close follow-up.

The mean diameter of rectal lesions was 1.1 cm, and mean distance from the anal verge was 8.4 cm, indicating the location of mid to high third of the rectum. For cases within 4 cm from the anal verge, retrospective studies noted trans-anal local excision might be more applicable due to several considerations: Firstly, for low rectal lesion, it seems easier for exposure with tractors instead of SILS channel; secondly, the installation of SILS required at least 2 cm normal anal mucosa, resulting in the awkward location-too close from the lesion to the port without guaranteed resection margin. Another concern for the TAMIS technique was the surgical duration, and the majority cases in our studies were finished approximately 60 min with a maximum blood loss of 20 mL, demonstrating the reproducibility of this technique. No severe post-operative complications (Clavien-Dindo 3A or over), such as bleeding or stenosis were observed during the hospital stay and short term

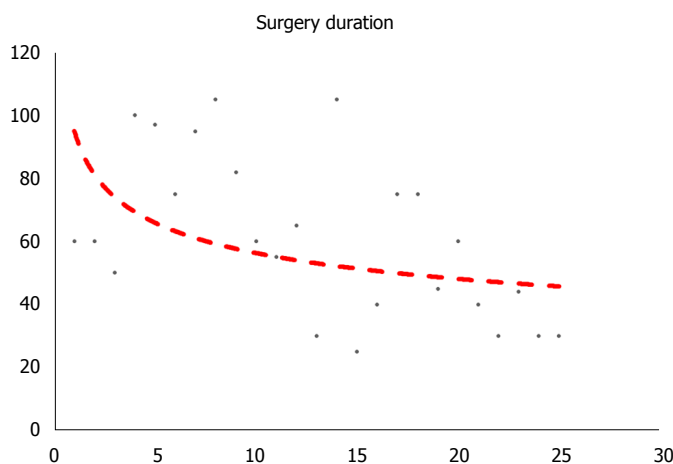


Figure 3 Correlation between cases and duration of trans-anal minimally invasive surgery surgeries. The X-axis represented individual case consequently, while the Y-axis was the duration of each surgery (min), indicating the learning curve of this technique.

(2 and 8 wk) follow-up. Previous literature mentioned the 3.3%-16.8% postoperative complication rate^[4,14,15], such as peritoneum perforation, urinary tract infection, subcutaneous emphysema, hemorrhoid thrombosis, etc.

Surgical duration and learning curve

Previous studies of TAMIS were majorly institutional experience with a small amount of cases by retrospective nature. Maya *et al*^[16] demonstrated that 4 cases might be necessary before the skillfulness obtaining by employing the CUSUM curve to assess competence in the surgical techniques of TAMIS. In this study, the routine protocol for TAMIS surgery were established following the literature. From the learning curve as shown above, we noted that surgery duration markedly varied within the first 10 cases and then stayed relatively stable (approximately 60 min), indicating that the proficient skills for TAMIS surgery required a minimal number of 10 cases. For surgeons with proficient laparoscopic technique, TAMIS would be easier since the share of similar instruments.

Positive resection margin

Previous studies reported that margin positivity was round 4% to 10%^[12], according to the so-far largest review on TAMIS. In our study, the rate of positive margin was 20%, which seemed higher than expected. Further reviewing of the data revealed that 2 of 5 cases with positive margin occurred in the first 10 cases, indicating so-called "trial and error" period in TAMIS surgery. Among the following cases, the rates of positive margin greatly lessened. It is important to notice that full thickness resection would merely be guaranteed by dissection of fat tissue in the mesorectum or even penetration into pelvic cavity. Interestingly, it was noted that all these cases were performed with patients in the Lloyd-Davies position, with tumors located either lateral (4/5) or anterior (1/5) wall. It was plausible that patients' position might had a significant effect on the exposure and dissection of the lesion and the

Lloyd-Davies position might not be appropriate for anterior lesions due to the rotated viewing angle^[17]. Additionally, correlation between types of positivity with final pathology demonstrated that for adenocarcinoma, 2 of 2 cases had lateral positive margin, while for NETs, 2 of 3 cases had basal positive margin as well as the other 1 with lateral positivity. It is believed that adenocarcinoma, originated from mucosa, would have intraluminal mucosa infiltration^[13]; conversely, NET has more mysterious features with various types of infiltration^[18]. The 3mm resection margin was recommended by "National Comprehensive Cancer Network" (NCCN) for the principle of local excision in terms of curative intent^[19], as implemented in our study. However, trans-anal local excision was performed using a variety of other standards of resection margin, from 5 mm to 10 mm^[20,21]. It is speculated that positivity of resection margin might be decreased if the 5 mm (or 10 mm) margin applied, indicating for rectal malignancy treated by the TAMIS surgery, an enlarged resection margin might be safer. On the other hand, the defect resulted from enlarged resection, might raise higher requirement for laparoscopic sewing, regarding longer incision, higher suture tension, increased probability of post-operative stricture or scarring^[22]. Whether enlarged margin would result in survival benefit might still be controversial and need more high quality of evidence.

Penetration into pelvic cavity

For neoplasia located on the anterior wall of upper third of rectum (above the peritoneal reflection), full-thickness resection inevitably results in the penetration into peritoneal cavity. In our study, there were 4 (16%) patients had the entering into peritoneal cavity intra-operatively with neoplasia located in the anterior wall. It is believed that full-thickness excision is mandatory when local excision performed for malignancy due to the probability containing an invasive component^[23]. However, not all published literature reported^[24]. It has been demonstrated that a partial thickness excision

would result in a dramatic increase in the rates of positive margin^[25], leading to enhanced risk of loco-regional recurrence.

Though with the well-established settings, TAMIS has its technical shortcomings. Firstly, it seems easier for instrumental manipulation compared with TEM, however, difficulties occurred with rectal masses which located over 10 cm from the anal verge, due to the existence of these transverse rectal folds as well as the physiological curvature of the pelvis. Secondly, the firm fixation of SILS Port platform to the anus required a minimal of 3-4 cm anal canal, therefore it would be difficult to get TAMIS done within 3-4 cm from the anal verge^[15]. Thirdly, through the single port apparatus, laparoscopic instruments roughly oriented in parallel, resulting in the failure of triangulation, making free bending and rotating more difficult. Fourthly, general anesthesia, as applied in our TAMIS surgeries, yet had the problem of peristalsis under autonomic innervation intraoperatively, which was a disturbing factor for steady surgical fields. For better relaxation effect, additional methods, such as low sacral anesthesia might worth a try.

This study has its own limitations by its retrospective nature, relatively small cohort size and selected cases. The short-term outcome demonstrated that TAMIS might be a feasible technique in terms of full-thickness resection and minimal sphincter injury. Recent studies demonstrated that total mesorectal excision (TME) by using the platform of TAMIS was increasingly performed, leading the advance in the management of distal rectal cancer^[26]. Long-term oncological safety needs to be investigated with further follow-up.

In summary, TAMIS is a feasible method of performing full thickness resection for rectal lesions with acceptable short-term outcome. Surgeon proficient with laparoscopic surgery would able to manage this technique after a training period of approximately 10 cases. TAMIS might be suggested as one of the alternative choices for the treatment of lesions located in the mid rectum of selected patients.

ARTICLE HIGHLIGHTS

Research background

Local excision is regarded as the standard treatment for mid-low rectal neoplasia, including benign tumors and early-stage malignancy. Due to the disadvantages in exposure, high quality of local excision could not be well guaranteed, though trans-anal endoscopic microsurgery (TEM) could merely provide solutions in certain conditions. Therefore, it is essential to call for another technique to fill the gap in-between. Recently, trans-anal minimally invasive surgery (TAMIS) has been introduced as an alternative choice for rectal lesions.

Research motivation

TAMIS surgery was reported by literature with relatively small amount of cases, however, there has been no published data on TAMIS surgery on the Chinese population. The safety and feasibility of TAMIS is still lack of evidence.

Research objectives

This study was designed to investigate the utility of TAMIS technique with both

favorable and unfavorable factors.

Research methods

TAMIS surgery was done by a standard laparoscopic platform (SILS Port). Patients' characteristics, surgery duration, pathological diagnosis and post-operative complications (Clavien-Dindo classification) were collected.

Research results

The research findings, their contributions to the research in this field, and the problems that remain to be solved should be described in detail. Among 25 patients enrolled, 10 (40%) patients were male, with the mean age of the patients 51.8 and the mean body mass index 23.9 kg/m². Mean diameter of the lesions was 1.1 cm (range from 0.5 to 2 cm) and the mean distance to anal verge was 8.4 cm (range from 5 to 10 cm). 3 (12%) patients was diagnosed benign lesions (adenomas), 22 (88%) were malignancies (16 with neuroendocrine tumors (NETs) and 6 with adenocarcinoma (5 patients pT1, and 1 pT3). Positive resection margin (less than 1 mm) was revealed in 5 patients and lymph-vascular invasion was seen in 1 patient. Eighteen (72%) TAMIS surgeries were performed in the Lloyd-Davies position, with the rest in jackknife position. The mean duration of was 61.3 min (ranger from 25 to 105 min), with mean blood loss 8.2 mL (range from 5 to 20 mL) and no conversion to laparoscopic surgery. No operative mortality or serious complication (over grade 3 by Clavien-Dindo grading system), and the mean length of hospital stay was 2.7 d post-operatively. A laparoscopic surgeon would be proficient to perform TAMIS surgery with around 10 cases.

Research conclusions

TAMIS could be safe and feasible technique to early stage rectal neoplasia. Laparoscopic surgeons would be proficient for TAMIS with approximately 10 cases. TAMIS might provide an alternative method with conventional laparoscopic apparatus, compared with TEM. This study demonstrated the first piece of evidence of peri-operative data and short-term outcome in patients treated with TAMIS in Chinese tertiary hospital. TAMIS is a safe method treating early stage rectal neoplasia. Surgical position might have a significant effect on the positivity of resection margin, and Lloyds-Davies position might not be appropriate for anterior lesions. TAMIS could offer full-thickness resection and minimal sphincter injury. TAMIS might be an alternative choice for patients with early stage rectal neoplasia.

Research perspectives

TAMIS could be feasible by utilizing laparoscopic apparatus. For lesion located anteriorly, it might be better with jackknife position. It might be essential to know the rate of positivity concerning resection margin with larger number of cases prospectively; and it worth a try to use TAMIS in down-stage rectal cancer patients underwent neoadjuvant chemoradiation for re-staging and curative intent. A prospective clinical trial might be a good choice.

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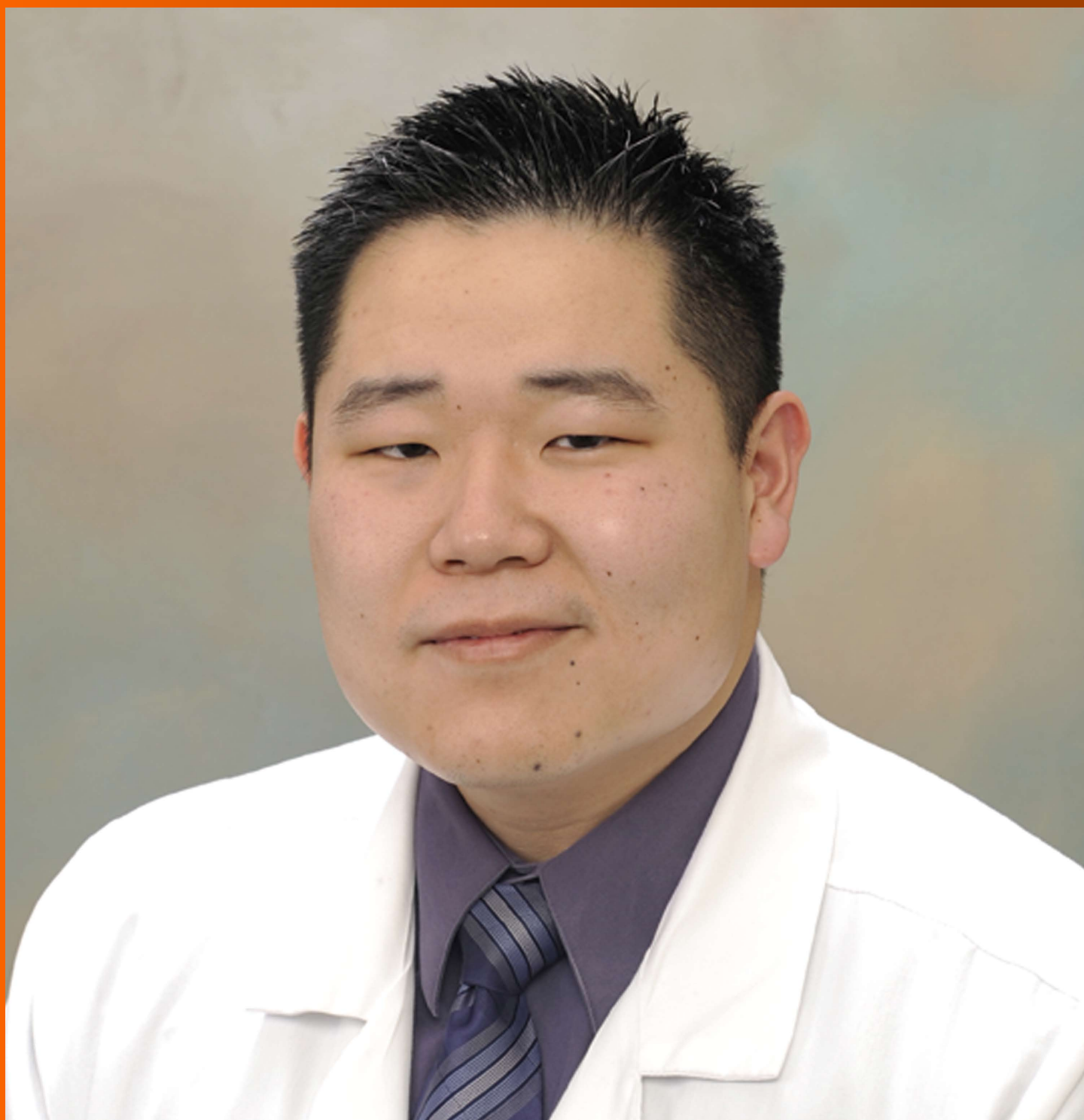


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Novel biomarkers for patient stratification in colorectal cancer: A review of definitions, emerging concepts, and data

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Abstract

Colorectal cancer (CRC) treatment has become more personalised, incorporating a combination of the individual patient risk assessment, gene testing, and chemother-

apy with surgery for optimal care. The improvement of staging with high-resolution imaging has allowed more selective treatments, optimising survival outcomes. The next step is to identify biomarkers that can inform clinicians of expected prognosis and offer the most beneficial treatment, while reducing unnecessary morbidity for the patient. The search for biomarkers in CRC has been of significant interest, with questions remaining on their impact and applicability. The study of biomarkers can be broadly divided into metabolic, molecular, microRNA, epithelial-to-mesenchymal-transition (EMT), and imaging classes. Although numerous molecules have claimed to impact prognosis and treatment, their clinical application has been limited. Furthermore, routine testing of prognostic markers with no demonstrable influence on response to treatment is a questionable practice, as it increases cost and can adversely affect expectations of treatment. In this review we focus on recent developments and emerging biomarkers with potential utility for clinical translation in CRC. We examine and critically appraise novel imaging and molecular-based approaches; evaluate the promising array of microRNAs, analyze metabolic profiles, and highlight key findings for biomarker potential in the EMT pathway.

Key words: Biomarker; Colorectal cancer; Epithelial-to-mesenchymal-transition pathway; Molecular biomarker; MicroRNA; Metabolic biomarker; Imaging biomarker; Tumour regression grade

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Core tip: Biomarkers are an emerging field that can potentially guide the diagnosis, prognosis, and treatment course in rectal cancer. Here, the current definitions, classifications, recent developments and emerging biomarkers with potential utility for clinical translation in colorectal cancer are reviewed by international experts for a better understanding in surgery.

Chand M, Keller DS, Mirnezami R, Bullock M, Bhangu A, Moran B, Tekkis PP, Brown G, Mirnezami A, Berho M. Novel biomarkers for patient stratification in colorectal cancer: A review of definitions, emerging concepts, and data. *World J Gastrointest Oncol* 2018; 10(7): 145-158 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i7/145.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i7.145>

INTRODUCTION

Colorectal cancer (CRC) is one of the most common types of cancer and cancer related deaths worldwide, with more than a third of the incidence involving the rectum^[1,2]. Historically, rectal cancer was associated with the worst oncological outcomes^[3]. The choice of treatment for rectal cancer was traditionally based upon the histologic type of malignancy, stage of the disease,

the tumour-node-metastasis (TNM) staging system, and circumferential resection margin (CRM) status^[2,4]. These variables provide clinical utility, help determine the need for neoadjuvant chemoradiotherapy (CRT) in patients with a threatened or involved CRM, post-operative adjuvant treatment in stage III disease, and are prognostic of oncological outcome. Nevertheless, they provide an incomplete picture, as many patients with predicted early-stage disease harbour lymph node and systemic micrometastases, which can ultimately result in local and/or distant disease recurrence. Administration of neoadjuvant CRT is also sub-optimal as this treatment modality has many side effects, some of which are fatal, while others impair quality of life (QOL). Response to CRT is also unpredictable; up to 30% of patients will have a complete pathological response (pCR = tumour regression grade 1, TRG1), and could have omitted surgery altogether^[5,6]. In 10% of cases however, no reduction in tumour volume is achieved, (tumour regression grade 5, TRG5); patients get no benefit from CRT, but are exposed to its side effects and may also experience cancer progression from delay to surgery^[7]. These observations underscore the limitations of current methods for accurate stratification of patients with rectal cancer, and highlight the pressing need to identify biomarkers indicative of aggressive disease and/or response to CRT, in order to avoid patient under- or over-treatment.

With the advent of the “holy plane”, standards for utilising chemoradiation, the application of minimally invasive surgery, and multidisciplinary tumour boards to guide care, the diagnosis, staging and management of rectal cancer has improved significantly in the past 25 years^[8-18]. However, considerable variation still exists in management and outcomes, and recurrence continues to be a problem, with 5-year survival rates stubbornly below 60% in most European countries^[19]. To further improve outcomes, there is a paradigm shift in the methods of diagnosis, staging, determining the patient’s prognosis, and developing a personalized therapeutic course using advances in molecular biology, genetics, biochemistry, imaging, and the individual patient’s personal risk assessment, neoadjuvant chemoradiotherapy, and adjuvant chemotherapy with surgery to optimise care^[20].

The routine evaluation of microsatellite instability (MSI) and KRAS/NRAS/BRAF mutational status in clinical practice, for risk stratification in stage II CRC and to determine the utility of monoclonal antibody-based adjuvant therapy, such as panitumumab or cetuximab, in metastatic disease, provides a clear proof-of-concept that more tailored therapeutic strategies can be translated to improve patient care through identification of biomarkers with functional activity. In this review, we explore the recent developments and emerging biomarkers with potential utility for clinical translation in CRC. We examine and critically appraise both novel imaging and molecular pathology based approaches; evaluating the promising array of microRNAs with biomarker potential; examining the developing techniques

and studies analysing metabolic profiles, and highlight key findings in the biomarker potential in the epithelial-to-mesenchymal-transition (EMT) pathway.

BIOMARKERS: TERMS OF REFERENCE, CONCEPTS, AND CLASSIFICATION

From the Biomarkers Definitions Working Group, the formal definition of a biomarker is a tumour characteristic that can be objectively measured and evaluated as an indicator(s) of normal biological or pathogenic processes, or pharmacologic responses to a therapeutic intervention that identify increased or decreased risk of patient benefit or harm^[21,22]. Biomarkers can take multiple forms when used to detect or confirm presence of disease or to identify affected individuals^[23]. Table 1 shows the categorisation of biomarkers. Most biomarkers applicable in CRC are prognostic - providing information about the likelihood of a condition, disease recurrence or progression; or predictive - providing information about the likelihood to respond to specific treatments. A cause of confusion around biomarkers has been the loose application of their definition and application. Distinguishing between predictive and prognostic biomarkers- which may not be mutually exclusive- has been another source of confusion in patient stratification and developing treatment strategies^[23]. Another source of confusion is the inconsistent terminology previously used, restricting the scope of biomarkers to describing biological molecules or monitoring the treatment response. The current definition laid out by Cancer Research United Kingdom provides a standardised vocabulary for investigators, explicitly stating, "molecular, histologic, radiographic or physiologic characteristics are examples of biomarkers"^[24]. With this progression, biomarkers may be used in a variety of situations and serve a number of purposes - as a diagnostic tool; for risk-stratification and staging of disease; as an estimator of prognosis; and, for prediction of disease response. The study of such biomarkers can be broadly divided into metabolic; miRNA; EMT; and imaging biomarkers. This review describes the current status of biomarkers in CRC within this framework.

MOLECULAR MARKERS ASSOCIATED WITH CARCINOGENESIS PATHWAYS

The search for molecular markers in CRC has been of significant recent interest. Extensive research has revealed that CRC develops through three major pathways: (1) chromosomal abnormalities that lead to mutations of oncogenes and tumour suppressor genes (classic pathway), characterised by the adenoma-carcinoma progression; (2) the microsatellite instability pathway that results from defects in the DNA repair system; and (3) the methylation pathway characterized by the epigenetic (post cellular division) methylation of numerous genes (methylator pathway). Hundreds of molecules

involved in the chromosomal instability pathway have been associated with prognosis, however, only 1 single marker- the epidermal growth factor receptor (EGFR) pathway-has successfully proven clinical utility to date, largely due to the complexity and redundancy of cellular pathways, as well as the lack of therapies that can target the different biomarkers.

The EGFR pathway is the most clinically relevant molecule involved in the chromosomal instability pathway, and the EGFR serves as the main target for treatment in locally advanced CRC. However, this treatment is only useful for patients with wild-type KRAS (wtKRAS)^[25]. Abnormal activation of the EGFR signalling pathways in CRC is mainly associated with three mutations in the mitogen-activated protein kinase and phosphatidylinositol-3-kinase (PI3K) pathways - KRAS, NRAS, and BRAF; these three mutations are reported to occur in more than half of all CRC cases^[26]. Mutation of some of the components of the EGFR pathway, specifically BRAF V600E, KRAS (exon 2, 3, 4), and NRAS mutation (exon 2, 3, 4) cause the malignant cells to become resistant to anti-EGFR therapy; thus, patients should not be treated with either cetuximab or panitumumab. As a result, all patients with metastatic CRC should have investigation of KRAS/NRAS and BRAF mutation status prior to the start of treatment. KRAS/NRAS and BRAF mutational status may be performed by a variety of techniques, detailed discussion of the different methodologies is out of the scope of this review, however it is essential to emphasize that several technical factors including tissue fixation and tumour volume amongst others may affect the accuracy of the test results leading to erroneous information with the consequent impact on the decision making process. Furthermore, any tumour molecular analysis should be performed only by a certified laboratory that can prove competency and proficiency to perform testing.

Microsatellite instability status (MSI) (high or low) is the primary molecular marker for stratification of stage II CRC. In node negative CRC, patients that are MSI-high have better outcomes than MSI-low tumours; therefore, adjuvant chemotherapy is usually not indicated in MSI-high tumours. MSI-high tumours arise in the setting of a defective DNA repair machinery, although several proteins have been implicated in DNA repair, abnormalities in MSH2, MSH6, PMS2 and MLH1 are the most commonly described. MSI-high tumours may be the result of an inherited mutation of the DNA repair genes (Lynch syndrome) or, more commonly, the abnormal epigenetic methylation of the *MLH1* promoter gene (sporadic MSI-high CRC). Analysis of the DNA repair system may be directly investigated by the tissue expression of MSH2, MSH6, PMS2 and MLH1 by immunohistochemistry, or alternatively by determination of microsatellite status by PCR.

The CpG Island Methylator Phenotype (methylator) pathway has been associated with a constellation of clinical (elderly patients, female, right-sided colon tumours) and histological features (poorly differentiated

Table 1 Biomarker types and definitions

Biomarker type	Objective
Diagnostic biomarker	These aim to identify the type of cancer, <i>e.g.</i> , PSA, CEA. They may also be used to monitor or detect disease recurrence
Pharmacological biomarker	These are used to measure response to a specific drug treatment. They are based on accurate pharmacokinetic data and measure treatment response in early drug trials, <i>e.g.</i> , drug therapy to angiogenesis
Predictive biomarker	These are used to identify individuals who will most likely show a survival benefit to a specific targeted treatment, <i>e.g.</i> , improvement in local recurrence risk following treatment for circumferential resection margin involvement
Prognostic biomarker	These indicate the progress of disease and to estimate the risk of disease recurrence for example. They are used to estimate survival outcome and are independent of treatment strategy, <i>e.g.</i> , nodal disease
Risk/predisposition biomarker	These aim to identify individuals who are at significant risk of developing tumours, <i>e.g.</i> , <i>MLH1</i> gene
Screening biomarker	These are used to identify disease at an early stage, <i>e.g.</i> , PSA
Surrogate response biomarker	These can be used as an alternative to a clinically meaningful endpoint. Therefore there must be correlation with a clinical endpoint, <i>e.g.</i> , CEA

tumours and advanced stage disease). This pattern seen in approximately 15%-20% of CRCs, and involves atypical methylation of the mismatch repair gene *MLH1*. The precursor lesions in CIMP cancers are serrated polyps, not adenomatous lesions, with the initial mutation occurring most often in the *BRAF* oncogene^[27]. *BRAF* mutations transform normal mucosa to aberrant crypt foci, hyperplastic, or sessile serrated polyps (SSP). With promoter methylation, loss of p16 occurs, allowing cells to progress to advanced polyps^[28]. Increasing activity leads to methylation of *MLH1*, silencing transcription. Loss of *MLH1* results in MMR deficiency and the MSI-H CRC phenotype. This is clinically important for diagnosis and therapeutic planning. An estimated 85% of MMR deficiency CRC is due to methylation of the *MLH1* promoter region. *BRAF* can be used to distinguish between *MLH1* promoter methylation and Lynch syndrome as the cause of CRC. A positive *BRAF* mutation is associated with the methylator pathway, and indicates *MLH1* down-regulation through somatic methylation of the gene's promoter region, not through a germline mutation. *BRAF* mutations are rare in Lynch Syndrome-related CRC. On the converse, *MLH1* promoter methylation in the absence of a *BRAF* mutation is consistent with Lynch Syndrome. Figure 1 shows a clinical algorithm for testing MMR deficiency. Several promising new therapies aimed at demethylation of genes are being developed.

METABOLIC PROFILING APPROACHES

In recent years the majority of molecular profiling approaches applied to the study of rectal cancer have focused on macromolecules (DNA, RNA, protein). While these avenues of research continue to offer significant insights into rectal cancer development and progression^[29,30], it is widely accepted that a macromolecular, "bottom up" view of system activity cannot provide all the answers to facilitate precision approaches for rectal cancer diagnosis, prognosis and therapeutic personalisation^[31]. Metabonomics (metabolomics/metabolic profiling) offers a dynamic "top down" view of system activity and is defined as the systematic, time-dependent measurement of metabolic shifts occurring in response to drugs,

environmental stimuli or disease^[32-34]. This approach provides rich *micromolecular* data downstream of the genome and proteome, offering a genuine functional "snapshot" of system activity^[33].

The basic concept of altered cancer metabolism is well described across a variety of cancer subtypes^[35-38]; the Warburg effect^[39] is central to our understanding of cancer metabolism and glycolytic flux forms the basis for [¹⁸F]-fluorodeoxyglucose enhanced positron emission tomography (FDG-PET) solid tumour imaging^[40]. Current and next-generation nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS)-based profiling platforms offer a means of interrogating the cancer metabolome in unprecedented detail and moving beyond the Warburg phenomenon to identify an entirely new pool of disease-relevant biomolecular data. These profiling approaches are likely to have three main areas of application in rectal cancer phenotyping: (1) to identify novel metabolic fingerprints for accurate and ultra-fast tumour tissue diagnosis, staging and grading; (2) to develop metabolite-based models for prediction of response to chemo and/or radiotherapy; and (3) to devise novel next-generation targeted therapies designed to disrupt specific metabolic pathways implicated in rectal cancer.

NMR spectroscopy techniques are highly versatile and have been developed and applied for metabolic profiling of liquid-state and solid-state systems^[41,42]. The technique of HR-MAS NMR has been introduced more recently to overcome spectral line-broadening effects seen with conventional NMR analysis of solids^[41]. This approach allows acquisition of tissue-specific high-resolution spectra, which in combination with chemometric data treatment methods have the capacity to identify novel molecular signatures within rectal cancer tissue^[43]. Recent work in this area has demonstrated increased abundance of taurine, glycine, lactate and scyllo-inositol in cancerous relative to healthy rectal mucosa, with a relative reduction in abundance observed for lipids and glucose^[44] (Figure 2). These findings can be used to determine tissue status (cancerous or healthy) by entirely biochemical means, and have also revealed strong differences in metabolite profiles according to tumour stage^[44]. From a pharmaco-

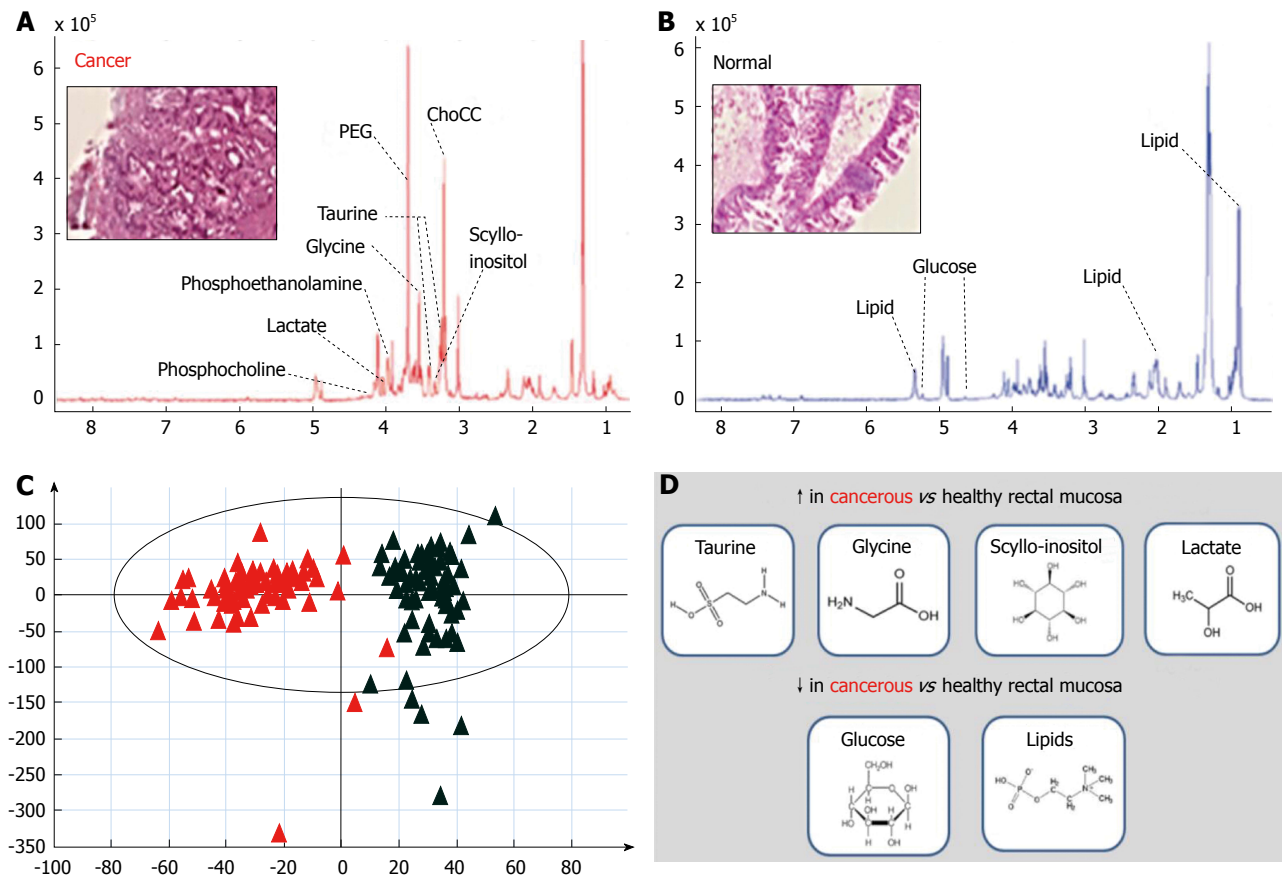


Figure 1 High-resolution magic angle spinning nuclear magnetic resonance spectroscopy of intact rectal cancer tissue biopsies. A and B: Annotated representative HR-MAS NMR spectral metabolite pattern for rectal cancer (A) and healthy rectal mucosa (B); C and D: Acquired data can then be subjected to supervised and un-supervised multivariate analysis using PCA and PLS-DA (C) to determine metabolic processes up- and down-regulated in cancerous tissue (D) (original data). NMR: Nuclear magnetic resonance; PCA: Principal component analysis; PLS-DA: Partial least squares discriminant analysis.

therapeutic perspective these discoveries offer the chance to develop novel anti-cancer agents; for example, taurine (2-aminoethane sulphonic acid), a common beta-amino acid has a known role in a number of fundamental physiological functions including cellular osmoregulation, cell-membrane stabilization and protein assembly^[45]. Exploiting this finding by disrupting taurine handling within the rectal cancer microenvironment may offer a means of developing next-generation targeted agents for rectal cancer down-staging^[46].

Mass spectrometry approaches have shown recent promise in the development of metabolite-based biomarker discovery for prediction of response to chemoradiotherapy. Crotti *et al.*^[47] described novel peptidomic methodology in an analysis of samples of serum collected pre- and post-CRT subjected to matrix-assisted laser desorption/ionisation-time of flight (MALDI-TOF) mass spectrometry. A comparison of pre-treatment serum fingerprints from responders [Mandard tumour regression grade (TRG) 1 and 2] and non-responders (Mandard TRG 3-5) identified three peptides (m/z 1082.552, m/z 1098.537 and 1104.538) that were capable of robust class separation. Kim and colleagues also used a MALDI-based approach, but specifically sought to evaluate the abundance of low-mass ions ($< m/z$ 1000) in serum

samples acquired from 73 patients with locally advanced rectal cancer, prior to CRT^[48]. A panel of nine low-mass ions were found to have discriminatory capacity, with hypoxanthine (HX; m/z 137.08) and phosphoenolpyruvic acid (PEP; m/z 169.04) highlighted as the most significant. Lower levels of HX and higher levels of PEP were shown to strongly correlate with improved response to CRT (TRG 1, 2). These studies indicate the exciting potential for the development of a circulating biomarker panel to predict chemoradiosensitivity prior to commencing therapy.

MiRNA AND RESPONSE TO TREATMENT

MicroRNAs (miRNA) are highly conserved, short, non-coding nucleotide segments that regulate gene expression post-transcriptionally through repressing translation or targeting mRNAs for degradation^[49]. miRNA genes account for between 2%-5% of the human genome and are commonly clustered within introns^[50]. Each miRNA is estimated to interact with multiple mRNA targets and, as a consequence, thus, these sequences may regulate more than 30% of all human genes^[51,52]. Oncogenes and tumour-suppressor genes are being discovered under miRNA control, with the majority of miRNA genes found within cancer-associated genomic regions^[53,54]. In CRC,

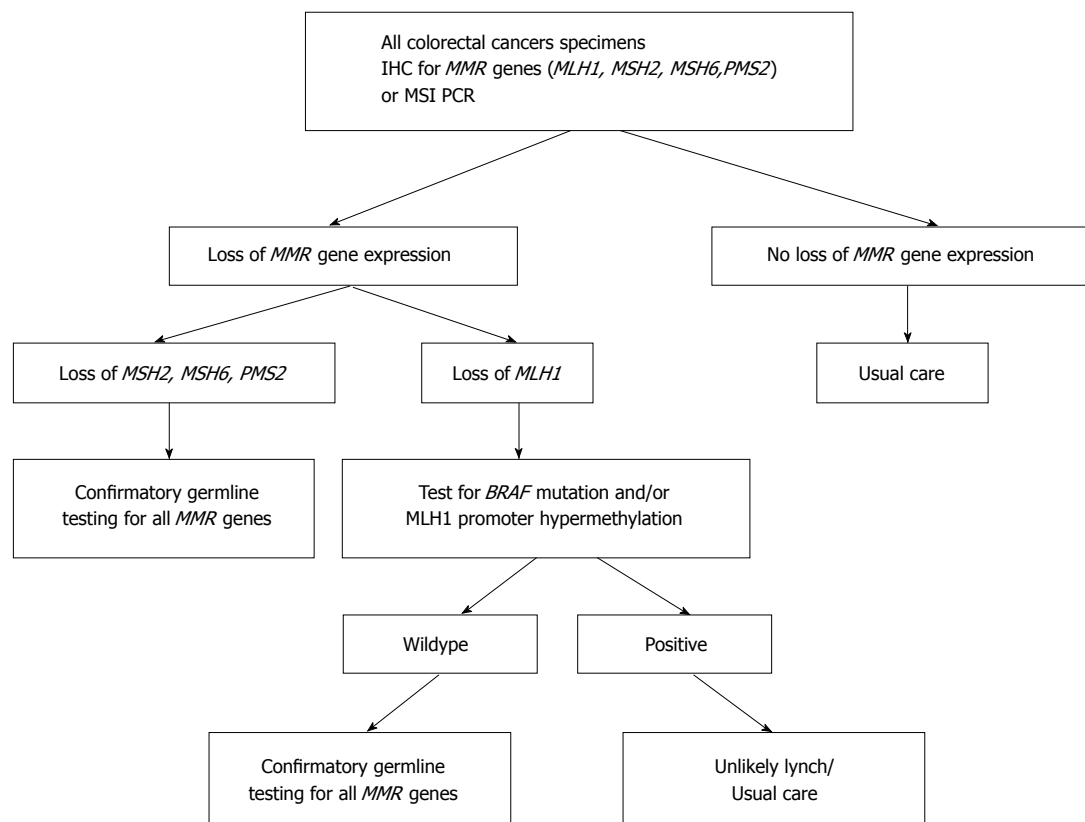


Figure 2 Algorithm for testing of mismatch repair genes in colorectal cancer for Lynch syndrome. MMR: Mismatch repair; MSI: Microsatellite instability.

abnormally expressed miRNAs disrupt cellular signal transduction and cell survival pathways, such as Wnt/ β -catenin, EGFR, and p53, linking miRNA to known events in the pathway of malignant transformation^[55].

Accumulating evidence suggests that miRNAs may also have powerful clinical applications. miRNA expression profiles are capable of discriminating tumours of different developmental origin^[56]. Furthermore, the expression of individual miRNAs may be used to predict patient survival, tumour stage, the presence of lymph node metastases and the response to therapy in CRC^[55,57,58].

Three studies have specifically examined the utility of miRNA expression signatures in predicting chemoradiotherapy response in rectal cancer^[59-61]. Della Vittoria Scarpato *et al.*^[59] examined miRNA expression in fresh-frozen pre-treatment tumour specimens from 38 patients with locally advanced (T3/T4 Node +ve) rectal cancer and compared miRNA profiles in patients with complete (Mandard TRG 1; $n = 9$) and incomplete (Mandard TRG > 1; $n = 29$) pathological responses to a standardised neoadjuvant chemoradiotherapy regime consisting of capecitabine, oxaliplatin and 45 Gy of pelvic conformal radiotherapy. Thirteen significantly differentially expressed miRNAs were subsequently validated using high sensitivity TaqMan[®] qRT-PCR, of which 2; miR-622 and miR-630, were found to predict chemoradiotherapy response with 100% sensitivity and specificity^[59].

A similar analysis of 20 patients undergoing combi-

ned radiotherapy and capecitabine/5-FU chemotherapy compared "responders", namely those displaying a positive response to treatment (Mandard TRG 1 and 2) with "non-responders" (Mandard TRG 3-5). TaqMan Low Density Arrays identified a miRNA signature consisting of 8 miRNAs capable of correctly classifying 90% (9/10) of responders and 90% (9/10) of non-responders^[60].

A third study, which used formalin fixed rather than fresh rectal cancer specimens identified a miRNA signature consisting of just 3 miRNAs (miR-153, miR-16 and miR-590-5p), capable of distinguishing patients with complete and incomplete responses to therapy, however the value of this data is unclear as patient demographics, tumour characteristics, study end-points and the neoadjuvant treatment strategy were not clearly described^[61].

As profiling methodology and the definition of tumour regression vary between these 3 studies, inter-study comparisons are of limited value; however it is important to note that no overlap is observed between the miRNA signatures described. This suggests that an miRNA based "therapy-response" prediction tool is some way from becoming a reality however; other studies have clearly established that miRNAs do play a role in regulating the tissue response to neoadjuvant therapy in CRC^[62-64]. Perhaps by focusing on the contribution of miRNAs within the biological pathways that govern resistance and/or sensitivity to neo-adjuvant therapy in rectal cancer, more clinically pertinent data will emerge on the role of miRNA as a potential biomarker in cancer treatment strategies^[65].

EMERGING TECHNOLOGY, LIQUID BIOPSIES

The term “liquid biopsy” in cancer arose when circulating tumor cells (CTC) were proposed as alternatives to conventional tissue biopsy in breast cancer for prognosis and evaluation of treatment responses^[66]. The theory has continued to grow experimentally and has gained particular traction in CRC. The clinical applications of liquid biopsy in CRC continue to grow, including detecting premalignant and early-stage cancers, identification of aggressive phenotypes and high-risk patients, assessing tumor heterogeneity, residual, and recurrent disease, and monitoring treatment response^[67]. In colon cancers, liquid biopsies may hold prognostic information beyond the nodal status for determining whether to administer adjuvant chemotherapy, while in rectal cancer, liquid biopsy may have roles for both primary disease evaluation and monitoring treatment response^[68]. Possible sources of liquid biopsies include blood, urine, saliva, and stool, which contain cancer-derived subcellular components, such as circulating tumor DNA (ctDNA) and circulating miRNAs.

Tumour-tissue remains the “gold standard”, but the advent of ctDNA analysis from blood samples has promise as a non-invasive biomarkers. Studies have reported a direct relationship between ctDNA levels and tumor burden, stage, vascularity, cellular turnover, and response to therapy^[69-71]. It can enable efficient temporal assessment of disease status, response to intervention, and early detection of recurrence superior to current strategies, such as CEA^[72]. ctDNA can monitor and recognize high-risk individuals, as the plasma tumour DNA levels are significantly higher in patients with increased advanced/stage IV disease, recurrence, or metastasis^[73,74]. ctDNA may be sensitive to detect with early, presumably curable CRC from common mutations, which could have implication for diagnostic testing^[75]. Meta-analysis has demonstrated high overall sensitivity and specificity for detecting the *KRAS* oncogene mutation in CRC, showing it may be a viable alternative to tissue analysis for the detection of *KRAS* mutations and subsequent therapeutic planning^[75]. Further, comparative analysis between CTCs and ctDNA in metastatic CRC has shown strong concordance between ctDNA and tissue for *RAS*, *BRAF*, and *ERBB2* mutations (84.6%) and greater detectability than CTCs with a smaller amount of blood sampling^[76]. ctDNA may hold specific promise as a biomarker to guide therapy in post-operative locally advanced rectal cancer, but further studies are needed for validation^[77]. There are limitations to ctDNA as a biomarker. Although ctDNA targets offer a high specificity, it is scarce in circulating biofluids- representing less than 1% of the total circulating free DNA and may be inadequate as clinically applicable diagnostic biomarkers. The best source of ctDNA is still uncertain and the size of the DNA released from dead cancer cells is longer than that of non-neoplastic DNA^[70,78]. Large scale controlled tr-

ials are needed for validation.

miRNA is an alternate for liquid biopsy. miRNAs have features making them ideal candidates for development as disease-specific biomarkers, and may offer superior sensitivity and specificity compared with ctDNA for diagnosing CRC^[79]. miRNAs are generally stable in blood and other body fluids due to their small size and their ability to escape from RNase-mediated degradation. miRNA expression levels are different in tumour compared to normal colon tissues^[80]. miRNA are actively secreted from living cells, while most ctDNA is dependent on release from apoptotic or necrotic cells^[81,82]. miRNA-based diagnostic markers and panels have been identified for early detection, risk of recurrence at the time of diagnosis, complement to CEA for identification of distant metastasis, and stratification of patients with poor prognosis and greater likelihood of metastasis to the lymph nodes, liver, and peritoneum^[80,83-88]. These miRNAs are detailed in Table 2. While a promising tool for “precision medicine”, there are limitations of circulating miRNAs as biomarkers in CRC. The existing studies use relatively small sample sizes, are retrospective in design, and utilized non-standardized sampling procedures. Larger, controlled studies are needed in order to validate the best purification method and clinical use of circulating miRNAs in CRC.

An example of a blood sample-based diagnostic biomarker that could make a clinical impact is methylated *Septin 9* (mSEPT9), which is validated to distinguish CRC from normal blood using real-time PCR^[89]. This non-invasive, blood-based tool for CRC could improve screening and surveillance compliance over colonoscopy and other screening methods^[90]. While monitoring of mSEPT9 may hold promise for CRC screening, a larger study population and more prospective studies are needed to validate mSEPT9 as a diagnostic biomarker in CRC.

ROLE OF EPITHELIAL MESENCHYMAL TRANSITION IN PRODUCING RECTAL CANCER CELLS WITH A RADIORESISTANCE PHENOTYPE

EMT is a physiological process resulting in transformation of stable epithelial cells into mobile mesenchymal cells^[91]. While EMT is a normal process during human development, it has also been shown to occur in carcinogenesis^[92]. In this situation, the resulting abnormal mesenchymal cells, which evade the influence of normal cellular control mechanisms, display an aggressive and invasive phenotype. These cells are increasingly linked to formation of micro-metastases, and causation of resistance to the effects of radiotherapy.

EMT cellular biology

Down-regulation of membranous E-cadherin is the classical finding of EMT. This results in loss of intercellular epithelial junctional complexes, promoting migration of

Table 2 Candidate liquid biopsy/circulating miRNA biomarkers^[145]

Expression level	Diagnostic biomarker	Prognostic biomarker (malignant potential, tumor recurrence)	Predictive biomarker (chemosensitivity)
High	miR-92a, miR-141, let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223, miR-23a, miR-378	miR-141, miR-320, miR-596, miR-203	miR-106a, miR-484, miR-130b
Low		miR-15a, miR-103, miR-148a, miR451	

Adapted from Tsutomu Kawaguchi *et al.* Circulating MicroRNAs: A Next-Generation Clinical Biomarker for Digestive System Cancers. *Int J Mol Sci* 2016; 17: 1459.

cells^[93-95]. The microRNA-200 family has been identified as a key post-transcriptional regulator of this process, through its targeting of E-cadherin transcriptional receptors^[96]. Subsequent escape from growth factor control, with uncontrolled proliferation, results from the EMT process^[94,95]. An end consequence of this pathway is tumour budding, defined as the presence of single cells or small cell clusters at the invasive front of tumour growth^[97]. Tumour budding is highly likely to be associated to EMT at the poorly differentiated invasive front^[97-100].

Current evidence

There is increasing evidence linking EMT to chemoresistance in ovarian, pancreatic and breast cancer cell lines^[101-104], and in human lung cancer specimens^[105]. Emerging evidence is also relating EMT to response to chemoradiotherapy in CRC. This initially arose from testing chemoresistance in colorectal cell lines^[106-108]. However newer human evidence is relating EMT as an independent biomarker of tumour budding, lymph node metastases, and radioresistance^[109]. The largest of these demonstrated that, in 103 patients with advanced rectal cancer, an EMT phenotype was associated with no-response to neoadjuvant therapy and reduced cancer specific survival^[110]. More evidence from human rectal cancer tissue is urgently needed to assess its potential as a biomarker.

Windows for intervention

A genetic predisposition to loss of E-cadherin and subsequent EMT may be causative, meaning that pre-treatment biopsy analysis presents a window for intervention. Radiotherapy may also be a traumatic triggering stimulus which forces some cells into an EMT phenotype, meaning other methods for patient selection may be required; overlap in causation is likely.

EMT as a prognostic and therapeutic biomarker

The biological action of metformin down-regulates the EMT transcription factors and up regulations E-cadherin^[110]. Its low toxicity profile makes it a feasible option in EMT prevention attempts, with subsequent improvements in response to neoadjuvant therapies^[111,112]. Additionally, cyclo-oxygenase (COX) inhibitors have shown potential to prevent EMT by reducing vimentin expression and increasing cell surface E-cadherin expression in cell line models^[113]. However, due to their serious associated ca-

rdiovascular side-effects, the particular COX agent and dose require optimisation before widescale use^[113,114]. The potential role of post-transcriptional microRNA-200 regulation presents a further potential therapeutic target^[96].

ROLE OF IMAGING BIOMARKERS IN DETECTION AND MONITORING DISEASE

The concept of an imaging biomarker is relatively new, but one which is becoming an increasingly important component of many phase II/III clinical trials as a surrogate endpoint. Imaging biomarkers may allow objective assessment of the tumour response to therapy and/or non-invasively detect early disease. Currently, the imaging techniques that seek to quantify treatment response in CRC can be broadly divided into those which measure tumour size and those which measure tumour activity. Whilst size criteria are the more commonly used biomarkers to assess radiological response in clinical trials because of their association with survival outcomes, it is the functional imaging techniques which are feted as having the greatest potential in uncovering the underlying biological processes which lead to cancer.

Measuring changes in tumour size

Reduction in tumour size has been shown to be a useful biomarker^[115]. This can be measured in one-, two- or three-dimensions by various routine imaging techniques such as CT and MRI^[116]. However, the two commonly used criteria - WHO^[117] and RECIST^[118] (Table 1); have contrasting characteristics, in particular in the technique used to measure tumour size - only one dimension using RECIST criteria. Further limitations to using size measurements have been deciding on what degree of tumour bulk reduction constitutes a significant clinical response. An example of this is has been shown by Morgan *et al.*^[119], who investigated the effect of a VEGF receptor inhibitor on colorectal metastases, whereby significant size reduction was not met with an equally significant overall response (< 10%). However the novel MRI-based tumour regression grade (mrTRG), which stratifies response on the degree of fibrosis visualised in the tumour following chemoradiotherapy, has been shown to be a useful clinical tool^[120]. The degree of fibrosis seen on MRI following CRT on a scale analogous to histopathological tumour regression grade (TRG)^[121] - tumour signal that has been completely replaced by radiological evidence of fibrosis is defined as radiological

complete response (mrTRG1-2)^[122]. These findings have been validated in a prospectively enrolled, multicentre study^[123] and used to influence treatment decisions in particular “deferral of surgery” programs. In the above study, multivariate analysis showed mrTRG hazard ratios (HR) were independently significant for overall and disease-free survival. Using fibrosis as a radiological feature is not limited to measuring tumour size but can be used to quantify other prognostic factors such as extramural venous invasion (EMVI), for example^[120]. A further study using prospectively collected data on EMVI response to neo-adjuvant chemoradiotherapy showed hazard ratio of 2.37 for DFS in tumours which had undergone more than 50% fibrosis of tumour signal in extramural vasculature^[124].

Measuring tumour activity

These techniques involve analysis of images to quantify the functional activity of tumours. The most common example of this is positron emission tomography (PET) with Fluorodeoxyglucose (18-FDG), which relies on the principle of a differential glycolytic rate seen in tumour cells. Using the glucose analogue 18-FDG gives an assessment of tumour metabolism^[125,126] by quantification of standard uptake values (SUV). However as timing of the scans from administration of the 18-FDG and subsequent clearance rates may vary between centres and patients, comparisons and standardisation of technique has been difficult. It is also important to note that until now, there has been no validation of response.

Dynamic contrast-enhanced (DCE) CT/MRI provides a detailed assessment of tumour bloodflow through acquisition of data as specific contrast material passes through the vasculature. DCE-CT has the potential to identify angiogenesis and has been shown to be able to distinguish from diverticular disease as well as detect early liver metastases^[127,128]. Although reports have identified a correlation between tumour blood flow, the development of metastases, and decreased survival outcomes^[129,130], this has not been translated to widespread clinical application. Vascular endothelial growth factor (VEGF) is upregulated in up to 78% of CRCs^[131,132] and is a potential target for functional imaging techniques. Bevacizumab is an anti-VEGF-A monoclonal antibody and DCE-MRI has been used in rectal cancer to evaluate treatment response using conjugation with a radiolabeled peptide^[133-135]. The analysis in DCE-MRI uses two compartments of plasma and extravascular-extracellular space to compare contrast agent - K^{trans} is the constant which is used to depict the bloodflow. Several studies have validated K^{trans} with expression of growth factors, such as VEGF and immunohistochemical confirmation of vessel architecture^[136-139]. Reduction in K^{trans} using Vatalanib (tyrosine kinase inhibitor which target VEGF receptor-2) for metastatic CRC with liver disease have shown promising results in the phase I / II setting^[119,140] but not been translated to survival benefit in phase III trials.

Diffusion weighted imaging (DWI) assesses the movement of water molecules within cells using diffusion-weighted gradients to T2 sequences. Quantitative analysis is possible by calculation of the apparent diffusion coefficients (ADC), which are inversely correlated with tumour cellularity. DWI has been effective in detecting small liver metastases and differentiation from inflammatory lesion^[141-143], as well as detecting lymph node metastases^[144], but application has been limited to mainly experimental work.

CONCLUSION

The interest in biomarkers relating to rectal cancer is clearly increasing. They form a new aspect of clinical and laboratory research which help translate these concepts to more meaningful applications in patient management. Much of the current literature is still in its embryonic stage, but as more results from clinical trials using biomarker endpoints and outcome measures become available, there will be a better understanding by clinicians of their potential, with possible future application to improve the predictive and prognosis of rectal cancer.

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HER2 inhibition in gastro-oesophageal cancer: A review drawing on lessons learned from breast cancer

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Abstract

Human epidermal growth factor receptor 2 (HER2)-inhibition is an important therapeutic strategy in HER2-amplified gastro-oesophageal cancer (GOC). A significant proportion of GOC patients display HER2 amplification, yet HER2 inhibition in these patients has not displayed the success seen in HER2 amplified breast cancer. Much of the current evidence surrounding HER2 has been obtained from studies in breast cancer, and we are only recently beginning to improve our understanding of HER2-amplified GOC. Whilst there are numerous licensed HER2 inhibitors in breast cancer, trastuzumab remains the only licensed HER2 inhibitor for HER2-amplified GOC. Clinical trials investigating lapatinib, trastuzumab emtansine, pertuzumab and MM-111 in GOC have demonstrated disappointing results and have not yet changed the treatment paradigm. Trastuzumab deruxtecan may hold promise and is currently being investigated in phase II trials. HER2 amplified GOC differs from breast cancer due to inherent differences in the HER2 amino-truncation and mutation rate, loss of HER2 expression, alterations in HER2 signalling pathways and differences in insulin-like growth factor-1 receptor and MET expression. Epigenetic alterations involving different microRNA profiles in GOC as compared to breast cancer and intrinsic differences in the immune environment are likely to play a role. The key to effective treatment of HER2 amplified GOC lies in understanding these mechanisms and tailoring HER2 inhibition for GOC patients in order to improve clinical outcomes.

Key words: Human epidermal growth factor receptor 2; Gastro-oesophageal cancer; Trastuzumab; Resistance; Biomarkers; Breast cancer

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Core tip: Human epidermal growth factor receptor 2 (HER2)-inhibition is an important therapeutic strategy in HER2-amplified gastro-oesophageal cancer (GOC). A significant proportion of GOC patients display HER2 amplification, yet HER2 inhibition in these patients has not displayed the success seen in HER2 amplified breast cancer. We evaluate current clinical and laboratory evidence surrounding HER2 inhibition in GOC. Inherent differences in the HER2 receptor, signalling pathways, associated microRNA signature and immune environment may partly explain the disappointing clinical trial outcomes seen in GOC. Only with improved understanding of HER2 inhibition can effective treatment be provided in order to improve clinical outcomes for patients.

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INTRODUCTION

Cancer therapy is becoming increasingly personalised and molecularly targeted, using biomarkers to identify patients most likely to respond to therapy. Human epidermal growth factor receptor 2 (HER2)-amplified cancer is defined as cancer with HER2 protein overexpression \pm HER2 gene amplification^[1]. It represents a molecularly-defined subgroup of malignancy and is known to exist in breast and gastro-oesophageal cancers (GOC), among others^[1]. Whereas the treatment for HER2-amplified breast cancer patients has been extremely successful, the treatment for GOC has been less so. In this review, we explore the mechanisms by which HER2 amplification contributes to cancer progression and prognosis, methods of targeting HER2 amplification, mechanisms of resistance to HER2 therapy, strategies to overcome resistance, biomarkers and future directions.

HER2 RECEPTOR AND ITS INTERACTIONS

HER2, encoded by the *ERBB2* oncogene on chromosome 17q21^[2], is a member of the epidermal growth factor receptor (EGFR) family associated with tumour cell proliferation, apoptosis, adhesion, migration and differentiation^[3]. All studies investigating HER2 receptor interactions have been conducted in breast cancer cells, and a literature search did not reveal any studies of HER2 receptor interactions conducted specifically in GOC. Given the relatively disappointing results seen in

GOC, we suggest it may be worthwhile exploring HER2 receptor interactions specifically in GOC, to investigate whether there are any mechanistic differences in HER2 binding and signalling between breast and GOC.

HER2 RECEPTOR OVEREXPRESSION AND ONCOGENIC MECHANISMS IN BREAST AND GOC

In both breast cancer and GOC, HER2 overexpression occurs in approximately 20%^[4,5]. The Gastric Cancer Genome Atlas [part of The Cancer Genome Atlas (TCGA)] recently classified gastric cancer into four subtypes and found that HER2 overexpression occurs only in Epstein-Barr virus (EBV)-positive tumours, genomically-stable (GS) tumours and tumours with chromosomal instability (CIN) but not in microsatellite unstable (MSI-high) tumours^[6]. Mechanisms by which HER2 overexpression can be oncogenic are complex, with activation of RAS-MAPK, c-jun and Akt-mTOR pathways^[3] (Figure 1). HER2 overexpression may lead to formation of HER2 homodimers and ligand-independent downstream signalling^[3]. The majority of studies investigating HER2 overexpression oncogenicity have been conducted in breast cancer, and mechanisms may differ in GOC.

INFLUENCE OF HER2 STATUS ON PROGNOSIS IN BREAST AND GASTRIC CANCER

In contrast to breast cancer, HER2 overexpression does not impact survival in GOC^[2]. Large phase III prospective randomised controlled trials such as ToGA^[5], LOGiC^[7] and TYTAN^[8] demonstrate that patients with HER2 amplified GOC who receive the control arm (chemotherapy alone) have an overall survival (OS) similar to all-comers (Table 1)^[5,7-9]. In the first-line ToGA and LOGiC trials, OS was 11.1 mo and 10.5 mo, respectively, in the control arms^[5,7], compared to OS in all-comers of 9.9 mo in the Phase III REAL2 trial^[10]. In the 2nd-line TYTAN trial, OS was 8.9 mo in the control^[8,9], which compared favourably to OS in all-comers treated with paclitaxel in the control arms of the RAINBOW (OS 7.4 mo)^[11] and GOLD trials (OS 6.9 mo)^[12]. This cross-trial comparison suggests that HER2 overexpression does not adversely affect GOC prognosis.

HER2 SCORING CRITERIA, DISCORDANCE AND HETEROGENEITY IN GOC AND BREAST CANCER

The HER2 scoring system in breast cancer was developed prior to the scoring system for GOC and was standardised in 2007 following an expert panel forum^[13].

Table 1 Summary of selected randomized phase III HER2 trials in HER2-amplified gastro-oesophageal cancer and breast cancer

Study title	Setting	n	Treatment arms	Primary endpoint	OS	PFS	HR and P value
Trastuzumab 1 st line metastatic ToGA ^[5]	1 st line metastatic GOC	594	Trastuzumab + chemotherapy <i>vs</i> chemotherapy alone	OS	Trastuzumab + chemotherapy: 13.8 mo (95%CI: 12-16) Chemotherapy alone: OS 11.1 mo (10-13)	Trastuzumab + chemotherapy: 6.7 mo (95%CI: 6-8) Chemotherapy alone: 5.5 mo (5-6)	HR = 0.74; 95%CI: 0.60-0.91; P = 0.0046
Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2 ^[19]	1 st line metastatic breast cancer	469	Trastuzumab + chemotherapy <i>vs</i> chemotherapy alone	PFS	Trastuzumab + chemotherapy: 25.1 mo Chemotherapy alone: 20.3 mo	Trastuzumab + chemotherapy: 7.4 mo Chemotherapy alone: 4.6 mo	P = 0.046
Lapatinib 1 st line metastatic LOGiC ^[7]	1 st line metastatic GOC	545	Lapatinib + CAPOX <i>vs</i> Placebo + CAPOX	OS	Lapatinib + CAPOX: 12.2 mo (95%CI: 10.6-14.2) Placebo + CAPOX: 10.5 mo (9.0-11.3)	Lapatinib + CAPOX: 6 mo (95%CI: 5.6-7.0) Placebo + CAPOX: 5.4 mo (4.4-5.7)	HR = 0.91; 95%CI: 0.73-1.12 P value not significant (exact value not given)
Randomized trial of lapatinib <i>vs</i> placebo added to paclitaxel in the treatment of human epidermal growth factor receptor 2-overexpressing metastatic breast cancer ^[29]	1 st line metastatic breast cancer	444	Lapatinib + paclitaxel <i>vs</i> Placebo + paclitaxel	OS	Lapatinib + paclitaxel: 27.8 mo (95%CI: 23.2-32.2 mo) Placebo + paclitaxel: 20.5 mo (17.9-24.3 mo)	Lapatinib + paclitaxel: 9.7 mo (95%CI: 9.2-11.1 mo) Placebo + paclitaxel: 6.5 mo (5.5-7.3 mo)	HR = 0.74; 95%CI: 0.58-0.94; P = 0.0124
Lapatinib 2 nd line metastatic Tytan ^[8]	2 nd line metastatic GOC	261	Lapatinib + Paclitaxel <i>vs</i> Paclitaxel alone	OS	Lapatinib + Paclitaxel: 11.0 mo Paclitaxel alone: 8.9 mo	Lapatinib + Paclitaxel: 5.5 mo Paclitaxel alone: 4.4 mo	HR = 0.84; 95%CI: 0.64-1.11 P = 0.1044
Lapatinib plus capecitabine for HER2-Positive advanced breast Cancer ^[30]	2 nd line metastatic breast cancer	324 included in preliminary analysis	Lapatinib + capecitabine <i>vs</i> capecitabine alone	PFS	Not reported	Lapatinib + capecitabine: 8.4 mo Capecitabine alone: 4.4 mo	HR = 0.49; 95%CI: 0.34 to 0.71; P < 0.001
T-DM1 2 nd line metastatic GATSBY ^[35]	2 nd line metastatic GOC	345	T-DM1 <i>vs</i> taxane	OS	T-DM1: 7.9 mo Taxane: 8.6 mo	T-DM1: 2.7 mo Taxane: 2.9 mo	HR = 1.15, 95%CI: 0.87-1.51; P = 0.86
EMILIA ^[33]	2 nd line metastatic breast cancer	991	T-DM1 <i>vs</i> lapatinib + capecitabine	PFS	T-DM1: 30.9 mo Lapatinib + capecitabine: 25.1 mo	T-DM1: 9.6 mo Lapatinib + capecitabine: 6.4 mo	HR = 0.65; 95%CI: 0.55 to 0.77; P < 0.001

HER2: Human epidermal growth factor receptor 2.

The ToGA trial used a new immunohistochemistry (IHC) scoring criteria developed by Hofmann^[14] for gastric cancer due to inherent biological differences compared to breast cancer, such as tumour heterogeneity and baso(lateral) membrane staining^[5,14]. Some criteria were the same as breast cancer: HER2 positivity was defined as an IHC score of 3+ and/or *erbB-2* amplification detected using fluorescent in-situ-hybridisation (FISH)^[5,14]. Notably, GOC patients with highly amplified HER2 gene experience better response and survival than patients with lower HER2 gene amplification levels when treated with 1st-line trastuzumab plus che-

motherapy for metastatic gastric cancer^[15].

HER2 expression in primary and metastatic sites demonstrates heterogeneity more frequently in GOC than in breast cancer^[16,17], and discordance between IHC and FISH results occur more frequently in GOC than in breast cancer^[18]. This may explain the limited success of targeted anti-HER2 therapy in GOC. If only a small proportion of GOC cells shows HER2 overexpression and if our detection methods are unreliable, GOC cancer cells that do not overexpress HER2 will not be effectively targeted with anti-HER2 therapy, and we may be failing to treat adequately some patients with

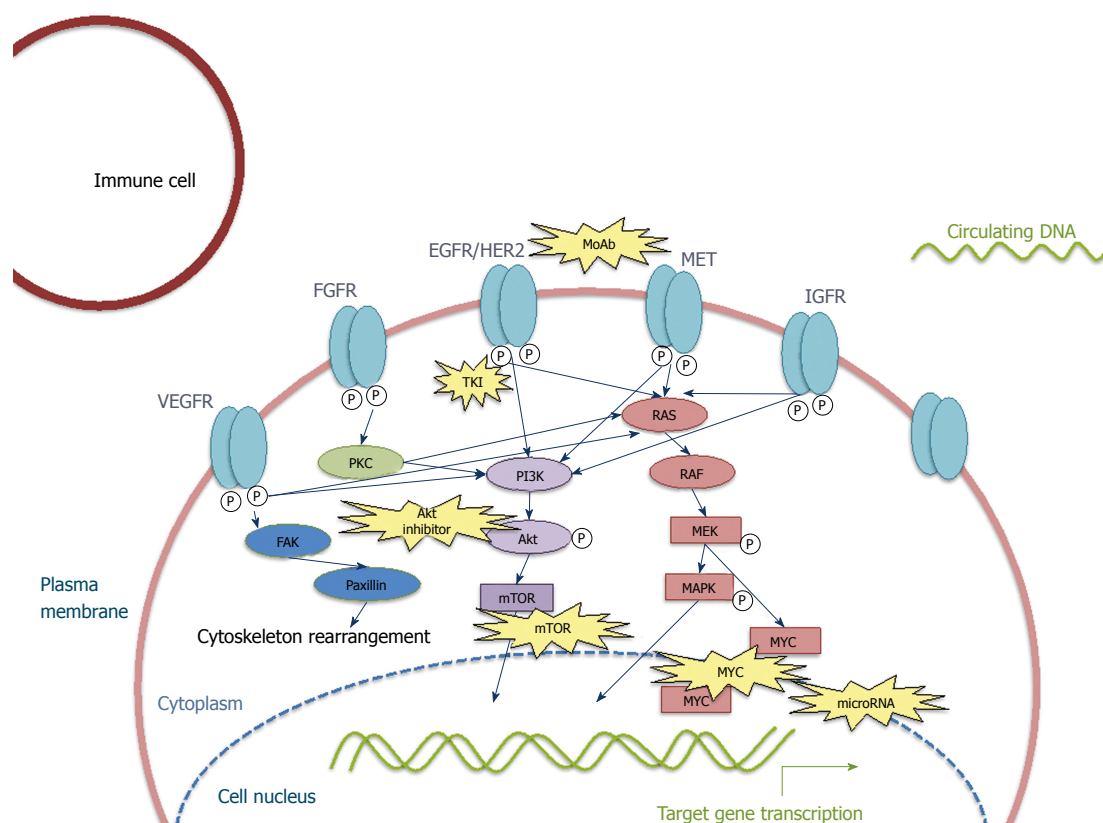


Figure 1 Simplified diagram showing signalling pathways related to human epidermal growth factor receptor 2 in gastric adenocarcinoma. VEGFR: Vascular endothelial growth factor receptor; FGFR: Fibroblast growth factor receptor; PKC: Protein kinase C; FAK: Focal adhesion kinase; HER2: Human epidermal growth factor receptor 2; MAPK: Mitogen-activated protein kinase; mTOR: Mammalian target of rapamycin.

HER2 overexpression.

THERAPEUTIC AGENTS TARGETING THE HER2 SIGNALLING PATHWAY

Trastuzumab

The efficacy of trastuzumab (a monoclonal antibody against HER2) in breast cancer in combination with chemotherapy has been convincingly demonstrated in both metastatic (OS 25.1 mo in patients receiving trastuzumab + chemotherapy vs 20.3 mo in those receiving chemotherapy alone, Table 1)^[19] and adjuvant settings^[20]. Breast cancer OS is, however, influenced by the greater number of treatment options in the 2nd-line setting and beyond.

In GOC, trastuzumab is the only licensed anti-HER2 treatment, following positive results from the ToGA trial, an open-label, international, phase 3, randomised controlled trial evaluating trastuzumab plus platinum-fluoropyrimidine chemotherapy for 1st-line treatment of HER2 positive GOC (Table 1)^[5]. Median OS was initially reported as 13.8 mo (95%CI: 12-16) in patients receiving trastuzumab plus chemotherapy vs 11.1 mo (10-13) in patients receiving chemotherapy alone (HR = 0.74; 95%CI: 0.60-0.91; *P* = 0.0046)^[5]. This led to trastuzumab plus platinum-fluoropyrimidine chemotherapy followed by trastuzumab maintenance

becoming the standard of care in 1st-line metastatic GOC patients^[5]. Updated OS (after a further 1 year of follow-up) released by the United States Food and Drug Administration (FDA) in 2016 showed median OS of 13.1 mo (95%CI: 11.9-15.1) in the trastuzumab plus chemotherapy arm and 11.7 mo (95%CI: 10.3-13.0) in the control arm (HR = 0.8, 95%CI: 0.67-0.97)^[21]. Subgroup analysis demonstrated that patients with IHC 3+ HER2 expression experienced the greatest benefit from trastuzumab (294 patients, HR = 0.66, 95%CI: 0.5-0.87). Patients with IHC 2+ HER2 expression gained less benefit from the addition of trastuzumab (160 patients, HR = 0.78, 95%CI: 0.55-1.10), and patients with IHC 1 or 1+ gained no benefit (133 patients, HR = 1.33, 95%CI: 0.92-1.92)^[21]. Recent data on two different doses of trastuzumab in combination with chemotherapy in GOC found that a higher trastuzumab maintenance dose does not convey additional survival benefit (OS 12.5 mo in the 8 mg/kg + 6 mg/kg group vs 10.6 mo in the 8 mg/kg + 10 mg/kg group)^[22].

It remains to be seen whether trastuzumab confers a survival benefit in the neo-adjuvant/perioperative/adjuvant setting in combination with chemotherapy + surgery ± radiotherapy, and several phase 2 trials are underway to address this question (UMIN 000016920, NCT01472029, NCT02250209, Table 2)^[23,24]. Perioperative trastuzumab appears to be safe and well tolerated^[25].

Table 2 Selected perioperative (neoadjuvant + adjuvant) clinical trials currently underway targeting HER2 in HER2-amplified localised gastro-oesophageal cancer

Official study title	Stage and study number	Treatment arms	Estimated enrollment	Primary endpoint
Trastuzumab A randomized phase II trial of systemic chemotherapy with and without trastuzumab followed by surgery in HER2-positive advanced gastric or esophagogastric junction adenocarcinoma with extensive lymph node metastasis: Japan Clinical Oncology Group study JCOG1301 (Trigger Study) ^[23]	Phase II UMIN 000016920	Preoperative S-1 + cisplatin + trastuzumab <i>vs</i> S-1 + cisplatin Followed by adjuvant chemotherapy with S-1 for 1 yr	130	OS
Multicenter, explorative phase II study of perioperative 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 (HerFLOT)	Phase II NCT01472029	Pre-operative 5-FU + leucovorin + docetaxel + oxaliplatin (FLOT) + trastuzumab Post-operative trastuzumab monotherapy	53	pCR
Trastuzumab plus XELOX for HER2-positive stage III gastric cancer after D2 gastrectomy: prospective observational Study ^[77]	Phase II NCT02250209	Trastuzumab + capecitabine + oxaliplatin after D2 gastrectomy	40	3-yr DFS
A phase III trial evaluating the addition of trastuzumab to trimodality treatment of HER2-overexpressing esophageal adenocarcinoma	Phase III NCT01196390	Radiotherapy + paclitaxel + carboplatin + trastuzumab <i>vs</i> Radiotherapy + paclitaxel + carboplatin	591	DFS
Lapatinib A randomised phase II / III trial of peri-operative chemotherapy with or without bevacizumab in operable oesophagogastric adenocarcinoma and a feasibility study evaluating lapatinib in HER-2 positive oesophagogastric adenocarcinomas and (in selected centres) MRI and PET/CT sub-studies (STO3 trial)	Phase II / III NCT00450203	Epirubicin + cisplatin + capecitabine (ECX) + lapatinib <i>vs</i> ECX	40 (within lapatinib sub-study)	Safety
Pertuzumab + Trastuzumab INtegrationN of trastuzumab, with or without pertuzumab, into perioperative chemotherapy of HER-2 positive stomach cancer: the INNOVATION-TRIAL ^[77]	Phase II NCT02205047	Cisplatin/capecitabine or cisplatin/5-fluorouracil <i>vs</i> cisplatin/capecitabine + trastuzumab or cisplatin/5-fluorouracil + trastuzumab <i>vs</i> cisplatin/capecitabine + trastuzumab + pertuzumab or cisplatin/5-fluorouracil + trastuzumab + pertuzumab	220	Near complete pathological response rate
FLOT <i>vs</i> FLOT/Herceptin/Pertuzumab for perioperative therapy of adenocarcinoma of the stomach and gastroesophageal junction expressing HER-2 A phase II / III trial of the AIO. (PETRARCA study)	Phase II / III NCT02581462	5-FU + leucovorin + docetaxel + oxaliplatin (FLOT) <i>vs</i> FLOT + trastuzumab + Pertuzumab	404	pCR OS
Feasibility study of chemoradiation, TRastuzumab and pertuzumab in resectable HER2+esophageal carcinoma: the TRAP study	Phase I / II NCT02120911	Pertuzumab + trastuzumab + standard chemoradiation with carboplatin and paclitaxel.	40	Safety

pCR: Pathological complete response; DFS: Disease-free survival.

One Phase II trial evaluating capecitabine + oxaliplatin with trastuzumab three cycles pre-operatively and post-operatively followed by 12 mo adjuvant trastuzumab reported an 18 mo DFS of 71% (95%CI: 53%-83%), a 24 mo DFS of 60% and a median follow-up of 24.1 mo (median DFS and OS not reached)^[26]. Although a phase III trial evaluating radiotherapy + chemotherapy ± trastuzumab is underway (NCT01196390, Table

2)^[24], it is notable that trastuzumab is not being investigated in phase III trials in the peri-operative GOC setting. This is likely due to the prohibitive number of patients (approximately 10000) that would require screening in order to recruit adequate numbers of patients for a sufficiently powered study, given that HER2 overexpression is around 20%^[5], and a relatively small proportion of patients in Western countries are diagnos-

Table 3 Selected clinical trials currently underway targeting HER2 in advanced and metastatic HER2-amplified gastro-oesophageal cancer

Official study title	Stage and study number	Treatment arms	Estimated enrollment	Primary endpoint
Trastuzumab in combination with targeted therapies Phase II study of docetaxel, oxaliplatin, capecitabine with bevacizumab and trastuzumab in case of HER2-positivity in patients with locally advanced or metastatic gastric cancer or adenocarcinoma of the gastro-oesophageal junction (B-DOCT study)	Phase II NCT01359397	Docetaxel, oxaliplatin, capecitabine, bevacizumab <i>vs</i> Docetaxel, oxaliplatin, capecitabine, bevacizumab, trastuzumab	Information not available	PFS
A phase II study of afatinib (BIBW 2992) and trastuzumab in patients with advanced HER2-positive trastuzumab-refractory advanced esophagogastric cancer	Phase II NCT01522768	Afatinib (BIBW 2992) + trastuzumab	40	ORR
Intraperitoneal trastuzumab Phase I trial of intraperitoneal ²¹² Pb-TCMC-trastuzumab for HER-2 expressing malignancy	Phase I NCT01384253	²¹² Pb-TCMC-trastuzumab + trastuzumab	36	Safety
T-DM1 A combination study of kadcyla (trastuzumab emtansine, T-DM1) and capecitabine in patients with HER2-positive metastatic breast cancer and patients with HER2-positive locally advanced/metastatic gastric cancer (TRAX-HER2 study) ^[78]	Phase II NCT01702558	Capecitabine + trastuzumab emtansine (T-DM1) <i>vs</i> T-DM1	235	Safety ORR
DS-8201 Phase I, two-part, multicenter, non-randomized, open-label, multiple dose first-in-human study of DS-8201A, in subjects with advanced solid malignant tumors ^[36]	Phase II NCT02564900	Trastuzumab deruxtecan (DS-8201a)	198	Safety ORR
Lapatinib Safety and clinical activity of lapatinib in patients with HER2-positive refractory advanced cancer: a phase II single arm prospective study	Phase II NCT02342587	Lapatinib	25	ORR
New HER2 inhibitors A phase I - II study to assess the safety, efficacy and pharmacokinetic profile of HM781-36B combined with paclitaxel and trastuzumab in patients with HER-2 positive advanced gastric cancer	Phase I / II NCT01746771	HM781-36B(Pozotinib) (Other Names: NOV120101) + paclitaxel + trastuzumab	48	Safety DLT
A phase 1, dose escalation study of MGAH22 in patients with refractory HER2 positive breast cancer and patients with other HER2 positive carcinomas for whom no standard therapy is available	Phase I NCT01148849	MGAH22 (margetuximab)	67	Safety
A phase I multicenter, open-label, dose-escalation, and dose-expansion study to evaluate the safety, pharmacokinetics, immunogenicity, and antitumor activity of MEDI4276 in subjects with select HER2-expressing advanced solid tumors	Phase I NCT02576548	MEDI4276	120	Safety MTD
A phase I study of pyrotinib in combination with docetaxel in patients with HER2 positive advanced gastric cancer	Phase I NCT02378389	Pyrotinib + docetaxel	28	Safety
A two-part phase I, open label, dose escalation study to evaluate the safety, tolerability and pharmacokinetics of pyrotinib in patients whose disease progressed on prior HER2 targeted therapy	Phase I NCT02500199	Pyrotinib	70	Safety MTD
Neratinib An open-label, multicenter, multinational, phase 2 study exploring the efficacy and safety of neratinib therapy in patients with solid tumors with activating HER2, HER3 or EGFR mutations or with EGFR gene amplification	Phase II NCT01953926	Neratinib	292	ORR
HER2-targeted immunotherapy A phase Ib/ II study of pembrolizumab and monoclonal antibody therapy in patients with advanced cancer (PembroMab) ^[77]	Phase I / II NCT02318901	Pembrolizumab + trastuzumab <i>vs</i> pembrolizumab + ado-trastuzumab emtansine (T-DM1) <i>vs</i> pembrolizumab + cetuximab	90	Safety and dose-finding
A phase I study to evaluate the antitumor activity and safety of DUKE-002-VRP (HUHER2-ECD + TM), an alphaviral vector encoding the HER2 extracellular domain and transmembrane region, in patient with locally advanced or metastatic human epidermal growth factor receptor 2-positive (HER2+) cancers including breast cancer	Phase I NCT01526473	AVX901	12	Safety

HER2-peptide vaccination of patients with solid tumors	Phase I NCT02276300	Cyclophosphamide sargramostim HER2-Peptid-Vakzine imiquimod	12	Safety
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ORR: Overall response rate; HER2: Human epidermal growth factor receptor 2.

ed with operable disease^[27]. Only a proportion of these patients would have an adequate performance status to enter a clinical trial; therefore, trastuzumab will likely never be investigated in phase III trials in the peri-operative setting.

In advanced GOC, trastuzumab is being investigated in combination with bevacizumab (NCT01359397, Table 3), afatinib (NCT01522768, Table 3) and *via* intraperitoneal delivery (NCT01384253, Table 3)^[24].

Lapatinib

Lapatinib is an oral tyrosine kinase inhibitor targeting EGFR and HER2^[7,28]. In breast cancer, lapatinib demonstrated significant clinical benefit and is now a standard line of treatment^[19,29,30]. In contrast, in GOC, although it showed promise in preclinical trials, lapatinib failed to translate into clinical benefit in both 1st-line (LOGiC)^[7] and 2nd-line settings (TYTAN) (Table 1)^[9]. The reasons for the disappointing results seen in GOC as compared to breast cancer may be related to lapatinib dosage, toxicities experienced, or different underlying HER2 signalling mechanisms in GOC and breast cancer. When lapatinib was combined with paclitaxel in a 1st-line breast cancer study and 2nd line GOC study (TYTAN), rates of AEs were broadly similar: 77% of patients in the lapatinib arm experienced diarrhoea in both the breast and TYTAN studies vs 29% of patients in the control arm in the breast study and 22% in the TYTAN study^[8,29]. There was, however, a slightly higher rate of treatment discontinuation seen in GOC patients as compared to breast patients, with AEs resulting in treatment discontinuation in 16% in the lapatinib plus paclitaxel group vs 13% in the breast study^[8,29]. In the 1st-line GOC LOGiC trial (Table 1)^[7], there were significantly higher toxicity rates in the lapatinib arm than the control arm, with 94% of patients experiencing adverse events (AEs) and 27% serious AEs (SAEs) in the lapatinib arm vs 88% AEs and 19% SAEs in the control arm. Diarrhoea occurred in 58% of patients receiving lapatinib vs 29% in the control arm, leading to lower relative drug exposure in the lapatinib arm^[7]. Again lapatinib treatment resulted in higher rates of treatment discontinuation in GOC than breast cancer patients: 21% of patients in the lapatinib arm of LOGiC required treatment discontinuation vs 13% of breast cancer patients in the 2nd-line breast study receiving lapatinib plus capecitabine^[30]. Overall, this suggests that the chemotherapy backbone with which to combine lapatinib is important, and chemotherapy drugs with overlapping toxicity may result in lower lapatinib dose-intensity and reduced efficacy in GOC. Additionally GOC patients frequently experience gastrointestinal si-

de-effects prior to treatment and may be less able to tolerate lapatinib treatment. Another possible reason for the poor efficacy of lapatinib in GOC is that HER2 and EGFR signalling mechanisms may differ as compared to breast cancer.

Lapatinib is currently being investigated in Phase II/III trials in the peri-operative setting (STO3 trial, NCT00450203, Table 2)^[24] and as monotherapy in the advanced setting (NCT02342587, Table 3)^[24]. Safety data from the STO3 trial was presented at ESMO 2016 and suggested that administration of lapatinib at a dose of 1250 mg/d in combination with ECX chemotherapy (capecitabine 1000 mg/m²) was feasible, although there was increased diarrhoea (21% in ECX + lapatinib group vs 0% in ECX group) and neutropenia (42% in ECX + lapatinib group vs 21% in ECX group), which did not appear to compromise operative management^[31].

T-DM1

Trastuzumab emtansine (T-DM1) is an antibody-drug conjugate that combines the HER2-targeted properties of trastuzumab with the cytotoxic activity of emtansine, enabling selective delivery of chemotherapy to HER2-overexpressing cells^[32]. Although T-DM1 demonstrated significant clinical benefit in the EMILIA breast cancer trial in the 2nd-line setting (Table 1)^[33], a similar study (GATSBY, Table 1) in GOC failed to meet its primary endpoint or any of its secondary endpoints^[34,35]. It is worth noting that nearly half of the patients in the GATSBY trial were from the Asia-Pacific region. These patients are generally fit with a good performance status; and, therefore, it is likely that a significant proportion will have received post-study treatment^[35]. T-DM1 monotherapy vs T-DM1 + capecitabine is being investigated in combination with capecitabine chemotherapy in GOC in pretreated patients (NCT01702558, Table 3) and recruitment has been completed^[24].

Trastuzumab deruxtecan

Trastuzumab deruxtecan (DS-8201) is an antibody-drug conjugate comprising a humanised antibody against HER2 and a topoisomerase I inhibitor "payload" bound together by an enzyme-cleavable linker^[36]. A phase I open label dose escalation study recently presented at ASCO^[37] demonstrated an overall response rate (ORR) of 46.7% in HER2+ breast cancer patients pretreated with T-DM1 and pertuzumab and an ORR of 44.4% in gastric cancer patients pretreated with trastuzumab^[37]. This high response rate demonstrates that the "payload" bound to the anti-HER2 antibody can make a significant

difference to treatment success. For the first time, similar response rates were seen in both breast and gastric cancers pretreated with HER2 inhibitors, and responses were seen even in low HER2-expressing tumours^[36]. Results of the currently planned phase 2 trials are eagerly awaited (NCT02564900, Table 3)^[24], and whether these response rates can translate into improved overall survival remains to be seen.

Pertuzumab

Pertuzumab is a humanised monoclonal antibody targeting a different HER2 epitope to trastuzumab^[38], preventing formation of HER2-HER3 heterodimers^[39]. It can be administered concurrently with trastuzumab^[40]. In the CLEOPATRA breast cancer study, pertuzumab demonstrated significant clinical benefit when added to trastuzumab plus taxane chemotherapy^[40]. Disappointingly, in advanced GOC, the phase III JACOB study of pertuzumab + trastuzumab failed to demonstrate a significant improvement in OS^[41].

Pertuzumab is currently being explored in combination with trastuzumab and chemotherapy in the perioperative GOC setting in INNOVATION (NCT02205047) and PETRARCA trials (NCT02581462) and with the addition of radiotherapy in the TRAP trial (NCT02120911) (Table 2)^[24].

Preclinical studies investigating pertuzumab in combination with T-DM1 in GOC cell lines and xenograft models found this combination caused growth inhibition but no tumour shrinkage^[42]. A literature search did not reveal any clinical studies investigating this combination in GOC.

MM-111

MM-111 is a bispecific antibody fusion protein designed by Merrimack to inhibit HER3-ligand binding and signalling in HER2-amplified tumours by preventing formation of HER2-HER3 heterodimers^[43,44]. Preclinical studies showed promise, leading to phase 1 and phase 2 studies in selected tumour types, including HER2-amplified breast and GOC^[43,44]. However, the phase 2 study investigating MM-111 in HER2-amplified GOC patients was terminated early by the independent data monitoring committee when it was found that the addition of MM-111 to chemotherapy + trastuzumab resulted in a significantly poorer PFS and OS^[43]. In light of the disappointing results seen in GOC^[43], all further studies investigating MM-111 were withdrawn, and Merrimack announced that it does not plan to invest further in MM-111.

New HER2 inhibitors

Pozotinib is an oral pan-HER2 inhibitor whose role in combination with trastuzumab and paclitaxel is currently under investigation in advanced gastric cancer (NCT01746771, Table 3)^[24]. Phase 1 studies in GOC are investigating MGAH22 (Margetuximab) (NCT01148849, Table 3)^[24], a chimeric anti-HER2 monoclonal antibody similar to trastuzumab but engineered for increased binding^[45]. Medimmune is investigating their HER2 inhibitor, MEDI4276, in a Phase 1 trial (NCT02576548, Table 3)

in both breast and gastric cancers^[24]. Pyrotinib is an oral tyrosine kinase inhibitor targeting both HER1 (EGFR) and HER2 and is currently being explored in phase 1 trials in GOC (NCT02378389, NCT02500199, Table 3)^[24].

MECHANISMS THAT MAY AFFECT HER2 INHIBITION IN GOC

Resistance to HER2 therapy can be one of two types: primary (intrinsic) resistance occurs when there is no response to HER2 inhibitors and secondary (acquired) resistance occurs when there is an initial response followed by cessation of response^[46]. Differentiating between these types of resistance is important, as it dictates the optimal timing of treatment strategies.

Alterations to the HER2 receptor

p95HER2: An aminotruncated form of HER2, known as p95HER2^[46], lacks the region to which trastuzumab binds and is expressed in 20%-37% of breast cancer patients^[47] and 60%-77% of GOC patients with HER2 amplified disease^[48,49]. This may partly explain the poorer response to trastuzumab in GOC as compared to breast cancer.

HER2 mutation: Within the TCGA, 15 cases of *ERBB2* mutation in GOC were detected using RNA evidence out of 215 non-hypermethylated tumours^[6]. Evaluation of HER2 mutation across an array of tumour types revealed HER2 mutations in around 5% of gastric cancer patients^[50]. Neratinib, a pan-HER tyrosine kinase inhibitor, is being explored in HER2-mutated cancer (NCT01953926, Table 3)^[24].

Loss of HER2 expression

A recent study presented at ASCO found that 35% of GOC treated with trastuzumab lost HER2 positivity^[51]. Similarly, in breast cancer, loss of HER2 positivity has been reported in patients treated with neoadjuvant trastuzumab + chemotherapy or chemotherapy alone, and loss of HER2 positivity was associated with an increased risk of disease relapse^[52].

Signalling pathways

PIK3CA/PTEN/PI3K/AKT/mTOR pathway: The antitumour activity of HER2 inhibitors requires downstream inhibition of *PI3K/AKT*^[46,53]. BOLERO-3 was a randomised, double-blind, placebo-controlled phase 3 trial in HER2 positive, trastuzumab-resistant, advanced previously-treated breast cancer patients that explored whether the mTOR inhibitor everolimus might restore sensitivity to trastuzumab^[54]. It demonstrated significant improvement in PFS with the addition of everolimus [7 mo (95%CI: 6.74-8.18) in the everolimus group vs 5.78 mo (5.49-6.9) in the placebo group]^[54]. The randomised phase 3 BOLERO-1 trial compared everolimus plus trastuzumab plus paclitaxel to placebo plus trastuzumab plus paclitaxel in order to assess whether addition of everolimus at tr-

eatment outset might prevent intrinsic resistance: primary endpoint (PFS) was not met^[55].

Phase 3 clinical trials have not been conducted specifically in HER2 positive GOC patients^[49]. The phase 3 GRANITE trial randomised 656 patients with advanced pretreated gastric cancer to either everolimus or matching placebo^[56]. HER2 status was not an inclusion or exclusion criteria, and we do not know the percentage of HER2 positive patients within this trial. The primary endpoint (OS) was not met, and everolimus was associated with significant side-effects: 21.5% of patients receiving everolimus required drug discontinuation and 55.4% required dose adjustments/interruptions^[56]. Such high rates of adverse events are concerning in the palliative setting, where quality of life is important.

IGF-1R expression

Insulin-like growth factor-1 receptor (IGF-1R) is involved in acquired resistance to HER2 blockade in breast cancer^[46,57] and GOC^[58] cells *in vitro* by forming heterodimers with HER2. Blockade of this heterodimer formation *in vitro* and *in vivo* restored sensitivity to HER2^[57], and combination studies of HER2 blockade in combination with IGF-1R inhibitors were more effective than either agent alone^[59]. Clinical studies exploring IGF-1R inhibitors in combination with HER2 inhibitors in breast cancer patients found no significant difference in PFS (NCT00684983)^[49]; other studies evaluating this strategy were withdrawn, and there are no GOC studies^[49].

MET overexpression

Clinical studies of MET inhibitors as monotherapy in HER2 negative breast cancer patients did not meet their primary endpoint^[49,60]. In GOC, a randomized double-blind phase 3 clinical trial exploring MET inhibition in HER2 negative, MET positive GOC patients found no benefit from the addition of the MET inhibitor onartuzumab to chemotherapy^[61]. Phase 2 results for an alternative MET inhibitor, tivantinib, similarly showed no survival advantage^[62]. In light of these disappointing results, it is unlikely MET inhibition will be explored in the clinical setting in HER2-overexpressing breast or GOC patients.

HSP90

Combining HER2- and Heat shock protein (HSP90)-inhibition to overcome resistance to HER2 inhibitors showed promise preclinically in cell lines and mouse models in breast and GOC cell lines^[63]. However, a phase 2 study in breast cancer has not yet released results^[64], and a phase 2 study in gastric cancer was terminated (NCT01402401)^[24].

MicroRNA

MicroRNAs (miRs) are small non-coding RNAs that control gene expression through messenger RNA degradation and post-transcriptional inhibition^[65]. MiRs are tissue-specific, and different microRNA signatures may occur during resistance to HER2 inhibition in breast and GOC. In HER2 positive breast and gastric cancer cells,

miR-21 overexpression leads to PTEN downregulation, suppression of trastuzumab-induced apoptosis and increased trastuzumab resistance^[66,67]. MiRNA-542-3p downregulation promotes trastuzumab resistance in breast cancer *via* AKT activation^[68]. MiR-7 functions as a suppressor of the oncogenic isoform of HER2, HER2^{Δ16}, and reverses HER2^{Δ16}-induced trastuzumab resistance in breast cancer^[69]. The use of miRs not only as biomarkers but as targets for anticancer therapy may allow new therapeutic miR silencing in the future^[70]. Inhibition of certain microRNAs may also enhance the effect of HER2 inhibition^[71].

Immune response

Natural killer (NK) cells are required in order to exert trastuzumab's therapeutic effect^[46]. Mice deficient in NK cells show trastuzumab resistance^[72] and when numbers of innate and adaptive immune cells in the tumour microenvironment increase, there is increased tumour eradication^[73]. Trials studying the immune environment in GOC are underway (NCT02318901, NCT01526473, NCT02276300, Table 3)^[24].

Biomarkers

Specific-uptake positron emission tomography (PET) scans: Targeted PET scans using radiolabelled trastuzumab (89Zr-Trastuzumab) to demonstrate HER2 uptake can give real-time information on HER2 expression levels, visually displaying the development of resistance with the advantage of being relatively non-invasive and, therefore, preferable for patients^[74].

Circulating DNA: Circulating DNA may represent a clinically useful biomarker that reduces the need for invasive biopsies. Plasma DNA digital PCR can detect HER2 status in metastatic breast cancer patients^[75]. A systematic review and meta-analysis has suggested that serum HER2 is a potential surrogate for tissue HER2 status in gastric cancer^[76].

CONCLUSION

Despite numerous HER2 inhibitors being investigated in a number of settings, trastuzumab in advanced disease is still the only HER2 inhibitor licensed for clinical use in the treatment of GOC. Even within this setting, the overall survival benefit is far less than that seen in breast cancer. Other HER2 inhibitors that have demonstrated success in breast cancer have failed to reach statistically significant endpoints in GOC clinical trials, and it remains to be seen whether clinical trials currently underway will show improved results. HER2 heterogeneity, amino-truncation loss of HER2 expression and differences in signalling pathways may contribute to the disappointing clinical trial outcomes seen in GOC. Different microRNA signatures and immune environments are also likely to play a role. Development of new HER2 inhibition strategies in conjunction with

further research into how the role of HER2 differs in GOC as compared to breast cancer is required. Clinical trials utilizing biomarkers such as specific uptake PET scans and circulating DNA may provide early insight into whether patients are responding to HER2 inhibition. Only with improved understanding of HER2 inhibition in GOC can effective treatment be provided in order to improve clinical outcomes for patients.

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Advances in molecular, genetic and immune signatures of gastric cancer: Are we ready to apply them in our patients' decision making?

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Abstract

In the last few years we have witnessed a vast expansion of our knowledge regarding the molecular and genetic profile of gastric cancer. The molecular subtypes described have shed light on the pathogenesis of the disease, thus prompting the development of new therapeutic strategies and favoring a more individualized approach for treatment. Most of the clinical trials for so called targeted therapies could be considered, at best, partially successful. In addition, checkpoint inhibitors have recently been added to our armamentarium in later stages of the disease, and combinations with chemotherapy and targeted agents are currently under development. In view of the rapid advances of molecular oncology, a new challenge for the clinical oncologist arises: The appropriate patient selection for each new therapy, which can be made possible only through the implementation of predictive biomarkers in our therapy decision making.

Key words: Gastric cancer; Cancer Genome Atlas; Asian Cancer Research Group; Targeted therapy

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Core tip: Despite recent advances in cancer therapeutics, the survival of gastric cancer patients with metastatic disease is dismal due to the complexity of the disease, the constant evolution of tumors and our still limited understanding of its biology. It is evident that a wide spectrum of prognostic and predictive biomarkers is needed in order to rationalize our decisions when managing patients with this specific tumor type and tailor our treatment to suit better the individual patient's unique needs.

Gkolfinopoulos S, Papamichael D, Papadimitriou K, Papanastasiopoulos P, Vassiliou V, Kountourakis P. Advances in molecular, genetic and immune signatures of gastric cancer: Are we ready to apply them in our patients' decision making? *World J Gastrointest Oncol* 2018; 10(7): 172-183 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i7/172.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i7.172>

INTRODUCTION

Gastric cancer (GC) is the fifth most common type of cancer and the third most common cause of cancer-related mortality worldwide^[1]. Despite recent advances in cancer therapeutics, driven by the application of the findings of basic science in cancer genetics and host-tumor immune interactions, the prognosis of most patients with metastatic disease is dismal^[2]. Indeed, in GC we seem to lack clear molecular targets based on key regulatory genes or the aberrant expression of growth factor receptors. Furthermore, the universal rise of immunotherapeutic approaches in various tumor types has only recently been incorporated in GC. It is evident that a wide spectrum of prognostic and predictive biomarkers is needed in order to rationalize our decisions when managing patients with this specific tumor type and tailor our treatment to suit better the individual patient's unique needs.

Genetic heterogeneity of GC

Our understanding in GC genetics was greatly expanded in 2014, when four main molecular subtypes of the disease were recognized in the context of the Cancer Genome Atlas (TCGA) project^[3]. Further efforts were undertaken in order to relate molecular subtypes with the known histological subtypes that Lauren had proposed roughly half a century ago as well as with the location of the primary tumor and prognosis^[4]. These efforts were met with moderate success, since it is now widely accepted that there is an important degree of overlap. Various basic studies and clinical trials followed, aiming to discover a clinically meaningful way of utilizing the findings of the TCGA project^[5]. Unfortunately, thus far, the results have fallen short of the initial high expec-

tations, although some success has been noted in subgroups of patients across trials that exhibited unique molecular characteristics. In 2015, another major molecular classification was proposed, this time from the Asian Cancer Research Group (ACRG), which shares similarities with TCGA yet has enough differences to be considered completely distinct (Table 1). The novelty with the ACRG was that the molecular subtypes discovered were associated with clinical outcomes^[6]. A short review and comparison of both classification systems will be presented, followed by a brief and non-exhaustive analysis of the most important clinical trials employing target or immunotherapeutic strategies in this expanding area of oncology.

MOLECULAR SUBTYPES OF GC ACCORDING TO TCGA

The first and most comprehensive molecular characterization of gastric adenocarcinoma was reported by the TCGA Network. In this study, 295 (therapy naive) primary gastric adenocarcinoma samples were characterized using six different molecular platforms, including array-based somatic copy number analysis, whole-exome sequencing, array-based DNA methylation profiling, messenger RNA sequencing, microRNA sequencing, and reverse-phase protein array. No survival or racial differences were found among patients from each subgroup^[3]. As mentioned before, there were four main subtypes discovered, which can roughly be categorized in the following groups.

Subtypes not inherently immunogenic

The following two subtypes are less likely to respond to immunotherapeutic strategies *per se*. Rather, combination approaches are probably required in order to attain a response using immunotherapy, such as adding chemotherapy to checkpoint inhibition or dual checkpoint inhibition. However, in cases with marked T-cells infiltration, we might expect that the checkpoints are probably up-regulated, and thus immunotherapy might still work. Apart from immunotherapy, targeted therapy with tyrosine kinase inhibitors (TKI) may prove to be another option in select subgroups of patients that carry specific driver mutations.

Chromosomal instability (50% of samples): The majority of the tumors analyzed in the project have fallen in this category. This subtype is found more frequently in the gastroesophageal junction (GEJ)/cardia (65%), is of intestinal histology, and affects mainly older (> 70 yo) individuals^[7]. Genetically, it is characterized by marked aneuploidy and high frequency of *TP53* mutations (73%). Consequently, it features a high number of focal amplification of receptor tyrosine kinases, most importantly *VEGFA*, *EGFR* (10%), *ERBB2* (24%), *ERBB3* (8%), and *c-Met* (8%) as well as amplification of genes encoding cell cycle mediators, such as *CCNE1*, *CCND1*,

Table 1 Molecular subtypes of gastric cancer according to the Cancer Genome Atlas and Asian Cancer Research Group

Molecular subtypes of gastric cancer	
TCGA	ACRG
CIN (50%)	MSS/TP53- (35.7%)
MSI-H (21%)	MSS/TP53+ (26.3%)
GS (20%)	MSI-H (22.7%)
EBV + (9%)	MSS-EMT (15.3%)

TCGA: Cancer Genome Atlas; ACRG: Asian Cancer Research Group; CIN: Chromosomal instability; MSI-H: Microsatellite-high; GS: Genomically stable; EBV: Epstein-Barr virus; MSS: Microsatellite stable; TP53: Tumor protein p53; EMT: Epithelial-mesenchymal transition.

and *CDK6*^[8]. These genetic aberrations contribute to making it the ideal candidate for application of targeted treatment, especially TKI inhibitors and monoclonal antibodies^[9].

Genomically stable (20% of samples): The trademark characteristics of this subtype are diploidy and somatic mutations in *CDH1* (37%), which is also the gene that is mutated in hereditary diffuse GC syndrome^[10]. Further common genetic aberrations are either *RHOA* mutations or *CLDN18-ARHGAP* rearrangements, both discovered in approximately 30% of tumors and usually mutually exclusive. All those mutations lead to disrupted intercellular cohesion and enhanced invasiveness, thus it is no surprise that most (73%) of these tumors belong to the diffuse histological variant. Most patients are of younger age (median 59 years), and there is no gender predominance^[3]. The inherent relative lack of immunogenicity and targetable driver mutations may lead to increased difficulty in applying individualized treatment in this subtype. Perhaps this is the single molecular subtype in TCGA classification where classic cytotoxic chemotherapy will continue to retain the primary role in treatment.

Highly immunogenic subtypes

The other two subtypes are characterized by extensive infiltration of PD-L1(+) immune cells, which are dispersed throughout the tumor instead of being located in the invasive margin, as is common with other malignancies^[11]. It is speculated that the patients who exhibit response to checkpoint inhibitors will belong to this particular subgroup, although this has not yet been proven^[12].

Microsatellite-high (21% of samples): The second most common subtype in the TCGA classification is characterized by extensive DNA methylation and multiple somatic mutations. These types of tumors are diagnosed at an older age (median age 72 years), with a slightly higher preponderance in female patients (56%). The various and dispersed mutations across the genome are mostly a consequence of *MLH1* promoter hypermethylation. Other important genes, with pote-

ntially targetable products, which are found mutated, are *PIK3CA*, *EGFR*, *ERBB2*, and *ERBB3*^[3].

The extensively mutated genetic material of these tumors creates an opportunity for immune system-oriented strategies. Indeed, the high amount of neoantigens, often presented in MSI-high tumors, elicit an immune response, manifested through extensive PD-L1 expression, which in this subtype reaches 33% and 45% on tumor and immune cells, respectively^[13,14].

Epstein-Barr virus-positive (9% of samples):

This subtype, whose main characteristic is the high Epstein-Barr virus (EBV) burden, was found to occur predominantly in the gastric fundus or body (62%), and is more common in men (81%). In TCGA, a recurrent amplification of 9p24.1 genetic locus is described, which is the site of genes *JAK2*, *CD274*, and *PDCD1LG2*. The first accounts for the aberrant activation of the JAK-STAT pathway, while the latter two encode PD-L1 and PD-L2, respectively. The 9p amplifications are found in at least 15% of EBV (+) tumors and lead to enhanced neoepitope presentation. It is also characterized by extreme DNA hypermethylation, most notably of the *CDKN2A* promoter, which leads to complete lack of p16 (p16INK4A) protein. It also features recurrent *PIK3CA* (80%), *ARID1A* (55%), and *BCOR* (23%) mutations^[3]. These molecular alterations characterizing this particular subtype hint at the therapeutic potential of JAK inhibition, *PI3K/MTOR* inhibition and immunotherapeutic approaches.

MOLECULAR SUBTYPES OF GC ACCORDING TO ACRG

The ACRG analyzed 300 GC samples using gene expression, genome-wide copy number microarray and targeted sequencing. Partially overlapping with the TCGA classification and sharing some similarities but also exhibiting enough differences to be categorized as a completely distinct classification, four molecular subtypes are described. In this case, the foundations of this molecular classification are based on the basis of MSI status, *TP53* function, and epithelial-mesenchymal transition (EMT). In this classification the subtypes were associated with relevant clinical outcomes and revealed survival differences that were validated in independent cohorts^[6].

The basis on which the first division took place was the loss of function of genes involved in the mismatch repair (MMR) system, thus distinguishing the MSI subtype. Then, the remaining tumors were divided depending on alterations in cell adhesion, angiogenesis, and motility, thus forming the MSS/EMT subtype. The rest were divided in two subtypes, depending on the loss of function of *TP53*, namely the microsatellite stable/*TP53* intact (MSS/*TP53*+) and microsatellite stable/*TP53* loss (MSS/*TP53*-) subtypes. Among these subtypes, the MSI showed the best overall prognosis, followed by

MSS/TP53+, MSS/TP53-, and MSS/EMT^[6]. More extensively, the molecular subtypes and their main specific characteristics are:

Microsatellite stable/TP53 loss (35.7% of samples)

This subtype is characterized by the highest rate of TP53 mutations (60%). Also, it features a greater aneuploidy and recurrent focal amplifications in *MDM2*, *ROBO2*, *GATA6*, *MYC*, *ERBB2*, *EGFR*, *CCNE1*, and *CCND1*^[6].

Microsatellite stable/TP53 intact (26.3% of samples)

Compared to the rest, this subtype is characterized by a higher prevalence of EBV infection. In addition to exhibiting an active TP53 pathway, it is associated with APC, *ARID1A*, *KRAS*, *PI3KCA*, and *SMAD4* mutations^[6].

Microsatellite-high (22.7% of samples)

This subtype occurred frequently in the antrum (75%), was mostly (> 60%) of intestinal-type histology, and was diagnosed more frequently at early stages (I or II), thus exhibiting the best overall survival. Genetically, it was associated with the presence of hypermutation, especially in genes encoding *KRAS* (23.3%), the PI3K-PTEN-mTOR pathway (42%), *ARID1A* (44.2%), *ERBB2* (16.3%), *ERBB3* (14%), and *ALK* (16.3%)^[6].

Microsatellite stable/epithelial-mesenchymal transition (15.3% of samples)

This subtype was associated with diffuse type histology, as it was expected considering that it features aberrations in genes responsible for cell adhesion and motility. It presents at a significantly younger age with most of the patients diagnosed at advanced stages (III/IV). Consequently, it carries the worst overall prognosis and a higher chance of recurrence. It is also characterized by higher rates of peritoneal spread, which can also be attributed to the above mentioned genetic changes^[6,15].

Comparison between TCGA and ACRG classifications

It is evident that, when comparing the two classifications, certain similarities exist between the different subtypes. Apart from the obvious association between the MSI subtypes in both classifications, it can be argued that roughly the equivalent of the genomically stable (GS) subtype in the ACRG classification is the microsatellite stable/epithelial-mesenchymal transition (MSS/EMT) subtype, while analogies exist between the EBV and chromosomal instability (CIN) subtypes on one hand, and MSS/TP53+ and MSS/TP53- on the other, respectively^[14]. However, as has been stated previously, there are certain major differences. For instance, while in the TCGA classification, EBV is a distinct subtype; ACRG EBV-infected tumors represent a part of the spectrum of the wider MSS/TP53+ subtype, which, moreover, is not characterized by hypermethylation or hypermutation. Another important difference is th-

at in ACRG classification, *CDH1* and *RHOA* mutations did not occur as frequently in the MSS/EMT as in its approximately equivalent GS subtype^[14]. It can be argued that these differences, among others, point also to the genetic heterogeneity of GC between different populations of different ethnic backgrounds, suggesting potentially different pathogenetic mechanisms for this disease in different parts of the globe.

CLINICAL TRIALS FOCUSING ON MOLECULAR AND IMMUNE BIOMARKERS

Targeting molecular pathways

HER2 inhibition: HER2 protein in GC is overexpressed mainly as a result of gene amplification. Its overexpression results in increased cell proliferation via its main target pathways, namely PI3K/Akt/mTOR and the RAS/MAPK^[16]. Consequently, its blockade may potentially halt tumor progression, at least temporarily, until an alternative pathway is switched-on driving resistance.

HER2 amplification is mainly a characteristic of GEJ tumors (15%-32%) rather than distal ones (10%-15%)^[14]. Also, the exact location of the protein in the cell differs, depending on the level of differentiation of the tumor. Well-differentiated tumors express the protein in the cell surface, whereas it is located mainly in the cytoplasm in poorly differentiated cancer cells^[17]. HER2 targeting has been implemented in various lines of therapy, with both monoclonal antibodies and TKIs with variable success (Table 2).

Trastuzumab, a chimeric monoclonal antibody targeting the domain IV of HER2, has gained approval in first-line therapy when combined with fluoropyrimidine/cisplatin chemotherapy doublet, after the positive results of the phase III ToGA trial. A subset analysis of this trial has indicated that the provided survival benefit is narrowed only to the group of patients where HER2 is clearly overexpressed, as manifested by combined immunohistochemistry (IHC) (+2) and fluorescent *in situ* hybridization (FISH) positivity, or IHC (+3) positivity. As a result, Trastuzumab should be administered to a specific subset of patients fulfilling the criteria mentioned above^[18].

In an attempt to replicate the positive results of CL-EOPATRA, where another HER2-targeting monoclonal antibody Pertuzumab gained approval in the treatment of advanced breast cancer, the phase III JACOB trial was initiated. In this trial, Pertuzumab was combined with chemotherapy doublet and Trastuzumab in stage IV treatment-naïve GC patients. Although the mOS was numerically superior in the Pertuzumab arm by 3.3 mo, with a 16% reduction in the risk of death, the trial missed statistical significance only just barely ($P = 0.0565$). Furthermore, as opposed to the ToGA trial, the majority of subgroups were consistent with the overall analysis. The combination therapy also resulted in more

Table 2 Main targeted agents evaluated in metastatic gastric cancer

Biologic target	Targeted agent	Name/type of trial	Line of therapy	Study arms	Results	Ref.
c-MET	Rilutumumab	RILOMET-1 Phase III	1 st	ECX + Ril	Negative effect	[58]
		EXPAND Phase III	1 st	XP ± Cet	No benefit	[48]
EGFR	Cetuximab	AIO Phase II	1 st	FOLFOX + Cet	> 4 <i>EGFR</i> gene copies: Increased OS (log-rank $P = 0.011$; HR = 0.2, 95% CI: 0-0.8; $P = 0.022$)	[50]
	Panitumumab	REAL-3 Phase III	1 st	EOX ± Pani	No benefit	[49]
	Trastuzumab	ToGA Phase III	1 st	XP/FP ± H	OS: 13.8 <i>vs</i> 11.1, $P = 0.0046$ OS (IHC+3, IHC+2/FISH+): 16 mo <i>vs</i> 11.8 mo, $P = 0.0036$	[18]
	Pertuzumab	JACOB Phase III	1 st	FP + H ± Pert	No benefit	[19]
HER-2	Lapatinib	TyTan Phase III	2 nd	Pac w ± Lap	No benefit (unselected population) OS (IHC: 3+): 14 mo <i>vs</i> 7.6 mo, $P = 0.0176$	[21]
	Trastuzumab emtansine	GATSBY Phase II-III	2 nd	TDM-1 <i>vs</i> taxane	No superiority	[22]
mTOR	Everolimus	GRANITE-1 Phase III	2 nd , 3 rd	Everolimus <i>vs</i> placebo	No benefit	[55]
	Bevacizumab	AVAGAST Phase III	1 st	XP ± Bev	Primary endpoint (OS) was not met PFS: 6.7 mo <i>vs</i> 5.3 mo, $P = 0.0037$ ORR: 46% <i>vs</i> 37.4%, $P = 0.0315$	[25]
VEGF, VEGFR	Ramucirumab	REGARD Phase III	2 nd	Ram <i>vs</i> placebo	OS: 5.2 mo <i>vs</i> 3.8 mo, $P = 0.047$	[26]
		RAINBOW Phase III	2 nd	Pac w ± Ram	OS: 9.6 mo <i>vs</i> 7.4 mo, $P = 0.017$	[27]
		Phase II	1 st	FOLFOX ± Ram	No benefit	[28]
	Apatinib	Phase III	beyond 2 nd line	Apa <i>vs</i> placebo	OS: 6.5 mo <i>vs</i> 4.7 mo, $P = 0.0149$ PFS: 2.6 mo <i>vs</i> 1.8, mo, $P < 0.001$	[30]

ECX: Epirubicin-Cisplatin-Capecitabine; Ril: Rilotumumab; XP: Cisplatin-Capecitabine; Cet: Cetuximab; EOX: Epirubicin - Oxaliplatin - Capecitabine; Pani: Panitumumab; FP: Cisplatin - 5Fu; H: Herceptin; Pert: Pertuzumab; Pac w: Paclitaxel weekly; Lap: Lapatinib; TDM-1: Trastuzumab emtansine; Bev: Bevacizumab; Ram: Ramucirumab; Apa: Apatinib; OS: Overall survival; PFS: Progression free survival; ORR: Overall response rate.

incidents of diarrhea and hypokalemia^[19].

Another attempt at HER2 inhibition in first line was the phase III TRIO-013/LOGIC trial, where, in a selected population of HER2 positive patients, the addition of Lapatinib, a small intracellular TKI of ERBB1 and ERBB2, was evaluated on whether it would improve the survival benefit derived by Oxaliplatin/Capecitabine doublet chemotherapy. Unfortunately, the trial failed to demonstrate a statistically significant survival benefit. However, it did raise the question of the accuracy of the current method of appreciating HER2 positivity, since the observed clinical benefit closely correlated with the degree of gene amplification as well as with HER2 protein levels, implying that implementing a different scoring system where HER2 over-expressing tumors are defined by an IHC score of more than 3 (IHC) or 2 (FISH) values, may be more precise^[20].

Lapatinib was also evaluated in the second line in the phase III Asian TyTAN trial, where it was added to weekly Paclitaxel. It is interesting to note that the trial was performed in an unselected population, with 31% demonstrating weak (IHC: 1+) or none at all HER2 positivity. No survival benefit was noted in the study

population, although in the subgroup with strong HER2 positivity (IHC: 3+), median survival improved to 14 mo *vs* 7.6 mo ($P = 0.0176$)^[21].

Another negative phase III trial compared a monoclonal antibody used in HER2(+) breast cancer, Trastuzumab Emtansine (TDM-1), and taxane monotherapy in HER2(+) patients (GATSBY trial). However, as in the TyTAN trial, HER2 expression was evaluated in archived samples, not taking into account the clonal heterogeneity and the possibility of tumoral evolution that may have occurred from the first to second line chemotherapy setting^[22].

An attractive hypothesis regarding the etiology of the negative results of the above mentioned trials, apart from using archival samples, is the downregulation of HER2(+) tumors as a result of our targeting the HER2 protein in the first line setting. It is possible that HER2-directed therapies should be implemented preferably in the beginning of the treatment algorithm, with continuation or switch to another HER2 targeting agent, beyond progression, remaining an option for the select few who retain HER2 positivity. However, this is currently hypothesis-generating and should be confir-

med within a clinical trial.

Inhibition of angiogenesis: Neoangiogenesis has an established role in GC pathogenesis, mainly through vascular endothelial growth factor (VEGF)/VEGFR2 signaling, as there is evidence that VEGF serum levels correlate with increased stage and worse prognosis^[23]. In animal models, VEGFR2 inhibition led to angiogenesis impairment and tumor regression^[24].

Based on these data, targeting this pathway, either the receptor or the ligand, with monoclonal antibodies and TKIs has been studied in various clinical trials. In this case, targeting VEGFA with Bevacizumab in combination with traditional chemotherapy in first line has not provided a substantial survival benefit in a phase III trial, although results showed a significant improvement in progression free survival (PFS) (6.7 mo vs 5.3 mo) and overall response rate (46% vs 37.4%)^[25].

On the contrary, targeting the receptor has been more effective. In the phase III REGARD trial, Ramucirumab, a monoclonal antibody blocking VEGFR2 demonstrated superior survival over placebo in second line^[26]. Also, the same drug, when combined with a taxane in second line, also led to a statistically significant survival benefit of 2.2 mo^[27]. The attempt to expand the use of Ramucirumab in first line in combination with FOLFOX in a phase II trial did not produce the required results^[28]. However, there is another ongoing phase III trial of Ramucirumab combined with Cisplatin and a fluoropyrimidine in HER2 negative patients in first line (RAINFALL; NCT02314117) that may clarify its efficacy in this setting^[29].

Inhibiting angiogenesis with TKIs also has a role in the management of advanced GC. Apatinib, a multitikinase inhibitor mainly targeting VEGFR2, significantly improved OS over placebo in a phase III trial in patients with heavily pretreated advanced GC, which led to its regulatory approval as monotherapy beyond second line^[30]. Also, Regorafenib, another multitikinase inhibitor targeting, among others, VEGFR2, is currently being tested in the same setting in a phase III trial after successfully achieving its primary endpoint of superior PFS in a relevant phase II trial^[31,32]. Sorafenib resulted in disease stabilization and moderately good PFS in chemo-refractory patients in first- and second-line, but its addition to chemotherapy did not provide adequately encouraging results to justify a phase III trial^[33-36]. Therefore, it appears that inhibition of angiogenesis has a definite role in advanced GC. Still, there are only hints regarding the potential predictive biomarkers that would help in individualizing its use. For instance, the two less immunogenic subtypes in the TCGA classification, namely the CIN and GS, were associated with VEGFA gene amplification and elevated expression of angiogenesis-related pathways, respectively, providing some clues regarding the importance of angiogenic pathways as a driving force of progression in tumors with these molecular signatures^[14]. It must also be noted that the positive results with angiogenesis inhibition have

been produced in the later lines of treatment, which may imply that in the early stages of GC progression, angiogenesis has a less substantial role, while it is more predominant in later stages of the natural course of the disease. Lastly, it is important to note that targeting the receptor rather than the ligand seems to be the appropriate strategy, a phenomenon for which we have not yet reached a clear and robust explanation but may prove crucial for future anti-angiogenic strategies.

EGFR inhibition: Epidermal growth factor receptor (EGFR) or Erb-B1 is a transmembrane receptor found overexpressed in 30% of GC, while the *EGFR* gene is amplified in nearly 5%^[37]. Increased EGFR signaling has been correlated with higher stage, poorly differentiated tumors, and increased invasiveness^[38-40]. In preclinical models, Cetuximab, a chimeric anti-EGFR antibody, induces antibody-dependent cell-mediated cytotoxicity (ADCC)^[41]. Phase II trials with Cetuximab, Panitumumab, or Erlotinib combined with cytotoxics have yielded responses ranging between 41% and 65%, while second line Gefitinib or Erlotinib monotherapy has provided less impressive results, with responses between 9% and 11%, limited mostly to proximal GC^[42-47].

These data have prompted testing of anti-EGFR targeting in phase III trials. However, both EXPAND and REAL3 phase III trials testing Cetuximab and Panitumumab in combination with Cisplatin-Capecitabine and EOX, respectively, did not show any PFS or OS benefit. Again, this may be attributed to poor patient selection, since the study population was not evaluated for EGFR expression or gene amplification^[48,49]. The potential importance of this parameter has been made clear in at least two studies: in the phase II study combining FOLFOX with Cetuximab, where the patients that exhibited greater than four *EGFR* gene copies demonstrated increased OS, and also in the TRANS-COG, where the subset of EGFR-amplified patients derived a statistically significant survival benefit with the addition of Gefitinib (HR = 0.19; *P* = 0.007)^[50,51].

This appears to have been taken into account in a phase III trial of second-line Nimotuzumab with Irinotecan (NCT01813253), which is currently recruiting patients that harbor EGFR-overexpressing (IHC: +2/3) tumors^[52].

PI3K/Akt/mTOR inhibition: Resistance to targeted therapies often appears as a result of activation of downstream effectors by alternative molecular pathways. The PI3K/Akt/mTOR pathway in GC may become constitutively activated either through mutations in the *PI3K* gene, which occurs most often in EBV(+) and MSI tumors, or through inactivation of *PTEN* gene, the main negative regulator of the pathway, which is mostly found in the MSI subtype^[3,53].

Targeting this pathway with an mTOR inhibitor, Everolimus, has produced encouraging results in a phase II trial, producing a median PFS of 2.7 mo and OS of 10.1 mo^[54]. However, the phase III GRANITE-1 trial

that compared Everolimus to placebo in an unselected patient population, as second- or third-line therapy, failed to demonstrate any survival benefit. Once again, the study population was unselected for PI3K pathway activation^[55]. Impairment of *Akt* function *via* allosteric inhibition in a phase II study of the small molecule MK-2206, in unselected patients, did not produce any positive results either^[56].

The above findings, rather than just annulling the findings of basic science, may be viewed as a further indication for the need of appropriate patient selection. PI3K/Akt/mTOR inhibition may still have a role where activation of this pathway is indeed the driver of cancer progression.

MET inhibition: The *MET* proto-oncogene encodes the c-MET receptor tyrosine kinase that has a crucial role in cell proliferation, angiogenesis, and migration. Its canonical activation pathway is *via* binding of its ligand, hepatocyte growth factor (HGF), but the activation can result independently of the binding through gene amplification or somatic mutation. The *MET* gene has been found amplified in 4%-10% of GC, while its protein product has been found overexpressed by IHC in up to 70%^[57]. The implications of this deviation between gene amplification and protein overexpression have been made evident in the *MET*-targeted clinical trials.

All phase II and III trials that included patients based on *MET* overexpression *via* IHC provided negative results. A probable explanation is the vague definition of *MET* positivity by IHC. In the phase III RILOMET study, the addition of Rilotumumab, an HGF-targeting monoclonal antibody, to triplet chemotherapy (ECX) proved detrimental. The study was terminated prematurely because of increased risk of death in the investigational arm^[58]. The main targeted agents evaluated in various clinical settings in GC are presented in Table 2.

Targeting cancer stemness

A possible way in which tumors survive complete elimination from cytotoxic chemotherapy is the presence of cancer stem cells. Cancer “stemness” is frequently manifested through the activation of the *STAT3* pathway, which induces the transcription of *Nanog* and *Myc* genes. The rationale for investigating this pathway in GC after failure of previous therapies in a large phase III trial (BRIGHTER) was provided by encouraging response and disease control data from phase I and II trials, where the small molecule BBI608 (Napabucasin) was combined with Paclitaxel. This trial is ongoing, however, interim analysis indicated diminished possibility of achieving the primary endpoint of OS^[59,60].

Targeting DNA damage repair pathway

Poly (ADP-ribose) polymerase (PARP) is essential in correcting single-strand DNA breaks induced by cytotoxic agents. Inhibition of PARP has provided significant benefit in the subgroup of patients with breast and

ovarian cancer that already exhibit a certain level of defect in the DNA repair mechanism, such as loss of function of *BRCA1/2* genes. Since *BRCA1/2* mutations in GC are rare, this strategy was implemented in tumors that are characterized by other defects in the repair pathway, like in the *ATM* gene, a quality termed “*BRCAness*”^[61,62]. Preclinical and early clinical trials on tumors with *ATM* deficiency and *TP53* mutations were completed with significant success^[63]. However, the phase III GOLD trial failed to reveal a statistically significant, according to predetermined criteria, survival benefit in patients treated with Olaparib and Paclitaxel. This failure might once again be attributed to poor patient selection, since the study population was not selected based on *TP53* mutations, while furthermore only 18% of patients were *ATM* negative^[64].

Targeting the tumor microenvironment

Andecaliximab, previously known as GS-5745, is a monoclonal antibody that targets matrix metalloproteinase (MMP) 9, an extracellular enzyme involved in matrix remodeling, angiogenesis, tumor growth, and metastasis. Encouraging results from the phase I study, where it was combined with FOLFOX in patients both treatment naive and pretreated, have secured its evaluation in a phase III trial (NCT02545504), where it is tested in first line in the same combination. The trial has completed accrual, and results are awaited. It is important to note that this strategy, if successful, has the potential to be implemented in a wide spectrum of patients with GC, without the need for a predictive biomarker. Also, since MMP inhibition affects the collagenous stroma of the tumor, not only will it clear the path for the chemotherapy drugs to reach cancer cells, but also it will enhance tumor immunogenicity, with obvious implications for a potential combination with immunotherapy^[65].

Manipulating immune responses

Immunotherapy, mainly through the form of checkpoint inhibitors, has over the last few years been added to the armamentarium of various cancer therapeutic approaches, with serial approvals for the treatment of a wide spectrum of solid and hematologic malignancies. Unfortunately, the only single predictive biomarker we currently have at our disposal is PD-L1, which is far from being the most efficient in the field. Indeed, patients without PD-L1 expression can still respond, while others who express the biomarker do not derive benefit. In GC, contrary to melanoma or lung cancer, PD-L1 is expressed mostly in myeloid-derived immune cells and not in tumor cells^[61]. The presence of MSI, as manifested through IHC or polymerase chain reaction (PCR), is considered predictive for response to immunotherapy, while other approaches, such as IFN- γ signature and immunoscore, have not yet been incorporated to clinical practice.

There is adequate evidence supporting the implementation of immunotherapy in GC management, both

preclinical and clinical. Firstly, there seems to be an association between PD-L1 and disease burden and, consequently, to limited survival^[66]. In addition, according to the data from TCGA, as previously mentioned, elevated PD-L1 expression has been noted in the EBV(+) GC subtype, which correlates with the significant amount of the neoantigens produced as an effect of viral infection, as well as of amplification of 9p24^[3]. Furthermore, it is well established that MSI-high tumors also mount a robust immune response, which predicts for clinical outcome and benefit of immune checkpoint blockade^[67-69]. Clinical trials thus far have focused on checkpoint inhibitors, especially anti-PD-1/anti-PD-L1 and anti-CTLA4 antibodies, with the best results having been produced by the former.

The first trial to test an anti-PD1 inhibitor in advanced disease was the Keynote-12, where the safety and activity of Pembrolizumab in this setting was assessed. Only patients with PD-L1 positive tumors were enrolled. PD-L1 positivity was deemed as membrane staining in $\geq 1\%$ of cells, or alternatively as the presence of a distinctive PD-L1 positive pattern at the interface between neoplastic cells and their adjacent stroma. In this trial, no association between PD-L1 levels and response was observed. The results were similar to other trials of anti-PD-1 in various solid malignancies, with a response rate of 22% (95%CI: 10-39) and manageable toxicity profile, prompting the initiation of two large phase III trials^[70]. The Keynote-061 is evaluating Pembrolizumab vs Paclitaxel in the second line^[71]. In the first-line setting, Keynote-062 has three arms comparing pembrolizumab as monotherapy and platinum/5-FU combination with or without pembrolizumab^[72]. Finally, following the most recent trend of combining immunotherapy with targeted therapies or chemotherapy, two multicenter phase IB/II studies are ongoing, determining activity and safety of Pembrolizumab in combination with anti-HER2 agents in patients with HER2 positive GC (NCT02901301 and NCT02689284)^[73,74]. Their results are eagerly awaited.

Continuing with PD-1/PD-L1 inhibition, Nivolumab, another anti-PD-1 agent, was the first to gain approval in the third line setting, following the positive results of the pivotal phase III trial ONO-4538/BMS-936558 (ATTRACTION 2). This trial, which employed an all-Asian study population, showed a statistically significant, albeit numerically small, survival benefit for Nivolumab over placebo in heavily pretreated patients with advanced/metastatic GC or GEJC. Median OS was 5.3 mo vs 4.1 mo (HR = 0.63, $P < 0.0001$,) and mPFS was 1.61 mo vs 1.45 mo (HR = 0.60, $P < 0.0001$) in the Nivolumab ($n = 330$) and placebo arms ($n = 163$), respectively^[75]. This resulted in the Food and Drug Administration (FDA) approval of Nivolumab for GC or GEJC, in third line or beyond, irrespective of PD-L1 expression.

Finally, in the field of PD-1/PD-L1 axis inhibition, another promising agent is the anti-PD-L1 Avelumab, which has provided promising clinical activity in unselected patients, treated as first-line maintenance or second-line after progression, in the phase Ib trial JAVELIN. In this

trial, patients were randomized after treatment with a first-line chemotherapy-based regimen by progression status: patients achieving disease control received Avelumab as switch maintenance, while those with progressive disease received the drug as second line. An acceptable safety profile, which was the primary endpoint of the trial, was demonstrated. Overall response rate was 9.0% and 9.7% in the two subgroups, respectively^[76]. Following these positive results, two randomized phase III trials were developed: JAVELIN Gastric 100, testing Avelumab as switch maintenance in the first line setting, and JAVELIN Gastric 300, in the third line^[77,78]. Unfortunately, it was recently announced that JAVELIN Gastric 300, comparing single-agent Avelumab with physician's choice of chemotherapy, did not meet its primary endpoint of superior overall survival. The other phase III trial is still ongoing.

Less encouraging has been the use of anti-CTLA4 inhibitors. Firstly, regarding Ipilimumab, the Phase II trial (NCT01585987) that compared the drug to placebo in the second line was stopped prematurely when it became evident that the final analysis would procure no PFS benefit^[79]. Also, no responses were reported with Tremelimumab, another anti-CTLA-4 inhibitor in the same setting^[80]. It should also be noted that higher toxicity was observed in these trials, as compared to anti-PD-1/PD-L1 blockade. These differences might be attributed to the different targeting of these two classes of checkpoint inhibitors. While those targeting the PD-1 axis have an immediate effect in the tumor microenvironment, the anti-CTLA-4 modulates the immune response mainly in the lymph nodes.

In an attempt to enhance the activity of anti-CTLA-4 agents, combination treatment with anti-PD-1 was tested. The CheckMate-32 was a phase I/II trial with three arms: 160 pretreated patients were randomized to receive either Nivolumab monotherapy in the dose of 3 mg/kg, or Nivolumab plus Ipilimumab in the doses of 3-1 mg/kg in the second arm or 1-3 mg/kg in the third arm of the study. In all three arms, notable responses were observed, with an overall disease-control rate of 38%. The responses differed between PD-L1-positive ($\geq 1\%$) and PD-L1-negative ($< 1\%$) tumors, reaching 27% and 12%, respectively. The highest overall response rate (26%) and overall survival (6.9 mo) were observed in arm 3 (Nivolumab 1 mg/kg and Ipilimumab 3 mg/kg), which prompted the launch of a phase III trial^[81]. The ongoing CheckMate-649 investigates Nivolumab plus Ipilimumab vs FOLFOX/XELOX in the first line, and a subgroup analysis regarding PD-L1 expression has already been planned^[82].

Conclusively, immunotherapy could have a role in GC management, although, as in the management of other cancers, better predictive biomarkers are required. Moreover, it remains to be seen whether there is rationale for combining immunotherapy with targeted therapies and/or chemotherapy.

CONCLUSION

Even though most clinical trials investigating targeted agents have not produced the desired results so far, their failures might be attributed mostly to erroneous study planning and unscrupulous patient selection. The value of recognizing distinct molecular cancerous pathways goes far beyond mere classification purposes, and shall be better appreciated when these results could be applied in everyday practice with the purpose of providing clinically meaningful outcomes for our patients. Unfortunately, it is still unclear whether the clinical benefits of implementing next-generation sequencing and targeted therapies in the clinic will outweigh the economic burden of such a practice. Perhaps a way to tackle this issue is to create a panel of the main molecular and immune signatures of implemented pathways in order to categorize appropriately the patients in distinct prognostic and predictive subgroups. The results of the TCGA and ACRG classifications, among others, may provide the basis of such a molecular/immune signature panel that remains to be validated prospectively in large clinical trials providing the basis for rational stratification and design.

Health economics concerns aside, if our goal is to optimize outcomes for our GC patients, we probably need to implement these new molecular signatures in our daily practice. Due to the complexity of the disease, the constant evolution of tumors, and our still limited understanding of its biology, our mission to provide the best therapy to our patients is extremely difficult and challenging. However, through targeting tumorigenic drivers and awakening the immune system through immune-oriented strategies, it might be possible that we will at least be able to achieve the goal of life prolongation, while, at the same time, effectively alleviate cancer-related symptoms. A potential, hopefully not overly idealized, glimpse to the future of managing this disease, entails its multidisciplinary management by a variety of experts from diverse scientific backgrounds, towards an individualized approach for each unique patient.

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Prediction of malignancy and adverse outcome of solid pseudopapillary tumor of the pancreas

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Abstract

Since solid pseudopapillary tumor of the pancreas (SPTP) was officially classified by the World Health Organization in 1996, SPTP has recently received special attention in the literature. Studies have shown that SPTP is a heterogeneous tumor, with a small percentage of patients harboring aggressive behaviors. However, criteria for malignancy grade in SPTP have not been well established. The prognosis of SPTP is generally good, with cases having a chance for long-term survival even with recurrence and/or metastasis after surgical resection. The current American Joint Committee on Cancer/Union for International Cancer Control tumor, node, metastasis staging system is not specific to SPTP. The lack of a predictive staging classification that accurately describes the heterogeneity of this disease hinders meaningful research into optimal individualized therapy. Here we summarize and discuss the associated factors proposed for appraisal of the malignant potential and adverse outcome of SPTP.

Key words: Pancreas; Recurrence; Solid pseudopapillary tumor; Malignancy; Metastasis

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Core tip: Solid pseudopapillary tumor of the pancreas (SPTP) is a heterogeneous tumor, with a small percentage of patients harboring aggressive behaviors. Its prognosis is generally good, with cases having a chance for long-term survival even with recurrence and/or metastasis after surgical resection. The lack of a predictive staging classification that accurately describes the heterogeneity of this disease hinders meaningful research into optimal individualized therapy. Here we summarize and discuss the associated factors proposed for appraisal of the malignant potential and adverse outcome of SPTP.

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INTRODUCTION

Since solid pseudopapillary tumor of the pancreas (SPTP) was officially classified by the World Health Organization (WHO) in 1996, SPTP has been accepted worldwide. It had also been called Frantz tumor, papillary cystic tumor/neoplasm/carcinoma, solid and papillary neoplasm, solid and papillary epithelial neoplasm, solid and cystic tumor, and solid and cystic papillary epithelial neoplasm. Most of the tumors are found in young women in their second or third decade while it is rare in male patients, accounting for 12.05% of all cases. More than half of the patients are under the age of 25 years^[1]. Occasionally, it occurs in children^[2]. There was no significant difference in age between male and female patients. Approximately one-third of patients were asymptomatic, with the tumors incidentally discovered during physical examination or in work-up for unrelated diseases^[1]. Although several genetic alterations such as somatic mutations in exon 3 of CTNNB1, and upregulated genes activated in Wnt/ β -catenin, Hedgehog, and androgen receptor signaling pathways have been identified^[3-5], the tumorigenesis of SPTP is still not clear. The incidence of SPTP seems to be increasing, and study of this rare tumor is thus of clinical significance.

Previously, SPTP was mostly considered as a benign tumor, but not until the 2010 version of the WHO classification was issued, all SPTPs are considered as low-grade malignant tumors. Studies have shown that SPTP is a heterogeneous tumor, with a small percentage of patients harboring aggressive behaviors^[6-8]. Even if the tumor has no evidence of malignant potential, such as perineural invasion, vascular invasion, invasion of pancreatic parenchyma, and infiltration of peripancreatic tissue, it may metastasize to the liver or recur after surgery. Long-term survival can be achieved in SPTP patients with advanced or metastatic disease, which reveals that SPTP is a relatively indolent disease compared with other pancreatic carcinomas. It is difficult to elucidate the natural course of SPTP and to predict its malignancy and outcome after surgery due to limited follow-up studies. As such, SPTP remains a pancreatic surgical enigma and studies have failed to identify prognostic factors predicting its malignant behavior.

EPIDEMIOLOGIC TREND

The incidence of SPTP has increased markedly in recent years, possibly due to the ready use of modern imaging, diagnostic endoscopy, and physician awareness. Although epidemiologic trends have been documented for

pancreatic cystic lesions^[9-11], the true incidence and epidemiologic trend for SPTP are less clear. An understanding of its epidemiology has been hampered by the pervasive tendency to report the incidence along with other pancreatic tumors.

As the incidence of pancreatic tumors in China increases year by year^[12], the number of patients with pancreatic diseases admitted to Huashan Hospital affiliated to Fudan University, Shanghai, China has continued to grow, so has the number of surgical procedures performed during the last decade. The number of patients with SPTP also increased during these years, with an average of more than six patients with this disease having been confirmed each year. Literature related to SPTP and the number of patients reported have rapidly grown since 1996 (Figure 1). A total of 390 cases were described in a previous systematic review of SPTP cases reported in China between 1996 and 2006^[1]. Law *et al.*^[13] conducted a systematic review of English literature concerning SPTP published up to 2012, and identified 2744 cases of SPTP. A nationwide survey from South Korea showed that SPTP ranked as the third most common pancreatic cystic tumors (18.3%)^[14]. These figures indicate that SPTP is not uncommon now worldwide. Given the population trend and the paucity of studies available to guide management of patients with SPTP, further research is imperative.

NATURE HISTORY AND TUMOR BIOLOGY

The origin, biological behavior and nature history of SPTP are not fully understood until now, leaving it as an enigmatic entity. SPTP was regarded as a borderline malignant tumor initially due to lack of evidence-based demonstration of true benign tumor. The WHO used the term "low-grade malignant" instead of "benign" in 2010. SPTP has a wide variability of tumor features from completely solid to almost completely cystic. Imaging studies have shown that smaller SPTPs usually appear as completely or mostly solid, while larger SPTPs typically appear as a large well-encapsulated heterogeneous mass with varying solid-cystic components due to necrosis, hemorrhage and degeneration^[15]. A recent report revealed that evolution of liver metastasis from SPTP was relatively slow, with the metastatic lesions having a similar growth pattern of primary tumor characterized by a solid-cystic mass with pseudopapillary structures^[16].

Parallel to the controversy regarding its histogenetic derivation, assessment of the malignant potential of SPTP remained a major controversial issue for decades. Although SPTP is considered as a tumor of low-grade malignancy, patients with this disease occasionally present with invasion into the portal/splenic vein (Figure 2) and/or adjacent organs or liver metastasis, mimicking pancreatic ductal adenocarcinoma. The prognosis of SPTP is generally good, with cases having a chance for long-term survival even with recurrence and/or meta-

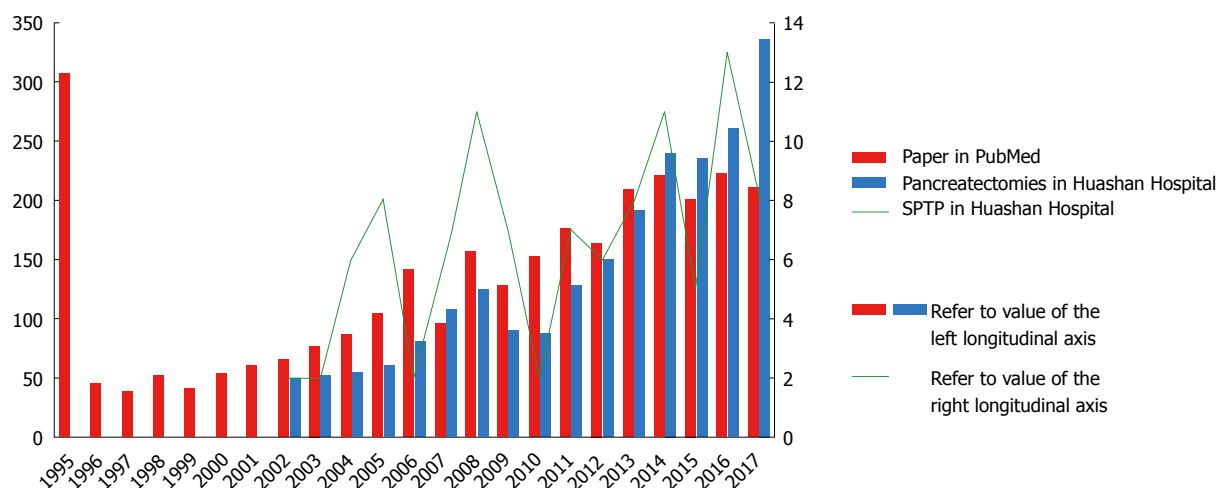


Figure 1 Publications concerning solid pseudopapillary tumor of the pancreas in PubMed, and number of pancreatectomies and patients undergoing surgery for solid pseudopapillary tumor of the pancreas in Huashan Hospital affiliated to Fudan University. Literature retrieved from PubMed (March 1, 2018) with the search terms “frantz tumor”, “solid and cystic papillary epithelial neoplasm”, “solid and cystic tumor”, “papillary cystic tumor”, “solid and papillary neoplasm”, “papillary cystic carcinoma”, “papillary and cystic tumor”, “papillary and solid neoplasm”, “solid and papillary epithelial neoplasm”, “papillary cystic neoplasm”, “solid pseudopapillary tumor”, “pancreas”, and “pancreatic” in “all fields”. SPTP: Solid pseudopapillary tumor of the pancreas.



Figure 2 Solid pseudopapillary tumor of the pancreas presenting with invasion into the portal splenic confluence. A: Enhanced computed tomography scan revealed intraluminal filling defect in the portal splenic confluence (arrow); B: An abnormal signal of the pancreatic head (arrow) and high signal foci in the right anterior lobe of the liver (yellow arrow) can be readily delineated from the coronal magnetic resonance imaging (MRI) section; C: Splenic vein tumor thrombus was noted by hematoxylin-eosin staining ($\times 100$).

stasis after surgical resection. Up to 10% of patients experienced a recurrence and/or metastasis of the disease after years of follow-up, and only a small subset of patients eventually died of this disease^[6-8,17-37] (Table 1).

DIAGNOSIS OF MALIGNANT SPTP

Studies showed that tumor markers such as carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) were usually within normal ranges in patients with this disease. Thus, routine tumor markers are of no value to predict malignant SPTP^[1]. Radiologically, SPTP typically appears as a well-capsulated heterogeneous mass with solid and cystic components, while small SPTP commonly represents a solid mass. Capsule and intratumoral hemorrhage are important clues to the diagnosis as they are rarely detected in other pancreatic neoplasms. In some cases, calcification may be present, whereas pancreatic duct dilatation is rarely found. Yang *et al.*^[16] reported that the liver metastatic lesions from

SPTP increased in sizes gradually with cystic change. The relatively slow evolution of liver metastasis indicates its classic growth pattern. Although the proportion of solid component^[38] and incomplete capsule^[39,40] were shown to be associated with malignancy by a few reports, no consistent results were demonstrated. Rastogi *et al.*^[41] reported that tumors with greater enhancement assessed by contrast-enhanced computed tomography (CT) had aggressive characteristics. However, no correlations between malignancy and other radiological features including calcification were found. These findings indicate that diagnosis of malignant SPTP is difficult with imaging studies. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has been shown to be useful for preoperative definite diagnosis^[42]. However, it may cause rupture of tumor and seeding of the needle tract by tumor cells during the procedure^[43,44]. Although EUS-FNA has been used more frequently than ever in SPTP^[13], its malignant nature is still difficult to confirm because of lack of specific markers.

Position emission tomography/computed tomogra-

Table 1 Reported series (> 20 cases) of solid pseudopapillary tumor of the pancreas in the English literature

Ref.	Country	Centers	F/M	Age (yr)	Size (cm)	Malignant, n (%)	Follow up (mo)	R/M (n)	Alive (n)
Peng <i>et al</i> ^[17] , 2006	China	Single	25/0	33 (11-65)	9.3 (2.5-25)	3 (12)	3-111	0	25
Yu <i>et al</i> ^[18] , 2007	China	Single	25/1	25.2 (13-57)	7.5 (3.8-15)	9 (34.6)	66 (10-237)	2	24
Machado <i>et al</i> ^[19] , 2008	Brazil	Single	27/7	23 (10-72)	7 (1.5-15)	13 (38.2)	84 (3-170)	2	33
Lee <i>et al</i> ^[20] , 2008	South Korea	Multi	57/5	30 (8-63)	6.5 (1.5-14)	9 (14.5)	47.5 (5.1-240.4)	2	62
Matos <i>et al</i> ^[21] , 2009	United States/ Germany	Multi	20/1	33 (13-60)	5.5 (2.5-19.3)	3 (14.3)	55 (7-176)	0	21
Nguyen <i>et al</i> ^[22] , 2011	Australia	Multi	30/4	33.3 (19.6-42.3)	6 (4.5-9)	9 (26.5)	70 (48-178)	2	32
Kim <i>et al</i> ^[23] , 2011	South Korea	Single	98/16	36 (11-75)	4.2 (1.2-15)	26 (22.8)	57 (11-177)	4	114
Butte <i>et al</i> ^[24] , 2011	United States	Single	38/7	38 (10-63)	4.9 (1.4-20)	9 (20)	44 (1-250)	5	38 ¹
Cai <i>et al</i> ^[25] , 2013	China	Single	30/3	29.2 (12-59)	4.9 (2-15)	17 (51.5)	45 (4-118)	1	32
El Nakeeb <i>et al</i> ^[26] , 2013	Egypt	Single	22/2	24.83 (12-52)	9.2 (3-25)	6 (25)	71.6 (1-180)	2	22
Raman <i>et al</i> ^[27] , 2013	United States	Single	43/8	29.3 (12.2-74.8)	5.3 (1.7-11.1)	11 (21.6)	37 (0-122)	1	50
Serrano <i>et al</i> ^[28] , 2014	Canada	Single	26/6	36 (13-64)	4.7 (1.5-14)	15 (46.9)	43 (3-207)	3	31
Suzuki <i>et al</i> ^[29] , 2014	Japan	Single	29/5	37.1 (15-68)	4.3 (1-11)	3 (8.8)	67 (3-326)	0	34
Kim <i>et al</i> ^[30] , 2014	South Korea	Single	85/21	36 (10-65)	4.5 (1-15)	17 (16)	56.9 (37-93.4)	2	105
Kang <i>et al</i> ^[6] , 2014	South Korea	Multi	317/34	36.8 ± 12.4	5.7 ± 3.3	98 (27.9)	> 6	9	316 ²
Estrella <i>et al</i> ^[7] , 2014	United States	Single	54/10	33 (9-62)	5 (1.4-20)	49 (76.6)	76 (2-203)	10	53 ³
Yu <i>et al</i> ^[31] , 2015	China	Multi	93/4	31.2 (16-57)	5.9 (1.5-14)	16 (16.5)	70.2 (3.5-221.5)	3	96
Zhang <i>et al</i> ^[32] , 2015	China	Single	56/6	26 (8-66)	7.2 (3-15)	3 (4.8)	46 (2-135)	0	62
Yang <i>et al</i> ^[8] , 2016	China	Single	58/13	31 (12-64)	5 (1-13)	13 (18.3)	45 (3-118)	3	70
Irtan <i>et al</i> ^[33] , 2016	France	Multi	41/10	13.1 (8.7-17.9)	7 (2-12)	22 (43.1)	65 (0.3-221)	7	51
Marchegiani <i>et al</i> ^[34] , 2016	Italy/United States	Multi	113/18	33 (7-68)	4 (0.7-20)	16 (12.2)	62 (12-304)	2	105 ⁴
Xu <i>et al</i> ^[35] , 2017	China	Single	93/28	33.7 (11-68)	5 (1-13)	35 (28.9)	42.7 (6-97)	3	100 ⁵
Song <i>et al</i> ^[36] , 2017	China	Single	46/7	35.4 (14-67)	6.4 (2-14)	10 (18.9)	48 (3-123)	2	45 ⁶
Lubezky <i>et al</i> ^[37] , 2017	Israel	Single	29/3	28.4 ± 12.2	5.9 (0.9-14)	13 (40.6)	49.2 (1-228)	4	31

Note: We included data from the latest or most complete study in the case of duplicate reports on overlapping patients from the same institutions; ¹Three patients died of SPTP, and four patients died of other causes; ²317 patients with more than 6 mo follow-up were reported for evaluation of oncologic outcome; ³Follow-up information was available for 59 patients; ⁴Follow-up information was available for 105 patients; ⁵Follow-up information was available for 103 patients; ⁶Follow-up information was available for 48 patients. F: Female; M: Male; R/M: Recurrence and/or metastasis.

phy (PET/CT) is a useful modality in the detection of malignant tumors and has been widely used in patients with pancreatic disease^[45]. Limited data are available on PET/CT characteristics of SPTP, making the value of this modality controversial. It has been reported that SPTP has significantly higher tumor size-adjusted metabolic tumor volume and total lesion glycolysis compared with pancreatic ductal adenocarcinoma^[46], which leads to a high rate of false positivity in F-18-fluorodeoxyglucose PET/CT when diagnosing this disease (Figure 3). However, this feature suggests that PET/CT may be helpful in detecting metastases of SPTP. Kang *et al*^[47] categorized SPTP into five types according to the PET images and found no association between the fluorodeoxyglucose uptake and malignant potential. Until now, no definitive conclusions can be drawn about the clinical significance of PET/CT in SPTP due to limited cases reported. Thus, the clinical application value of PET scan in SPTP needs further investigation.

TREATMENT OF MALIGNANT SPTP

Surgical resection is curative in most of the patients with SPTP resulting in a five-year disease-specific survival rate of 98.5%^[8]. Long-term survival can be achieved

even in those with advanced or metastatic disease. It is interesting to note that patients who underwent limited resection with microscopically positive margins had similar outcomes as those who underwent extensive surgery with R0 resection^[7]. The generally good prognosis of SPTP attributes to its relatively low malignant biological behavior. Therefore, aggressive surgical intervention is the optimal therapy for patients with advanced SPTP, even with metastasis. Wang *et al*^[48] reported four patients with liver metastases undergoing aggressive surgery. All the patients received surgical resections for both the primary and metastatic lesions as completely as possible, and had good clinical outcomes during follow-up.

Adjuvant therapies such as chemotherapy (5-fluorouracil and gemcitabine as the main chemotherapeutic drugs) and radiotherapy have been reported in a few patients with a mean survival of 51.1 mo^[13]. Sporadic reports found that neoadjuvant chemotherapy or radiation therapy could benefit some patients with unresectable tumors^[49-51]. Other therapeutic methods including radiofrequency ablation^[52], transcatheter arterial chemoembolization^[53], selective internal radiotherapy (SIRT)^[54] and liver transplantation^[55] have also been reported to achieve good results for patients with

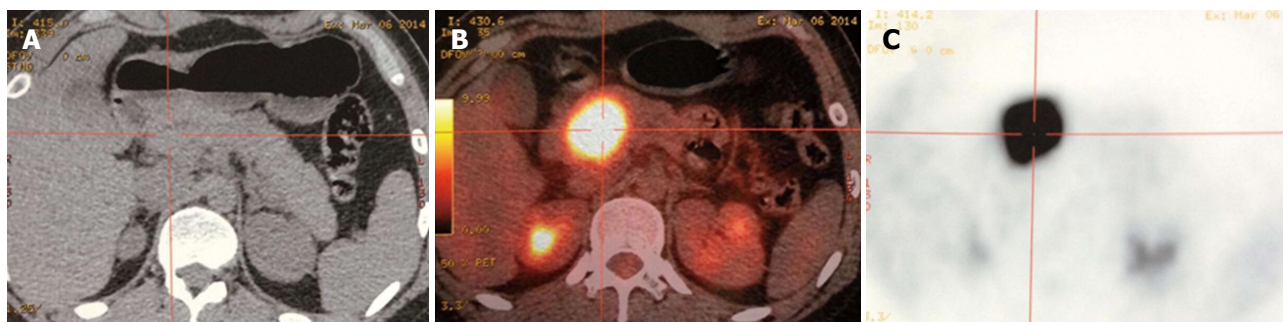


Figure 3 High uptake of F-18-fluorodeoxyglucose in a patient with solid pseudopapillary tumor of the pancreas. SPTP in a 25-year-old female patient with a T2 stage tumor. A: CT scan revealed a 5-cm isodense mass in the pancreatic head. B and C: Transaxial PET/CT (B) and PET (C) showed a hypermetabolic lesion with the maximum standardized uptake value of 33. She was disease free for 32 mo after surgical resection. SPTP: Solid pseudopapillary tumor of the pancreas; PET/CT: Position emission tomography/computed tomography.

liver metastasis from SPTP. However, despite a better understanding of this disease, individual treatment of unresectable or metastatic SPTP requires further study.

PREDICTORS OF MALIGNANCY

Malignant SPTP occurs in 18.3% of adult patients and in 43.1% of pediatric patients^[8,33]. Preoperative differential diagnosis between benign and malignant SPTP is usually very difficult except in patients with tumor invasion to adjacent organs or with distant metastasis. There has been no consistency about the diagnostic criteria of malignant SPTP until today. Criteria for malignancy in SPTP have not been well established. Many researchers used the WHO-defined criteria for classification of solid pseudopapillary carcinoma, such as angioinvasion, perineural invasion, or deep infiltration into the surrounding tissue or metastasis to confirm the diagnosis of malignant SPTP^[30]. Butte *et al.*^[24] defined malignant SPTP as locally unresectable tumor with macrovascular invasion, metastatic disease to regional or distant sites, or recurrence of disease after surgery. Ye *et al.*^[56] considered SPTP with incomplete capsules as malignant.

Due to the arbitrary criteria of malignancy used, and rarity of the disease with small proportion of malignancy, conflicting results have been reported about factors associated with malignant potential across institutions^[8,20,23,24,26,27,30,31,35,36,38,39,56-58] (Figure 4). Butte *et al.*^[24] found that patients with malignant SPTP presented with larger tumor size ($P < 0.005$). Chung *et al.*^[39] explored differential imaging features between malignant and benign SPTP, and found that malignant SPTP more frequently had focal lobulated margins ($P = 0.027$) and focal discontinuity of capsule ($P = 0.005$). The study by Ye *et al.*^[56] revealed that SPTP with incomplete capsule had larger tumor size ($P = 0.0015$) and mainly exophytic growth pattern ($P = 0.0194$). Yu *et al.*^[31] and Xu *et al.*^[35] showed that positive status for Ki-67 correlated with malignancy of SPTP, while Yang *et al.*^[8] did not demonstrate any association between the Ki-67 index and malignant SPTP. Most other studies^[20,23,26,27,57,58] found no significant differences between benign and malignant

SPTP, including age, sex, symptomatology, laboratory data, tumor marker, tumor size and location, tumor composition, growth pattern, and histopathology. Thus, malignancy cannot be easily predicted on the basis of preoperative findings and immunohistochemical patterns.

PREDICTORS OF ADVERSE OUTCOME

Most of the patients with SPTP have a good prognosis, while some have a less favorable prognosis because of recurrence and/or metastases. Studies on SPTP were characterized by case reports and small case series lacking of long-term follow-up. Kang *et al.*^[6] reported a low recurrence rate (2.8%) and excellent disease free survival and overall survival for SPTP after surgical resection in South Korea. The patients had a 5-year disease free survival of 95.4% and an overall survival of 98.8%. In a recent systematic review, the 5-year and 10-year recurrence free survival was 89.5% and 86.3%, respectively, with the 5-year and 10-year disease specific survival of 92.3% and 86.5%, respectively^[8]. It is unclear whether factors associated with malignant potential are statistically significant predictors of adverse outcomes. Although a few recent studies have gathered significant series of SPTP, results are inconclusive with regard to predictors of prognosis^[3,6-8,28,33-38,59,60] (Figure 5).

Estrella *et al.*^[7] showed that recurrent/metastatic SPTP was significantly associated with larger tumor size, invasion of muscular vessels, and the European Neuroendocrine Tumour Society (ENETS) tumor stage, but not with other clinicopathologic factors. In addition, muscular vessel invasion, ENETS T4 disease, and stage IV were important predictors of poor disease-specific survival after surgical resection. Kang *et al.*^[6] demonstrated that tumor size larger than 8 cm, microscopic malignant features, and stage IV were significant prognostic factors for tumor recurrence by multivariate analysis. Irtan *et al.*^[33] confirmed that the significant risk factors for recurrence in pediatric cases of SPTP were age < 13.5 years at diagnosis and positive surgical margins at initial tumor resection. It is interesting to note that many other studies^[6-8,22,28,34,60] have shown that patients who

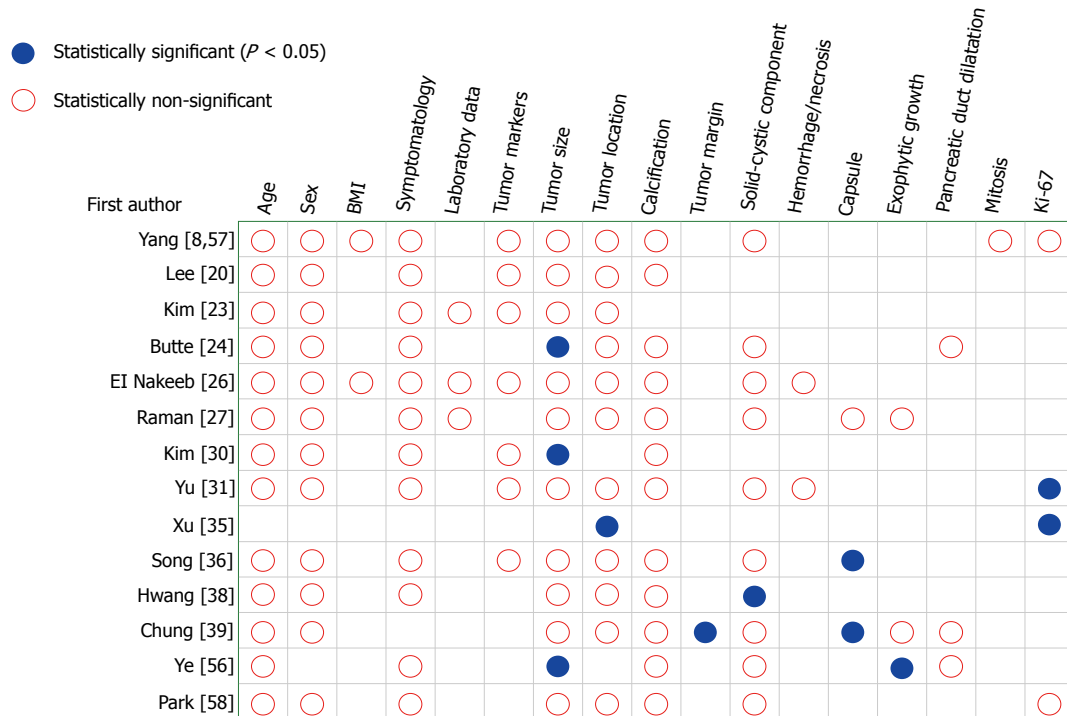


Figure 4 Factors associated with malignant solid pseudopapillary tumor of the pancreas by univariate analysis.

underwent limited resection and those with R1 resection had the same clinical outcomes as those who received more extensive resection with negative margin. Serrano *et al*^[28] clarified that patients with stage IV or lymphovascular invasion more commonly developed recurrence. Both studies of Marchegiani *et al*^[34] and Hwang *et al*^[38] revealed that recurrence was more common in patients with malignant SPTP which fulfilled the WHO criteria. The study by Zhang *et al*^[59] indicated that recurrence in malignant SPTP correlated with family malignant tumor history.

Several studies^[3,57,61,62] have proposed Ki-67 as an additional support to histology for predicting tumor outcome, but conflicting results do exist. Yang *et al*^[8] identified the most discriminating value of Ki-67 index using receiver operating characteristic curve analysis and demonstrated that the prognostic value of Ki-67 was maintained in both the Huashan cohort and the new historical cohort from literature. The result was consistent with a latest study by Kim *et al*^[3]. However, similar to most studies, multivariate analysis could not be performed due to the small number of events. Nevertheless, a much larger number of patients is needed to validate the prognostic relevance of Ki-67.

CHALLENGES AND PERSPECTIVES

Recent studies have analyzed the biological behavior of SPTP, however reliable data on long-term follow-up are still needed. Case reports, small retrospective case series, and subjective views rather than facts dominate the available data. These studies have limitations

including a small number of cases or events, no uniform parameters studied, lack of a gold standard for judging malignancy, and short length of follow-up. Although some studies adopted the WHO definition of malignancy, a considerable number of studies did not specify the proportion of malignant patients. The excellent prognosis makes overall survival difficult to be assessed, even if several studies have evaluated disease/recurrence free survival. In the light of these limitations, multicenter large-scale studies with long-term follow-up are needed to determine prognostic factors.

To date, no staging systems have been used to stratify patients in any international guidelines for management and follow-up of SPTP^[63-65]. The American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) tumor, node, metastasis (TNM) staging system is a generally accepted standard for cancer staging with the principal aim of facilitating a uniform and standardized analysis of malignant tumors. While the current TNM staging system applies well to pancreatic carcinoma, it is not specific to SPTP. Tumors considered for the TNM system have potentials of local invasiveness (T-categorization) and spread via the lymphatic and blood vessels (N- and M-categories). In view of the rarity of lymphatic and hematogenous metastasis from SPTP, its usefulness in this condition was evidently limited.

The relative rarity of SPTP has delayed the development of evidence-based treatment guidelines. Patients with benign SPTP are still at risk of tumor recurrence or metastasis after surgical resection. Contemporary evidence supports surgery as the primary treatment for patients with operable metastatic SPTP^[48,66]. One ob-



Figure 5 Predictors for adverse outcome of solid pseudopapillary tumor of the pancreas after surgical resection by univariate analysis. WHO: World Health Organization; SPC: Solid pseudopapillary carcinoma; AJCC: American Joint Committee on Cancer; ENETS: European Neuroendocrine Tumour Society.

stale to better management of patients is the lack of a predictive classification that accurately describes the complexity and heterogeneity of this disease. In order to provide proper information to predict prognosis, a more specific and standardized histopathological evaluation of SPTP is needed. It is obvious that we urgently need an international consensus for collecting standardized data on SPTP. Better understanding of molecular mechanisms involved in SPTP tumorigenesis is important for improved management. It is probable that novel molecular prognostic variables for SPTP, which may be incorporated

into classification systems, will emerge in future.

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Retrospective Study

Atypical anastomotic malignancies of small bowel after subtotal gastrectomy with Billroth II gastroenterostomy for peptic ulcer: Report of three cases and review of the literature

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Abstract

AIM

To present patients who developed small-bowel malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy for ulcer, to review relevant literature, and to attempt to interpret the reasons those cancers developed to these postsurgical non-gastric sights.

METHODS

For the current retrospective study and review of literature, the surgical and histopathological records dated from January 1, 1993 to December 31, 2017 of our department were examined, searching for patients who have undergone surgical treatment of small-bowel malignancy to identify those who have undergone subtotal gastrectomy for benign peptic ulcer. A systematic literature search was also conducted using PubMed, EMBASE, and Cochrane Library to identify similar cases.

RESULTS

We identified three patients who had developed small-intestine malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy with Billroth II gastroenterostomy for benign peptic ulcer—two patients with adenocarcinoma originated in the Braun anastomosis and one patient with lymphoma of the efferent loop. All three patients were submitted to surgical resection of the tumor with Roux-en-Y reconstruction of the digestive tract. In the literature review, we only found one case of primary small-intestinal cancer that originated in the efferent loop after Billroth II gastrectomy because of duodenal ulcer but none reporting Braun anastomosis adenocarcinoma following partial gastrectomy for benign disease. We also did not find any case of efferent loop lymphoma following gastrectomy.

CONCLUSION

Anastomotic gastric cancer following distal gastrectomy for peptic ulcer is a well-established clinical entity. However, malignancies of the afferent or efferent loop of the gastrointestinal anastomosis are extremely uncommon. The substantial diversion of the potent carcinogenic pancreaticobiliary secretions through the Braun anastomosis and the stomach hypochlorhydria, allowing the formation of carcinogenic factors from food, are the two most prominent pathogenetic mechanisms for those tumors.

Key words: Anastomotic cancer; Efferent loop; Braun anastomosis; Adenocarcinoma; Anaplastic large cell lymphoma

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Core tip: Anastomotic gastric cancer following distal gastrectomy for peptic ulcer is a well-established clinical entity. However, malignancies of the afferent or efferent loop of the gastrointestinal anastomosis are extremely uncommon. In this paper, three patients who developed small-bowel malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy for ulcer are presented. The two most prominent pathogenetic mechanisms for those tumors are the stomach hypochlorhydria, allowing the formation of carcinogenic factors from food, and the substantial diversion of the potent carcinogenic pancreaticobiliary

secretions through the Braun anastomosis.

Kotidis E, Ioannidis O, Pramateftakis MG, Christou K, Kanellos I, Tsalis K. Atypical anastomotic malignancies of small bowel after subtotal gastrectomy with Billroth II gastroenterostomy for peptic ulcer: Report of three cases and review of the literature. *World J Gastrointest Oncol* 2018; 10(7): 194-201 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i7/194.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i7.194>

INTRODUCTION

Small-bowel malignancies are among the rarest cancers, accounting for only 2% of all gastrointestinal cancers, even though the organ makes up more than 70% of the length and 90% of the surface area of the gastrointestinal tract^[1]. Approximately 60% of small-bowel tumors are malignant, and among those, adenocarcinomas comprise 35% to 50% of all cases, carcinoid tumors 20% to 40%, sarcomas 15%, and lymphomas 10% to 15%^[2-5]. Anastomotic gastric cancer following distal gastrectomy for peptic ulcer disease has long been recognized. However, malignancies of the afferent or efferent loop of the gastrointestinal anastomosis are extremely uncommon.

In this paper, we present three patients who developed small-bowel malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy for ulcer, and we attempt to interpret the reason that those cancers developed to these postsurgical non-gastric sights.

MATERIALS AND METHODS

For the current retrospective study and review of literature, the surgical and histopathological records dated from January 1, 1993 to December 31, 2017 of our department were examined, searching for patients who have undergone surgical treatment of small-bowel malignancy to identify those who have undergone subtotal gastrectomy for benign peptic ulcer. A systematic literature search was also conducted using PubMed, EMBASE, and Cochrane Library to identify similar cases.

RESULTS

Case 1

A 79-year-old white male presented at our hospital because of chronic anemia appearing as syncope episodes for the last 4-5 mo. He also developed early satiety during this period. In the past, the patient had undergone a subtotal gastrectomy followed by Billroth II gastroenterostomy and Braun anastomosis for the treatment of peptic ulcer disease 22 years ago. His history also included hepatitis C, hypertension, type II diabetes,

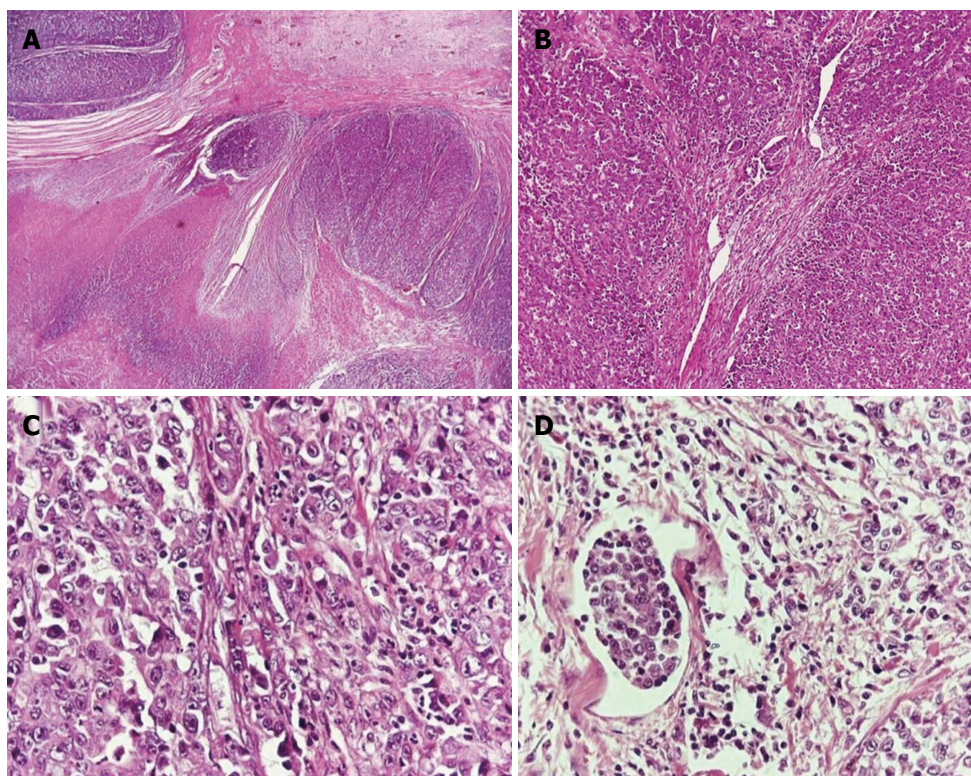


Figure 1 Adenocarcinoma of the small intestine. A: Low-power view shows the nodular formations of the carcinoma and foci of necrosis (H and E, $\times 25$); B: High-power view shows the diffuse growth pattern and the presence of few tubular structures (H and E, $\times 100$); C: High-power view shows the neoplastic cells with the hyperchromatic, irregular nuclei with prominent nucleoli; D: The presence of lymphatic tumor emboli (H and E, $\times 400$).

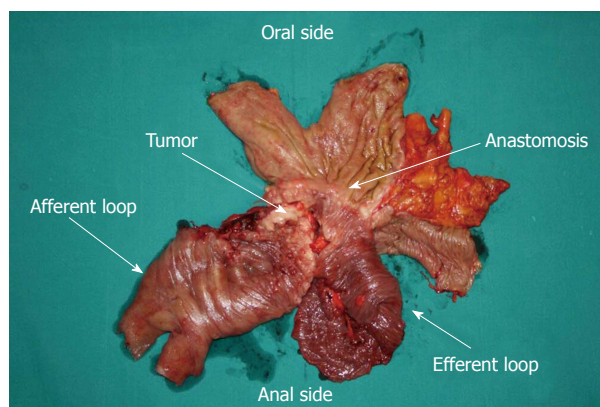


Figure 2 Surgical specimen after partial gastrectomy of the gastric pouch with resection of the Braun anastomosis.

and myocardial infarction. His physical examination revealed an enlarged liver, and his blood tests showed a hypochromic anemia and slightly deranged liver function. The upper gastrointestinal endoscopy showed a lesion with an uneven surface at the Braun anastomosis. The computed tomography (CT) scan demonstrated liver cirrhosis and a tumor at the level of Braun anastomosis.

Hematoxylin- and eosin-stained sections from the lesion revealed the presence of a high-grade adenocarcinoma infiltrating the entire bowel wall and extending into the surrounding mesenteric fat tissue. Fibrotic bands in the histological sample resulted in the formation of nodular configurations. Only focally few tubular structures

were identified. Foci of necrosis and lymphatic tumor emboli were also present. The neoplastic cells had hyperchromatic, irregular nuclei with prominent nucleoli, and they arranged mainly in a diffuse growth pattern (Figure 1).

The tumor, about 4 cm in diameter, was resected en bloc with the previous gastrojejunal anastomosis, and the gastrointestinal continuity was restored with a Roux-en-Y gastrojejunal anastomosis. The patient was discharged and is free of disease until today, 9 mo after surgery.

Case 2

A 76-year-old man presented at our hospital with undefined abdominal discomfort and relapsing melenas for the last 2-3 mo. He also experienced a drop in hematocrit during this period. Prior surgical history included a subtotal gastrectomy because of a bleeding pyloric ulcer, followed by a Billroth II Hofmeister-Finsterer anastomosis and a jejunojejunostomy (Braun), approximately 30 years ago. His physical examination and laboratory tests were unremarkable except for the presence of anemia. Endoscopy showed a tumor at the Braun anastomosis that ended up being an adenocarcinoma. The abdominal CT showed only the Braun anastomosis tumor. He underwent a partial gastrectomy of the gastric pouch with resection of the Braun anastomosis and tumor, measuring 5 cm \times 2 cm, and a Roux-en-Y reconstruction (Figure 2). The patient had a smooth postoperative course and 2.5 years after treatment is free of relapse.

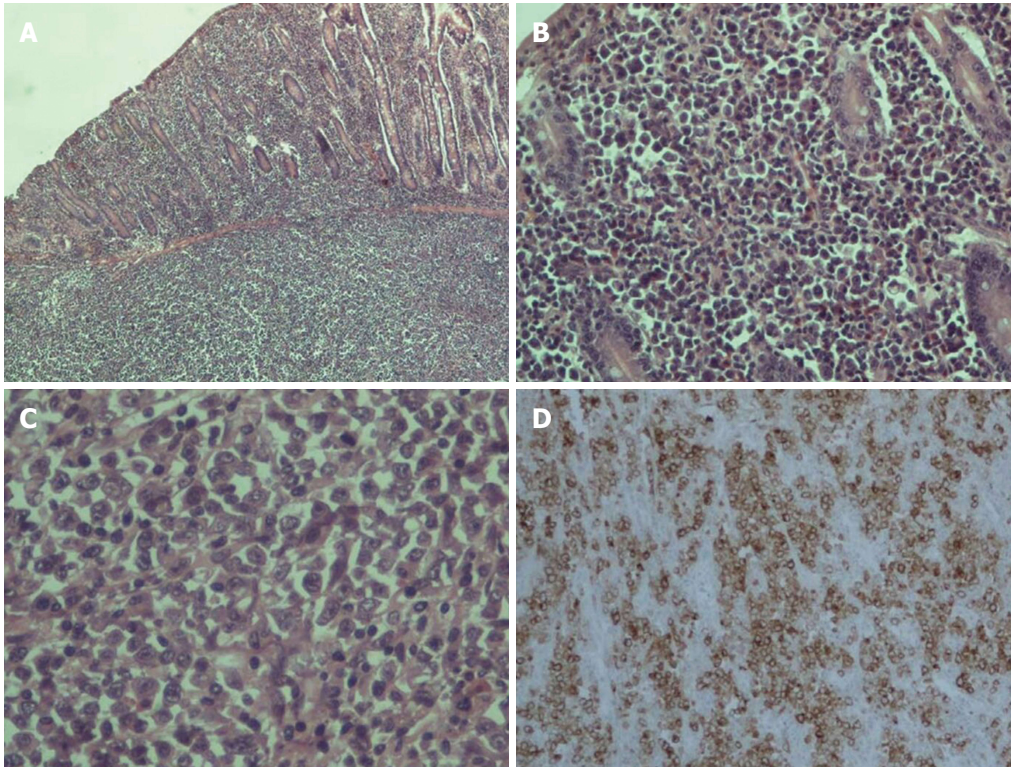


Figure 3 Anaplastic large cell lymphoma of the small bowel. A: H and E stain $\times 40$; B: H and E stain $\times 200$; C: H and E stain $\times 400$; D: Ki-1 antigen (CD30) staining (+).

Case 3

A 78-year-old man was referred to our hospital for hematemesis and melena. The patient had undergone a partial gastrectomy with Billroth II gastroenterostomy because of duodenal ulcer disease 30 years ago. His history, however, included splenectomy caused by trauma, cholecystectomy, and hypertension. His physical exam and blood tests were unremarkable except for the presence of a normocromic anemia. He underwent an upper-gastrointestinal endoscopy, which identified a sizable ulcer crater at the beginning of the efferent jejunal loop, about 4 cm from the anastomosis, with unsuccessful attempts of permanent hemostasis. A laparotomy was decided upon. A large tumor of the efferent jejunal loop was identified with multiple small infiltrations in the afferent loop of 15–20 cm. The rest of the small intestine was free. The *Helicobacter pylori* examination was positive. A segmental resection of the gastric pouch and the infiltrated jejunal loops was performed, followed by a Roux-en-Y reconstruction.

On histopathologic examination, the reported ulcer was part of a grayish intramural lesion that infiltrated the entire wall of the intestine. Microscopically, large undifferentiated neoplastic cells were widely disseminated. The cells contained a moderate amount of cytoplasm and sizable, oval, frequently irregular pleomorphic nuclei with multiple prominent nucleoli. Binucleate, abnormal multinucleate, and multilobed nuclei formats were observed (Figure 3). By immunohistochemistry, the large cells were strongly positive for CD30. They were also po-

sitive for vimentin, epithelial membrane antigen (EMA), CD7, CD43, and MUM1. Partial positivity was for the antigens CD138, p53, CD38, CD45RO (LCA), perforin, and AE1/AE3 (cytokeratin). The large cells were negative for the expression of CD2, CD3, CD5, CD4, CD8, ALK, CD56, CD20, CD79a, PAX5, CD45RA, TIA1, CD15, myeloperoxidase (MPO), lysozyme, and EBV-LMP1.

The findings are consistent with anaplastic large cell lymphoma (ALCL) ALK-negative (anaplastic lymphoma kinase), a rare type of non-Hodgkin lymphoma. The resection boundaries were free of neoplastic infiltration, and no lymph node involvement (17 in total) was found. The patient has been referred to the hematology department for further treatment and follow-up.

DISCUSSION

Small-bowel adenocarcinomas are relatively uncommon and have a slight male preponderance (3:2), and their peak incidence is the seventh decade of life^[1]. They are believed to arise from premalignant adenomas^[6]. They also have a predilection for the duodenum, with a marked decrease in frequency moving axially along the small bowel^[1]. Knowing from physiology that this is exactly the effect of the distribution of ingested chemicals and the effect of gastric and pancreaticobiliary secretion on intestinal mucosa may indicate that these substances may have carcinogenic properties^[4,5]. Furthermore, small-bowel adenocarcinoma is associated with Crohn's disease (up to 100-fold risk), celiac disease,

and familial polyposis syndrome, none of which were included in our patients' history. There is not a specific complex of symptoms diagnostic for small-bowel cancer, but the most common are abdominal pain, nausea, obstruction symptoms, and weakness. Bleeding, either occult as melena like our first and second case or acute in the form of hematemesis like our 3rd case, is more uncommon^[2,6].

Following distal gastrectomy for peptic ulcer disease, gastric cancer can develop, usually after years, in the gastric remnant^[7]. A gastric remnant carcinoma is defined as a primary carcinoma arising in the stomach, remnant at least 5 years after previous partial gastrectomy for benign disease, most frequently peptic ulcer disease. The 5-year interval is necessary to avoid confusion with cancer recurrence after initial misdiagnosis. Several large prospective studies with long-term follow-up indicate that the relative risk for this gastric neoplasm development is not increased for up to 15 years after gastric resection^[8,9], likely because of surgical removal of mucosa at risk for gastric cancer development, followed by modest increases in cancer risk (three times the control value) observed only after 20 years^[10-12]. Recently, conservative medical therapy has displaced partial gastrectomy for the treatment of ulcer. Nevertheless, since surgical therapy was still used frequently for the treatment of gastroduodenal ulcer disease until the late 1970s and early 1980s and gastric remnant carcinoma develops with a time interval of 20–40 years, the surgeon will be confronted with this disease regularly until at least 2020^[13]. Stage for stage, the prognosis for gastric stump cancer is similar to proximal gastric cancer^[14].

Additionally, Ravi Thiruvengadam *et al.*^[15] had attempted to estimate the risk of cancer at gastrointestinal spots other than the stomach, such as the small and large intestines, the esophagus, and the gallbladder, after gastric surgery for benign disease. There was no strong evidence for an increased risk of any gastrointestinal cancer following gastric surgery. However, after 10 years, Staël von Holstein *et al.*^[16] showed that there is an increased risk for nongastric gastrointestinal cancer, but similar to gastric remnant carcinoma, that risk emerges only 20 years postoperatively. The abovementioned studies concluded that all patients should be screened after an interval of 15–20 years after the distal gastrectomy.

With regard to the pathogenesis of gastric remnant cancer, the predominant factors presumed to be responsible for it are duodenogastric reflux and hypochlorhydria. Chronic duodenogastric reflux causes various histological alterations at the gastric stump, such as intestinal metaplasia, dysplasia, and adenoma. The gastrojejunal anastomosis is considered the most common site of gastric remnant carcinoma because the quantity and concentration of gastroduodenal reflux are highest here. Both bile acids and pancreatic juice seem to be carcinogenic factors, even though we do not know exactly which components are responsible. Bile acids,

such as deoxycholic bile acid and nitrated derivatives of glycocholic and taurocholic bile acids, seem to have a carcinogenic influence at the gastric stump mucosa^[5,17-19]. Braun, in 1893, introduced the jejunojejunal anastomosis between the afferent and efferent small intestine loops immediately distal to a gastrointestinal anastomosis. Using radionuclide biliary scanning, Vogel *et al.*^[20] found that Braun enteroenterostomy adequately diverts a substantial amount of bile from the stomach in patients undergoing gastroenterostomy or Billroth II resection. Hence, because of the skipping of the ascending (or afferent) and descending (or efferent) jejunal loop (approximately 50 cm), the pancreaticobiliary fluids come less in contact with the gastric stump and more with the Braun anastomosis and the efferent limb distal to it. Therefore, the increased exposure of the latter surfaces to carcinogenic bile acids, not only for the gastric stump mucosa but also for the small intestine^[1,4], is most likely the underlying pathophysiologic mechanism that enables the Braun anastomosis mucosa to become dysplastic and neoplastic before the gastric stump mucosa does.

Because of the resection of the gastrin-producing cells after a distal gastrectomy, the gastric stump mucosa usually becomes atrophic. This atrophy causes hypochlorhydria, and thus, the pH value rises, resulting in bacterial population growth. Some of these bacteria reduce dietary nitrates to nitrites, which, in the presence of substrates, such as food proteins, can lead to the formation of potent carcinogens^[13,21,22]. If those carcinogens are absorbed systemically, then that supports the observation of Staël von Holstein *et al.*^[16] that after a gastrectomy for ulcer, there is an increased risk of developing a carcinoma in a location other than the gastric stump, just like in our patients.

We reviewed the literature and found some similar cases of gastrointestinal cancer near but not on the anastomosis after partial gastrectomy for benign disease. Takebayashi *et al.*^[23] presented a case of primary small-intestinal cancer that originated in the efferent loop after the Billroth II gastrectomy that occurred 32 years earlier because of duodenal ulcer. Rose *et al.*^[24] reported a case of gastric adenocarcinoma arising at the duodenal stump 40 years after a Billroth II partial gastrectomy for benign condition. Table 1 summarizes the reported cases in the literature and our cases with atypical anastomotic malignancies of small bowel after subtotal gastrectomy with Billroth II gastroenterostomy for peptic ulcer. To our knowledge, our patients are the first reported cases of Braun anastomosis adenocarcinoma following partial gastrectomy for benign disease.

Lymphomas affect the small bowel as a manifestation of systemic disseminated disease, or they may be primarily present^[3]. Non-Hodgkin lymphomas (NHLs) of the gastrointestinal tract represent 4% to 20% of all NHLs^[25]. Of all gastrointestinal NHLs, 25% to 35% of cases occur within the small bowel, in which lymphomas parallel the distribution of lymphoid follicles, resulting in

Table 1 Synopsis of reported cases with atypical anastomotic malignancies of small bowel after subtotal gastrectomy with Billroth II gastroenterostomy for peptic ulcer

Case	Sex	Age	Tumor	Origin	Clinical data	Laboratory data	Treatment	Outcome	Ref.
1	M	79	Small intestine adenocarcinoma	Braun anastomosis after 22 yr from gastrectomy	Syncope episodes, early satiety	Hypochromic anemia	En block resection and Roux-en-Y gastrojejunal anastomosis	Disease free 9 mo	Kotidis, 2018
2	M	76	Small intestine adenocarcinoma	Braun anastomosis after 30 yr from gastrectomy	Abdominal Discomfort, Melena	Anemia	En block resection and Roux-en-Y gastrojejunal anastomosis	Disease free 2.5 yr	Kotidis, 2018
3	M	78	Anaplastic large cell lymphoma	Efferent loop after 30 yr from gastrectomy	Hematemesis, melena	Normochromic anemia	En block resection and Roux-en-Y gastrojejunal anastomosis	Referred to hematology department	Kotidis, 2018
4	M	79	Small intestine adenocarcinoma	Efferent loop after 32 yr from gastrectomy	Asymptomatic	Anemia	Jejunectomy	Disease free at 17 mo	[23]
5	F	79	Duodenal adenocarcinoma	Duodenal stump 40 yr after gastrectomy	Fatigue and weakness for 3 mo	Anemia	Resection of afferent limb	Disease free at 12 mo	[24]

the ileum being the most common site of involvement^[1]. Anaplastic large cell lymphoma (ALCL) is a distinctive subtype of NHL. It accounts for approximately 2% of all cases of NHL. It belongs to the NHL subcategory of peripheral T-cell lymphomas (PTCL). It is made up of either malignant T-cells or “null-lymphocytes” (lack both B- and T-cell markers). The presence of the protein CD30 antigen on the surface of lymphoma cells is the hallmark of the disease^[26]. Usually, ALCL is negative for cytokeratin. The positive cytokeratin AE1/AE3 cells in our case were considered remnant epithelial cells. The ALK-negative subtype of ALCL appears more commonly in the elderly, is more aggressive, and belongs to the systemic form of ALCL^[27], which typically presents with painless enlarged lymph nodes and extranodal site involvement, most commonly including the skin, bones, soft tissues, and lungs. The gastrointestinal tract being involved in our case is rare^[27], and even though, in the literature, rare cases of gastrointestinal ALCL at various spots, including the small intestine, have been documented^[28-30], as far as we know, this is the first case reported at the efferent loop of a Billroth II gastroenterostomy decades after operation.

In conclusion, the “by-pass” path of the bile made by a Braun anastomosis added to a Billroth II gastrectomy is the most prevalent hypothesis for the development of the two adenocarcinomas at the specific spot. Much more needs to be discovered about the ALCL ALK-negative type of lymphoma to make assumptions since it has not been studied for more than 20 years. However, we cannot be certain that the appearance of those small-

intestinal tumors at those spots were directly related to the operations that occurred decades ago.

ARTICLE HIGHLIGHTS

Research background

Despite the fact that the small intestine makes up more than 90% of the surface area and 70% of the length of the gastrointestinal tract, small bowel malignancies are among the rarest cancers. Anastomotic gastric cancer following distal gastrectomy for peptic ulcer is a well-established clinical entity. However, malignancies of the afferent or efferent loop of the gastrointestinal anastomosis are extremely uncommon.

Research motivation

To present patients who developed small-bowel malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy for ulcer.

Research objectives

In this paper, we present three patients who developed small-bowel malignancy at the level of the gastrointestinal anastomosis decades after a subtotal gastrectomy for ulcer, to review relevant literature, and to interpret the reason that those cancers developed to these postsurgical nongastric sights.

Research methods

For the current retrospective study and review of literature, the surgical and histopathological records of our department were examined, searching for patients who have undergone surgical treatment of small-bowel malignancy to identify those who have undergone subtotal gastrectomy for benign peptic ulcer. A systematic literature search was also conducted using PubMed, EMBASE, and Cochrane Library to identify similar cases.

Research results

We identified three patients who had developed small-intestine malignancy at the level of the gastrointestinal anastomosis decades after a subtotal

gastrectomy with Billroth II gastroenterostomy for benign peptic ulcer-two patients with adenocarcinoma originated in the Braun anastomosis and one patient with lymphoma of the efferent loop. All three patients were submitted to surgical resection of the tumor with Roux-en-Y reconstruction of the digestive tract. In the literature review, we only found one case of primary small-intestinal cancer that originated in the efferent loop after Billroth II gastrectomy because of duodenal ulcer but none reporting Braun anastomosis adenocarcinoma following partial gastrectomy for benign disease. We also did not find any case of efferent loop lymphoma following gastrectomy.

Research conclusions

Anastomotic gastric cancer following distal gastrectomy for peptic ulcer is a well-established clinical entity. However, malignancies of the afferent or efferent loop of the gastrointestinal anastomosis are extremely uncommon. The substantial diversion of the potent carcinogenic pancreaticobiliary secretions through the Braun anastomosis and the stomach hypochlorhydria, allowing the formation of carcinogenic factors from food, are the two most prominent pathogenetic mechanisms for those tumors.

Research perspectives

The "by-pass" path of the bile made by a Braun anastomosis added to a Billroth II gastrectomy is the most prevalent hypothesis for the development of the two adenocarcinomas at the specific spot. Much more needs to be discovered about the ALCL ALK-negative type of lymphoma to make assumptions since it has not been studied for more than 20 years. However, we cannot be certain that the appearance of those small-intestinal tumors at those spots had a direct relation to the operations that occurred decades ago.

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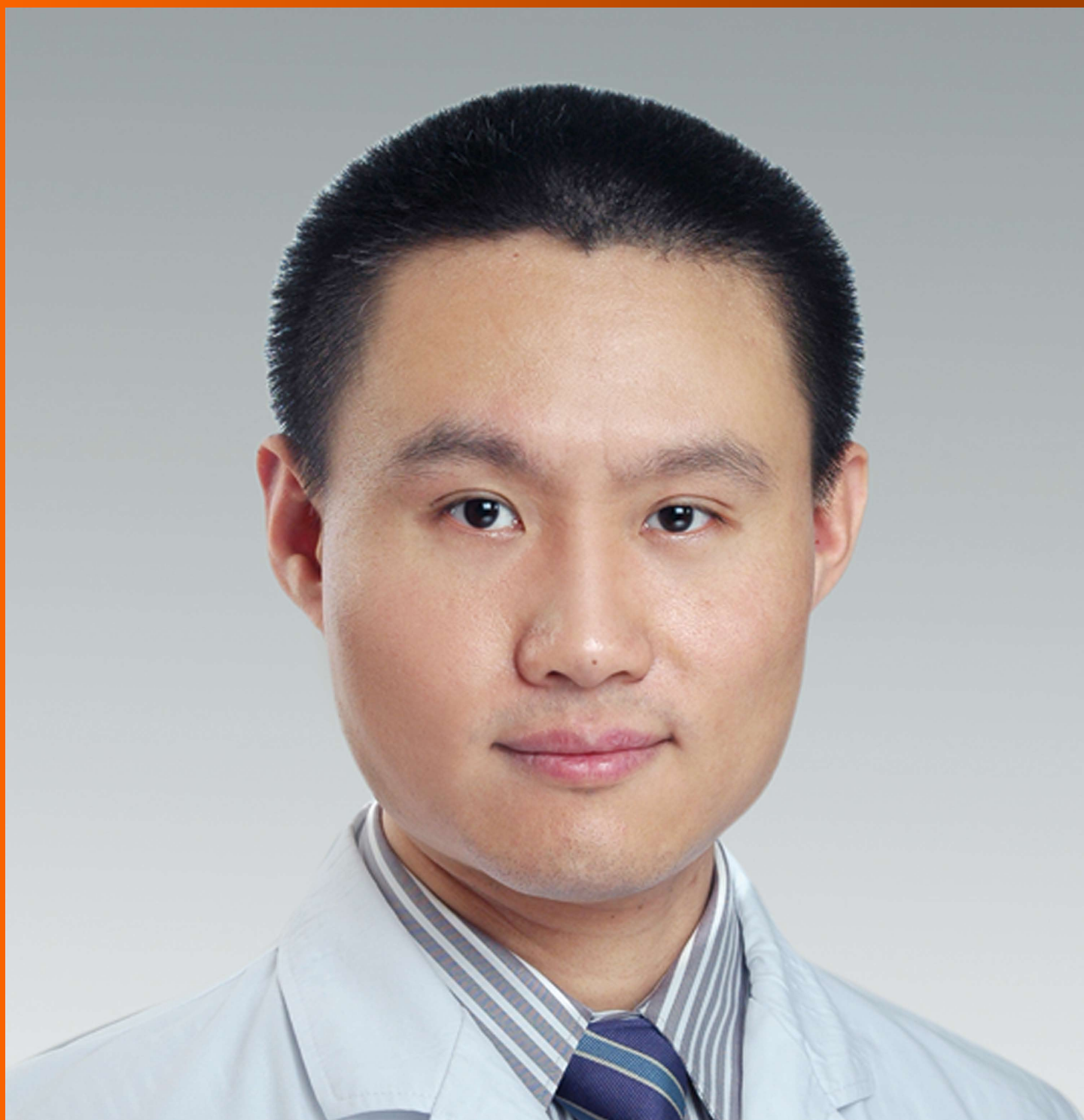


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Shattering the castle walls: Anti-stromal therapy for pancreatic cancer

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(PC) remain unsatisfactory. The presence of an abundant fibrous stroma in PC is considered a crucial factor for its unfavorable condition. Apparently, stroma acts as a physical barrier to restrict intratumoral cytotoxic drug penetration and creates a hypoxic environment that reduces the efficacy of radiotherapy. In addition, stroma plays a vital supportive role in the development and progression of PC, which has prompted researchers to assess the potential benefits of agents targeting several cellular (e.g., stellate cells) and acellular (e.g., hyaluronan) elements of the stroma. This study aims to briefly review the primary structural properties of PC stroma and its interaction with cancer cells and summarize the current status of anti-stromal therapies in the management of metastatic PC.

Key words: Pancreatic cancer; Stroma; Stellate cells; Hyaluronan; Secreted protein acidic and rich in cysteine

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Core tip: The primary characteristic of pancreatic adenocarcinoma is the presence of an extensive desmoplastic stroma around neoplastic cells. In this study, we aim to briefly review the primary structural properties of pancreatic cancer (PC) stroma and its interaction with cancer cells and summarize the current status of anti-stromal therapies in the management of metastatic PC.

Kanat O, Ertas H. Shattering the castle walls: Anti-stromal therapy for pancreatic cancer. *World J Gastrointest Oncol* 2018; 10(8): 202-210 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i8/202.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i8.202>

Abstract

Despite the availability of potent chemotherapy regimens, such as 5-fluorouracil, folinic acid, irinotecan, and oxaliplatin (FOLFIRINOX) and nab-paclitaxel plus gemcitabine, treatment outcomes in metastatic pancreatic cancer

INTRODUCTION

The primary characteristic of pancreatic adenocarcinoma is the presence of an extensive desmoplastic stroma

around neoplastic cells in both primary and metastatic lesions^[1]. The structural organization of stroma is not entirely different from those in other solid tumors; in fact, it is a mixture of cellular and acellular [extracellular matrix (ECM) proteins] elements^[2]. However, in contrast to several other solid tumors, stromal elements can occupy $\geq 80\%$ of the total tumor volume in most pancreatic cancer (PC) cases^[3].

Abundant accumulation of fibrous proteins, primarily collagen (types I and III), fibronectin, and secreted protein acidic and rich in cysteine (SPARC) in the ECM offers exceptional mechanical properties of pancreatic adenocarcinoma stroma, including considerably enhanced stiffness and reduced elasticity^[4]. In addition, increased deposition of another crucial ECM element hyaluronan (HA) in the tumor stroma creates substantial swelling stress, which progressively increases the interstitial fluid pressure^[5]. The occurrence of this condition besides increased tissue stiffness compresses intratumoral blood vessels, resulting in tumor hypoperfusion and hypoxia. Reportedly, hypoperfusion drastically reduces intratumoral delivery of chemotherapy drugs and, consequently, their efficacy^[6,7]. Hypoxia confers a survival advantage for neoplastic cells and potentiates their invasion, stemness, and metastatic capacity primarily through the hypoxia-inducible factor-1 α -mediated hepatocyte growth factor/c-Met pathway activation^[8,9]. Moreover, hypoxia compromises the efficacy of radiotherapy.

In PC, ECM proteins are primarily produced by a distinct type of stromal cells called activated pancreatic stellate cells (PSCs). PSCs phenotypically resemble myofibroblasts and exhibit the α -smooth muscle actin expression. However, in contrast to myofibroblasts, PSCs are positively stained for selective markers such as desmin and glial fibrillary acidic protein. They also demonstrate increased proliferation and migration ability relative to myofibroblasts, and can produce large amounts of collagen and other ECM proteins^[10,11]. PSCs possess the adequate capacity to interact with cancer cells and other stromal cells (*i.e.*, immune cells, inflammatory cells, and endothelial cells) to extend stroma and promote cancer progression. Thus, both cellular (especially PSCs) and acellular (especially HA) components of PC stroma have been held accountable for unsatisfactory treatment outcomes in patients with PC. This condition has encouraged PC researchers to elucidate the potential beneficial effects of stroma disrupting agents alone or in combination with standard chemotherapy in the treatment of PC (Figure 1).

ROLES OF PSCS IN PC PROGRESSION

Despite being debatable, activated PSCs are deliberated to originate from their inactive (quiescent) forms that are primarily found in the periacinar space of the exocrine pancreas^[12]. Reportedly, inflammatory (*i.e.*, interleukin-1 and interleukin-6, and tumor necrosis factor- α) and

mitogenic (*i.e.*, transforming growth factor and platelet-derived growth factor) cytokines secreted by cancer cells are accountable for the PSC activation^[13-18]. Perhaps, some intracellular pathways, including p38 mitogen-activated protein kinase, RhoA/Rho kinase, and cyclooxygenase-2, could play a vital role in this process^[18-22].

In pancreatic carcinogenesis, activated PSCs seemingly serve two primary functions, to produce ECM molecules and regulate the formation of desmoplastic reaction and enable cancer cell proliferation and invasion^[13]. The direct cell-to-cell contact between PSCs and PC cells has been demonstrated to result in the activation of the Notch signaling pathway in both cell types^[23]. The Notch signaling plays a vital role in the proliferation, migration, differentiation, and apoptosis of cancer cells^[24,25]. Apparently, PSCs can activate the mitogen-activated protein kinase and Akt pathways in tumor cells, causing enhanced tumor growth and metastasis^[26]. PSCs secrete matrix metalloproteinase-2 into the tumor microenvironment in response to extracellular matrix metalloproteinase inducer (EMMPRIN) secreted by cancer cells to facilitate the tissue invasion and metastasis^[27]. In addition, PSCs can accompany cancer cells to distant sites, where they stimulate angiogenesis, cancer cell seeding, survival, and proliferation and, thus, facilitate the metastasis formation^[28]. Furthermore, PSCs can indirectly protect cancer cells from the immune system attack. A study demonstrated that PSCs secreted CXCL12 chemokine and sequestered CD8⁺ T cells to reduce their accumulation in the juxtatumoral compartments^[29]. Mace *et al.*^[30] suggested that PSC-derived cytokines, such as interleukin-6, cause myeloid-derived suppressor cell expansion in the stroma, thereby indirectly inducing immune cell dysfunction.

Preclinical data indicated that PSCs might enhance stem-cell like phenotypes in PC cells^[31]. Indirect co-culture of PSCs with PC cells increased the spheroid-forming capacity of tumor cells, and induced the expression of stem cell-related genes including Nestin, ABCG2 and LIN28^[31]. Lonardo *et al.*^[32] showed that the secretion of transforming growth factor- β superfamily members Nodal and Activin from PSCs significantly promotes the self-renewal capacity and invasiveness of PC stem cells.

Recent studies have shown that extracellular vesicles (also known as exosomes) derived from PSCs may play a role in the progression of PC^[33,34]. Takikawa *et al.*^[34] reported that immortalized human PSCs produce exosomes containing numerous microRNAs (miRNAs) that can induce chemokine gene expression in PC cell lines resulted in increased proliferation and migration. Leca *et al.*^[35] found that annexin 6A/receptor-related protein 1/thrombospondin-1 complex-containing exosomes released by PSCs could increase PC cell aggressiveness under physiopathologic conditions. In addition, exosomes have been suggested to contribute to chemoresistance of PC cells by promoting reactive oxygen species detoxification and by decreasing gemcitabine-metabolizing enzyme activity^[36].

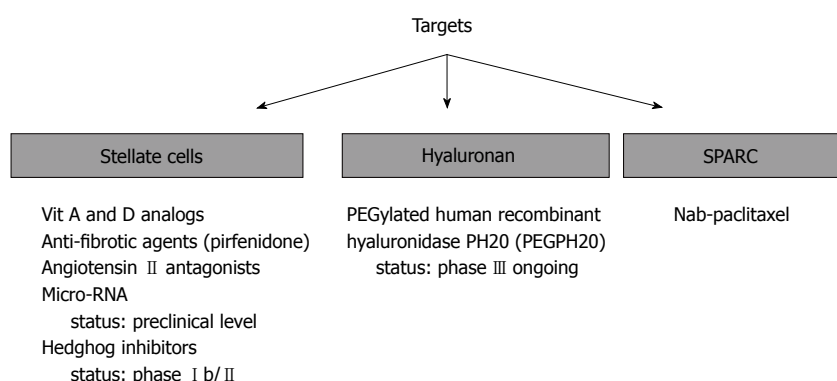


Figure 1 Stroma-targeting treatment strategies in pancreatic cancer. SPARC: Secreted protein acidic and rich in cysteine.

POTENTIAL THERAPEUTIC STRATEGIES TARGETING PSCS

Vitamin D and A analogs

Preclinical studies have reported that the PSC activation can be restricted or reversed by pharmacological interventions leading to substantial modulation of the tumor stroma^[37,38]. Apparently, PSCs express higher levels of vitamin D receptors^[37]. Sherman *et al.*^[37] reported that a potent vitamin D analog calcipotriol treatment decreased the expression of activation and cancer signature genes in cultured PSCs, stimulated lipid droplet formation, and reduced the α -smooth muscle actin expression, signifying their inactivation; this correlated with a decline in stromal inflammation and fibrosis. In addition, the authors compared the efficacy of gemcitabine plus calcipotriol treatment with gemcitabine alone in the KPC model of PC and reported that the combination therapy increased the intratumoral uptake of gemcitabine, reduced tumor volume, and exhibited 57% improvement in animal survival compared with gemcitabine monotherapy. These findings suggested that the tumor stromal modulation (reprogramming) by inactivating PSCs could be a reasonable treatment strategy for PC. Paricalcitol, a synthetic vitamin D analog is currently being tested in combination with conventional chemotherapy or immunotherapy in the treatment of metastatic PC (Table 1).

Quiescent PSCs store vitamin A-containing lipid droplets in their cytoplasm, which are lost during the activation process. Research has revealed that restoring vitamin A in PCs by using vitamin A metabolites could reprogram these cells to a quiescent phase^[39]. Jaster *et al.*^[40] reported that all-trans-retinoic acid (ATRA) could impede the proliferation and collagen synthesis of PSCs isolated from rat pancreas by hindering the AP-1 activation. Of note, AP-1 is a transcription factor that regulates cell growth, differentiation, and survival. McCarroll *et al.*^[41] described that ATRA and 9-cis retinoic acid could avert the activation of cultured activated PSCs by inhibiting the mitogen-activated protein kinase signaling pathway, and

decreased collagen I, fibronectin, and laminin expression in these cells. In addition, a study reported that the reduction of Wnt-B-catenin signaling by ATRA in PC cells resulted in slower tumor progression^[42]. Furthermore, Chronopoulos *et al.*^[43] determined that ATRA could reduce the actomyosin-dependent contractility, mechanosensing, and migration of PSCs in a retinoic acid receptor (RAR)- β -dependent manner. Likewise, Sarper *et al.*^[39] also reported similar findings. Overall, reprogramming of PSCs using vitamin A metabolites, such as ATRA or selective RAR- β agonists, in a clinical setting could open new avenues in the treatment of PC (Table 1).

Antifibrotic agents

Kozono *et al.*^[44] reported that the antifibrotic anti-inflammatory agent pirfenidone could impede the proliferation, invasiveness, migration, and ECM protein synthesis ability of PSCs in vitro. In mice bearing orthotopically implanted PC and PSCs, pirfenidone was shown to suppress the tumor growth and metastasis formation and displayed a synergistic antitumor effect with gemcitabine. In addition, Suklabaidya *et al.*^[45] reported that the effects of pirfenidone could be potentiated when co-administered with antioxidant N-acetyl cysteine. Thus, the potential effects of pirfenidone alone or in combination with N-acetyl cysteine in PC necessitate further assessment in human subjects.

Angiotensin II inhibitors

Previously, preclinical studies have demonstrated that angiotensin II plays a promoting role in the PSC proliferation, which seems to be controlled by induction of the Smad7 expression through a protein kinase C-dependent pathway, resulting in the inhibition of TGF- β 1 signaling^[46]. On the basis of these findings, several angiotensin II receptor antagonists have been investigated as a potential strategy to reduce PSC-mediated stromal fibrosis. Yamada *et al.*^[47] reported that candesartan considerably reduces the PSC proliferation and decreases the histological score of experimental

Table 1 Summary of existing studies evaluating the efficacy of anti-stromal agents in the treatment of metastatic pancreatic cancer

Agent	Target	Treatment arm (s)	Type of study	National clinical trial number	Status	Results
Paricalcitol	Vitamin D metabolic pathway	Gemcitabine and nab-paclitaxel plus paricalcitol or placebo	Phase I / II	NCT03520790	Recruiting	
		Nab-paclitaxel, cisplatin and gemcitabine plus paricalcitol	Phase II	NCT03415854	Recruiting	
		Nivolumab ¹ , nab-paclitaxel, cisplatin, and gemcitabine plus paricalcitol	Phase II	NCT02754726	Recruiting	
		Pembrolizumab ¹ plus paricalcitol or placebo	Phase II	NCT03331562	Recruiting	
All trans retinoic acid	Pancreatic stellate cells	Gemcitabine and nab-paclitaxel plus all trans retinoic acid	Phase I	NCT03307148	Recruiting	
Vismodegib	Hedgehog signaling	Gemcitabine plus vismodegib or placebo	Phase I / II	NCT0106422	Completed	Vismodegib did not improve ORR, PFS and OS
IPI-926	Hedgehog signaling	FOLFIRINOX plus IPI-926	Phase I	NCT01383538	Completed	The combination treatment was safe but IP-926 was not beneficial
		Gemcitabine plus IPI-926 or placebo	Phase I / II	NCT01130142	Completed	The combination treatment was well tolerated, and showed promising activity
PEGPH20	Hyaluronic acid	Gemcitabine and nab-paclitaxel plus PEGPH20 vs chemotherapy alone	Phase II	NCT01839487	Completed	PEGPH20 significantly improved PFS, especially in patients having tumors with high-level hyaluronic acid
		Gemcitabine and nab-paclitaxel plus PEGPH20 or placebo ²	Phase III	NCT02715804	Recruiting	
		Modified FOLFIRINOX plus PEGPH20 vs chemotherapy alone	Phase I / II	NCT01959139	Closed	PEGPH20 was found to have a detrimental effect on OS

¹Nivolumab and Pembrolizumab: PD-1-targeted T-cell checkpoint inhibitors; ²This study included only patients whose tumors had high levels of hyaluronic acid. ORR: Overall response rate; PFS: Progression-free survival; OS: Overall survival; FOLFIRINOX: 5-fluorouracil, irinotecan and, oxaliplatin.

pancreatic inflammation and fibrosis formation by avoiding the activation of TGF- β 1 signaling. In addition, Masamune *et al*^[48] investigated the effects of another angiotensin II antagonist, olmesartan, on PC-associated fibrosis in a subcutaneous xenograft model. Apparently, olmesartan could inhibit the PSC proliferation and collagen I production, resulting in the tumor growth suppression. Nevertheless, further preclinical data are warranted before advancing these agents to clinical trials.

Upregulation of microRNAs in PSCs

miRNAs are small noncoding RNA molecules involved in RNA silencing and post-transcriptional gene expression regulation. A study reported that miR-21, a profibrotic miRNA, is upregulated in cancer-associated myofibroblasts and PSCs isolated from resected PC tissues^[49]. In addition, PC cells have been assumed to induce miR-21 upregulation in these cells, expediting their invasion and metastasis^[49]. Donahue *et al*^[50] reported that a high stromal miR-21 level correlated with worse overall survival in patients with PC who received adjuvant 5-fluorouracil but not gemcitabine. A meta-analysis showed that miR-21 upregulation in tumor tissue and blood samples of patients with PC was significantly associated with poorer overall survival, disease-free survival, and progression-free survival. A significant correlation was detected between miR-21 expression and lymph node status and tumor grade^[51]. Frampton *et al*^[52] reported that, in addition to miR-21, other miRNAs, such as miR-10b, miR-34, miR-155, and

miR-203 also appear to have prognostic significance in pancreatic ductal adenocarcinoma. The dysregulation of miR-320a, miR-365, miR-200, and miR-210 has been found to be involved in tumor invasion, epithelial to mesenchymal transition development, and chemotherapeutic drug resistance in PC^[53]. Thus, silencing of specific miRNAs by chemically modified antisense oligonucleotides could be a novel therapeutic intervention for PC.

Inhibition of hedgehog signaling in PSCs

Bailey *et al*^[54] were the first to report that sonic hedgehog (Hh) ligands secreted by PC can activate the canonical Hh signaling pathway in PSCs, resulting in their activation, differentiation, and proliferation. In addition, sonic Hh has been shown to promote desmoplasia in orthotopic mouse models of PC, and inhibiting sonic Hh with monoclonal antibody 5E1 markedly decreased the degree of desmoplasia^[54].

In their groundbreaking preclinical study, Olive *et al*^[55] assessed the effects of orally administered smoothened antagonist IPI-926 (or saridegib, a derivative of Hh inhibitor cyclopamine) on the tumor stroma and intratumoral uptake of gemcitabine in pancreatic tumor-bearing KPC mice. The result revealed that IPI-926 treatment considerably reduced the proliferation of stromal myofibroblasts, considerably depleted stromal components, and resulted in a transient increase in the intratumoral vascular density and intratumoral concentration of gemcitabine, facilitating transient disease stabilization. On the basis, in part, of

these findings, a phase I/III clinical study was commenced to assess the safety and efficacy of IPI-926 and gemcitabine combination treatment in metastatic PC^[56] (Table 1). The initial outcomes revealed that this combination was well tolerated and resulted in a partial response in 5 of 16 patients in the phase 1b portion of the study.

In another phase I study, IPI-926 was used in combination with 5-fluorouracil, folinic acid, irinotecan, and oxaliplatin (FOLFIRINOX), a potent and intensive chemotherapy regimen, in the first-line treatment of advanced PC^[57]. The preliminary outcomes revealed that the unsubstantiated overall response rate was 66.7%, and that treatment-related toxicities were acceptable and tolerable. However, the initial findings of a phase I b/II study conducted by Catenacci *et al.*^[58] questioned the efficacy of Hh inhibition in advanced PC. The authors evaluated the synergistic activity of vismodegib, a small-molecule inhibitor of smoothened, and gemcitabine in patients with metastatic PC. They observed no safety concerns in the phase 1b portion of the study. In the phase II portion of the study, they randomized 106 patients into gemcitabine plus vismodegib or gemcitabine plus placebo groups, but observed no significant differences in the progression-free ($P = 0.30$) and overall survival ($P = 0.84$) between the two treatment groups. Moreover, the response rates were not significantly different (Table 1).

OTHER TARGETABLE ELEMENTS OF STROMA

Hyaluronan

Reportedly, the PC stroma might comprise a considerable amount of HA, which is a high-molecule glycosaminoglycan comprising repeating units of D-glucuronic acid and N-acetyl-glucosamine^[59,60]. Reportedly, HA levels in PC tissue might reach 12-fold higher than that found in healthy pancreatic tissue^[61]. In addition, PC cells typically express high levels of the primary HA receptor, CD44^[62,63]. When HA binds to CD44, four major signaling pathways activated in PC cells are as follows: RAS, Rac, MAPK, and phosphatidylinositol-3-kinase. In fact, signaling through these pathways accelerates the proliferation, epithelial-to-mesenchymal transition, stemness, and metastatic capacity of PC cells and increases their resistance against chemotherapeutic drugs^[64-70]. Besides its significant tumor-promoting effects, HA is a crucial contributor to the impaired blood perfusion of tumor cells, increased tumor hypoxia, and, more crucially, insufficient drug delivery to the tumor, as mentioned previously^[1,60,69,70].

Some preclinical studies have reported that the enzymatic degradation of HA using PEGylated human recombinant hyaluronidase PH20 (PEGPH20) in genetically engineered mouse models of PC could prompt the re-expansion of collapsed tumor vessels and promote doxorubicin and gemcitabine delivery. Furthermore, the combined use of gemcitabine and PEGPH20 exhibited a synergistic effect and substantially inhibited the tumor

growth, resulting in the upgraded survival of animals. Conversely, gemcitabine monotherapy only modestly affected the tumor growth compared with PEGPH20 alone^[60]. Provenzano *et al.*^[71] reported similar findings and observed that PEGPH20 effectively ablated HA from metastatic deposits as with primary tumors and reinstated the vascular pattern.

Consequently, a phase 1b study by Hingorani *et al.*^[72] evaluated the safety and efficacy of escalating doses of intravenous PEGPH20 combined with gemcitabine in patients with metastatic PC. The treatment was well tolerated by patients ($n = 28$) and exhibited a promising clinical activity. However, patients with tumors comprising higher HA levels seemingly benefited more from this treatment than those whose tumors had lower HA levels. In addition, the median progression-free and overall survival durations were 7.2 and 13 mo for patients with high HA levels and 3.5 and 5.7 mo for patients with low HA levels, respectively. Notably, these results encouraged further clinical research.

The final outcomes of phase 2 HALO-109-202 study, in which PEGPH20 was administered together with nab-paclitaxel plus gemcitabine regimen, were presented at the 2017 American Society of Clinical Oncology Annual Meeting^[73]. The study randomized 279 patients with untreated metastatic PC to receive either PEGPH20 plus chemotherapy (100 patients treated) or chemotherapy alone (160 patients treated). The combination therapy substantially improved the median progression-free survival (primary endpoint: 6.0 mo vs 5.3 mo; $P = 0.045$) in unselected patients. In HA-high patients (34% of enrolled patients), a significant increase was again noted in the progression-free survival with PEGPH20 plus chemotherapy compared with chemotherapy alone (median: 9.2 mo vs 5.2 mo; $P = 0.48$). However, no significant difference was observed between the two treatment arms regarding the overall survival (median: 11.5 mo vs 8.5 mo; HR, 0.96). Apparently, thromboembolic events pose a primary complication of PEGPH20 treatment. In the first stage of this phase 2 study, none of the patients randomized to PEGPH20 arm was provided thromboprophylaxis, and 43% of these developed thrombosis, causing a temporary cessation in the treatment. However, in the second stage, the rate of this complication was decreased to 28% with the administration of enoxaparin prophylaxis. PEGPH20 treatment was also associated with increased incidence and severity of other manageable side effects, such as painful muscle spasms, arthralgia, peripheral edema, and neutropenia. Overall, PEGPH20 is the first stroma-targeting agent that has demonstrated its efficacy in a clinical setting. Currently, a phase III study (HALO Pancreatic 301; NCT02715804) is recruiting patients with stage IV PC whose tumors have a high level of HA to validate phase II results.

In contrast, a recently presented randomized phase I/II study evaluating the efficacy of PEGPH20 and modified FOLFIRINOX in patients with metastatic PC who have a good performance status suggested that PEGPH20 can

have a detrimental effect on OS (HR = 0.48). Therefore, further studies are needed to clarify whether the benefit from the use of PEGPH20 is restricted to patients treated with gemcitabine and nab-paclitaxel^[74].

Secreted protein acidic and rich in cysteine

SPARC (also known as osteonectin or basement membrane protein 40) is a member of the matricellular proteins group and plays regulatory roles in cellular proliferation and adhesion. Guweidhi *et al.*^[75] described that primary and metastatic lesions of PC expressed SPARC 31-fold more compared with normal pancreatic tissue. In addition, PC cells fail to produce SPARC because of aberrant hypermethylation in their *SPARC* gene. Thus, almost all SPARC in PC tissue is produced by PSCs^[75-78]. Reportedly, SPARC can increase the migration ability and invasive properties of PC cells^[78-80]. In addition, SPARC can stimulate the MMP production in neoplastic cells, thereby enhancing their metastatic potential^[75,77,81,82]. Accordingly, patients with PC whose tumors contain elevated amounts of SPARC have been reported to have worse survival compared with those whose tumors contain lower SPARC levels following radical surgery or chemoradiotherapy^[80,83-85].

Owing to its high affinity for albumin, stromal SPARC, perhaps, increases the intratumoral delivery and efficacy of the chemotherapeutic drug albumin-bound paclitaxel (nab-paclitaxel) in patients with PC^[86]. In their phase I/II study, Von Hoff *et al.*^[86] examined the efficacy of escalating doses of nab-paclitaxel in combination with fixed doses gemcitabine in 67 patients with previously untreated metastatic PC. The treatment resulted in an overall response rate of 48%, and the median overall survival duration of 12.2 mo. In the study, the SPARC status was assessed in 36 patients, and patients whose tumors had high SPARC expression ($n = 19$) exhibited better overall survival than patients whose tumors displayed low SPARC expression (median: 17.8 mo vs 8.1 mo; $P = 0.0431$). In addition, the study established a significant correlation between the stromal SPARC level and the patients' survival ($P = 0.013$). However, SPARC in tumor cells did not exert any effect on survival ($P = 0.15$). Besides, the authors assessed the treatment-related stromal changes and intratumoral penetration of the drugs in a patient-derived xenograft mouse model of PC and demonstrated that tumors resected from mice treated with gemcitabine alone demonstrated an extensive desmoplastic stroma. However, tumors in mice treated with nab-paclitaxel alone or in combination with gemcitabine exhibited the reduced stromal content, which was accompanied by dilated tumor blood vessels. Thus, the intratumoral concentration of gemcitabine was determined to be 2.8-fold higher in nab-paclitaxel plus gemcitabine-treated mice compared with mice receiving gemcitabine alone.

On the basis of these results, Von Hoff *et al.*^[87] conducted a phase III study in which 861 patients with metastatic PC were randomly allotted to receive either nab-paclitaxel

plus gemcitabine or gemcitabine alone. Their findings established the superiority of the combination regimen over gemcitabine monotherapy. In addition, patients receiving nab-paclitaxel plus gemcitabine exhibited longer median overall survival compared with those receiving gemcitabine alone (8.5 mo vs 6.7 mo; $P < 0.001$). Furthermore, they demonstrated a better response rate (23% vs 7%; $P < 0.001$). Hence, it could be speculated that the tumor SPARC level could be used as a predictive marker to determine patients with advanced PC most likely to benefit from nab-paclitaxel-based chemotherapy.

CONCLUSION

Despite the determination of active chemotherapeutic regimens, such as nab-paclitaxel plus gemcitabine and FOLFIRINOX, in metastatic PC, the overall treatment outcomes remain inadequate. Perhaps, stroma-depletion strategies could provide novel treatment opportunities for patients with this formidable disease. Among them, the enzymatic degradation of stromal HA by PEGPH20 is currently the only effective method in the clinical setting. After the announcement of the final outcomes of the phase III HALO Pancreatic 301 study, PEGPH20 could be incorporated into standard-of-care treatment regimens in metastatic PC. Of note, promising preclinical effects of Hh inhibitors await clinical confirmation; however, these could exhibit a stronger activity and synergy when they are combined with potent chemotherapy combinations rather than gemcitabine monotherapy. Moreover, agents that have demonstrated promising anti-stromal activity in preclinical models, especially vitamin A and D analogs, warrant clinical testing and could extend the therapeutic armamentarium in the future.

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Pancreatic, periampullary and biliary cancer with liver metastases: Should we consider resection in selected cases?

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Abstract

AIM

To analyse the safety and efficacy of curative intent surgery in biliary and pancreatic cancer.

METHODS

An extensive literature review was performed using MEDLINE, Google Scholar and EMBASE to identify articles regarding hepato-pancreatoduodenectomy or resection of liver metastasis in patients with pancreatic, biliary tract, periampullary and gallbladder cancers.

RESULTS

A total of 19 studies were identified and reviewed. Major hepatectomy was undertaken in 391 patients. The median overall survival for pancreatic cancer ranged from 5-36 mo and for biliary tract/gallbladder cancer, it was 8-38 mo. The 30 d mortality rate was only 1%-9%. Overall Survival was significantly better for patients, who had good response to neoadjuvant chemotherapy, underwent metachronous liver resection and who had intestinal type tumours.

CONCLUSION

Resection of liver metastases in pancreatic and biliary cancers may provide survival benefit without compromising safety and quality of life in a very select group of patients. These data may be utilised to formulate selection criteria that may allow future investigation of resection in the era of more effective systemic therapy.

Key words: Pancreas; Liver resection; Gall bladder; Cholangiocarcinoma; Review

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Core tip: Hepatic resection may be feasible for highly selected pancreatic and biliary tract cancer patients with a propensity towards improved outcomes and provide a chance for long term survival. The longer disease free interval between primary tumour and the liver metastases, response to the neoadjuvant treatment and other prognostic markers may also facilitate better selection of patients with more favourable tumour biology and prognosticate individual patient.

Lee RC, Kanhere H, Trochsler M, Broadbridge V, Maddern G, Price TJ. Pancreatic, periampullary and biliary cancer with liver metastases: Should we consider resection in selected cases? *World J Gastrointest Oncol* 2018; 10(8): 211-220 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i8/211.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i8.211>

INTRODUCTION

Hepatic metastases are 18 to 40 times more common than primary liver cancers^[1]. Better understanding of tumour biology, improved techniques for liver resection^[2,3] and multidisciplinary treatments have led to new algorithms for managing metastatic disease in the liver. For selected patients, surgical resection of liver metastases in colorectal cancer has shown 5-year survival rates as high as 40% to 71%^[4-8] and for neuroendocrine tumours, the 5-year survival ranges between 61% to 76% can be achieved^[9-12]. Fewer studies have also shown benefit of liver resection in noncolorectal non-neuroendocrine liver metastases with 5-year survival of 36%^[13-15]. Very similar long term outcome was recently reported after liver resection for non-colorectal non-neuroendocrine liver metastases in an Australian setting^[16].

There is existing evidence in literature including a national registry based study in Sweden which documents feasibility and benefit of hepatectomy for hepatobiliary (pancreas, gall bladder and cholangiocarcinoma, ampullary) liver metastases^[17-25]. Prior reviews of gastric and oesophageal cancer have been published and suggest that in highly selected patients, prolonged survival can be achieved^[26]. However, resection of liver metastases from other primary tumours still remains controversial because of the heterogeneity of the data and fewer patients are referred for assessment of resectability.

Evolution of new neoadjuvant chemotherapy regimens has a significant potential to downstage cancers to potentially resectable state. This coupled with increased safety of liver resections has led to expansion of indications for patients being suitable for resection of metastatic disease, particularly in colorectal cancer^[27-31]. Importantly

significant advances have been made in systemic therapy in recent times for hepatobiliary cancers with regimens such as infusional FU, leucovorin, oxaliplatin, and irinotecan (FOLFIRINOX) in pancreatic cancer having response rates over 30%^[32]. Furthermore, staging of disease has improved greatly and therefore identifying oligometastatic disease, in particular isolated liver metastasis is far more accurate. With these changes in mind we undertook a review and analysed data in the literature related to the role of curative surgery for hepatic metastasis in periampullary, biliary and pancreatic cancers.

MATERIALS AND METHODS

A comprehensive literature search of MEDLINE, EMBASE along with Google Scholar was performed by using the following key words: Pancreatic cancer, biliary cancer, ampullary cancer, liver metastasis, metastasectomy, metastasis resection, pancreatoduodenectomy. The key words were identified either independently or in various combinations in order to retrieve the maximum number of relevant search results. Conference abstracts were also included due to limited studies available for analysis. Furthermore, the references of all selected articles were reviewed to identify any additional, potentially eligible studies. All the published studies conducted from 1996 to 2017 were included in analysis. Heterogeneity of studies was evaluated by analyzing comparability of the following items: Number of patients, grade or stage of disease, type of surgery performed, type of adjuvant treatment applied.

Study inclusion criteria

All prospective or retrospective studies reporting outcomes post liver metastasectomy for pancreatic and biliary tract cancer were included. The primary goal of this study was better evaluation of the safety and clinical efficacy of hepatectomy for synchronous and metachronous liver metastases of pancreatic and biliary cancer hepatic metastases. Outcome measures of interest included the post-operative mortality, median overall survival, 5-year survival rate and prognostic factors associated with survival.

Studies were restricted to those in English only and were excluded if outcome measures of interest especially survival data was not reported or could not be extracted. Studies limited to cell lines or animal models were also excluded from this review.

All studies meeting selection criteria were reviewed by the first and last author to determine eligibility. The data and studies available were too small and heterogeneous for a systematic review to be carried out (Figure 1).

RESULTS

Pancreatic ductal adenocarcinoma

A total of 11 studies were identified with 281 patients. Four studies evaluated benefit of both synchronous and

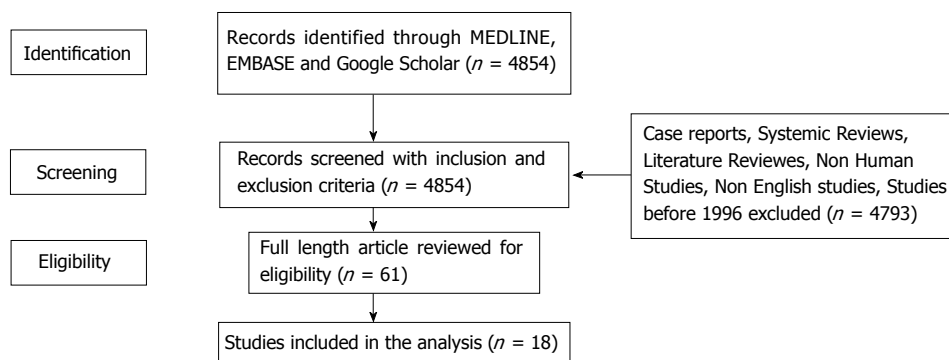


Figure 1 Literature review.

metachronous liver resection while 7 studies included only synchronous liver resection. Morbidity rate varied from 20% to 68%^[33-38]. For patients with synchronous liver metastases, most common type of pancreatic resection was pancreatoduodenectomy ($n = 125$) followed by distal pancreatectomy ($n = 75$) and total pancreatectomy ($n = 27$) and most common type of liver resection performed were atypical resection ($n = 61$), wedge resection ($n = 32$) and segmentectomy ($n = 25$) with hepatectomy ($n = 5$) being less common. Synchronous liver resection had higher morbidity than metachronous liver resection (33%-45% vs 0%-21%)^[39,40]. Common complications were infection, bleeding and pancreatic fistula. The 30 d post-operative mortality was between 0% to 9.1%^[36-39]. Sixty percent of patients had disease recurrence in liver after curative resection^[34,35] (Tables 1 and 2). A few case series have showed favourable results with regards to overall survival (OS). They have reported one-year survival rates of 36%-41% after synchronous resection of solitary liver metastases in patients with metastatic pancreatic cancer with proper patient selection^[33,36,41].

In a recently published retrospective multi-center analysis of six European centers consisting of 69 patients^[38], the 5-year survival was 0% in the non-resection group versus 5.8% in the group that underwent combined liver and pancreas resection (median OS was 14.5 in resected group vs 7.5 mo in non resected group, $P < 0.001$).

Some studies have reported a dismal survival of 5.6-8 months for patients with adenocarcinoma of the pancreas who underwent synchronous liver resection^[35,37,40,42]. Most of them died of recurrent disease within 12 mo of surgery^[37,40]. Zanini *et al.*^[35] reported median disease free survival (DFS) of 5.2 mo for 11 patients with 57% having disease recurrence in liver. All patients had moderate or poorly differentiated pancreatic ductal adenocarcinoma (PDAC). Chemotherapy may play an important role in selection of patients for liver resection and also to down-stage the tumour. Some series have suggested that response to neoadjuvant chemotherapy either radiological or biochemical (CA 19-9) may serve as a useful tool for careful selection of patients for aggressive surgery^[34,39].

Crippa *et al.*^[34] reported an impressive median OS of 36 mo for 11 patients who underwent surgical resection and 11 mo in chemotherapy only group ($n = 116$), similar to median OS seen in FOLFIRINOX group in ACCORD11 trial^[32]. In their study, patients were considered for liver metastasectomy only if they achieved complete or partial response after neoadjuvant chemotherapy. Seven percent had complete response and 37% had partial response to chemotherapy. The different chemotherapy regimen used were FOLFIRINOX, PEXG/PDXG: Cisplatin, capecitabine, gemcitabine plus either epirubicin (PEXG) or docetaxel (PDXG) and PEFG: Cisplatin, epirubicin, fluorouracil and gemcitabine.

In case series by Hackert *et al.*^[39], 85 out of 128 patients with liver metastases showed survival benefit of radical surgery with 5 year survival of 8.1%. Of these, 16% ($n = 20$) received neoadjuvant and 57% ($n = 73$) completed adjuvant chemotherapy. 79.5% received gemcitabine, 8.2% 5-fluorouracil and 12.3% other schemes. In a recent retrospective study^[43], 24 out of 535 patients achieved complete radiological response of the liver metastatic lesions post neoadjuvant chemotherapy. The chemotherapy administered consisted of single-agent gemcitabine, combination of gemcitabine and nab-paclitaxel or FOLFIRINOX regimen.

Prognostic factors and patient selection

In general longer survival has been reported after resection of metachronous disease when compared to synchronous resection of liver metastases in pancreatic cancer and this may be a potential factor in patient selection^[35,40,44]. Overall survival was better in metachronous group which was 11.4 mo against 8.5 mo for synchronous group^[40].

Several case series determined prognostic factors associated with worse outcome however results differ. In some studies, independent predictors of OS for patients with metastatic pancreatic cancer included resection status, use of multiple agents of chemotherapy, reduction in CA 19-9 level less than 50% of baseline value and > 5 liver metastases^[34,38,43]. In contrast other studies have not confirmed that survival is influenced by tumour location (head/body/tail), size and number of liver metastases,

Table 1 Studies for pancreatic cancer

	<i>n</i>	Age	No. of hepatic metastases	Median size of liver metastases	Chemotherapy	Mortality rate
Hackert <i>et al</i> ^[39] , 2017	85	60	96% had 3 lesions 3 had > 3 lesions	31% had 1-2 cm 43% had < 1 cm	74% received Adjuvant gemcitabine or 5 FU	2.90%
Crippa <i>et al</i> ^[34] , 2016	11	65 (35-80)	10% had 1 28% had 1-5 61% had > 5	NA	Neoadjuvant gemcitabine (14%), 30% gemcitabine + nab-paclitaxel while 66% had FOLFIRINOX, PEFG, PEXG or PDXG	0
Tachezy <i>et al</i> ^[38] , 2016	69	65 (31-83)	2 (1-11)	NA	Neoadjuvant gemcitabine in 4% or FOLFIRINOX in 14%. Adjuvant in 80%, 80% got gemcitabine and 7% FOLFIRINOX	1%
Zanini <i>et al</i> ^[35] , 2015	15	55 (52-64)	2 (1-3) 60% had 1 lesion	2.2 cm (1.8-2.5)	Adjuvant gemcitabine	0
Klein <i>et al</i> ^[33] , 2012	22	57.5 (31-78)	NA	NA	Adjuvant gemcitabine	0
Dünschede <i>et al</i> ^[40] , 2010	9	55 (39-72)	3 (1-5)	3.5 (1-9)		0
Gleisner <i>et al</i> ^[37] , 2007	17	64.7 ± 11.4	1 (1-1)	0.6 (0.3-1.2)	6 received 5FU or gemcitabine	9.10%
Shrikhande <i>et al</i> ^[36] , 1996	11	65 (60-74)	2 (1-3)	NA	Adjuvant Gemcitabine or 5FU or radiation	0

5FU: Fluorouracil; FOLFIRINOX: Oxaliplatin, irinotecan, fluorouracil and leucovorin, cisplatin; PEXG: Gemcitabine plus capecitabine and epirubicin; PDXG: Capecitabine and docetaxel; PEFG: Epirubicin and fluorouracil; NA: Not available.

Table 2 Results by outcome for pancreatic cancer

	<i>N</i>	Median OS(mo)	95%CI	<i>N</i>	Median OS (mo)	95%CI	<i>P</i> -value
		Resection			No resection		
Positive studies							
Hackert <i>et al</i> ^[39]	85	12.3		NA			
Tachezy <i>et al</i> ^[38]	69	14	10.8-18.2	69	7.5	4.9-10.2	< 0.001
Crippa <i>et al</i> ^[34]	11	39		116	11		< 0.0001
Klein <i>et al</i> ^[33]	22	16.6		NA			
Yamada <i>et al</i> ^[75]	11	10.1		28	6.8		NS
Shrikhande <i>et al</i> ^[36]	11	11.4	7.8-16.5	118	5.9	5.4-7.6	0.04
Negative studies							
Zanini <i>et al</i> ^[35]	15	9.1	8.6-9.7	NA			
Dünschede <i>et al</i> ^[40]	9	8 (4-16)		5	11 (10-12)		
Gleisner <i>et al</i> ^[37]	22	5.9		66	5.6		0.46
Takada <i>et al</i> ^[42]	11	6 (2-10)		33	3 (2-9)		

NA: Not available; NS: Not significant.

preoperative CA 19-9 levels and resection margin status^[35,37,39,42].

Biliary tract and ampullary cancers

Eight studies were identified with 110 patients. Two studies evaluated synchronous resection of the primary as well as metastatic liver lesions^[37,45], 2 studies only included staged resection in their analysis^[46,47] while remaining 3 studies evaluated efficacy of both synchronous and metachronous resection^[13,48,49] (Tables 3 and 4). Few case series^[46,48] have reported morbidity rate of 30%, infection being most common and post-operative mortality rate of 1%-21%^[37,45,48]. About 60%-70% had disease recurrence mainly in liver^[46-48].

In a study from Japan, 10 out of 64 patients who underwent radical resection for gall bladder cancer with liver metastases, had median survival of 17.2 mo, in contrast to 4.4 mo in palliative surgery group (*n* = 12)

and 7 mo in non-curative resection group^[50]. Fujii *et al*^[46] reported an impressive survival of 3 years for patients with periampullary carcinoma (*n* = 7; cholangiocarcinoma *n* = 2, ampulla of vater *n* = 2, duodenal cancer *n* = 3) following liver resection who had longer interval between treatment of primary cancer with pancreatoduodenectomy and occurrence of solitary liver lesion. Some studies failed to show any survival benefit with hepatectomy for biliary tract cancers^[37,45,49]. The median survival ranged from 5 to 15 mo with 3 years survival rate of 6%^[37] and 5-year survival rates lower than 20%^[45] after liver resection.

The only prospective study by Kurosaki *et al*^[47] showed that hepatectomy for a solitary metastasis in distal common bile duct cancer and ampulla of vater cancer was associated with improved overall survival of 44.9% at 5 years compared to patients with unresectable liver disease with shorter survival rate of less than 2 years. 13 patients underwent liver resection for

Table 3 Studies for biliary tract cancer

	N	Age (yr)	No. and size of hepatic metastases	Treatment	Median OS (mo)	Mortality Rate	Survival rate %
Kurosaki <i>et al</i> ^[47] , 2011	Distal bile duct (<i>n</i> = 7) Ampullary cancer (<i>n</i> = 6)	65 ± 10	Median no = 2 (1-3) Median size 3 cm (1.8-6 cm)	Adjuvant cisplatin + 5 FU or gemcitabine or S1 (<i>n</i> = 10)	Bile duct = 14 Ampullary = 20	-	5-yr = 44.9%
Bresadola <i>et al</i> ^[49] , 2011	Gall bladder (<i>n</i> = 5) Papilla of Vater (<i>n</i> = 3) Biliary tract (<i>n</i> = 1)	56 (46-64)	-	-	Gall bladder = 5 (1-12) Papilla of Vater = 7 (5-71) Biliary tract = 17	3%	
de Jong <i>et al</i> ^[48] , 2010	Ampullary (<i>n</i> = 10) Duodenal (<i>n</i> = 5) Biliary (<i>n</i> = 5) Pancreas (<i>n</i> = 20)	63.0 ± 10.6	Median no 1(1-5) and median size 0.7 (0.2-5.9)	Neoadjuvant chemotherapy (pancreatic <i>n</i> = 4 ampullary <i>n</i> = 2 duodenal <i>n</i> = 1) Adjuvant chemotherapy <i>n</i> = 22 (55%) Gemcitabine (<i>n</i> = 14) 5-fluorouracil (<i>n</i> = 4), cyclophosphamide (<i>n</i> = 2) Combination irinotecan (<i>n</i> = 3)	Intestinal type = 23 Pancreatobiliary = 13	5%	3-yr survival Intestinal tumours = 33% Pancreatobiliary tumours = 8%
Wakai <i>et al</i> ^[45] , 2008	Extrahepatic cholangiocarcinoma; adeno- carcinoma (<i>n</i> = 2) Gall bladder; adeno-squamous (<i>n</i> = 1)	63 (35-79)	-	-	Bile duct = 8 and 15 gall Bladder = 9	21%	5 yr = Extra hepatic 12% Gall bladder 9%
Gleisner <i>et al</i> ^[37] , 2007	Ampullary (<i>n</i> = 1) Duodenal (<i>n</i> = 2) Distal bile duct (<i>n</i> = 2) Histology Adenocarcinoma	65(53-82)	Median no = 1 and median size 0.6 cm (0.3-1.2)	FOLFIRI given to duodenal cancer	9.9	9.10%	3 yr = 6.7%
Adam <i>et al</i> ^[13] , 2006	Ampullary (<i>n</i> = 15) Pancreatic (<i>n</i> = 41) Gallbladder (<i>n</i> = 23) Biliary (<i>n</i> = 5)	53 (10-87)	-	-	Ampullary = 38	-	5 yr Ampullary = 46% The entire cohort = 27%
Fuji <i>et al</i> ^[46] , 1999	Bile duct (<i>n</i> = 2) (adenocarcinoma) Ampulla of vater (<i>n</i> = 2) Duodenal cancer (<i>n</i> = 3)	58 (36-67)	Median no = 1	-	20	-	3 yr = 28%

metachronous liver metastases for adenocarcinoma of distal cholangiocarcinoma (*n* = 7) and adenocarcinoma of ampullary cancer (*n* = 6). In subgroup analysis, patients with solitary lesion, R0 resection and who received adjuvant chemotherapy consisting of cisplatin + 5 fluorouracil or gemcitabine or S1 benefitted the most with longer overall survival. Whereas those with multiple hepatic lesions, R1 resection and did not receive adjuvant chemotherapy had early tumour recurrence and a short survival period of less than 2 years following the operation. The pattern of re-recurrence after hepatectomy was favoured the remnant liver.

Prognostic factors and patient selection

In some studies resection of liver metastasis proved advantageous in a subset of patients with intestinal-type tumours compared to those with pancreatobiliary lesions. de Jong *et al*^[48] analysed patients by tumour origin and

by presentation (synchronous vs metachronous). Among the 40 patients in the study, 50% had pancreatic cancer (*n* = 20), with fewer patients having an ampullary (*n* = 10), duodenal (*n* = 5) or biliary (*n* = 5) tumour. 5-fluorouracil, gemcitabine and irinotecan based regimens was offered as neoadjuvant therapy to 7 patients and adjuvant treatment to 22 patients. Survival was affected by tumour origin. Specifically, patients with a pancreatobiliary tumour (*i.e.*, pancreas or distal cholangiocarcinoma) had worse survival compared with patients with intestinal-type tumours (*i.e.*, ampullary or duodenal); 23 mo vs 13 mo, respectively; *P* = 0.05; 3 years survival of 33% vs 8%). Post-operative mortality was only 5% in contrast to other studies.

Similar findings were reported by Adam *et al*^[13] where in a cohort of patients with ampullary primary tumours that presented with metachronous liver disease, benefitted the most from resection with 5-year overall rate of

Table 4 Results by outcome for biliary tract cancer

	N	Median OS (mo)	95%CI		N	Median OS (mo)	95%CI
		Resection				No resection or palliative surgery	
Positive studies							
Fujii <i>et al</i> ^[46]	7	20			NA		
Kurosaki <i>et al</i> ^[47]	13	28-60			9	6-12	
Niguma <i>et al</i> ^[50]	10	17.2			12	4.4	
de Jong <i>et al</i> ^[48]	8	17-19			7	7	< 0.01
Adam <i>et al</i> ^[13]	15	38			NA		
Negative studies							
Gleisner <i>et al</i> ^[37]	5	9.9				6	0.43
Wakai <i>et al</i> ^[45]	3	9			NA		
Bresadola <i>et al</i> ^[49]	7	15			NA		

NA: Not available.

46% compared to those with pancreatic cancer with 5-year survival rate of 27%. For biliary tract cancers, survival was not affected by number or size of liver metastases and disease presentation (synchronous or metachronous)^[48] but tumour origin had a major effect on long-term outcome^[13,48]. Survival benefit was seen in patients with longer duration of disease free survival between primary surgery and occurrence of solitary liver lesion^[13,46], with R0 resection and received chemotherapy^[47].

Systemic therapy in the future

Recent progress in systemic therapy may play a role in increasing surgical options. In particular for pancreatic cancer response rates have increased from under 10% to now over 30% in some trials. FOLFIRINOX^[32] and gemcitabine with nab paclitaxel^[51] have substantial activity in metastatic PDAC with response rate of 31% and 23%. Furthermore, these regimens may convert a substantial number into resectable tumours. Few case series have demonstrated efficacy of these regimen in locally advanced and borderline resectable pancreatic cancer^[52,53]. With FOLFIRINOX, overall response rate reported range from 30% to 50%^[54,55], resection rates 40%-50%^[56-58] with 40%-90% having R0 resection^[57-59]. In similar patient groups gemcitabine and nab paclitaxel, has a response rate of 30%^[60,61] with resection rate of 56% and R0 resection rate of 80%^[61].

New treatment modalities are being evaluated using genomics-driven precision medicine for advanced pancreatic ductal carcinoma. COMPASS is a prospective study which showed that patients with an "unstable" genomic subtype responded well to m-FOLFIRINOX while tumours that displayed basal-like RNA expression signature were chemotherapy resistant^[62]. Pancreatic cancer tissues have a higher expression of CD40 as compared to adjacent normal tissues. A combination of CD40 agonist antibody with gemcitabine showed tumour regression in advanced PDAC with liver metastasis^[63].

Similarly in biliary tract cancer, gemcitabine and cisplatin is now the treatment of choice in metastatic setting with response rate of 36%^[64]. A retrospective analysis also evaluated the activity of gemcitabine-platinum-based regimen in 37 locally advanced gall bladder cancer patients

showing an overall response rate (ORR) of 67.5% with 17 patients (46%) that underwent R0 resection^[65].

Unlike pancreatic cancer, clinical data have suggested an encouraging future for targeting checkpoint pathways in biliary tract tumours^[66]. A phase 1b trial using PDL1 inhibitor monotherapy for PDL1 positive advanced biliary tract cancers (BTC) demonstrated modest antitumor activity with an overall response rate of 17.4% with 4 patients having a partial response^[67]. An additional group of BTC with mismatch-repair deficiency have shown impressive durable responses with checkpoint inhibitor therapy in a phase 2 study. Four cases of BTC had an objective response in 71% and PFS in 67% of these patients to pembrolizumab^[68]. There are clinical trials that are using combination immunotherapy or immunotherapy with chemotherapy in advanced biliary tract cancers. Like pancreatic cancer, genomic alterations in BTC may serve as biomarkers in predicting response to chemotherapy and immunotherapy^[69].

Potential biomarkers

Development of more efficacious approaches for pancreatic cancer treatment would require identification of biomarkers that can predict the response and toxicity to various therapeutic agents. In this regard, the predictive value of CA 19-9 was demonstrated in a retrospective cohort study^[39]. It was suggested that CA 19-9 predicts resectability as well as survival in PDAC patients. Highly elevated preoperative or increasing postoperative CA 19-9 levels were associated with low resectability and poor survival rates. Recently, pharmacogenomics profiling of circulating tumour and invasive cells (CTICs) isolated from patients with PDAC was evaluated as a predictor of tumour response, progression, and resistance^[70].

As 95% of PDACs harbour KRAS mutations (mKRAS), circulating tumour DNA (ctDNA) has potential utility in this setting. Recent study demonstrated that positive ctDNA KRAS in metastatic disease has been associated with lower PFS and OS^[71]. In a study by McDuff *et al*^[72], undetectable preoperative ctDNA following neoadjuvant treatment for locally advanced pancreatic cancer is associated good surgical outcome. This approach is worthy of further study also in stage 4 setting for incorporating

ctDNA with the goal of improving patient selection for surgery.

DISCUSSION

The resection of the primary tumour and synchronous liver metastases is not recommended under current national and international guidelines for the treatment of PDAC and survival data at this time for hepatic resection of metastatic pancreatobiliary adenocarcinomas is mixed.

PDAC represents one of the most aggressive tumours with a poor prognosis with 5-year survival of 1% in stage 4. The liver is the most common site of metastatic disease^[73]. Currently, the standard of care for PDAC patients with stage IV disease is systemic therapy with palliative intent. Surgical resection is hardly ever considered.

The studies on the surgical management of PDAC liver metastasis are all retrospective studies involving a small number of patients without well-defined indications for resection. The analysed groups were heterogeneous and information on parameters, such as the general condition of the patients, comorbidities, tumour-related symptoms and quality-of-life were also lacking. Few studies lacked control groups. In few case series of liver metastasectomy, the median overall survival was comparable in the patients who under underwent liver resection to that achieved with the standard chemotherapy regimen for stage 4 PDAC without surgery.

Regrettably, most studies were conducted long time back and did not include chemotherapy as part of neoadjuvant strategy. Also most studies did not include details of utilized chemotherapy regimens and the combination of FOLFIRINOX and metastasectomy has yet to be evaluated. The significantly higher response rate of this regimen and the increasing experience of its use in down-staging prior to resection may see a greater role for selected liver resection.

Despite these limitations, the data inferred from all the trials suggests that hepatic resection can be safe and may be appropriate for highly selected PDAC patients with a propensity towards improved outcomes and provide a chance for long term survival. A longer survival has been reported for patients who underwent curative intent surgery after neoadjuvant gemcitabine and with use of FOLFIRINOX or combination of gemcitabine and nab Paclitaxel, better response rate can be achieved with promising results as demonstrated in in the setting of locally advanced PDAC. Bile duct cancer and gallbladder cancer are aggressive diseases with poor prognosis with median survival time of 8-11 mo with chemotherapy in advanced setting^[64,74].

The evidence to support liver resection for biliary tract tumour is even more limited due to the paucity of cases of surgical treatment of biliary carcinoma, the diversity of surgical procedures and the surgical outcomes of the procedure have not been adequately analysed. In all the studies, there was no defined control group and lack of standard chemotherapy may have impacted long term outcome.

Adams *et al.*^[13], Kurosaki *et al.*^[47] and de Jong *et al.*^[48] revealed that liver metastasis from duodenal or ampullary-

origin tumour was accompanied by improved survival after surgery as compared with that from pancreatobiliary tumour with an impressive 5-year overall rate of 46%. This needs to be interpreted with caution due to small study population in each study. However this finding seems to reflect the differences in the behaviour of the primary tumour with periampullary cancers having better prognosis than pancreatic cancers. Promising outcomes of conversion pancreatectomy for locally advanced pancreatic cancer has been reported with better treatment regimens consisting of either chemotherapy or immunotherapy or combination and this may be a major change in the future. Multi-institutional prospective trials are required to fully delineate the potential therapeutic utility and operative indications of liver metastasectomy in the setting of modern interdisciplinary management of hepatobiliary tract tumours. The use of neoadjuvant and adjuvant chemotherapy with FOLFIRINOX or combination of gemcitabine and nab Paclitaxel for pancreatic cancer and with cisplatin and gemcitabine for gall bladder, cholangiocarcinoma and ampullary cancer in setting of synchronous or metachronous liver metastases should be standardized to avoid confounding results.

The disease free interval between primary tumour diagnosis and the discovery of a metachronous liver metastases and response to the neoadjuvant treatment may also facilitate the selection of patients with more favourable tumour biology and prognosticate individual patient. Incorporation of genomic profiling in clinical practice should be carried out for improved patient stratification and treatment selection. Furthermore the use of liquid biopsies and assessment of ctDNA may have a major role here in allowing selection of patients with the lowest risk of systemic involvement being considered for surgical intervention.

Hepatic resection is safe and can be effective, with outcomes mainly dependent on primary tumour site and histology. Hence a decision for a resection must be made on a highly individual basis and is multifactorial, including the, age, performance status, favourable tumour biology, valid prognostic markers, local resectability patient preference and the individual risk of complications. Application of a possible statistical model based on key prognostic factors may provide further guidance for better patient selection for curative liver resection by predicting long-term survivals. Further prospective, adequately powered studies with appropriate control arms are warranted for external validation of existing prognostic markers for more accurate selection, stratification of patients for these procedures and confirm the benefit of hepatic metastasectomy for selected group of patients.

ARTICLE HIGHLIGHTS

Background

Hepatic metastasectomy is well established for colorectal and neuroendocrine cancer with survival benefit. The overall prognosis for advanced pancreas and biliary tract cancers remains dismal. The resection of the primary tumour and synchronous liver metastases is not recommended under current national and

international guidelines for the treatment of stage 4 pancreaticobiliary cancer and survival data at this time for hepatic resection under such circumstances is mixed.

Research frontiers

The studies on the surgical management of pancreaticobiliary liver metastasis are all retrospective studies involving a small number of patients. There are inconsistent results with regards to benefit of liver metastasectomy on overall survival. Hence why we conducted extensive literature review to analyse and consolidate findings from all the studies to evaluate the safety and feasibility of liver metastasectomy in setting of stage 4 pancreatic and biliary tract cancers.

Research Innovations

This paper showed that resection of liver metastases in pancreatic and biliary cancers may provide survival benefit without compromising safety and quality of life in a very select group of patients. Patients with metachronous liver metastases and with good response to neoadjuvant chemotherapy derived the most benefit. However most studies included in our review were conducted long time back and did not include chemotherapy as part of neoadjuvant strategy or used biomarkers to select patients. Evolution of new neoadjuvant systemic treatment such as FOLFIRINOX and immunotherapy may have significant potential to downstage cancers to potentially resectable state. This coupled with increased safety of liver resections and discovery of potential biomarkers can aid in better population selection for resection of metastatic disease under such circumstances, with hope to improve the survival outcome.

Research perspectives

Our review highlights the need for multi-institutional prospective trials to fully delineate the potential therapeutic utility of liver metastasectomy for hepatobiliary tract tumours in era of modern systemic treatment and for further validation of prognostic markers used for patient selection. Comprehensive genomic profiling and use of ctDNA should also be considered for improved patient stratification and treatment selection.

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AIM AND SCOPE

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Immune blockade inhibitors and the radiation abscopal effect in gastrointestinal cancers

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Abstract

The field of tumor immunology has produced in the recent years a revolution in cancer therapeutics putting an end in the long lasting frustration of investigators in the area stemming from largely unsuccessful strides to develop cancer vaccines. This progress has come from the introduction of immune checkpoint inhibitors, monoclonal antibodies blocking ligand/receptor pairs with inhibitory effects for immune cells. Through this blockade immune checkpoint blockers are able to activate the immune system and create an anti-tumoral effect. A significant sub-set of patients with various types of cancers such as melanoma, lung carcinomas and urothelial cancers benefit from treatment with these drugs and survivals have improved in some cases. However other cancers are primarily resistant to immune blockers and secondary resistance is also the norm. Radiation therapy is often used in the palliative treatment of patients with advanced cancers and, in addition to the local effect in the irradiated field, it may in rare cases produce a systemic antitumor effect, termed "abscopal". This effect has been suggested to be produced by immune mechanisms. Thus an opportunity presents for a synergistic effect of immune stimulation between radiation and immune blockade inhibitors. The therapeutic opportunities presented with the combination of radiation and these drugs for gastrointestinal cancers will be discussed in this editorial overview.

Key words: Abscopal effect; Radiation; CD28/cytotoxic T-lymphocyte antigen-4; Immune blockade inhibitors; Programmed death 1; Programmed death ligand-1

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Core tip: Immune checkpoint inhibitors activate the immune response to tumors by blocking inhibitory

receptor pairs. Radiation treatment may also promote anti-tumor immune response. Thus, there exist an opportunity for synergy between the two treatment modalities that may be exploited therapeutically in gastrointestinal and other cancers.

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INTRODUCTION

Immune blockade inhibitors are a new class of anti-cancer drugs introduced over the last few years and moved to the first line treatment of some metastatic cancers as well as later line treatment of several others. Their indications expand with a quick pace and they are currently actively studied in the adjuvant setting. Their effectiveness has improved the outcomes of cancers such as metastatic melanoma and lung carcinomas, prolonging survival by several months^[1-3]. Most impressively there is a significant minority of metastatic patients treated with immune blockade inhibitors who obtain long-term disease control^[1-4].

The currently approved immune blockade inhibitors are monoclonal antibodies targeting CD28/cytotoxic T-lymphocyte antigen-4 (CTLA-4) or the programmed death-1/programmed death ligand-1 (PD-1/PD-L1) pair of immune blockade molecules^[5]. CTLA-4 inhibitors include ipilimumab and tremelimumab while inhibitors of the PD-1/PD-L1 pair include pembrolizumab, nivolumab (anti-PD-1), durvalumab, avelumab and atezolizumab (anti-PD-L1). Each one of these drugs has its own approved indications^[6]. The mechanism of action of these inhibitors involves re-enforcement of the cytotoxic activity of immune effector cells [cytotoxic T lymphocytes (CTLs) and NK cells] against tumor cells, by neutralizing inhibitory immune receptors expressed by tumor cells and antigen presenting cells. Both CTLs and NK cells may have cytotoxic effects that include targeting of cancer stem cells, believed to be at the root of cancer resistance to various therapies^[7,8]. Immune blockade inhibitors are overall well-tolerated and many patients are able to receive treatment without adverse effects for several months or even years. Immune adverse effects are not uncommon, though, and they have to be recognized and treated promptly. The most common such effects reported in phase III trials include pneumonitis, colitis, hepatitis, endocrinopathies and immune-mediated nephritis.

Radiation therapy is often used in metastatic cancers to control disease threatening vital organs such as the spinal cord or to palliate intractable symptoms such

as pain. Radiation treatment schedules in the palliative and metastatic setting tend to be shorter than definite or adjuvant treatments. Single fractionation treatments have become popular in the palliation of bone metastases due to their efficacy, convenience for the patient and cost effectiveness^[9].

It has been recognized for some time that, besides the local tumoricidal effect that takes place within the field applied, radiation therapy may have a systemic anti-cancer effect that affects cancer deposits outside the radiation field. This is termed the abscopal (off-target) effect. This effect is produced by the local radiation treatment which leads to production of new antigens through its tumoricidal effect. These antigens stimulate incoming immune effector cells and promote the systemic immune response to tumors through augmentation of the immune killing of tumor deposits in locations other than the irradiated tumor^[10].

Despite impressive results in some cancers, most patients, including the majority of patients with gastrointestinal (GI) cancers, do not respond to immune blockade inhibitor treatments. This paper will briefly discuss immune blockade inhibitors in GI cancers and explore ways to increase their responsiveness to the drugs using the abscopal effect of radiation.

IMMUNE BLOCKADE INHIBITION IN GI CANCERS

Immune blockade inhibitors have been studied in clinical trials for all major GI cancers. Representative results of the most advanced stage trials in different GI cancers are discussed in this section. A randomized phase III trial of nivolumab versus placebo in metastatic gastroesophageal cancer patients that had received or were intolerant to two previous lines of chemotherapy was conducted in three Asian countries (Japan, South Korea and Taiwan) and showed an 1-year overall survival (OS) of 26.6% with nivolumab versus 10.9% with placebo^[11]. Median OS increased by about a month from 4.14 mo in the placebo arm to 5.32 mo in the nivolumab arm. This difference, although modest, was statistically significant.

An initial phase Ib study of pembrolizumab focused on patients with PD-L1-positive metastatic gastric and gastroesophageal junction adenocarcinoma, defined as 1% or more positive cells (both cancer and inflammatory cells countable)^[12]. Partial responses were observed in eight of the 36 (22%) evaluable patients. The median OS was 11.4 mo and a subset of patients remained on treatment for more than 6 mo. Pembrolizumab was also investigated in an extensive phase II study that included a cohort of 259 gastroesophageal cancer patients from both Asian and western countries in the third line metastatic setting^[13]. Response rate (RR) was 11.6% and an additional 16.2% of

patients had stable disease. The study included both PD-L1-positive and PD-L1-negative patients and RR was higher in PD-L1-positive patients. In addition, in a small subset of patients that had tumors with microsatellite instability (MSI), RR was 57%^[13]. The anti-PD-L1 antibody avelumab was investigated in a phase Ib trial in patients with metastatic gastric cancer in the second line setting^[14]. This study which has only been published in an abstract form included also an arm with 89 patients receiving avelumab treatment as maintenance after chemotherapy. The median duration of treatment in this arm was about 3 mo with a range from 2 wk to over a year.

In hepatocellular carcinoma, nivolumab has been approved by the American Food and Drug Administration in the fall of 2017 for patients with disease not amenable to curative surgery or local treatments, with or without hepatitis B or C and treated previously with sorafenib. Approval was based on a phase I /II escalation/expansion trial that showed an overall RR of 14.3%^[15]. In most responders the duration of response was longer than 6 mo and in half of the responders it lasted for over a year. The anti-CTLA-4 antibody tremelimumab was shown in a phase I study of 20 patients with hepatocellular carcinoma and hepatitis C-associated liver cirrhosis Child-Pugh grade A or B to produce partial responses in 17.6% of evaluable patients (3 of 17)^[16]. Median OS was 8.3 mo. Trials with other immune checkpoint inhibitors are ongoing as well as a randomized trial of nivolumab versus sorafenib in the first line setting.

A small phase Ib/II study of pembrolizumab with chemotherapy (nab-paclitaxel and gemcitabine) in metastatic pancreatic cancer has included 17 patients of whom eleven were evaluable for response in the phase II part^[17]. A partial response or stable disease was obtained in all patients for a disease control rate of 100%. Median OS was 15 mo. Several other trials are ongoing in pancreatic cancer to clarify the clinical benefit of immune blockade inhibitors in this disease which still has grim prognosis.

Results of immune blockade inhibition as monotherapy in colorectal cancer as a whole are not encouraging. However, the subset of colorectal cancers with mismatch repair defects (dMMR) and MSI display a higher sensitivity to immune blockade inhibitors. In a phase II trial of pembrolizumab in patients with metastatic colorectal cancer the objective RR was 40% in patients with dMMR and 0% in patients with proficient mismatch repair (pMMR)^[18]. Median OS was not reached in dMMR patients and was 5 mo in pMMR patients. Similarly in a phase II study of nivolumab that included only metastatic dMMR or MSI colorectal cancer patients the objective RR was 31% and the disease control rate at 12 wk or longer was 51%^[19]. The combination of nivolumab with ipilimumab was even more effective in metastatic dMMR or MSI colorectal cancer patients

producing a RR of 55% and disease control rate at 12 wk or longer of 80%^[20].

Given these results and similar encouraging efficacy in non-colorectal cancers with dMMR and high mutation load tumors^[18], the American FDA has granted pembrolizumab with the first indication for use in any solid tumor with MSI/dMMR independently of primary site. Mutation load arises, thus, as a marker of effectiveness to checkpoint inhibitors independently of the underlying defect that creates this increased load. Besides MSI/dMMR, other genetic defects, such as mutations in polymerases ϵ (POLE) and $\delta 1$ (POLD1) may result in high tumor mutation load^[21]. These results and the fact that even among MSI/dMMR patients only a subset derive clinical benefit from immune checkpoint inhibitors illustrate the point that there is a need for further prognostic markers development in immune checkpoint inhibitors therapeutics. Improvement in characterization of responsive tumors may also help in discovering ways of inducing sensitivity in initially resistant tumors and tumors with acquired resistance.

MOLECULAR PATHOGENESIS OF THE ABS COPAL EFFECT

Double strand DNA (dsDNA) released in the cytoplasm of irradiated cells activates cGAS (cGMP-AMP synthase), an enzyme that synthesizes cyclic GMP-AMP (cGAMP). This and other dinucleotides activate protein STING (stimulator of interferon response) which results in production of type I interferons (type I IFNs) through the action of transcription factors IRF3 and NF- κ B and concomitant up-regulation of MHC I molecules and danger signals^[22,23]. Type I IFNs act in an autocrine and paracrine manner to promote the inflammatory environment that may result in an anti-tumor response, if additional conditions are fulfilled. These conditions include tumor antigen presentation by the cancer cells and absence of inhibitory signals that inhibit incoming immune effectors. Activated immune effector cells can kill tumors locally but also by travelling to other locations where tumor cells expressing the same antigens exist.

Ligation of PD-1 inhibitory immune receptor may have a negative effect in the development of abscopal effect^[24]. In a pre-clinical study in mice bearing tumors in two different locations, of which only one was irradiated with a single dose of 15 Gy, the abscopal effect observed in the non-irradiated tumor was stronger in mice receiving an anti-PD-1 antibody or mice that were knock-out for the PD-1 receptor than in control mice. Interestingly, PD-1 knock-out or antibody-treated mice had also a better response in the primary tumor site. In contrast, no difference was observed in a secondary tumor consisting of a different cell line from the one at the irradiated site, suggesting that activation of immune cells has to take place in the context of the relevant

antigen presentation and not in the context of antigens from a different tumor^[24]. Thus, the combination of the two treatments, radiation and PD-1 inhibitors, presents an opportunity of synergy, mediated by the local radiation-induced activation of immune cells and in parallel neutralization of inhibitory receptors by immune blockade inhibitors.

The dose of radiation treatment has been proposed to be of importance in the production or lack of abscopal effect. Experiments in mice showed that radiation treatment at a dose of 24 Gy in three fractions of 8 Gy each, in combination with an anti-mouse CTLA-4 monoclonal antibody was effective in inducing an abscopal effect in breast cancer xenografts^[25]. In contrast, a single fraction of 20 Gy was ineffective in inducing an abscopal effect, despite synergistic effects in control of the irradiated site that were similar. Use of five fractions of 6 Gy each was also successful in inducing an abscopal effect^[26]. Colon cancer xenografts exhibited similar behavior, responding in remote sites only when the radiation was fractionated. The differential effectiveness of different radiotherapy fractionations was traced to induction of DNA exonuclease Three prime Repair Exonuclease 1 (TREX1) by the higher radiation dose. Activated TREX1 cleaves cytoplasmic dsDNA, preventing cGAS activation and induction of the type I IFNs response. A single dose of 12 to 15 Gy or above was shown to induce TREX1 in different cell lines and a decrease in dsDNA production post-irradiation was observed in parallel^[25,27].

The timing of radiation treatment in relation to immune checkpoint inhibitors administration may be also relevant for obtaining an optimal abscopal effect^[28]. For example, when radiation precedes immune checkpoint inhibitors administration, it may help produce antigens that could serve as targets for the revitalized immune system. Conversely radiation therapy in patients already receiving immune checkpoint inhibitors may diversify antigens available for presentation and prevent immune exhaustion^[29]. These scenarios remain speculative as timings of the two treatments has not been directly examined and compared and further study of the optimal timing of the combination and whether it is critical is warranted.

ABSCOPAL EFFECT OF RADIATION THERAPY IN GI CANCER PATIENTS RECEIVING IMMUNE CHECKPOINT INHIBITORS

A few clinical studies that had included patients with GI cancers and described the abscopal effect of radiation therapy in patients receiving also immune checkpoint inhibitors have been published.

A radiation- ipilimumab combination phase I study of 35 patients with various cancers included seven patients

with GI cancers (4 colorectal, 2 gastroesophageal and 1 cholangiocarcinoma)^[30]. Patients received stereotactic ablative radiation therapy (SABR) to a total dose of 50 Gy in four fractions or 60 Gy in 10 fractions starting after the first or second dose of ipilimumab which was given at a dose of 3 mg/kg every 3 wk for 4 doses. Organs irradiated included the liver and the lung. Among the 31 assessable patients, three patients (10%) had an abscopal partial response and four additional patients had stable disease in non-irradiated lesions lasting at least 6 mo for a clinical benefit rate of 23%^[30]. Patients who derived clinical benefit from the radiation-immunotherapy treatment had an increased ratio of circulating CD4+/CD8+ lymphocytes as well as an increase in expression of receptors 4-1BB and PD-1 in CD8+ lymphocytes. It is not possible from the published report to derive the specific responses of patients with GI cancers.

A report of patients with metastatic cancers that were treated in a trial of the monoclonal anti-PD-L1 antibody durvalumab and concomitantly received radiation therapy for palliation of pain or compression symptoms or for dissociated progression of their disease included four patients with colon cancer^[31]. No evidence of abscopal activity was observed in any of these patients or any of the six patients with other cancers that were included in this report.

The tolerance of combination of immune checkpoint inhibitors and radiation therapy has been documented and no unexpected adverse effects have been observed^[30-32]. The irradiated organs do not show increased incidence of immune mediated adverse effects with either anti-CTLA-4 or anti-PD-1 monoclonal antibodies^[32]. Acceptable tolerance notwithstanding, these very early data suggest that there is significant room for improvement in the efficacy of combinations of immune checkpoint inhibitors and radiation therapy in GI cancers.

PERSPECTIVE: RADIATION ABSCOPAL EFFECT TO IMPROVE EFFICACY OF CHECKPOINT INHIBITORS IN GI CANCERS

Given that the radiation abscopal effect has an immunologic pathogenesis, and concomitantly checkpoint molecules are up-regulated in irradiated tissues, the combination of inhibitors blocking PD-L1, PD-1 or CTLA-4 has the potential to act synergistically with radiation^[33]. The combination of radiation therapy with immune blockade inhibitors to boost immune-mediated effects is under investigation^[34,35]. The potentiation of the efficacy of checkpoint inhibitors in GI cancers with high mutation burden, such as MSI-H colorectal cancers, by radiation therapy would be derived from a better priming of effector immune cells in the inflammatory environment

of irradiated tumors where the absence of checkpoint inhibition would negate the restrain of immune cells normally produced by immune checkpoint molecules up-regulation, as well as activation of incoming effector immune cells by inhibition of inhibitory receptors in other tumor locations^[36]. In the other hand, tumors with low mutation burden may require additional treatments such as chemotherapy to boost the radiation effect and increase their immunogenicity and presentation of antigens to the activated immune effectors. Mutation burden may not be the only determinant of immune blockade inhibitors efficacy. For example, EBV-positive gastric cancers are MSS and possess low mutation burden but display a high immune infiltration and a tumor environment with high expression of PD-L1^[37]. In addition, immune cells in EBV-positive as well as MSI-H gastric cancers were observed to penetrate in tumors, in contrast to MSS, EBV-negative gastric cancers where immune cells remained in the periphery of tumors^[38]. A case of an EBV-positive gastric cancer patient who derived benefit from treatment with the checkpoint inhibitor avelumab was recently reported illustrating the above points^[37]. Additional predictive biomarkers for response to immune checkpoint inhibitors combinations with radiotherapy would certainly help optimize treatment.

The aforementioned fractionation dose of radiation effect on production of abscopal effect deserves to be more studied in clinical trials for fractionation optimization. Moreover, besides effects on tumor cells, radiation has direct effects on lymphocytes that happen to be in the radiation field and thus may be adversely affected or killed. Lymphocytes are more sensitive in doses of radiation lower than those that have tumoricidal effect and inadvertent immunologic effect of radiation will have to be taken into consideration when designing optimal schedules. This could be more critical if more extensive fields or fields including significant amount of lymphatic tissue are used.

The organ irradiated may also have implications for an optimal production of an abscopal effect. Some studies have shown that a greater abscopal effect may be produced when liver is irradiated compared to irradiation of lung^[30]. The mechanism may involve enhanced production of cytokines from irradiated hepatic resident cells that could produce a systemic immune-promoting effect. As a result, a systemic abscopal effect would not be expected to be identical in different clinical radiation scenarios and this would have to be incorporated in the design of combination studies.

Clearly further studies will be needed for the optimal determination of indications for use of immune checkpoint inhibitors in GI cancers, based on biomarkers and the optimal incorporation of radiation treatment parameters in order to harness the abscopal effect.

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Real practice studies in oncology: A positive perspective

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Abstract

In the majority of phase III clinical trials, patients are generally excluded on the basis of specific comorbidities, performance status Eastern Cooperative Oncology

Group ≥ 2 , age ≥ 65 years, previous malignancies, brain metastases, active infections, psychiatric disorders, non-measurable disease, number and type of previous lines of chemotherapies or biologic therapies. A question is raised: Can results of phase III studies be extended to the general population? There is increasing attention to and a resurgence of some terms as "real world" or "real practice" which are wrongly viewed as contrary to clinical trial protocols. In fact, the general perception is that a contraposition exists between "wrong" (retrospective and biased) and "right" (prospective, randomized, well statistically designed) clinical research. We have to change this perspective. Real practice studies, generally retrospective in their nature, deserve to be reevaluated; biases are physiologically present but their punctual and rigorous description and analysis can help the interpretation of and in some cases reinforce results and their hypothesis-generating power. The correct and balanced interaction between clinical trials and real practice reports can help the scientific community to improve the knowledge on anti-cancer drug efficacy.

Key words: Clinical trials; Real practice; Methodology; Gastrointestinal oncology

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Core tip: Oncologic patients enrolled in phase III pivotal trials are usually selected on the basis of specific characteristics and they are quite different from the real practice populations: this could account for low reproducibility of results in the clinics real world. In this Editorial, differences between prospective clinical trials and real practice studies are discussed giving a critical and positive perspective on the results of real practice studies also through specific examples. The correct and balanced interaction between clinical trials and real practice results can help the scientific community to improve the knowledge on anti-cancer drug therapies.

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Anti-cancer drugs are evaluated through a process involving different phases of clinical research. The methodological pathway is based on: (1) "early" clinical trials (phase I) in unselected patients designed to find dosages and toxicities; (2) "intermediate" disease-oriented trials (phase II) designed to define activity and toxicity; and (3) "late" randomized trials (phase III) comparing the new versus standard treatment for efficacy (progression-free survival, overall survival, quality of life) in highly selected cohorts. In recent years the introduction of biologic therapies has partially changed some methodological issues further refining patient selection on the basis of specific molecular alterations and biomarkers. In the "post-marketing" period, phase IV studies can be pursued in order to evaluate predominantly the long-term safety in a greater number of patients; these are observational studies in their nature.

Usually, competent Authorities refer to phase III trials to register a specific drug or combination of drugs for a particular clinical use^[1]. Some exceptions to this process exist, but their descriptions are beyond the scope of the present Editorial. In phase III studies, the patients gain the same chance to undergo different treatments through randomization. The power of prospective, randomized phase III trials is to normalize any factor that could influence final results, so that treatment arms are quite equivalent with regard to known prognostic and predictive factors. However, one of the most important pitfalls of these trials resides in clinical criteria for patient selection^[2-5]. In the majority of phase III studies, patients are generally excluded if they present one or more of the following conditions: specific comorbidities (unstable diabetes, chronic liver or kidney diseases, cardio-vascular diseases, etc.), performance status Eastern Cooperative Oncology Group (ECOG) ≥ 2 , age ≥ 65 years, previous malignancies, brain metastases, active infections, psychiatric disorders, non-measurable disease. In some cases, other selection criteria such as number and type of previous lines of chemotherapies or biologic therapies are added. A question is raised: Can results of phase III studies be extended to the general population? This deserves some reflection because in clinical practice few patients present with the characteristics required by a phase III clinical trial; one or more excluding conditions are frequently present.

We have recently presented at European Society of Medical Oncology (ESMO) Congress 2018 (abstract 1927, "Folifiri-Aflibercept vs Folifiri-Bevacizumab as second-line treatment of RAS mutated metastatic colorectal cancer in real practice") a study reporting an efficacy comparison (overall survival) between two different second-line therapies (Folifiri-Bevacizumab,

arm A vs Folifiri-Aflibercept, arm B) in advanced RAS mutated oxaliplatin and bevacizumab-pretreated colorectal cancer patients in a "real world" population. There is actually need to clarify therapy of this clinical setting, and prospective randomized trials on these different sequences of therapy do not exist [Folfox-Bevacizumab first followed by Folifiri-Bevacizumab (arm A) or Folfox-Bevacizumab first followed by Folifiri-Aflibercept (arm B)]. In arm A, after an induction phase of 6 mo, maintenance with bevacizumab was permitted; by contrast no maintenance therapy in arm B was applied. Interestingly, in arm B we found a lower risk of cancer-related death vs arm A (HR: 0.42; 95%CI: 0.15 to 1.15; $P = 0.0425$) during the induction phase. Three important biases were present consisting of: (1) the predominance of more extended disease (> 2 metastatic sites) in arm B [26/43 (60.5%) vs 10/31 (32.2%) arm A; $P = 0.0414$]; (2) the duration of first-line chemotherapy which was significantly shorter in patients treated in arm B (12 patients < 6 mo arm B vs 1 patient in arm A; $P = 0.0278$); and (3) the lack of a maintenance treatment with aflibercept. These biases do not stultify even if they reinforce the positive impact of Folifiri-Aflibercept in RAS mutated advanced colorectal cancer.

Real practice studies may also have a hypothesis-generating role. Until now, after a long period of skepticism still resisting in some parts of the scientific community, many preclinical and clinical studies have demonstrated that the interactions between immune system and tumor cells can be exploited for therapeutic scopes. The issue is extremely complex, innovative and largely unknown and oncologists have just "started" to apply in clinics basic knowledges from the immunology. Very recently, we have collected information about a cohort of 47 multi-organ oligo-metastatic colorectal cancer patients refusing metastasectomies and treated with depotentiating courses of chemotherapy and stereotactic radiotherapy (SRT) finding high disease control, with median survival of 44 mo (95%CI: 39.9-52.1) and two patients still alive at 82 and 86 mo from diagnosis with stable disease. In that, a possible role is played by abscopal effect of SRT: First described in 1953 as an effect of radiotherapy, the abscopal effect was observed in the clinical practice when a localized treatment produced also the shrinking of untreated distant tumor masses. Evidences demonstrate that this phenomenon is mediated by the immune system leading to tumor cell recognition and destruction, a specific and regulated process involving lymphocytes, dendritic cells, T regulatory subset cells, and other suppressor cells^[6-8]. Based on that, many prospective clinical and translational trials in advanced lung, melanoma and colorectal cancer are now recruiting patients through protocols based on SRT and immunotherapies with different mechanisms of action (pembrolizumab, durvalumab, tremelimumab, dabrafenib, trametinib, MK-3475, etc.) (Clinicaltrials.gov).

There is increasing attention to and a resurgence of some terms as "real world" or "real practice" which are wrongly viewed as contrary to clinical trial protocols.

In fact, the general perception is that a contraposition exists between “wrong” (retrospective and biased) and “right” (prospective, randomized, well statistically designed) clinical research. We have to change this perspective. Real practice studies, generally retrospective in their nature, deserve to be reevaluated; biases are physiologically present but their punctual and rigorous description and analysis can help the interpretation of and in some cases reinforce results.

This perspective should be adopted also by editors, reviewers, clinicians and researchers when evaluating results of studies. Sometimes real practice study results are not consistent with those of phase III studies; this happens as much as the fraction of treated patients does not meet the eligibility criteria of the corresponding phase III trial. One recent example in colorectal oncology is represented by the clinical benefit obtained with trifluridine/tipiracil in refractory metastatic colorectal cancer patients in real life^[9] vs the phase III study^[10]. The correct and balanced interaction between clinical trials and real practice reports can help the scientific community to improve the knowledge on anti-cancer drug efficacy.

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Priming the seed: *Helicobacter pylori* alters epithelial cell invasiveness in early gastric carcinogenesis

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Abstract

Helicobacter pylori (*H. pylori*) infection is a well-established risk factor for the development of gastric cancer (GC), one of the most common and deadliest neoplasms worldwide. *H. pylori* infection induces chronic inflammation in the gastric mucosa that, in the absence of treatment, may progress through a series of steps to GC. GC is only one of several clinical outcomes associated with this bacterial infection, which may be at least partially attributed to the high genetic variability of *H. pylori*. The biological mechanisms underlying how and under what circumstances *H. pylori* alters normal physiological processes remain enigmatic. A key aspect of carcinogenesis is the acquisition of traits that equip preneoplastic cells with the ability to invade. Accumulating evidence implicates *H. pylori* in the manipulation of cellular and molecular programs that are crucial for conferring cells with invasive capabilities. We present here an overview of the main findings about the involvement of *H. pylori* in the acquisition of cell invasive behavior, specifically focusing on the epithelial-to-mesenchymal transition, changes in cell polarity, and deregulation of molecules that control extracellular matrix remodeling.

Key words: *Helicobacter pylori*; Plasminogen activation system; Invasion; Epithelial-to-mesenchymal transition;

Cell polarity; Gastric carcinogenesis

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Core tip: *Helicobacter pylori* (*H. pylori*) infection induces chronic inflammation in the gastric mucosa that, in the absence of treatment, may progress through a series of steps to gastric cancer (GC). GC is only one of several clinical outcomes associated with this bacterial infection, which may be at least partially attributed to the high genetic variability of *H. pylori*. Accumulating evidence implicates *H. pylori* in the manipulation of cellular and molecular programs that are crucial for conferring the cells with invasive capabilities, including reprogramming of the epithelial-to-mesenchymal transition signaling programs, changing of the cell apicobasal polarity, and remodeling of the extracellular matrix.

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INTRODUCTION

Persistent *Helicobacter pylori* (*H. pylori*) infection induces chronic inflammation in the gastric mucosa, which in susceptible individuals may progress to gastric cancer (GC)^[1,2]. The final clinical outcome of the infection depends on complex interactions among the infecting strain of the bacterium, the host, and the environment^[3]. The biological mechanisms underlying how and under what circumstances *H. pylori* alters normal physiological processes in such a way that sequential events culminate in the development of GC remain largely unknown.

A key feature of malignant transformation and progression is the invasion of malignant cells locally and then to distant sites (metastasis)^[4]. Invasion and metastasis occur through a series of events in which several processes take place, including reprogramming of signaling pathways that drive the epithelial-derived malignant cells into a mesenchymal-like phenotype, the so-called epithelial-to-mesenchymal transition (EMT), changing of the cell polarity, and remodeling of the extracellular matrix (ECM)^[5,6]. Several of these events are activated in gastric epithelial cells by *H. pylori* directly or as a result of the inflammatory reaction mounted in response to this bacterial infection. This review summarizes the current evidence implicating *H. pylori* in the activation of molecular and cellular mechanisms related to invasion in the early stages of the pathogenic series of events leading to GC. Specifically, we address the role of *H. pylori* in the deregulation of molecules that control EMT, cell polarity, and ECM remodeling.

EPIDEMIOLOGY

GC is the fifth most common and the third death-causing cancer worldwide^[7]. Incidence rates vary considerably depending on age and sex; however, the most substantial variation is connected to geographic location, with very well-established high- and low-risk areas across the world^[8,9]. GC incidence is steadily declining worldwide; and although the reasons are not clear, this may be at least partially linked to the concomitant decrease in *H. pylori* prevalence^[8]. The decrease, however, is not of the same magnitude in GC of different histological subtype or anatomical location^[10]. Similarly, mortality rate varies geographically, being particularly high in developing countries but declining globally^[8,9]. The 5-year survival rate remains below 30% in most countries, which is mainly connected to the fact that most of the cases are diagnosed at advanced stages, when therapeutic interventions are likely to fail.

HISTOPATHOLOGY

Several schemes are used for classifying GC according to microscopic and histological characteristics. The Lauren classification system is probably the most commonly used^[11,12]. The Lauren system divides GC into intestinal, diffuse and mixed subtypes, with important differences at the epidemiological, pathological and molecular levels^[11,13].

Marked epidemiological and etiological differences have been revealed for malignant tumors located in the distal part of the stomach and those of the proximal region^[14,15]. Therefore, anatomical location of the lesions is regarded as an important parameter in the classification of GC.

PATHOGENESIS

The pathogenesis of GC is a complex and multifactorial process in which environment and lifestyle, host genetics, and *H. pylori* infection play a role^[2,16-21]. As already mentioned, the pathogenesis of GC substantially differs depending on the histological and anatomical subtype. The intestinal subtype of GC, for instance, arises through a sequential series of steps known as the Correa cascade^[22], in which *H. pylori* plays a pivotal role. The infection is usually established early in life and persists lifelong in the absence of treatment, which in combination with environmental factors leads to sustained chronic inflammation characterized by infiltration of inflammatory cells in the gastric mucosa and expression of inflammatory mediators.

Intriguingly, most of the infected individuals remain asymptomatic, while others develop pathologies that are not related to GC. In a minority of infected people, the inflammation evolves into a chronic atrophic gastritis, which is regarded as a pre-neoplastic lesion^[22,23]. This may subsequently progress to intestinal metaplasia, dysplasia, and invasive carcinoma^[22]. Much less is known

about the pathogenesis of the diffuse subtype of GC^[24,25] and the malignant lesions arising in the most proximal segment of the stomach^[26].

H. PYLORI

Infection with *H. pylori* is one of the most prevalent bacterial infections worldwide^[27]. This bacterium utilizes several strategies for colonizing and surviving in the hostile environment of the stomach. Some of these are common bacterial mechanisms of acid resistance, such as proton pump activation, decarboxylases, and membrane lipid modification^[28]. More specific adaptations to the acidic environment include the enzyme urease, which is encoded by the *ure* gene cluster and catalyzes the conversion of urea into ammonium and carbon dioxide. Urease was, in fact, the first protein identified in *H. pylori* with a role in neutralizing gastric acid, and it is considered a virulence factor^[29] since it has proven essential to the survival of the bacterium in the gastric mucosa^[30,31]. Besides the *ure* gene cluster, transcriptional regulation in response to acid extends to other genes related to motility, chemotaxis, and virulence^[32].

The genetic variability of *H. pylori* is high, and it probably explains in part the association of this infection with several gastric and extra-gastric pathologies, in addition to GC. Some strains, however, are more strongly associated with GC, namely those harboring particular polymorphic variants in the gene encoding the vacuolating cytotoxin A (VacA) and the ones expressing the Cag pathogenicity island (Cag-PAI)^[3,33-36]. Despite no physical or functional relation known for *vacA* and *cag-PAI* loci, strains that express virulent VacA usually contain functional Cag-PAI^[35,37]. In addition to VacA and Cag-PAI, other virulence factors of *H. pylori* have been associated with gastric pathology, including BabA, SabA, OipA, and DupA (Figure 1)^[3,36].

INVASION AND METASTASIS

The dissemination of cancer cells from primary lesions to form new tumor colonies at distant sites is a key feature of cancer^[4]. This occurs in a multistep process, termed the invasion-metastasis cascade: cancer cells locally invade, intravasate into the vascular system, travel in the circulation, extravasate at distant sites, form micrometastatic nodules of cancer cells, and, finally, grow into overt metastatic lesions^[5,6]. Importantly, early in this series of events, malignant cells acquire traits that equip them with the ability to invade, leave, and travel to distant tissues. A centrally important process that confers epithelial-derived malignant cells with increased motility and invasiveness is the EMT program^[38,39].

In order to become invasive, cells commonly lose their apico-basal polarity due to rearrangements in the cytoskeleton, which maintains the shape and internal organization of the cells, and modifications in the intercellular unions that hold them together^[39,40]. Also, the degradation of ECM components is essential

in several phases of the invasion-metastasis cascade. ECM remodeling is primarily mediated by proteases that belong to the plasminogen activation (PA) system and the matrix metalloproteinase (MMP) family^[41,42]. The cellular and molecular mechanisms underlying these processes, as well as their regulation, have been reviewed in depth^[39,40,43-45]. Accumulating experimental evidence has implicated *H. pylori* in all these aspects (Figure 2), as discussed below.

EMT and *H. pylori*

EMT is an evolutionary conserved, reversible process in which polarized epithelial cells acquire a mesenchymal phenotype through phenotypical and biochemical changes, thereby resulting in increased capacities of migration, invasion, and apoptosis resistance as well as ECM production and remodeling^[38]. Transcription factors such as Snail, Slug, zinc-finger E-box binding (ZEB1/2) and FOXC2 are activated at the beginning of the process. This is accompanied by the expression of specific microRNAs (miRs), for instance the miR-200 family, changes in the expression of particular cell surface proteins, cytoskeletal reorganization, and activation of Wnt/ β -catenin and Notch signaling^[38,46,47].

A critical feature of EMT is the down-regulation of E-cadherin^[48], a surface glycoprotein expressed in epithelial cells that is a key component of the adherent junctions in epithelial tissues^[49]. Expression of E-cadherin can be repressed directly or indirectly by multiple transcription factors, including ZEB1/2, Snail, Slug, nuclear factor-kappa B (NF- κ B), E47 and KLF8, but also by the proteins SIX1 and FOXC2^[50-52]. Furthermore, various signaling pathways can influence the expression of E-cadherin, including TGF β , hypoxia-induced response, Wnt/ β -catenin, Notch and PI3K/Akt, and therefore play a role in EMT^[53,54]. Although EMT is usually depicted as a binary switch that shifts cells from a fully epithelial to a fully mesenchymal state, this is a misrepresentation of this process. Frequently, the EMT program drives cells from a fully epithelial state to a partially mesenchymal one in which some epithelial markers are retained. Nonetheless, this subset of mesenchymal traits has profound effects on the cell biology^[55].

Activation of EMT programs in neoplastic cells is usually connected to their dedifferentiation and acquisition of stem cell-like properties^[56]. The existence of cancer cells with stem-like properties, the so-called cancer stem cells (CSCs), was first described in breast cancer and subsequently documented in various malignancies, including GC. One of the first studies about CSCs in GC showed that these cells have enhanced capability of invasion and tumorsphere formation. Also, it was found that CSCs have distinctive features of the EMT, such as reduced expression of E-cadherin and increased levels of vimentin and MMP2^[57]. In primary GC tissue, it was demonstrated by immunohistochemistry that the combination of Snail-1, vimentin, E-cadherin and CD44 predicts tumor aggressiveness^[58]. Furthermore,

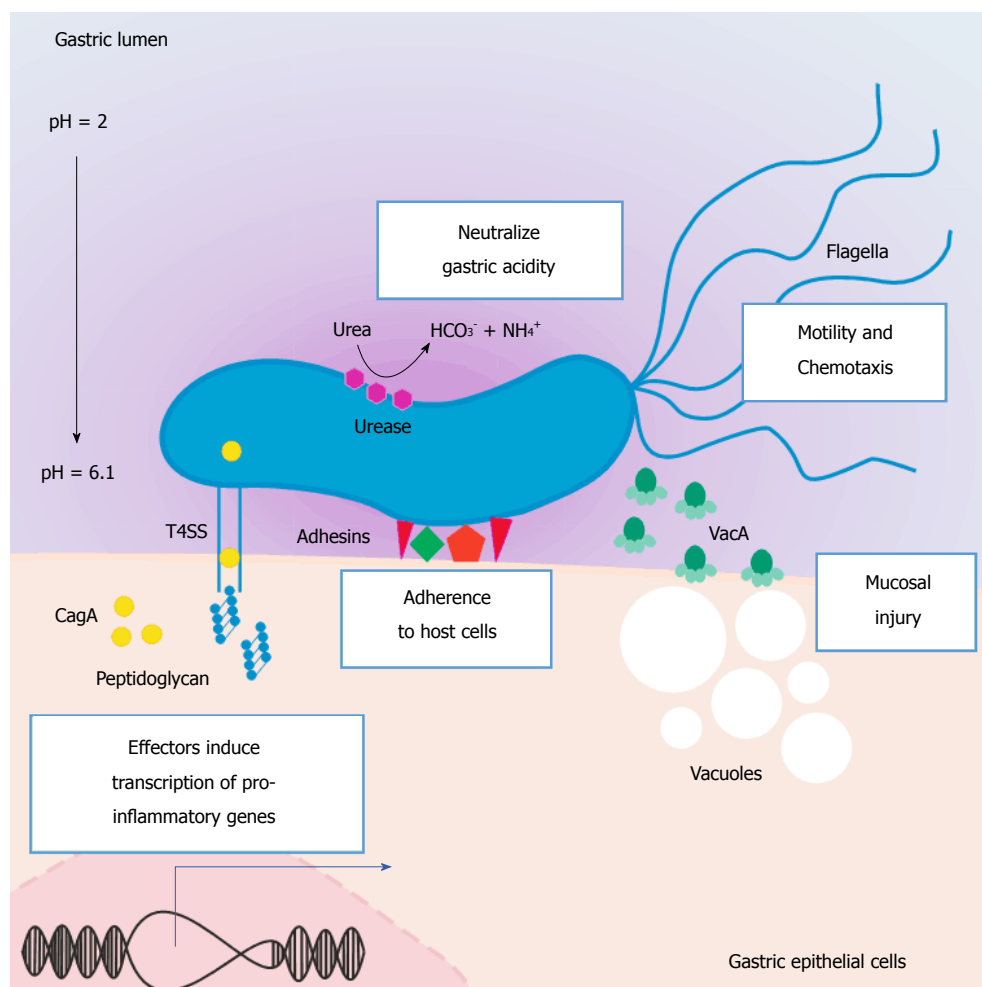


Figure 1 *Helicobacter pylori* virulence factors associated with gastric pathogenic processes. *H. pylori* is genetically highly variable, and some strains are more strongly associated with gastric pathologies, including GC. The most prominent are those that express the virulence factors VacA and Cag-PAI. VacA is a cytotoxic protein expressed by the polymorphic gene *vacA* that induces the formation of vacuoles, thus generating damage in the gastric epithelium. The Cag-PAI-positive strains (approximately 60%) possess a functional genetic region, which contains approximately 30 genes that code for proteins, that together make up a T4SS. The secretion system introduces a number of molecules, including the virulence factor CagA and peptidoglycans, into the cytoplasm of epithelial cells of the gastric mucosa. Once in the cytoplasm, CagA is phosphorylated, and this triggers downstream intracellular events, such as cytoskeletal rearrangement, alterations in cellular polarity, expression of inflammatory mediators, and activation of signaling pathways that promote cellular proliferation. The conversion of urea into ammonium and carbon dioxide by urease is essential to the survival *H. pylori* in the stomach. Flagella play an important role in the colonization of the gastric mucosa, as they produce differential motility depending on the pH of the stomach lumen and the concentration of compounds such as urea, thus enabling *H. pylori* bacteria to swim across the mucous layer towards the epithelial lining. Other less well-characterized virulence factors of *H. pylori* associated with gastric pathology are the adhesins, which include BabA, SabA, and OipA. Cag-PAI: Cag pathogenicity island; GC: Gastric cancer; *H. pylori*: *Helicobacter pylori*; T4SS: Type IV secretion system; VacA: Vacuolating cytotoxin A.

it has been reported that MKN7 GC cells undergoing Wnt5a-induced EMT acquire CSC properties^[59], similar to what has been observed in hypoxia-driven EMT *in vitro* models with the BGC823 and SGC7901 GC cell lines^[60].

Using *in vitro* systems, it was revealed that *H. pylori* infection results in the activation of EMT programs and the emergence of CD44^{high} cell populations with CSC properties in the AGS, MKN45 and MKN74 GC cell lines^[61]. These cells acquire elongated shape and show enhanced expression of mesenchymal markers (*i.e.*, Snail1, ZEB1, and vimentin). Compared to the CD44^{low} cells, the CD44^{high} GC cell population gained the ability to migrate and invade and was better at forming tumorspheres *in vitro* and tumors in immunodeficient mice. According to that study, the induction of the EMT

and CD44^{high} cell population was dependent on the CagA oncoprotein. CagA induces the EMT in a number of ways, as exemplified by a recent *in vitro* study showing that this bacterial protein up-regulates MMP3, which is also part of the EMT program, through EPIYA motifs in a phosphorylation-dependent manner^[62]. Immunohistochemistry staining of human and murine gastric tissue have confirmed that *H. pylori* infection is correlated with high expression of CD44 and EMT markers^[61]. Presumably, ERK and JNK are involved in the described EMT-like changes, CD44 overexpression and the ability to form tumorspheres *in vitro* that is triggered by *H. pylori* cagA-positive strains^[61].

Other studies addressing the potential induction of CSC-like properties by *H. pylori* concluded that Wnt/

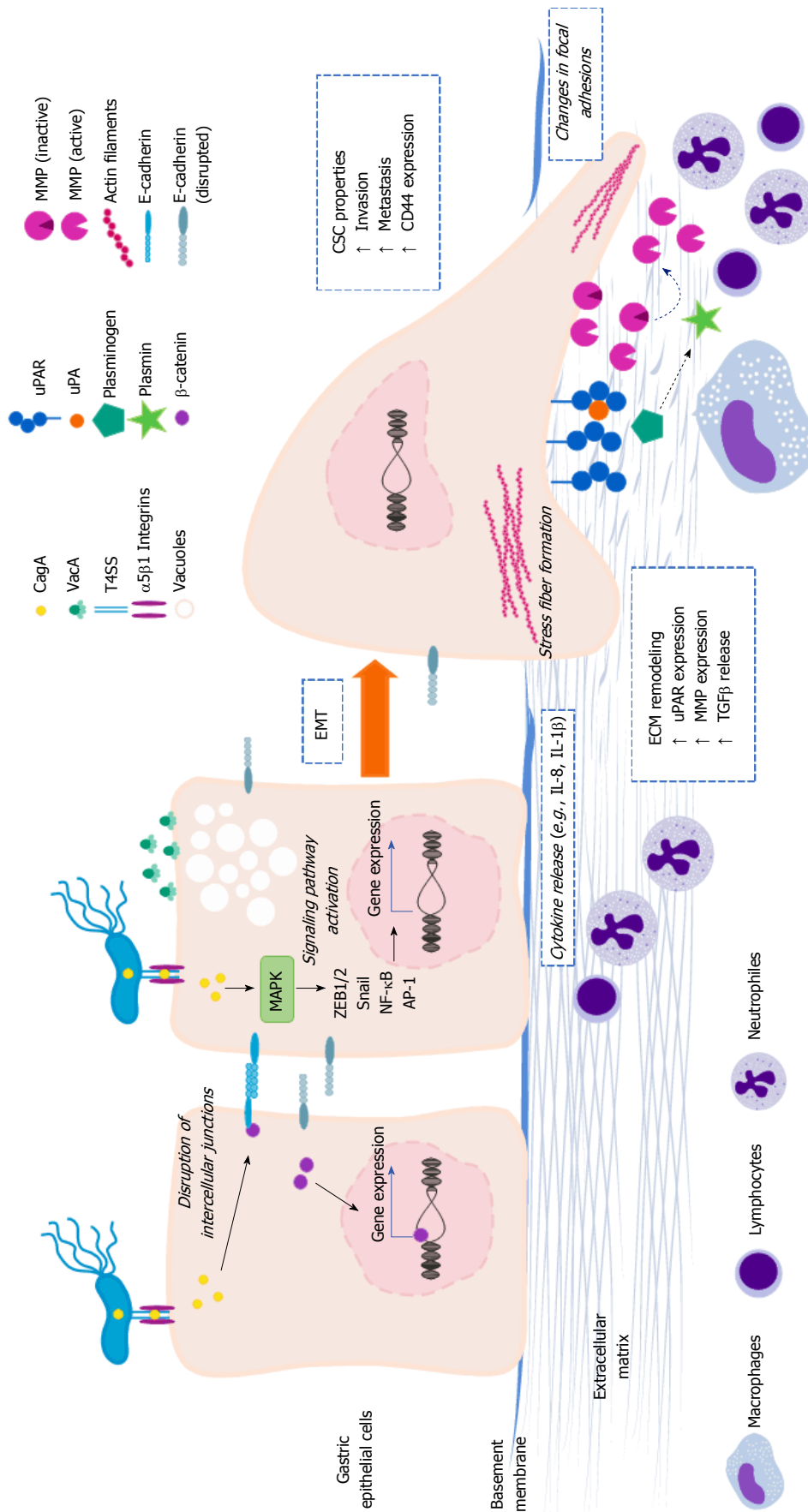


Figure 2 Role of *Helicobacter pylori* in the reprogramming of cellular and molecular programs related to invasive behavior. Upon infection, *H. pylori* induces a number of intracellular processes in gastric epithelial cells, some of them can result in the reprogramming of cellular and molecular mechanisms underlying the invasive cell behavior. Several of these events take place after phosphorylation of CagA, others may be independent of the translocation and phosphorylation of CagA, and a portion are not connected to the Cag-PAI at all. Although not exclusively, CagA plays an important role in the activation of transcription factors, such as β-catenin, Snail, ZEB1/2, NF-κB, and AP-1. Some of these transcriptional regulators (e.g., β-catenin, Snail, ZEB1/2) modify the expression of genes encoding for key effectors of the EMT and stem cell programs, including up-regulation of TGFβ and CD44 and down-regulation of E-cadherin. Changing of the gastric epithelial cell polarity is another downstream intracellular event connected to *H. pylori*, which is primarily attributed to the physical interaction of components of the T4SS (especially CagL) and β1 integrins. In addition to this translocation-independent mechanism, alteration of the cell polarity can also result from CagA translocation and phosphorylation. Another consequence of the manipulation of the invasive properties by *H. pylori* is the induction of the expression of ECM remodeling effectors, namely uPAR, uPA, MMP7, MMP2, MMP3, and MMP9. This is presumably linked to the activation of the MAPK signaling pathway, which in turn leads to the activation of NF-κB and AP-1. It is not yet clear whether plasminogen and MMP activation (dashed arrows) takes place, or what are the functional implications of the enhanced expression of these ECM-related molecules in this particular context. Cag-PAI: Cag pathogenicity island; ECM: Extracellular matrix; EMT: Epithelial-to-mesenchymal transition; *H. pylori*: *Helicobacter pylori*; MMP: Matrix metalloproteinase; NF-κB: Nuclear factor-kappa B; T4SS: Type IV secretion system; uPA: Plasminogen activator; uPAR: Plasminogen activator receptor.

β -catenin activation in response to this bacterial infection is a necessary event for the acquisition of such properties in GC cell lines^[63]. Finally, observations on patients with gastric dysplasia and early GC, before and after eradication of *H. pylori*, showed a connection between the mRNA expression levels of TGF- β 1, EMT markers, and immunohistochemical expression of CD44, suggesting that *H. pylori* infection may trigger a TGF- β 1-induced EMT and the emergence of CSCs^[64].

Cell polarity alterations induced by *H. pylori*

Epithelial cells in the gastrointestinal tract are normally found in organized layers or epithelia. Their polygonal shape and functional organization in an apico-basal polarized manner allow them to lay in an orderly fashion, and the unions they form with each other and with the basal membrane give the epithelium a barrier function. As already mentioned, invasiveness is enhanced when cells lose their polarity due to alterations in the cytoskeleton and intercellular unions. *H. pylori* has been implicated in changing the polarity of the gastric epithelial cells, which may have important consequences in the context of gastric carcinogenesis. Of the three types of filaments that compose the cytoskeleton, the actin microfilaments are the most affected during *H. pylori* infection.

The actin cytoskeleton is a very dynamic structure whose assembly is finely tuned by complex signaling networks and involves numerous regulatory proteins. Actin microfilaments form a wide variety of structures: contractile rings, phagocytosis- and endocytosis-related structures, microvilli, cortex, adherens belts (associated with adherens junctions), filopodia, lamellipodia, and stress fibers. The ability of *H. pylori* to promote rearrangements of the actin cytoskeleton is well-established^[65-67]. The most evident demonstration of this is the so-called hummingbird phenotype, comprising a change in the epithelial cell shape to the characteristic elongated morphology of *H. pylori*-infected cells *in vitro*. This phenotype is thought to be linked to cancer cell migration and invasive growth *in vivo*^[68]. The hummingbird phenotype involves the formation of stress fibers and protrusions, the disruption of cell-to-cell adhesions, and the deregulation of focal adhesions between the cell and the ECM.

The basic mechanisms by which *H. pylori* changes the dynamics of the actin cytoskeleton during cell migration have been reviewed in depth by Wessler *et al.*^[69]. Briefly, *H. pylori*, via cag-PAI type-4 secretion system (T4SS; especially CagL) and CagA, is able to modify the host cell's signaling networks. On the one hand, CagL binds β 1 integrins, thereby stimulating the focal adhesion kinases (FAKs) and the Src-family kinases (SFKs); meanwhile, CagA activates the Abl-kinase. FAK, SFK, and Abl activate Crk, which in turn activates Rac1, which then promotes the assembly of actin filaments *via* activation of the Arp2/3 complex, contributing to cell motility. On the other hand, upon injection into the host-cell cytosol,

CagA is phosphorylated by SFK (c-Src) and binds Shp-2 and Csk, which then inhibit SFK in a negative feedback loop. Inhibition of SFK induces dephosphorylation of actin regulatory proteins, such as ezrin, vinculin, and cortactin. Cortactin stimulates the actin nucleation activity of Arp2/3 and, upon *H. pylori*-induced dephosphorylation, accumulates at the tip of the cellular protrusions and colocalizes with F-actin^[70].

The serine/threonine kinase polarity-regulating kinase partitioning-defective 1b (PAR1) participates in the CagA-mediated remodeling of the actin cytoskeleton. PAR1 inhibits the formation of stress fibers and cortical actin in the cell periphery. Kikuchi *et al.*^[71] showed that the physical interaction between the CagA multimerization sequence and PAR1b, the isoform present in gastric epithelial cells, is crucial for the stable binding of CagA and Shp-2. In fact, a second study found that CagA indirectly activates RhoA-dependent formation of stress fibers by impairing PAR1b-mediated inhibition of RhoA^[72]. These results were elegantly combined in a model that proposes a link among cell polarity regulation, the hummingbird phenotype, and actin cytoskeleton^[73]. More specifically, upon cell polarity loss of the epithelial cell, PAR1b and aPKC are relocated, resulting in the establishment of a front-to-rear polarity in which these two molecules are asymmetrically distributed, with PAR1b localized in the rear part of the migrating cell.

The binding of CagA to PAR1b modifies this program by perturbing PAR1b localization, which translates into loss of its kinase activity, lifting of the repression of RhoA, and formation of stress fibers; the salient manifestation of this is the hummingbird phenotype^[73]. The affinity of CagA for PAR1b and formation stress fibers increases proportionally to the number copies of the CagA-multimerization (CM) domain present in CagA, which is seemingly higher in East Asian CM than in Western CM and differs in five amino acid residues^[74].

Podosomes are dot-like structures of densely packed F-actin and serve as regulatory proteins by their capacity to degrade ECM components due to the presence of MMPs within. It has been shown in a model of primary hepatocytes and hepatoma cell lines that *H. pylori* can enhance the formation of podosomes by the induction of inflammatory cytokines such as TGF β ^[75], thus providing additional evidence of the capacity of *H. pylori* to modify actin structures. Actin-remodeling activity has also been described in another *Helicobacter* species, *H. pullorum*. Its cytolethal distending toxin, responsible for the cytopathological effects observed upon infection, induces actin cytoskeleton remodeling that is accompanied by delocalization of vinculin and up-regulation of cortactin in large, cortical actin-rich lamellipodia^[76].

Another important component of the cellular cytoskeleton is the microtubules. Structurally, they are formed by tubulins and regulated by microtubule-associated proteins. The microtubular network organizes the cell movement of organelles and is part of specialized structures such as cilia, flagella, mitotic spindles, cen-

osomes, and basal bodies. During cell migration, the small Rho-GTPase protein Cdc42 controls the actin microfilaments in the cell migration front and binds PAR6. The Cdc42-PAR6 dimer recruits the microtubule-regulating dynein/dynactin complex, which directs the machinery of the secretory pathway to the migration front. Importantly, this facilitates the delivery of integrins and other proteins that mediate the interaction with the ECM to the sites of migration.

Although not many, some studies have addressed the role of *H. pylori* infection in microtubule regulation in the context of cell migration. Slomiany and colleague^[77], for example, concluded that *H. pylori* lipopolysaccharide induced the secretion of MMP9 in a primary culture of murine gastric mucosal cells. In that study, the authors also found an accompanying increase of microtubule stabilization. Presumably, those changes are modulated by ghrelin and involve the activation of PKC δ and SFK.

Focal adhesions provide the structural link between the stress fibers and the ECM. They need to be dynamically assembled and disassembled in order to allow cell migration. *H. pylori* impairs focal adhesion release during cell migration, which leads to the characteristic elongation of infected cells^[68]. Paxillin, a multidomain protein that acts as an adaptor between the cytoplasmic tail of integrins and the actin cytoskeleton, has been designated as the convergent point of the epithelial growth factor receptor, FAK/Src, and PI3K/Akt signaling pathways in the context of *H. pylori* infection^[78]. Paxillin phosphorylation is dependent on the presence of a functional Cag-PAI or OipA. The phosphorylated paxillin was localized along the elongations, suggesting a role in the formation of stress fibers^[78].

PA system and *H. pylori*

The PA system comprises a few proteins that, by acting in sequence, lead to the conversion of zymogenic plasminogen into its active enzymatic form, plasmin. Extravascular activation of plasminogen is controlled by the urokinase-type plasminogen activator (uPA), its receptor (uPAR), its inhibitor PAI-1, and α_2 -antiplasmin. Besides degrading major ECM proteins (e.g., fibrin, fibronectin, laminins, and vitronectin), the generated plasmin also releases latent growth and angiogenic factors sequestered in the matrix^[79,80]. The expression of uPA, uPAR, and PAI-1 under normal homeostatic conditions is almost undetectable; however, in cancer and other pathologies, their expressions increase significantly^[81]. An important body of evidence correlates uPAR expression in cancer lesions with invasive and metastatic disease. Accordingly, high levels of uPAR in tissue and plasma are associated with poor patient survival in various types of cancer, including GC^[82-84]. Most of these reports have focused on uPAR, since this receptor is crucial for the initiation of the sequential series of events that ultimately result in the activation of plasminogen.

As already mentioned, *H. pylori* has been linked to

the induction of members of the PA system, primarily uPAR. Part of the experimental evidence supporting this phenomenon comes from *in vitro* studies, for example global gene-expression analyses ranking uPAR among the top up-regulated genes in AGS and T84 cell lines, when co-cultured with *H. pylori*^[85-87]. This has been confirmed by more specific *in vitro* studies, showing that in co-cultures, the bacterium rapidly induces uPAR expression in GC cell lines^[88-91]. A few of these reports indicate that uPAR induction is predominantly linked to CagA-positive strains^[87,89]. The potential connection between *H. pylori* and uPAR induction has also been documented in non-neoplastic tissue adjacent to GC lesions^[92] and in gastric biopsies from healthy patients who are infected with the bacterium^[93]. Interestingly, it has been reported that the expression of uPAR in neoplastic tissue may be correlated with the presence of *H. pylori* in adjacent non-neoplastic tissue^[94].

The link between *H. pylori* and uPAR has been systematically investigated in a mouse model of *H. pylori*-induced gastritis (Figure 3)^[95]. In this model, uPAR expression is up-regulated very early in response to the infection and increases progressively during the course of infection, and this is reverted to its physiological baseline levels if *H. pylori* is eradicated by antimicrobial therapy^[95]. Additional experiments in this model suggest that uPAR expression is directly induced by the bacterium (and Alpizar-Alpizar, unpublished results)^[95]. It is not possible to rule out, however, that uPAR induction in murine gastric epithelium is a consequence of the inflammatory reaction against *H. pylori*.

A few signaling pathways and transcription factors have been proposed as potential inducers of uPAR in cancer; however, much less is known about the mechanisms of induction in response to *H. pylori*. Studies in cancer cell lines have found that the NF- κ B can drive uPAR expression by direct binding to specific sequences within the regulatory region of the gene encoding uPAR^[96] or indirectly via HIF1 α activation^[97]. It is well known that *H. pylori* infection can lead to the activation of NF- κ B^[36,98]. Therefore, NF- κ B is a likely transcriptional inducer of uPAR in epithelial cells of *H. pylori*-colonized mucosa, both in human and mouse. *In vitro* evidence supports this idea^[88], but no experimental data have been generated *in vivo*.

AP-1 is another transcriptional regulator that is activated by *H. pylori* infection^[36] and has been implicated in the induction of uPAR in cancer^[99,100]. Thus, AP-1 may explain the potential connection between these two parameters^[90]. Both NF- κ B and AP-1 can be activated via the Ras-ERK MAPK signaling pathway^[99,101]. This pathway is often manipulated by *H. pylori*^[36], which makes it an interesting study target to gain further insight about the mechanism of induction of uPAR in the gastric epithelium colonized with *H. pylori*.

MMPs and *H. pylori*

The MMP family comprises more than 23 zinc-depen-

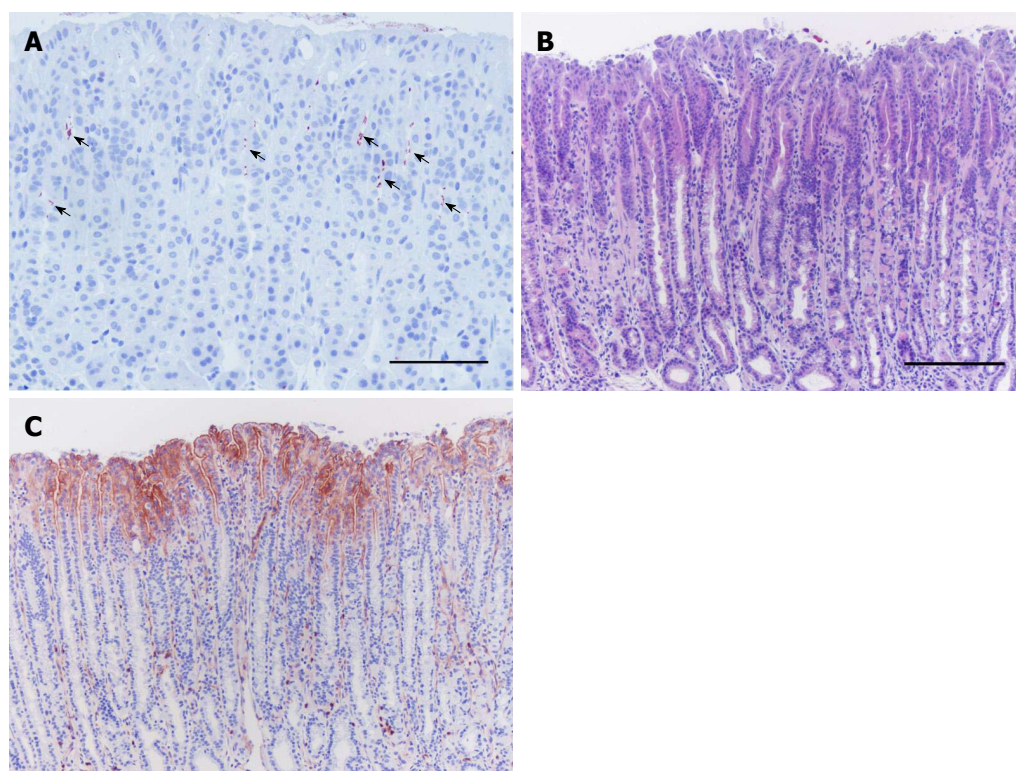


Figure 3 Plasminogen activator receptor induction in gastric epithelial cells in response to *Helicobacter pylori* infection. Stomach tissue sections of a mouse infected with *H. pylori* and euthanized 14 wk after inoculation processed for immunohistochemistry against *H. pylori* (A) and uPAR (C), and with H&E staining (B). Clusters of *H. pylori* bacteria (arrows) are observed in the upper third of the gastric glands along the gastric epithelium of the mouse stomach (A). Histopathological alterations are seen, including inflammation and mucous metaplasia (B). uPAR expression becomes evident at the apical membrane of foveolar epithelial cells in the corpus epithelium of *H. pylori*-colonized mice, such as the representative immunohistochemistry staining shown here (C). uPAR-positive scattered neutrophils are seen in the microphotograph (C) since they constitutively express this molecule. Scale bars: A: 100 μ m; B and C: 200 μ m. *H. pylori*: *Helicobacter pylori*; H&E: Hematoxylin and eosin; uPAR: Plasminogen activator receptor.

dent endopeptidases, subdivided into eight groups according to structural characteristics^[42,102]. MMPs are synthesized in the form of zymogens (pro-MMPs) by several cell types of the tumor microenvironment; and, when released to the extracellular space, they become activated by other proteases, including MMPs themselves and plasmin^[42]. Besides their role in invasion and metastasis, MMPs are involved in other aspects of tumor biology. The degradation of ECM constituents results in the liberation of sequestered growth, proliferative and angiogenic factors but also in the generation of ECM-derived peptides with similar biological properties to those factors. Some MMPs can cleave membrane-bound growth factor precursors, thus releasing their active form, for example TGF β ^[42]. Elevated expression of several MMPs has been consistently correlated with poor cancer patient survival in several types of cancer, including GC. Of note, a few MMPs actually inhibit malignant transformation and tumor growth, including MMP8, MMP12, and MMP26^[103-107].

The possible connection between *H. pylori* infection and induction of MMPs (*e.g.*, MMP2, MMP3 and MMP9) in gastric epithelial cells has been suggested; however, the most compelling evidence is probably for MMP7. MMP7 enhances tumor formation in rodents^[108], and it is particularly interesting in the context of the gastric

carcinogenesis because its expression is increased in human GC lesions^[109,110]. In human gastric cell lines co-cultured with *H. pylori*, it was found that cag-PAI-positive strains augment the levels of MMP7 up to 7-fold compared to uninfected controls or to cells incubated with specific isogenic mutant strains^[111]. According to that report, the induction of MMP7 in the *in vitro* system was dependent on the activation of ERK 1/2 and required an active interplay between viable bacteria and epithelial cells^[111]. That study also evaluated the expression of MMP7 in gastric biopsies of human patients and found that it was over-expressed in epithelial cells of gastritis-affected individuals infected with CagA-positive strains^[111], which has also been previously documented by an independent report^[112].

These observations served as the driving force for conducting subsequent investigations in MMP7 knockout mouse models. Such studies concluded that gastric inflammation and epithelial cellular turnover are substantially increased in MMP7-deficient mice infected with *H. pylori*, compared to their wild-type counterparts^[113,114]. It is speculated that over-expression of MMP7 in response to *H. pylori* colonization may be a mechanism to protect the gastric mucosa from damage and development of lesions that could ultimately result in GC^[112,113]. Nevertheless, it is also proposed that sus-

tained expression of MMP7 in the gastric epithelium could lead to malignant transformation^[112]. In fact, a few studies suggest that MMP7 proteolytically cleaves specific pro-apoptotic molecules, such as Fas ligand, from tumors cells, thus promoting tumor survival^[115,116].

CONCLUSION

H. pylori is a determining factor in the development of GC, due to the multiple ways in which it manipulates the host gastric epithelial cells. A key aspect of carcinogenesis is the acquisition of invasive capacities, and *H. pylori* could modulate several factors associated with invasion. A number of bacterial virulence factors may be of relevance in the manipulation of cellular and molecular programs that lead to increased invasive behavior; however, Cag-PAI stands out as a major orchestrator in hijacking these host cell pathways.

A number of key effectors of the EMT and cell polarity are deregulated in response to *H. pylori* infection. The two processes enhance cell motility and regulate the attachment of preneoplastic cells to the ECM and to other cells. This finally translates into an increased versatility of the cells to initiate the invasive process and adapt to the physiological changes suffered by the cell through the dedifferentiation induced by EMT. The acquisition of stem-like properties is a pivotal event that results from the activation of the EMT programs in response to *H. pylori*, since it confers the cells with augmented capability of survival and proliferation.

The induction of members of the PA system and MMPs by *H. pylori* could have important implications in the genesis of GC, given the wide array of aspects in which these molecules participate. Particularly interesting is the fact that this bacterium up-regulates the expression in non-neoplastic gastric mucosa of the uPAR, a protein that until now has been implicated in processes related to late stages of cancer development and progression, and has been correlated with the prognosis of cancer patients in general.

Altogether, the findings reviewed here show that *H. pylori* alters a fundamental process in gastric malignant transformation and invasiveness. Although we have discussed aspects related to EMT, cell polarity and ECM remodeling as independent processes, there are several points of interconnection among them (Figure 2). For instance, some factors implicated in the activation of EMT programs that are deregulated by *H. pylori* lead to the induction of MMPs and changes in cytoskeletal reorganization. Some of these proteinases, in the meantime, are capable of activating mediators of the EMT and cell polarity programs.

Therefore, the link between *H. pylori* and cell invasive properties is complex and an exciting open area of research where many aspects remain far from being clear. For instance, there is a need to gain further insight on how and under what circumstances *H. pylori* manipulates regulatory networks controlling the EMT and stem-cell programs. Also, it is important to unravel

the cellular and molecular mechanisms underlying the induction of members of the PA system and MMPs in *H. pylori*-colonized gastric epithelium.

Elucidation of the key orchestrators governing these invasion-related programs is crucial to understanding the implications that these processes may have for the survival of the bacterium or in the pathological context. All this information could be of relevance for identifying individuals with an increased risk of GC, who may require *H. pylori* eradication therapy, especially in countries with limited resources and high prevalence of this bacterial infection. Finally, this may contribute to prediction of pre-neoplastic lesions that are more likely to progress in the pathogenic series of steps to malignancy, which may be of relevance to reducing GC burden.

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Colorectal carcinogenesis: Insights into the cell death and signal transduction pathways: A review

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Abstract

Colorectal carcinogenesis (CRC) imposes a major health burden in developing countries. It is the third major cause of cancer deaths. Despite several treatment strategies, novel drugs are warranted to reduce the severity of this disease. Adenomatous polyps in the colon are the major culprits in CRC and found in 45% of cancers, especially in patients 60 years of age. Inflammatory polyps are currently gaining attention in CRC, and a growing body of evidence denotes the role of inflammation in CRC. Several experimental models are being employed to investigate CRC in animals, which include the APC^{min/+} mouse model, Azoxymethane, Dimethyl hydrazine, and a combination of Dextran sodium sulphate and dimethyl hydrazine. During CRC progression, several signal transduction pathways are activated. Among the major signal transduction pathways are p53, Transforming growth factor beta, Wnt/ β -catenin, Delta Notch, Hippo signalling, nuclear factor erythroid 2-related factor 2 and Kelch-like ECH-associated protein 1 pathways. These signalling pathways collaborate with cell death mechanisms, which include apoptosis, necroptosis and autophagy, to determine cell fate. Extensive research has been carried out in our laboratory to investigate these signal transduction and cell death mechanistic pathways in CRC. This review summarizes CRC pathogenesis and the related cell death and signal transduction pathways.

Key words: Colorectal cancer; Cell death; Apoptosis; Autophagy; Inflammation; Hippo signalling; Nuclear factor erythroid 2-related factor 2; Wnt signaling

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Core tip: Colorectal carcinogenesis (CRC) imposes a major health burden. This review addresses the cell death mechanisms and major signal transduction pathways involved in CRC. Regulated cell death is important for maintaining normal homeostasis, and the dysregulation of cell death processes leads to a spectrum of diseases including cancer. It is interesting to note that cell death pathways collaborate with each other, so understanding the various cell death mechanisms are therefore essential. CRC is orchestrated by various signal transduction pathways, which are used as drug targets. This review highlights the key concepts concerning cell death mechanisms and signal transduction in CRC.

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INTRODUCTION

Cancer is a dreadful disease caused to an anomalous growth of cells, which leads to an irregular balance of cell proliferation and death. Cell death is a physiological process where normal cells are regulated by "touch contact-inhibition". However, proliferating tumor cells metastasize to distant sites and invade other tissues, often causing morbidity^[1,2]. In recent years, colorectal carcinogenesis (CRC) has imposed a major health burden in developing countries^[3,4]. CRC is the second highest cause of cancer deaths in women, and the third highest cause of cancer deaths in men^[5]. Due to environmental factors, a sedentary lifestyle and diet, the risk of CRC has been growing over the past few years. In many cases, the symptoms are not recognized by the individual. Although awareness *via* cancer screenings and the knowledge of therapy modalities has increased, the burden of CRC is much more pronounced in developing countries. The mortality rate of CRC is particularly high in Asian and African populations. Recently, mortality rates are declining in Western countries because of early screening and better treatment procedures^[6]. An increase in mortality has been reported in several Latin American countries, the Caribbean and Asia, likely due to inadequate health infrastructure and the lack of awareness about cancer screenings^[7]. It is well-known that dietary factors influence the incidences of CRC^[8]. Diets that are rich in fiber and that have low fat content tend to prevent CRC. The food stuffs we

intake determine our quality of health. Fried foods, red meat, and processed foods all increase CRC risk^[9,10].

ROLE OF POLYPS IN COLORECTAL CANCER

The cells in the lining of the colon change morphologically and proliferate uncontrollably. Benign (non-cancerous) polyps are often found lining the bowels. They occur in several areas of the gastrointestinal tract, but predominantly arise in the colon. They appear as small protrusions in the lumen. As aging progresses, the number of polyps increases. Malignant polyps indicate an adenoma that appears benign. Adenomas are precursor lesions in CRC that arise through the adenoma-carcinoma sequence. CRC develops due to the formation of malignant neoplasms within the lining of the large intestine^[11]. Malignancy risk has been linked to the site, size, and histological characteristics of polyps. Polyps < 5 mm in diameter are harmless and pose an insignificant risk of malignancy, whereas those with a diameter > 25 mm pose a significant risk^[12]. Colonic polyps are aberrant growths that appears in the colon. Polyps, in principle, can be diagnosed by screening the colon via endoscopy or colonoscopy. Three types of colonic polyps include hyperplastic polyps, adenomatous polyps and malignant polyps^[13]. These small colorectal polyps vary in size, ranging from small (< 10 mm) to diminutive (< 6 mm), and develop into cancer in 3%-5% of cases^[14]. The larger polyps have a greater chance of developing into a tumor. Among polyps, the most common ones are adenomas, which have the potential to become cancerous and can be removed during screening tests. Hyperplastic polyps must be differentiated from adenomatous polyps, as they have less cancerous potential unless localized in the proximal colon^[15]. Inflammatory polyps are gaining attention and often contribute to ulcerative colitis. Ulcerative colitis therefore increases the overall risk of CRC^[16,17]. A recent article highlights the importance of both managing these complex polyps and resecting colonic tumors^[18]. It is known that 5% of all CRC cases are attributed to two specific inherited syndromes, which include hereditary nonpolyposis colorectal cancer and familial adenomatous polyposis^[19,20].

SYMPTOMS AND RISK FACTORS OF COLORECTAL CANCER

Common symptoms of CRC are rectal bleeding, significant changes in the colour of stool (especially dark or black-colored stools), irregular bowel habits, pain or discomfort in the lower abdomen, weakness or fatigue, and certain types of anemias^[21]. Several risk factors are thought to cause CRC. Age is a major risk factor. About 90% of CRC patients are above the age of 50. The median age of CRC diagnosis is 68 in men and 72 in women. CRC risk also increases due to environmental

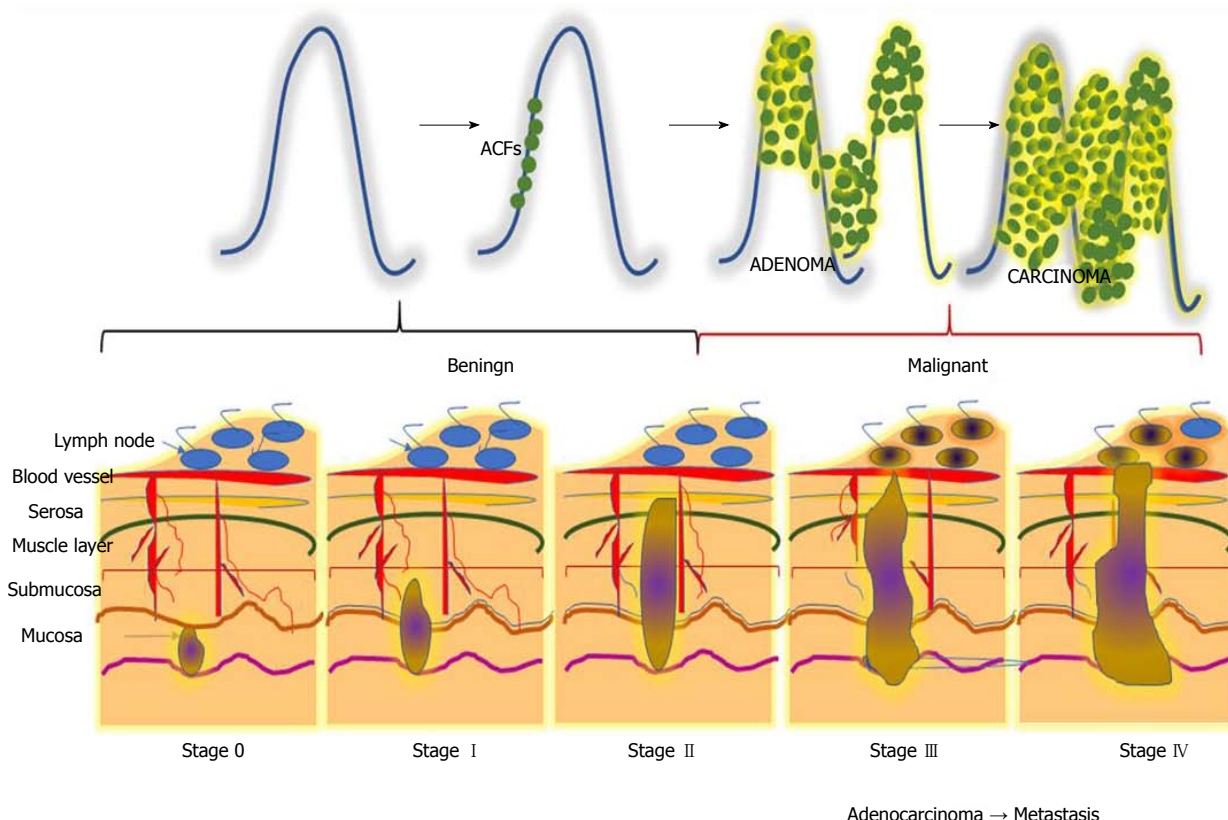


Figure 1 Different stages during the progression of colorectal carcinogenesis. Stage 0: The cancerous cells grow within the inner lining of the mucosa; Stage I: The cancerous cells grow throughout the mucosa and submucosa. The cancerous growth invades into the muscular layer of the colon; Stage II: The cancerous growth penetrates through the wall of the colon without spreading to neighbouring tissues or lymph nodes; Stage III: The cancer penetrates through layers of muscle into the serosa, the layer of visceral peritoneum. The cancer begins to spread to the lymph nodes; Stage IV: A tumour nodule forms in the tissue surrounding the colon, cancer cells appears within the lymph nodes, and the cancer begins to metastasize.

factors, which include consuming a diet rich in red meat and fat, poor intake of dietary fiber, sedentary life style, obesity, diabetes mellitus, smoking and consumption of alcohol^[22,23]. One possible mechanism of diet-associated CRC is the production of heterocyclic amines during the cooking of meat, as well as higher levels of fecal bile acids^[24]. Conversely, the consumption of fish oil rich in omega 3 - fatty acids (Omega 3 PUFA) reduces CRC risk. Personal history of sporadic tumours is also known to increase the risk of CRC. A previous history of colonic polyps, small bowel, endometrial, breast or ovarian cancers are additional factors that contribute to CRC^[25,26]. In recent years, there has been an increasing interest in evaluating the genetic pathways that contribute to CRC. The current research trend has been diverted towards chromosome instability pathways, which correlate with sporadic CRC *via* mutations arising in K-ras, p53 and adenomatous polyposis coli (APC). The microsatellite instability pathway describes hereditary non-polyposis through frequent mutations in DNA mismatch repair pathway genes^[27,28].

STAGES OF COLORECTAL CANCER

CRC is a horrendous disease that progresses gradually through three precisely-connected stages: Initiation - a process that alters the molecular message of the normal

cell, promotion - aberrant signal transduction cascades and progression - phenotypically-altered, transformed cells. CRC can be divided into five stages, stage 0 to IV (Figure 1). Disease severity and the corresponding therapeutic options depend on the stage^[29]. Stage 0 can be characterized by a tumour at the region of the mucosa or inner lining of the colon. CRC stage I is when cancer cells grow in the mucosa, yet their invasive capacity is restricted to the muscular region and not present in the neighbouring tissues of the colon^[30]. Stage II can be subcategorized into three types based on invasive growth into: the walls of the colon, the muscular layer of abdomen lining, and nearby tissues^[31]. Depending upon the growth of the cancer, stage III can be further divided into three types. During this stage, the cancer grows into the inner lining of the colonic muscular layer and forms lymph nodules in surrounding tissues. Based on the number of nodule formations, this stage can be named IIIA, IIIB or IIIC. Stage IV describes the worst stage of the disease where the cancer has spread to distant parts of the body, such as the liver, lungs, *etc.*^[32].

MURINE MODELS OF COLORECTAL CANCER

Basic CRC research using animal models has grown^[33,34].

Especially in recent times, animal models have contributed towards our understanding of CRC pathogenesis and yielded insights into the development of novel chemotherapeutic drugs. In spite of this, murine models have become a key tool in understanding the effects of genetic modifications that occur during the process of CRC formation^[35,36]. Researchers have developed and modified murine models of CRC, which is a resource with immense potential. Murine models have been segregated into three different classifications, namely genetically-modified, western diet-induced, and chemically-induced models^[37].

APC^{min/+} mouse model

Studies over the past few decades involving preclinical CRC research utilize the APC^{min/+} mouse^[38]. The APC^{min/+} mouse is a genetically-engineered model of mouse colon carcinogenesis. When these mice reach the age of 4 wk old, they spontaneously develop tumors in the intestine and colon. It is a well-known phenomenon that about 80% of CRCs arise due to mutations in the APC gene. Researchers removed one allele of the APC gene, thus creating the APC^{min/+} mouse model. The APC^{min/+} model of intestinal/colorectal cancer has been extensively studied in the context of developing novel chemotherapeutic drugs^[39,40].

Dimethyl hydrazine and azoxymethane

Azoxymethane (AOM) and 1,2 dimethyl hydrazine (DMH) are the two notorious chemical carcinogens used to induce and study CRC in rat and mouse models^[41,42]. AOM and DMH are alkylating agents that produce free radicals that bind to DNA and cause mutations. These accumulating mutations will develop into tumours. These agents are injected either intraperitoneally (i.p.) or subcutaneously (s.c.) into animals for several weeks to induce colonic tumors^[43]. Detailed analysis of colonic tumours from these chemically-induced rodents harbour mutations in the β -catenin gene, which is quite similar to Human Non Polyposis Colorectal Cancer^[44]. In our laboratory, we extensively use this model to develop many natural chemotherapeutic agents^[45].

DSS/DMH model of ulcerative colitis-induced CRC

Chronic inflammatory bowel disease, where the colon is extensively injured over a prolonged period of time due to inflammation, increases the risk of CRC. The most common forms of inflammatory bowel disease are ulcerative colitis and Crohn's disease^[46]. A combination of Dextran sodium sulfate (DSS) and DMH are now used to induce CRC in Fisher rats^[47]. A single dose of AOM and three cycles of 2% DSS in drinking water for seven days results in tumor formation within 8 wk. These AOM/DSS or DMH/DSS mouse models are largely used by researchers to screen drugs.

N-methyl-N-nitro-N-nitrosoguanidine and N-methyl-N-nitrosourea

Chemically-induced N-methyl N-nitro-N-nitrosoguanidine and N-methyl-N-nitrosourea are non-specific colon

cancer models. These carcinogens induce neoplasia in multiple organs when administered to rodents^[48-51]. N-methyl-N-nitrosourea injection into rodents also induces prostate and breast cancer^[52]. When N-methyl-N-nitrosourea is administered through the rectum, it not only causes a greater incidence of CRC, but also induces thymic lymphoma and lung cancers^[53]. Since this is considered to be a non-specific colon cancer model, it is not frequently used to study colorectal cancer.

Western diet-induced rodent CRC model

Epidemiologic studies indicate that diet plays a vital role in the development of colorectal cancer risk in humans^[54]. Many studies have examined the influence of typical western diets on the incidence of colorectal cancer. About 12 wk of feeding these western diets to rats and mice promotes hyperplasia in colonic crypts^[55,56]. Approximately 70% of the mice fed with this Western diet exhibited nuclear atypia in their colonic epithelia, and 40% of the mice showed features of dysplastic crypts at the end of two years^[57,58]. These reports suggest the involvement of a Western diet in eliciting CRC.

EPITHELIAL-MESENCHYMAL TRANSITION IN COLORECTAL CANCER

Epithelial cells: targets of colorectal cancer

CRC is believed to originate in epithelial cells that line the colon and rectum. The epithelium is highly vulnerable to mutation and carcinogenesis, as the replication rate of cells in the epithelium of the colon and rectum is relatively high, with a replication rate of 10^{10} cells every day^[59]. The abnormal accumulation of epithelial cells can cause mutations in oncogenes and tumour suppressor genes, which may lead to neoplastic growth. Thus, these abnormal changes in cells of the colon and rectum, which form benign lesions, have the potential to further develop into cancer and metastasize to other organs^[60].

Epithelial-to-mesenchymal transition: a complex mechanism in cancer metastasis

Epithelial-to-mesenchymal transition (EMT) represents a well-organized mechanism in which epithelial cells alter their cellular characteristics and behaviour, and reform into a mesenchymal-like phenotype^[61]. Polarized epithelial cells are tightly-packed through tight junction molecules such as claudin, occludin, and zonula occludens; adherens junction molecules such as E-cadherin and desmosomes form a sheet-like structure in the normal epithelium^[62]. In contrast to epithelial cells, mesenchymal cells do not possess cell-cell adhesion molecules, which give mesenchymal cells migratory capacity and invasiveness. The dissolution of cell adhesion molecules results in loss of apical-basolateral cell polarity in mesenchymal cells. Another important

feature of these mesenchymal cells is resistance to cellular senescence and apoptosis. Mesenchymal cells are characterized by the enhanced expression of extracellular proteases and transcription factors, such as snail, slug and twist, which activate the cells to produce collagen, fibronectin, vimentin, α -smooth muscle actin (α -SMA), etc^[63]. Interestingly, the shift from an epithelial to mesenchymal state is complex. Upon triggering by mediators, these events begin with the dissolution of cell-cell adhesion, which results in the loss of microvilli and cilia at the apical surface of epithelial cells. At this stage, cytoskeletal reorganization takes place, which releases α -smooth muscle actin and matrix metalloproteinases. These secreted matrix metalloproteinases degrade the extracellular matrix, which facilitates the dissolution of cells from the basement membrane and allows cells to move along the matrix^[64].

EMT plays a key role in the spreading of cancer throughout distant parts of the body. Newly-produced cells by EMT display several properties associated with cancer metastasis. Reports suggest that EMT cells can avoid cellular senescence by inhibiting tumour-suppressor proteins^[65]. Furthermore, research evidence shows that high levels of vimentin in EMT cells makes these cells more resistant to chemotherapeutic drugs^[66]. The mechanism of EMT in colorectal cancer metastasis is depicted in Figure 2. The mechanism of EMT is considered to be complex because of the heterogeneity within this population. Interestingly, not all epithelial cells in a mutated epithelium undergo EMT. Moreover, not all EMT cells facilitate metastasis. Several environmental factors as well as signalling cascades regulate these mechanisms of EMT^[67]. A successful metastasis is achieved through the involvement of another mechanism called mesenchymal-to-epithelial transition. The invasive mesenchymal cells produced by EMT travel through systemic circulation and anchor themselves in other distant parts. For this, cells must regain their epithelial features and thereby undergo a mesenchymal-to-epithelial transition. The modulation of cells between EMT and mesenchymal-to-epithelial transition states facilitates cancer metastasis^[68].

Interestingly, E-cadherin, a hallmark for EMT, is reported as a biomarker for colorectal cancer^[69]. Recently, a research group reported that the silencing of ubiquitin-specific protease 47, a deubiquitinating enzyme, augments the proteasomal degradation of Snail, the transcription factor involved in EMT, to prevent the progression of colorectal cancer^[70]. A molecular genetic approach towards the involvement of EMT in colorectal cancer revealed that the epithelial nature of colon cancer cells might be sensitive to several drugs, including Src, Notch, and epidermal growth factor receptor inhibitors^[71]. Further studies are warranted to identify novel regulators of EMT in order to find novel cellular targets of colorectal cancer.

CELL DEATH IN COLORECTAL CANCER: “CUTS TWO WAYS” PROCESS FROM WOMB TO TOMB

Although Carl Vogt reported the incidence of cell death in metamorphic toads in 1842, the mechanisms of cell death was recognized in the middle of the 19th century^[72]. However, research attempts have yet to come out with a clear picture of the phenomena of cell death, and confusions still remain between the alternative forms of cell death. As an essential physiological process required to maintain tissue homeostasis, the different modalities of cell death are intensively studied^[73]. The decision of a cell to live or die is important and can be the determining factor in the progression of cancers^[74]. Chemotherapies targeting cell death mechanisms are highly encouraged in order to prevent cancer progression and metastasis^[75,76]. Dysregulated cell death signalling cascades are considered to be fundamental to the progression and worsening of CRC. Considering this notion, a conceptual understanding of the involvement of different modes of cell death in colon carcinogenesis and its progression would shed light on novel cellular targets against colorectal cancer.

Death-triggering environmental cues in the colorectum

The urogenital system and hindgut, which include the colon and rectum, begin to divide in the 4th week of human gestation and become separate units by the 7th week^[77]. Cell death, particularly apoptosis in the mesenchyme, plays a predominant role in this process. Research evidence shows that apoptotic cells are concentrated in the mesenchyme of the terminal rectum during the formation of the anal canal in the 7th week of gestation^[78]. A number of developmental regulatory signalling molecules such as Wnt 5a, Cdx1, Hoxd-13, Tcf4 and Shh actively participate in the up- and downregulation of apoptotic cell death during the formation of the colorectum^[79,80]. Interestingly, researchers have reported the decisive role of autophagy in the activation of cellular signals that are required for the phagocytic engulfment of apoptotic cells during embryonic development^[81]. Yet another research group has reported that alternative cell death mechanisms such as autophagy, cornification, entosis, and necroptosis exist when apoptosis machinery fails during embryogenesis^[82]. Previous reports clearly point out that cell death mechanisms are not only important in shaping the embryo, but also for maintaining adult tissue homeostasis, and can therefore be considered as key machinery from womb to tomb.

TYPES AND CHARACTERIZATION OF CELL DEATH

According to the 2018 nomenclature committee on cell death, all cell death processes taken together can be

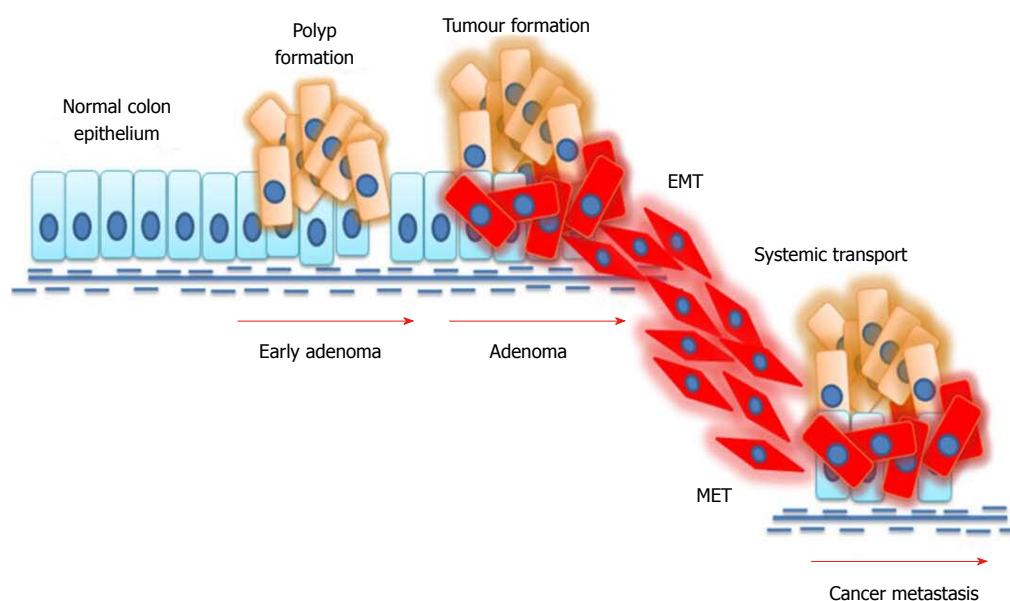


Figure 2 Mechanism of epithelial-to-mesenchymal transition in colorectal cancer. External stimuli or mutations in cancer cells induce epithelial-to-mesenchymal transition (EMT), where epithelial cells undergo phenotypic changes and transit into an invasive mesenchymal cell state. Mesenchymal cells invade the systemic circulation and undergo a mesenchymal-to-epithelial transition (MET) in distant organs, thus facilitating metastasis.

classified into fourteen or more subgroups based on their morphological characteristics, enzymological criteria, functional phases, and immunological reactions. These subgroups include apoptosis, necrosis, necroptosis^[83], ferroptosis^[84], pyroptosis^[85], parthanatos^[86], entosis^[87], NETosis^[88], autophagy^[89], and mitotic catastrophe^[90]. Genetically-programmed mechanisms for the targeted eradication of permanently-damaged and destructive cells or organs are collectively termed as regulated cell death mechanisms. The major classifications of different cell death modalities with each of their functional aspects are depicted in Figure 3.

Targeting cell death in colorectal cancer: implications for therapy

An interesting finding about cancer is that several genes that are responsible for cancer development are very much active during embryogenesis and fetal development, particularly regulating embryonic growth and organ formation. These genetic programs remain silent throughout the rest of the life of an organism; however, they are turned on in cells during cancer formation^[91]. The genetic paradigm of colorectal cancer reveals that APC or β -catenin is responsible for the initial changes in normal mucosa that form dysplastic aberrant crypt foci. COX-2 mainly regulates the formation of early adenomas, and K-RAS regulates the formation of intermediate adenomas. CPC4/SMAD4 is responsible for late adenomas and p53 is majorly responsible for carcinomas^[92]. During these sequential events from benign polyp formation to adenomas and finally carcinomas, cell death plays an essential role.

A low rate of apoptosis in the base of the crypt, where stem cells are expected to reside, is fundamental

to the function of the normal intestine. It is interesting to note that epithelial cells residing in the villi of the small intestine and colon are resistant to apoptosis^[93]. Changes in the expression patterns of several apoptotic proteins during the transformation of adenomas into carcinomas reveal the importance of apoptosis during colon cancer progression^[94]. Since 70% of reported CRCs are associated with mutations in the tumour suppressor p53 gene, the transition from adenomas to carcinomas in the colorectal region is considered to involve a mechanism whereby apoptosis machinery fails^[95]. Therefore, chemotherapies intended to stimulate apoptosis in colon cells would be central to controlling disease progression^[96]. With this notion, our laboratory is interested in elucidating the apoptosis-inducing effect of certain phytochemicals in order to eradicate cancer cells and provide protection against CRC progression. We have provided evidence that the bioflavonoid luteolin has strong anti-proliferative activity. Luteolin inhibits the Wnt/ β -catenin signalling cascade, which induces apoptosis and cell cycle growth arrest in the G2/M phase in HCT-15 colon cancer cells^[97]. In addition, azoxymethane induces colon carcinogenesis in BALB/c mice^[98]. Our reports suggest that apoptosis is an efficient parameter in preventing malignant transformation since it eradicates harmful cells. On the contrary, apoptosis can also promote cancer growth by preventing the removal of certain genetic variants that have a high potential to induce malignancy. Yet another interesting hypothesis about cancer is that tumour tissues possess a higher apoptotic index than normal tissues. Notably, a higher apoptotic index in tissue indicates more a malignant tumour^[99]. Therefore, apoptosis can be considered as a double-edged sword in cancer progression. However, the

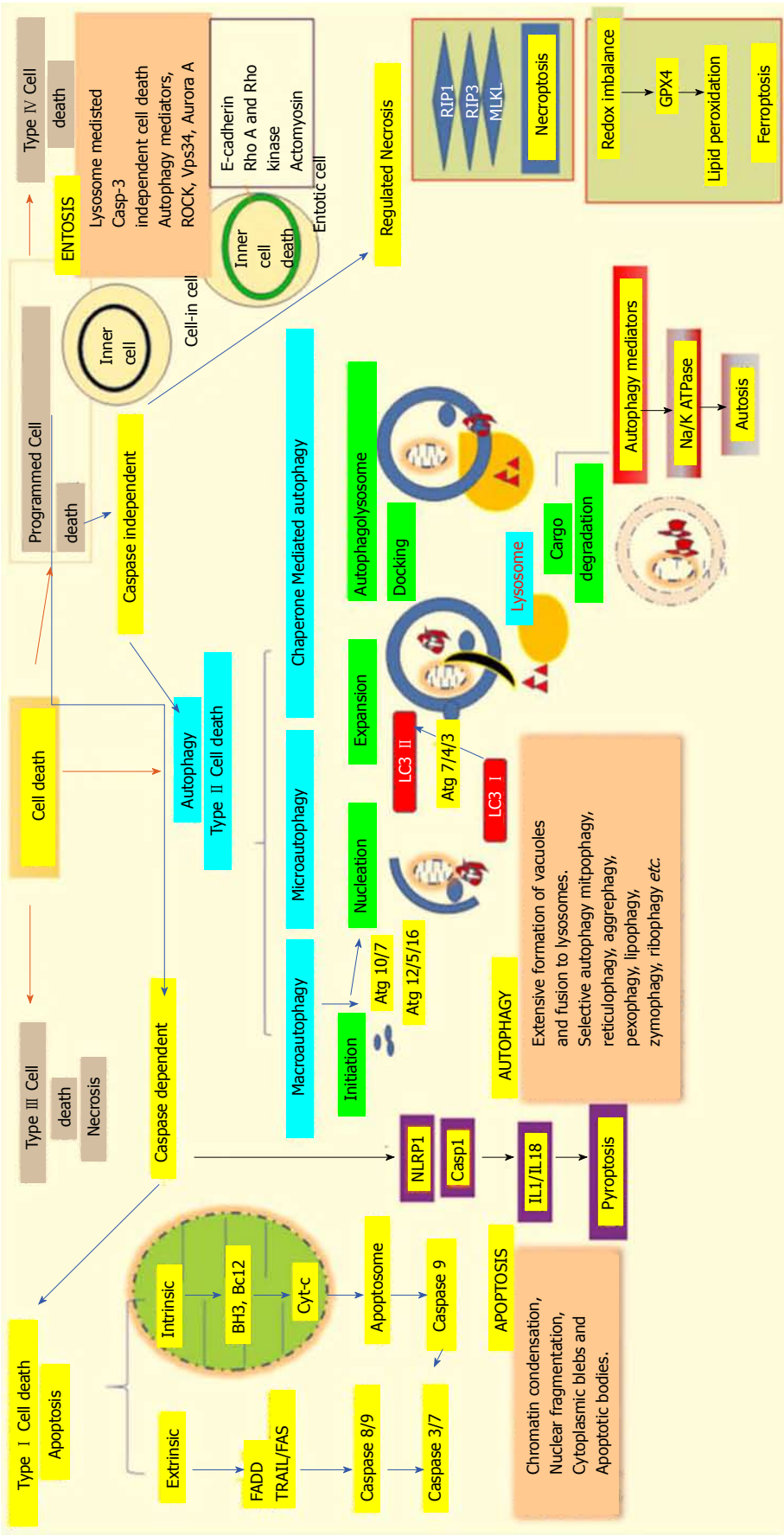


Figure 3 Different modalities of cell death. Unpredictable perturbations in the extracellular or intracellular microenvironments of a cell can activate several signal transduction cascades that ultimately lead to various forms of cell death. Type I cell death apoptosis: the extrinsic pathway of apoptosis is mediated by Fas-associated death domain protein (FADD). Caspase 8, in turn, triggers caspase 3 and 7, which then activates caspase 9. Both intrinsic and mitochondrial pathways of apoptosis are mediated through the inhibition of anti-apoptotic Bcl2, which in turn activates Bax/Bak and induces release of cytochrome c from the mitochondria. The activation of caspase 9 in the apoptosome induces apoptotic cell death. Type II cell death autophagy: autophagy is an active lysosomal degradative flux, which can be divided into three distinct types: macroautophagy, microautophagy and chaperone-mediated autophagy. Macroautophagy involves four different steps: initiation, autophagosome nucleation, phagosome expansion and completion, and autolysosome docking. The tightly-regulated autophagy machinery is mediated through several autophagy-related (Atg) molecules. Regulated necrosis/necroptosis: regulated necrosis is mediated through the interaction of receptor interacting protein 1 (RIP1) with RIP3 upon caspase 8 inhibition. RIP3 and mixed-lineage kinase domain-like (MLKL) are phosphorylated and assembled into complex 1b, which is then translocated to the plasma membrane to mediate membrane permeabilization. Ferroptosis: this regulated form of cell death is driven by the loss of glutathione peroxidase 4 (GPX4) activity, a lipid repair enzyme, followed by the accumulation of lipid hydroperoxides. Autosis: a plasma membrane Na⁺/K⁺-dependent autophagy form of cell death. Entosis: internalized cells undergo entotic cell death through the formation of entotic vacuoles, which is mediated by autophagy proteins like Vps34, etc. Pyroptosis: a caspase-dependent cell death mechanism that is an intermediary variation of apoptosis and necrosis. Caspase 1 is activated by the NLRP3 inflammasome, which activates the inflammatory cytokines interleukin 1 β and interleukin 18, which in turn mediate the lytic cycle.

mechanism linking a high apoptotic rate with increased cancer cell proliferation and metastasis needs to be further elucidated.

Apart from apoptosis, other cell death modes are also gaining attention in cancer research in order to find better therapeutic targets. From this point of view, the pro- and anti-metastatic effects of autophagy have been studied in several cancers including brain, liver, pancreas, colon *etc.* Several signalling cascades are known to regulate autophagy. Among these, PI3K/Akt/mammalian target of rapamycin (mTOR) is an important signalling pathway that acts as a checkpoint in autophagy and promotes cancer progression. Interestingly, PI3K/Akt hyperactivation, PIK3CA mutations, and both PTEN mutations and deletions have been reported in the incidence of CRC^[100]. Autophagy is reported as an anti-metastatic mechanism in the early stages of cancer metastasis by preventing both the infiltration of inflammatory cells as well as tumour cell necrosis, thus helps reduce cancer cell invasion and metastasis. However, autophagy may act as a promoter of metastasis during advanced cancer stages by enhancing EMT, cell survival and metastasis^[101]. Moreover, high expression of LC3I/II, which is a key regulator of autophagosome nucleation and is known to downregulate Beclin 1, has been reported in the advanced stages of CRC^[102]. This research evidence points out that autophagy machinery influences all stages of cancer progression, including initiation, proliferation and metastasis, while its effect on inhibiting or promoting cancer metastasis seems to be context-dependant.

Targeted therapies for necroptosis, a caspase-independent, receptor-interacting protein kinase-mediated form of regulated cell death, has recently been postulated as a novel strategy for cancer prevention. Very few reports are available concerning the role of necroptosis in regulating CRC progression. Moriwaki and colleagues have shown a significant downregulation in RIPK1 and RIPK3 expression in colon cancer tissues when compared with normal colon tissues^[103]. Interestingly, dimethyl fumarate, an approved drug for the treatment of multiple sclerosis, is reported to induce necroptosis through the depletion of glutathione in colon cancer cells^[104]. Colon cancer cell resistance against the 5-fluorouracil drug is sensitized by the usage of pan-caspase inhibitors, which facilitate 5-fluorouracil-induced necroptosis in CRC cells^[105]. However, more research should be conducted to identify the possible regulatory role of necroptosis in the prevention of CRC. Altogether, these reports shed a limelight on colon cancer research by revealing a promising therapeutic target against cancer progression.

SIGNALLING PATHWAYS IN COLORECTAL CANCER

The development of colorectal involves various signalling pathways that regulate cellular proliferation, differentiation and immortalization. Signalling activation of Wnt/

β -catenin, inactivation of transforming growth factor β (TGF β) and epidermal growth factor receptor, and mutation in k-ras signalling all play a vital role in the progression of CRC^[106,107].

Wnt/ β -catenin signalling in colon cancer

Wnt signaling plays divergent biological roles, such as regulating cellular homeostasis and maintaining cell self-renewal throughout embryogenesis and adulthood. This pathway particularly promotes intestinal epithelial proliferation and differentiation of the intestinal crypt^[108]. In the presence of Wnt ligand, the receptor frizzled inhibits the phosphorylation of Glycogen synthase kinase-3 beta, thus impeding the degradation of β -catenin by ubiquitins. Accumulated cytoplasmic β -catenin translocates to the nucleus and transcribes target genes (Figure 4). The activity of this signalling pathway depends on the cellular localization of β -catenin. Among 90% of colonic tumours have a mutation in the APC and β -catenin genes^[109]. Mutations in the cluster region of APC leads to the generation of truncated protein, which fails to prevent complex formation. This mutational dysregulation in Wnt signalling stabilizes cytoplasmic β -catenin, and its nuclear translocation promotes β -catenin-dependent transcription of Wnt target genes, which therein contributes to CRC progression^[110]. Nuclear β -catenin favours peripheral cellular changes that impact cell adhesion and migration. Interestingly, Wnt signaling is necessary for the initial activation of intestinal stem cells. This plays a crucial role not only for stem cell maintenance but also for crypt homeostasis. Research evidence shows that experimental abolition of Wnt signalling in cells leads to the specific loss of proliferative crypts^[111,112].

PI3K/Akt/mTOR signalling in colorectal cancer

PI3K/Akt/mTOR is the second most frequently mutated oncogenic signalling network in human cancers. The dysregulation of PI3K is observed in almost 30% of human cancers, making this signalling cascade an important therapeutic target in controlling cancer progression^[113]. The involvement of PI3K/Akt/mTOR signalling in colon carcinogenesis has been intensively studied. Overexpression of p-Akt and impaired expression of PTEN, a tumor suppressor negative regulator of Akt, have been reported in 70% of colorectal cancer patients^[114]. The carotenoid Lycopene has been reported to suppress leptin-mediated cell invasion in CRC HT-29 cells through the inhibition of Akt phosphorylation^[115]. Yet another research group has reported that aspirin, an inhibitor of mTOR and activator of AMP-activated protein kinase, induces autophagy and protects against the progression of colorectal cancer^[116].

TGF β / Smad signalling in colorectal cancer

TGF β and related bone morphogenetic proteins belong to the family of cytokines involved in the governing of various cellular processes, including proliferation, differentiation, and apoptosis^[117]. The TGF β superfamily

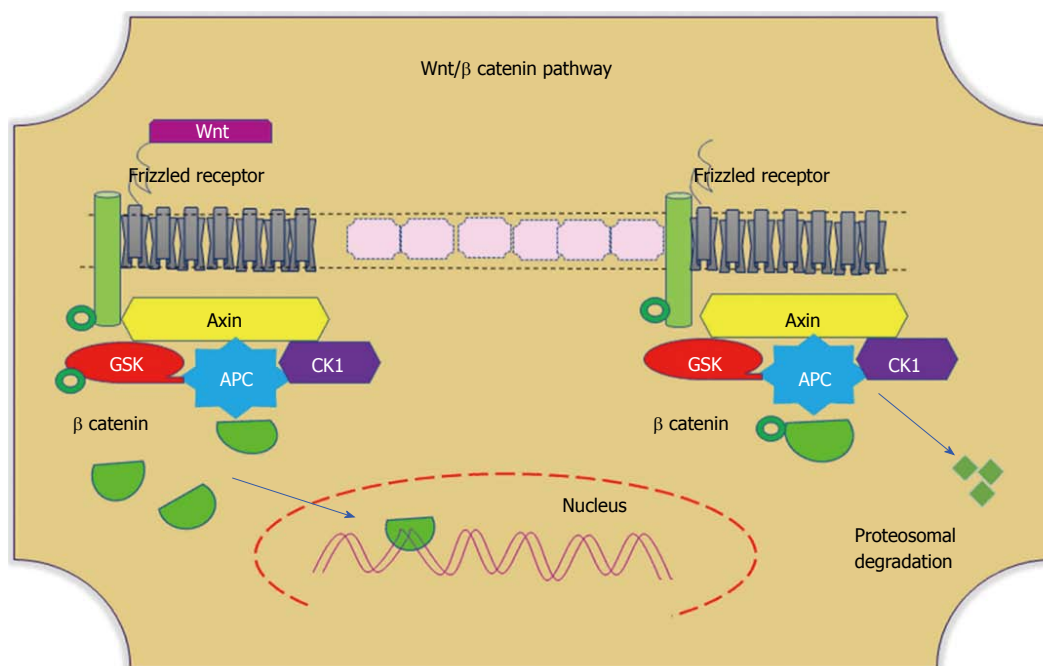


Figure 4 Wnt/β-catenin pathways. In the absence of Wnt, cytoplasmic β-catenin forms a complex with Axin (yellow), APC (blue), GSK3 (red), and CK1 (purple). Phosphorylated β-catenin undergoes ubiquitin-mediated proteosomal degradation. In the presence of Wnt, Wnt binds to the frizzled receptor, which in turn recruits the Axin complex. This disrupts Axin-mediated proteosomal degradation of β-catenin. Cytoplasmic β-catenin then travels to the nucleus and functions as a co-activator with TCF to activate Wnt-regulated gene expression. GSK: Glycogen synthase kinase; APC: Adenomatous polyposis coli; CK1: Casein kinase 1.

of cytokines contains many proteins, including TGFβ1, TGFβ2, TGFβ3, and activins. TGFβ conducts its signals *via* numerous intracellular signalling molecules, including the Smad family of proteins, mainly Smads 2 and 3^[118,119]. TGFβ enhances the expression of several fibrogenic and pro-inflammatory cytokines, such as platelet-derived growth factor, tumor necrosis factor α or interleukin 1β, and promotes the development and progression of the fibrotic reaction^[120]. Three major isoforms of TGFβ have been identified in mammals, namely TGFβ1, TGFβ2, and TGFβ3. In general, TGFβ is secreted in an inactivated form through its attachment to a latent TGFβ-binding protein^[121]. The downstream regulation of TGFβ signalling is activated upon ligand binding to type II receptors, which phosphorylates the type I receptor, which then further phosphorylates Smads 2 and 3. The phosphorylated Smads heterodimerize with Smad4 and translocate into the nucleus to promote gene transcription (Figure 5)^[122]. TGFβ plays a dual role in early cancer progression. TGFβ can perform as a tumor-suppressor pathway in normal colon epithelial cells by regulating cell proliferation and apoptosis. In later stages of cancer, however, TGFβ promotes cell migration by increasing EMT events and suppressing the immune response^[123,124]. The involvement of TGFβ signalling in CRC was described earlier^[125-127].

Epidermal growth factor receptor and Ras-Raf-MEK-ERK signalling

Epidermal growth factor receptor, a membrane-bound receptor tyrosine kinase, plays a vibrant role in the development and progression of many cancers. Ligand-

activated receptors form homo and heterodimers with the other ErbB family members and autophosphorylate their tyrosine residues^[128]. Once ligand binds to the receptor, it triggers the activation of downstream signalling such as Ras, MAPK, ERK, NFκB and PI3K/Akt. These pathways are very critical to CRC development. The overexpression of epidermal growth factor receptor and its ligands correlates with the development of human cancer and its poor prognosis^[129].

P53 AND COLORECTAL CANCER

p53 is well known gene for its tumor suppressor role and is one of the most mutated genes in all forms of human cancer. Activation of the *p53* DNA damage stress response induces DNA repair and regulates the cell cycle to prevent oncogenic mutation^[130]. Alteration of *p53* signalling in colon cancer, which results in the loss of apoptosis and cellular checkpoints while altering genetic integrity, ultimately leads to malignancy. Accumulation of mutations in cancer-related genes, such as *K-ras*, *p53* and *APC*, instigates the transition from normal epithelium to adenomatous to colorectal cancer^[131].

NOTCH SIGNALLING IN COLORECTAL CANCER

In mammals, the major components of Notch signalling include five ligands (Delta like ligands 1, 3 and 4, and Jagged 1 and 2 (Sterrate-like ligands)), four Notch

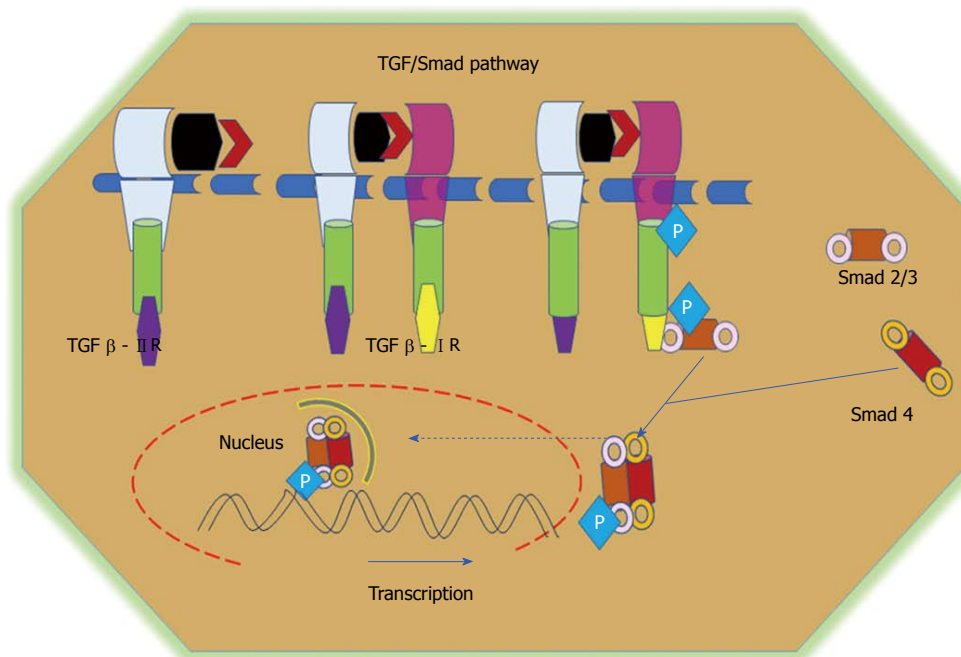


Figure 5 TGF/Smad pathway. TGFβ signalling is initiated via the binding of the TGFβ1 ligand to receptor II, which promotes dimerization between receptor II and receptor I and the subsequent transphosphorylation of TGFβRI. Activated TGFβRI therein phosphorylates and activates receptor-regulated Smads (Smad2 and Smad3). Phosphorylated Smads, along with co-Smads, form a trimeric complex and translocate to the nucleus to induce the transcription of target genes and promote cell growth and survival. TGF: Transforming growth factor.

receptors (Notch 1-4), and several downstream target genes^[132]. Signal-transduction is initiated by the interaction of a notch ligand that is present on one cell with the transmembrane Notch receptor that is present on a neighbouring cell. This binding interaction activates metalloproteases that cleave the transmembrane domain of the Notch receptor, resulting in the release of the constitutively-active Notch intracellular domain. Translocation of this domain to the nucleus regulates transcriptional complexes to induce expression of target genes (Figure 6)^[133]. Although currently available reports provide little information about cell-specific functions of Notch signalling in CRC when compared with other solid tumours, aberrant activation of Notch signalling has been reported in CRC. In a recent study, the superior therapeutic effect of targeting both Notch and MAPK signalling on colon cancer growth, as well as its role in regulating tumor cell plasticity, has been reported^[134]. Notch signalling has been reported to induce cellular resistance to chemotherapeutic drugs. It was demonstrated that Notch signalling is significantly upregulated in SW480 cells that are resistant to the experimentally-generated Regorafenib drug, a multi-kinase inhibitor. Interestingly, the inhibition of Notch signalling in resistant cells restored their sensitivity to Regorafenib, thus suggesting the important role of Notch in promoting resistance to chemotherapeutic drugs^[135]. The dysregulation of Notch signalling in colon cancer metastasis has been studied in detail^[136]. These reports strongly suggest the importance of Notch signalling in the pathogenesis and progression of CRC.

Nrf2/Keap signalling in colorectal cancer

Oxidative stress is denoted as an imbalance between oxidant production and antioxidant defences, where oxidants dominate and lead to cellular dysfunction and tissue damage. Oxidative stress caused by harmful reactive oxygen species are involved in colorectal cancer. Reactive oxygen species cause cellular damage, leading to the progression of many diseases such as cancers, fibrosis, neurodegenerative disorders *etc.* In turn, cells possess detoxification genes (Phase II) and antioxidant genes to counterbalance the lethal effects of reactive oxygen species^[137]. In many disease settings, NF-E2-related factor 2 (Nrf2), which is a basic leucine zipper transcription factor, plays a crucial role in protecting tissues against free radical-mediated insults including carcinogens, drugs, inflammation, *etc.*^[138]. Nrf2 is a member of the Cap-N-collar transcription factor family. It recognizes the antioxidant response element in the promoter of target genes^[139,140]. Under basal conditions, Nrf2 is restricted to the cytoplasm by Kelch like ECH associated protein 1. Kelch like ECH associated protein 1 is very critical, as it serves as a linker protein substrate between the Cul3-based E3-ubiquitin ligase complex and Nrf2, leading to the ubiquitination and proteosomal degradation of Nrf2^[141]. Certain conditions, such as the induction of the antioxidant response element, promote the detachment of Nrf2 from its partner Kelch like ECH associated protein 1, thereby facilitating the translocation of Nrf2 to the nucleus. Inside the nucleus, Nrf2 dimerizes and associates with small Maf proteins, leading to the binding of Nrf2 to antioxidant response elements, which

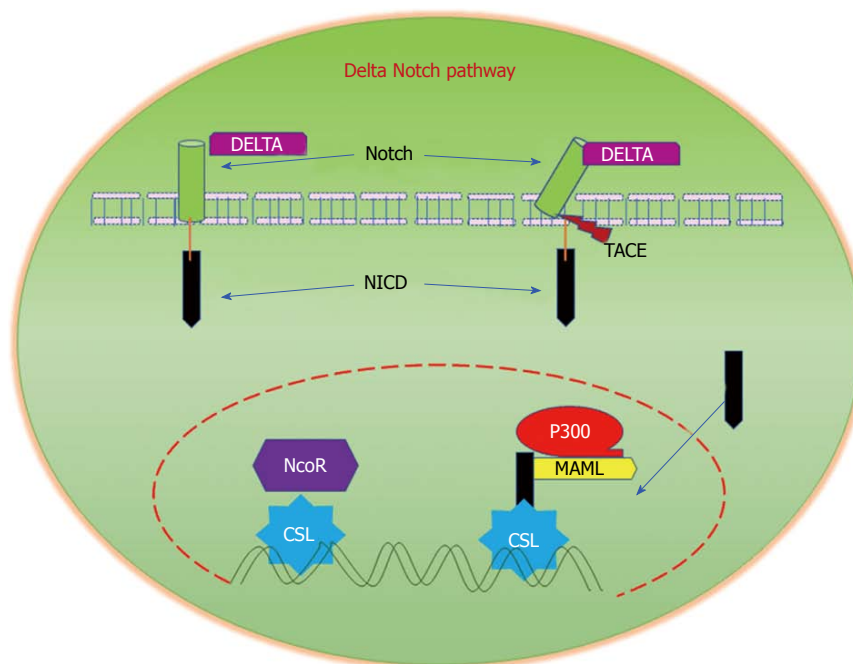


Figure 6 Delta/Notch Signalling. Notch signalling is initiated by the binding of Notch on one cell to the transmembrane ligands Delta or Jagged on a neighbouring cell. This binding interaction promotes cleavage of the Notch receptor and releases the Notch intracellular domain (NICD), which travels to the nucleus and controls the transcription of Notch responsive genes. In the nucleus, NICD binds to the transcriptional repressor CBF1, which recruits Mastermind-like (MAML) and other co-activators to initiate the transcription of downstream Notch-regulated genes.

therein promotes transcriptional activation of these genes. In colorectal cancer, the chemopreventive effect of many drugs greatly depends on this signalling^[142-144].

Hippo signalling and colorectal cancer

The origin of the hippo pathway began with observations in *Drosophila melanogaster* flies with concomitant mutations that led to tissue overgrowth^[145]. Hippo signalling has gained attention in cancer biology because of its crosstalk with oncogenic signalling pathways^[146]. Yes associated protein 1 is the key transcriptional regulator of the Hippo pathway. This protein, along with its partner PDZ-binding domain taffazin, orchestrate the Hippo pathway^[147]. In principle, hippo signalling plays an important role in the regulation of tissue homeostasis, development, regeneration, and cancer^[148]. Three protein components in mammals are depicted: Mammalian Ste 2 like kinase 1 and 2, and large tumor suppressor kinase 1 and 2. These kinases phosphorylate Yes associated protein 1 and PDZ-binding domain taffazin, which leads to their nuclear exclusion and ubiquitin-mediated proteosomal degradation in the cytoplasm, thus promoting the suppression of Yes associated protein 1/ PDZ-binding domain taffazin-targeted genes^[149,150]. Recently, a huge body of evidence suggests the critical role of Hippo signalling in CRC^[151,152]. The Hippo signalling pathway has been reported to crosstalk with other signalling pathways^[153,154].

MiRNAs AND COLORECTAL CANCER

Over the years, several molecular mechanisms have

been identified to be involved in CRC^[155]. In recent years, the discovery of microRNAs (miRNAs) has attracted considerable attention in different disease conditions. An understanding of the roles of miRNAs in development and disease, especially in cancer, have made miRNAs both an attractive tool and novel therapeutic target^[156]. Generally, miRNAs are non-coding RNAs that are 20-24 nucleotides in length and were classified into Oncomirs, including the tumor-suppressor miRNAs that are related to cancer. According to recent research relating miRNAs and cancer, miRNAs impact several vital processes such as the cell cycle, proliferation, differentiation, metabolism and apoptosis^[157]. It was reported that miRNAs such as miR-21, miR-181b1, miR-101, the let7 family, miR-133b, and miR-126 were dysregulated in CRC^[158,159]. Recently, miR-760 was found to suppress human colorectal cancer growth by targeting BATF3/AP-1/cyclinD1 signalling^[160]. MiR-422a acts as a tumor-suppressor in colorectal cancer, and its expression is limited to CRC tumours. Increasing the expression of miR-422a could inhibit CRC cell growth and promote cell apoptosis in colorectal cancer cells. It was also reported that miR-422a restricts colorectal cancer by inhibiting the p38/MAPK pathway^[161]. Therefore, miRNAs are emerging as potential targets in CRC.

CONCLUSION

Research attempts to develop more effective therapies against CRC progression are of outstanding importance, as the effectiveness of mono-therapeutic approaches in CRC treatment are very limited. However, combinational

therapies are gaining attention due to their ability to manipulate certain signalling cascades and induce different modalities of cell death to prevent cancer metastasis. The regulation of both cell signalling pathways and cell death represents a promising tool to improve patient responses to chemotherapy. When the normal orchestra of cellular signalling is dysregulated, cells become pathological and ultimately decide whether to die or survive. A subset of novel signalling pathways, and their association with colorectal cancer progression and metastasis, was discussed in this review. A better understanding of anticancer agents that target these cellular pathways and induce cell death modalities will hopefully provide more insights into the complicated molecular mechanisms that underlie colorectal cancer, thus facilitating the development of more effective treatments.

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Emerging role of long non-coding RNA in the development of gastric cancer

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and has a poor prognosis due to late diagnosis. Long non-coding RNAs (lncRNAs) are a significant subtype of RNA molecules with a length longer than 200 nucleotides (nt) that rarely encode proteins. In recent decades, deregulation of lncRNAs has been shown to be involved in tumorigenesis and tumor progression in various human carcinomas, including gastric cancer. Accumulating evidence has shown that some lncRNAs may function as diagnostic biomarkers or therapeutic targets for gastric cancer. Thus, exploring the specific functions of lncRNAs will help both gain a better understanding of the pathogenesis and develop novel treatments for gastric cancer. In this review, we highlight the expression and functional roles of lncRNAs in gastric cancer, and analyze the potential applications of lncRNAs as diagnostic markers and therapeutic targets.

Key words: Function; Tumorigenesis; Gastric cancer; Therapeutic target; Long non-coding RNAs; Diagnostic marker

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Core tip: Gastric cancer is a common, worldwide malignancy that has a poor prognosis. The promising regulatory potential of long non-coding RNAs (lncRNAs) in tumorigenesis and cancer development, including gastric cancer, has been widely demonstrated. Thus, exploring the function of lncRNAs can help to both gain a better understanding of the pathogenesis and develop novel treatments for gastric cancer. In this review, we aim to elucidate the expression patterns and functional roles of lncRNAs in gastric cancer, and analyze the latent applications of lncRNAs as diagnostic markers and therapeutic targets.

Abstract

Gastric cancer is a common, worldwide malignancy

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INTRODUCTION

Gastric cancer is one of the most common malignancies of the digestive tract^[1]. Furthermore, there has been consistent growth in the incidence and mortality rates of gastric cancer due to late diagnosis. The 5-year survival rate could reach 90%-97% if patients are diagnosed early and get prompt treatment, either by endoscopy or surgery. Nevertheless, the 5-year survival rate is currently under 20% for terminal cancer patients^[2-8]. As a result, prompt diagnosis of gastric cancer would significantly improve prognosis. An exploration of the molecular mechanisms involved in the initiation and development of gastric cancer is needed to help discover credible markers, and further reduce mortality rate, decrease disability and improve prognosis.

In the past, most non-coding RNAs were considered "junk RNAs" of the transcriptome. Nevertheless, with the rapid evolution of whole-genome analysis of gene expression, it has been demonstrated that most of the genome is transcribed into RNAs that have no protein-coding functions^[9,10]. Although non-coding RNAs do not encode proteins, they regulate gene expression through various mechanisms. Non-coding RNA-mediated gene silencing is an important part of epigenetic changes, which have been demonstrated to be involved in human carcinogenesis^[11]. During the last decade, more attention has been paid to the functional significance of non-coding RNAs in oncogenesis and tumor progression^[12]. Long non-coding RNAs (lncRNAs), defined as transcripts > 200 nt in length, are an important group of non-coding RNAs^[13]. It has been revealed that in various carcinomas, lncRNAs are frequently deregulated, which may indicate their potential role in the initiation of cancers^[14-16]. Thus, understanding the roles of lncRNAs will help elucidate the underlying biological events in different cancers, including gastric cancer, and ultimately lead to the development of novel diagnostic tools and targeted therapies. Furthermore, multiple lncRNAs have been shown to be related to diverse biological processes associated with gastric cancer, which enable lncRNAs to serve as diagnostic biomarkers and therapeutic targets. Here, we aim to review the recent progress made in elucidating the function of gastric cancer-related lncRNAs, and to also explore their potential capacity to serve as diagnostic or prognostic biomarkers.

Structure of lncRNAs

The length of transcript > 200 nts and the limited protein-coding potential are two of the main characteristics that distinguish lncRNAs from others^[17]. Researchers first identified lncRNAs when trying to sequence full-length cDNAs in mice^[18]. Thereafter, a 2.2 kilobase functional lncRNA termed "HOTAIR" was shown to be involved in

multiple processes of epigenetic regulation^[19]. In the past decade, with the development of transcriptomics, more lncRNAs have been recognized as important functional products of the genome^[20]. The polyadenylation and transcription of lncRNAs are commonly performed by RNA polymerase (RNAP) II^[21-23]. The length of lncRNAs typically varies from 1000 to 10000 nts, and some lncRNAs can reach 100000 nts^[20]. To date, the sequence and molecular structure of many lncRNAs still need to be elucidated. For sequence elements, some lncRNAs may perfectly match Watson-Crick base-pairing in order to function properly, while others would utilize imperfect pairing, which is when Watson-Crick base pairs are interspersed with non-Watson-Crick pairs^[17]. In a previous study, an analysis of 204 lncRNAs and their comparison to protein-coding transcripts showed that a paucity of introns, low GC content, and lack of start codons were some of the sequence traits of lncRNAs. Some of the biological features of lncRNAs, including positioning within the nucleus and low transcription levels, are generated from the sequence traits formerly mentioned^[24]. The secondary elements and three-dimensional structures of RNA also play a vital role in their action mode, but structural studies of lncRNAs have not been performed.

Category of lncRNAs

Thus far, there has been no systematic classification of lncRNAs. In fact, lncRNA entries are a mixture of multiple functions and mechanisms, only a small proportion of which has been functionally annotated. Many lncRNAs cannot be classified into any particular category. As a result, it is difficult to classify lncRNAs based on only one principal.

Different classification methods are used according to different features of lncRNAs. For example, based on genomic location and context relative to protein-coding genes, lncRNAs can be divided into five broad categories: (1) Sense lncRNA is transcribed from the sense strand and contains several overlapping exons; (2) Antisense lncRNA, on the contrary, is transcribed from the antisense strand; (3) Bidirectional lncRNA is transcribed on one strand, while an adjacent protein-coding gene initiates expression on the same strand, simultaneously; (4) Intronic lncRNA is transcribed entirely from within the introns of protein-coding genes; and (5) Intergenic lncRNA is transcribed within genomic intervals of neighboring protein-coding genes^[21]. According to their effects exerted on DNA sequences, lncRNAs can be classified into cis-lncRNAs (cis-acting lncRNAs) and trans-lncRNAs (trans-acting lncRNAs). The expression levels of adjacent genes can be regulated by cis-lncRNAs, while those of remote genes by trans-lncRNAs^[25].

Recent advances in high-throughput transcriptome sequencing technologies have made it feasible to conduct deep mining of both the function and mechanism of more lncRNAs, which will eventually enable us to optimize the

arbitrary classifications of lncRNAs.

MECHANISMS AND FUNCTION OF LNCRNAS

With the rapid development of experimental and computational technologies, more and more lncRNAs have been identified, among which only a small proportion has been functionally annotated. However, researchers have shown that the process of chromatin remodeling, transcription and post-transcriptional modification could be regulated by lncRNAs^[20,26-28].

Chromatin remodeling

Chromatin remodeling was one of the first identified functions of lncRNAs. It has been elucidated that lncRNAs could alter the structure of chromatin and modulate the expression level of genes^[29]. lncRNAs can confine chromatin remodeling complexes to particular regions in the genome, which is frequently achieved by interaction with polycomb repressive complex 2 (PRC2), so as to epigenetically regulate gene expression^[20,26]. In association with PRC2, small interfering RNAs (siRNAs) have been shown to mediate deletion of specific lncRNAs and thus further alter their expression levels^[30]. In addition to acting through PRC2, some lncRNAs directly recruit DNA methyltransferases or other complexes to modify chromatin conformation^[31-33].

Transcriptional regulation

lncRNAs regulate transcription by interfering with the transcription of enhancers and promoters^[34,35]. Some lncRNAs are transcribed within adjacent gene promoters. These lncRNAs can modulate the function of specific genes by interfering with the binding of protein factors. For example, the non-coding RNA SRG1 is transcribed across the promoter of the *SER3* gene, and the expression of SRG1 can remarkably repress *SER3*. Mechanistically, the transcription of SRG1 in the promoter area disturbs the binding of activators^[36]. lncRNAs can also be transcribed within distal enhancers and recruit transcription factors to these loci to regulate the expression level of neighboring genes^[37]. Furthermore, lncRNAs can act by regulating RNAP II activity^[38]. Some lncRNAs could regulate the transcription of key apoptotic genes, which is one of the vital pathways for carcinogenesis control^[39]. For instance, the lncRNA *INXS* is transcribed from the intron of the *BCL-X* gene. Under the regulation of *INXS*, *BCL-X* can splice into *BCL-XS*, which is a pro-apoptosis isomer of *BCL-X*^[40].

Post-transcriptional regulation

lncRNAs can recognize complementary sequences, and thus can regulate multiple procedures in the post-transcriptional modification of messenger RNAs (mRNAs). For instance, the complementarity of lncRNA *Xist* and *Tsix* can form complex dimers *in vivo*. These dimers are then spliced into small RNAs, which can balance the

effect of X-chromosome inactivation through RNAi-mediated silencing^[41]. Some lncRNAs could also act as competing endogenous RNAs (ceRNAs). Studies showed that these lncRNAs were able to bind miRNAs (sponging) and diminish the inhibitory effect on their natural targets^[42]. lncRNA sponges are widely involved in cancer tumorigenesis. For example, in hepatocellular carcinoma, the lncRNA *CCAT1* could act as a molecular sponge for *let-7* and de-repress the function of its endogenous targets *HMG2* and *c-Myc*^[43].

By exploring the function of lncRNAs in various aspects of cell transformation and metastasis, we will finally gain a better understanding of cancer biology. Nevertheless, many other functions of lncRNAs remain to be discovered.

ROLES OF LNCRNAS IN CANCER

Aberrant gene expression is the foundation of cancer pathogenesis. Intensive study of the genetic causes of cancer has found that variation in non-coding sequences is responsible for a large proportion of cancer susceptibility^[44]. In fact, most single nucleotide polymorphisms (SNPs) associated with malignant tumors are found to be located in non-protein-coding loci. Recent studies have shown that many cancer risk loci are transcribed into non-coding RNAs, particularly lncRNAs, which play vital roles in the process of tumorigenesis and progression.

The underlying mechanisms of the regulatory function of lncRNAs in the progression of cancer remain largely unknown. Evidence to date shows that some lncRNAs can recruit protein factors to particular regions of the genome in order to epigenetically modify chromatin, while others can regulate the protein signaling pathways underlying carcinogenesis. lncRNAs can functionally control cellular growth, division and differentiation, thus making them the focus of current cancer research.

As mentioned above, lncRNAs are key regulators of cancer initiation and progression, suggesting they may have applications in diagnosis and therapeutics. Many lncRNAs are highly correlated with particular cancer states and are useful as diagnostic and prognostic markers. For instance, the lncRNA prostate cancer non-coding RNA 1 (*PRNCR1*) is upregulated in both prostate cancer and precursor lesion prostatic intraepithelial neoplasia. In addition, *PRNCR1* expression levels are elevated in patient urine samples, thus making it a fine noninvasive indicator of prostate cancer^[45].

Deregulations of lncRNAs in gastric cancer

The above data showed that lncRNAs have strong correlations with cancer state, and their deregulation can lead to cancer initiation and progression. Many lncRNAs have also been shown to be involved in gastric cancer. Among lncRNAs associated with gastric cancer, some of them function as oncogenes and are upregulated during tumorigenesis, while others are downregulated and

serve as tumor suppressors. In this section, we briefly review some of the well-studied lncRNAs involved in gastric cancer.

HOX transcript antisense RNA

Located on chromosome 12, the HOX transcript antisense RNA (HOTAIR) contains 6232 nts and encodes 2.2 kbs of long non-coding RNA. HOTAIR is a non-protein-coding RNA with significant regulatory potential via gene remodeling^[46]. High levels of HOTAIR expression are associated with cancer cell proliferation, apoptosis, invasion, and progression in a variety of malignancies, making it a significant predictor of subsequent metastasis and death^[47-50].

In gastric cancer tissues, HOTAIR expression levels are remarkably elevated, which suggests that HOTAIR functions as an oncogene in gastric cancer. Song *et al.*^[51] observed that HOTAIR was overexpressed in gastric cancer, and that by inhibiting miR-152, HOTAIR was responsible for the elevation of human leukocyte antigen G. Furthermore, Endo *et al.*^[52] elucidated that upregulation of HOTAIR was correlated with lymph node metastasis, invasion into vessels, and reduction of survival time in gastric cancer. Chen *et al.*^[53] also found that HOTAIR was significantly upregulated in gastric cancer tissues, and that its overexpression was associated with migration and invasion.

The mechanism of HOTAIR overexpression in gastric cancer is currently unknown. Previous studies have proposed several potential mechanisms of how deregulated HOTAIR functions in tumorigenesis. Epithelial-to-mesenchymal transition (EMT) is generally considered to be the foundation of metastasis. Liu *et al.*^[54] found that by suppressing HOTAIR, the EMT process could be reversed in gastric cancer cells. Other research showed that HOTAIR promoted gastric cell EMT and metastasis by inhibiting E-cadherin expression through its interaction with EZH2^[53]. The functional SNP rs4759314 of HOTAIR had strong associations with gastric cancer susceptibility. SNP rs4759314, which resides in the promoter region of an intron, has been demonstrated to influence the expression of HOTAIR by interfering with this promoter^[55].

H19

The lncRNA H19, discovered in 1991 by Bartolomei^[56], was the first imprinted lncRNA gene identified. H19, residing on chromosome 11p15.5, is transcribed from the H19/IGF2 gene^[57,58]. Similar to mRNA, the H19 gene contains five exons and is transcribed by RNA polymerase II. However, it does not contain a common open reading frame. In general, the high conservation in H19 structure is considered to be responsible for the universality of its functions^[59]. Deregulation of H19 has been reported in various malignancies, such as breast cancer, bladder cancer and cervical carcinomas, which indicates its oncogenic role^[60-64]. H19 has also been reported to function as an oncogene in gastric cancer, and its overexpression may contribute to gastric

carcinogenesis. Li *et al.*^[58] demonstrated the upregulation of lncRNA H19 in gastric cancer tissues compared with paired normal tissues, and its positive correlation with lymph node metastasis and clinical stage. *In vitro*, upregulation of H19 could accelerate the proliferation, migration and invasion of gastric cancer cells, while knockdown of H19 caused apoptosis^[61,65-67]. Moreover, Hashad *et al.*^[68] demonstrated that H19 was upregulated in the plasma of gastric cancer patients, making it a potential non-invasive diagnostic biomarker for gastric cancer.

Multiple previous researchers have presented the potential functional mechanisms of H19 as an oncogene in gastric cancer. Studies have shown that H19 and miR-675, the primary precursor of H19, act together as oncogenes by promoting cell growth and malignant transformation in gastric cancer^[58]. H19 expression was negatively related to the expression of miR-141 in gastric cancer. The proliferation and invasion of gastric cancer could be accelerated by H19, but suppressed by miR-141. The competitive inhibitory relationship between H19 and miR-141 plays significant roles in the development of gastric cancer^[69]. Other research demonstrated that the H19-PEG10 axis is involved in EMT, and that the knockdown of this axis could induce tremendous changes in the expression of EMT-associated proteins, making it a potential therapeutic target in gastric cancer^[70].

Growth arrest-specific transcript 5

Growth arrest-specific transcript 5 (GAS5), a long non-coding RNA of approximately 650 nts, was originally isolated when screening for potential tumor suppressor genes during growth arrest^[71]. The aberrant expression of GAS5 has been found in a variety of human malignancies, including prostate cancer, renal cell carcinoma, and breast cancer. Furthermore, by regulating apoptosis and the cell cycle, GAS5 managed to arrest the growth of many cancer cell lines^[72-74]. Given the statistics above, the potential tumor suppressor role of GAS5 is clear. In a study that retrospectively analyzed the expression of GAS5 in 89 patients with gastric carcinoma, Sun *et al.*^[75] found that the decreased GAS5 expression was a common event, and that downregulation of GAS5 was positively correlated with tumor size, tumor stage, invasion depth and regional lymph nodes. Another study also demonstrated lower expression levels of GAS5 in gastric cancer tissues vs non-cancerous tissues, and its positive relation to tumor size and clinical stage^[76].

The downregulation of GAS5 in gastric cancer has been generally proven, however the functional mechanisms of it remain to be elucidated. Accumulating evidence shows that GAS5 could function by binding with miRNA during the process of tumorigenesis. Li *et al.*^[77] found that overexpression of GAS5 could suppress cell proliferation in gastric cancer cells by negatively regulating miR-222, which was proven to be an oncogenic miRNA. Liu *et al.*^[78] showed that GAS5 expression in gastric cancer cells was inversely correlated with upregulated expression

of miR-23a, indicating that GAS5 affected the biological behavior of gastric cancer by negatively regulating miR-23a expression. GAS5 has also been reported to be further downregulated in Adriamycin (ADM)-resistant gastric cancer cells. Nevertheless, when ADM-resistant gastric cell lines were transfected to promote GAS5 overexpression, they were more sensitive to ADM treatment, suggesting that GAS5 may act as a potential therapeutic target in gastric cancer treatment^[79].

Maternally expressed gene 3

Located on chromosome 14q32.3, maternally expressed gene 3 (*MEG3*) is downregulated in multiple cancer tissues and cells^[80,81]. It has been proven that *MEG3* is a tumor suppressor gene involved in various types of cancers, including gastric cancer. A previous study that detected *MEG3* expression in 31 patients with gastric cancer showed that *MEG3* was significantly downregulated in gastric cancer tissues vs adjacent non-cancerous tissues. Furthermore, it demonstrated that *MEG3* expression was negatively-related to tumor size and positively-related to overall survival rates of gastric cancer patients^[82]. Accumulating studies demonstrated that overexpression of *MEG3* could inhibit proliferation and metastasis, and that *MEG3* was strongly correlated with deep tumor invasion, advanced metastasis and poor gastric cancer prognosis^[82-84].

Increasing evidence reveals that lncRNA might play a crucial role in the occurrence and development of gastric cancer by interacting with miRNAs and promoting signaling pathways^[85,86]. Studies showed that *MEG3* could act as a competing endogenous RNA that sponges different miRNAs, such as miR-148a, miR-770, miR-181 and miR-141, to regulate the malignant activity of gastric cancer^[83,87-89]. Other studies showed that overexpression of *MEG3* promoted the expression of p53 in gastric cancer cell lines, indicating that *MEG3* may suppress the proliferation and metastasis of gastric cancer *via* p53-dependent transcription pathways^[82].

Long intergenic non-coding RNA 00152

Located on chromosome 2p11.2, long intergenic non-coding RNA 00152 (LINC00152) has an 828 nt-long transcript^[90]. In a study in which the expression level of LINC00152 was detected in 71 gastric cancer tissues and their paired non-cancerous tissues, Pang *et al.*^[91] found remarkable overexpression of LINC00152 in gastric carcinoma, making it a potential novel biomarker for predicting gastric cancer. Moreover, high expression of LINC00152 was positively-correlated with tumor size, invasion depth and prognosis^[92].

Functional analysis showed that silencing LINC00152 could inhibit cell proliferation, arrest cell cycle at the G1 phase, induce late apoptosis, suppress EMT and inhibit cell migration and invasion^[93]. Another study demonstrated that gastric cancer cell proliferation could be remarkably inhibited by knocking down LINC00152. Moreover, LINC00152 could exert its function by binding

to the oncogenic driver epidermal growth factor receptor (EGFR), leading to subsequent EGFR activation, which is a significant step in the tumorigenesis of gastric cancer^[94]. Huang *et al.*^[92] discovered that LINC00152 was inversely-related to miR-193a-3p, which could significantly reduce gastric cancer cell proliferation and inhibit tumor growth by targeting MCL1. Thus, LINC00152 exerts its biological effects in gastric cancer development through the LINC00152/miR-193a-3p/MCL1 pathway^[92]. LINC00152 could also bind to the enhancer of zeste homolog 2 (EZH2), which might lead to the repression of p15 and p21, thereby inducing gastric cancer cell cycle progression^[95].

Urothelial carcinoma-associated 1

Researchers first discovered urothelial carcinoma-associated 1 (UCA1) in urinary bladder carcinoma. UCA1 was then shown to be an oncogenic long non-coding RNA^[96,97]. Dereglulation of UCA1 has been reported in a variety of additional human malignancies as well, such as melanoma, breast cancer, colorectal cancer, and tongue squamous cell carcinoma^[98-100]. Recently, UCA1 has consistently been proven to play significant roles in the pathogenesis of gastric cancer. In a previous study, which analyzed UCA1 expression in 112 tumor and adjacent normal tissue samples from gastric cancer patients, researchers found that UCA1 was dramatically overexpressed in gastric cancer tissues and cell lines. Further clinicopathological analysis showed that the expression level of UCA1 was positively related to tumor size, invasion depth, TNM stage and poor overall survival^[101].

Functional studies revealed that UCA1 expression could enhance cell proliferation, colony formation, and invasion of gastric cancer cells, and that silencing of UCA1 inhibits tumor growth. Gu *et al.*^[102] found that UCA1 might function by both negatively regulating miR-590-3p expression and activating the expression of its downstream target CREB1. UCA1/miR590-3p/CREB1 may be a potential target for the treatment of gastric cancer^[102]. Another study indicated that knockdown of UCA1 reduced EMT-related protein levels, and that this effect could be partially rescued by treatment with transforming growth factor β 1 (TGF β 1). Hypothetically, UCA1 might promote the proliferation, invasion and metastasis of gastric cancer upon TGF β 1 induction^[103]. Moreover, Shang *et al.*^[104] demonstrated that chemotherapeutic resistance to ADM in gastric cancer cells was depressed, and that the half maximal inhibitory concentration (IC50) of ADM was also strongly decreased by silencing UCA1, thus making it a potential chemotherapeutic target for gastric cancer.

Metastasis-associated lung adenocarcinoma transcript 1

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), encoded on chromosome 11q13.1, is a long non-coding RNA with a length of more than 8000 nts. In response to growth signals, MALAT1 could bind to unmethylated PRC2 proteins and thus activate the

Table 1 Deregulations of long non-coding RNA associated with gastric cancer in this review

LncRNA	Deregulation	Biological roles	Ref.
HOTAIR	Upregulated	Induces EMT and promotes metastasis	[46-55]
H19	Upregulated	Promotes cell growth, proliferation, invasion	[56-70]
		Promotes EMT	
GAS5	Downregulated	Suppresses cell proliferation	[71-79]
		Sensitizes cells to ADM treatment	
MEG3	Downregulated	Suppresses cell proliferation and metastasis	[80-89]
LINC00152	Upregulated	Promotes cell proliferation and tumor growth	[90-95]
UCA1	Upregulated	Promotes cell proliferation, invasion, metastasis	[96-104]
		Depresses resistance to ADM treatment	
MALAT1	Upregulated	Promotes cell proliferation and invasion	[105-118]
		Promotes chemo-induced autophagy and chemoresistance	
ANRIL	Upregulated	Promotes tumor growth and metastasis	[119-121]
FENDRR	Downregulated	Inhibits migration and invasion	[122]
AFAP1-AS1	Upregulated	Promotes cell proliferation and cell cycle progression	[123, 124]
Sox2ot	Upregulated	Promotes cell growth and motility	[125]
CCAT2	Upregulated	Promotes EMT	[126]
Linc00261	Downregulated	Represses metastasis	[127]
		Inhibits EMT	
SNHG5	Downregulated	Suppresses cell proliferation and metastasis	[128]
LincRNA717	Downregulated	Inhibits tumor growth and invasion	[129]

EMT: Epithelial-to-mesenchymal transition; ADM: Adriamycin; LncRNA: Long non-coding RNA.

growth control program^[105]. At first, researchers found that MALAT1 could function as a metastatic biomarker for early-stage lung carcinoma^[106]. Recently, MALAT1 overexpression has been observed in a variety of solid carcinomas, including gastric cancer, indicating that MALAT1 plays an important role in cancer development and metastasis^[107-112]. By analyzing expression levels of MALAT1 in gastric cancer tissues and paired non-cancerous tissues, researchers revealed the upregulation of MALAT1 and the positive correlation between expression level and local invasion, lymph node invasion, peritoneal metastasis and short overall survival time^[113,114] {Feng, 2017 #301; Okugawa, 2014 #329; Li, 2017 #333}. Another study showed that MALAT1 was aberrantly highly expressed in gastric cancer patients with distant metastasis compared to those without metastasis. Furthermore, functional studies demonstrated that EMT could be prevented by epigenetically silencing MALAT1, thus inhibiting cancer cell migration and invasion^[115,116]. According to this evidence, the diagnostic potential of MALAT1 for gastric cancer is unequivocal.

An *in vitro* study confirmed that MALAT1 was negatively-correlated with miR-1297 expression, which promotes cell proliferation and invasion by targeting HMGA2. Moreover, silencing MALAT1 could reduce HMGA2 protein levels by eliminating miR-1297 inhibition, thus indicating that MALAT1 functions as an oncogenic lncRNA in part by modulating HMGA2 expression^[113]. Another report illustrated that MALAT1 inhibited the expression of tumor suppressor PCDH10 by binding to EZH2, leading to gastric cancer cell migration and invasion^[117]. MALAT1 could also serve as a competing endogenous RNA for miR-23b-3p and diminish its inhibitory effect on ATG12, which is a significant regulator of autophagy. This would thus promote chemo-induced

autophagy and chemoresistance in gastric cancer cells. These findings revealed that MALAT1 could function as a therapeutic target for gastric cancer^[118].

In addition to the well-documented lncRNAs discussed above, many other lncRNAs play important pathological roles in gastric cancer (Table 1). ANRIL is an antisense lncRNA located in the *INK4* gene area. ANRIL has been shown to be overexpressed in gastric cancer and positively-related to tumor size, TNM stage and decreased survival. ANRIL regulates the development of gastric cancer by modulating miR-99a/miR-449a through the mTOR and CDK6/E2F1 pathways^[119-121]. FENDRR is one of the lncRNAs that plays significant roles in tumorigenesis. Researchers have demonstrated the downregulation of FENDRR and its correlation with invasion depth, metastatic lymph nodes and poor patient prognosis. FENDRR exerts its function by targeting FN1 and MMP2/MMP9^[122]. Other lncRNAs found to be overexpressed in gastric cancer include AFAP1-AS1, Sox2ot and CCAT2, while Linc00261, SNHG5 and LincRNA717 were downregulated in gastric cancer^[123-129].

CONCLUSION

In summary, with the rapid development of various bioinformatic techniques, thousands of lncRNAs have been discovered. Thus far, various studies have proven the significant functions of lncRNAs in tumorigenesis of gastric cancer. Aberrantly-expressed lncRNAs might be used as diagnostic biomarkers, prognostic markers, and therapeutic targets for gastric cancer. However, our current understanding of lncRNAs in relation to gastric cancer remains limited. As a result, more investigations are necessary to gain a better understanding of lncRNAs and their mechanisms in gastric cancer.

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Between evidence and new perspectives on the current state of the multimodal approach to gastric cancer: Is there still a role for radiation therapy?

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Abstract

In patients affected by gastric cancer (GC), especially those in advanced stage, the multidisciplinary approach of treatment is fundamental to obtain a good disease control and quality of life. Although many chemotherapeutics in combination to radiotherapy are adopted in the peri- or postoperative setting, the most optimal timing, regimens and doses remains controversial. In the era of radical surgery performed with D2-lymphadenectomy, the role of radiation therapy remains to be better defined. Categories of patients, who could benefit more from an intensified local treatment rather than more toxic systemic therapy, are still under investigation. Evidence and recent updates of the randomized trials, meta-analysis and prospective trials show that the postoperative radiotherapy plays a fundamental role in reducing the loco-regional recurrence and in turn the disease-free survival in operable advanced GC patients, also after a well performed D2 surgery. Therapeutic decisions should be taken considering the individual patients, but the multimodal approach is necessary to guarantee a longer survival and a good quality of life. Ongoing randomized trials could better define the timing and the combination of radiotherapy and systemic therapy.

Key words: Adjuvant chemoradiation; Locally advanced; Perioperative chemotherapy; Gastric cancer; Combined treatment

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Core tip: This is a review of recent updates from rand-

omized data and prospective phase I/II trial regarding the role of radiotherapy in the multimodal approach of gastric cancer (GC). The actual state of art is still controversial and in particular adjuvant therapy for locally advanced disease remains undefined in different countries. Recent efforts show that a more intensified local therapy such as radiation therapy could have a benefit in increasing the disease-free survival, especially in the category of patients with positive pathological lymph nodes. A carefully multidisciplinary evaluation of the patients with GC is then recommended in the clinical practice.

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INTRODUCTION

In Europe, gastric cancer (GC) remains one of the most common causes of death from cancer, affecting more than 100000 people per year^[1]. The prognosis in patients affected by GC is poor, with longer survival in the Asiatic population^[2]. Surgery is the only known radical treatment, but in a locally advanced setting, a multimodal approach is necessary to improve outcome. D2- vs D1-lymphadenectomy is still controversial because of different findings in the Western and Eastern countries^[3,4]. However, recently available data suggest that D2-lymphadenectomy is the most optimal surgical standard.

A combined therapy including chemotherapy (CT) and/or radiotherapy (RT) is often recommended in a perioperative and/or postoperative setting to improve local control and disease-free survival (DFS). In clinical practice, postoperative chemoradiation therapy (post-CRT) or CT alone can follow radical surgery in patients that have received a previous perioperative CT (peri-CT). In addition, in a postoperative setting, a sequential scheme of RT and CT is sometimes preferred to the combined concomitant regimen. A recent meta-analysis aimed to provide more evidence on the role of post-CRT compared to CT alone after a D2 node dissection and conducted a systematic review of randomized controlled trials by extracting data on survival and toxicity^[5]. A significant reduction of loco-regional recurrence rate ($P = 0.0005$) and prolonged DFS ($P = 0.002$) were demonstrated in the post-CRT group, but no differences in overall survival (OS) and toxicity rates were reported.

In radiation treatment, indications, doses and techniques are often based on the experience of a single center rather than a predetermined guideline. A worldwide consensus on modality, timing, and combination of RT and CT has not been reached yet.

The aim of this review is to explore the current role of radiation therapy according to a definitive multidisciplinary treatment in the era of modern technology, new systemic agents, and radical surgery with D2-lymphadenectomy.

KNOWN EVIDENCE AND UPDATES ON COMBINED THERAPY

Adjuvant therapy

In resectable locally advanced GC, multimodal therapy has been considered a necessity since the American SWOG/INT-0116 trial demonstrated that surgery alone was inferior to surgery and associated adjuvant CRT in terms of survival and disease progression, concluding that resection alone was not enough to obtain acceptable oncological control of the disease^[6]. Subsequently, post-CRT became the standard of care in the United States for locally advanced resected GC patients, and the results of the original trial were also confirmed by updated analysis after long-term follow up^[7]. A significant survival benefit in contrast to surgery alone ($P < 0.001$) was demonstrated for post-CRT, despite the reported high rates of severe toxicity probably due to the conventional two-dimensional (2D) radiation techniques, which include a larger volume of normal tissue in the irradiated target volume. The high incidence of toxicity was also likely accounted for by the high dose bolus schedule of 5-fluorouracil (5-FU) that was previously implemented but is no longer recommended in treatment guidelines such as those of the National Comprehensive Cancer Network. This trial was highly criticized for the antiquated surgical procedure using D0- or D1-lymphadenectomy instead of the radical D2 node dissection (90% vs 10%). However, there is conflicting evidence regarding the choice of D2 vs D1 node dissection; lower loco-regional recurrence rates and cancer-related deaths are demonstrated after a D2 procedure, but no survival advantage and higher complication rates are related to the same approach^[8,9]. As D2-lymphadenectomy is widely used and integrated into current surgical practice for advanced stage GC^[10], does post-CRT still have value in a combined treatment, or is post-CT alone sufficient to obtain the same disease control with less toxicity?

The randomized Korean trial ARTIST investigated post-CRT vs post-CT alone in resected GC patients who received D2-lymphadenectomy^[11]. The primary endpoint was DFS and they did not find any significant difference between the two arms. The trial was limited by the lower rates of enrolled patients with locally advanced tumors (only 41%), and the higher rates of patients presenting early stage disease with lower risk of loco-regional recurrence after a well-conducted radical surgery such as D2-lymphadenectomy. However, a significant advantage of post-CRT in terms of higher DFS was observed in the subgroup of patients with pathologically positive lymph nodes ($P = 0.0365$). An

update of the data confirmed that post-CRT significantly reduced loco-regional recurrence rates ($P = 0.03$) after D2 resection, especially among the subgroup of patients with lymph node metastases ($P = 0.009$)^[12]. Moreover, in a recent analysis of the above trial, the influence of the metastatic lymph node-ratio, also called the N-ratio (number of positive lymph nodes/total number of resected nodes), was investigated as a possible prognostic factor in terms of DFS in both arms^[13]. On the multivariate analysis, the N-ratio was found to be an independent prognostic factor for DFS. In particular, 5-year DFS rates were 55% and 28% ($P = 0.02$) for N-ratio $> 25\%$ in the post-CRT and post-CT arm, respectively, suggesting an advantage of CRT for selected D2-resected GC patients.

The ongoing ARTIST II trial would further examine the role of post-CRT in the category of patients with resectable GC and pathologically positive lymph nodes after radical surgery. Furthermore, in the multicenter phase III trial CRITICS, patients with resectable GC were randomized to receive either peri-CT followed by gastrectomy with a D1+ lymphadenectomy (minimum of 15 removed lymph nodes, stations 1- 9 and 11) and post-CT, or preoperative CT followed by surgery and post-CRT^[14]. Recently, the surgico-pathological quality and protocol adherence for lymphadenectomy were accurately evaluated in the CRITICS trial, and the surgical quality and centralization were found to be excellent in the Netherlands^[15]. The definitive findings from the CRITICS trial showed a median OS of 43 mo (95%CI: 31-57) in the post-CT group and 37 mo (30-48) in the post-CRT group (95%CI: 0.84-1.22; $P = 0.90$) at a median follow-up of 61.4 mo^[16]. There were 368 (47%) grade 3 adverse events, 130 (17%) grade 4 adverse events, and 13 (2%) deaths. No survival benefits were added with the use of RT in an adjuvant setting. Of the 788 enrolled patients, 233 (59%) of 393 patients started post-CT and 245 (62%) of 395 started post-CRT. Due to the poor postoperative patient compliance in both arms, the authors concluded that the preoperative therapies should be further optimized.

In 2012, a phase III study from South Korea conducted in the National Cancer Center (NCC) reported a longer DFS in locally advanced (98%) D2-resected (100%) GC patients in the arm receiving adjuvant CRT following the Macdonald scheme vs post-CT alone ($P = 0.056$)^[17]. Unfortunately, the study was closed prematurely due to poor accrual. A small multicenter Chinese study, also published in 2012, randomized D2-resected GC patients to receive post-CRT ($n = 56$) delivered with intensity modulated RT (IMRT) or CT alone ($n = 59$)^[18]. In both arms, survival rates and related side effects were not significantly different, but in the CRT group, the median recurrence-free survival was significantly longer (50 mo) than that in the CT-alone arm (36 mo, $P = 0.029$).

Despite clear evidence in favor of adjuvant-combined CRT after a curative-intent surgery, open questions regarding the optimal timing and benefit of RT, and

its combination to systemic therapy remain, especially in the era of extended and well-performed D2-lymphadenectomy.

Two other randomized trials, the Japanese ACTS-GC trial and the Korean CLASSIC trial, demonstrated that the addition of post-CT vs surgery alone offered a tangible benefit in terms of OS and DFS, respectively, in D2-resected patients with stage II/III GC^[19,20]. The first trial showed a significant DFS improvement at 3 years and was subsequently stopped after the interim efficacy analysis, while the second trial was closed after the first interim analysis showed a significantly higher OS in the post-CT arm ($P = 0.002$). Both trials have influenced the type of adjuvant treatment administered in Asian countries, where the use of RT might not be considered fundamental and the related side effects could be synergic to those from CT. The main randomized studies evaluating the postoperative therapy are summarized in Table 1.

While data from these randomized trials gave unclear indications regarding the most optimal adjuvant regimen, a meta-analysis by Fiorica *et al.*^[21] published this year highlighted the importance of post-CRT in increasing survival through the analysis of 10 randomized controlled trials. An increase not only in DFS but also in OS was found in favor of combined CRT, supporting once again the advantages of local therapy in addition to systemic therapy.

Preoperative therapy

It has been proven that both peri-CT and post-CRT have a significant survival benefit over surgery alone^[22], but there has not been a specific randomized controlled trial to test which of these methods is the best choice in the treatment of patients with resectable GC. According to the results of randomized trials, surgery should be performed 3-6 wk from the last day of preoperative therapy. A recent analysis on 5058 patients with resectable gastric/gastroesophageal junction adenocarcinoma published in 2017 by Fitzgerald *et al.*^[23] evaluated the impact of peri-CT vs post-CRT on survival in patients selected from the United States cancer registry treated between 2004 and 2013. They found a 72% survival advantage in patients receiving peri-CT compared with those treated with post-CRT ($P < 0.0001$). Moreover, the survival benefit was higher among patients with positive lymph nodes in the preoperative state which subsequently converted to negative lymph nodes after being treated with peri-CT, suggesting significant tumor downstaging with peri-CT and an important role of the N-status as a significant prognostic factor^[24].

Peri-CT could control micrometastases, increase the chance of a good pathological response, and improve the performance of a curative surgery. In Northern Europe, GC treatment was influenced by the findings described in the MAGIC trial, a major trial regarding the use of preoperative CT published in 2006^[25]. Pa-

Table 1 The main randomized trials in gastric cancer that evaluate the postoperative therapy

Trial	Year	Randomization scheme	OS	DFS, PFS	Limits
SWOG/INT-0116 ^[6]	2001	S-alone vs S + CRT	3-yr: 50% vs 41% (<i>P</i> = 0.005)	3-yr: 48% vs 31% (<i>P</i> < 0.001)	Low rates of D2 node dissection, 2D RT technique
Update SWOG/INT-0116 ^[7]	2012	S-alone vs S + CRT	HR = 1.32 (95%CI: 1.10-1.60; <i>P</i> = 0.0046)	HR = 1.51 (95%CI: 1.25-1.83; <i>P</i> < 0.001)	Low rates of D2 node dissection, 2D RT technique
ARTIST ^[11]	2012	S + CT + CRT + CT vs S + CT	NR	3-yr: 78% vs 74% (<i>P</i> = 0.086)	Planned events not reached, lower % of locally advanced tumors
CRITICS ^[16]	2018	CT + S + CT vs CT + S + CRT	Median OS 43 vs 37 mo (<i>P</i> = 0.09)		Poor postoperative patient compliance in both treatment arms
NCC, South Korea ^[17]	2012	S + CRT vs S + CT	NR	5-yr: 73.5% vs 54.6%, (<i>P</i> = 0.056)	Poor accrual Sometimes 2D RT technique
Chinese Study ^[18]	2012	S + CRT vs S + CT	5-yr: 48.4% vs 41.8% (<i>P</i> = 0.122)	5-yr: 45.2% vs 35.8% (<i>P</i> = NS)	Small series
ACTS-GC ^[19]	2007	S-alone vs S + CT	3-yr: 80.1% vs 70.1% (<i>P</i> = 0.003)	3-yr: 59.6% vs 72.2% (<i>P</i> < 0.001)	Closed earlier due to significant survival benefit in the CT-arm
CLASSIC ^[20]	2012	S-alone vs S + CT	NR	3-yr: 59% vs 74% (<i>P</i> < 0.0001)	Stopped after the interim efficacy analysis

OS: Overall survival; DFS: Disease-free survival; PFS: Progression-free survival; RT: Radiotherapy; S: Surgery; CT: Chemotherapy; CRT: Chemoradiation; NR: Not reported; HR: Hazard ratio; CI: Confidence interval; NS: Not significant.

tients with potentially resectable GC were randomly assigned to receive preoperative epirubicin, cisplatin, and infused fluorouracil (ECF) or surgery alone. Surgery was scheduled 5–6 wk after the last day of the final preoperative CT cycle. Downstaging and downsizing of the disease were observed in the preoperative CT arm. Also, a significant improvement in 5-year OS (36% vs 23%, *P* = 0.009) and progression-free survival (*P* < 0.001) were reported in the CT group. The main limitations of the study were the low adherence to post-CT (42% received the entire treatment, including post-CT as already planned), and the enrolment of patients with malignancies of the esophagogastric junction or lower esophagus in addition to those with GC. Subsequently, a smaller trial conducted in France, the FNCLCC/FFCD study, demonstrated the advantage of peri-CT over surgery alone in terms of 5-year OS (38% vs 24%; *P* = 0.02) and 5-year DFS (34% vs 19%; *P* = 0.003)^[26]. Although patients with lower esophagus and gastroesophageal junction adenocarcinoma were enrolled, peri-CT was found to be particularly favorable in stomach tumor localization (*P* < 0.01) in a multivariate analysis.

Currently, there are many systemic agents, including immunotherapy or targeted therapies that could reinforce the effect of well-known and widely used chemotherapeutics. Towards this purpose, the multicenter phase II/III MAGIC B trial aimed to evaluate

efficacy, safety, and OS after randomizing operable patients with GC, gastroesophageal junction cancer or lower esophageal cancer to either combination CT with bevacizumab or lapatinib (for HER-2 positive tumors) or to CT alone^[27]. The primary analysis after phase III concluded showed no significant difference in 3-year OS between the two arms (50.3% in the CT alone group vs 48.1% in the CT plus bevacizumab group; *P* = 0.36)^[28]. More than 70% of the patients in both groups also received post-CT as previously planned, but the main limitation remained the inclusion of both gastric and esophageal tumors.

The intensification of systemic therapy could bring more postoperative complications without any benefit in terms of survival. For this reason, a local approach associated to a well-tolerated CT could compensate for disease control, conserving at the same time a good quality of life with fewer side effects. To date, there is still no randomized trial evaluating peri-CT in a large homogeneous cohort of only resectable locally advanced GC patients. The use of combined therapy (CT + RT) in a preoperative setting has been investigated even less, despite many efforts to evaluate this modality of treatment in other gastrointestinal tumors.

In the German POET trial, patients with locally advanced adenocarcinoma of the lower esophagus or gastric cardia were randomized to receive induction peri-CT or CT followed by CRT before surgery^[29].

Table 2 The main randomized trials in gastric cancer that evaluate the preoperative therapy

Trial	Year	Randomization scheme	OS	DFS, PFS	Limits
MAGIC ^[25]	2006	S-alone vs CT + S + CT	5-yr 23% vs 36% (<i>P</i> = 0.009)	3-yr 26% vs 38% (<i>P</i> < 0.001)	Low adherence to post-operative CT, inclusion of gastroesophageal junction or lower esophagus cancer
FNCLCC/ FFCD ^[26]	2011	S-alone vs CT + S + CT	5-yr 24% vs 38% (<i>P</i> = 0.02)	5-yr 19% vs 34% (<i>P</i> = 0.003)	Inclusion of gastroesophageal junction or lower esophagus cancer, small series
MAGIC-B ^[28]	2017	CT/Beva + S + CT/ Beva vs CT + S + CT	3-yr 48.1% vs 50.3% (<i>P</i> = 0.36)	NR	Inclusion of gastroesophageal junction or lower esophagus cancer
POET trial ^[29]	2009	CT + S vs CT + CRT + S	3-yr 27.7% vs 47.4% (<i>P</i> = NS)	NR	Gastroesophageal junction tumors, closed earlier

OS: Overall survival; DFS: Disease-free survival; PFS: Progression-free survival; RT: Radiotherapy; S: Surgery; CT: Chemotherapy; CRT: Chemoradiation; NR: Not reported; NS: Not significant; Beva: Bevacizumab.

Surgery was performed 3-6 wk after induction therapy. The primary end-point was OS, and at 3 years, the median survival was higher in favor of the preoperative CRT group (47.4% vs 27.7%, *P* was not significant), but the predicted statistical survival advantage was not achieved and the study was finally closed prematurely. The authors concluded that a benefit in terms of pathologic complete response was reported in the CRT arm compared to the CT arm (15.6% vs 2.0%). A long-term update of the data in 2017 showed only a trend of significance in OS in favor of preoperative CRT (*P* = 0.055), suggesting an advantage in local control with the addition of RT^[30]. The main randomized studies evaluating preoperative therapy are summarized in Table 2.

Further randomized trials in a preoperative setting are needed to compare the best therapy options. In the meantime, the ongoing phase III randomized TOPGEAR trial (peri-CT vs induction CT followed by CRT) could provide insightful data regarding resectable GC^[31]. An interim analysis showed good adherence to preoperative therapy in both arms, and the treatment could be safely delivered without a significant increase in toxicity or surgical morbidity^[32].

Efforts have been made to improve the surgical approach to a less invasive method with less postoperative complications. With the development of laparoscopic techniques in recent decades for the treatment of localized and locally advanced GC, peri-CT has been more recommended principally in Asiatic countries. However, the safety and efficacy of laparoscopic techniques following preoperative CT still need to be verified prospectively^[33]. Surgical randomized trials have reported less postoperative complications such as the occurrence of excessive bleeding after a well-conducted laparoscopic approach in contrast to an open gastrectomy at experienced centers^[34,35]. Unfortunately, these were short-term results and the oncological efficacy in the locally advanced GC remains to be validated in combination with other treatments

necessary for the local and distant control of the disease.

RECENT EVIDENCE OF NOVEL CHEMOTHERAPEUTIC REGIMENS AND MODERN RT TECHNIQUES

In current clinical practice, the multimodal treatment strategy is based on both peri-CT and post-CRT to obtain better outcomes as demonstrated by randomized studies. The role of RT remains controversial and limited due to higher severe toxicity rates, particularly when combined with systemic agents. In the previously mentioned study by Macdonald *et al*^[6], 273 (97.1%) patients in the CRT arm developed grade ≤ 3 toxicities, mainly represented by hematological and gastrointestinal side effects. Furthermore, 54 (17%) patients discontinued the protocol treatment due to unacceptable toxicity, but this toxicity was due to the use of old RT techniques. Toxicity rates could be reduced with modern RT techniques, which are able to spare normal tissues from higher radiation doses. Also, new chemotherapeutic agents could be combined with RT to obtain better disease control. Meanwhile, data from recent phase I - II studies could be considered in place of randomized data, which is currently lacking.

An American database analysis compared OS between patients who underwent peri-CT to those receiving post-CRT^[36]. A significantly improved OS was found with adjuvant RT on the univariate (*P* = 0.013) and multivariate (*P* = 0.009) analyses in 3656 patients; also, RT had greater benefit among patients with positive surgical margins (*P* < 0.001).

Despite the literature, open questions persist. Could innovative scheduling of radiosensitizers be adopted to increase local control and maintain acceptable levels of toxicity? Could the toxicity profile in a post-CRT setting be improved in locally advanced GC using modern RT techniques? An overview of the phase I - II trials published in the last 5 years has been reported.

Preoperative therapy

Two multicenter phase II trials evaluated by Michel *et al.*^[37] explored the role of CRT pre- ($n = 42$, 50 Gy/2 Gy) or postoperatively ($n = 21$, 45 Gy/1.8 Gy) following induction CT with 4 courses of folinic acid, 5-FU and irinotecan (FOLFIRI). The planned feasibility rates of both approaches were $> 88\%$, while considering unremarkable feasibility rates under 70% . Both studies failed in the primary end-point; in particular, the post-CRT study showed a lower feasibility rate (42.9%) and was prematurely closed. The preoperative study failed with a 73.8% feasibility rate. These results are comparable to the 64% feasibility rate reported by INT-0116, but not to that of post-CRT with capecitabine reported by the ARTIST trial, where the feasibility rate was $> 80\%$, probably reflecting different sensitivities to CT or RT^[11]. The findings from the above phase II studies seem to suggest a higher tolerability for preoperative CRT, but the interpretation of these data should be made with caution due to the use of old RT techniques. The disappointing 8.6% rate of pathologic complete response in the preoperative study would probably suggest a poor effect of irinotecan in contrast to more active agents like cisplatin or paclitaxel.

Effectively, the addition of paclitaxel and carboplatin to a course or preoperative RT showed interesting results in a phase II study by Trip *et al.*^[38]. The design of the study was based on the results of the MAGIC trial^[25], although the treatment protocol was performed in accordance to the CROSS trial^[39], where RT with concurrent weekly paclitaxel and carboplatin was shown to be feasible and improved surgical results in esophageal cancer patients, but was not investigated in GC patients. In the above phase II study, feasibility was 92% and tolerability was good, with 12% severe acute gastrointestinal toxicity and 12% grade 3 leukopenia. Relative high rates of initially unresectable patients (48%) were enrolled, followed conversely by 72% of R0-surgery rates (67% in the unresectable group only). Additionally, this study was limited to a small population ($n = 25$) and despite encouraging results, definitive conclusions on preoperative combined therapy could not be reached.

Another phase II study by Wydmanski *et al.*^[40] enrolled only 13 patients with unresectable GC who were treated with fluoropyrimidine-based preoperative CRT. The study was prematurely closed due to slow accrual (13/40 planned patients). Final results were published due to the favorable outcome in a population that was frequently a candidate only to best supportive care, even if only a small population of limited scientific value was evaluated. Surprisingly, median actuarial OS was 17.1 mo and 1- and 3-year OS were 59% and 48% , respectively; toxicity was mainly characterized by grade ≥ 3 thrombocytopenia (92.3%). The main phase I - II studies evaluating preoperative therapy are summarized in Table 3.

The current studies regarding preoperative CRT

are conducted in small cohorts using inhomogeneous therapy regimens and mostly closed prematurely. This treatment setting needs to be evaluated in larger controlled trials with well-selected GC patients and DFS as the primary endpoint.

Postoperative therapy

Many phase I - II studies have been conducted to evaluate the efficacy of post-CRT. In a phase I study by Wang *et al.*^[41], 18 patients with stage II - III GC were treated with 6 cycles of postoperative oxaliplatin, folinic acid and 5-FU (FOLFOX4) before or after capecitabine-based CRT (45 Gy/1.8 Gy + boost 10.8 Gy/1.8 Gy for R+) delivered with IMRT. Severe toxicity was mainly gastrointestinal (33.4%) and hematological (16.7%), and maximum tolerable dose (MTD) for capecitabine was 800 mg/m^2 twice daily. The use of modern RT techniques may increase the safety and tolerance of the treatment, but the real survival benefit of adjuvant CRT remains controversial and could depend on the type of lymphadenectomy (D0, D1 or D2) performed. In the study, the authors themselves admitted to the inferior performance of D2-lymphadenectomy, as compared to that reported in Japanese and Korean trials. Therefore, they encourage the introduction of post-CRT as a standard of care in their center, emphasizing the general concern regarding high rates of side effects when post-CRT was delivered with out-of-date RT techniques.

Thus, it is well known that irradiation techniques could determine the safety of the treatment, especially in a postoperative setting, as demonstrated by many recent phase I - II studies. Zhai *et al.*^[42] treated 30 patients with 2 cycles of adjuvant FOLFOX6 before or after a fluoropyrimidine-based CRT following D2-lymphadenectomy. Acute severe toxicity was characterized generally by neutropenia (40%) and nausea/vomiting (33%). Another study by Wang *et al.*^[43] administered an intensified post-CT-CRT-CT regimen consisting of 1 cycle of FOLFOX, followed by 2 cycles of FOLFOX on days 1 and 22 of RT and 5 additional cycles of FOLFOX after RT in 110 patients with R0 gastrectomy and D2-lymphadenectomy. The most experienced severe toxicities were nausea/vomiting (14.5%) and leukopenia (9.1%). Nevertheless, 3-year OS and recurrence-free survival (RFS) were 77.6% and 67.8% , respectively, and were not superior to those of other trials.

Recently, Liu *et al.*^[44] evaluated the effect of an intensified post-CT with docetaxel, cisplatin, and 5-fluorouracil (DCF) plus CRT, with docetaxel as a radiosensitizer, in a population of 55 resected GC patients. The outcome of this phase II study was promising, showing 3- and 5-year OS of 72% and 61% , respectively. The use of the dose-attenuated DCF regimen employed by Liu *et al.*^[45], which is less aggressive than standard DCF and other CT regimens, and the use of a single agent, docetaxel, as radiosensitizer combined to IMRT could explain the relatively low rate of severe toxicity, when compared to the traditional results of old randomized

Table 3 The main phase I/II trials in gastric cancer that evaluate the preoperative therapy

Trial	Year/type	N° of patients	Treatment schedule	Median FU	Severe toxicity	Clinical efficacy	Survival	Limits/ characteristics
Matsuda ^[51]	2014/ Phase I	9	SP q15 + RT	NR	Diarrhea (11.1) Anorexia (11.1)	PR (78) SD (22)	NR	MTD: CDDP 25 mg/m ²
Michel ^[37]	2014/ Phase II	42	FOLFIRI4→CRT	38.1 mo	During FOLFIRI (26.2) During RT (19.1)	CR (8.6) Median PFS: 12.3 mo	Median OS: 26.4 mo	Reduced feasibility, 73.8% of patients completed the schedule
Trip ^[38]	2014/ Phase I / II	25	CBDCA-PTX + RT	NR	Nausea (4) Anorexia (4) Esophagitis (4) Leukopenia (12) Febrile neutropenia (4) Thrombosis (4) Fatigue (4)	CR (16) PR (52)	Median OS: 15 mo	
Wydanski ^[40]	2014/ Phase II	13	5FU + RT	30.1 mo	Nausea (7.7) Vomiting (7.7) Thrombocytopenia (92.3) Leukopenia (7.7)	NR	Median OS: 17.1 mo 3-yr OS: 48%	Inoperable patients. High rate of severe thrombocytopenia, with 5FU 325 mg/m ² d1-5 and 29-33
Liu ^[52]	2017/ Phase II	40	SOXx1→S-1 + RT → SOXx1 → surgery → SOXx4	26.5 mo	Leukopenia (10) Neutropenia (10) Thrombocytopenia (2.5)	CR (7.5) PR (30) SD (40) PD (12.5) 2-yr DFS: 47%	2-yr OS: 56%	Treatment compliance: 87.5%

CRT: Chemoradiotherapy; RT: Radiotherapy; CR: Complete response; OS: Overall survival; NR: Not reported; PR: Partial response; SD: Stable disease; MTD: Maximum tolerated dose; PD: Progressive disease; DFS: Disease-free survival; PFS: Progression-free survival.

data. The same authors reported a comparable toxicity when the same treatment schedule was administered in a population of 36 medically inoperable GC patients where, as expected, survival was lower. The main phase I - II studies evaluating postoperative therapies are summarized in Table 4.

Despite lower toxicity, good outcomes could also be observed in patients with advanced GC treated with post-CRT and modern radiation techniques, even after D2-lymphadenectomy. Randomized data are needed to support these hypotheses.

Novel chemotherapy

Cisplatin is known to be an active agent against GC and is safely used concomitantly with RT to improve outcome in several cancers. A phase I / II study by Goody *et al*^[46] investigated the effect of the addition of cisplatin to a fluoropyrimidine-based post-CRT with the aim of identifying the MTD, which was established to be 40 mg/m² weekly. Overall, the acute toxicity rate for all dose levels was 29.1% (37% for MTD group), and 2-year OS and DFS for patients treated at the MTD were 88% and 77%, respectively. Moreover, a very advanced RT technique with 4D-computed tomography planning, daily cone beam-CT, and IMRT were used; compliance was assessed with the European Organization for Research and Treatment of Cancer Quality-of-Life (EORTC) C-30 questionnaire. Unfortunately, the principal limitation of the study was the use of D2-lymphadenectomy only in < 50% of patients and the incomplete planned accrual

that contributed to the reduced power of the study.

In Eastern countries, more evidence is emerging regarding the role of S-1, an oral fluoropyrimidine derivative, in the treatment of advanced GC. Due to the specific characteristics of S-1, it is expected to be well-tolerated by patients and be more effective than 5-FU, as recently demonstrated by a meta-analysis^[47]. In addition, the ARTIST II trial appears to be using S-1 as the concurrent CT agent during radiation. Nevertheless, this drug is not available in Western countries. In some recent studies, S-1 was administered concomitantly to RT, alone^[48-50] or in combination with cisplatin, both in a preoperative and postoperative setting^[51,52]. No excessive toxicities and encouraging outcomes were reported, and an MTD ranging between 70 and 80 mg/m² was identified. Further studies are needed to clearly assess the potential role of this molecule, whose characteristics seem well-suited to be used in combination with RT.

Further molecular characterization of tumors and understanding of disease biology may identify biomarkers and specific markers for trials to optimize radiation timing and the choice of target-oriented therapy^[53]. Similarly, the identification of specific prognostic factors could identify subgroups of patients who could benefit from intensified therapy.

CONCLUSION

In Europe, peri-CT is considered the standard of care in locally advanced GC, while in the United States, pos-

Table 4 The main phase I/II trials in gastric cancer that evaluate the postoperative therapy

Trial	Year/type	N° of patients	Treatment schedule	Median FU	Severe toxicity	Clinical efficacy	Survival	Limits/ characteristics
Michel ^[37]	2014/Phase I	21	FOLFIRIx4→RCT	26.6 mo	During FOLFIRI (23.8) During RT (9.5)	Median PFS: 22.8 mo	Median OS: 32.9 mo	Parallel study with a neoadjuvant schedule (see above). Study closed for futility (42.9% completed the schedule)
Wang ^[41]	2014/Phase I	18	5FU + RT→FOLFOX4 (8) FOLFOX4→5FU+RT (7) 5FU + RT (3)	45 mo	Nausea (11.1) Vomiting (5.6) Esophagitis (5.6) Leukopenia (11.1) Neutropenia (5.6)	4-yr LRC: 93.8%	4-yr OS: 68.1%	MTD: 5FU 800 mg/m ² twice daily
Zhai ^[42]	2014/Phase II	30	FOLFOX6x2→5FU + RT	21 mo	Nausea (33.3) Vomiting (33.3) Diarrhea (6.7) Hepatic (3.3) Cutaneous (3.3) Neutropenia (40) Sensory (23.3)	3-yr DFS: 65%	3-yr OS: 72.7%	
Wang ^[43]	2014/Phase II	110	FOLFOXx1→FOLFOXd1, 22 + RT→FOLFOXx5	43 mo	Nausea and vomiting (14.5) Diarrhea (0.9) Anorexia (11.8) Fatigue (6.4) Abdominal pain (2.7) Leuko-/neutropenia (9.1) Hemorrhage (0.9)	3-yr RFS: 67.8%	3-yr OS: 77.6%	Stage ≤ IIIA significant factor predicting more favorable OS
Qiu ^[48]	2015/Phase I	21	SOXx1→S-1 + RT	26 mo	Nausea (19) Vomiting (19) Fatigue (4.7) Anorexia (14.2) Leukopenia (4.7)	2-yr DFS: 66.7%	2-yr OS: 90.4%	MTD: S-1 70 mg/m ² d
Shim ^[49]	2016/Phase II	46	SPx1→S-1 + RT→SPx2	56.5 mo	Nausea (17.4) Vomiting (8.7) Diarrhea (4.3) Anorexia (15.2) Fatigue (6.5) Neutropenia (28.2) Anemia (6.5) Thrombocytopenia (4.3)	3-yr DFS: 65.2%	3-yr OS: 76.1%	Treatment compliance: 73.9% Intestinal-type tumor showed better DFS and OS
Goody ^[46]	2016/Phase I / II	55	5FU-CDDP + RT	36.4 mo	Hematological (36.3) Constitutional (9) Dermatologic (3.6) Gastrointestinal (18.1) Infection (5.4) Musculoskeletal (1.8)	2-yr LRR: 16.8% 2-yr RFS: 74%	2-yr OS: 85%	MTD: CDDP 40 mg/m ² w1,3,5,7 Treatment compliance: 85.5%
Liu ^[44]	2017/Phase II	55	mDCFx2→TXL + RT→mDCFx2	61 mo	Nausea (63) Vomiting (49) Diarrhea (12) Anorexia (34) Fatigue (31) Neutropenia (60) Thrombocytopenia (51) Thrombocytopenia (15) Anemia (13) Febrile neutropenia (10)	3-yr PFS: 75% 5-yr PFS: 59%	3-yr OS: 72% 5-yr OS: 61%	Treatment compliance 76%

Liu ^[45]	2017/ Phase II	36	mDCFx2→wTXL + RT →mDCFx2	35.6 mo	Nausea (63) Vomiting (48) Diarrhea (9) Anorexia (33) Stomatitis (44) Fatigue (27) Neutropenia (53) Thrombocytopenia (62) Thrombocytopenia (16) Anemia (13) Febrile neutropenia (9)	RR: 83% CR: 36% 3-yr PFS: 32%	3-yr OS: 42%	Inoperable patients. RT was delivered with IMRT technique
Wang ^[50]	2018/ Phase I / II	73	S-1 + RT Various adjuvant CT before or after RT	37.6 mo	Nausea (9.6) Vomiting (5.7) Anorexia (9.6) Esophagitis (3.8) Stomatitis (1.9) Fatigue (1.9) Leukopenia (11.5) Neutropenia (3.8)	3-yr LRFS: 92.2%	3-yr OS: 70%	MTD: S-1 80 mg/ m ²

CRT: Chemoradiotherapy; RT: Radiotherapy; CR: Complete response; OS: Overall survival; NR: Not reported; MTD: Maximum tolerated dose; PD: Progressive disease; DFS: Disease-free survival; PFS: Progression-free survival; LRC: Loco-regional control; RFS: Relapse-free survival; RR: Response rate; LRFS: Local relapse-free survival.

toperative CT has traditionally been used in common clinical practice. As demonstrated by the evidence and the recent updates of randomized trials, meta-analyses and prospective trials, postoperative RT plays a fundamental role in reducing the loco-regional recurrence and in turn, the DFS, in patients with resectable advanced GC, even after a well-performed D2 surgery. A major benefit is noticed in patients with lymph node metastases, suggesting that careful multidisciplinary evaluation of this subgroup is needed. The current results recommend that therapeutic decisions should be made considering individual patients, but a multimodal approach is necessary to guarantee a longer survival and a good quality of life. Ongoing randomized trials could better define the timing and the combination of RT and systemic therapy.

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Optimizing outcomes for patients with gastric cancer peritoneal carcinomatosis

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Abstract

Peritoneal carcinomatosis (PC) from gastric cancer has

traditionally been considered a terminal progression of the disease and is associated with poor survival outcomes. Positive peritoneal cytology similarly worsens the survival of patients with gastric cancer and treatment options for these patients have been limited. Recent advances in multimodality treatment regimens have led to innovative ways to care for and treat patients with this disease burden. One of these advances has been to use neoadjuvant therapy to try and convert patients with positive cytology or low-volume PC to negative cytology with no evidence of active peritoneal disease. These strategies include the use of neoadjuvant systemic chemotherapy alone, using neoadjuvant laparoscopic heated intraperitoneal chemotherapy (NLHIPEC) after systemic chemotherapy, or using neoadjuvant intraperitoneal and systemic chemotherapy (NIPS) in a bi-directional manner. For patients with higher volume PC, cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) have been mainstays of treatment. When used together, CRS and HIPEC can improve overall outcomes in properly selected patients, but overall survival outcomes remain unacceptably low. The extent of peritoneal disease, commonly measured by the peritoneal carcinomatosis index (PCI), and the completeness of cytoreduction, has been shown to greatly impact outcomes in patients undergoing CRS and HIPEC. The uses of NLHIPEC and NLHIPEC plus NIPS have both been shown to decrease the PCI and thus increase the opportunity for complete cytoreduction. Newer therapies like pressurized intraperitoneal aerosol chemotherapy and immunotherapy, such as catumaxomab, along with improved systemic chemotherapeutic regimens, are being explored with great interest. There is exciting progress being made in the management of PC from gastric cancer and its' treatment is no longer futile.

Key words: Peritoneal carcinomatosis index; Peritoneal carcinomatosis; Gastric cancer; Cytoreductive surgery; Heated intraperitoneal chemotherapy; Neoadjuvant intraperitoneal and systemic chemotherapy

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Core tip: Peritoneal carcinomatosis (PC) from gastric cancer, along with positive peritoneal cytology, are associated with poor overall outcomes. The treatment of patients with this disease burden has greatly improved and new multimodality treatment regimens have been introduced. Some of these include neoadjuvant laparoscopic heated intraperitoneal chemotherapy and bidirectional therapies like neoadjuvant intraperitoneal and systemic therapy. Appropriate patient selection remains crucial for optimal outcomes but we can be optimistic about the prospects for carefully selected patients with PC from gastric cancer.

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INTRODUCTION

Gastric cancer, more than any other malignancy, has a particular predilection for peritoneal dissemination. The incidence of peritoneal carcinomatosis (PC) at diagnosis ranges anywhere from 5%-30% depending on the staging modality used^[1,2]. Furthermore, PC is the most common form of relapse after undergoing curative resection as 30% of all recurrences are in the peritoneum and up to 60% of patients have PC at their time of death^[3,4]. Imaging is inadequate with computed tomography (CT) scans having a sensitivity of only 33% and specificity of 99% for detecting PC and 2-[18F]-Fluoro-2-Deoxy-D-Glucose ([18F]FDG) and positron emission tomography (PET) scans having a sensitivity of 28% and specificity of 97%^[5]. Therefore, diagnostic laparoscopy and peritoneal cytology is indicated for clinical stage T1b or higher gastric cancer as a vital step to detect radiologically occult PC in nearly 40% of patients^[6,7]. The presence of microscopic cancer cells within the peritoneal cavity can be identified in up to 6% of patients with no other evidence of metastatic disease^[8]. Patients without visible peritoneal metastases but with positive cytology are considered to have stage M1 disease according to the most recent American Joint Committee on Cancer (AJCC) staging as the outcomes are more similar to patients with gross peritoneal metastasis than those with local disease only^[9-11].

PC from gastric cancer has generally been considered a terminal progression of disease and has worse outcomes than PC from other malignancies such as ovarian cancer or appendiceal cancer^[9,10,12]. Survival for patients with PC is limited but varies based on the burden of disease. A recent series from MD Anderson of patients treated with modern systemic chemothe-

rapy reported 1 year survivals of 24%, 57% and 84% for patients with radiographic PC, PC identified on diagnostic laparoscopy only and positive cytology only, respectively^[13]. A similar report from Memorial Sloan-Kettering confirmed a poor overall survival (OS) for patients with gastric cancer and peritoneal cytology with a median OS of 1.3 years compared to 0.8 years for patients with radiographic evidence of peritoneal disease^[7].

PERITONEAL CYTOLOGY

The management of patients with positive peritoneal cytology is an evolving field. The role for gastrectomy in patients with limited primary disease and positive cytology without any other peritoneal disease has been debated. Some small studies have shown a survival benefit with a gastrectomy in this subset of patients^[14,15]. However, gastrectomy in the setting of untreated positive peritoneal cytology invariably leads to recurrence. National Comprehensive Cancer Network (NCCN) guidelines recommend peritoneal cytology be managed similar to other patients with metastatic gastric cancer with systemic chemotherapy and no surgery^[16].

The need to overcome this seemingly small volume and yet unfavorable disease burden has led investigators to seek ways to convert patients with positive cytology to negative cytology so they can proceed to a curative intent gastrectomy (Table 1). The use of neoadjuvant chemotherapy is one of these methods. Aizawa *et al*^[17] found that 23 of 47 patients (48.9%) with positive cytology converted to negative cytology after neoadjuvant systemic chemotherapy. R0 resections were able to be performed on all patients. The patients who had a conversion to negative cytology and underwent salvage gastrectomy had a survival benefit of 30.4 mo vs 15.0 mo ($P = 0.03$) when compared to those who had persistently positive cytology treated with gastrectomy^[17]. Similarly, a study from Memorial Sloan-Kettering demonstrated that 21 of 48 (44%) patients with initially positive peritoneal cytology treated with systemic chemotherapy achieved negative cytology on repeat laparoscopy^[7]. Unfortunately, the Aizawa *et al*^[17] study reported that 19% of patients progressed on systemic chemotherapy and the MSKCC study reported that 56% had disease progression while receiving systemic chemotherapy. Therefore, better induction treatments are needed^[7,17].

One potential induction treatment is neoadjuvant laparoscopic hyperthermic intraperitoneal chemotherapy (NLHIPEC). In a small phase 2 study, Badgwell *et al*^[18] found that 7 of 19 patients (36.8%) with positive peritoneal cytology or low volume PC had resolution in their peritoneal disease and 5 were able to proceed to gastrectomy. Of note, all patients had undergone systemic chemotherapy before being enrolled in the study. Median OS from the time of diagnosis for the entire cohort was 30.2 mo and median OS for the patients who

Table 1 Studies with positive cytology or low volume peritoneal carcinomatosis

Ref.	Patient No.	Treatment group(s)	Intraperitoneal regimen	Systemic regimen	Outcomes
Aizawa <i>et al</i> ^[17] , 2015	47	NA systemic chemo	--	Variable	48.9% converted to negative cytology Negative cytology Positive cytology Median OS: 30.4 mo Median OS: 15.0 mo
Badgwell <i>et al</i> ^[18] , 2017	19	NA systemic chemo, then NLHIPEC, then gastrectomy if peritoneal disease cleared	MMC and cisplatin	Variable	36.8% converted to negative cytology or had clearance of PC Entire cohort median OS: 30.2 mo
Fujiwara <i>et al</i> ^[19] , 2011	25	NA systemic and IP chemo → gastrectomy if peritoneal disease cleared	MMC and cisplatin	IV docetaxel, 5-fu, cisplatin	56% converted to negative cytology or had clearance of PC Negative Positive Median OS: 27.1 mo Median OS: 9.6 mo
Ishigami <i>et al</i> ^[20] , 2009	40	NA systemic and IP chemo	Paclitaxel	IV paclitaxel and oral S-1	Median OS: 22.5 mo

NA: Neoadjuvant; chemo: Chemotherapy; OS: Overall survival; NLHIPEC: Neoadjuvant laparoscopic hyperthermic intraperitoneal chemotherapy; MMC: Mitomycin C; PC: Peritoneal carcinomatosis; IP: Intraperitoneal; IV: Intravenous; 5-FU: 5-fluorouracil.

proceeded to gastrectomy was 29 mo from the time of their resection^[18]. This approach utilized systemic chemotherapy first, followed by direct intraperitoneal therapy, with encouraging results. Unfortunately, 63.2% of patients had persistently positive cytology or residual PC and did not go onto salvage gastrectomy.

Neoadjuvant intraperitoneal and systemic chemotherapy (NIPS) is another method that utilizes systemic chemotherapy and intraperitoneal chemotherapy, but performs this at the same time in a bidirectional design. Fujiwara *et al*^[19] reported 14 of 25 patients (56%) had resolution of their peritoneal disease with either negative cytology or complete regression of PC. Median OS rate for the group with resolution of peritoneal disease was 27.1 mo vs 9.6 mo ($P < 0.0001$) in patients with persistently positive cytology or residual PC^[19]. Ishigami *et al*^[20] looked at the safety and efficacy of bidirectional treatment for patients with positive cytology or PC. They showed a median OS of 22.5 mo and 1-year survival rates of 78%.

PC

The role of gastrectomy in patients with peritoneal disease was addressed in the REGATTA trial^[21]. This phase 3 trial enrolled 175 patients with a single incurable factor and randomized them to systemic chemotherapy alone or gastrectomy plus systemic chemotherapy. PC was the incurable factor in three-quarters of the patients enrolled. The authors reported no survival benefit to patients undergoing gastrectomy in addition to systemic chemotherapy^[21]. This confirmed that removing the primary tumor without addressing the metastases is not beneficial to the patient.

Cytoreductive surgery (CRS) and hyperthermic intraperitoneal chemotherapy (HIPEC) attempt to address both the primary and the peritoneal metastases simultaneously (Table 2). This aggressive approach has been investigated for gastric cancer since the late 1980's^[22-24]. It includes resection of all visible tumor

from the peritoneal cavity, followed by the instillation of HIPEC^[22]. For the past 30 years, CRS combined with HIPEC has remained the only potentially curative treatment for this advanced stage of gastric cancer^[25,26]. A recent meta-analysis that included 11 randomized controlled trials and 21 high quality prospective studies demonstrated an increased median survival of 4 mo in patients with gastric cancer PC treated with HIPEC^[27], however, the HIPEC group did experience a higher risk of severe complications. Similarly, CRS and HIPEC have shown a significant improvement in survival for patients with PC from other primaries like appendiceal and ovarian cancer^[28,29].

Furthermore, the recent CYTO-CHIP study investigated whether CRS alone was beneficial compared to CRS with HIPEC^[30]. They found a significantly improved OS in the CRS with HIPEC group (18.8 mo vs 12.1 mo), suggesting that it is the combination of CRS and HIPEC that improves survival^[30]. Yang *et al*^[31] reported similar results with improved survival for CRS and HIPEC when compared to CRS alone. Median OS for patients undergoing CRS and HIPEC was 11.0 mo compared to 6.5 mo ($P = 0.046$) for CRS alone. Lastly, in a large retrospective study, Glehen *et al*^[32] reported a 9.2 mo median OS for 159 patients undergoing CRS with HIPEC or EPIC, with improvement to 15 mo if the cytoreduction was complete.

The benefit of CRS and HIPEC over systemic chemotherapy alone was shown by Rudloff *et al*^[33]. In a small cohort of 16 patients, those that underwent CRS, HIPEC, and systemic chemotherapy had an overall median survival rate of 11.3 mo compared to 4.3 mo in the systemic chemotherapy alone group^[33].

Unfortunately, although these studies all demonstrated a modest benefit to CRS and HIPEC, OSs remain unacceptably low. It appears that not all patients benefit from CRS and HIPEC and that appropriate patient selection is vital in order to optimize outcomes. The two most commonly found prognostic factors for survival are consistently the extent of disease, most commonly

Table 2 Studies for peritoneal carcinomatosis with cytoreductive surgery

Ref.	Patient No.	Treatment group(s)	Intraperitoneal regimen	Systemic regimen	Outcomes	
Bonnot <i>et al</i> ^[30] , 2018	277	CRS alone <i>vs</i> CRS + HIPEC	¹	¹	CRS Alone Median OS: 12.1 mo	CRS + HIPEC Median OS: 12.1 mo
Yang <i>et al</i> ^[31] , 2011	68	CRS alone <i>vs</i> CRS + HIPEC	Cisplatin and MMC	-	CRS Alone Median OS: 6.5 mo	CRS + HIPEC Median OS: 11.0 mo
Glehen <i>et al</i> ^[32] , 2010	159	CRS with PIC (HIPEC or EPIC)	Variable	-	Median OS: 9.2 mo	
Rudloff <i>et al</i> ^[33] , 2014	16	CRS/HIPEC/SC <i>vs</i> SC alone	Oxaliplatin	FOLFOXIRI	SC Alone 4.3 mo	CRS/HIPEC/SC Median OS: 11.3 mo
Canbay <i>et al</i> ^[34] , 2014	194	NA systemic and IP chemo, then CRS and HIPEC if responsive	Docetaxel and cisplatin	Oral S-1	78.3% had negative cytology and underwent CRS and HIPEC No response (no CRS or HIPEC) Median OS: 7.5 mo	Response (CRS with or HIPEC) Median OS: 15.8 mo
Yonemura <i>et al</i> ^[38] , 2017	105	NLHIPEC → CRS or NLHIPEC → NIPS → CRS	Docetaxel and cisplatin	Oral S-1, IV docetaxel and cisplatin	NLHIPEC + CRS Median OS: 14.1 mo PCI: 14.2 → 11.8	NLHIPEC + NIPS + CRS Median OS: 19.2 mo PCI: 14.8 → 9.9

¹Abstract only, agents used not included. CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemotherapy; OS: Overall survival; MMC: Mitomycin C; PIC: Perioperative chemotherapy; EPIC: Early postoperative intraperitoneal chemotherapy; SC: Systemic chemotherapy; NA: Neoadjuvant; IP: Intraperitoneal; NLHIPEC: Neoadjuvant laparoscopic HIPEC; NIPS: Neoadjuvant intraperitoneal and systemic chemotherapy; PCI: Peritoneal carcinomatosis index.

measured by the peritoneal carcinomatosis index (PCI), and the completeness of cytoreduction. Glehen *et al*^[32] showed that the PCI was the only independent prognostic factor in patients with a complete cytoreduction. No patient survived more than 3 years if their PCI was > 12^[32]. A meta-analysis confirmed this with no patients being alive after 3 years if their PCI was > 12^[4]. A lower threshold of PCI ≤ 6 was an independent prognostic factor for patients undergoing CRS and HIPEC after bidirectional chemotherapy (HR 2.16, 95%CI: 1.17-3.98, *P* = 0.013) in a recent Japanese study^[34]. Similarly, Chia *et al*^[35] found that a PCI of < 7 was a significant predictor of survival. Those with PCI < 7 had a median OS of 26.4 mo compared to 10.9 mo in those who had a PCI ≥ 7 (HR 2.67, 95%CI: 1.54-4.64, *P* < 0.001). All the patients who were considered cured as defined by being disease-free at 5 years had a PCI < 7. This same PCI cut-off was seen in a study by Yonemura *et al*^[36] who found that a PCI < 7 was associated with improved survival (median survival 2.8 years vs 1.1 years, *P* = 0.0001).

With a lower volume of disease, there is a higher probability of being able to completely remove all the metastatic disease. This is the only population that can be expected to have a chance at long-term survival. A meta-analysis showed that cytoreductive scores of 0 or 1 significantly improved survivals in patients with gastric PC^[4]. Glehen *et al*^[37] showed that patients undergoing a complete cytoreduction with a CC score of 0 or 1 achieved a median OS of 21.3 mo compared to only 6 mo for those with an incomplete cytoreduction. The 5-year OS was 29.4% for those who attained a complete cytoreduction with no survivors in the incomplete cytoreduction group^[37]. Canbay *et al*^[34] used bidirectional therapy (neoadjuvant systemic and

intraperitoneal therapy) to reduce the volume of disease before CRS and HIPEC for patients that responded to treatment. They found better OS in patients who responded to the neoadjuvant treatment and were able to undergo CRS and HIPEC (15.8 mo vs 7.5 mo)^[34].

There is substantial interest in novel and innovative ways to reduce the PCI prior to cytoreduction. This is crucial because PCI is a determinant in achieving a complete cytoreduction and only patients with a low volume of disease who undergo a complete cytoreduction have a long-term survival benefit from the procedure. Yonemura *et al*^[38] used NLHIPEC and NLHIPEC plus NIPS to try and reduce PCI levels before CRS. They found that while NLHIPEC alone reduced PCI levels (14.2 ± 10.7 to 11.8 ± 11.0, *P* = 0.023), NLHIPEC plus NIPS doubled the PCI reduction (14.8 ± 11.4 to 9.9 ± 11.3, *P* < 0.0001). This may provide more patients with the opportunity for a complete cytoreduction when this would have otherwise not been possible due to a high PCI.

UNRESECTABLE PC

Even with all the advances in therapy for patients with PC from gastric cancer, there are still a large number of patients who are not eligible for these therapies given their high tumor burden or conditional status. Palliative treatment for these patients includes chemotherapy, chemoradiation, or supportive care. None of these regimens treat the peritoneal disease burden and patients generally have very limited survivals.

A new experimental therapy that has emerged to treat these patients is pressurized intraperitoneal aerosol chemotherapy, or PIPAC^[39]. This method delivers aerosolized chemotherapy to the peritoneum. The benefit of this method is that the pressure allows

Table 3 Immunotherapy studies

Ref.	Patient No.	Treatment group(s)	Intraperitoneal regimen	Systemic regimen	Outcomes	
Heiss <i>et al</i> ^[45] , 2010	66	Paracentesis + catumaxomab <i>vs</i> Paracentesis alone	Catumaxomab	-	Paracentesis Alone Median OS: 44 d	Paracentesis + Catumaxomab Median OS: 71 d
Bokemeyer <i>et al</i> ^[46] , 2015	54	NA chemotherapy, surgery, intra- and post-op catumaxomab	Catumaxomab	Variable	4 yr DFS: 38% 4 yr OS: 50%	

OS: Overall survival; NA: Neoadjuvant; DFS: Disease free survival.

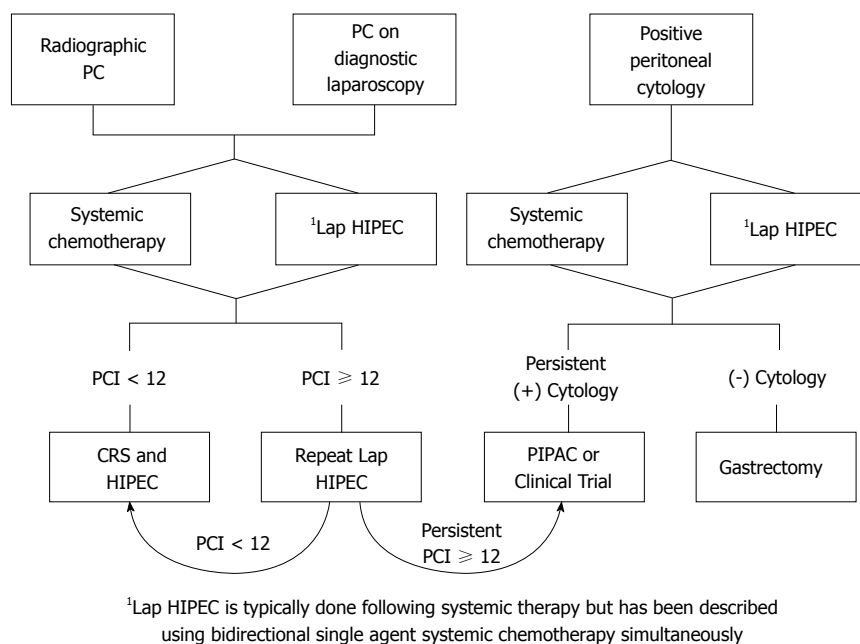


Figure 1 Treatment algorithm for gastric cancer peritoneal carcinomatosis. PC: Peritoneal carcinomatosis; CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemotherapy; PCI: Peritoneal carcinomatosis index.

for greater lesion penetration as well as allowing for diffuse and even coverage throughout the abdomen^[40]. This deeper penetration is likely more critical in these patients with advanced bulky peritoneal metastases. Nadiradze *et al*^[39] recently published data on 24 patients with end stage gastric cancer with PC. These patients underwent 1 or more rounds of PIPAC with doxorubicin and cisplatin. The median OS for these patients was 15.4 mo with 52% alive at one year^[39]. A multi-center study of PIPAC for advanced PC from a variety of histologies including gastric cancer demonstrated that 63.5% of patients achieved resolution of symptoms^[41]. This therapy may prove to be beneficial for more than just end stage gastric cancer patients but additional research is needed.

FUTURE EFFORTS

Innovative discoveries and continued efforts to optimize treatment for patients with PC from gastric cancer are needed. This includes improved systemic chemotherapy options such as FLOT, which has been demonstrat-

ed to be effective in patients with limited metastatic disease^[42]. The AIO-FLOT3 trial reported a median OS of 31.3 mo and a 60% radiographic response rate for patients who were treated with perioperative FLOT systemic chemotherapy and surgical resection of all metastatic disease^[42].

Another innovative approach is the use of immunotherapy, like catumaxomab, as an intraperitoneal treatment (Table 3). Catumaxomab is an antibody that binds to both epithelial cells through epithelial cell adhesion molecule (EpCAM) and T-cells through CD3^[43]. Gastric cancer expresses high levels of EpCAM so the intraperitoneal administration of EpCAM provides targeted therapy to peritoneal implants^[44]. In patients with malignant ascites from PC of gastric origin, it was found to significantly prolong OS from 44 to 71 d^[45]. Bokemeyer *et al*^[46] conducted a phase 2 study where patients underwent intra- and post-operative intraperitoneal catumaxomab administration after undergoing neoadjuvant chemotherapy and resection. These patients had four-year disease-free survival rates of 38% and four-year OS rates as high as 50%. Though catumaxomab is no lon-

ger available, the use of intraperitoneal immunotherapy remains promising and is under continued investigation^[47].

There remain many areas related to the management of PC from gastric cancer that can be improved. Better detection of early occult peritoneal metastases would allow the clinician to select more appropriate patients for these multidisciplinary treatments. This may be in the form of improved imaging modalities like fluorescence and antibody-labelled imaging^[48] or the use of RT-PCR with cytology to improve the sensitivity of detecting cancer cells in peritoneal washings^[49]. The optimal chemotherapeutic agent, or agents, to use is unclear, both systemically and in the peritoneal cavity. Many of the studies discussed here used different treatment regimens with some varying even within the same study, so it is difficult to compare outcomes from one study to the next. Also, the ideal sequence, route, and duration of treatment for these patients that will deliver the greatest long-term benefit with manageable side-effects is unknown, though there are many promising options.

Appropriate patient selection remains crucial for optimal outcomes in patients with gastric cancer, but patients with PC or positive cytology should no longer be immediately excluded from potentially curative multimodality treatment regimens. There are treatment options that can be offered to suitable patients with PC from gastric cancer that have the possibility of extended survival (Figure 1). We are finally seeing progress in the management of a disease that has traditionally been thought of as terminal and it is time to change our approach. We are not yet at a point where we can offer these patients a cure, but the treatment of PC from gastric cancer is no longer a futile endeavor and can be approached with careful optimism.

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Inhibiting focal adhesion kinase: A potential target for enhancing therapeutic efficacy in colorectal cancer therapy

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Abstract

Focal adhesion kinase (FAK) is a major integrin-dep-

endent tyrosine phosphorylated protein, recently, FAK association with colorectal cancer (CRC) has gained attention. The various cancer-promoting mechanisms that associated with FAK can be implicated in the progression of CRC. The interactions between structural features of FAK and various kinases could be closely related to growth, survival, and metastasis in CRC cells. These interactions include human epithelial growth factor receptor, c-Met, platelet-derived growth factor receptor, vascular endothelial growth factor receptor, and Src. Such interactions can trigger the survival signaling of CRC cells and are also involved signaling downstream of phosphatidylinositol 3-kinase, AKT, and the extracellular regulated kinase. Based on this scientific background, many pharmaceutical companies are taking efforts to develop FAK inhibitors to treat solid cancer including CRC. Although the anti-cancer efficacies have been noted in many studies, the commercial drugs have not been developed yet. Therefore, the FAK research on CRC is expected to gain momentum and be highly appreciated as a potential field for developing the new drugs. Therefore, the studies on FAK that effect on the progression of human CRC s would be possible to suggest various approaches to CRC treatment, and FAK could be a potential target as an anticancer candidate for CRC therapies.

Key words: Colorectal cancer; Focal adhesion kinase; Focal adhesion kinase inhibitor; Anticancer effect

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Core tip: Despite ongoing development in treatment for colorectal cancer (CRC), effective markers for treatment of CRC have not been elucidated. FAK association with various kinases for progression and invasion of CRC has recently gained attention. The possibility for this association is accounted that FAK is interactions with integrins, growth factor receptors, and adjacent kinase domain. Targeting FAK is possible to explain the mechanism at

the upstream level by which can mediate the expression of various survival signaling and inhibition of onco-suppressor genes as well as inducing migration and invasion of the CRC cells. Therefore, FAK could be a prognostic marker and a potential candidate target for CRC therapies.

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Focal adhesion kinase (FAK) is a major integrin-dependent tyrosine phosphorylated protein and a non-receptor tyrosine kinase that is localized to cellular focal adhesions^[1]. Although there have been many studies on the role of FAK in breast cancer, its association with colorectal cancer (CRC) has recently gained attention. FAK, known as protein tyrosine kinase 2, is related to other tyrosine kinases, such as Src kinase^[2]. FAK comprises a central kinase domain between an N-terminal FERM domain and a C-terminal domain that includes the focal adhesion sequence. The construction of the N-terminal FERM domain is similar to that of cytoskeletal proteins and several tyrosine phosphatases and tyrosine kinases. This domain mediates FAK interactions with integrins and growth factor receptors and interacts with the adjacent kinase domain in FAK. The C-terminal domain contains proline-rich sequences for SH3 domain-containing proteins and acts to recruit additional signaling proteins^[3,4].

The interactions between structural features of FAK and various kinases could be closely related to cancer growth, survival, and metastasis. FAK is activated by the direct interaction of the Src kinase with the integrin β cytoplasmic domain^[4]. Integrin can trigger the survival signaling of cancer cells at locations further downstream of phosphatidylinositol 3-kinase (PI3K), AKT, and the extracellular regulated kinase (ERK)^[1,5]. The kinase complex with Src is reportedly affected in the activation of these survival pathways. In addition, FAK interacts with several receptor tyrosine kinases, including human epithelial growth factor receptor, c-Met, platelet-derived growth factor receptor, and vascular endothelial growth factor receptor (VEGFR), which also mediates the survival pathway of cancer cells^[2,6]. The detailed mechanism of PI3K signaling is as follows. The PI3K/AKT pathway induces the expression of apoptosis inhibitory proteins through nuclear factor kappa (NF- κ) B and protects the cells from stress-induced apoptosis. It is also associated with expression of cancer suppressor genes^[5,6]. FAK promotes cell survival via suppression of p53 activation. This is mediated by the kinase-independent FAK FERM domain, and it suppresses the transcriptional activation of target genes

that is mediated by p53 activation. Therefore, FAK can enhance cell survival through both kinase-dependent and-independent mechanisms^[7]. Further, the expression of an active mutant of ERK has indicated a direct role of FAK in promoting cancer growth. It is suggested that FAK signaling through the ERK pathway is needed to maintain cancer cell development^[8]. Furthermore, the kinase activity of FAK is estimated to be significant for the invasive phenotype and for cancer metastasis. FAK reportedly promotes cancer cell invasion through the regulation of matrix metalloproteinases (MMPs)^[1,9]. In v-Src transformed cells, the Rac1 and JNK is activated in FAK/Src complex and is induced the MMP2 and MMP9 expression. Thus, FAK promotes increased invasiveness of cancer cells^[10].

Of course, the various cancer-promoting mechanisms associated with FAK described above could also be implicated in the progression of CRC. Colon cells including epithelial and fibrous cells increases the FAK expression at early stages of carcinogenesis, even before the cancer has formed^[1,11]. The up-regulation of FAK promotes the adhesive properties of CRC cells and their survival^[11]. FAK signaling is associated with the binding of the Rho guanine nucleotide exchange factor, and this signaling complex promotes the local invasion of colon carcinoma. The increase in FAK activation is thus related to elevated tyrosine phosphorylation and an adaptor protein, such as paxillin, involved in the growth of the CRC cells^[1,2,12]. Further, FAK signaling contributes to epithelial-mesenchymal transition (EMT) profile change in CRC cells. FAK scaffolding increases, thus leading to alterations in EMT markers, including MMP-induced motility of CRC cells. Therefore, FAK acts to affect the dynamic internalization of E-cadherin in CRC cells^[2,13]. Furthermore, FAK FERM overexpression can reduce steady-state p53 levels in CRC cells, particularly HCT-116 cells. As increased FAK expression is often found in early-stage CRCs, the FAK FERM-mediated cell survival pathway is expected to have an important function in the survival of CRC cells^[7,14]. During cancer progression and metastasis, an anchorage-independent pathway can facilitate the spread of cells from the primary cancer site. Under these conditions, the cancer cells that show higher levels of FAK may be more resistant to apoptosis by non-integrin-associated FAK to translocate to the nucleus and prevent excessive p53 activation^[2,7,15]. It is associated with that alternative-spliced transcripts encompassing the N-terminal FERM domain without the FAK kinase or C-terminal regions would be related to the progression of CRC^[2,7].

Based on this scientific background, many pharmaceutical companies are taking efforts to develop FAK inhibitors. TAE-226 by Novartis exhibits nanomolar inhibitory activity toward FAK and protein tyrosine kinases and has anti-cancer activity. It particularly blocks cell proliferation and invasion and showed increased apoptosis in many xenograft animal models^[7]. Further, TAE-226 in combination with docetaxel, a microtubule stabi-

zer, significantly decreases angiogenesis and cancer cell invasion^[15]. Pfizer has developed PF-228 that shows more specific FAK inhibitory activity. It inhibits cancer cell migration *in vitro*. Pfizer has also developed PF-573, 228 compound, and the results indicated cancer growth inhibition in the colon xenograft cancer model^[16]. In addition, several other FAK inhibitors have been developed, including GSK2256098 by GlaxoSmithKline as a formulation for oral intake and VS-4718 by Poniard as an improved version of the previous product, PND-1186^[17,18]. Although efficacy has been noted in non-clinical and early-stage clinical trials, the drugs have not been commercialized yet. Therefore, the FAK research on CRC is expected to gain momentum and be highly appreciated as a potential field for developing the new drugs.

The kinase-dependent function and kinase-independent ability of FAK are essential for cancer development^[19]. Multifunctional characteristics of FAK have been highlighted as modulators of numerous signal transductions in CRC cells. The established role of FAK in cancer progression and metastasis has obviously proposed that increase in FAK expression contributes a very important part in CRC development. Various inhibitors by small-molecules for targeting inhibition of FAK kinase and autophosphorylation have been produced by many pharmaceutical companies. Although some clinical trials have already been undergoing and potential efficacy has been noted, further studies must be needed to confirm if FAK expression has important role in a progression of human CRC and elaborates on the clear mechanisms and downstream effectors in the context of carcinogenicity. Taken together, based on the clinical observations, the over-expression of FAK at both transcriptional and translational levels in human CRCs would imply that targeting FAK could be a prognostic marker and a potential anticancer candidate for CRC therapy.

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Simultaneous curative resection of double colorectal carcinoma with synchronous bilobar liver metastases

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Abstract

Synchronous colorectal carcinoma (SCRC) indicates more than one primary colorectal carcinoma (CRC) discovered at the time of initial presentation, accounts for 3.1%-3.9% of CRC, and may occur either in the same or in different colorectal segments. The accurate pre-operative diagnosis of SCRC is difficult and diagnostic failures may lead to inappropriate treatment and poorer prognosis. SCRC requires colorectal resections tailored to individual patients, based on the number, location, and stage of the tumours, from conventional or extended hemicolectomies to total colectomy or proctocolectomy, when established predisposing conditions exist. The overall perioperative risks of surgery for SCRC seem to be higher than for solitary CRC. Simultaneous colorectal and liver resection represents an appealing surgical strategy in selected patients with CRC and synchronous liver metastases (CRLM), even though the cumulative risks of the two procedures need to be adequately evaluated. Simultaneous resections have the noticeable advantage of avoiding a second laparotomy, give the opportunity of an earlier initiation of adjuvant therapy, and may significantly reduce the hospital costs. Because an increasing number of recent studies have shown good

results, with morbidity, perioperative hospitalization, and mortality rates comparable to staged resections, simultaneous procedures can be selectively proposed even in case of complex colorectal resections, including those for SCRC and rectal cancer. However, in patients with multiple bilobar CRLM, major hepatectomies performed simultaneously with colorectal resection have been associated with significant perioperative risks. Conservative or parenchymal-sparing hepatectomies reduce the extent of hepatectomy while preserving oncological radicality, and may represent the best option for selected patients with multiple CRLM involving both liver lobes. Parenchymal-sparing liver resection, instead of major or two-stage hepatectomy for bilobar disease, seemingly reduces the overall operative risk of candidates to simultaneous colorectal and liver resection, and may represent the most appropriate surgical strategy whenever possible, also for patients with advanced SCRC and multiple bilobar liver metastases.

Key words: Colorectal surgery; Synchronous colorectal liver metastases; Major hepatectomy; Parenchymal-sparing hepatectomy; Intraoperative ultrasonography; Simultaneous colorectal and liver surgery; Synchronous colorectal carcinoma; Ablative therapies

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Core tip: Simultaneous colorectal and liver resection represents an appealing surgical strategy in selected patients with colorectal cancer and resectable synchronous liver metastases (CRLM). Synchronous colorectal carcinoma may represent an adequate indication to simultaneous resections, even though it may require more complex colorectal resections. In patients with multiple bilobar synchronous CRLM, major hepatectomies performed simultaneously with colorectal surgery have been associated with increased perioperative risks compared to major hepatectomies alone. Conservative or parenchymal-sparing hepatectomies reduce the extent of hepatectomy while preserving oncological radicality, and may represent the best option to reduce the perioperative risks of simultaneous colorectal and liver resection.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most frequent causes of cancer-related death in Western countries^[1,2]. The development of at least two different neoplasms is defined as multiple primary CRC (MPCRC), which

represents 5% to 10% of all CRCs^[3,4]. Synchronous colorectal carcinoma (SCRC) indicates more than one primary CRC discovered in a single patient at the time of initial presentation, while neoplasms diagnosed some time after the resection and/or diagnosis of the first lesion are called metachronous CRC^[3,4]. Compared with solitary CRC, SCRC possess distinctive features that need to be extensively investigated in preoperative evaluation to ensure adequate diagnosis and treatment^[5]. SCRC account for 3.1% to 3.9% of CRCs^[3,6], and may occur either in the same segment of the large intestine or separately in different colon segments^[3,5]. Multiple factors, including inflammatory bowel diseases, hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome, and familial adenomatous polyposis (FAP)^[3,7], predispose to CRC and have also been associated with a higher risk of SCRC, though predisposing factors only account for a minority of cases^[8]. Patients with SCRC have in most cases an overall oncological prognosis similar to those with solitary CRC, at least when the pathological stages of tumours are comparable and the resections are curative^[4,6,8-13]. Nonetheless, the accurate preoperative diagnosis of SCRC remains difficult and diagnostic failures may lead to inappropriate treatment and poorer prognosis^[5]. The presence of SCRC or multiple neoplasms requires operative techniques tailored to individual patients, based on the number, location, and stage of the tumours. Patients with SCRC and established predisposing conditions such as HNPCC, FAP, and ulcerative colitis require extensive surgery, usually total colectomy or proctocolectomy. In the other cases the optimal surgical strategy is still debated. Conventional hemicolectomies or extended hemicolectomies can be indicated if multiple tumours are located in adjacent segments^[12]. When SCRC are located in distant colonic segments, some authors suggest total or subtotal colectomy^[14,15], while others suggest more conservative surgical strategies with resection of two intestinal segments, either open or laparoscopic-assisted^[13,16-18], seemingly resulting in a higher risk of anastomotic dehiscence^[6]. However, overall perioperative results of colorectal resections for SCRC seem to be worse than those of solitary CRC with more postoperative complications and reinterventions and longer hospital stays^[6]. As a consequence, an accurate preoperative workup and adequate surgical strategies are required for SCRC especially when adjunctive simultaneous surgical procedures are needed to obtain potential cure.

Synchronous liver metastases (CRLM) are evident in nearly 15% to 25% of patients with CRC at the time of diagnosis^[1]. Radical liver resection (LR) is presently considered the only curative therapy capable of achieving long-term survival with more recent series describing 5-year overall survival (OS) rates of 37% to 58% after hepatectomy^[19,20]. Nonetheless, the management of patients who present with CRC and synchronous metastases is more complex because they are considered to have less favourable cancer biology

and expected long-term results than those with metachronous liver disease^[2,21]. The optimal timing for surgical resection in case of synchronous presentation of CRC and liver metastases is still controversial. Most surgeons usually prefer a staged approach with initial resection of the colorectal primary followed by hepatectomy^[19], presuming that this strategy avoids increased perioperative complications associated with simultaneous procedures^[20,22], and avoids also inappropriate hepatic surgery in patients with progression of the liver disease after colectomy especially if occurred during interval chemotherapy (CHT)^[22]. More recently an increasing number of studies have shown satisfactory perioperative outcomes for simultaneous procedures comparable to those of staged strategies^[19,23-30]. Simultaneous colorectal and liver procedures have the obvious advantage of avoiding a second surgical procedure, along with the chance of an earlier initiation of adjuvant CHT. However, an adequate evaluation of the cumulative risks of the two procedures is mandatory. In the last decade, the paradigm of surgical strategies for synchronous presentation of primary CRC and liver metastases is progressively changing, even though a consensus is far from being reached. Simultaneous colorectal resection and minor hepatectomy have perioperative results similar to minor hepatectomy alone, and are at present considered the treatment of choice in most patients with limited liver disease^[19,23-30]. In patients requiring simultaneous colorectal and major LR the perioperative results are much more conflicting. Most investigators have reported worse perioperative outcomes than for major LR alone^[20], while others remark that simultaneous colorectal and major hepatic resection can be performed safely in selected cases, with perioperative risks comparable to major LR alone^[31-33]. Also simultaneous resection of rectal primaries and major hepatic resections have been considered reasonable in carefully selected patients^[33,34].

Major hepatectomies have been traditionally preferred in the past to obtain radical resection of CRLM, especially in the case of large and/or multiple nodules. However, extensive hepatectomies have been associated with significant morbidity and mortality rates, usually related to posthepatectomy liver failure^[35,36]. Several strategies have been developed to improve the feasibility of LR without increasing the risk of postoperative liver failure. Different systemic and locoregional chemotherapy protocols may significantly reduce the neoplastic burden in the liver with the aim of converting initially unresectable to resectable CRLM^[37], but also of limiting the extension of LR^[38]. Some technical innovations have permitted an increase in the amount of the future remnant liver (FRL) in candidates for major hepatectomy at increased risk of posthepatectomy liver failure based on the preoperative hyperplasia of the estimated remnant liver parenchyma, including preoperative portal vein embolization (PVE) and two-stage hepatectomy (TSH)^[39]. An alternative strategy is to remove liver tumours with the minimum sufficient oncological margin to preserve as much non-

tumorous liver parenchyma as possible, to limit the risk of liver failure in the perioperative period even for patients with advanced neoplastic liver disease^[35], but also to preserve the major intrahepatic vessels whenever possible in order to increase the chance of resection in case of hepatic recurrence (salvageability)^[40,41]. In fact, resection of relapsed CRLM has been widely demonstrated to have the potential for cure in selected patients with recurrent disease^[20,42], with comparable morbidity and mortality rates than those of initial resection^[43,44]. An accurate preoperative planning and an expert use of intraoperative ultrasonography (IOUS) are of paramount importance to achieve adequate oncological and surgical results. This strategy has been termed "conservative" or "parenchymal-sparing" liver resection (PSLR)^[40,41]. A progressive shift toward more conservative hepatectomies has been observed in the last decade also for multiple and/or bilobar CRLM, and has been correlated with decreased morbidity and mortality rates and similar oncological results compared to major hepatectomies^[45-47].

There is growing evidence, at least in numerous experimental studies, that surgical procedures for primary and metastatic CRC can activate multiple local and systemic events, such as hypoxia, inflammation, immune depression, release of multiple factors after the resection of the primary tumour and/or the CRLM, and release of tumour cells during surgical manipulation^[48]. These events can exert local tumour-promoting effects, such as favouring the implantation and the proliferation of the residual neoplastic cells (predisposing the patient to local recurrences), activating dormant tumour cells in distant organs, and/or establishing a pre-metastatic niche (predisposing the patient to the occurrence of distant metastases)^[48]. The real impact of these events in the clinical setting is still uncertain. On the other hand, LR activates within few hours multiple molecular changes (upregulation of several cytokines and growth factors) with subsequent activation and proliferation of mature hepatocytes, hepatic progenitor cells, and non-parenchymal liver cells to restore the optimal liver volume. These specific regenerative factors determine a complex microenvironment, which has been demonstrated to promote either the proliferation of residual cancer cells or tumour propagation in the remnant liver and also at distant sites, at least in various experimental models^[48-52]. In patients with multiple bilobar CRLM, extended hepatectomies are traditionally considered to achieve potentially curative LR. In selected patients, PVE with or without TSH is proposed to induce preoperative hyperplasia of the FRL and increase the resectability rate. As for liver regeneration, several experimental and clinical studies have demonstrated that also PVE promotes tumour progression, either through an upregulation of cytokines and growth factors or by haemodynamic changes in the blood supply to the liver, which may adversely influence the subsequent management of the neoplastic disease^[49,53-55]. Taken together, these experimental and clinical observations

support the theoretical advantages of simultaneous colorectal and liver resection, to prevent the drawbacks of multiple surgical procedures, and of conservative hepatectomies, to limit the impact of liver regeneration on tumour growth and metastatization.

The aim of the present review is to critically analyse the available data to determine whether complex colorectal resections for synchronous CRC are compatible with the simultaneous resection of CRLM, even in the case of multiple and/or bilobar CRLM.

SYNCHRONOUS COLORECTAL CARCINOMA

Epidemiology and predisposing conditions

The overall prevalence of SCRC ranges from 1% to 8% in different studies^[3,6]. In four large multicentric studies including a study population between 13000 and 25000 patients with CRC, the prevalence ranged from 3.1% to 3.9%^[6,10,11,56], while a recent systematic review pooling data from 39 series reported an overall prevalence of 3.5%^[3]. In these series, SCRC had a higher male to female ratio when compared to solitary carcinoma, ranging between 1.5 and 2.2^[3,6,10,11,56]. The mean age at presentation was 63 years in a systematic review pooling data from 32 series^[3], usually higher than in patients with solitary CRC^[3,6], even though this point is somewhat controversial^[5,11]. Preferred locations of SCRC are still debated. Some authors have reported that many SCRC occur in the same segment of the large intestine, while others believe that most SCRC occur separately in different colon segments^[3,5]. Moreover, SCRC are located in the ascending colon probably more often than described for solitary CRC^[3,5,6], but also this point is controversial^[5]. A minority of patients develop more than two SCRC^[7,16], with a maximum of seven simultaneous colorectal lesions described in a single patient^[56].

Possible predisposing factors, including inflammatory bowel diseases, HNPCC or Lynch syndrome, and FAP, to CRC have also been associated with a higher risk of SCRC^[3,7]. SCRC has been diagnosed in up to 20% of patients with CRC associated with inflammatory bowel disease^[57,58] (more frequently ulcerative colitis than Crohn's disease^[59]), and in 21% of patients with CRC associated with FAP^[57]. Patients with known predisposing factors might account for about 12% of SCRC^[8]. Dysplasia induced by chronic inflammation and adenomas are involved in the development of SCRC in these patients^[8,60]. Colorectal serrated polyps have more than a two-fold increase risk of detection of advanced CRC, with proximal and large serrated polyps having the highest risk^[61]. Also the serrated neoplastic pathway may predispose to MPCRC^[62]. Higher incidence rates of associated benign neoplasms have been described for SCRC than for single cancers^[5,11,13]. The higher incidence of mucinous carcinoma in SCRC is still controversial^[3,7].

Metachronous CRC can also occur after resection of SCRC, especially in patients with inflammatory bowel disease^[59].

Mechanisms of carcinogenesis and molecular biology

MPCRC usually develop on a common etiologic substrate, either hereditary or environmental. Multiple recent studies on molecular carcinogenesis have demonstrated that chromosomal instability, microsatellite instability, and gene methylation are all mechanisms implicated in multiple lesions or events predisposing to SCRC. This may be due either to familial predisposition or more frequently to individual factors (mainly environmental exposure). Factors involved in the development of MPCRC have been recently reviewed^[3-5,62]. CRC has a substantial heritable component^[63]. Based on multicentric data derived from almost 45000 pairs of twins, the estimated effect of heritability on CRC is up to 35%^[64], even though involved genetic factors are still incompletely understood. Well-known hereditary CRC syndromes, including HNPCC and FAP (which account for 3% to 5% of all CRC^[65]), present germline mutations and promote the development of several neoplasms over time^[8]. Other diseases and conditions, such as inflammatory bowel diseases, may extensively involve colorectal mucosa, thus promoting the formation of multiple foci of dysplasia and cancer^[57]. In most cases however, the origin of SCRC is unknown, likely due to the coexistence of genetic predisposition and environmental factors^[4]. As for other neoplasms, also for SCRC the concept of a field defect has been proposed to explain tumour multiplicity through a generalized cellular or molecular disorder in the entire colorectal mucosa^[66]. Because only a minority of all SCRC are related to hereditary diseases, an important proportion of SCRC lack a clear basis of inheritance^[4,9], being possibly related to individual predisposition to MPCRC. As for sporadic CRC, the prevalence of SCRC increases with age^[9,10], indicating the possible role of cumulative environmental damage, even though this point has not been confirmed in other studies^[8,11]. Alcohol intake and tobacco smoke, which consists of different genotoxic substances, have been related to an augmented risk of MPCRC^[4,5].

Molecular biology and mechanisms of development of SCRC are heterogeneous. The majority of CRC follows the classical adenoma-carcinoma sequence of tumour progression, and dysplastic adenomas are the most common form of premalignant precursor lesions^[63,67]. However, more than 15% of sporadic CRC develop through alternative pathways of molecular events, including cancers originating from serrated precursor lesions^[63,68]. Molecular pathways of development of SCRC have been recently reviewed^[3,5,9,66,68-70] and are out of scope for this review. Nonetheless, the complex mechanism of carcinogenesis involved in the development of SCRC is still largely unknown and only partially related to known genetic mutations commonly found in CRC.

Prognosis

The prognosis of patients with SCRC compared to solitary CRC is still debated. Even though the first prospective study on the outcome of SCRC reported worse long-term results than solitary CRC^[15,69], most recent studies could not demonstrate different survival rates between SCRCs and CRCs when the pathological stages of tumours were matched and the resections were curative. However, some authors have reported marginal survival benefits of patients with SCRC^[3,5,6,8-13].

Diagnosis

The preoperative diagnosis of multiple SCRC remains difficult (Table 1). Additional tumours may be ignored or missed at the time of diagnosis of the first cancer, with diagnostic failure leading to inappropriate treatment and poorer prognosis^[5]. Routine preoperative colonoscopy is mandatory to identify synchronous neoplasms^[71]. Because preoperative evaluation of the colon during colonoscopy is often incomplete due to bowel obstruction, poor bowel preparation, or technical reasons, double-contrast barium enema and computed tomographic (CT) colonography, magnetic resonance (MR) colonography, and/or positron emission tomography/computer tomography (PET/CT) colonography are advisable^[5,63,72-74]. Also the use of intraoperative colonoscopy has been recommended in selected cases^[5,16,75]. At the time of operation, it is also important to palpate the entire colon and check pathological specimens thoroughly^[5,16]. An adequate combination of these imaging techniques with the traditional colonoscopy usually permits an accurate definition of number and location of synchronous colorectal neoplasms and an appropriate plan of the optimal surgical procedures^[6]. Patients with mid and low rectal adenocarcinoma should routinely receive endorectal ultrasound and pelvic magnetic resonance imaging because the quality of preoperative imaging for local staging is essential to pursue an appropriate therapeutic strategy^[76-78], which includes perioperative chemoradiotherapy and surgical resection for locally advanced extraperitoneal tumours^[77,78].

Surgical treatment strategies

The standard surgical procedure for the treatment of rectal cancer is total mesorectal excision consisting of the removal of the rectum together with the mesorectum, which contains most of the involved lymph nodes and tumour deposits, and the mesorectal fascia^[76] along with clear circumferential margins^[77]. The appropriate removal of the rectal cancer reduces the risk of local recurrence and the development of distant metastases^[77,78]. Surgical procedures for colon cancer entail resection of the tumour with the corresponding lymph nodes. The extent of colonic resection is determined by the tumour location and the supplying blood vessels. The presence of SCRC or multiple neoplasms requires operative te-

chniques tailored to individual patients based on the number, location, and stage of the tumours. Patients with SCRC and established predisposing conditions such as HNPCC, FAP, and ulcerative colitis require extensive surgery, usually total colectomy or proctocolectomy. In the other cases, the optimal surgical strategy is still debated. Early-stage lesions can be removed during colonoscopy with endoscopic mucosal or submucosal resection. Hemicolectomy or extended hemicolectomy can be indicated if multiple tumours are located in adjacent segments^[12]. When SCRC are located in distant colonic segments, some authors suggest total or subtotal colectomy to remove synchronous tumours or polyps eventually undetected at preoperative imaging and to prevent the development of metachronous neoplasms^[14,15]. In the same circumstances, other authors suggest more conservative surgical strategies, with resection of two intestinal segments (either open or laparoscopic-assisted)^[13,16-18] and two anastomoses, seemingly resulting in a higher risk of anastomotic dehiscence^[6].

Perioperative results of colorectal resections for SCRC are also debated. In a multicentric study of 884 patients who were operated for SCRC between January 2009 and December 2011 and were registered in the Dutch Surgical Colorectal Audit^[6], extended surgery (e.g., subtotal colectomy, proctocolectomy, or combined resection) was performed in more than 35% of cases. The application of neoadjuvant chemoradiation for rectal tumours was lower for synchronous than for solitary CRC (20% vs 38%), laparoscopic resections were less frequent, and more (permanent and deviating) stomas were constructed during surgery than for solitary tumours. Overall, the perioperative outcomes of SCRC were worse than for solitary CRC: postoperative complications, reinterventions, 30-day mortality, and time of hospital stay were significantly increased in patients with SCRC. After adjustment for patient- and tumour-related factors, having SCRC was still associated with a higher risk of severe postoperative complications and reinterventions, but not with higher 30-d mortality. The authors concluded that the higher risk of unfavourable perioperative outcomes could be explained by the more extended surgical resection often required for SCRC. Holubar *et al.*^[17] reported 69 patients who underwent multiple colonic anastomoses, laparoscopic-assisted in ten (17%) cases, with a 44% conversion rate. Length of stay was seven (5-10) days, overall 30-day morbidity was 36% without anastomotic leaks or fistulas, and 30-day mortality was 3%. Li *et al.*^[18] examined a personal series of 11 patients and 52 adjunctive patients collected from six previous reports of the literature who underwent laparoscopic-assisted combined bowel anastomoses for SCRC, and concluded that combined bowel anastomoses are potentially feasible and safe procedures for SCRC when performed by experienced surgeons.

Table 1 Diagnostic evaluation of synchronous colorectal cancer**Local tumour staging**

Preoperative colonoscopy with histological assessment of all colorectal lesions
CT of the abdomen and pelvis

In case of rectal cancer include

Endorectal ultrasound
Pelvic magnetic resonance imaging

If preoperative evaluation during colonoscopy is incomplete (bowel obstruction, poor bowel preparation, technical reasons, *etc.*)

Double-contrast barium enema
CT colonography, if available
MRI colonography, if available
PET-CT colonography, if available

Intraoperative assessment

Intraoperative colonoscopy
Palpation of the entire colon
Thorough examination of pathological specimens

Evaluation of metastatic disease

CT of the chest, abdomen, and pelvis
MRI of the chest, abdomen, and pelvis, in selected cases
18FDG-PET-CT, in selected cases

Patient performance status

Thorough evaluation of coexisting morbidities
Pulmonary function tests, in selected cases
Echocardiography, in selected cases

CT: Computer tomography; MRI: Magnetic resonance imaging; PET-CT: Positron emission tomography/computer tomography.

SURGICAL STRATEGIES FOR SYNCHRONOUS CRLM

Surgical strategies in patients with resectable CRC and upfront resectable synchronous metastases limited to the liver have been widely debated in the last decades. The traditional “staged” or “classic” approach with resection of the colorectal tumour followed by hepatectomy is probably still favoured in most cases because the risks of the colorectal and the liver surgery are not cumulated^[20,22,79,80], and CHT can be selectively administered between the two procedures^[22]. In the case of large synchronous CRLM and uncomplicated primary tumour, a reversed therapeutic strategy with LR followed by colorectal resection has been proposed, to minimize the risk of progression of the metastatic liver disease to unresectability. This strategy is termed “reverse” or “liver-first” approach^[22,81,82] and has become more widely used, either in patients with borderline resectable liver involvement and uncomplicated primary tumour or in patients with resectable CRLM and locally advanced rectal cancer that can be treated with neo-adjuvant chemoradiotherapy and subsequent rectal surgery^[22,81,83-85]. Moreover, in a small proportion of patients, a complete clinical, endoscopic, and radiological response of the primary tumour to chemoradiotherapy subsequent to initial radical LR has been reported, thus delaying or even avoiding bowel surgery^[85]. However, simultaneous colorectal and liver resection remains the most appealing approach and is obtaining a growing consensus due to the advances in oncological concepts and continued development of anaesthesia, critical

care, radiological imaging, and techniques of hepatobiliary surgery favouring the expansion of resectability criteria^[40]. Simultaneous resections have clear advantages because the patient experience is improved and psychological stress is limited by decreasing the time to removal of the disease, the total number of surgical procedures, the duration of perioperative CHT^[19,29]. Also the cumulative costs of hospitalization are substantially decreased in selected cases^[86]. Nonetheless, the real impact on the oncological outcome and on the perioperative results are still debated^[2,20].

Preoperative assessment

The accurate preoperative staging of advanced CRC is of paramount importance (Table 2) and can be obtained with cross-sectional imaging by CT or MRI^[1,2,87,88]. The current guidelines of the North American National Comprehensive Cancer Network (NCCN) suggest the use of CT or MRI of the chest, abdomen, and pelvis. 18FDG-PET-CT imaging is reserved for patients who may undergo potentially curative surgical resection^[2]. Preoperative liver imaging should be accurately evaluated to define the number and the site of CRLM, the tumour-vessels relationship, the pattern of intrahepatic vasculature, the presence of anatomical variations, and the FRL volumes^[35,89-91]. Recent studies underline the favourable impact of preoperative MRI on the overall oncological outcome of patients with multiple CRLM^[92]. The accurate assessment of patient performance status is mandatory to determine suitability for more complex therapies, especially those including liver surgery. Coexisting morbidities and liver steatosis should be

Table 2 Diagnostic evaluation of synchronous colorectal liver metastases**Local tumour staging**

CT and/or MRI of the liver, to evaluate
 Number and location of CRLM
 Tumour-vessels relationship
 Pattern of the hepatic vasculature
 Presence of anatomical variations
 Future remnant liver volumes

Intraoperative assessment

Intraoperative ultrasonography

Evaluation of metastatic disease

CT of the chest, abdomen, and pelvis
 MRI of the chest, abdomen, and pelvis, in selected cases
 18FDG-PET-CT

Patient performance status

Thorough evaluation of coexisting morbidities
 Pulmonary function tests
 Echocardiography

In the case of suspected liver disease/steatosis include (elderly patients, metabolic syndrome, previous systemic CHT, *etc.*)

Liver function tests
 Evaluation of the grade of steatosis, in selected cases

CT: Computer tomography; CRLM: Colorectal cancer and synchronous liver metastases; CHT: Chemotherapy; MRI: Magnetic resonance imaging; PET-CT: Positron emission tomography/computer tomography.

adequately assessed. Accurate stratification of the perioperative risks should include liver function tests with evaluation of the grade of steatosis in selected cases, and pneumological and cardiological evaluation with pulmonary function tests and echocardiography^[88]. Even though up to 70% of the normal adult human liver can be removed, previous systemic CHT may seriously alter liver function and the consequent ability to tolerate extended resections^[93-96]. Oxaliplatin-based regimens are associated with augmented risks of vascular lesions, including the sinusoidal obstruction syndrome (SOS), which has been reported to increase morbidity after major LR, especially after administration of more than six cycles^[97]. Irinotecan-based regimens are associated with the occurrence of various degrees of steatosis up to the chemotherapy-associated steatohepatitis (CASH), which may worsen perioperative morbidity and mortality rates after LR^[97]. The impact of adding targeted molecular therapies, including cetuximab or bevacizumab, to conventional systemic chemotherapy on perioperative morbidity or mortality rates after hepatectomy is still controversial^[97].

Simultaneous vs staged colorectal and liver resection

Many recent systematic reviews and meta-analyses have compared the perioperative and long-term outcomes of simultaneous versus delayed hepatectomy for synchronous CRLM. In a systematic review of the literature including 16 controlled trials comparing simultaneous resection of synchronous CRLM and of the primary cancer with a staged approach, where the metastases were resected at a later stage, there was a tendency towards shorter hospital stays and lower perioperative morbidity after simultaneous resection^[23]. Perioperative mortality seemed to be lower with the

staged approach, and five-year survival rates seemed to be similar in the two groups. The authors underlined that all studies were retrospective and had a general bias because staged procedures were significantly preferred in patients with left-sided primary CRC and larger, more numerous and bilobar metastases. They concluded that simultaneous resections might be selectively undertaken. In a meta-analysis evaluating 14 comparative studies comprising 2204 patients^[24], those undergoing simultaneous resection had similar operative time and intraoperative blood loss, shorter hospital stay, and lower morbidity rate. One-, three- and five-year survival rates were similar between groups. The authors concluded that simultaneous resection is a safe and effective treatment for patients with synchronous CRLM and might be considered as the preferred treatment in appropriately selected patients. Another systematic review and meta-analysis of 19 non-randomized controlled trials including 2724 patients came to similar conclusions^[25]. Yin *et al*^[26] conducted a systematic review and meta-analysis of 17 retrospective studies including 2880 patients, of whom 1015 with simultaneous resection and 1865 with delayed resection. The simultaneous group had lower postoperative complications, whereas postoperative mortality within 60 d and overall and recurrence-free survival (RFS) were similar between groups. Moreover, the authors proposed precise selection criteria for patients suitable for a simultaneous resection, including LR of no more than three segments, colon resection (especially the right-sided colectomy), age < 70 years, and exclusion of severe comorbidities.

Somewhat different conclusions were drawn in a wider meta-analysis including 24 studies published between 1991 and 2010, which comprised 3159 patients, of whom 1381 had simultaneous resections and 1778

had delayed resections^[27]. Significantly fewer patients received neoadjuvant CHT in the simultaneous resection group. The bilobar distribution ($P = 0.01$), the size of CRLM ($P < 0.001$), and the proportion of major LR ($P < 0.001$) were found to be higher in the delayed resection group. Operative blood loss and length of surgery were similar between groups, and length of hospital stay was significantly reduced in simultaneous resections ($P = 0.007$). Post-operative complications, OS, and disease-free survival (DFS) were similar between groups. The authors concluded that delayed resections may result in better outcomes because patients undergoing delayed resection had intraoperative parameters, postoperative complications, and survival rates comparable to those of patients undergoing simultaneous resection, despite more extensive metastatic liver disease. A subsequent meta-analysis evaluating 4494 patients from 22 studies published between January 2000 and April 2013^[28] questioned the reliability of some previously published meta-analyses because important biases of the examined retrospective studies, mainly the fact that significantly more patients with mild conditions underwent simultaneous procedures, were not corrected. Summarized baseline analyses to find imbalanced factors between simultaneous and staged groups showed that patients were more likely to undergo simultaneous resection when they had less CRLM (single nodule, $P = 0.002$; ≤ 3 nodules, $P < 0.0001$), of smaller size (diameter ≤ 5 cm, $P = 0.04$; smaller mean diameter, $P < 0.00001$), with unilobar distribution ($P = 0.0002$), requiring minor LR rather than major LR for curative resection ($P < 0.00001$), and a right-sided CRC rather than left-sided ($P = 0.0006$). After correction of baseline imbalance, simultaneous and staged resections had comparable safety and efficacy, with similar postoperative morbidity and mortality, and overall and disease-free survivals. Similar results were found in another recent systematic review and meta-analysis of 30 studies including 5300 patients, of whom 2235 patients received simultaneous resections and 3065 patients received staged resections^[26]. Patients undergoing delayed surgery were more likely to have received neoadjuvant treatment, have bilobar disease, or undergo major LR. Parameters relating to safety and efficacy were similar between the two groups. The average length of hospital stay was six days shorter with the simultaneous approach ($P < 0.001$). Long-term survival was similar for the two approaches.

The discordant results of the numerous meta-analyses published in recent years is due to the limitations intrinsic to meta-analysis of retrospective studies, mainly due to the fact that compared to RCTs retrospective studies are not randomized. As a consequence, experimental and control groups are often poorly comparable, and the baseline imbalances may significantly compromise the accuracy of the results. Without adequate correction of baseline imbalances before pooled analyses, ideally using methods based on the individual patient data analysis (which however is not always available), meta-analyses

can only improve the precision, not the accuracy, of the pooled results, which should be interpreted and applied with great prudence^[28]. The copious studies comparing simultaneous and classical staged resections, where the colorectal resection is followed by hepatectomy usually with interval CHT, must be interpreted cautiously because at least two major confounding factors are usually present. Candidates to simultaneous resection were usually younger, in better clinical conditions, with right-sided primary cancer, and more limited liver involvement usually necessitating minor hepatectomies^[23,26-30]. On the other hand, patients enrolled in the staged groups included significantly more patients who received pre-operative CHT^[27-29], and only those who had received successful staged resections, while patients who developed progressive liver disease during the interval were excluded. For these reasons, the overall survival of patients selected for staged approaches could be overestimated by including only patients with more favourable cancer biology or responsive to perioperative (neoadjuvant and/or interval) CHT. Future studies should avoid this selection bias by including patients with progressive metastatic disease after colorectal resection that missed the subsequent hepatectomy^[28].

More recent studies have compared all the available surgical strategies, the staged primary-first vs the staged liver-first vs the simultaneous resection. In a small series of 57 patients with rectal cancer and synchronous CRLM, the authors compared the traditional staged resections with the simultaneous resections and the liver-first approach^[98]. The overall morbidity rate was 24.6%, without in-hospital mortality. The median in-hospital stay was significantly shorter for the simultaneous approach. The five-year OS rate was 38%, with an estimated median survival of 47 mo. The authors concluded that long-term survival can be achieved using an individualized approach in patients with rectal cancer and synchronous CRLM and that simultaneous procedures as well as the liver-first approach are attractive alternatives to traditional staged procedures. In another series of 156 consecutive patients with synchronous CRLM, Brouquet *et al.*^[81] compared the results of the three different surgical strategies, and found comparable three- and five-year OS rates. The only factors independently associated with the OS were a liver tumour size > 3 cm and the cumulative perioperative morbidity. Similar conclusions have been drawn in a multi-institutional study including over 1000 patients from four major hepatobiliary centres^[82]. The median OS was 50.9 mo and the cumulative one-, three- and five-year survivals were 89%, 60%, and 44%, respectively, without significant differences between simultaneous and staged surgical procedures. The cumulative recurrence rate was 57%, and was similar between patients undergoing simultaneous and staged procedures. Independent factors of worse long-term prognosis were being male, a rectal primary, and combined LR plus ablation. The authors concluded that tumour biology rather than surgical strategy was the main effector of the oncological

outcome. A systematic literature review of 18 studies comparing the different surgical approaches in patients with synchronous CRLM concluded that none of the three surgical strategies appeared inferior to the others^[99]. Similarly, a network meta-analysis review of 3605 patients comparing classic staged, simultaneous, and liver-first surgical strategies could not demonstrate significant differences of 30-day mortality, postoperative complications, and five-year OS rates^[100]. In a systematic review of three cohort studies comprising a pooled population of 1203 patients who underwent surgical treatment of CRC with synchronous CRLM between 1982 and 2011 and where the different treatment modalities were reported separately^[101], 62.2% of patients received bowel-first surgery, 6.2% of patients received liver-first surgery, and 31.6% of patients received simultaneous surgery. Perioperative outcomes were similar between the three methods with low overall treatment-related mortality and similar survival rates.

Neoadjuvant CHT in resectable liver disease

Strategies including different CHT protocols to augment resectability in the case of initially unresectable synchronous CRLM are out of scope for this review. The role of neoadjuvant CHT in patients with resectable CRLM is still controversial. The EORTC Intergroup trial 4098386 was a randomized comparison of perioperative oxaliplatin-based CHT administered either before or after LR vs LR alone in patients with limited CRLM (≤ 4) classified as resectable at baseline assessment^[102]. Thirty-five percent of patients had synchronous disease. The overall results revealed an absolute increase in the rate of progression-free survival at three years in the patients randomized to receive perioperative CHT, but significantly more frequent reversible postoperative complications in the same group. However, the absolute differences in outcomes observed between groups were small and the study received much criticism^[28,30]. Moreover, a long-term follow-up report of this trial could not find any difference in survival between the groups^[103]. A systematic review of 23 trials evaluating the clinical response and outcomes of neoadjuvant systemic CHT for resectable CRLM suggested that preoperative CHT may achieve objective response with improvement in DFS^[104]. However, also this study was considered to have enough limitations to affect the final conclusions^[30]. Another systematic literature review concluded that, while combination regimens resulted in enhanced tumour response and resectability rates in up to 30% for unresectable CRLM, studies on neoadjuvant CHT failed to convincingly demonstrate a survival benefit for resectable lesions, with most reports describing increased postoperative complications in a subset of patients due to parenchymal alterations associated with CHT^[97]. A recent analysis of a multi-centric cohort from the LiverMetSurvey International Registry, which included patients who had received curative LR for synchronous CRLM, compared 693 patients who received

neoadjuvant CHT prior to liver surgery with 608 patients treated by surgery alone, and could not find any survival advantage between the groups^[105]. Discouraging results were also obtained associating the targeted molecular agent cetuximab with conventional neoadjuvant CHT protocols^[106].

CONSERVATIVE OR PARENCHYMAL-SPARING LIVER SURGERY

Resectability of CRLM has significantly improved over the last decades. The traditional criteria related to the features of liver tumours to evaluate resectability have been replaced by an accurate preoperative estimation of what remains after LR. Tumours should be considered resectable if complete liver tumour excision can be obtained with curative intent (macroscopically uninvolved surgical margins), in the absence of unresectable extrahepatic disease, and the estimated FRL parenchyma is sufficient to prevent liver failure^[107]. Major liver resections, including conventional major hepatectomies and more recently described two-stage procedures, with or without PVE, are traditionally preferred by most surgeons to obtain radical resection of CRLM, especially in the case of large and/or multiple nodules. However, extensive hepatectomies have been associated with significant morbidity and mortality rates, usually related to posthepatectomy liver failure^[35,36]. "Conservative" or "parenchymal-sparing" hepatectomies are based on the expert use of IOUS, which permits removal of liver tumours with the minimum sufficient oncological margin to preserve as much non-tumourous liver parenchyma as possible, to limit the risk of perioperative liver failure^[35], but also to preserve the major intrahepatic vessels whenever possible with the aim of increasing salvageability in case of hepatic recurrence^[40,41]. The progressive diffusion of conservative strategies of LR is related to at least three factors: The increasing evidence that CRLM have different intrahepatic diffusion patterns than hepatocellular carcinoma, so that anatomical resections per se have no impact on the oncological outcome; the evolution of the concept of adequate surgical resection margin (RM), where the "1-cm rule" proposed by Ekberg *et al*^[108] has been progressively abandoned in favour of the concept of "negative margin" without considering margin width; and the increasing evidence that also patients with large numbers of CRLM are potential candidates for curative liver surgery in the context of multimodal treatment strategies of advanced CRC.

Anatomic vs non-anatomic resection

Adequate resection of liver tumours should involve resection of the tumour with enough margin to prevent recurrence and to achieve potentially curative treatment. Hepatocellular carcinoma has a high propensity for vascular invasion and metastatic spread through the portal venous system. As a consequence, anatomic

resection (AR) is considered the optimal surgical strategy because it eradicates portal tributaries close to the tumour, possibly reduces the risk of local tumour spread, and may ultimately determine a survival benefit compared to non-anatomic resection (NAR)^[35,109]. Multiple surgical strategies which limit the extension of LR while respecting the segmental or subsegmental distribution of intrahepatic vessels have been described over the last 30 years and successfully performed due to the expert use of IOUS, either for primary or for metastatic liver tumours^[40,110-114]. Metastatic tumours can spread within the liver by different pathways. Neoplastic cells might disseminate within and outside the liver through portal and hepatic veins, lymphatic vessels, bile ducts, and perineural spaces^[115]. Sasaki *et al.*^[116] defined portal vein, hepatic vein, and bile duct invasion as the growth of cancer cells into blood vessels or bile duct branches in the liver parenchyma, and defined intrahepatic lymphatic invasion as the growth of cancer cells in luminal structures located in the portal spaces and lined by endothelial cells. Korita *et al.*^[117] described intrahepatic lymphatic invasion as the presence of isolated cancer cells or cell clusters within vessels with immunoreactivity for D2-40 antibody^[117,118]. Other studies about the prognostic role of different patterns of intrahepatic diffusion of CRLM did not describe the method used to define vascular invasion, so that differentiation between invasion of blood vessels and of lymphatic vessels was uncertain^[115]. With these limitations, the prognostic role of the portal vein and the hepatic vein invasion is still uncertain^[115,118], while migration of tumour cells from CRLM through intrahepatic lymphatic vessels has a documented adverse impact on survival^[116-119]. For these reasons, AR including portal tributaries close to the tumour and the corresponding liver parenchyma should not be theoretically justified for CRLM, and NAR with adequate surgical margin is presently considered an appropriate surgical strategy^[35,90,120-125]. A recent meta-analysis of seven non-randomized controlled studies including 1662 patients with CRLM, compared 989 patients who underwent AR and 673 who underwent NAR^[121]. NAR reduced the operation time and blood transfusion requirements whereas postoperative morbidity and mortality were similar between groups. Also oncological outcomes, including surgical margins, OS, and DFS survival were similar between the groups. Another systematic review of 12 studies included 2005 patients, who underwent either PSLR (1087 patients) or AR (1418 patients) for CRLM^[122]. Most studies included a large subset of patients with solitary tumours and a reported median tumour number of one to two regardless of surgical strategy. While there was considerable inter-study variability regarding RM status, there was no difference in the incidence of R0 resection between groups. Median postoperative length-of-stay was similar; also OS was similar after PSLR (five-year OS: mean 44.7%, range 29%-62%) and AR (five-year OS: mean 44.6%, range 27%-64%). The authors concluded that PSLR had

comparable safety and efficacy profiles compared with AR without compromising oncological outcomes.

Since the early 2000s, the systematic use of conservative procedures of LR, either for primary or for metastatic liver tumours, has been considered of paramount importance in some Japanese studies to achieve zero mortality and low morbidity rates. Meticulous attention to the balance between the hepatic functional reserve and the hepatic volume to be removed, the routine use of NAR with adequate surgical margin for resection of liver metastases whenever possible, and the attitude to perform simultaneous colorectal and liver resections for synchronous CRLM were among the most important criteria to perform safe hepatectomies without perioperative mortality^[35]. Kokudo *et al.*^[123] retrospectively evaluated 115 patients with unilobar single or double tumours undergoing major AR (64 patients) or limited NAR (51 patients) and found that survival rates were similar between the groups. Anatomical major hepatectomy was unnecessary in 80.4% of the cases if the tumours were resectable by limited NAR, and 90% of the ipsilateral recurrence, which could have been avoided if the first operation was anatomical hemihepatectomy, could undergo a second hepatectomy with a five-year survival rate of 58.3%. The authors concluded that limited NAR should be a basic surgical procedure for CRLM to minimize surgical stress and operative risks. Mise *et al.*^[124] have recently evaluated a series of 300 patients with a solitary CRLM ≤ 30 mm undergoing PSLR (156 patients) or more extended hepatectomy (144 patient), including right hepatectomy, left hepatectomy, or left lateral sectionectomy. The rate of PSLR increased during the 20-year study period. PSLR did not negatively impact OS, RFS, and liver-only recurrence-free survival compared to non-PSLR. Repeat LR was more frequently performed in the PSLR group (68% vs 24%, $P < 0.01$). Subanalysis of patients with recurrence limited to the liver revealed better five-year OS from initial LR (72.4% vs 47.2%; $P = 0.047$) and from hepatic recurrence (73.6% vs 30.1%; $P = 0.018$) in the PSLR group. Upon multivariate analysis, non-PSLR was an independent significant risk of non-candidacy for repeat hepatectomy. The authors concluded that conservative resections did not increase recurrence in the liver remnant while increasing the opportunity of salvage resection and the five-year survival rate in case of recurrence. These results have been subsequently confirmed in a multicentric cohort of 1720 patients from the LiverMetSurvey registry, with a single CRLM ≤ 30 mm located in the right hemiliver^[125]. Eight-six percent of patients underwent PSLR and fourteen percent underwent right hepatectomy. PSLR was associated with lower major complication rates (3% vs 10%; $P < 0.001$) and 90-day mortality rates (1% vs 3%; $P = 0.008$). Hepatic recurrence was similar between groups (20% vs 22%; $P = 0.39$), as well as the five-year OS and RFS rates. However, in patients with liver-only recurrence, repeat LR was more frequently performed after PSLR than after right hepatectomy (67% vs 31%;

$P < 0.001$), and the five-year OS rate was significantly higher after PSLR than after right hepatectomy (55% vs 23%; $P < 0.001$). Taken together, these results indicate that a combination of conservative NAR followed by liver reresection in the case of recurrence limited to the liver offers superior oncological benefits than major LR in most patients with limited hepatic disease, and should be considered at present the most appropriate surgical strategy^[123-125].

Similar results have been recently reported in patients with two or more CRLM. Karanjia *et al.*^[126] evaluated 283 consecutive patients who underwent successful LR for CRLM over ten years and compared 128 patients who had right and extended right hepatectomy with 155 patients who had other types of LR. Operative mortality was 3.9% and 0.7% after right hepatectomy and after other types of LR, respectively ($P = 0.04$). Morbidity was 31.3% and 18% after right hepatectomy and after other types of LR, respectively. The one-, three- and five-year OS rates were 84.1%, 54.3%, and 38.9% after right hepatectomy and 95.4%, 65.9%, and 53.3% after other types of LR, respectively ($P = 0.03$). The one-, three- and five-year DFS rates were 69.5%, 34.4%, and 25.5% after right hepatectomy and 68.4%, 34.91%, and 34.91% after other types of LR, respectively ($P = 0.46$). The authors concluded that in patients with CRLM, right and extended right hepatectomy have greater operative morbidity and mortality and significantly worse OS compared to all other types of LR. In a more recent series of 917 consecutive patients who received LR for CRLM from 2000 to 2010, Lordan *et al.*^[127] compared 238 patients who underwent PSLR case-matched with 238 patients who had major hepatectomy using a propensity scoring system. Fewer PSLR patients received perioperative blood transfusions ($P < 0.0001$). PSLR patients had a lower incidence of complications ($P = 0.04$), grade III/IV complications ($P = 0.01$), 90-day mortality ($P = 0.03$), and a shorter hospital stay ($P = 0.04$). OS and DFS rates were similar. The authors concluded that patients with resectable CRLM should be offered PSLR if technically feasible because PSLR is safer than major hepatectomy without compromising long-term survival. Parenchymal-sparing hepatectomies are effective also for CRLM deeply placed where major hepatectomies have been traditionally preferred. Matsuki *et al.*^[128] evaluated 63 patients who received first curative LR for deeply placed CRLM whose centre was located > 30 mm from the liver surface. PSLR and major hepatectomy were performed in 63% and 37% of patients, respectively. Resected volume was smaller after PSLR than after major hepatectomy (251 g vs 560 g) ($P < 0.01$). Total operation time, amount of blood loss, rate of major complications, and positive operative margins were similar. OS, RFS, and liver recurrence-free survivals did not differ between the two groups. The authors underlined that direct major hepatectomy without PVE was unfeasible in 40% of the PSLR group because of the small FLR and concluded that PSLR for deeply placed

CRLM can be performed safely without compromising oncologic radicality and can also increase the number of patients eligible for a direct surgical treatment by limiting the resection volume.

Resection margin

There has long been controversy over the impact of the width of the resection margin on the oncological outcome of LR candidates for CRLM. Since the 1980s surgeons have advocated for R0 resection margins of 10 mm or greater, the so-called "1-cm rule", in order to prevent local recurrence and optimize overall survival^[38,108,129]. The presence of residual microscopic deposits of neoplastic cells after removal of metastatic nodules is considered an important source of remetastasis and a significant factor of adverse prognosis^[115,129]. As for the primary tumour, micrometastases may occur in CRLM. Intrahepatic micrometastases are defined as detectable microscopic tumour nests within the liver parenchyma or portal tracts surrounding the dominant tumour, but separated by a rim of non-tumourous parenchyma, are predominant within 4 mm to 10 mm of the tumour margin, and are considered the morphological expression of remetastasis from existing liver metastases^[40,119,130,131]. Their role as a prognostic factor in the oncological outcome of patients with CRLM is however still controversial. One study reported that patients with intrahepatic micrometastases had higher incidence of intrahepatic recurrence and worse survival, with ten-year survival rates of 21.9% compared to 64.3% for patients without micrometastases^[132]. In another study, intrahepatic micrometastases were less frequently detected in patients treated with neoadjuvant CHT than in those untreated^[133]. A 2 mm RM is however considered acceptable to significantly reduce the incidence of local recurrence in the series where the role of intrahepatic micrometastases has been evaluated^[119,130]. In a small series based on the detection of tumour-specific mutant DNA in liver tissue surrounding metastases, mutant DNA was detectable in surrounding liver tissue within 4 mm of the tumour border, while biopsies at 8 mm, 12 mm, and 16 mm from the macroscopically visible margin were free from microscopically visible tumour cells and detectable mutant DNA, even in patients whose tumours were larger before CHT^[131]. Also the presence of fibrotic tissue between the tumour and the surrounding hepatic parenchyma has been recognized as a favourable prognostic factor in CRLM and may be relevant in the evaluation of the RM. Yamamoto *et al.*^[134] reported that the five-year survival rate was 71% in patients with a thick pseudocapsule, 63% in those with a thin pseudocapsule, and only 19% in the absence of a pseudocapsule. Similar results were reported in the study by Okano *et al.*^[135], where five-year survival rates were 88% in patients with a thick pseudocapsule, 64% in patients with a thin pseudocapsule, and 31% in those without a pseudocapsule. Taken together, these data show that CRLM are usually well circumscribed, with very low incidences of satellite nodules or micrometasta-

ses, so that limited negative resection margins may have a limited impact on recurrence and survival rates, even though RM width of 10 mm should be achieved whenever possible^[38].

R1 resection

The presence of residual macroscopic or microscopic tumour on RM after surgery for CRLM is traditionally considered a significant factor of adverse prognosis^[108] due to increased local and intrahepatic recurrence as well as decreased OS and DFS. As a consequence, the adequate evaluation of the RM is of paramount importance to define the postoperative oncological prognosis. However, the accurate assessment of margin status depends on multiple factors. Different techniques of liver transection create different extensions of tissue loss^[38]. The thermal effects of energy devices and of the argon-beam coagulation on the cut surface of the liver causes extensive cell killing within 2-5 mm of the RM^[130,136]. Also pathologic assessment of the exact distance between the excised tumour and the end of the liver parenchyma has multiple limitations^[137]. With these limitations, there is strong evidence that microscopically positive RM (R1) negatively impacts overall oncological results. R1 resection has been associated with an increased risk of recurrence at the surgical margin^[119,131,138-140] and of intrahepatic recurrence^[139,141]. Tranchart *et al.*^[142] found that R1 resection was an independent adverse predictor of OS and DFS, and the use of postoperative CHT was the only independent predictor of improved DFS in patients with R1 resection. The adverse effect of R1 LR on survival has been confirmed by other studies^[138,143,144]. However also the protective effect of postoperative CHT after R1 resection has been recently confirmed^[141,145].

The role of neoadjuvant CHT on the oncological outcome of R1 resection is controversial. Ayez *et al.*^[146] found that R1 resection remained an adverse prognostic factor in OS and DFS in patients receiving LR for CRLM not treated with neoadjuvant CHT, but not in those who had undergone neoadjuvant CHT. Different results were obtained in a study of 378 patients treated with neoadjuvant CHT and subsequent LR, where the effect of positive margins on OS was analysed in relation to response to CHT^[147]. Fourteen percent of resections were R1 (tumour-free RM < 1 mm). The five-year overall survival rates were 55% for patients with R0 resection (tumour-free RM ≥ 1 mm) and 26% for those with R1 resection ($P = 0.017$). R1 resection and a minor pathologic response to CHT at histology were independently correlated with worsened survival upon multivariate analysis. The survival advantage correlated with negative resection margins (R0 vs R1 LR) was higher in patients with suboptimal morphologic response at CT scans after CHT (five-year OS: 62% vs 11%; $P = 0.007$) than in those with optimal response (three-year OS: 92% vs 88%; $P = 0.917$), and higher in patients with a minor pathologic response at histologic evaluation

(five-year OS: 46% vs 0%; $P = 0.002$) than in those with a major response (five-year OS: 63% vs 67%; $P = 0.587$). The authors concluded that with the current neoadjuvant CHT protocols, negative resection margins still represent a crucial prognostic factor and should remain the principal purpose of LR, and that the adverse influence of positive RM is most evident in the presence of suboptimal response to neoadjuvant CHT. In a similar study of 227 patients who received neoadjuvant oxaliplatin and/or irinotecan and 5-FU and subsequent curative LR^[148], positive margins (tumour-free RM < 1 mm) significantly increased the risk of death without postoperative CHT ($P = 0.0077$), but not with postoperative CHT. Negative RM sizes of ≥ 1– < 5, ≥ 5– < 10, and ≥ 10 mm were not significant predictors of OS. The authors concluded that patients undergoing LR for CRLM should receive postoperative CHT if negative margins cannot be achieved, and that negative margins wider than 1 mm do not improve OS for patients receiving neoadjuvant CHT. It should be noted however that when neoadjuvant CHT is interrupted, regardless of previous response, regrowth may occur at the periphery rather than in the centre of the metastasis, with clustering of viable cancer cells infiltrating the liver tissue for several millimetres at the periphery of the metastasis, irrespective of any signs of response in its centre, a phenomenon called “dangerous halo”^[136]. Similarly, it has been found that neoadjuvant CHT may determine irregular borders of CRLM, particularly evident in lesions with significant contraction, and sometimes discrete islands of viable tumour cells outside of the main tumour, but all close to the peripheral margin of the tumour mass^[149]. The possible progression of the dangerous halo is particularly worrying, and the planned surgical margin should be wide enough to limit the risk of local recurrence, especially if CHT has been interrupted for a relatively long time. It has been suggested that the argon-beam coagulation of the cut surface of the liver might reduce the risk of recurrence by providing a layer of necrosis of 2 mm to 5 mm^[136].

Also submillimetric clear margins have been considered adequate for resection of CRLM. A total of 2368 patients undergoing LR for CRLM at Memorial Sloan Kettering Cancer Center between 1992 and 2012 were examined to evaluate the impact of margin width on OS^[144]. The median OS of the R1, 0.1-0.9 mm, 1-9 mm, and ≥ 10 mm groups was 32 mo, 40 mo, 53 mo, and 56 mo, respectively ($P < 0.001$). Compared with R1 LR, all RM widths, together with submillimetric margins, were associated with increased OS ($P < 0.05$). The significant association of RM width and OS remained when adjusted for all the other pathological and clinical factors of prognosis. The authors concluded that RM width is independently predictive of better survival rates, so that adequate margins should be obtained whenever possible. However, LR should be performed also in patients where narrow RM are anticipated because submillimetric margin clearance may improve

survival. The authors also suggested that the favourable outcome observed with submillimetric margins could be the expression of the biological behaviour of the tumour rather than the result of the surgical technique. Detachment of CRLM from intrahepatic vessels has been proposed as part of IIOUS-guided PSLR^[113,150]. Even though this kind of resection implies formally R1 resection margins, oncological outcomes seem to be similar to those described for R0 resections. In a recent series of 627 resection areas in 226 consecutive patients with CRLM, Viganò *et al.*^[151] compared the outcomes of R1 surgery (RM < 1 mm), distinguishing standard R1 resection and R1 resection with detachment of CLM from major intrahepatic vessels (R1 vascular). Five percent of recurrences at surgical RM occurred in 12.4% of patients. Local recurrence risk was similar between the R0 and R1 vascular groups but increased in the standard R1 resection group ($P < 0.05$ for both). Standard R1 resection had a higher rate of hepatic-only recurrences ($P = 0.042$) and was an independent negative prognostic factor of OS on multivariate analysis ($P = 0.034$). Conversely, R1 vascular resections had oncological outcomes similar to those of R0 resections suggesting that CRLM detachment from intrahepatic vessels can be safely pursued to increase resectability. Similar strategies of conservative IIOUS-guided LR sparing intrahepatic vessels have been used in simultaneous colorectal and liver resection of advanced CRC with synchronous CRLM to limit the extension of LR with the aim of reducing the overall risk of the simultaneous procedures^[91].

The data on whether R1 margin status is an independent predictor of survival have been conflicting because some authors have found that R1 margin status was not associated with survival after controlling for competing risk factors on multivariate analyses^[138,139,141]. Tumour biology might play a determinant role in the impact of RM status on oncological outcome, where R1 resections could not have a prognostic value per se but reflect a more severe disease^[38,40,129,138,141,145]. Recent changes in the prognostic value of R1 resections could reflect in part the beneficial effect of perioperative CHT^[142,145-148]. In a recent series of 1784 hepatectomies analysed from a multicentric retrospective cohort of hepatectomies performed for CRLM in 32 French centres from January 2006 to December 2013^[152], positive primary tumour lymph nodes at colorectal resection ($P = 0.02$), operative time > 240 minutes ($P = 0.05$), synchronous CRLM ($P = 0.02$), clamping of the hepatic pedicle > 40 min ($P = 0.001$), tumour size > 50 mm ($P = 0.001$), recurrent hepatectomy ($P = 0.001$), > 3 nodules ($P = 0.0001$), and bilateral nodules ($P = 0.0001$) were recognized as risk factors for R1 resection upon multivariate analysis. After a propensity score matching according to Fong criteria, however, R1 resection still maintained an adverse impact on OS and DFS, with one-, three-, and five-year OS of 94%, 81%, and 70% in R0 LR vs 92%, 75%, and 58% in R1 LR, respectively ($P = 0.008$), and with one-, three-, and five-year DFS

of 64%, 41%, and 28% in R0 LR versus 51%, 28%, and 18% in R1 LR, respectively ($P = 0.0002$).

R0 resection: the optimal free resection margin

Determining the optimal free RM in surgery of CRLM is much more controversial, since the traditional 1-cm rule to consider oncologically adequate the RM has been widely debated in the last decades. Pawlik *et al.*^[138] in 2005 demonstrated that OS, DFS, recurrence risk, and site of recurrence were not significantly different among patients undergoing resection of CRLM with RM of 1-4 mm, 5-9 mm, and ≥ 10 mm, and suggested that predicted margin of < 1 cm after LR should not contraindicate LR. A similar study including 1019 patients from the Memorial Sloan Kettering Cancer Center showed that patients undergoing LR with RM > 10 mm had better survival than those with RM < 10 mm. However, within the latter group there was no significant difference in survival when stratified according to RM width, and patients with subcentimetric RM had an overall survival of 42 months (significantly better than similar patients treated with systemic CHT or ablative therapies)^[153]. In another multicentric study of 2715 patients who received primary resection of CRLM, a 1-mm tumour-free RM was sufficient to obtain 33% five-year overall DFS, while extra RM width did not further increase DFS. After the propensity case-match analysis, the authors did not find a statistical difference in DFS between patients with negative narrow RM and wider RM clearance^[143]. Recent meta-analyses however support the need of achieving adequate resection margins whenever possible. Dhir *et al.*^[154] examined 4821 patients with negative RM from 18 studies and found that the five-year OS for the ≥ 1 cm negative RM subgroup was 46% when compared with 38% for < 1 cm negative RM subgroup ($P = 0.009$). In another meta-analysis based on 18 studies including 6790 patients^[155], R1 resection had a negative impact on OS and DFS rates and was associated with more frequent recurrences. The use of current protocols of CHT did not alter the adverse oncological outcome of R1 resection. Notably, ≥ 1 cm negative RM obtained the best overall survival rates. Margonis *et al.*^[156] evaluated 34 studies including a cohort of 11147 LR. Wider RM (> 1 cm vs < 1 cm) was significantly associated with improved OS and DFS at three years, five years, and ten years. Also > 1 mm vs < 1 mm RM was significantly associated with improved OS. Meta-regression analyses did not reveal any significant impact of perioperative CHT. The authors concluded that even though a > 1 mm RM determines better prognosis than a submillimetric RM, obtaining a RM > 1 cm may determine even better oncologic results and should be attempted whenever possible. Taken together, these data suggest that the 1-cm rule still has prognostic importance in the oncological outcome of resection of CRLM and should be pursued whenever possible. However, the likelihood of local and intrahepatic recurrences seem to be frequently independent

of margin width, where tumour biology seems to be a more decisive predictive factor of both intrahepatic recurrence and poorer long-term survival. Even though R1 resections should be avoided, the actual margin width of R0 resections seems to have a limited impact on the postoperative oncological outcome. For all these reasons, failure to comply with the 1-cm rule should no longer contraindicate liver resection of colorectal metastases.

Surgical strategies for multiple bilobar metastases

In 1984 Adson *et al.*^[157] reported a study of 141 patients who had resection of CRLM between 1948 and 1982 and found similar five-year survival rates between patients with single metastases and those with multiple lesions. They concluded that removal of multiple hepatic metastases was advisable in selected cases. This study was contradicted by Ekberg *et al.*^[108] in a series of 72 LR for CRLM between 1971 and 1984, where poor prognostic factors contraindicating surgical resection of CRLM included more than four lesions, impossibility to achieve a RM ≥ 1 cm, and evidence of extrahepatic disease. These data were confirmed by Hughes *et al.*^[158] in a series of 100 patients who survived for more than five years after resection, where patients with ≥ 4 metastases were considered to be contraindicated for LR. The considerable improvements achieved in the 1990s in the knowledge and treatment of colorectal metastases led to substantial changes in the surgical strategies for multiple CRLM^[89]. In 1995 Scheele *et al.*^[159] reported their experience with 32 patients undergoing LR of ≥ 4 CRLM. According to their study, five or more independent metastases had an adverse effect on resectability. However, if a radical excision of all detectable disease could be obtained, the number of metastases (1-3 vs ≥ 4) was not significantly predictive of either OS or DFS. Subsequently Weber *et al.*^[160] reported a study of 155 patients who received LR for ≥ 4 CRLM with a five-year OS of 23%. As the number of tumours increased, the five-year survival rate diminished from 33% to 14%. However, in this study there were twelve five-year survivors, including two patients with nine or more nodules. Also the potential benefits of neoadjuvant CHT were delineated. Tanaka *et al.*^[161] reported 71 patients who had received LR for ≥ 5 bilobar CRLM and compared the outcome of 48 patients who received neoadjuvant CHT followed by LR with that of 23 patients treated by LR alone. Patients with neoadjuvant CHT experienced better three- and five-year survival rates from the time of diagnosis than those without CHT (67.0% and 38.9% vs 51.8% and 20.7% respectively; $P = 0.039$), and required fewer extended LR (four segments or more) (81.3% vs 100.0%; $P = 0.027$). Multivariate analysis demonstrated that neoadjuvant CHT independently predicted survival. The authors concluded that in patients with bilateral multiple CRLM, neoadjuvant CHT before LR was associated with improved survival.

For patients with extensive bilobar disease, multiple

strategies combining TSH and neoadjuvant CHT were described by the surgeons from the Paul Brousse Hospital^[162-164]. In selected patients with multiple CRLM not eligible for a curative one-stage resection, even when downstaged by CHT, after PVE, or combined with local ablation techniques, Adam *et al.*^[162] proposed a TSH strategy, where the highest possible number of nodules was resected in a first non-curative procedure, and the remaining tumours were resected after an adequate period of hepatic regeneration. The three-year survival rate of the 16 patients who completed the procedure was 35%, with four patients (31%) disease-free at 7 mo, 22 mo, 36 mo, and 54 mo. The same group subsequently examined a series of 33 patients with bilobar CRLM where a right or extended right LR was planned. The first-stage hepatectomy consisted of a clearance of tumours of the left FRL by resection or radiofrequency ablation (RFTA) to prevent the growth of metastatic nodules in the estimated FRL after PVE, followed by a right PVE to induce atrophy of the right hemiliver and hyperplasia of the left hemiliver. The second-stage hepatectomy, a right or extended right hepatectomy, was performed in patients with adequate left FRL hyperplasia and without disease progression. The one- and three-year survival rates were 70.0% and 54.4%, respectively, in the 25 patients in whom the procedure was completed^[164].

In all these Western studies, patients with multiple CRLM were candidates for major or extended hepatectomies in most cases. In the same period the surgeons from the Cancer Institute and the University of Tokyo were following a different approach to multiple CRLM^[35,89,130]. Kokudo *et al.*^[89] reported a series of 183 patients who received LR with curative intent for CRLM from 1980 to 2000 with five-year OS of 41.9%. The overall outcome of 21 patients who had ≥ 4 tumours in the liver was not significantly different from that of patients with ≤ 3 tumours. In the same study the authors delineated the principles of conservative LR strategy for multiple CRLM: Accurate preoperative evaluation of the tumour number and their proximity to the major intrahepatic vasculature, careful intraoperative inspection and palpation of the liver and use of IIOUS, multiple partial resections whenever possible instead of extended hepatectomies, with resection of large intrahepatic vessels only if tumour invasion was present, non-anatomical resection even with a minimum surgical margin, and preoperative PVE when the estimated volume of the remnant liver was under 40% in case of major hepatectomy. In the overall series the remnant liver was the most common site of recurrence, and repeated liver resection was carried out in approximately half of the patients after recurrence, with a five-year survival rate of 44.7% starting from the first hepatectomy. With these diagnostic and therapeutic strategies the same group performed over 1000 hepatectomies without mortality^[35]. A similar approach to multiple bilobar CRLM was reported by Torzilli *et al.*^[165] in a series of 29 patients

with multiple (≥ 4) bilobar CRLM where the surgical strategy was based on tumour-vessel relationships at IOUS and on findings at colour-Doppler IOUS. Tumour removal was feasible in 89.7% of patients. There was no in-hospital mortality and the overall morbidity rate was 23%. After a median follow-up of 14 mo (range 6-54), three patients had died from systemic recurrence, twelve were alive without disease, and eleven were alive with recurrence. However, no local relapses were observed at the surgical RM. The authors concluded that IOUS-guided resection based on strict criteria allows one-stage LR in selected patients with multiple bilobar CRLM, and thus decreasing the need for a TSH.

In the past decade, ablative techniques, including RFTA and microwave ablation (MWA), have emerged as an appealing option for the local treatment of primary and metastatic liver tumours, including CRLM, alone or in combination with LR. The role of ablation in patients with CRLM is unclear since ablative techniques have usually shown significantly lower rates of complications, but also lower survival rates and higher rates of recurrence as compared to LR^[166-168], even though RFTA might have a role equivalent to liver surgery in the treatment of small (≤ 2 cm) CRLM^[167]. Recent studies have shown that LR combined with intraoperative ablation techniques is effective in the treatment of multiple bilateral CRLM, with adequate perioperative outcomes and without compromising overall oncological results compared with bilateral resection or with TSH. It may represent an excellent option to pursue effective parenchymal-sparing treatments for extensive CRLM^[169-172].

A progressive shift toward more conservative hepatectomies for bilobar CRLM has been reported also by surgeons traditionally inclined to more extensive LR. In a series of 443 LR in 440 patients who received resection of bilateral CRLM at the Memorial Sloan-Kettering Cancer Center^[145], a major hepatectomy including three segments, hemihepatectomy or more extended resection in most cases, was performed as part of 380 operations. Major complications were 29% and 90-day mortality was 5.4%. Estimated five-year disease-specific and recurrence-free survivals were 30% and 18%, respectively. However, the surgical technique changed over time toward parenchymal-sparing techniques based on the wider use of multiple simultaneous liver resections, wedge resections, and local ablations, which correlated with decreased mortality rates without changes in disease-specific survival or liver recurrence. The authors concluded that resection of bilateral CRLM can be achieved with reasonable morbidity, mortality, and oncologic results, and that increased use of parenchymal-sparing approaches is associated with decreased mortality without compromising oncological outcomes. The favourable results of PSLR have been recently confirmed in a multicentric retrospective series of patients who had received LR for multiple (> 3) bilobar CRLM, comparing 331 patients who had received PSLR with 360 who had received non-PSLR, defined as the

resection of three or more consecutive liver segments, excluding TSH^[146]. PSLR was associated with lower complications (25% vs 34%; $P = 0.04$) and fewer Dindo-Clavien grade III and IV complications (10% vs 16%; $P = 0.03$). Liver failure was less frequent after PSLR (2% vs 7%; $P = 0.006$), with a shorter ICU stay (0 days vs 1 day, $P = 0.004$). OS and DFS were similar between the two groups. The authors concluded that PSLR for multiple bilobar CRLM represents an appropriate alternative to non-PSLR in selected patients, with lower morbidity and comparable oncological outcomes. Recent studies have further demonstrated the positive impact of PSLR in the treatment of multiple bilobar CRLM, bringing into question also the consolidated role of the TSH in these cases^[147]. A bi-institutional study compared the outcome of patients with multiple bilobar CRLM who had received TSH or PSLR. The inclusion criteria were ≥ 6 CRLM, ≥ 3 CRLM in the left liver, and ≥ 1 lesion with vascular contact. A total of 74 TSH and 35 PSLR were compared. Drop-out rate of TSH was 40.5%. PSLR had significantly lower blood loss, overall morbidity, severe morbidity, and liver-specific morbidity than TSH. R0 resection rate was similar between groups. PSLR and completed TSH had similar five-year OS (38.2% vs 31.8%), three-year RFS (17.6% vs 17.7%), and recurrence sites. The authors concluded that parenchymal-sparing hepatectomies are a safe alternative to TSH for multiple, bilobar, deeply located CRLM, and that PSLR should be preferred whenever achievable because of better safety and oncological results comparable to completed TSH without the drop-out risk.

Recent reports have demonstrated that also patients with large numbers of CRLM are potential candidates for liver surgery. In a bi-institutional Japanese study of 736 patients who underwent LR for CRLM over a 16-year period^[173], the authors compared 493 patients with 1-3 tumours, 141 with 4-7 tumours, and 102 with ≥ 8 tumours. Major hepatectomies had been performed in a minority of patients (21.6%). The five-year OS and DFS rates were 51% and 21%, respectively, for the entire patient cohort, 56% and 29% for patients with 1-3 tumours, 41% and 12% for those with 4-7 tumours, and 33% and 1.7% for those with ≥ 8 tumours. Positive lymph node metastasis of the primary CRC, the presence of extrahepatic metastases, a maximum tumour size > 5 cm, and tumour exposure during LR were associated with decreased survival upon multivariate analysis. The authors concluded that in patients with multiple CRLM, the number of CRLM has less prognostic impact than other factors, and that complete LR may offer a chance of cure even in patients with numerous CRLM, including those with eight or more nodules. In another bi-institutional study of 849 patients undergoing LR for CRLM^[174], 743 patients with 1-7 metastases were compared to 106 with ≥ 8 metastases. The overall perioperative mortality rate was 0.4%. Patients with 1-7 metastases had higher five-year OS (44.2% vs 20.1%; $P < 0.001$) and DFS (28.7% vs 13.6%; $P < 0.001$) rates. In patients with ≥ 8 metastases, OS and

DFS were similar for patients with 8-10, 11-15, or > 15 metastases. In this group, multivariate analysis identified three preoperative factors of adverse prognosis, including extrahepatic disease ($P = 0.010$), no response to preoperative CHT ($P = 0.023$), and primary rectal cancer ($P = 0.039$). Patients with two or more risk factors had very poor outcomes, while those with no risk factors had survival rates similar to patients with 1-7 metastases (five-year OS rate 44.0% vs 44.2%). The authors concluded that LR is safe in selected patients with ≥ 8 metastases, and offers reasonable five-year survival independent of the number of metastases. A recent French multicentric study examined the outcome of 529 patients undergoing liver surgery for ≥ 10 CRLM from 2005 to 2013, prospectively collected in the LiverMetSurvey registry^[92]. The five-year OS was 30%. A macroscopically complete (R0/R1) resection was achieved in 72.8% of patients and was associated with a three- and five-year OS of 61% and 39%, compared to 29% and 5% for R2/no resection patients ($P < 0.0001$). Upon multivariate analysis, R0/R1 resection resulted as the strongest favourable factor of OS ($P < 0.0001$). Other independent favourable factors were maximal tumour size < 40 mm ($P = 0.02$), age < 60 years ($P = 0.005$), preoperative MRI ($P = 0.007$), and adjuvant CHT ($P = 0.04$). Of the 346 patients who underwent R0/R1 resection, 74.6% had developed a recurrence at last follow-up, with three- and five-year primary DFS rates of 23% and 7%, respectively. When hepatic recurrence and extrahepatic recurrence were surgically treated, the secondary DFS rates (taking into account the impact of repeat surgery) at three years and five years were 42% and 31%, respectively. The authors concluded that, even though the oncological outcome of patients with ≥ 10 CRLM is obviously worse compared to patients exhibiting fewer lesions, surgery remains the only hope of prolonged survival, especially if complete resection can be performed, and that the number of CRLM should not be considered per se as contraindication to surgery.

The impact of PSLR on simultaneous colorectal and liver surgery

Simultaneous colorectal resection and minor hepatectomy have perioperative results similar to minor hepatectomy alone, and are at present considered the treatment of choice in most patients with limited liver disease suitable for minor LR^[1,19,24]. The results are much more conflicting in patients requiring simultaneous colorectal and major LR because most investigators have reported worse perioperative outcomes than for major LR alone also in experienced hepatobiliary centres^[20,79,80,82,175], while others remark that simultaneous colorectal resection and major hepatectomy can be performed safely in selected cases with perioperative risks comparable to major LR alone^[31-33]. Most studies comparing simultaneous and staged procedures are retrospective, with patients undergoing simultaneous procedures having more limited hepatic involvement, which could explain these

discordant outcomes^[19,22,26]. At present, most authors suggest combined resections in the case of easily accessible, uncomplicated colorectal tumours with CRLM requiring minor hepatectomies^[26,27,176], while these criteria could be selectively extended in units experienced in both hepatobiliary and colorectal surgery^[23]. In a recent survey reporting the opinion of colorectal and liver surgeons about simultaneous resection of CRC and liver metastases^[177], most surgeons of both groups perceived that simultaneous procedures were appropriate in adequately selected patients, especially in candidates to any type of colorectal surgery with minor LR. Restorative rectal resections coupled with a major LR were considered inappropriate due to the risk of leakage of the colorectal anastomosis. Some concern did exist as well, especially among liver surgeons, about the risk of leakage also for colo-colic anastomoses if combined with major LR.

As a matter of fact, even though surgeons experienced in colorectal and hepatobiliary surgery should carefully select candidates to simultaneous resection to minimize perioperative complications, the planned extent of LR seem to represent the most important determinant of whether simultaneous procedures are individually appropriate for CRC with synchronous CRLM^[19,24,178,179]. As previously discussed, IOUS-based conservative techniques of liver surgery substantially decrease the need for major hepatectomies also for multiple bilobar CRLM, with a substantial reduction of perioperative related risks and may represent an appropriate solution even for potential candidates to simultaneous colorectal and liver resection for bilobar synchronous CRLM. In a small retrospective series of 39 consecutive patients with synchronous CRLM, who underwent curative simultaneous "one-stage" hepatectomy and resection of the colorectal primary, Tanaka *et al.*^[178] observed that only the volume of the resected liver was a significant risk factor for postoperative complications (350 g mean resected liver volume in patients with postoperative complications vs 150 g in those without complications; $P < 0.05$). The systematic application of the criteria of conservative liver surgery have been associated with higher rates of feasibility of simultaneous colorectal and liver resections also in patients with multiple hepatic nodules. Minagawa *et al.*^[180] in 2006 reported 148 patients admitted with CRC and synchronous CRLM since January 1989, evaluated for simultaneous resection regardless of the location of the primary cancer and the extent of CRLM. A simultaneous resection was performed in 142 cases (feasibility rate 96%), without perioperative mortality. Fifty-one percent of patients had the primary tumour located in the rectum. With the systematic application of their principles of conservative IOUS-based liver surgery^[89], only 11.3% of patients required a hemihepatectomy, while the others received limited resections (74.6%) or the resection of one or two segments (14.1%). In a more recent study of 150 patients who underwent resection of primary CRC

and synchronous CRLM between 1993 and 2011^[181], the proportion of simultaneous resections was 84.7%. Among the 127 patients who had received a simultaneous colorectal and hepatic resection, there was no postoperative mortality, postoperative complications were 61.4%, major complications were 18.2%, and anastomotic failure occurred in 1.6% of patients. The three-, five- and ten-year OS was 74%, 64%, and 52%, respectively. In a small series of 45 patients who underwent elective resection of primary CRC and synchronous CRLM, a simultaneous colorectal resection with anastomosis and conservative one-stage LR was feasible in 75.6% of patients. It was possible to avoid a right hepatectomy in all the patients undergoing simultaneous restorative colorectal resection^[91]. Seven patients had synchronous CRC at presentation (unpublished data), and two of them had rectal cancer within diffuse colorectal poliposis and received restorative proctocolectomy with ileoanal J-pouch and temporary diverting loop ileostomy. One patient with multiple CRLM of the right hemiliver underwent the restorative proctocolectomy after neoadjuvant CHT, with a subsequent resection of liver segments S6–S7–S8. The other had a single metastasis in segment S8 and underwent simultaneous restorative proctocolectomy and liver segmentectomy. Two patients had a simultaneous cancer proximal to a rectal cancer, with multiple bilobar CRLM. One received neoadjuvant chemoradiotherapy and subsequent resection of the sigmoid colon and of the rectum with simultaneous one-stage PSLR. In the other patient a TSH was planned to treat the hepatic disease. The patient received neoadjuvant chemoradiotherapy and a subsequent rectal resection with a first-stage LR consisting of multiple wedge resection in the left hemiliver with right portal vein ligation. At re-exploration for the second-stage LR a massive diffusion of the cancer at the hepatic hilum was found and the planned right hepatectomy was not performed. Finally, three patients had SCRC in distant colonic segments, and we opted for a restorative subtotal colectomy. One patient underwent simultaneous liver bisegmentectomy of S2–S3 with splenectomy and interaortocaval lymphadenectomy because of splenic and interaortocaval lymph node metastases. The other two underwent PSLR for multiple bilobar CRLM, associated with intraoperative RFTA in one patient. Therefore, five patients received simultaneous potentially curative colorectal and one-stage liver resection without postoperative mortality and complications requiring reoperation.

CONCLUSION

In conclusion, simultaneous procedures represent an attractive surgical option in selected patients with resectable CRC and resectable synchronous CRLM. Simultaneous resections should only be considered by surgical teams experienced in both fields. Staged procedures are still advisable in the case of complicated

CRC requiring urgent colorectal resection. In all other cases, simultaneous resections should be theoretically considered whenever possible, including patients with SCRC. In these cases, if the synchronous tumours are located in distant colorectal segments, an extended restorative colectomy should be considered to prevent the risks related to multiple colorectal anastomoses, especially if prolonged hepatic pedicle clamping is planned for extensive PSLR and/or CRLM adjacent to major intrahepatic vessels. When rectal cancer is diagnosed, the indication to preoperative chemoradiotherapy and its potential benefits should be adequately considered. A systematic approach to liver resection that focuses on the need of reducing the extent of hepatectomy while preserving oncological radicality may represent the best strategy to limit the perioperative risks in candidates to simultaneous colorectal and liver resection.

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Histo-molecular oncogenesis of pancreatic cancer: From precancerous lesions to invasive ductal adenocarcinoma

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Abstract

Pancreatic cancer is a lethal malignancy, whose precursor lesions are pancreatic intraepithelial neoplasm, intraductal papillary mucinous neoplasm, intraductal tubulopapillary neoplasm, and mucinous cystic neoplasm. To better understand the biology of pancreatic cancer, it is fundamental to know its precursors and to study the mechanisms of carcinogenesis. Each of these precursors displays peculiar histological features, as well as specific molecular alterations. Starting from such pre-invasive lesions, this review aims at summarizing the most important aspects of carcinogenesis of pancreatic cancer, with a specific focus on the recent advances and the future perspectives of the research on this lethal tumor type.

Key words: Oncogenesis; Intraductal papillary mucinous neoplasm; Mucinous cystic neoplasm; Pancreatic ductal adenocarcinoma; Pancreatic intraepithelial neoplasm; *KRAS*; Carcinogenesis; Pancreatic cancer; Intraductal tubulopapillary neoplasm

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Core tip: Pancreatic intraepithelial neoplasm, intraductal papillary mucinous neoplasm, intraductal tubulopapillary neoplasm, and mucinous cystic neoplasm are precursor lesions of invasive pancreatic cancer. Each of these precursors displays peculiar histological and molecular features, which have been summarized in this review along with the most important aspects of pancreatic carcinogenesis. The most recent advances and the future perspectives of the research on this topic have also been highlighted.

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INTRODUCTION

Precursor lesions of pancreatic cancer

Precursor lesions of pancreatic ductal adenocarcinoma (PDAC) are non-invasive lesions, which can progress to infiltrating carcinoma^[1]. Following the 2010 World Health Organization classification, different consensus conferences and international meetings have provided the basis for the current view of the definition and classification of pancreatic precursor lesions^[2-4].

One major issue in classification is in regards to the grading of dysplasia of pre-invasive lesions, which should be restricted to the two categories of "low-grade" and "high-grade" with the elimination of the poorly reproducible intermediate entity of "moderate dysplasia"^[5]. Furthermore, small intraductal papillary mucinous lesions (PanIN) ranging from 0.5 to 1.0 cm would be better defined as "incipient intraductal papillary mucinous neoplasm (IPMN)"^[5].

Three PDAC precursors have been recognized: PanIN, IPMN, and mucinous cystic neoplasm (MCN). In addition, intratubular papillary neoplasms (ITPNs) display the features of pre-invasive lesion. Each of these entities has distinct clinical, histological, and molecular profiles. Through a multi-step carcinogenesis, with the accumulation of cellular and molecular alterations, each of these precursors may lead to the development of invasive ductal adenocarcinoma.

CLINICAL FEATURES OF PRECURSOR LESIONS OF PANCREATIC CANCER

PanIN

PanIN represents the most common PDAC precursor, affecting both men and women equally. Its incidence increases directly with age^[5]. The strict correlation with PDAC is suggested first of all by the fact that these lesions can be found in more than 80% of pancreas with invasive

carcinoma^[1,6], and by a reported multifocality in patients with PDAC familial history^[7,8]. Usually, due to their small size (by definition < 0.5 cm), these lesions are classically found incidentally during histological examination and are not associated with clinical symptoms or specific signs. From the radiological point of view, PanINs are more often associated with acinar atrophy and/or fibrosis, but this correlation is not specific^[9,10].

IPMN

IPMNs are grossly visible lesions with intraductal growth, papillary architecture, and mucous producing cells. The first definition of IPMNs was reported in 1994^[11]. The median age of IPMN patients ranges from 60 to 66 years. They are more common in men than in women (ratio: 3/1.3 in Europe, 3/2.1 in United States, and 3/1.8 in Asia) and arise most frequently in the proximal pancreatic head and the "uncinatus" process^[12]. Although rare, IPMN involving more than a pancreatic segment or even the entire pancreas have been described (Figure 1)^[12]. It is estimated that IPMNs may require up to six years to become invasive, although such estimation may be affected by multiple biases, including the specific IPMN histotype^[13-15]. Similarly to PanINs, IPMNs are found more frequently in patients with PDAC familial history, thus highlighting the importance of a genetic predisposition to carcinogenesis of IPMN patients. In fact, such precursors have been found also in the context of multi-organ syndromes such as Peutz-Jeghers, familial adenomatous polyposis, Lynch, and McCune-Albright syndromes^[16-18]. Moreover, patients with IPMNs have an increased risk of developing other extrapancreatic cancers^[19].

One of the most important distinctions between IPMNs and PanINs is the possibility that IPMNs may be detected with imaging techniques. Patients with IPMNs should be followed-up according to specific protocols based on radiological examination^[20]. The typical intraductal growth of IPMNs leads to cystic dilation of the pancreatic tree ducts^[21]. Based on their location, IPMNs may be classified from the topographic point of view in: (1) main duct IPMNs where Wirsung's duct is involved; (2) branch duct IPMNs in the case of the involvement of secondary ducts; or (3) mixed IPMNs in the case of contemporaneous involvement of the main and the branch ducts^[1,20,21]. Although this distinction cannot always be confirmed by histopathological examination due to some branch duct IPMNs displaying some degree of Wirsung's duct involvement^[22], this topographic definition has an important clinical impact. Indeed, main duct IPMNs are more often associated with PDAC development and patients with this type of lesion must follow stringent surveillance protocols^[20]. IPMN-associated carcinomas usually display a better prognosis than conventional PDACs^[1].

MCN

MCNs are typically reported in perimenopausal women, with few cases described in men^[4]. They usually arise



Figure 1 Extensive involvement of the pancreas by an intraductal papillary mucinous neoplasm. This neoplasm involves almost all the pancreatic ductal tree. The asterisks indicate Wirsung's duct along its course.

in the distal part of the pancreas (body and/or tail), and by definition do not communicate with the pancreatic ductal tree^[1]. It has been hypothesized that the females may be predisposed to MCNs due to embryogenesis or by a carcinogenetic process stimulated by female hormones^[23,24]. This theory is also corroborated by their histological aspect because under the mucinous, non-papillary epithelium is a classic ovarian-like stroma^[1]. The mean age of patients with MCNs is about 44 years. The mean age of patients with MCNs with an associated adenocarcinoma is about 55 years^[20]. This observation is in line with the status of MCN as a PDAC precursor lesion. The association with PDAC is present in up to one third of MCNs^[25,26]. In contrast to PanINs, but similar to IPMNs, MCNs can be detected by imaging. MCN patients must undergo strict follow-up or pancreatic resection because there is a non-negligible risk of PDAC development^[21].

ITPN

ITPNs are rare intraductal neoplasms of the pancreas composed of mucinous cells displaying a tubule-papillary architecture^[1,5]. The incidence is similar in women and men^[1]. These lesions are more commonly located in the head of the pancreas^[1], and their symptoms are unspecific, including undefined abdominal pain and vomiting. Notably, about 40% of ITPNs harbor an associated invasive carcinoma^[21]. PDACs arising in association with ITPNs usually have a better prognosis than that of conventional PDACs with a 5-year survival rate of more than 30%^[21].

HISTOPATHOLOGY OF PRECURSOR LESIONS OF PANCREATIC CANCER

PanIN

PanINs are non-infiltrating microscopic intraductal lesions with a diameter < 0.5 cm^[1,3]. From the histological point of view, they are composed of cuboid to columnar mucinous cells with varying degrees of dysplasia, reflecting the different degrees of cytologic and/or

architectural atypia^[1,3]. In the vast majority of cases, PanINs show gastric/foveolar differentiation^[21]. Hruban *et al.*^[4] classified PanINs into a three-tiered scale, based on the degree of dysplasia. In this scheme, PanIN-1 shows low-grade dysplasia, PanIN-2 shows intermediate dysplasia and PanIN-3 shows high-grade dysplasia characterized by marked cell atypia, presence of mitotic figures, loss of polarity, and complex architecture. To improve inter-observer agreement and in order to report only the most important histological information, a recent consensus suggested a new classification system, distinguishing low-grade PanINs, which includes the previously called PanIN-1 and PanIN-2, and high-grade PanINs that includes PanIN-3 (Figure 2)^[5]. In high-grade PanINs, cribriform structures, atypical mitosis, tufting of epithelial cells in the lumen, and even necrosis may be present, but in case these features are concomitant with a PDAC, the most important differential diagnosis of high-grade PanINs is with non-dysplastic ducts, which have been colonized by PDAC cells^[27]. Notably, high-grade PanINs have been reported almost exclusively in association with an infiltrating PDAC^[1,21]. However, a recent report pointed out that high-grade dysplasia PanINs may be found without concomitant infiltrating PDAC, and, when they involve the main duct, they may cause stenosis with extensive upstream duct dilation^[28]. At the immunohistochemical level, PanINs show an increased expression of mucin 1 and mucin 5AC (MUC1 and MUC5AC) and a decreased expression of mucin 6 (MUC6)^[29-32].

IPMN

IPMNs are non-infiltrating neoplasms > 1.0 cm with intraductal growth composed of mucinous cells with a papillary architecture^[1,5]. Lesions with such features but with a size > 0.5 cm but < 1.0 cm should be classified as "incipient IPMN"^[5]. Similarly to PanINs, a recent consensus suggested grading IPMNs into low-grade and high-grade and to avoid the intermediate-grade dysplasia, which should be included into the low-grade category^[5]. IPMNs can be classified not only basing on topography (main duct, branch duct, or mixed type), but also from the histological point of view, based on the histotype of the predominant epithelium, which also influences their biological behavior: Gastric, pancreatobiliary, intestinal, and oncocytic^[1,33-41] (Figure 3).

Gastric-type IPMNs usually do not involve the Wirsung's duct. They are composed of cells with the features of the gastric foveolar epithelium. There is a single layer of mucinous cells with polarized nuclei located at the basis of the cells. Usually this epithelium is associated with low-grade dysplasia. It can show a mixture of papillary, pseudopapillary, and flat structures^[1]. When high-grade dysplasia is present in a gastric-type IPMN, with complex structure and atypical cells, the lesion becomes histologically very similar to a pancreatobiliary-type IPMN^[33]. Questions are still open if these aspects represent different degrees of gastric-epithelial dyspla-

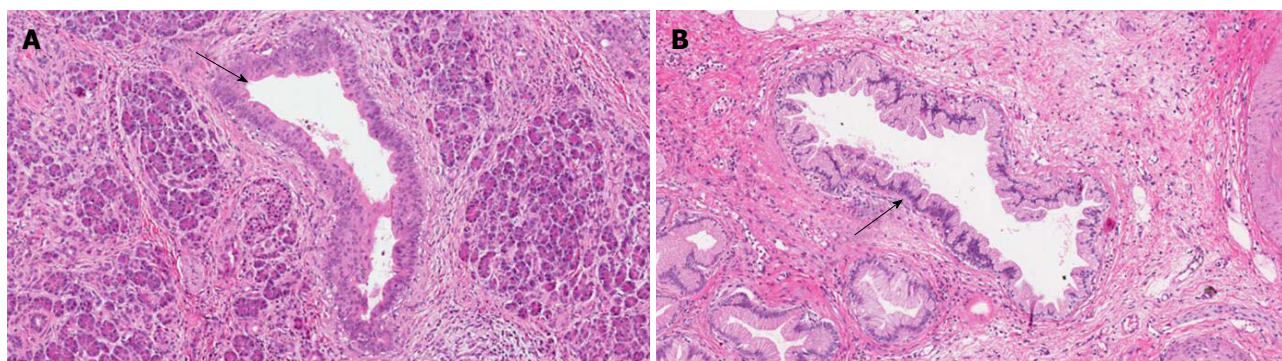


Figure 2 Pancreatic intraepithelial neoplasm precursor lesions. A: High-grade pancreatic intraepithelial neoplasm (PanIN); B: Low-grade PanIN. Original magnification: $\times 10$. Black arrows indicate ducts involved by PanINs.

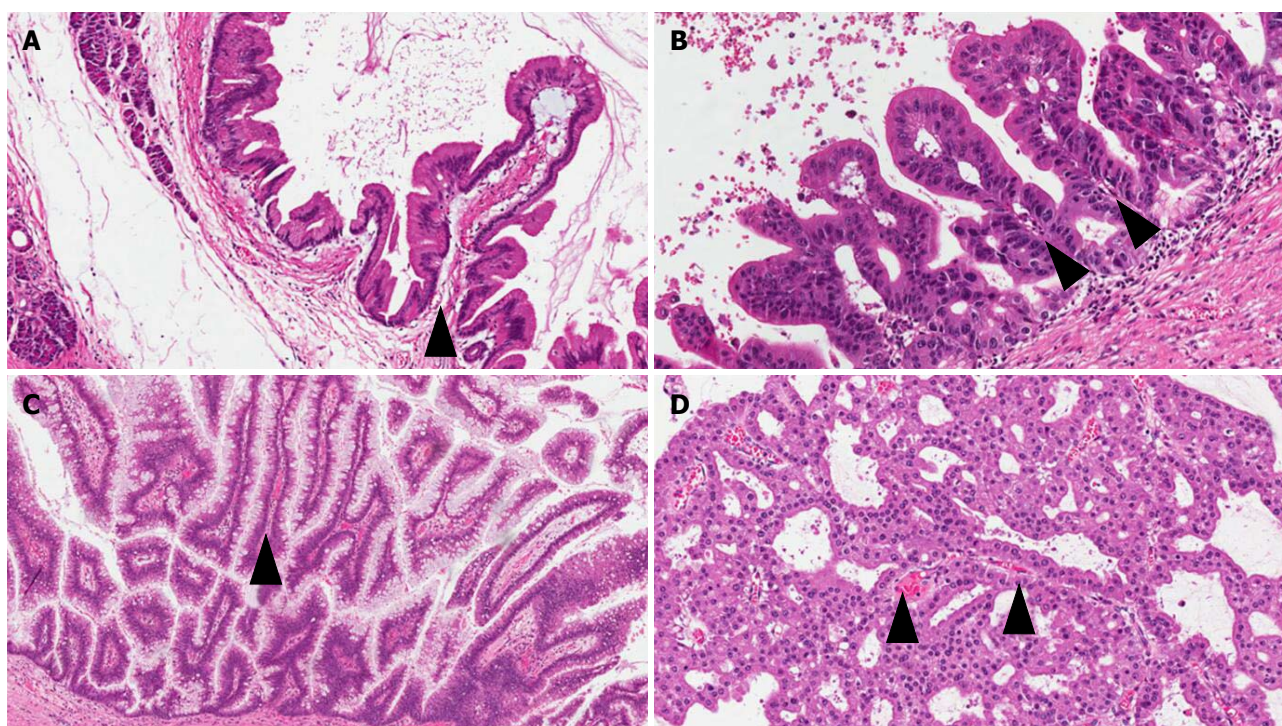


Figure 3 The four different types of intraductal papillary mucinous neoplasm. A: Gastric; B: Pancreatobiliary; C: Intestinal; D: Oncocytic. Original magnification: A and C: $\times 10$; B and D: $\times 20$. Black arrowheads indicate the fibro-vascular axis of the papillary structures.

sia, or could represent intratumor heterogeneity of IPMNs with low-grade gastric epithelium and high-grade pancreatobiliary epithelium coexisting in the same lesion^[33-36].

Pancreatobiliary IPMNs usually involve the Wirsung's duct. They are composed of irregular cells usually with enlarged nuclei and prominent nucleoli. Typically, the dysplasia in this type of lesion is high-grade. Among the different IPMN subtypes, they have the highest risk to progress into PDAC^[21,33-41]. Indeed, a recent meta-analysis including 14 studies for a total of 1617 patients, showed that pancreatobiliary IPMNs are associated with the most aggressive behavior, while gastric IPMNs display the lowest risk of cancer progression^[37].

Intestinal IPMNs usually involve Wirsung's duct. They are histologically similar to villous adenoma of the lar-

ge bowel. Their most evident morphological feature is represented by the presence of goblet cells, the papillae are long and sometimes branching, and the nuclei of the cells are hyperchromatic, elongated, and show different degrees of pseudostratification^[1,21]. Although their risk is lower than pancreaticobiliary type, intestinal IPMNs can progress into invasive adenocarcinoma as well. Interestingly, the latter is not usually represented by a conventional adenocarcinoma but by a colloid carcinoma (Figure 4), which displays a better prognosis than conventional PDAC^[1].

Oncocytic IPMNs are rare lesions, which may involve both main and branch ducts, or even the entire pancreatic ductal tree. They are composed of cells with a typical eosinophilic and granular cytoplasm due to the abundance of accumulated mitochondria^[32]. Not only

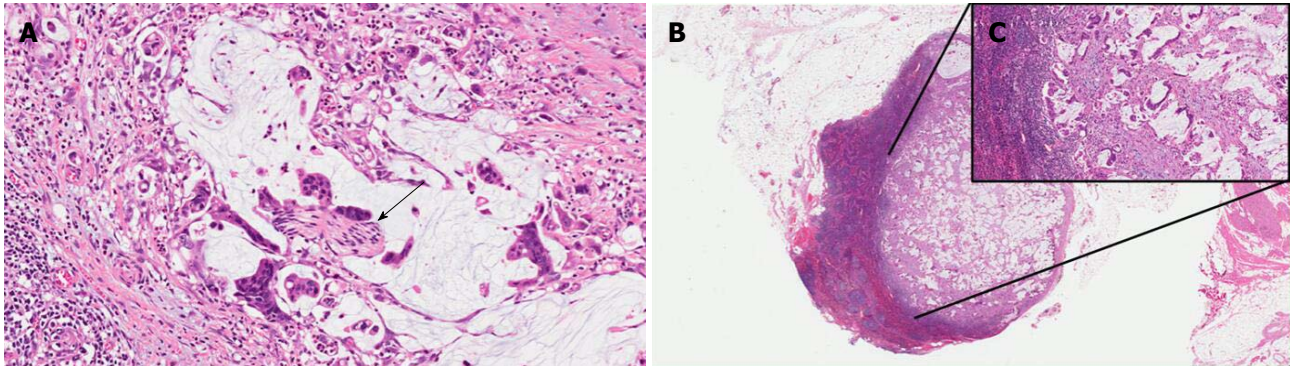


Figure 4 Colloid carcinoma with perineural invasion (A, black arrow) and nodal metastasis (B: low magnification; C: higher magnification of the same metastasis). Original magnification: A: $\times 10$; B: $\times 4$; C: $\times 20$.

is the cytological appearance peculiar, but so is the architecture. Oncocytic IPMN form arborizing papillae, lined by one to five layers of cuboidal cells. A specific feature is represented by punched-out spaces in the epithelium^[1,21,32].

The best strategy for pathologists to classify IPMN histotypes is coupling morphology with immunohistochemistry, particularly in the case of high-grade dysplasia. Immunohistochemistry based on mucin staining appears of great importance in this setting (Table 1)^[1,33-41]. However, even with this integrated approach about 25% of cases are difficult to classify, mainly due to the presence of phenotypic heterogeneity or dedifferentiated areas^[42].

MCN

MCNs are composed of columnar cells with abundant mucin located in the luminal part of the cells. The dysplasia of MCNs should be classified with a two-tiered scale (MCNs with low-grade including the previously called intermediate dysplasia, vs MCNs with high-grade dysplasia), following the recommendation of the latest consensus conference^[5]. MCNs with low-grade dysplasia show mild cell atypia and lack of architectural complexity. MCNs with high-grade dysplasia are composed of atypical cells often with enlarged nuclei and multi-layer stratification (Figure 5). The diagnostic clues for the diagnosis of MCNs are represented by the lack of communication with the pancreatic ductal tree (always present in IPMNs), and the presence of an ovarian-like stroma located under the mucinous epithelium (Figure 5)^[1,5,43]. These stromal cells usually exhibit immunostaining for progesterone and estrogen receptors as well as for α -inhibin^[44,45]. In the case of an associated invasive adenocarcinoma, the latter is usually represented by a conventional PDAC^[21].

ITPN

ITPNs are composed of relatively uniform and cuboidal cells, without a significant amount of mucin, arranged in densely packed tubules and back-to-back glands, with a typical intraductal, tubulopapillary growth (Figure 6)^[1]. In this type of lesion, extracellular mucin production may

be lacking or very focal with less common cyst formation as a direct consequence. An intestinal-type necrosis may also be present^[46]. ITPNs are typically negative for MUC5AC, while MUC6 is often strongly positive (Table 1)^[1,46]. Because of their potential progression to invasiveness as well as for their non-negligible association with PDAC, ITPNs are also considered a PDAC precursor lesion^[1,21].

MOLECULAR PROFILES OF PRECURSOR LESIONS OF PANCREATIC CANCER

The study of the molecular landscape of PDAC precursor lesions has generated a growing body of knowledge, very useful not only to the comprehension of its oncogenesis but also to plan future strategies for their early detection. From the molecular point of view, *KRAS*, *TP53*, *CDKN2A*, and *SMAD4* represent the four major driver genes of PDAC^[1,27], and it is of great interest the timing in which its precursors acquire alterations in such genes during their specific carcinogenesis. The most important aspect in this process, which is common to each precursor, is that a *KRAS* mutation is a fundamental and early event.

PanIN

The generally accepted definition of PanIN as a true precursor lesion of PDAC has been necessarily confirmed through their molecular characterization. Seminal research on this topic has showed that there is molecular evidence of the progression of PanIN towards PDAC. Early lesions (low-grade PanINs) display *KRAS* somatic mutations, and high-grade PanINs harbor *CDKN2A*, *TP53*, and *SMAD4* mutations^[47-53]. In PanIN carcinogenesis, *TP53* and *SMAD4* inactivation appear as the latest events^[53].

IPMN

A recent whole-exome sequencing study on IPMNs has showed an average of 26 mutated genes per case^[54]. The most frequently mutated genes in IPMNs are *GNAS* and *KRAS*, which are altered in up to 60%

Table 1 Immunohistochemical markers for intraductal papillary mucinous neoplasm/intraductal tubulo-papillary neoplasm histopathological classification

Type of lesion	Subtype	MUC1	MUC2	MUC5AC	MUC6	CDX2
IPMN	G	Negative	Negative	Positive ¹	Negative	Negative
	PB	Positive ¹	Negative	Positive ¹	Positive	Negative
	INT	Negative	Positive ¹	Positive ¹	Negative	Positive ¹
	ONC	Positive	Negative	Positive	Positive ¹	Negative
ITPN		Positive	Negative	Negative	Positive ¹	Negative

IPMN: Intraductal papillary mucinous neoplasm; ITPN: Intraductal tubulo-papillary neoplasm; G: Gastric; PB: Pancreaticobiliary; INT: Intestinal; ONC: Oncocytic. ¹The positivity of a marker is very intense at the immunohistochemical level (++).

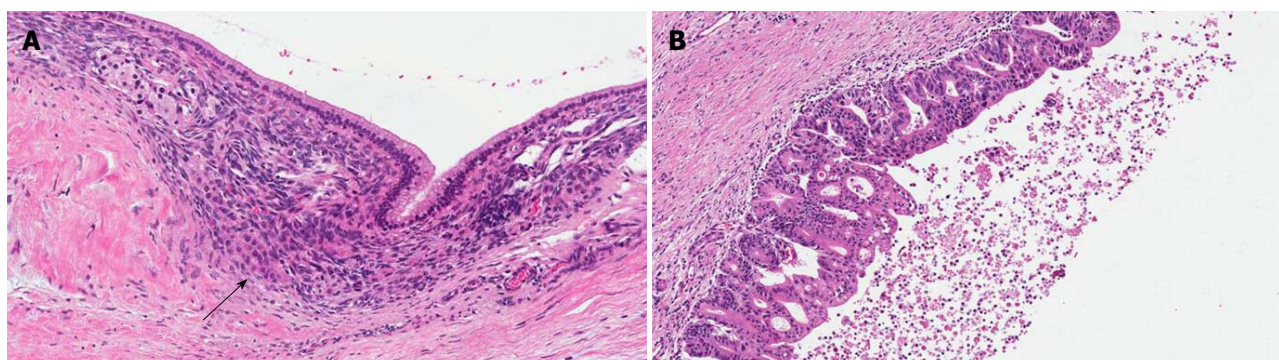


Figure 5 Mucinous cystic neoplasm precursor lesions. A: Low-grade mucinous cystic neoplasm (MCN); B: High-grade MCN. The black arrow indicates the ovarian-like stroma, a typical component of this type of lesion. Original magnification: $\times 10$.

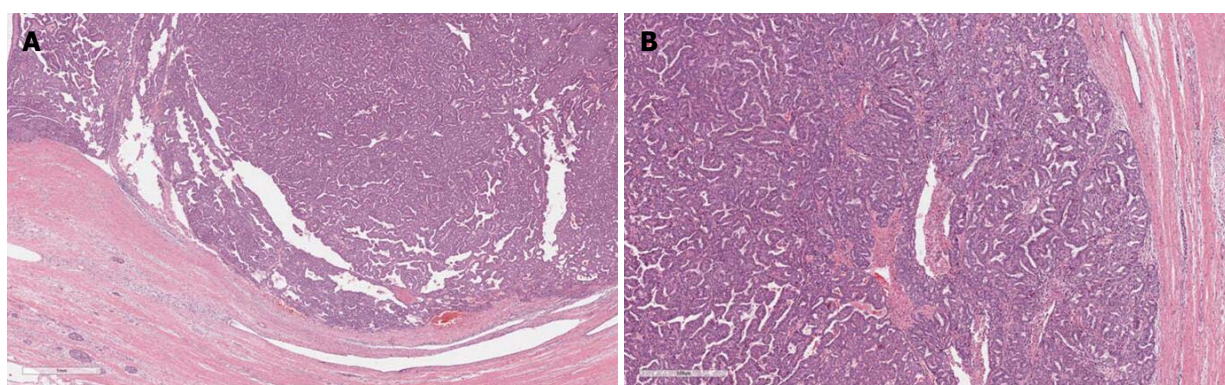


Figure 6 Intraductal papillary neoplasm precursor lesions. A: Low magnification showing an extensive intraductal growth; B: Higher magnification. Original magnification A: $\times 1$; B: $\times 4$.

and to 80% of cases, respectively^[54,55]. Notably, recent studies pointed out that the carcinogenesis of IPMNs may follow two distinct pathways: The first, linked to *GNAS* mutations, are intestinal IPMNs progressing to colloid adenocarcinomas, and the second, linked to *KRAS* mutations, are typical of pancreatobiliary IPMNs and leads to conventional PDAC^[56-59]. Another frequently mutated gene in IPMNs is *RNF43*, an E3 ubiquitin-protein ligase, which functions as a negative regulator of the Wnt-signaling pathway^[54,60]. Lastly, *BRAF*, *TP53*, and *SMAD4* mutations can be found in IPMN with high-grade dysplasia. *TP53* and *SMAD4* mutations, similar to high-grade PanINs, are the latest molecular events in IPMN

carcinogenesis^[60].

MCN

A recent whole-exome sequencing study of MCNs revealed an average of 16 somatic mutations per case^[54], and compared to IPMNs there was a lower percentage of cases with loss of heterozygosity events, a molecular feature associated to poor prognosis^[54,61]. The fewer number of mutations and chromosomal alterations in MCNs could explain the lower frequency of progression to PDAC of this type of precursor when compared with IPMNs. In MCNs, somatic mutations involving the four classic PDAC driver genes (*KRAS*, *CDKN2A*, *TP53*, and

SMAD4) and *RNF43* have also been reported^[54].

ITPN

This type of lesion has a peculiar molecular profile. Particularly, mutations involving *KRAS*, *NRAS*, and *GNAS* are very rare^[60,62,63]. At the same time, *PIK3CA* mutations and *AKT* alterations (and consequently the involvement of the druggable mTOR pathway) are seen in 21% to 27% of ITPN cases^[62,63].

RECENT ADVANCES AND FUTURE PERSPECTIVES ON PANCREATIC CARCINOGENESIS

Recent molecular advances in pancreatic carcinogenesis have given new interesting insights into the biological behavior of PDAC. The study of the genetic landscape of its precursor lesions has highlighted important implications for the early detection and for the clinical management of patients with pre-invasive and invasive pancreatic tumors.

PanIN

The most recent advances on the genetics of PanINs come from the study by Hosoda *et al.*^[64] of a series of isolated PanINs, *i.e.* those occurring in the absence of a concomitant PDAC. Whole-exome or targeted sequencing of 23 isolated high-grade PanINs found that *KRAS* mutations were present in the vast majority of lesions (> 90%), and *CDKN2A* and *RNF43* mutations were relatively frequent (about 20%-25% of cases), but other genes previously considered important in high-grade PanINs, *i.e.*, *TP53*, *SMAD4*, *GNAS*, *ARID1A*, *PIK3CA*, and *TGFBR2* were very rarely mutated or not altered^[64]. In the same study, 16 adjacent low-grade PanINs were sequenced showing very frequent *KRAS* mutations (> 90% of cases) and lack of mutations in *TP53*, *CDKN2A*, and *SMAD4* tumor suppressor genes^[64]. The main conclusion of this paper was that mutations of *TP53* and *SMAD4* are events mainly associated with invasive PDAC and not with PanIN precursor lesions. Also the inactivation of chromatin remodeler genes, such as *ARID1A* tumor suppressor gene, previously thought to be important in PDAC and other invasive malignancies^[65-68], appeared to be associated with infiltrating cancers rather than precursor lesions in the pancreas. The refinement of our knowledge on the morphological and molecular alterations of PanINs should be taken into account by future researchers in order to improve the possibilities of PDAC early detection.

IPMN

The clinical management of IPMNs has changed in the last decade. The most recent guidelines indicate the need of combining clinical and radiological information in order to define the best therapeutic choice. Particular features, whose presence has different implications,

have been distinguished in IPMN patients and indicated as "high-risk stigmata" and "worrisome features"^[69]. The "high-risk stigmata" are represented by: (1) obstructive jaundice in a patient with cystic lesion of the head of the pancreas; (2) enhancing mural nodule > 5 mm; and (3) main pancreatic duct > 10 mm. The "worrisome features" comprise of: (a) clinical pancreatitis; (b) cyst > 3 cm, (c) enhancing mural nodule < 5 mm; (d) thickened/enhancing cyst walls; (e) main duct size 5–9 mm; (f) abrupt change in caliber of pancreatic duct with distal pancreatic atrophy; (g) lymphadenopathy; (h) increased serum level of CA19-9; and (i) cyst growth rate > 5 mm/2 years^[69]. Thus, patients with IPMN should be followed-up with a stringent protocol, which integrates imaging (endoscopic ultrasonography, computed tomography, and magnetic resonance) and clinical data, on the basis of their importance and their specific risk of progression to invasive cancer. From the molecular point of view, the recent advances in this field have provided interesting information from the genetic analysis of cyst fluids^[70]. Future protocols should integrate clinical-radiological information with molecular data, to obtain for each patient an integrated estimation of the risk of PDAC development. This approach, however, should also take into account the issue of field-effect carcinogenesis of PDAC. Indeed, IPMNs and PDACs are not necessarily genetically related as recently reported by Felsenstein *et al.*^[71], who demonstrated that about 20% of coexisting IPMNs and PDACs are molecularly unrelated, indicating the possibility of PDAC development independent from a coexisting IPMN. The main implication of this research regards the strategy of surveillance of patients with IPMN^[72]. Also, the local recurrence of IPMN or PDAC after pancreatic resection for a IPMN may be genetically unrelated, highlighting the existence of a field-effect carcinogenesis of PDAC^[73].

MCN and ITPN

The clinical management of patients with MCNs and ITPNs should take into account recent molecular knowledge. MCNs and ITPNs represent true PDAC precursor lesions, thus an integrated approach with clinical-radiological information and molecular data should be implemented in order to define stringent protocols for the surveillance of low-risk subjects as well as precise parameters indicating the need of pancreatic resection in high-risk patients.

Cell of origin of pancreatic cancer

Another recent fascinating advance in pancreatic carcinogenesis are the putative cells of origin of this tumor type. Indeed, recent evidence from engineered mice-models suggests that PanIN development seems to be the result of the transdifferentiation of acinar cells, while IPMNs seem to arise from the progenitor niche of the pancreatic ductal epithelium^[74-76]. These new concepts have totally changed the previous convictions, which indicated the differentiated ductal cells as the progenitor of

Table 2 Precursor lesions and their most important histopathological and molecular features

Precursor lesions	Main histopathological features	Molecular hallmarks
PanIN	Non-infiltrating lesions involving pancreatic ducts and < 0.5 cm, composed of cuboid to columnar mucinous cells, with two degrees of dysplasia: (1) Low-grade PanINs include the previously called PanIN-1 and PanIN-2; and (2) high-grade PanINs include PanIN-3	<i>KRAS</i> somatic mutations are early molecular events (Low-grade PanINs); <i>CDKN2A</i> , <i>TP53</i> , and <i>SMAD4</i> mutations are late molecular events (High-grade PanINs)
IPMN	Non-infiltrating intraductal neoplasms > 1.0 cm composed of mucinous cells with papillary architecture. IPMNs have two degrees of dysplasia: (1) Low-grade IPMNs; and (2) High-grade IPMNs. IPMNs can be classified based on topography (main duct, branch duct or mixed) and also on histology (gastric, pancreaticobiliary, intestinal, or oncocytic type, see Table 1)	<i>GNAS</i> and <i>KRAS</i> are altered in up to 60% and to 80% of IPMNs, respectively There are two possible carcinogenetic processes: (1) <i>GNAS</i> mutations cause progression to colloid carcinomas; and (2) <i>KRAS</i> mutations lead to conventional PDAC. Other frequently mutated genes in IPMNs are <i>RNF43</i> , <i>BRAF</i> , <i>TP53</i> , and <i>SMAD4</i>
MCN	Composed of columnar cells with abundant mucin located in the upper part. There are two degrees of dysplasia: (1) Low-grade MCN; and (2) High-grade MCN. The histopathologic clues for MCN diagnosis are the lack of communication with the pancreatic ductal tree and the presence of an ovarian-like stroma under the mucinous epithelium	There are fewer mutations and chromosomal alterations in MCNs compared with other precursors, and this could explain the lower frequency of progression of MCN to PDAC. Frequently altered genes are <i>KRAS</i> , <i>CDKN2A</i> , <i>TP53</i> , <i>SMAD4</i> , and <i>RNF43</i>
ITPN	Composed of uniform cuboidal cells without a significant amount of mucin, arranged in densely packed tubules and back-to-back glands with a typical intraductal, tubulopapillary growth	<i>PIK3CA</i> mutations and <i>AKT</i> alterations are frequently seen in ITPNs. Mutations involving <i>KRAS</i> , <i>NRAS</i> , and <i>GNAS</i> are very rare in ITPNs

PanIN: Pancreatic intraepithelial neoplasm; IPMN: Intraductal papillary mucinous neoplasm; MCN: Mucinous cystic neoplasm; ITPN: Intratubular papillary neoplasm; PDAC: Pancreatic ductal adenocarcinoma.

PDAC. Understanding these two different pathways of PDAC carcinogenesis, one starting from acinar epithelium and one from ductal epithelium, could also partly explain the different biological behaviors of PanINs and IPMNs and their progression into an overt PDAC^[74-76].

CONCLUSION

The histopathological and molecular features of PDAC precursor lesions have been summarized in Table 2 to provide a complete vision on this important topic. They represent a fundamental issue for the comprehension of PDAC carcinogenesis and its biological behavior. Only an integrated approach coupling histopathology and molecular analysis may guarantee a decisive step for the early detection of PDAC and to design more effective therapeutic strategies.

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Facing the challenge of venous thromboembolism prevention in patients undergoing major abdominal surgical procedures for gastrointestinal cancer

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Abstract

Venous thromboembolism (VTE) refers to a hypercoagulable state that remains an important and preventable factor in the surgical treatment of malignancies. VTE includes two identical entities with regards to deep vein thrombosis and pulmonary embolism. The incidence of VTE after major abdominal interventions for gastrointestinal, hepato-biliary and pancreatic neoplastic disorders is as high as 25% without prophylaxis. Prophylactic use of classic or low-molecular-weight heparin, anti-Xa factors, antithrombotic stocking, intermittent pneumatic compression devices and early mobilization have been described. Nevertheless, thromboprophylaxis is often discontinued after discharge, although a serious risk may persist long after the initial triggering event, as the coagulation system remains active for at least 14 d post-operatively. The aim of this review is to evaluate the results of the current practice of VTE prevention in cancer patients undergoing major abdominal surgical operations, with special attention to adequately elucidated guidelines

and widely accepted protocols. In addition, the recent literature is presented in order to provide an update on the current concepts concerning the surgical management of the disease.

Key words: Deep vein thrombosis; Pulmonary embolism; Gastro-intestinal cancer; Thromboprophylaxis; Venous thromboembolism

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Core tip: Venous thromboembolism (VTE) refers to a hypercoagulable state that remains an important and preventable factor in the surgical treatment of malignancies. The incidence of VTE after major interventions for gastro-intestinal, hepatobiliary and pancreatic neoplastic disorders is as high as 25% without prophylaxis. Prophylactic use of classic or low-molecular-weight heparin, anti-Xa factors, antithrombotic stocking, intermittent pneumatic compression devices and early mobilization have been described. The aim of this review is to evaluate the results of the current practice of VTE prevention in cancer patients undergoing major abdominal surgical operations, with attention to adequately elucidated guidelines and widely accepted protocols.

Mastoraki A, Mastoraki S, Schizas D, Patras R, Krinos N, Papanikolaou IS, Lazaris A, Liakakos T, Arkadopoulos N. Facing the challenge of venous thromboembolism prevention in patients undergoing major abdominal surgical procedures for gastrointestinal cancer. *World J Gastrointest Oncol* 2018; 10(10): 328-335 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i10/328.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i10.328>

INTRODUCTION

Venous thromboembolism (VTE) refers to a hypercoagulable state that remains an important and preventable factor in the surgical treatment of malignancies. VTE includes two identical entities with regards to deep vein thrombosis (DVT) and pulmonary embolism (PE)^[1]. The incidence of VTE after major abdominal intervention for gastrointestinal (GI), hepatobiliary and pancreatic (HPB) neoplastic disorders is as high as 25% without prophylaxis^[2]. Associated immobility, the Trendelenburg position, abdominal surgical procedure, potential compression of the vena cava, placement of intravenous catheters and chemotherapy have been proposed as major determinants of hypercoagulation and VTE prevalence. Neoadjuvant chemoradiotherapy followed by surgical resection as well as laparoscopic techniques have also been implicated. Recent surveys suggest that mechanical and pharmacological prophylaxis is effective in preventing post-operative VTE^[3]. Prophylactic use of classic or low-molecular-weight heparin (LMWH), anti-Xa factors, antithrombotic stocking, intermittent pneumatic compression devices and early mobilization

have been alternatively described^[4]. Nevertheless, thromboprophylaxis is interrupted early in many cases, while relevant risk may exist long after discharge, as the activation of the coagulation system persists for at least 2 wk post-operatively. In 2007, the American Society of Clinical Oncology (ASCO) suggested an evidence-based clinical practice for the prophylactic and therapeutic approach to VTE. A subsequent update has recently been reported. However, there is still debate about the choice and duration of the appropriate anticoagulation therapeutic approach. Both guidelines recommend consideration of extended prophylaxis in high-risk patients, despite the lack of a relevant, specific definition^[5]. The aim of this study was to elucidate the results of the current practice of VTE prevention in cancer patients undergoing major abdominal surgical operations, with special attention to adequately evaluated guidelines and widely accepted protocols. In addition, recent literature is presented to provide an update on current concepts in surgical management of the disease.

HISTOLOGY AND PATHOGENESIS

Although several predisposing factors in DVT have been meticulously investigated, mechanisms of thrombus development remain unclear. The classic Virchow triad refers to the combination of blood flow restriction, a hypercoagulable state and prothrombotic alterations in the vessel wall, and plays a pivotal role in thrombosis initiation^[6]. Traditionally, a blood clot contains a mishmash of platelets, red blood cells and fibrin. Arterial clots are usually created under high shear stress after rupture of an atherosclerotic plaque or other vascular destruction. As they are platelet-rich, administration of antiplatelet drugs is often implemented. In contrast, venous clots are fibrin-rich and develop under lower shear stress on the surface of a macroscopically intact endothelium. The therapeutic approach always involves anticoagulant drug administration^[7]. Disturbed blood flow remains a significant risk parameter, as it can provoke DVT due to long-term immobilization^[8]. Hypoxia activates the endothelium, promotes the release of Weibel-Palade bodies (storage granules in endothelial cells), and facilitates blood coagulation. Weibel-Palade bodies are also responsible for the production of the von Willebrand factor, which has an important pathogenetic role in platelet recruitment.

The blood coagulation cascade is well-defined and divided into the extrinsic and intrinsic pathways. Deficiencies in the anticoagulants antithrombin and proteins C and S constitute significant genetic risk factors that contribute to the development of a hypercoagulable condition. Mild genetic alterations in von Leiden factor, prothrombin G20210A and fibrinogen C10034T predispose patients to decreased fibrinolysis^[9]. It is common knowledge that the most frequent site of thrombus formation is the valve pocket sinus due to its vertical blood flow and inadequate oxygen tension. Therefore, small thrombi initiated within the valve pocket develop slowly and extend along the inside of the vein wall, resu-

ling in vascular occlusion. It has been proposed that, under abnormal conditions, tissue factor (TF) is expressed on both circulating leukocytes and activated endothelial cells together with the platelet inhibitors nitric oxide and prostacyclin^[10]. In addition, recent surveys demonstrate that neutrophils accelerate thrombosis by releasing serine proteases that inactivate the anticoagulant TF pathway inhibitor, suggesting that interfering with the binding of leukocytes to the activated endothelium may represent a promising therapeutic strategy against DVT. Finally, post-thrombotic syndrome describes chronic venous insufficiency following DVT and is attributed to venous hypertension, which may result from persistent thrombotic occlusion or venous valvular reflux due to a previous thrombotic condition^[11]. Additionally, inflammation may contribute to successive venous valvular damage.

CLINICAL PRESENTATION

Considering that VTE encompasses two clinical conditions, including DVT and PE, clinical findings refer to both nosologic entities. DVT typically presents with pain and lower limb oedema, the latter being the most specific symptom. If the thrombus is located in the iliac bifurcation, pelvic veins or the inferior vena cava, bilateral rather than unilateral oedema is usually apparent. Moreover, high partial obstruction often causes moderate oedema imitating that of heart, liver or renal insufficiency^[12]. Pain with tenderness occurs in the majority of affected patients. Relevant clinical signs are considered nonspecific and remain independent of the size, location and extent of the thrombus. Warmth of the related limb, locoregional erythema, or discoloration and dilation of superficial veins may also be apparent. Homan's sign (calf pain on dorsiflexion of the foot) also presents in 50% of patients with DVT^[13]. Furthermore, DVT should be differentially diagnosed from various other diseases including cellulitis, Baker's cyst, musculoskeletal injury, neoplasm, lymphedema, hematoma, sarcoma, venous or arterial aneurysms, and connective tissue disorders^[14]. Finally, a very uncommon but hazardous form of DVT is Phlegmasia Cerulea Dolens, which is the consequence of extensive thrombotic occlusion of the major and collateral veins of a lower extremity, including the iliac and femoral veins. It is characterized by acute onset of pain, oedema, blue discoloration and swelling of the affected limb, which, if left untreated, will result in foot gangrene^[15].

As far as PE is concerned, aetiology refers to air, septic and amniotic fluid emboli. Relevant clinical findings may vary from deadly hemodynamic collapse to progressive dyspnoea, and most patients with PE present with obscure symptoms. Taking into consideration the aforementioned clinical evidence, common signs of PE include sudden dyspnoea (73%) that worsens with exertion, pleuritic chest pain (66%) deteriorating with inhalation, or exertion and a productive cough (37%) that may lead to haemoptysis (13%)^[16]. Similar findings

upon physical examination include tachypnoea, rales, tachycardia, fever, cardiac galloping, lower limb oedema and cyanosis.

DIAGNOSTIC MODALITIES

Several imaging studies have been proposed for DVT diagnosis. Duplex Ultrasonography (B-mode and Doppler) remains the current first line examination performed, due to non-invasiveness and absence of irradiation or contrast material^[17]. B-mode is based on the principle that normal venous structures easily collapse with the pressure applied by the transducer, while veins harboring thrombi will not compress and will therefore be visible. The Doppler color-flow imaging technique can reveal the potential adequacy of blood flow in an area where an isoechoic clot might not be depicted. Sensitivity and specificity are as high as 95% in symptomatic patients, but diminish with obesity, small and peripheral thrombi, as well as asymptomatic disease^[18].

Venography with pedal vein cannulation, injection of contrast material, and serial limb radiographs remains the diagnostic modality of choice for DVT verification, with sensitivity and specificity reaching 100%. However, this technique is invasive and may induce serious consequences, such as hypersensitivity reactions, superficial phlebitis and renal toxicity. Another modality applied is Impedance Plethysmography, which is sensitive and specific in proximal vein thrombosis. It measures the electrical resistance of the calf, which reflects changes in blood volume^[19]. Spiral multidetector-row CT venography from the popliteal fossa provides adequate diagnostic accuracy in association with sonographic assessment. Finally, magnetic resonance imaging remains the modality of choice for suspected iliac vein or inferior vena cava thrombosis, especially when CT venography is contraindicated or technically difficult^[20]. Radiolabelled peptides that tend to connect to various thrombus components have also been studied. Apcitide, a technetium-labelled platelet glycoprotein IIb/IIIa receptor antagonist, is proposed for diagnostic investigations of DVT. Diagnostic modalities related to DVT detection are summarized in Table 1.

With regard to PE, common electrocardiographic abnormalities, including tachycardia, nonspecific ST-T disorders, right heart strain, atrial fibrillation and S₁ Q₃ T₃ pattern, are encountered in the minority of affected patients^[21]. CT pulmonary angiography (CTPA) is considered as the initial imaging modality of choice for stable patients^[22]. CTPA reveals emboli as an intraluminal filling defect after injection of contrast material, is non-invasive and widely available, and provides invaluable information for differential diagnosis^[23]. Sensitivity and specificity are disproportionate to the size of the affected pulmonary artery. Nevertheless, PA is the criterion standard for diagnosing PE. With the use of contrast material, a filling defect or a sharp cut-off of the problematic artery is detected in anterior, posterior and lateral studies^[24]. Essential to verifying PE, Ventilation/

Table 1 Diagnostic modalities applied for deep vein thrombosis detection

Deep vein thrombosis	U/S (B-mode)	U/S (Doppler)	Venography	Impedance plethysmography	CTV	MRI	Radiolabeled peptides
Mechanism of action	Veins with thrombi do not compress	Absent or abnormal blood flow when a thrombus is present	Pedal vein cannulation and injection of contrast material	Measures electrical resistance of the calf reflecting blood volume change	Spiral multidetector CT venography from popliteal fossa to the pelvis	-	Radiolabeled peptides that bind to various components of a thrombus
Sensitivity and specificity	95%	95%	100%	Sensitive and specific in proximal vein thrombosis	-	-	-
Advantages	Non-invasiveness Absence of radiation or contrast material	Non-invasiveness Absence of radiation or contrast material	High sensitivity and specificity	-	-	Ileac vein or inferior vena cava thrombosis, when CT venography is contraindicated or technically inadequate	Apcitide, a technetium-labeled platelet glycoprotein IIb/IIIa receptor antagonist
Disadvantages	Obesity, small peripheral thrombi, asymptomatic disease	Obesity, small peripheral thrombi, asymptomatic disease	Invasiveness Hypersensitivity reactions Renal toxicity	-	Correlation with sonographic findings	-	Expensive

CTV: Computed tomography venography; MRI: Magnetic resonance imaging.

Perfusion Scanning may be used when CTPA or Pulmonary Angiography are contraindicated^[25]. Finally, PE demonstrates increased signal intensity within the pulmonary artery during magnetic resonance angiography with intravenous administration of gadolinium. Sensitivity and specificity are high for central, lobar, and segmental emboli, while sub-segmental emboli render magnetic resonance angiography inadequate^[26] (Table 2).

THERAPEUTIC APPROACH

VTE remains the second most common cause of death in cancer patients and constitutes an independent prognostic factor for mortality^[27]. Moreover, recurrent VTE and major bleeding complications are higher in cancer patients, even if they receive anticoagulation therapy. Patients with upper GI malignancies, such as hepatobiliary and gastroesophageal cancer, are in great danger of VTE, with pancreatic cancer presenting the highest VTE prevalence. In advanced pancreatic cancer patients, relevant risk is as high as 25%, and asymptomatic VTE incidence is up to 60%. GI cancers are frequently treated with antiangiogenic or chemotherapeutic agents, such as cisplatin and irinotecan, which are associated with increased risk for VTE as well as combined neoadjuvant chemoradiotherapy^[28]. It is estimated that chemotherapy provokes an inflammatory response due to endothelial disruption. In particular, IL-1 and TNF- α , among other cytokines, diminish the concentration of anticoagulant proteins, such as antithrombin III and protein C. The procoagulant reaction is reinforced by increased TF expression, and is maintained for up to 6 mo after induction of chemoradiation, thus implying an in-

creased long-term risk for VTE^[29]. Major abdominal cancer surgery is also a risk factor for VTE, even after hospital discharge and discontinuation of the usual perioperative prophylaxis^[30]. As for laparoscopic surgery, there is still no consensus as to whether the laparoscopic or open techniques abate morbidity related to VTE. Some investigators state that the risk is lessened due to overall reduction in postoperative morbidity, while others claim that the impact of pneumoperitoneum increases the risk, due to compression of the inferior vena cava and iliac veins. However, existing trials are not adequate to reliably evaluate these findings^[31].

Pharmacologic thromboprophylaxis is strongly recommended in patients with GI cancer undergoing major surgery, as risk reduction up to 80% has been proven for VTE. Current guidelines suggest LMWH as the standard of care. ASCO recommendations propose unfractionated heparin, fondaparinux or LMWH as a first-line treatment, unless contraindicated due to high bleeding risk or active bleeding. Prophylactic dosages at levels of 3000-5000 anti-Fxa units per day have proven more effective than and as safe as lower doses. Treatment should begin 12-24 h pre- or 6-24 h postoperatively and last 7-10 d. The combination of pharmacologic and mechanical prophylaxis, such as compression stockings and intermittent pneumatic compression devices, may be more efficient, especially in the high-risk group of patients. Extended thromboprophylaxis up to 28 d should be taken into serious consideration only in high-risk patients who fulfill the following criteria, including cancer-related stage III/IV, upper GI cancer, histological features of adenocarcinoma, thrombocytosis, leucocytosis, elevated D-dimer and CRP. Patient-related factors refer

Table 2 Imaging modalities for pulmonary embolism verification

PE	ECG	CTPA	V/Q Scan	MRA
Findings	Sinus tachycardia Non-specific ST-T disorders S1Q3T3 pattern Atrial fibrillation Right heart strain	Intraluminal filling defect of pulmonary artery after injection of contrast material	Ventilated area not perfused	Increased signal intensity of pulmonary thrombi within pulmonary artery after injection of gadolinium
Advantages	Immediate Costless	Criterion standard for diagnosis	Radiation dose lower than CTPA	High sensitivity and specificity for central, lobar, and segmental emboli
Disadvantages	Low sensitivity and specificity	Invasiveness Hypersensitivity reactions Renal toxicity	-	Inadequate for subsegmental emboli

CTPA: CT pulmonary angiography; V/Q: Ventilation/perfusion; MRA: Magnetic resonance angiography.

to ages older than 60 years, obesity, previous history of VTE, surgery lasting 2 h or longer, prolonged postoperative immobilization, and presence of infection or fever. Treatment-related determinants include chemotherapy, central-line or port catheter, parenteral nutrition and radiation therapy. On the other hand, European Society of Molecular Oncology (ESMO) guidelines do not suggest fondaparinux as the first line of treatment and recommend extended prophylaxis up to 28 d for all cancer patients undergoing abdominal or pelvic surgery. Given these differentiations, the newest ESMO and ASCO guidelines consort with each other. American Society of Hematology and Australian Government National Health and Medical Research Council recommendations coincide with ASCO guidelines, while Mayo Clinic VTE Prevention and Management and German guidelines go along with ESMO proposals.

As far as long-term prevention of VTE is concerned, ASCO guidelines suggest the use of LMWH as the standard of care. If this is unavailable, vitamin K antagonists (VKA) are used. Novel Oral Anticoagulants (NOACs) are currently not suggested for patients with GI cancer and VTE due to the limited data available in patients with malignancy. Treatment should last for 6 mo. ESCO and American Society of Haematology guidelines recommend the use of LMWH for 6 mo. ESCO specifically proposes an initial dose of LMWH 100% for 1 mo and 75%-80% of the initial dose for 5 mo thereafter. Additionally, the Mayo Clinic suggests that anticoagulants should be continued until there is no evidence of active malignancy, either as evidence of imaging or cancer-related treatment, while German guidelines propose LMWH for 3-6 mo and highlight that prophylaxis could last for a lifetime in persistent cancers^[32].

Non-vitamin-K NOACs have been introduced in the treatment of VTE associated with GI cancer. As the aforementioned guidelines state, the use of NOACs are currently not recommended due to limited data in cancer patients. On the other hand, available anticoagulants exhibit certain disadvantages. Unfractionated heparin requires platelet monitoring and daily injections, which are highly inconvenient. Also, it is associated with heparin-induced thrombocytopenia, types I and II, bleeding

and osteoporosis. LMWH is contraindicated in renal impairment, adding to its drawbacks. VKA, such as warfarin, require INR monitoring and have multiple drug and food interactions. A narrow therapeutic window, delayed onset of action and bleeding risk render VKA inadequate and inferior to LMWH^[28].

The family of NOACs includes dabigatran etexilate, rivaroxaban, apixaban and edoxaban, each one with their own special pharmacokinetics and pharmacodynamics^[33]. Dabigatran etexilate is a direct thrombin (factor IIa) inhibitor. It is administered orally and presents a half-life of 12-14 h and a rapid onset of action. Its bioavailability does not exceed 10% (3-7%), and its absorption is facilitated by acids. Its excretion is primarily in urine (80%), so caution is required for patients with renal impairment, as its half-life can be increased up to 34 h. More specifically, it is contraindicated when creatinine clearance (CrCl) is under 30 mL/min. Its clearance is also dependent on the P-glycoprotein transport pathway. In addition, routine monitoring is not required because of predictable pharmacokinetics. In the RECOVER, RE-SONATE and RE-MEDY phase III clinical trials, dabigatran showed non-inferiority to warfarin and superiority to placebo. It is FDA approved for VTE prophylaxis after hip and knee arthroplasty, stroke prevention in patients with non-valvular atrial fibrillation, and VTE treatment. As for adverse effects, dyspepsia is the only one that occurs more frequently with dabigatran than with warfarin^[28,33].

Rivaroxaban is a direct inhibitor of factor Xa. It is orally administered and has a half-life of 7-11 h, with a rapid onset of action as well^[34]. Its bioavailability is excellent (80%-100%). Significant food interactions have not been reported. It is a substrate of the cytochrome P450 system, especially CYP3A4 and P-glycoprotein, and is excreted by both the renal and hepatic systems, demanding extreme caution in patients with renal or hepatic insufficiency^[33]. More specifically, it is contraindicated when CrCl is under 30 mL/min in haemodialysis and in patients with Child-Pugh B or C cirrhosis. Additionally, monitoring is not required. In the EINSTEIN-DVT and EINSTEIN-Extension phase III clinical trials, rivaroxaban presented non-inferiority to VKA/LMWH

Table 3 Comparative evaluation of mechanism of action and contra-indications of novel oral anticoagulants

Novel oral anticoagulants	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Mechanism of action	Direct thrombin (factor IIa) inhibitor	Direct factor Xa inhibitor	Direct factor Xa inhibitor	Direct factor Xa inhibitor
Route of administration	Per os	Per os	Per os	Per os
Half-life	12-14 h	7-11 h	12 h	8-10 h
Bioavailability	3%-7%	80%-100%	50%	62%
Metabolism	P-glycoprotein	P-glycoprotein Cytochrome P450 system (CYP3A4)	P-glycoprotein Cytochrome P450 system (CYP3A4)	P-glycoprotein Cytochrome P450 system (CYP3A4)
Excretion	Urine (80%)	Urine and HBR	Urine (25%)	Primarily: HBR Secondarily: Urine
Contraindication	CrCl < 30 mL/min	CrCl < 30 mL/min Hemodialysis Child Pugh B and C stage cirrhosis	CrCl < 15 mL/min	
FDA approval	VTE prophylaxis after hip and knee arthroplasty, Non valvular atrial fibrillation VTE treatment	VTE prophylaxis after hip and knee arthroplasty, Non valvular atrial fibrillation VTE treatment	Non valvular atrial fibrillation VTE treatment and prevention after major orthopedic surgery	Non valvular atrial fibrillation VTE treatment and prevention after major orthopedic surgery
Clinical trials	Non-inferiority to warfarin Superiority to placebo	Non-inferiority to VKA/ LMWH Superiority to placebo	-	
Dosage	100-150 mg × 2/24 h	10-30 mg × 1/24 h	2.5-5 mg × 2/24 h	15-30 mg × 1/24 h

HBR: Hepatobiliary route; VTE: Venous thromboembolism; VKA: Vitamin K antagonists; LMWH: Low molecular weight heparin.

and superiority to placebo, respectively. It is also FDA approved with the same indications as dabigatran^[28].

Apixaban appears to have the same mode of action and route of administration as rivaroxaban. It has a half-life of 12 h, and a bioavailability of about 50%. It is metabolized by P-glycoprotein, the cytochrome 450 system, and the CYP3A4 pathway. Its excretion is in urine (25%), and the drug is contraindicated when CrCl is under 15 mL/min. Edoxaban inhibits factor Xa and is orally administered. It has a half-life of 8-10 h and good bioavailability (62%). It is excreted primarily by the hepatobiliary route and secondarily in the urine. Also, it is metabolized by both P-glycoprotein and the CYP3A4 pathway. As mentioned for the other NOACs, monitoring is not required. Both apixaban and edoxaban are FDA approved for non-valvular atrial fibrillation, VTE treatment and prevention after major orthopaedic surgery^[28,33].

As far as dosing frequency is concerned, dabigatran and apixaban require 110-150 mg and 2.5-5 mg twice a day, respectively, while rivaroxaban and edoxaban necessitate 10-30 mg and 15-30 mg once daily, respectively^[33]. Apixaban remains the safest of the NOACs, showing reduced risk of major or clinically-relevant minor bleeding at a statistically significant level, with dabigatran taking the second place. Additionally, apixaban and rivaroxaban pose a significantly lower risk for major bleeding compared with LMWH or VKA, a fact which may be of particular clinical importance^[35]. In conclusion, differences between doses of dabigatran and apixaban, as well as the correlations between the safety and timing differences between dabigatran or apixaban and

rivaroxaban or edoxaban, are summarized in Table 3.

CONCLUSION

VTE refers to both DVT and PE and is highly associated with malignancy, with HPB and gastric cancer ranking first^[36]. CTPA is the initial diagnostic modality, while ultrasonography is preferred for DVT^[37]. LMWH is used pre- or 6-24 h postoperatively and should continue for 7-10 d. Extension up to 28 d is highly recommended for major abdominal or pelvic surgical procedures^[38-42]. NOACs are promised to revolutionize current treatment and bring together efficacy and many benefits for patients. However, the use of NOACs for VTE prophylaxis is certainly debatable. Potential drug interactions with chemotherapeutic components, GI abnormalities, and hepatic and renal insufficiency remain significant determinants of NOAC administration^[43-45]. Therefore, bioavailability may not reach desirable levels^[46]. The lack of rapid reversal agents also prevents the use of these agents for invasive procedures and thrombocytopenia. Furthermore, cancer patients are at a greater risk of bleeding than non-cancer patients due to chemotherapy-induced thrombocytopenia and antiangiogenic therapy. Moreover, a reduction in circulating proteins and albumins could influence the binding levels of NOACs. Thus, a comparative study of NOACs with the current curative approach, LMWH, may clarify this dispute.

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Role of pre-transplant ¹⁸F-FDG PET/CT in predicting hepatocellular carcinoma recurrence after liver transplantation

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Abstract

The last two decades have seen a paradigm shift in the selection of patients with hepatocellular carcinoma (HCC) for liver transplantation. Microvascular invasion and differentiation have been the most significant factors affecting post-transplant recurrence; however, because of inherent disadvantages of pre-transplant biopsy, histological criteria never gained popularity. Recently, the selection criteria evolved from morphological to biological criteria, such as biomarkers and response to loco-regional therapy. With the introduction of multi-modality imaging, combination of computed tomography with nuclear medicine imaging, particularly, ¹⁸F-fluorodeoxyglucose positron emission tomography fulfilled an unmet need and rapidly became a critical component of HCC management. This review article will focus on the use of ¹⁸F-fluorodeoxyglucose positron emission tomography combined with computed tomography in the pre-transplant evaluation of HCC patients with special discussion on its ability to predict HCC recurrence after liver transplantation.

Key words: ¹⁸F-fluorodeoxyglucose positron emission tomography; Hepatocellular carcinoma; Recurrence; Liver transplantation

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Core tip: The last two decades have seen a paradigm shift in the selection of patients with hepatocellular carcinoma (HCC) for liver transplantation. With the introduction of multimodality imaging, combination of computed tomography with nuclear medicine imaging

fulfilled an unmet need and rapidly became a critical component of HCC management. This review article will focus on the use of 18F-fluorodeoxyglucose positron emission tomography in the pre-transplant evaluation of HCC patients with special discussion on its ability to predict HCC recurrence after liver transplantation.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Currently, HCC is the sixth most common cancer with more than a half million new cases diagnosed annually, and it is the second leading cause of cancer-related mortality in the world^[1]. The global risk of HCC has been largely associated with hepatitis B and C virus infection. In addition, improved survival from cirrhosis and increasing rates of obesity and non-alcoholic fatty liver disease are expected to contribute to the ever-increasing incidence of HCC^[2,3]. Because of the strong link between cirrhosis and HCC, liver transplantation (LT) is the best treatment option, since it removes the tumor and the underlying tumor-generating cirrhosis. Recently, HCC has been reported as the most common indication for LT in the United States^[4].

Until the landmark study by Mazzaferro *et al*^[5] in 1996, the liberal selection of HCC patients for LT resulted in high recurrence rates and poor survival. With the introduction of Milan criteria (MC), excellent long-term outcomes have been achieved that were not different from those of patients without HCC. The MC have been validated in several studies and widely accepted as the benchmark for selection of patients with HCC for deceased donor LT (DDLT). Subsequent studies searching for more liberal morphological criteria have shown that it was possible to extend the size and number of tumors without compromising post-transplant outcome^[6-11] (Table 1). Despite being continually expanded, aforementioned morphological criteria have been criticized for a variety of reasons: they were restrictive and precluded numerous patients who otherwise would have benefited from LT with a low risk of HCC recurrence; they relied solely on tumor burden (defined as the size and number of tumors at a certain point) and excluded the factors related to tumor behavior (*i.e.*, tumor differentiation, molecular markers, and response to bridging therapy); they depended on imaging parameters that were inconsistent: in patients within MC, up to 40% had explant pathology that exceeded the MC, and in those beyond MC, up to

34% had explant pathology that was within the MC^[12,13]. An earlier study investigating the correlation between pathologic and radiologic staging according to the morphological criteria have found that the accuracy of imaging classification for both Milan and (University of California San Francisco (UCSF) criteria was only 60%^[14].

In patients with HCC, vascular invasion has been defined as one of the major determinants of the outcome after LT^[15]. Further studies have shown that tumor differentiation has also been an independent predictor of recurrence and survival after the transplant^[16,17]. Despite initial hesitancy against the use of pre-transplant tumor biopsy, Toronto criteria have led the way to the use of histological criteria in selection of patients with HCC for LT^[12]. However, pre-transplant tumor biopsy has not gained popularity because of its limitations: In spite of the invasive biopsy procedures, the presence of vascular invasion and tumor differentiation may not be detected reliably; the sensitivity of biopsy varies depending on location of the tumor, needle size, and tumor size. Moreover, preoperative needle biopsy may increase tumor seeding and post-transplant recurrence^[18]. Nevertheless, this was the beginning of a new era when there was a shift in selection criteria from morphological to the combination of biological and histomorphological criteria^[19].

Meanwhile, major transplant centers in Asia started to expand aggressively the morphological criteria with the addition of biomarkers to the patient selection process. While in the West, alpha-fetoprotein (AFP) has been traditionally used as a reference biomarker to screen and support the diagnosis of HCC; in the East, des-gamma-carboxy prothrombin (DCP) was introduced as a significant marker for assessing the biological behavior of HCC, particularly in Japan. Shirabe *et al*^[20] reported that selection of HCC patients for LT might improve with the use of DCP measurement because pre-transplant DCP level has been shown to be a significant predictor of microvascular invasion (MVI).

The utilization of a combination of biological and morphological data has been a perfect fit for living donor LT (LDLT), which was not restricted by deceased donor organ allocation system. The Kyoto group reported their selection criteria to include no more than 10 tumors, all less than 5 cm in diameter with DCP levels less than 400 ng/mL^[21], while the Kyushu group suggested more extended criteria to include a tumor size of less than 5 cm and DCP levels less than 300 ng/mL with no limitation on the number of tumors^[22]. Both centers achieved outstanding post-transplant outcomes. The criteria that incorporated biomarkers with expanded morphological criteria are shown in Table 2^[21-24].

As the selection criteria have been continuously expanded, search for new criteria to predict the biological behavior of HCC also continued. To this end, response to loco-regional therapy (LRT) has been suggested as a surrogate marker of tumor biology^[19]. Bridging therapies primarily focused on reducing the tumor burden and has

Table 1 Morphological criteria used in selection of patients with hepatocellular carcinoma for liver transplantation

Ref.	Year	Size and number
Milan ^[5]	1996	1 lesion \leq 5 cm, or 2 to 3 lesions each \leq 3
University of California San Francisco ^[6]	2001	1 lesion \leq 6.5 cm, 2-3 lesions each \leq 4.5 cm with total tumor diameter \leq 8 cm
Tokyo University ^[8]	2008	Up to 5 tumors, each $<$ 5 cm
Asan Medical Center ^[9]	2008	The largest tumor diameter $<$ 5 cm, tumor number \leq 6
Alberta ^[10]	2008	Total tumor volume $<$ 115 cm
Valencia ^[11]	2008	Up to 3 tumors, each $<$ 5 cm, and a cumulative tumor burden \leq 10 cm
Up-to-seven ^[7]	2009	7 as the sum of the size of the largest tumor and total number of tumors

Table 2 The use of biomarkers with expanded morphological criteria

Ref.	Year	No. of patients	Criteria	Overall survival	
				Within criteria	Beyond criteria
Kyoto ^[21]	2007	136	Up to 10 tumors, all \leq 5 cm; DCP \leq 400 ng/mL	87% (5-yr)	37% (5-yr)
Kyushu ^[22]	2007	40	Any number, tumor diameter \leq 5 cm; DCP $<$ 300 ng/mL	77% (3-yr)	40% (3-yr)
Seoul ^[23]	2007	140	Any number, tumor diameter \leq 5 cm; AFP \leq 400 ng/mL	87% (5-yr)	23% (5-yr)
Hangzhou ^[24]	2008	195	Total tumor diameter \leq 8 cm; or total tumor diameter $>$ 8 cm and grade I / II and AFP \leq 400 ng/mL	71% (5-yr)	19% (5-yr)

Table 3 The criteria used for prediction of biological behavior of hepatocellular carcinoma in the pre-transplant setting

Biomarkers (AFP, DCP) ^[21-24]
The neutrophil-lymphocyte ratio ^[27]
Pre-transplant liver biopsy ^[12]
Response to loco-regional therapy ^[19]
Test of time (3-mo waiting period) ^[19,26]
Dynamic evaluation (tumor doubling time and change in AFP) ^[19]
FDG-PET scan

AFP: Alpha-fetoprotein; DCP: Des-gamma-carboxy prothrombin; FDG-PET: Fluorodeoxyglucose positron emission tomography.

been recommended to downstage the HCC patients who exceeded the morphological selection criteria to within the MC to become eligible for DDLT^[25]. In addition, long waiting times for DDLT and high dropout rates have led to an active approach to the treatment of HCC with LRT to prevent progression while awaiting LT. The LRTs have also been used in LDLT to exclude patients with unfavorable tumor behavior, such as the patients who are unresponsive to treatment or those with progression upon observation. The interval between therapy and LT was found to help in identifying the patients who have HCC with poor tumor biology with an increased risk of post-transplant recurrence^[26].

Despite the ability of cross-sectional imaging studies to reliably diagnose HCC, neither computed tomography (CT), nor magnetic resonance imaging (MRI) have been instrumental as a marker of tumor biology^[27] (Table 3). With the introduction of multimodality imaging, combination of CT with nuclear medicine imaging, particularly 18F-fluorodeoxyglucose positron emission tomography (18F-FDG-PET/CT), fulfilled an unmet need and rapidly became a critical component of HCC management^[28]. This review article will focus on the use of 18F-FDG PET/

CT in the setting of LT for HCC with special discussion on its ability to predict HCC recurrence after LT.

18F-FDG PET/CT IMAGING IN HCC

The successful application of 18F-FDG to a growing number of oncological indications has led to the widespread use of 18F-FDG-PET/CT in the diagnosis, staging and follow-up of patients with distinct types of cancer. Oncological imaging using 18F-FDG is based on the principle of enhanced glucose metabolism in tumors as compared with normal tissues. However, in normal hepatic parenchyma, where the concentration of glucose-6-phosphatase is high, the rapid clearance of 18F-FDG leads to a reduced discrimination between normal tissue and well-differentiated HCC. Because of the fact that low-grade HCC exhibits a lower FDG avidity, the general reported false-negative rate of 18F-FDG-PET/CT approaches 50% in the imaging of HCC^[29]. The 18F-FDG uptake in HCC ranges from 38% to 70% with an overall sensitivity of only about 60%^[29-32].

In the liver, PET/CT positivity is determined by examining whether the FDG uptake in tumor is significantly higher than that in the surrounding liver parenchyma. Standardized uptake values (SUV) of the lesions are calculated by plotting a circular region of interest (ROI) at the area of the maximum FDG uptake in the PET images. Numerous studies have defined PET/CT positivity vs PET/CT negativity by using the maximum SUV (SUVmax) within ROI. In a retrospective study of 280 patients undergoing LDLT for HCC, Lee *et al.*^[33] defined the SUVmax values for PET/CT positivity and negativity as 4.46 and 3.08, respectively ($P < 0.001$). However, SUV measurements are prone to be influenced by a variety of factors, including high glucose metabolism in the normal liver tissue, as well as the factors related with scanner and reconstruction parameters. Therefore,

Table 4 The standardized uptake values used to define clinically significant 18F-fluorodeoxyglucose positron emission tomography/computed tomography positivity for hepatocellular carcinoma

Ref.	Year	No. of patients	Study model	SUV values		
				SUVmax	TSUVmax-to-LSUVmax	TSUVmax-to-LSUVmean
Lee <i>et al</i> ^[34]	2009	59	LT	3	1.15	1.35
Song <i>et al</i> ^[35]	2012	83	LRT	4	1.45	1.9
Lee <i>et al</i> ^[36]	2015	280	LDLT	4.4		
Hsu <i>et al</i> ^[37]	2016	147	LDLT	4.8		2
Hong <i>et al</i> ^[38]	2016	123	LDLT		1.1	
Boussouar <i>et al</i> ^[39]	2016	28	LT		1.15	
Bailly <i>et al</i> ^[40]	2016	34	LT		1.15	
Lin <i>et al</i> ^[41]	2017	65	LT	3.8	1.49	1.69

SUV: Standardized uptake values; TSUVmax: Tumor SUVmax; LSUVmax: Normal-liver SUVmax.

many researchers suggested using either tumor SUVmax to normal-liver SUVmax (TSUVmax/LSUVmax) or tumor SUVmax to normal-liver SUVmean (TSUVmax/LSUVmean) values instead of SUVmax to identify PET/CT positivity^[34-41] (Table 4).

While 18F-FDG-PET/CT has demonstrated standard sensitivity in discovering new HCC, it has been useful in detecting extra-hepatic metastases, with detection rates reported as high as 100%^[42,43]. 18F-FDG-PET/CT has also been reported to detect post-treatment recurrences earlier and at higher rates than conventional imaging modalities^[44]. The sensitivity of 18F-FDG-PET/CT is size-dependent in both extra-hepatic metastases and recurrences. Sugiyama *et al*^[42] reported a detection rate of 83% for extra-hepatic metastases > 1 cm, which was only 13% for lesions ≤ 1 cm in diameter. In patients with post-transplant HCC recurrence, Kim *et al*^[45] reported that a detection rate of > 90% has been achieved for extra-hepatic metastases when the lesions were larger than 1 cm in diameter. However, 18F-FDG-PET/CT was not able to detect any of the extra-hepatic lesions under 1 cm and demonstrated a low detection rate of less than 10% for intrahepatic recurrences. They reported a detection rate of 100% in bone, 60% in the lungs, and 100% in lymph nodes. 18F-FDG-PET/CT has also been used in the evaluation of patients with unexplained AFP elevation after surgical or interventional treatment^[46]. In HCC patients presenting with portal vein thrombosis, 18F-FDG-PET/CT was found more valuable than conventional imaging studies in differential diagnosis of tumor thrombus^[47,48].

Considering the limited role of 18F-FDG-PET/CT in the detection of HCC because of its low overall sensitivity, Ho *et al*^[49] advocated for the use of 11C-acetate, which showed better detection sensitivity of 87.3% compared to 47.3% using 18F-FDG. In another study from Hong Kong, which evaluated the accuracy of dual-tracer PET/CT in HCC patients who underwent either partial hepatectomy or LT, the sensitivity of 11C-acetate PET/CT was significantly higher than those of 18F-FDG-PET/CT and contrast-enhanced CT for the detection of small HCCs (87.0% vs 17.4% and 43.5%, respectively)^[50]. Recent studies have concluded that in patients undergoing LT

for HCC, although 11C-choline PET had a better detection rate for well-differentiated lesions and the addition of 11C-acetate to 18F-FDG-PET/CT significantly increased the overall sensitivity and specificity for the detection of HCC, the complementary role of 18F-FDG should not be underestimated as a marker of poorly differentiated tumor pathology^[51-53].

CORRELATION BETWEEN 18F-FDG PET/CT AND HISTOLOGICAL FINDINGS

In HCC, the growth rate and the activity of glycolytic enzymes are related^[54]. Therefore, contrary to well differentiated HCC, poorly differentiated HCC cells have low glucose-6 phosphatase activity and high uptake of 18F-FDG^[30]. Recent studies have suggested that maximum standardized uptake values in 18F-FDG PET/CT imaging demonstrated strong correlation with histopathological characteristics of HCC, such as MVI and tumor grade^[28,55-57]. The reported accuracy rate of 18F-FDG-PET/CT for detection of MVI invasion and tumor differentiation in HCC ranged between 68.3% to 88.1% and 57.4% to 71.4%, respectively^[55].

Considering the risk of tumor seeding and limitations related to multifocality and microscopic heterogeneity within tumor, 18F-FDG-PET/CT is a more valuable tool in the prediction of tumor biology. The maximum standardized uptake value (SUVmax) and ratio of tumor-to-normal liver SUVmax value (SUVmax T/L) have been recognized as objective indices for the definition of 18F-FDG-PET/CT positivity. In a recent study on 65 HCC patients who underwent 18F-FDG-PET/CT before LT, Lin *et al*^[41] have found that the SUVmax T/L ratio was an independent predictor of vascular invasion. The optimal cutoff values for SUVmax of the tumor and SUVmax T/L ratio for the prediction of HCC vascular invasion were 3.80 and 1.49, respectively. In another study that reviewed 18F-FDG-PET/CT findings of 34 patients with HCC who underwent LT, Bailly *et al*^[40] reported that none of the patients with SUVmax L/T ratio > 1.15 had well differentiated HCC.

A study from Seoul National University investigated the association of the gadoteric acid-enhanced MR and

Table 5 The use of 18F-fluorodeoxyglucose positron emission tomography/computed tomography in predicting post-transplant hepatocellular carcinoma recurrences

Ref.	Year	Follow-up (mo)	Recurrence		Disease-free survival	Risk of recurrence (95%CI)
			PET/CT (+)	PET/CT (-)		
Yang <i>et al</i> ^[28]	2006	19	13/8	25/3	2-yr, 46.1% vs 85.1%	OR = 7.6 (1.9-28.9)
Kornberg <i>et al</i> ^[56]	2009	11.5	19/9	36/1	3-yr, 46.9% vs 93.3%	OR = 23.9 (2.1-268.5)
Lee <i>et al</i> ^[34]	2013	26.1	55/22	136/16	3-yr, 57.1% vs 86.8%	HR = 3.9 (1.1-13.0)
Hsu <i>et al</i> ^[37]	2016	25.8	30/9	117/9	5-yr, 68.3 vs 84.8%	HR = 13.5 (4.7-38.2)
Kornberg <i>et al</i> ^[57]	2017	74	41/24	75/5	5-yr, 38.1% vs 93.3%	HR = 22.8 (6.3-83.0)
Ye <i>et al</i> ^[63]	2017	25.7	78/46	25/7	5-yr, 21.9% vs 76%	HR = 3.6 (1.3-9.6)

PET/CT: Positron emission tomography/computed tomography.

the 18F-FDG-PET/CT findings with the MVI in patients who underwent LT for HCC^[58]. Multivariate analysis revealed that peritumoral enhancement and the ratio of tumor maximum standardized uptake value (SUV) to normal liver mean SUV (TSUVmax/LSUVmean) ≥ 1.2 had a statistically significant association with MVI, with an odds ratio of 10.6 and 14.2, respectively. With regard to predicting MVI, the sensitivity and specificity was 35.7% and 93.3% for MRI and 64.3% and 86.7% for PET/CT, respectively. For the prediction of MVI, a sensitivity of 78.6% and a specificity of 80% were achieved when both imaging modalities were combined.

CORRELATION BETWEEN 18F-FDG PET/CT AND MORPHOLOGICAL CRITERIA

As the selection criteria for LT shifted towards biological criteria, MC as the current gold standard and other morphological criteria have been challenged with a number of studies using 18F-FDG PET/CT. Kornberg *et al*^[59] was the first to investigate the prognostic value of preoperative 18F-FDG PET/CT in liver transplant candidates with HCC. They concluded that PET/CT negative patients with HCC beyond MC might achieve excellent post-transplant disease-free survival (DFS). In a more recent study, they combined the pre-transplant 18F-FDG-PET/CT assessments with Up-to-seven criteria^[60]. Among 116 patients with HCC who underwent 18F-FDG-PET/CT prior to LT, 5-year DFS was comparable between patients within Up-to-seven criteria ($n = 85$) and those beyond Up-to-seven criteria with negative PET/CT ($n = 16$) (81.0% vs 87.1%, $P = 0.5$).

A Japanese multicenter study including 182 LDLT recipients from 16 Japanese LT centers investigated the significance of pre-transplant 18F-FDG-PET/CT at a much larger scale. While patients beyond MC had a significantly higher recurrence rate at 5 years compared with those within MC (38% vs 7%, $P < 0.001$), a subgroup of "beyond MC" patients with negative PET/CT and low AFP (< 115 ng/mL) showed similar recurrence rate with

"within MC" patients (19%, $P = 0.1$)^[61]. Similar data were recently published by the Taiwan group who combined pre-transplant PET/CT results with UCSF criteria for predicting the risk of post-transplant HCC recurrence. In a group of 147 patients with HCC who underwent 18F-FDG-PET/CT and proceeded to LDLT, patients within UCSF criteria and those beyond UCSF criteria with a low FDG uptake had similar post-transplant recurrence rates (3.6% vs 11.1%)^[37].

Another study from Korea investigated the clinical impact of 18F-FDG-PET/CT in patients undergoing LDLT for advanced HCC, where more than half of the patients were beyond MC. In patients beyond either MC ($n = 147$) or UCSF ($n = 136$) criteria, PET/CT negative patients had 5-year DFS rates of 73.3% and 72.8%, respectively. Despite the fact that these figures were significantly lower than those of patients within MC (89.8%), the outcome is highly acceptable when the discussion shifts from "zero recurrence" towards targeting 50% 5-year survival as an acceptable goal in advanced HCC^[33].

ROLE OF 18F-FDG PET/CT IN PREDICTING POST-TRANSPLANT HCC RECURRENCE

Seoul National University Hospital was the first to report the effectiveness of pre-transplant 18F-FDG-PET/CT to predict post-transplant HCC recurrence^[28]. Further studies have shown that a high 18F-FDG uptake on pre-transplant PET/CT was a strong predictive factor for MVI and tumor recurrence after LT^[56,33,62] (Table 5).

In a cohort of 116 liver transplant patients with HCC, Kornberg *et al*^[60] reported a 5-year DFS rate of 93.3% in PET/CT negative patients vs 38.1% in PET/CT positive patients. PET/CT positive patients showed a recurrence rate of 58.5%, while only 6.7% of the PET/CT negative patients had recurrence. Ye *et al*^[63] also investigated the clinical value of pre-transplant PET/CT in the selection and prognostic prediction of patients with advanced

HCC in the LT setting. Patients with a positive 18F-FDG-PET/CT had significantly increased risk of post-transplant recurrence compared to PET/CT negative patients (59.0% vs 28.0%, $P = 0.007$). In patients with positive PET/CT, they reported a significantly lower 5-year DFS rate than that of patients with negative PET/CT (76.0% vs 21.9%, $P < 0.001$). In another study investigating the role of PET/CT as a prognostic factor for early HCC recurrence after LT, Lee *et al.*^[62] have shown that median SUVmax of PET/CT-positive tumors in the early, late, and no recurrence groups was 5.2, 3.7, and 3.2, respectively. They concluded that preoperative 18F-FDG-PET/CT was an independent and significant prognostic factor for early tumor recurrence after LT for HCC.

Hong *et al.*^[38] further developed the concept, hypothesizing that the combination of 18F-FDG PET/CT positivity and serum AFP level might improve the prediction of post-LT outcome for patients with HCC. Using cut-off values of 200 ng/mL for AFP and 1.1 for SUVmax T/L ratio for the definition of “high-risk” HCC, they found that the rate of MVI and poor differentiation was 33% and 92%, respectively in the high-risk group. They reported 5-year DFS rates of 49.1% vs 93.4% in PET/CT positive vs negative patients and 47.7% vs 88.3% in high AFP vs low AFP patients. In the high-risk group ($n = 12$), 5-year DFS rate was only 8.4%.

CONCLUSION

In patients with HCC, LT is the best treatment option. The selection criteria for LT have been shifting from morphological to the combination of biological and histomorphological criteria. When combined with serum markers, 18F-FDG-PET/CT represents the “new generation” of biological criteria, which has the potential to further improve the prediction of tumor behavior and to provide a better risk stratification model for HCC.

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Basic Study

miR-122-5p as a novel biomarker for alpha-fetoprotein-producing gastric cancer

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Abstract

AIM

To investigate the clinical utility of alpha-fetoprotein (AFP)-producing gastric cancer (AFPGC)-specific micro-RNA (miRNA) for monitoring and prognostic prediction of patients.

METHODS

We performed a comprehensive miRNA array-based approach to compare miRNA expression levels between AFP-positive and AFP-negative cells in three patients with primary AFPGC. We next examined the expression levels of the selected miRNAs in five AFPGC and ten non-AFPGC tissue samples by quantitative reverse transcription-polymerase chain reaction to validate their utility. We also investigated the expression levels of the selected miRNA not only in tissue but also in plasma

samples. Moreover, we investigated the relationship between plasma AFP levels and plasma selected miRNA expression levels, and also investigated the correlation of the selected miRNA expression levels and malignant potential.

RESULTS

Among the five miRNAs selected from the miRNA array results, the expression levels of *miR-122-5p* were significantly higher in the AFPGC patients than in the non-AFPGC patients ($P < 0.05$). In tissue samples, *miR-122-5p* expression level tended to be lower in the non-AFPGC tissue than the normal gastric mucosa. Conversely, in the AFPGC tissue, *miR-122-5p* expression level was significantly higher in the AFPGC tissue than both the normal gastric mucosa and the non-AFPGC tissue samples ($P < 0.05$). Plasma *miR-122-5p* expression levels were also significantly higher in the AFPGC patients than the health volunteers and the non-AFPGC patients ($P < 0.05$) and were strongly correlated with plasma AFP levels ($r = 0.7975$, $P < 0.0001$). Moreover, the correlation of *miR-122-5p* expression in tissue samples with malignant potential was stronger than that of plasma AFP level in the AFPGC patients. In contrast, no correlation was found between *miR-122-5p* expression levels and liver metastasis in the non-AFPGC patients.

CONCLUSION

miR-122-5p might be a useful biomarker for early detection and disease monitoring in AFPGC.

Key words: Gastric cancer; Alpha-fetoprotein; Alpha-fetoprotein producing gastric cancer; MicroRNA; *miR-122-5p*

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Core tip: We examined the microRNAs (miRNA) expression in alpha-fetoprotein (AFP)-producing gastric cancer (AFPGC) tissue samples using a comprehensive miRNA array-based approach, and also investigated the clinical utility of the identified AFPGC-specific miRNAs. We found the expression of *miR-122-5p* was significantly higher in the AFPGC tissues and plasma samples. Moreover, tissue *miR-122-5p* expression levels exhibited a stronger correlation with malignant potential than plasma AFP level in AFPGC patients. *miR-122-5p* might be a useful biomarker for early detection and disease monitoring in AFPGC.

Maruyama S, Furuya S, Shiraishi K, Shimizu H, Akaike H, Hosomura N, Kawaguchi Y, Amemiya H, Kawaida H, Sudo M, Inoue S, Kono H, Ichikawa D. *miR-122-5p* as a novel biomarker for alpha-fetoprotein-producing gastric cancer. *World J Gastrointest Oncol* 2018; 10(10): 344-350 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i10/344.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i10.344>

INTRODUCTION

Gastric cancer (GC) is one of the most common solid tumors and is the third leading cause of cancer-related deaths worldwide^[1,2]. Despite improvements in treatment approaches, prognosis of patients with advanced GC remains poor even after curative resection.

Among various tumor subtypes, alpha-fetoprotein (AFP)-producing GC (AFPGC) is recognized as one of the most aggressive tumors, with a high propensity for liver metastasis and subsequent poor prognosis compared with other GC subtypes^[3-7]. The incidence of AFPGC is low, ranging from 1.3% to 15% of all GCs^[8-12]. Therefore, recent comprehensive molecular analyses have not yet referred to this minor subtype.

MicroRNAs (miRNAs) are endogenous, small, non-coding, single-stranded RNAs of 20-25 nucleotides that regulate the expression of target genes at post-transcriptional level by binding to complementary sequences^[13]. Various miRNAs were shown to play crucial roles in cancer as well as normal cells were reported to act as tumor suppressors or oncogenes in a cell type-dependent manner in various cancers^[14]. In addition, certain miRNAs have been used for cancer detection, monitoring of tumor dynamics, and predicting prognosis and chemoresistance^[15-20].

In the present study, we examined the miRNAs expression in AFPGC tissue samples using a comprehensive miRNA array-based approach. We also investigated the clinical utility of the identified AFPGC-specific miRNAs in monitoring and prognostic prediction of patients with AFPGC and evaluated their potential as universal biomarker for liver metastases.

MATERIALS AND METHODS

Patients and samples

A total of 492 patients underwent gastrectomy for GC at the University of Yamanashi Hospital between 2012 and 2018. Tumor specimens and resected lymph nodes obtained at the time of surgery were immediately fixed in 10% neutral-buffered formalin and embedded in paraffin after fixation. None of the patients underwent preoperative chemotherapy or radiotherapy. Tissue samples of all five patients with primary AFPGC and those from ten patients with primary non-AFPGC at various stages as controls from the same cohort were selected. The selected AFPGC samples contained all AFPGC patients who were operated in our hospital.

Pre-operative plasma samples were also obtained from four AFPGC patients and twenty non-AFPGC patients with GC who underwent surgical resection at the University of Yamanashi Hospital between 2017 and 2018. Control plasma samples were collected from 12 healthy adult volunteers. A total of 5 mL blood samples were collected into ethylenediaminetetraacetic acid-coated tubes and immediately spun at 3000 rpm at 4°C for 10 min to separate serum, which was stored at

-80°C for further processing. AFPGC was defined based on a plasma AFP level above 10 ng/mL or positive AFP immunoreactivity in tissue samples. This study was approved by the Ethics Committee of Yamanashi University and performed in accordance with the ethical standards of the Declaration of Helsinki and its amendments.

RNA extraction

Formalin-fixed, paraffin-embedded tissue samples were cut into 10-μm-thick sections, and total RNA was extracted from tumor and normal gastric mucosa in each patient using RNeasy FFPE kit (Qiagen, Valencia, CA), according to the manufacturer's protocol. In plasma samples, total RNA was extracted from 100 μL plasma using RNeasy Serum/Plasma kit (Qiagen), according to the manufacturer's protocol.

miRNA microarray analysis

Microarray analyses of the GC tissue samples were performed using 3D-Gene miRNA oligo chips (Toray Industries, Kamakura, Japan), with 2565 genes mounted onto each DNA chip. Results were compared between the AFP-positive and AFP-negative cells among AFPGC patient samples using macro-dissection. Tissue samples from the three AFPGC patients who underwent curative surgery were mixed equally. RNAs were labeled with the 3D-Gene miRNA labeling kit (Toray Industries). Fluorescent signals were scanned using a 3D-Gene scanner 3000 (Toray Industries) and analyzed with the 3D-Gene Extraction software (Toray Industries). In the current study, expression level of each miRNA was normalized using the median signal intensity of the all genes in each chip, and median signal intensity was adjusted to 25.

Quantification of miRNA by quantitative reverse transcription-polymerase chain reaction

Levels of miRNAs were quantified by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) using a Human TaqMan MicroRNA Assay kit (Applied Biosystems, Foster City, CA), according to standard procedures. Reverse transcription was conducted with a TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems). Tissue miRNA levels were normalized to the endogenous control *RNU6B*, and plasma miRNA levels were normalized to a synthetic RNA oligonucleotide, cel-miR-39-3p (Qiagen), by spiking the samples with the oligonucleotide which does not exist in human genome. The following primers were used for the Taqman assay (Thermo Fisher Scientific, CA, United States): human *hsa-miR-122-5p* (cat #002245), *hsa-miR-144-5p* (cat #002148), *hsa-miR-20a-5p* (cat #000580), *hsa-miR-20b-5p* (cat #001014), *hsa-miR-106a-5p* (cat #000578), *RNU6B* (cat #001093), and cel-miR-39-3p (cat #000200). ΔCt values for all miRNAs relative to the control gene *RNU6B* and cel-miR-39-3p were determined. $\Delta\Delta\text{Ct}$ values were calculated using

mean ΔCt values in non-AFPGC tissue, normal gastric mucosa, or healthy volunteer plasma samples. Plasma *miR-122-5p* expression was calculated using $\log_{10}(2^{-\Delta\text{Ct}})$.

Statistical analysis

Statistical significance was determined using GraphPad Prism® version 5 (San Diego, CA). Quantitative values were expressed as means \pm SD unless noted otherwise. Statistical significance was evaluated using the Student's *t* test and one-way analysis of variance for each time point, followed by Tukey's *post hoc* test. Pearson's correlation coefficient was determined to assess the correlation between plasma AFP and plasma *miR-122-5p* levels. *P* values < 0.05 were considered to indicate statistical significance.

RESULTS

Identification of miRNA candidates from a comprehensive miRNA array-based approach in AFPGC tissue

We selected miRNA candidates using a miRNA array-based approach. We compared the expression levels of each miRNA between the AFP-positive and AFP-negative cells in AFPGC patients. Of the 2565 candidates analyzed, we selected the following five miRNAs: *miR-122-5p*, *miR-20a-5p*, *miR-20b-5p*, *miR-106a-5p*, and *miR-144-5p*. The expression levels of these selected miRNAs were significantly different in AFP-positive cells compared with the AFP-negative cells, and the signal intensity of each miRNA was sufficient (Table 1).

Validation of the expression levels of five miRNAs in AFPGC and non-AFPGC tissue samples

We examined the expression levels of the five selected miRNAs in five AFPGC and ten non-AFPGC tissue samples by qRT-PCR to validate their utility (Figure 1). Among these five miRNAs, the expression of *miR-122-5p* was significantly higher in the AFPGC patients than the non-AFPGC patients. Therefore, we selected *miR-122-5p* for further analyses in this study.

miR-122-5p expression levels in tissue and plasma samples

Next, we investigated the expression levels of *miR-122-5p* not only in tissue but also in plasma samples. In tissue samples, *miR-122-5p* expression levels tended to be lower in the non-AFPGC tissue samples than in the normal gastric mucosa samples. Conversely, *miR-122-5p* expression levels were significantly higher in the AFPGC tissue samples compared with the normal gastric mucosa and the non-AFPGC tissue samples (Figure 2A). The plasma expression levels of *miR-122-5p* were also significantly higher in the AFPGC patient samples than the samples from health volunteers and the non-AFPGC patients (Figure 2B).

Table 1 Summary of five miRNA candidates selected by microarray analysis

Gene ID	Signal intensity		Fold change AFPGC/non-AFPGC
	AFPGC	Non-AFPGC	
<i>hsa-miR-122-5p</i>	492	105	4.7
<i>hsa-miR-20a-5p</i>	245	113	2.2
<i>hsa-miR-20b-5p</i>	198	94	2.1
<i>hsa-miR-106a-5p</i>	304	195	1.6
<i>hsa-miR-145-5p</i>	712	1367	0.5

Expression level of each miRNA was normalized using the median signal intensity of the all genes in each chip, and median signal intensity was adjusted to 25. AFPGC: Alpha-fetoprotein-producing gastric cancer.

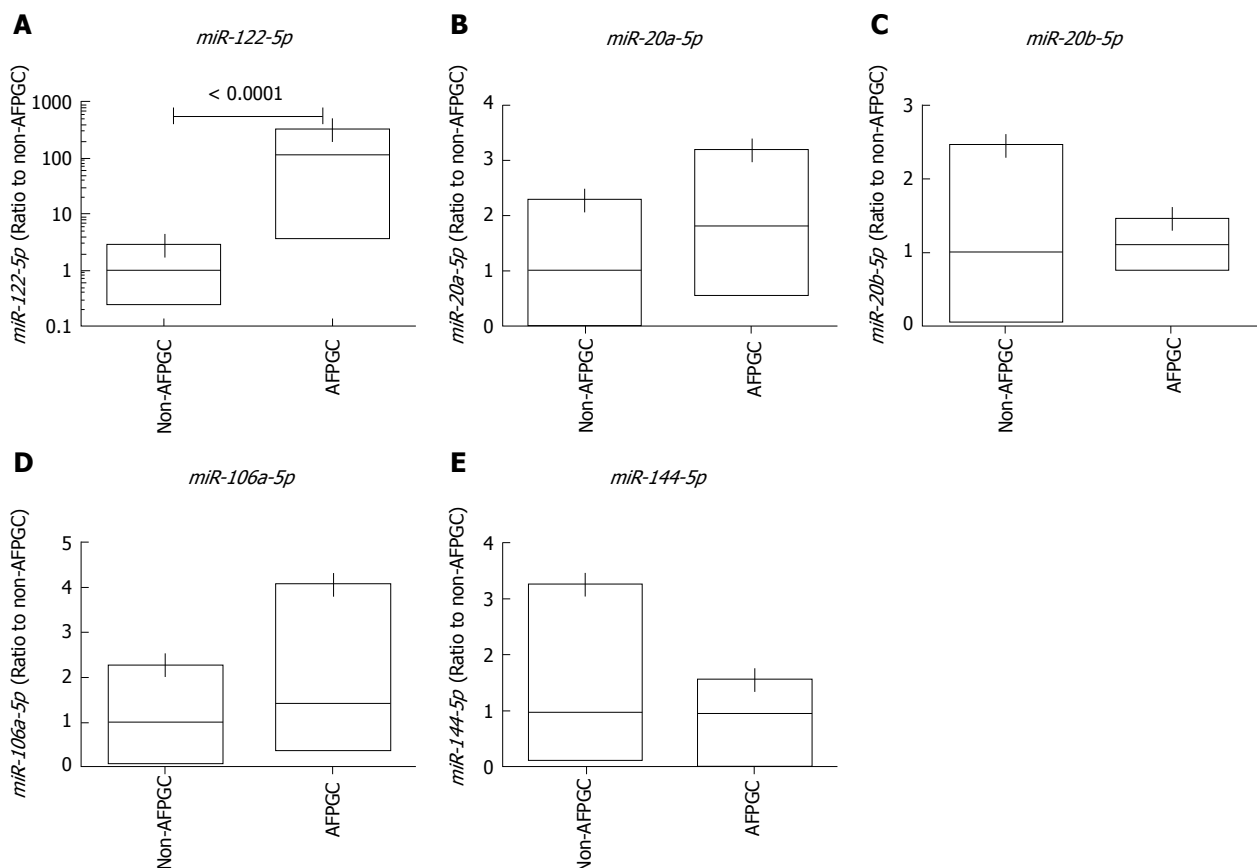


Figure 1 Validation of five microRNAs in non-alpha-fetoprotein producing gastric cancer and alpha-fetoprotein producing gastric cancer tissue samples performed by quantitative reverse transcription-polymerase chain reaction. A: *miR-122-5p*; B: *miR-20a-5p*; C: *miR-20b-5p*; D: *miR-106a-5p*; E: *miR-144-5p*. The lines inside the box plot represent the average size. AFPGC: Alpha-fetoprotein-producing gastric cancer.

Plasma *miR-122-5* levels are strongly correlated with plasma AFP levels in GC patients

We next investigated the relationship between plasma AFP levels and plasma *miR-122-5p* expression levels in the AFPGC and non-AFPGC patients and found that *miR-122-5p* expression level in plasma was strongly correlated with plasma AFP level ($r = 0.7975$, $P < 0.0001$; Figure 3).

Prognostic utility of tissue *miR-122-5p* expression in AFPGC patients

Figure 4 shows the correlation between malignant potential, all biomarkers, tissue *miR-122-5p* expression, and plasma AFP level in the AFPGC patients. We found

that the expression level of *miR-122-5p* in tissue exhibited a stronger correlation with malignant potential (*i.e.*, liver metastasis) than plasma AFP level in the AFPGC patients. Two patients with malignant potential were diagnosed morphologically as poorly differentiated adenocarcinoma and mucinous adenocarcinoma, and the other current alive patients were diagnosed as poorly differentiated adenocarcinoma and hepatoid adenocarcinoma.

DISCUSSION

AFPGC has been reported to be more likely to metastasize to liver and is therefore associated with extremely

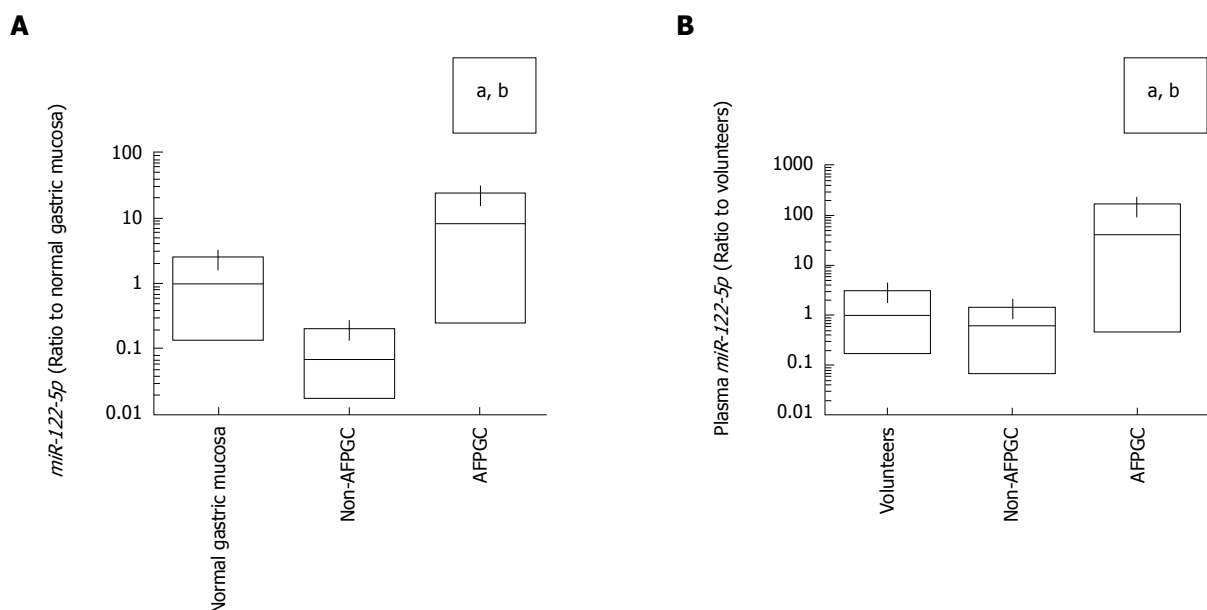


Figure 2 Quantification of *miR-122-5p* expression levels by quantitative reverse transcription-polymerase chain reaction. A: Comparison of *miR-122-5p* expression levels between normal gastric mucosa, non-alpha-fetoprotein-producing gastric cancer (AFPGC) and AFPGC in tissue samples. ^a $P < 0.05$, compared to normal gastric mucosa; ^b $P < 0.05$, compared to non-AFPGC; B: Comparison of *miR-122-5p* expression levels between health volunteers, non-AFPGC and AFPGC in plasma sample. ^a $P < 0.05$, compared to health volunteers; ^b $P < 0.05$, compared to non-AFPGC. The lines inside the box plot represent the average size.

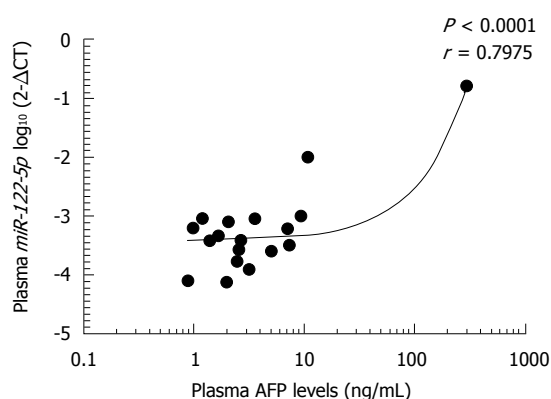


Figure 3 Relationship between plasma alpha-fetoprotein levels and plasma *miR-122-5p* expression levels. Plasma *miR-122-5p* expression level was strongly correlated with plasma alpha-fetoprotein (AFP) levels in gastric cancer patients ($r = 0.7975$, $P < 0.0001$).

poor prognosis^[8,11]. However, no genomic analyses have been conducted for AFPGC due to its rarity. Therefore, AFPGC-specific genomic and/or epigenomic alterations are not well known, which have urged us to examine the molecular characteristics specific to AFPGC. The current study investigated the molecular characteristics of AFPGC with a comprehensive analysis, with particular focus on miRNA expression.

The findings of the present study clearly demonstrated that the expression of *miR-122-5p* was significantly higher in the AFPGC tissues than the normal and non-AFPGC tissues. The expression levels of this miRNA were also higher in the plasma samples of patients with AFPGC compared with those of healthy volunteers and non-AFPGC patients and correlated with plasma

AFP levels to a certain extent. Interestingly, the tissue expression level of *miR-122-5p* exhibited a stronger correlation with malignant potential than plasma AFP level in AFPGC patients, suggesting that *miR-122-5p* might have utility as a prognostic biomarker especially for liver metastasis in this small GC subgroup.

miR-122-5p expression has been increasingly examined in various normal and cancer tissue types. Several studies reported that *miR-122-5p* was specifically expressed in human liver and that hepatocyte-specific *miR-122-5p* regulated hepatocyte differentiation and metabolism^[21-23]. Taken together, AFPGC might show characteristics of hepatocytes not only morphologically but also in its miRNA expression patterns. In fact, AFPGC was not necessarily hepatoid adenocarcinoma in this series, and two patients with aggressive development of liver metastasis were diagnosed morphologically as poorly differentiated adenocarcinoma and mucinous adenocarcinoma. Conversely, *miR-122-5p* was previously shown to function as a tumor suppressor and was reported to be downregulated in several cancer types such as hepatocellular carcinoma^[24], non-small-cell lung cancer^[25], gallbladder carcinoma^[26], bladder cancer^[27], and breast cancer^[28]. In GC, the expression of *miR-122-5p* was reported to be lower in tumor tissue than the adjacent non-cancerous tissue. Furthermore, several studies reported that *miR-122-5p* inhibited proliferation, migration, and invasion in GC^[15,29,30]. It's not known exactly why *miR-122-5p*, which is known as suppressor gene, is higher in AFPGC. Some miRNA was reported that decreased in early cases and elevated again in staged-advanced cases^[31]. Therefore, *miR-122-5p* decreased in carcinogenesis might be elevated during tumor evolution

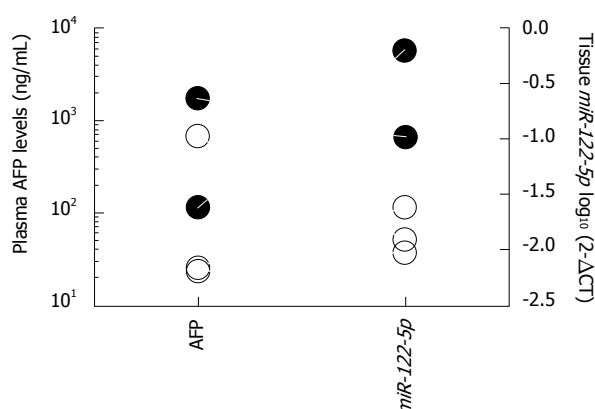


Figure 4 Correlation between malignant potential and tissue *miR-122-5p* expression levels in alpha-fetoprotein-producing gastric cancer patients. White symbols indicate current alive and black symbols indicate current death. AFP: Alpha-fetoprotein.

to AFPGC. However, the exact mechanism is unknown at the present time.

In the current study, we did not see a correlation between *miR-122-5p* in patients with non-AFPGC and development of liver metastasis, suggesting that the mechanism underlying liver metastasis might be distinct between AFPGC and non-AFPGC. We assume that AFPGC is completely different from non-AFPGC, and the mechanism of liver metastasis between AFPGC and non-AFPGC is also distinct.

Several reports demonstrated that the clinical behavior of AFPGC was distinct from that of non-AFPGC^[32]. Recently, Lu *et al.*^[33] demonstrated that AFP contributed to invasion and metastasis directly. We speculate AFPGC has specific ability of liver metastasis, and correlated with *miR-122-5p*. Therefore, *miR-122-5p* might directly facilitate tumor proliferation, migration, and invasion, which raises the possibility of *miR-122-5p* as a potential therapeutic target in AFPGC. However, future studies are warranted to demonstrate the biological function underlying altered expression of *miR-122-5p* in AFPGC. The current study revealed *miR-122-5p* as a potentially useful biomarker for early detection, disease monitoring, and prognostic prediction in patients with AFPGC, which warrant further investigation.

ARTICLE HIGHLIGHTS

Research background

Alpha-fetoprotein (AFP)-producing gastric cancer (AFPGC) is recognized as one of the most aggressive tumors, with a high propensity for liver metastasis and subsequent poor prognosis compared with other GC subtypes. Recent comprehensive molecular analyses have not yet referred to this minor subtype because of its rareness.

Research motivation

To discover universal biomarkers for liver metastasis by researching AFPGC-specific microRNAs (miRNAs).

Research objectives

To investigate the clinical utility of AFPGC-specific miRNA for monitoring and

prognostic prediction of patients.

Research methods

We performed a comprehensive miRNA array-based approach to compare miRNA expression levels between AFP-positive and AFP-negative cells, and also investigated the clinical utility of the identified AFPGC-specific miRNAs.

Research results

We found the expression of *miR-122-5p* was significantly higher in the AFPGC tissues than the normal and non-AFPGC tissues. The expression levels of this miRNA were also higher in the plasma samples of patients with AFPGC compared with those of healthy volunteers and non-AFPGC patients and correlated with plasma AFP levels. Moreover, the tissue expression level of *miR-122-5p* exhibited a stronger correlation with malignant potential than plasma AFP level in AFPGC patients.

Research conclusions

miR-122-5p as a potentially useful biomarker for early detection and disease monitoring in patients with AFPGC.

Research perspectives

We identified *miR-122-5p* as AFPGC-specific miRNA. *miR-122-5p* might be a clinical useful biomarker in AFPGC. Although studies are warranted to demonstrate the biological function underlying altered expression of *miR-122-5p* in AFPGC, the *miR-122-5p* might be a potential therapeutic target for liver metastasis in AFPGC.

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Retrospective Study

Prognostic value of vascular endothelial growth factor receptor 1 and class III β -tubulin in survival for non-metastatic rectal cancer

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Abstract**AIM**

To assess the long-term prognostic value of vascular endothelial growth factor receptor 1 (VEGFR1) and class III β -tubulin (TUBB3) mRNA expression in non-metastatic rectal cancer.

METHODS

A total of 75 consecutive patients with non-metastatic rectal cancer from March 2004 to November 2008 were analyzed retrospectively at our institute. The mRNA expressions of VEGFR1 and TUBB3 were detected by multiplex branched DNA liquid-chip technology. The Cut-off Finder application was applied to determine cutoff point of mRNA expression. SPSS software version 22.0 was used for analysis.

RESULTS

The median follow-up was 102.7 mo (range, 6-153.6). The χ^2 and Fisher's exact tests showed that VEGFR1 expression was related to lymph node metastasis ($P = 0.013$), while no relationships between TUBB3 and clinicopathological features were observed. Univariate analysis showed that T stage, lymph node metastasis, tumor differentiation, VEGFR1 and TUBB3 mRNA expression were correlated to overall survival (OS) ($P = 0.048$, $P = 0.003$, $P = 0.052$, $P = 0.003$ and $P = 0.015$, respectively). Also, lymph node metastasis and VEGFR1 expression independently influenced OS by multivariate analysis ($P = 0.027$ and $P = 0.033$). VEGFR1 expression was positively correlated with TUBB3 ($P = 0.024$). The patients with low expression of both TUBB3 and VEGFR1 presented a better OS ($P = 0.003$). In addition, the receiver operating characteristic analysis suggested that the combination of lymph node metastasis and VEGFR1 had a more favorable prognostic value ($P < 0.001$).

CONCLUSION

VEGFR1 expression and lymph node metastasis independently and jointly affect survival. Moreover, low expression of VEGFR1 and TUBB3 presented a better OS in patients with non-metastatic rectal cancer, which might serve as a potential prognostic factor.

Key words: Rectal cancer; Class III β -tubulin; Vascular endothelial growth factor receptor 1; Overall survival

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Core tip: Nowadays, personalized and precision medicine becomes vital in cancer treatment. Herein, we focus on the long-term prognostic value of vascular endothelial growth factor receptor 1 (VEGFR1) and class III β -tubulin (TUBB3) mRNA expression in non-metastatic rectal cancer. In the 75 consecutive patients enrolled, we found that VEGFR1 expression and lymph node metastasis were independent factors influencing overall survival, and the combination of them showed a favorable prognostic value. Also, VEGFR1 expression was significantly related to lymph node metastasis. In addition, VEGFR1 expression was positively correlated with TUBB3 expression.

Kong XQ, Huang YX, Li JL, Zhang XQ, Peng QQ, Tang LR, Wu JX. Prognostic value of vascular endothelial growth factor receptor 1 and class III β -tubulin in survival for non-metastatic rectal cancer. *World J Gastrointest Oncol* 2018; 10(10): 351-359 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i10/351.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i10.351>

INTRODUCTION

Rectal cancer is one of the most diagnosed malignan-

cies among both males and females worldwide with worse outcomes than colon cancer^[1,2]. Clinically, patients showed various outcomes to multimodality therapies. Nowadays, personalized and precision medicine has become essential in the treatment of rectal cancer. Recent studies conducted gene expression profiling to predict the response and long-term prognosis of malignancies^[3,4]; however, no consensus was achieved on prognostic gene profiling for rectal cancer.

Vascular endothelial growth factor (VEGF) possesses a significant role in angiogenesis by binding to VEGFR1 and VEGFR2, which is required for cancer progression and metastasis^[5,6]. A phase II trial indicated that VEGF could predict the pathological response to locally advanced rectal cancer patients treated with neoadjuvant cetuximab-based chemoradiation^[7]. In addition, class III β -tubulin (TUBB3) has been reported to play a critical role in tumor development and malignant transformation as a β -tubulin isotype. The variable levels of expression of the gene have been reported in colon, lung, ovary, kidney, prostate, and throat cancer with solid tumors^[8-10]. However, only a few studies focused on its role in rectal cancer.

Herein, our study attempted to explore the potential prognostic value of VEGFR1 and TUBB3 for long-term survival in non-metastatic rectal cancer.

MATERIALS AND METHODS

Patients

Eighty cases of well-preserved formalin-fixed and paraffin embedded tumor tissue specimens that had undergone total mesorectal excision (TME) at the Fujian Cancer Hospital from March 2004 to November 2008 were retrospectively examined. Among these, two patients with previous malignancy and three with distant metastasis were excluded. Finally, 75 patients who fulfilled the following inclusion criteria were enrolled in the study: (1) Pathologically confirmed as primary rectal adenocarcinoma; (2) underwent TME; (3) no evidence of distant metastasis; (4) no previous or concurrent malignancy; and (5) complete follow-up information was obtained.

The variables such as gender, age, preoperative carcino-embryonic antigen (pre-CEA), pre-operative hemoglobin (pre-Hb), distance to the verge, T stage, lymph node metastasis, venous invasion, and tumor differentiation were considered. The T stage and lymph node metastasis were re-diagnosed based on the 8th Edition of the American Joint Committee on Cancer (AJCC)^[11].

Treatments and follow-up

All patients underwent TME, including abdominoperineal resection and low anterior resection. Of these, eight cases received neoadjuvant chemoradiotherapy followed by TME. A total of 66 cases received 5-fluorouracil (5-FU)-based chemotherapy. The overall survival (OS)

Table 1 Patient characteristics

Characteristics	Data, n (%)
Gender	
Female	36 (48)
Male	39 (52)
Age (yr)	
median (range)	52 (29-74)
≤ 60	58 (77.3)
> 60	17 (22.7)
Pre-CEA (ng/mL)	
≤ 5	36 (63.2)
> 5	21 (36.8)
Pre-Hb (g/L)	
≤ 120	26 (34.7)
> 120	49 (65.3)
Distance to verge (cm)	
≤ 5	46 (61.3)
> 5	29 (38.7)
T stage	
T1 + T2	13 (17.3)
T3 + T4	63 (82.6)
Lymph node metastasis	
Negative	22 (29.3)
Positive	53 (70.6)
Venous invasion	
Negative	68 (90.7)
Positive	7 (9.3)
Tumor differentiation	
Poorly differentiated	20 (26.7)
Moderately-well differentiated	55 (73.3)
Chemotherapy	
No	9 (12)
Yes	66 (88)
TUBB3 expression	
Low-expression	39 (52)
High-expression	36 (48)
VEGFR1 expression	
Low-expression	53 (70.7)
High-expression	22 (29.3)
TUBB3 and VEGFR1	
Both low expression	32 (42.6)
Others	43 (57.3)

Pre-CEA: Preoperative carcino-embryonic antigen; Pre-Hb: Preoperative hemoglobin.

was defined as the duration from the date of diagnosis to the last follow-up or the date of death due to any cause, which was obtained from the medical records and telephonic interviews.

Multiplex branched DNA liquidchip technology

The formalin-fixed and paraffin embedded (FFPE) tumor tissue specimens containing more than 70% of tumor cells were selected. The Multiplex branched DNA liquidchip (MBL) technology (Guangzhou SurExam Bio-Tech Co., Ltd., China) was implemented to determine the mRNA expression levels of VEGFR1 and TUBB3. The FFPE tissue samples were lysed in the presence of proteinase K, at 56°C for 2 h. Then, the lysate was transferred to a 96-well plate containing the blocking reagent, capture beads with probes for VEGFR1 and TUBB3, and target gene-specific probe sets. The sandwich nucleic acid hybridization was carried out for 16 h. The unbound RNA was removed by three washes

with buffer under a vacuum system. The signal bound to the target mRNA was amplified with a streptavidin-conjugated phycoerythrin solution at 50°C for 30 min. The fluorescence values of the samples were identified and analyzed using Luminex 200 system (Luminex, Austin, TX, United States), which were regarded as the RNA expression levels of each gene. The cutoff point of mRNA expression affecting the survival was determined by the Cutoff Finder application^[12].

Statistical analysis

The end point of our analysis was OS. The association of gene expression level and clinicopathological features was studied by the χ^2 and Fisher's exact tests. The association between the mRNA expressions of VEGFR1 and TUBB3 was studied by the Spearman correlation test. The Kaplan–Meier test was used to analyze the OS, and Cox regression model (LR forward) was employed for univariate and multivariate analysis. Receiver operating characteristic (ROC) analysis was employed for assessing the specificity as well as the sensitivity of predicting OS by specific parameters. The statistical significance of area under the ROC (area under curve, AUC) was calculated by Delong's test^[13]. *P*-values < 0.05 were deemed significant. The statistical analysis was conducted by SPSS version 22.0 (IBM Corporation, Armonk, NY, United States). The statistical methods of our study were reviewed by Qian-yu Ni from The First Affiliated Hospital of Fujian Medical University.

RESULTS

Patient characteristics

A total of 75 patients were enrolled in the present study. The characteristics of non-metastatic patients are summarized in Table 1. Median follow-up time was 102.7 mo (range: 6.0-153.6). The cohort comprised of 39 (52%) male and 36 (48%) female cases with the median age 52 years (range, 29-74). Among these patients, 21 (36.8%) cases presented pre-CEA records that were higher than 5 ng/mL, while they could not be accessed for 18 cases. In the case of pre-Hb, 26 (34.7%) patients were ≤ 120 g/L and the remaining were > 120 g/L. In terms of the tumor location, 46 (61.3%) patients had low rectal cancer (0-5 cm distance to verge), while the other 29 (38.7%) patients were > 5 cm. In all, 22 (29.3%) with lymph node metastasis positive and 53 (70.6%) were negative. Twenty (26.7%) patients were identified as poorly differentiated and 55 (73.3%) as moderate-to-well differentiated. According to the Cutoff Finder software, 0.0575 and 0.2025 were considered as the optimal cutoff point for the VEGFR1 and TUBB3 expression value, respectively (Figure 1). In addition, 36 (48%) and 22 (29.3%) patients showed a high expression of VEGFR1 and TUBB3, respectively.

Associations between mRNA expression and clinicopathological features

The correlations between VEGFR1/TUBB3 mRNA expres-

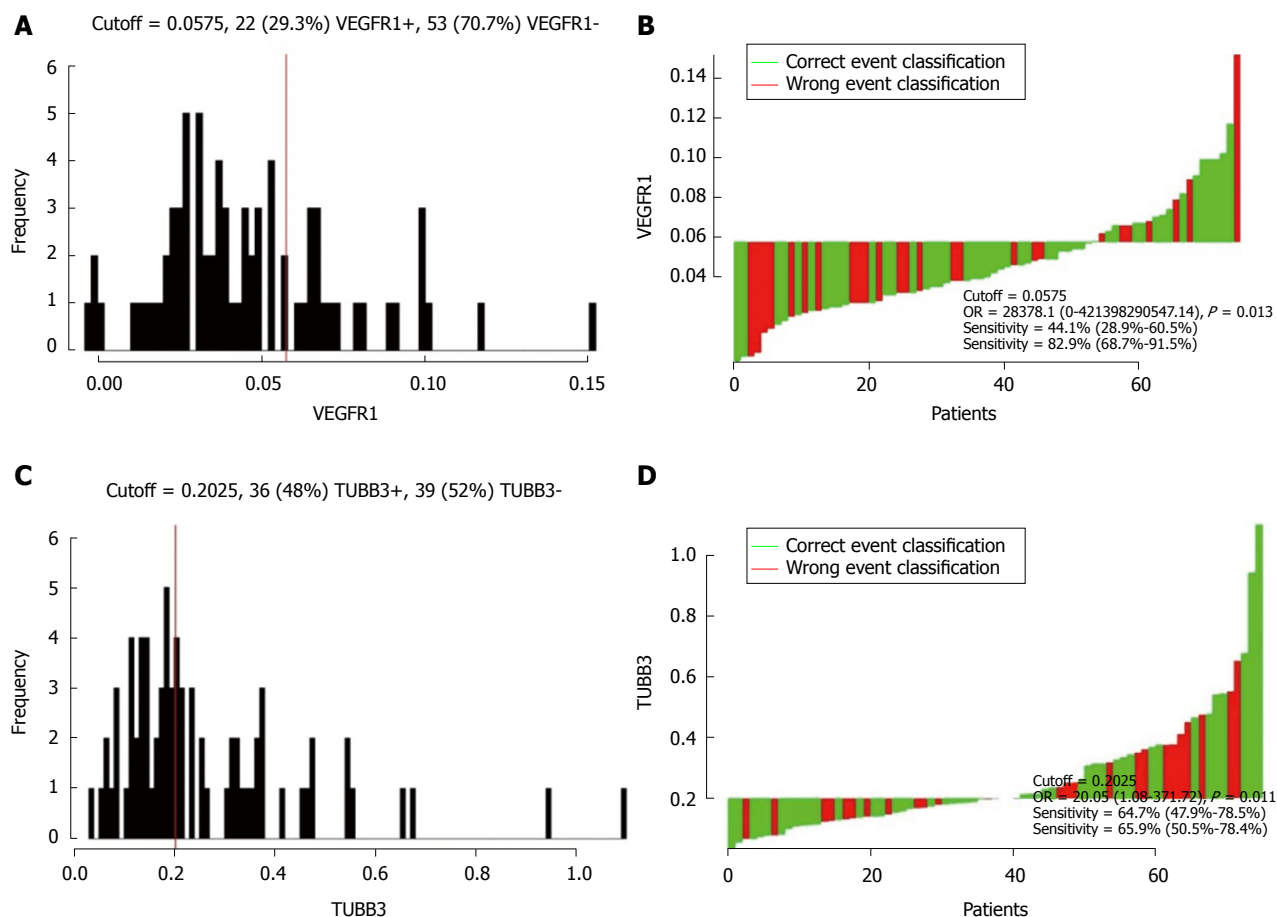


Figure 1 Distribution-based cutoff optimization of vascular endothelial growth factor receptor 1 and class III β -tubulin expression value in 75 non-metastatic rectal cancer patients. A: Histograms of vascular endothelial growth factor receptor 1 (VEGFR1) expression value; B: Waterfall plot of optimal dichotomization for VEGFR1 expression value; C: Histograms of class III β -tubulin expression value; D: Waterfall plot of optimal dichotomization for VEGFR1 expression value.

ssion and clinicopathological features were analyzed (Table 2). A majority of the patients displayed positive lymph node metastasis in the high-expression group of VEGFR1 ($P = 0.013$). However, no significant difference was found between the expression level of TUBB3 expression and clinicopathological features (gender, age, pre-CEA, pre-Hb, distance to the verge, T stage, lymph node metastasis and venous invasion, all $P > 0.05$).

Impact of VEGFR1 and TUBB3 on OS

The Cox regression analysis of OS influencing factors was shown in Table 3. Univariate analysis showed that T stage, lymph node metastasis, tumor differentiation, and VEGFR1 and TUBB3 expression were significantly related to OS ($P = 0.048$, $P = 0.003$, $P = 0.052$, $P = 0.003$ and $P = 0.015$, respectively) (Figures 2, 3 A and B). Moreover, Kaplan-Meier analysis showed that the rates of 1-, 3-, and 5-year OS in the TUBB3 low- and high-expression groups were 94.9% vs 94.4%, 76.9% vs 52.8%, and 71.8% vs 47.2%, respectively ($P = 0.017$). The rates of OS in the VEGFR1 low- and high-expression groups were 98.1% vs 86.4%, 77.4% vs 36.4%, and 69.8% vs 36.4%, respectively ($P = 0.003$).

Moreover, lymph node metastasis (HR = 3.042, 95%CI: 1.137-8.142, $P = 0.027$) and VEGFR1 (HR = 2.151, 95%CI: 1.062-4.355, $P = 0.033$) were independent factors influencing OS, as evaluated by the multivariate Cox regression model.

Prognostic value of different combinations on survival

VEGFR1 and TUBB3 expression were positively correlated ($P = 0.006$, $r = 0.315$) by the Spearman's correlation test. Both low expression of VEGFR1 and TUBB3 were observed in 32 (42.6%) cases. Moreover, the Kaplan-Meier analysis showed that the 1-, 3-, and 5-year OS of both low-expression patients vs others were 96.9% vs 93.0%, 84.4% vs 53.5%, and 78.1% vs 46.5%, respectively ($P = 0.003$, Figure 3C). Meanwhile, Kaplan-Meier analysis showed that the rates of 1-, 3-, and 5-year OS in positive lymph node metastasis patients with high expression of VEGFR1 vs others were 90.0% vs 98.2%, 35.0% vs 78.2%, and 30.0% vs 70.9%, respectively ($P < 0.001$) (Figure 3D).

Finally, we combined the two independent prognostic factors, lymph node metastasis and VEGFR1 expression, to construct a prognostic model and supplemented the VEGFR1 expression to the lymph node metastasis

Table 2 Correlation between vascular endothelial growth factor receptor 1 and class III β -tubulin expression with clinicopathological features

Parameter	TUBB3		<i>P</i>	VEGFR1		<i>P</i>
	Low (<i>n</i>)	High (<i>n</i>)		Low (<i>n</i>)	High (<i>n</i>)	
Gender			0.426			0.081
Female	17	19		22	14	
Male	22	17		31	8	
Age (yr)			0.31			1
≤ 60	32	26		41	17	
> 60	7	10		12	5	
Pre-CEA			0.203			0.244
≤ 5	20	16		26	10	
> 5	8	13		12	9	
Pre-Hb			0.801			0.206
≤ 120	13	13		16	10	
> 120	26	23		37	12	
Distance to verge (cm)			0.608			0.792
≤ 5	25	21		32	14	
> 5	14	15		21	8	
T stage			0.883			0.744
T1 + T2	7	6		10	3	
T3 + T4	32	30		43	19	
Lymph node metastasis			0.071			0.013
Negative	15	7		20	2	
Positive	24	29		33	20	
Tumor thrombus			0.25			1
Negative	37	31		48	20	
Positive	2	5		5	2	
Tumor differentiation			0.754			0.939
Poorly	11	9		14	6	
Moderately-well	28	27		39	16	
Chemotherapy			0.156			0.051
No	7	2		9	0	
Yes	32	34		44	22	
VEGFR1			0.024			
Low	32	21				
High	7	15				

Pre-CEA: Preoperative carcino-embryonic antigen; Pre-Hb: Preoperative-hemoglobin.

by ROC analysis to assess the improvement of the model for OS. The lymph node metastasis (AUC: 0.688, 95%CI: 0.567–0.808, $P = 0.005$) showed a better prognostic value than VEGFR1 expression (AUC: 0.635, 95%CI: 0.507–0.764, $P = 0.045$). Furthermore, a better prognostic value was shown when combining the lymph node metastasis and VEGFR1 expression (AUC: 0.748, 95%CI: 0.637–0.859, $P < 0.001$) (Figure 4).

DISCUSSION

Firstly, we evaluated the long-term prognostic value of VEGFR1 and TUBB3 expression after the diagnosis of non-metastatic rectal cancer with a median follow-up of 102 mo. Here, we found that VEGFR1 and TUBB3 expression affected OS in non-metastatic rectal cancer by univariate analysis. Moreover, a favorable OS in both low expression of VEGFR1 and TUBB3 was noted as compared to others. In addition, the association between VEGFR1 expression and lymph node metastasis was also assessed. The combination of lymph node metastasis and VEGFR1 expression might also provide a promising tool for the prognosis of non-metastatic rectal cancer.

Reportedly, VEGFR correlates with poor prognosis, metastasis, and recurrence in various tumor types, including breast and lung cancers^[14,15]. Moreover, previous studies demonstrated that VEGF plays a crucial role as a potent angiogenic factor in both experimental and human studies with respect to colorectal cancer progression and metastasis^[16–18]. The co-expression of VEGF and VEGFR1/2 in the nucleus stimulates the proliferation and migration of endothelial cells, thereby providing nutrition for growing tumors and establishing a continuity between tumor cells and host vasculature^[19].

VEGFR1 is primarily localized in the nucleus of endothelial cells; As the predominant receptor of the tumor microenvironment, it is essential for the survival of endothelial cells^[20]. Tsai *et al.*^[21] reported that the overexpression of VEGF is a significant positive predictor for early postoperative relapse in stage I–III colorectal cancer patients, leading to poor OS ($P = 0.002$). Similarly, Nriagu *et al.*^[22] reported that the overexpression of VEGF mRNA was an independent factor affecting OS as assessed by multivariate analysis (HR = 1.94, $P = 0.005$). Herein, we found that the low expression of VEGFR1 might positively affect OS with a 5-year OS of 69.8% for low

Table 3 Cox regression analysis for overall survival

Variables	Univariate			Multivariate		
	HR	95%CI	P	HR	95%CI	P
Gender						
Female/male	1.018	0.519-1.997	0.958			
Age						
≤ 60/> 60	1.175	0.548-2.518	0.679			
Pre-CEA						
≤ 5/> 5	1.067	0.496-2.298	0.868			
Pre-Hb						
≤ 120/> 20	0.651	0.328-1.290	0.219			
Distance to verge (cm)						
≤ 5/> 5	1.265	0.642-2.491	0.497			
T stage						
T1 + T2/T3 + T4	4.221	1.011-17.632	0.048	4.05	0.968-116.93	0.055
Lymph node metastasis						
Negative/positive	6.247	1.905-20.491	0.003	3.042	1.137-8.142	0.027
Tumor thrombus						
Negative/positive	1.303	0.458-3.705	0.62			
Tumor differentiation						
Poorly/moderately-well	0.503	0.251-1.006	0.052	-	-	0.18
Chemotherapy						
No/yes	1.407	0.430-4.605	0.572			
TUBB3 expression						
Low/high	2.407	1.188-4.877	0.015	-	-	0.1
VEGFR1 expression						
Low/high	2.817	1.424-5.570	0.003	2.151	1.062-4.355	0.033

Pre-CEA: Preoperative carcino-embryonic antigen; Pre-Hb: Preoperative hemoglobin.

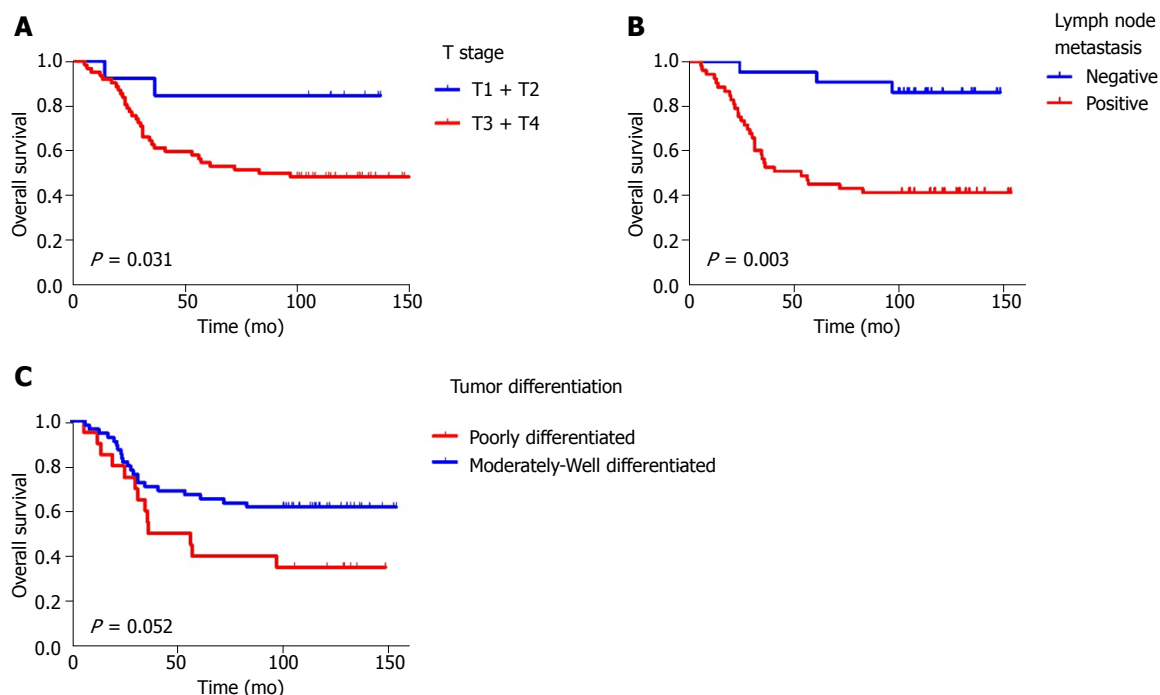


Figure 2 Kaplan-Meier survival curves of overall survival. A: T stage (T1 + T2 vs T3 + T4, $P = 0.031$); B: Lymph node metastasis (negative vs positive, $P = 0.003$); C: Tumor differentiation (poorly differentiated vs moderately-well differentiated, $P = 0.052$).

vs 36.4% for the high-expression group (HR = 2.151, $P = 0.033$). These results indicated that VEGFR1 functions as a positive regulator of angiogenesis^[23], which might lead to poor survival in cancer patients.

A previous study evaluated VEGF expression in 117 colorectal adenocarcinoma patients, and confirmed

that lymph node metastasis (positive vs negative, $P < 0.001$) and TNM stage (stage III vs I/II, $P < 0.001$) were related to increased VEGF expression. Moreover, the mean number of metastatic nodes was significantly associated with VEGF expression (1.06 ± 2.84 for low expression vs 2.45 ± 4.03 for high expression, $P =$

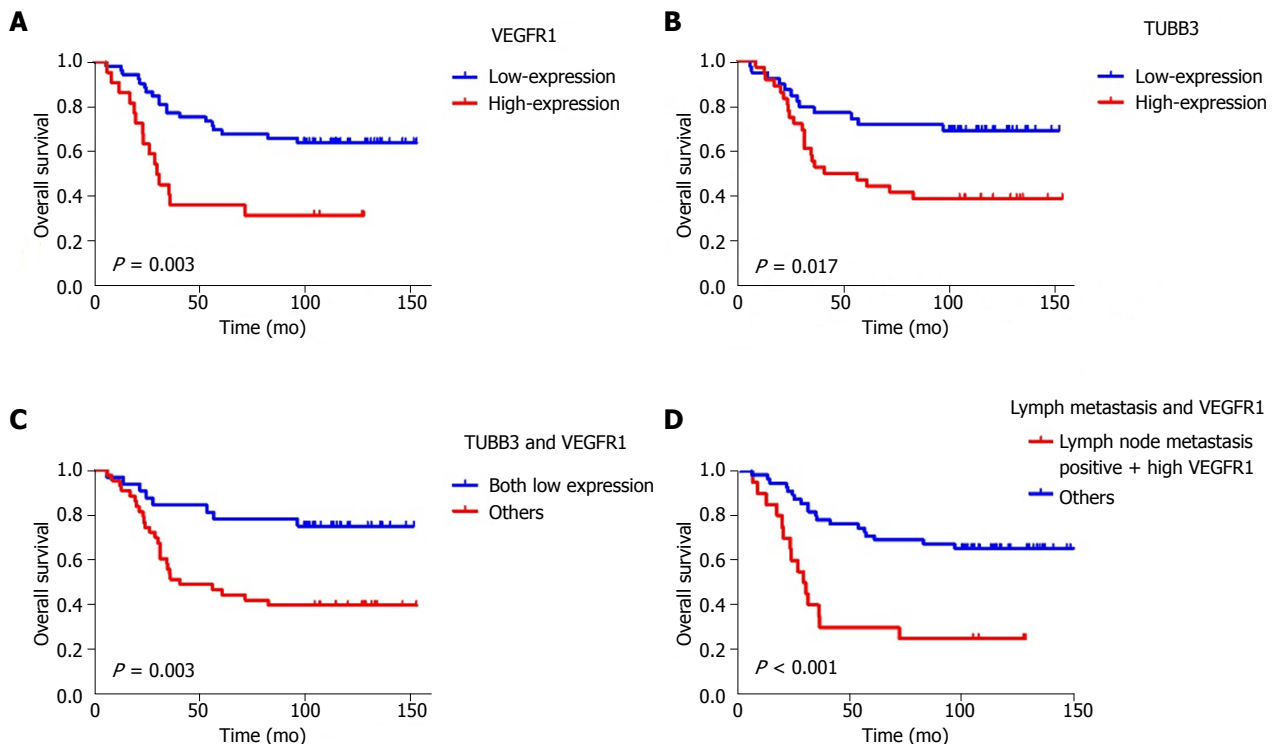


Figure 3 Kaplan-Meier survival curves of Overall Survival. A: Vascular endothelial growth factor receptor 1 (VEGFR1) expression (low vs high, $P = 0.003$); B: Class III β -tubulin (TUBB3) expression (low vs high, $P = 0.017$); C: TUBB3 and VEGFR1 (both low expression vs others, $P = 0.003$); D: TNM stage and VEGFR1 (stage III + high VEGFR1 expression vs others, $P < 0.001$).

0.031)^[24]. Similarly, our study implied that VEGFR1 expression was related to lymph node metastases ($P = 0.013$). However, whether the function of VEGF/VEGFR1 affects lymph node metastasis is yet unclear. Nagy *et al*^[25] hypothesized that tumor cells in the circulation directly reached the regional lymph nodes through the supply vessels or blood vessel-lymph vessel junctions.

A retrospective study reported that VEGF expression could identify an unfavorable subgroup of patients with stage II colon cancer for optimal treatment strategy (the recurrence rate was 50% for VEGF-positive vs 11.7% for VEGF-negative, $P = 0.001$)^[26]. As shown by ROC curves in our analysis, though low sensitivity of VEGFR1 (44.1%), the specificity was high with 82.9%, which exerted a similar effect on prognosis as lymph node metastasis. Moreover, the sensitivity increased when combined with lymph node status, and a superior prognostic value was noted for the combination. Further identification of a group of lymph node metastasis-positive with high VEGFR1 expression allows for selective treatment with adjuvant chemotherapy using antiangiogenic therapy, including VEGFR1 antisense and monoclonal antibodies, as well as postoperative follow-up.

Several clinical studies demonstrated that the increased expression of TUBB3 in various human malignancies was related to low response rate and poor survival in patients treated with taxane-based chemotherapies^[27-30]. However, studies focusing on the relationship between TUBB3 and non-metastatic rectal cancer are limited. The

current study showed that the low expression of TUBB3 had better OS in non-metastatic rectal cancer patients as assessed by univariate analysis (5-year OS, 71.8% vs 47.2%), although no significant difference was observed by multivariate analysis.

Furthermore, Makarchenko *et al*^[31] and Widow *et al*^[32] reported that VEGFR1 regulated the chemo-resistant genes such as TUBB3, which might result in the poor prognosis of lung and gastroesophageal cancers. The current study established a positive correlation between VEGFR1 and TUBB3 ($r = 0.315$, $P = 0.006$), and a favorable OS was observed in both low expression groups ($P = 0.003$). Paradiso *et al*^[33] had investigated the combination of TUBB3 and VEGFR1 in advanced breast cancer. Hypoxia in the tumor microenvironment promotes angiogenesis, and VEGFR1 is known to be related to angiogenesis^[23]. TUBB3 was found to be involved in an adaptive response to low oxygen levels and poor nutrient supply in solid tumors^[34,35]. Therefore, we speculate that the underlying mechanism of the two correlations might be related to anoxic environments.

Notably, this study was limited to a small-sample retrospective analysis. Thus, additional mRNA expression data might help to establish a superior predictor. Finally, prospective data and large sample size are essential for further substantiation of the results.

We confirmed that the increased expression of VEGFR1 and TUBB3 might be negatively correlated with long-term prognosis of non-metastatic rectal cancer.

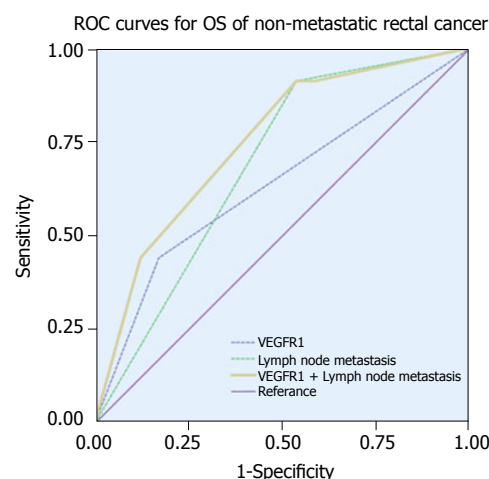


Figure 4 Receiver operating characteristic analyses in non-metastatic rectal cancer patients. *P*-values show the area under the receiver operating characteristic (ROC) curves in the three models. ROC analyses of the prediction of overall survival by vascular endothelial growth factor receptor 1 (VEGFR1) expression model, lymph node metastasis, and the combined VEGFR1 expression-lymph node metastasis model.

Furthermore, VEGFR1 expression and lymph node metastasis affected the survival independently as well as synergistically. These results might provide additional prognostic information compared to the conventional tumor histopathological factors.

ARTICLE HIGHLIGHTS

Research background

Rectal cancer is one of the most common form of cancer in both men and women. Gene expression profiling for predicting the response and long-term prognosis of malignancies has been reported in recent decades. Vascular endothelial growth factor (VEGF) and class III β -tubulin (TUBB3) have been reported to play a vital role in cancer progression. However, few studies focused on their role in rectal cancer.

Research motivation

We try to explore the potential prognostic value of VEGFR1 and TUBB3 for long-term survival in non-metastatic rectal cancer.

Research objectives

A total of 75 patients diagnosed with primary rectal adenocarcinoma without metastases were retrospectively analyzed.

Research methods

Multiplex branched DNA liquidchip technology was applied to detected mRNA expressions of VEGFR1 and TUBB3. The cutoff point of mRNA expression was determined by Cutoff Founder.

Research results

VEGFR1 expression was positively correlated to TUBB3. Patients with both low expression of TUBB3 and VEGFR1 presented a better overall survival (OS). In addition, VEGFR1 and lymph node metastasis had potential as prognostic factors for OS in non-metastatic rectal cancer patients, and the combination of

them showed a favorable prognostic value.

Research conclusions

We confirmed that the increased expression of VEGFR1 and TUBB3 might be negatively correlated with long-term prognosis of non-metastatic rectal cancer. Furthermore, VEGFR1 expression and lymph node metastasis affected the survival independently, as well as synergistically. These results might provide additional prognostic information compared to the conventional tumor histopathological factors.

Research perspectives

VEGFR1 has the potential to contribute to decision making regarding individual treatment in rectal cancer. A larger sample size and additional mRNA expression data are warranted to establish a superior prognosis model.

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Retrospective Study

Predictive factors for lymph node metastasis and defining a subgroup treatable for laparoscopic lymph node dissection after endoscopic submucosal dissection in poorly differentiated early gastric cancer

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Author contributions: Li H, Huo ZB and Fan-Ting Kong contributed equally to this work. Liu DX, Li H, designed the research; Li H, Huo ZB and Fan-Ting Kong analyzed the data and drafted the manuscript; He QQ revised the manuscript critically for important intellectual content and contributed to the data analysis; Gao YH and Liang WQ helped draft the manuscript; all authors read and approved the final manuscript.

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Institutional review board statement: This study is a retrospective study for the data of patients collected from the Department of Surgical Oncology, Affiliated Xing Tai People's Hospital of Hebei Medical University during 1990-2015. No human body was involved in this study. In our hospital policy, this study does not require approval by the hospital.

Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: No conflict of interest was declared by the authors.

Data sharing statement: No additional data are available.

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Abstract

AIM

To investigate the predictive factors of lymph node metastasis (LNM) in poorly differentiated early gastric cancer (EGC); to guide the individual application of a combination of endoscopic submucosal dissection (ESD) and laparoscopic lymph node dissection (LLND) in a suitable subgroup of patients with poorly differentiated EGC.

METHODS

We retrospectively analyzed 138 patients with poorly differentiated EGC who underwent gastrectomy with lymphadenectomy between January 1990 and December 2015. The association between the clinicopathological factors and the presence of LNM was retrospectively analyzed by univariate and multivariate logistic regression analyses. Odds ratios (OR) with 95% confidence interval (95%CI) were calculated. We further examined the relationship between the positive number of the significant predictive factors and the LNM rate.

RESULTS

The tumor diameter (OR = 13.438, 95%CI: 1.773-25.673, $P = 0.029$), lymphatic vessel involvement (LVI) (OR = 38.521, 95%CI: 1.975-68.212, $P = 0.015$) and depth of invasion (OR = 14.981, 95%CI: 1.617-52.844, $P = 0.024$) were found to be independent risk factors for LNM by multivariate analysis. For the 138 patients diagnosed with poorly differentiated EGC, 21 (15.2%) had LNM. For patients with one, two and three of the risk factors, the LNM rates were 7.7%, 47.6% and 64.3%, respectively. LNM was not found in 77 patients that did not have one or more of the three risk factors.

CONCLUSION

ESD might be sufficient treatment for intramucosal poorly differentiated EGC if the tumor is less than or equal to 2 cm in size and when LVI is absent upon postoperative histological examination. ESD with LLND may lead to the elimination of unnecessary gastrectomy in poorly differentiated EGC.

Key words: Poorly differentiated cancer; Laparoscopic lymph node dissection; Lymph node metastasis; Early gastric cancer; Endoscopic submucosal dissection

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Core tip: The new technique combines endoscopic submucosal dissection (ESD) with laparoscopic lymph node dissection (LLND), which may lead to the elimination of "unnecessary" gastrectomy in poorly differentiated early gastric cancer (EGC) patients that have a potential risk of lymph node metastasis (LNM). ESD followed by LLND enables the complete resection of the primary tumor and the histologic determination of the lymph node status. In this study, we determined the risk factors that were predictive of LNM in poorly differentiated EGC patients. Our results provided some suggestions to guide the application of combination of ESD and LLND for selected patients with poorly differentiated EGC.

Li H, Huo ZB, Kong FT, He QQ, Gao YH, Liang WQ, Liu DX. Predictive factors for lymph node metastasis and defining a subgroup treatable for laparoscopic lymph node dissection after endoscopic submucosal dissection in poorly differentiated early gastric cancer. *World J Gastrointest Oncol* 2018; 10(10): 360-366 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/>

INTRODUCTION

Endoscopic submucosal dissection (ESD) has become widely accepted, as it provides *en bloc* resection and histologically complete resection and is a valuable alternative to gastrectomy for treating early gastric cancer (EGC)^[1-4]. The accurate assessment of the potential presence of lymph node metastasis (LNM) is required for ESD. ESD can be used for EGC but it does not have the risk of LNM^[5-7]. Because the risk of LNM is negligible (0%), ESD is often applied to well or moderately differentiated EGC confined to the mucosa without ulceration and smaller than or equal to 2 cm^[8]. For undifferentiated EGC, the risk of LNM is higher so the usage of ESD has been limited. Thus, for patients with undifferentiated EGC, gastrectomy was accepted as a standard treatment. Undifferentiated carcinomas of gastric cancer consist of mucinous adenocarcinoma, primary signet ring cell carcinoma and poorly differentiated adenocarcinoma^[8]. However, approximately 96.6% of poorly differentiated EGC cases with potential risk of LNM are eventually found to have no LNM after "unnecessary" gastrectomy, suggesting that it may be overtreatment for these cases^[9]. The new technique combines ESD with laparoscopic lymph node dissection (LLND), which may lead to the elimination of "unnecessary" gastrectomy in EGC patients having a potential risk of LNM^[10-13]. ESD followed by LLND enables the complete resection of the primary tumor and the histologic determination of the lymph node status.

In this retrospective study, we determined the risk factors that were predictive of LNM in poorly differentiated EGC patients. Our results provided some suggestions to guide the application of combination of ESD and LLND for selected patients with poorly differentiated EGC.

MATERIALS AND METHODS**Patients**

EGC is considered to be a lesion confined to the mucosa or submucosa regardless of the presence or absence of LNM, according to the Japanese Classification of Gastric Carcinoma (JCGC)^[8]. This retrospective study enrolled patients who had undergone radical gastrectomy due to EGC. The patients were from the Department of Surgical Oncology, Affiliated Xing Tai People's Hospital of Hebei Medical University (Xingtai, China). Time points were from January 1990 to December 2015.

For this current study, inclusion criteria included: (1) Diagnosed with poorly differentiated EGC depending on JCGC by pathological analyses through specimens and lymph nodes; (2) Lymph node dissection beyond limited (D1) dissection; (3) Over sixteen lymph nodes dissected; and (4) Available medical record from database.

Table 1 Univariate analysis of potential risk characteristics for lymph node metastasis *n* (%)

Factor	Lymph node metastasis	
	Positive	<i>P</i> -value
Age (yr)		
< 60 (<i>n</i> = 95)	16 (16.8)	0.494
≥ 60 (<i>n</i> = 43)	5 (11.6)	
Sex		
Male (<i>n</i> = 87)	14 (16.1)	0.748
Female (<i>n</i> = 51)	7 (13.7)	
Macroscopic type		
I (<i>n</i> = 6)	0 (0)	0.564
II (<i>n</i> = 82)	12 (14.6)	
III (<i>n</i> = 50)	9 (18.0)	
Family medical history		
Positive (<i>n</i> = 11)	2 (18.2)	0.809
Negative (<i>n</i> = 127)	19 (15.0)	
Location		
Upper (<i>n</i> = 29)	4 (13.8)	0.497
Middle (<i>n</i> = 8)	0 (0)	
Lower (<i>n</i> = 101)	17 (16.8)	
Number of tumors		
Single (<i>n</i> = 133)	20 (15.0)	0.799
Multitude (<i>n</i> = 5)	1 (20.0)	
Tumor size in diameter		
≤ 2 cm (<i>n</i> = 78)	5 (6.4)	0.005
> 2 cm (<i>n</i> = 60)	16 (26.7)	
Ulceration		
Negative (<i>n</i> = 109)	18 (16.5)	0.474
Positive (<i>n</i> = 29)	3 (10.3)	
Lymphatic vessel involvement		
Negative (<i>n</i> = 122)	11 (9.0)	< 0.001
Positive (<i>n</i> = 16)	10 (62.5)	
Depth of invasion		
Mucosa (<i>n</i> = 83)	5 (6.0)	0.002
Submucosa (<i>n</i> = 55)	16 (29.1)	

During the 25 years, a total of 138 patients (87 men and 51 women) with histopathologically poorly differentiated EGC were included for analyses. The ages of the patients ranged from 29 to 81 years (mean 49).

Dissection and classification of lymph nodes

For each patient, lymph nodes were dissected from the *en bloc* specimens. The classification was performed according to the JCGC^[8]. After careful review of specimens, an experienced surgeon gave the classification of the dissected lymph nodes^[8]. After that, the lymph nodes were sectioned and the histopathologic and immunohistochemical features were detected by eosin and hematoxylin staining and immunohistochemistry. Pathological examination for metastasis and lymphatic vessel involvement (LVI) was detected by immunohistochemistry with D2-40. We used uniform measurement standards to guarantee uniformity of treatment among the sample over the 25 years. Histologic slides were re-read in a blind manner by one pathologist. The main clinical and pathological data could be obtained from archival documents, including surgical report, conclusions of the pathologist, and the patient card.

Association between clinicopathological parameters and LNM

In this current study, we included clinicopathological

parameters according to JCGC^[8] for analysis. These parameters included family medical history of gastric cancer, gender (female, male), age (≥ 60 years, < 60 years), lymphatic vessel involvement, depth of invasion (mucosa, submucosa), macroscopic type, ulceration, tumor size (maximum dimension ≤ 2 cm, or > 2 cm), location of tumor (lower, middle, or upper stomach), number of tumors (single or multiple). As described below, the relationship between LNM and clinicopathological factors was explored.

Statistical analysis

Chi-squared test was performed to determine differences between patients with and without LNM in clinicopathological parameters. After that, multivariate stepwise logistic regression analysis was carried out to identify independent risk factors for LNM. Hazard ratio and 95% confidence interval (CI) were calculated. A *P* value < 0.05 was considered to have statistical significance. All statistical analyses were performed using SPSS v21.0 software (IBM Corp, Armonk, NY, United States).

RESULTS

Association between clinicopathological parameters and LNM

Table 1 showed the relationship of LNM and clinicopathological factors using a χ^2 test. Results showed that tumor diameter > 2 cm, the presence of LVI, and submucosal invasion were associated with a high LNM rate (*P* < 0.05). On the other hand, no significant association was observed between LNM and family medical history, macroscopic type, ulceration, location, number, age or gender.

Potential independent risk clinicopathological parameters for LNM

Univariate analysis results demonstrated that there are three significantly associated characteristics with LNM. Multivariate analysis showed that for LNM, all three characteristics were independent and significant risk factors (*P* < 0.05, Table 2).

LNM in poorly differentiated EGC

Twenty-one (15.2%) of 138 patients diagnosed with poorly differentiated EGC had LNM. The relationship between the three risk clinicopathological factors (tumor diameter > 2 cm, LVI, and submucosal invasion) and LNM was studied in poorly differentiated EGC. In poorly differentiated EGC, for patients with one, two or three risk factors, LNM rates were 7.7% (2/26), 47.6% (10/21) and 64.3% (9/14), respectively. For the other 77 patients without any of the risk factors, we did not find any LNM (Table 3).

DISCUSSION

Endoscopic treatments, such as EMR and ESD, are standard treatments for EGC. ESD is superior in allowing

Table 2 Multivariate analysis of potential risk factors for lymph node metastasis

Characters	Hazard ratio	95%CI	P-value
Tumor size			
≤ 2 cm	13.438	1.773-25.673	0.029
> 2 cm			
Lymphatic vessel involvement			
Positive	38.521	1.975-69.212	0.015
Negative			
Depth of invasion			
Mucosa	14.981	1.617-52.844	0.024
Submucosa			

CI: Confidence interval.

Table 3 Association between the three identified risk factors and lymph node metastasis in poorly differentiated early gastric cancer

Number of positive risk factors	Lymph metastasis rate
None	0% (0/77)
One	9.1% (2/26)
Two	22.2% (10/21)
Three	57.1% (9/14)

en bloc resection at the submucosal location, leading to accurate pathologic assessment of specimens^[14-16]. The dominance of ESD over surgery is less invasive, less expensive, and it better preserves physiological function^[17,18]. ESD is applied to EGC without LNM, and the indication criteria for differentiated cancer. On the other hand, even though the gastric lesions can be completely removed with ESD for patients with poorly differentiated EGC, standard gastrectomy with lymph node dissection is usually performed. However, gastrectomy may be not necessary for poorly differentiated EGC patients, of which approximately 96.6% patients with surgically treatment actually do not have LNM^[9]. Complications from gastrectomy are rare and not serious, including postoperative reflux esophagitis, dumping syndrome and impaired food intake^[19,20]. If gastric lesions can be completely removed and lymph node status can be histologically determined before gastrectomy, unnecessary surgery could be obviated. The new technique combines ESD with LLND, and not only completely resects the primary tumor but also determines the histologic status of the lymph node.

A precise prediction of the presence of LNM plays a vital role in choosing ESD for EGC. The factors that can help to predict LNM have been verified by previous studies in EGC. However, few studies have tried to explore whether ESD can be used in poorly differentiated EGC. Thus, we would like to seek a possible way to expand ESD in poorly differentiated EGC. In this study, we retrospectively examined the poorly differentiated EGC cases to confirm whether LNM could be predicted. Our data indicated that LNM has significant predictive factors, including tumor diameter > 2 cm, presence of LVI, and submucosal invasion. This present study

demonstrates that poorly differentiated EGC are in accordance with some published studies, indicating the existence of a significant correlation between the presence of LVI, submucosal invasion and large tumor size with high LNM incidence^[21-29].

During the analysis of this study, numerous relevant subgroup analyses were also done to identify patients of whom the potential LNM can be excluded and then find the candidates who are potentially curable by ESD treatment. Interestingly, we found that patients whose tumor is confined to the intramucosa, and is less than or equal to 2 cm without LVI did not have LNM, indicating that for these cases, ESD could be sufficient and over-treatment may be avoided.

In addition, the association between the positive number of the three risk factors (presence of LVI, tumor diameter > 2 cm, and submucosal invasion) and LNM rate were further studied to discuss management strategies for the treatment of poorly differentiated EGC. From the results of this study, we have determined that there is a certain association between LNM rate and number of significant risk factors. When the number of factors is one, two or three, LNM rates were 7.7%, 47.6% and 64.3%, respectively. Therefore, gastrectomy with lymphadenectomy is preferable for these patients with risk factors.

Standard gastrectomy with lymphadenectomy remains of value as standard therapy for the potential presence of LNM in poorly differentiated EGC patients. However, the combination of ESD and LLND could avoid unnecessary gastrectomy. Studies have been reported that some patients with EGC received ESD, but the surgery could not meet standard or expanded resection. Salvage treatment of LLND showed overall survival benefits^[30,31]. ESD has a high complete resection rate for localized primary tumor, and LLND has complementary surgical benefits, which could enable the confirmation of negative LNM^[32]. Thus, this combination was a survival effective strategy compared to conventional treatment. Indeed, previous data have shown that this combination has a significantly greater effect on overall survival during the long-term follow-up period^[33]. The combination of ESD and LLND has fewer complications (such as perforation, etc.) and can be used in any areas in the stomach. Therefore, the combination of ESD and

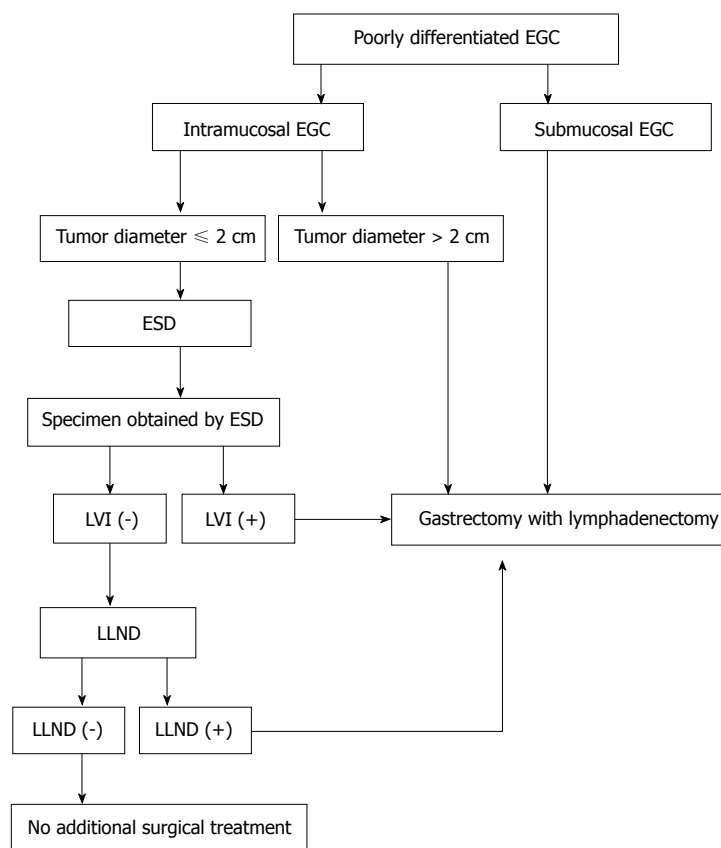


Figure 1 Flow chart of the therapeutic strategy for cases with poorly differentiated early gastric cancer. EGC: Early gastric cancer; ESD: Endoscopic submucosal dissection; LLND: Laparoscopic lymph node dissection; LVI: Lymphatic vessel involvement.

LLND may be an effective, minimally invasive treatment and beneficial for long-term quality of life in poorly differentiated EGC patients.

However, this study has several limitations. It was a single center study, and the sample size was relatively small. Moreover, our study was performed retrospectively, and the data collected were not randomized and could have been subject to associated bias. Therefore, our findings and conclusions may be not very informative to make robust conclusions. Randomized, prospective studies are needed to verify these results.

In this study, we proposed a novel treatment strategy for patients with poorly differentiated EGC (Figure 1). For patients with a tumor less than or equal to 2 cm in size or when LVI is absent upon postoperative histological examination, ESD might be sufficient treatment. The combination of ESD and LLND enables complete resection for not only the primary tumor but also the potentially metastatic lymph node. When LLND reveals LNM or specimens of ESD shows with LVI, gastrectomy with lymphadenectomy may be a better choice to achieve R0 resection. We believe that LLND may lead to the elimination of ESD in poorly differentiated EGC patients having a potential risk of LNM.

with lymphadenectomy is usually performed even though the gastric lesions can be completely removed with endoscopic submucosal dissection (ESD) due to the higher risk of lymph node metastasis (LNM). However, many surgical EGC cases actually do not have LNM, indicating that this surgery may not be necessary for many cases of EGC. To avoid this unnecessary surgery, the new technique combines ESD with laparoscopic lymph node dissection (LLND), which may lead to the elimination of unnecessary gastrectomy in poorly differentiated EGC patients having a potential risk of LNM.

Research motivation

We attempted to identify a subgroup of poorly differentiated EGC patients in whom the risk of LNM can be ruled out and treated them with ESD and LLND, which may serve as a breakthrough treatment for poorly differentiated EGC.

Research objectives

In this study, we intended to determine the risk factors that were predictive of LNM in poorly differentiated EGC patients and to provide some suggestions to guide the application of the combination of ESD and LLND for selected patients with poorly differentiated EGC.

Research methods

We retrospectively analyzed 138 patients with poorly differentiated EGC who underwent gastrectomy with lymphadenectomy (between January 1990 and December 2015). We also retrospectively analyzed (by univariate and multivariate logistic regression analyses) the association between the clinicopathological factors and the presence of LNM. We further examined the relationship between the positive number of the significant predictive factors and the LNM rate.

Research results

Tumor size, depth of invasion and lymphatic vessel involvement were found to be independently risk clinicopathological factors for LNM in poorly differentiated

ARTICLE HIGHLIGHTS

Research background

For patients with poorly differentiated early gastric cancer (EGC), gastrectomy

EGC. Furthermore, we established a simple criterion to expand the possibility of using ESD and LLND for the treatment of poorly differentiated EGC.

Research conclusions

ESD might be sufficient treatment for intramucosal poorly differentiated EGC if the tumor is less than or equal to 2 cm in size, and when lymphatic vessel involvement is absent upon postoperative histological examination. We found that the ESD with LLND may lead to the elimination of unnecessary gastrectomy in poorly differentiated EGC.

Research perspectives

The minimization of therapeutic invasiveness in order to preserve quality of life is a major topic in the management of EGC. One of the critical factors in choosing minimally invasive surgery for EGC would be the precise prediction of whether the patient has LNM. Therefore, in the future, we will carry out this retrospective study to determine the clinicopathological factors that are predictive of LNM in EGC and to guide the individual application of minimally invasive surgery in a suitable subgroup of patients with EGC.

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Upgraded role of autophagy in colorectal carcinomas

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Abstract

Autophagy is a basic catabolic process closely associated with degradation of cellular components. The role of autophagy in colorectal cancer (CRC) remains controversial. The mechanism of autophagy has been identified as protecting mechanism against tumorigenesis by isolation of damaged organelles or as cytoprotective provides energy in hypoxic regions of CRC tumors. Mutations in proto-oncogenes, such as *RAS* and *BRAF*, have been associated with autophagy initiation through signaling pathways of BRAF/MEK/ERK and PI3K/AKT/mTOR. A combination therapy of chemotherapeutic agents and autophagy inhibitors such as hydroxychloroquine or immunotherapy might represent a major step that could be evaluated as a putative novel therapeutic strategy in CRC patients.

Key words: Autophagy; Tumorigenesis; Clinical trials; Autophagy inhibitors; Colorectal cancer

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Core tip: The significant role of autophagy in maintaining the balance of tumorigenesis and cancer cell death remains controversial. The last decade grown body of evidence support the notion that autophagy is a promising target for many malignant tumors, including colorectal cancer (CRC). A novel therapeutic approach which could involve autophagy inhibitors or immunotherapy plus chemotherapeutic drugs could open a new field for treating patients with CRC.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most commonly diagnosed malignancies leading to many cancer-related deaths worldwide. Some patients are initially diagnosed with metastatic CRC (mCRC), while 20% of CRC patients will eventually develop metastases, thus emphasizing the importance of novel effective treatment options^[1].

Many studies have shown that CRC is closely associated with the cytoprotective mechanism of autophagy, a self-digesting process in cells. The last decade, many studies have identified and characterized autophagy as an important mechanism in mammalian systems, in healthy state and during carcinogenesis^[2]. Cancer cells have the ability to use autophagy mechanism in trafficking of many oncogenic factors, such as chemotactic, pro-invasive or pro-inflammatory molecules and/or angiogenic molecules. Malignant tumors that use autophagy have the ability to change their micro-environment through the regulation of crosstalk between cancerous and stromal cells. This is a significant property which has been described in many chemotherapeutic treatment approaches^[3]. Three different types of autophagy have been so far identified; macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy has been closely associated with the formation of phagophore which engulfs cytosolic proteins for degradation in lysosomes^[4].

ROLE OF ONCOGENES IN AUTOPHAGY INITIATION

It is well experienced that the majority of mCRC patients eventually develop acquired resistance during their chemotherapy-based treatment. Oncogenes such as *EGFR*, *RAS* and *BRAF* have been characterized as key elements in the modulation of resistance mechanisms in mCRC. Additionally, these oncogenes regulate the cytoprotective mechanism of autophagy. *EGFR* is responsible for activation of signaling pathways that affect autophagy, among them PI3K-AKT-mTOR^[5]. This pathway inhibits autophagy through the formation of PI3K-Beclin-1 homodimers. On the other hand, *BRAF*-depend signaling pathway (*BRAF*/MEK/ERK) has been shown to trigger autophagy *via* up-regulation of Beclin-1^[6]. Moreover, several studies support the idea that *BRAFV600E* mutation induces the expression of autophagic markers; light chain 3 and Beclin-1 in CRC cells. Additionally, anti-EGFR MoAbs (such as cetuximab and panitumumab) induce autophagy which acts as a protective response in CRC cells. Several studies have described that mutant *RAS* can prevent the formation of autophagophore in autophagy machinery through the reduction of *BECN1* expression^[7].

CONTROVERSIAL ROLE OF AUTOPHAGY IN CRC

The controversial role of autophagy in CRC development has been supported by a plethora of data. Cancer cells have been found to require high basal levels of autophagy for cell proliferation^[8]. In already established tumors, autophagy has been associated with the hypoxic tumor regions where the metabolic demands are increased. The increasing levels of autophagy in hypoxic regions of tumors have also been associated with the modulation of immunosurveillance and immunosuppression in tumor microenvironment^[9]. In addition, advanced tumors appear to be addicted in autophagy to maintain their energy balance. Through autophagy, cancer cells recycle intracellular components and build pro-tumorigenic factors. *KRAS*-dependent tumors also use autophagy machinery to maintain basic components to support cancer cells' growth under stressful condition^[10].

AUTOPHAGY IN CLINICAL PRACTICE

The mechanism of autophagy has been suggested as a crucial modulator that can be targeted to improve the effect of anti-neoplastic drugs in several tumors, including mCRC. This notion has led to the development of agents that inhibit autophagy, thereby improving treatment outcome. The last decade many molecules that inhibit autophagy have been developed. Autophagy inhibitors, such as chloroquine and its analog hydroxychloroquine (HCQ), have been shown to decrease autophagy through the disruption of lysosomal function^[6]. The anti-antineoplastic effect of these agents has been assessed in the clinical setting. Phase I and II clinical trials have already evaluated the efficacy of the combination of HCQ and chemotherapy (e.g., oxaliplatin, fluorouracil) and anti-angiogenic agents (e.g., bevacizumab) in mCRC patients. Furthermore, mCRC patients have achieved disease stabilization after combining HCQ with vorinostat^[11]. Further elucidation of the effect of the currently existed as well as developing autophagy inhibitors in CRC patients is of paramount importance due to the dual role of autophagy in CRC.

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Ampulla of Vater carcinoma: Molecular landscape and clinical implications

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Abstract

Ampulla of Vater is a peculiar anatomical structure, characterized by the crossroad of three distinct epithelia: Intestinal, ductal pancreatic and biliary. Adenocarcinomas arising in this area represent an opportunity to understand the comparative biology of all periampullary malignancies. These neoplasms can exhibit intestinal, pancreaticobiliary or mixed features, whereas the sub-classification based on morphology and immunohistochemical features failed in demonstrating a robust prognostic reliability. In the last few years, the molecular landscape of this tumor entity has been uncovered, identifying alterations that may serve as prognostic and predictive biomarkers. In this review, the histological and genetic characteristics of ampullary carcinomas are discussed, taking into account the main clinical and therapeutic implications related to this tumor type as well.

Key words: Pancreatobiliary; Intestinal; Mixed; *ELF3*; *TP53*; *KRAS*; Ampullary; Vater; Histotype

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Core tip: Ampulla of Vater carcinomas comprise tumors with intestinal and/or pancreaticobiliary differentiation, but such histotypical classification is of little help for their prognostic stratification. Integration of the recently reported molecular profiles with histopathological and clinical information furnishes novel keys for fostering the development of a more efficient prognostic stratification and the identification of novel therapeutic strategies.

Pea A, Riva G, Bernasconi R, Sereni E, Lawlor RT, Scarpa A, Luchini C. Ampulla of Vater carcinoma: Molecular landscape and clinical implications. *World J Gastrointest Oncol* 2018; 10(11): 370-380 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i11/370.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i11.370>

INTRODUCTION

Ampullary neoplasms represent a wide array of tumors arising in the ampulla of Vater, the most common of which is represented by ampulla of Vater carcinoma (AVC), although other rare malignancies, such as neuroendocrine tumors, may be encountered in this location^[1-3]. AVC comprises 30% of pancreaticoduodenectomies and 20% of all tumor-related obstructions of the common bile duct^[4-6]. Data from the surveillance, epidemiology, and end results registries have indicated an increased number of new diagnoses in the last years, with the average age at diagnosis ranging from 60 to 70 years old^[6-8]. The etiology of ampullary carcinoma has not been clearly defined and an association with a noninvasive component displaying the adenoma-to-carcinoma sequence similar to colorectal carcinoma may be present^[9,10].

The ampulla of Vater region presents very peculiar histological aspects, as it represents a crossroad of three different epithelia: Intestinal, ductal pancreatic and biliary. This kind of structure characterizes this area, with a unique complexity and morphological heterogeneity^[1]. From the histological point of view, coupling morphological and immunohistochemical analyses, AVCs have been subgrouped into intestinal and pancreatobiliary subtypes based on the epithelium of origin; in case of coexistence of aspects of both subtypes, the mixed category has been introduced for a more precise classification^[1,11-14]. However, the former classification has been challenged by lines of evidence showing a significant interobserver variability upon the interpretation of these patterns, and the mixed subtype being the predominant subgroup of AVCs, representing up to 40% of cases^[15-17]. In addition, poorly differentiated tumors can further confound the histological classification^[1]. The prognostic significance of this histological classification has been subjected to investigation with inconsistent results^[15-18] that will be briefly discussed in this review.

In recent years, much progress has been made in characterizing the molecular alterations underlying AVC tumorigenesis, showing a complex mutational spectrum that supports only in part the distinction in different histological subtypes^[14,17]. Molecular analysis showed alterations in overlapping pathways that may serve as foundation for developing new therapeutic approaches and may improve early prognostication models. In this review, we will discuss the histological and genetic landscape of AVCs and its clinical implications, with a specific focus on the treatment of choice and on the future perspectives related to this important topic.

HISTOPATHOLOGY

Gross appearance and location

According to the gross appearance and location, AVCs can be divided into three different categories: (1) intraampullary neoplasms, characterized by a intraluminal growth pattern, without extension out of the Oddi's sphincter; (2) periampullary neoplasms, with a significant vegetating component on the duodenal surface of the ampulla, usually adenomatous, noninvasive, and frequently characterized by an ulcerating part corresponding to the invasive component; and (3) mixed neoplasms, which show both intraampullary and vegetating growth^[18-21]. In all of these cases, the ampullary region has a typical enlarged macroscopic appearance (Figure 1).

Histology and immunohistochemistry

In 2010, the World Health Organization revised the criteria for the pathological diagnosis of ampullary carcinoma to include three distinct histopathological subtypes on the basis of morphology and immunohistochemical characteristics: (1) the intestinal-type AVCs; (2) the pancreatobiliary-type AVCs; and (3) the mixed-type AVCs^[1].

The intestinal type is frequently associated with a noninvasive component (duodenal adenoma). Its morphology is characterized by a colorectal-like architecture, with tubular or cribriform glands and central necrosis (Figure 2)^[11,22]. The invasive component is usually smaller than in the pancreatobiliary type and less frequently exhibits adverse pathological factors and lymphovascular and perineural invasion^[23-26]. This AVC subtype usually expresses intestinal immunomarkers, such as caudal-related homeodomain transcription factor 2 (CDX2), mucin2 (MUC2) and cytokeratin 20 (CK20)^[27].

The pancreatobiliary type is morphologically similar to pancreatic ductal adenocarcinoma or to the cancer of the extra-pancreatic bile duct. Complex tubular glands composed of atypical cells and associated with a prominent desmoplastic stroma characterized this subtype (Figure 3)^[11,22]. At immunohistochemistry, those cells stain positively for MUC1, MUC5AC and CK7^[27].

A significant proportion of AVCs, ranging between 18% and 40%, presents a hybrid phenotype charac-

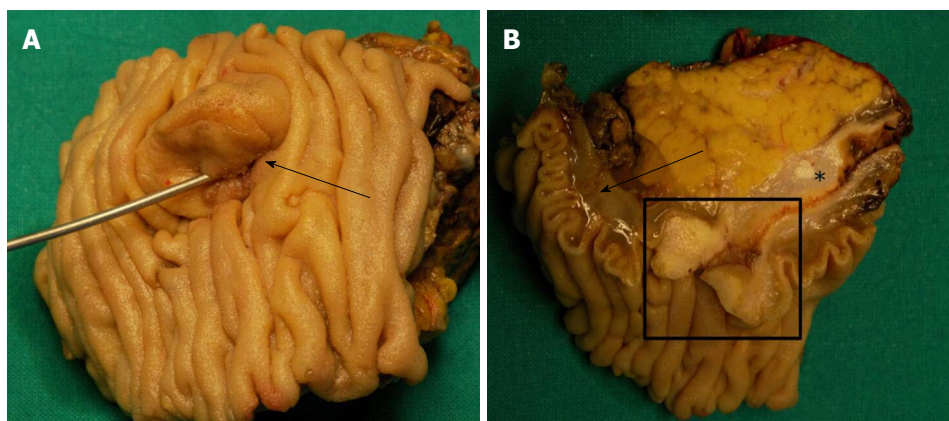


Figure 1 A classic example of the macroscopic appearance of a case of ampulla of Vater carcinoma. A: The ampullary area is markedly enlarged (black arrow); B: On the section surface, the ampulla of Vater carcinoma (black box), the adjacent duodenal wall (black arrow) and bile duct (asterisk) are clearly visible.

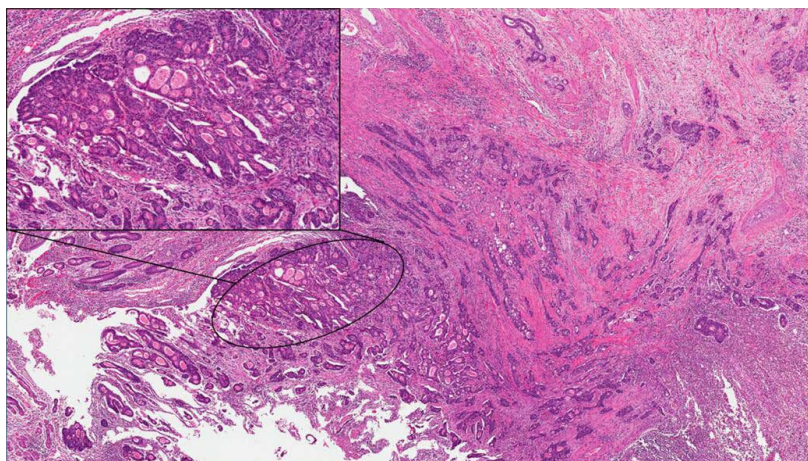


Figure 2 A classic example of intestinal-type ampulla of Vater carcinoma. At low magnification ($2 \times$ original magnification) and at higher magnification (the box in the upper left corner, $10 \times$ original magnification) to better show its histological features. The lesion is composed of a colorectal-like architecture, with glands characterized by comedo-like necrosis.

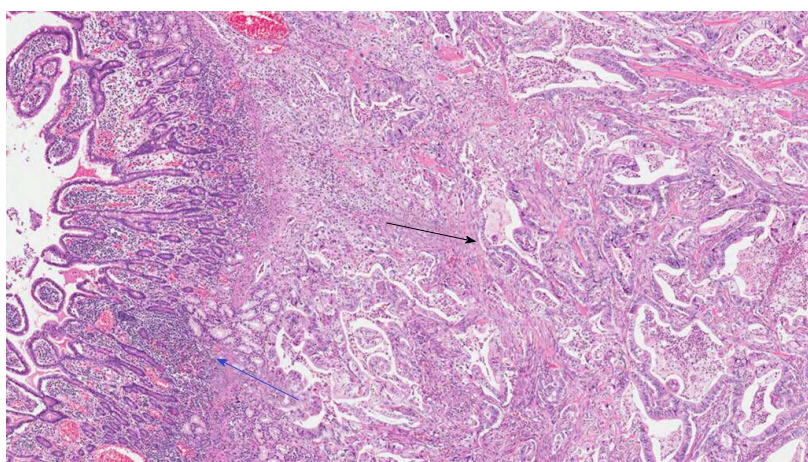


Figure 3 A classic example of pancreaticobiliary-type ampulla of Vater carcinoma (original magnification: $20 \times$). The lesion is composed of ductal adenocarcinoma-like glands (black arrow) invading the duodenum (blue arrow).

terized by overlapping intestinal and pancreatobiliary features^[28,29] and frequently by a nondistinctive immunohistochemistry (Figure 4)^[28]. These aspects partially

explain the high interobserver variability among pathologists in classifying AVCs subtypes^[15,16,28].

Different immunohistochemical panels have been

Table 1 Proposed immunohistochemical markers for ampulla of Vater carcinoma histological classification (adapted from Mafficini *et al.*^[16])

Immunohistochemical marker criteria present	Intestinal type	Pancreatobiliary type positive	Mixed/Ambiguous type	Note
Ang <i>et al.</i> ^[12] (MUC1, MUC2, CDX2, CK20)	Positive CK20 or CDX2 or MUC2, and negative MUC1 Positive CK20 and CDX2, and MUC2 and any MUC1	Positive MUC1 and negative CDX2, and negative MUC2 and any CK20	All other combinations	
Chang <i>et al.</i> ^[13] (MUC1, CDX2)	Positive CDX2 or negative MUC1	Negative CDX2 and positive MUC1	Not applicable	CDX2 positivity based on H score (percentage of positive cells × intensity of staining) > 35 MUC1 positivity based on any staining
Gingras <i>et al.</i> ^[17] (MUC1, CDX2)	Ratio of the CDX2/MUC1 H score ≥ 2	Ratio of the CDX2/MUC1 H score < 0.5	Ratio of CDX2/MUC1 H score ≥ 0.5 and < 2	Use only MUC1 and CDX2 as per Chang <i>et al.</i> ^[13] , with H scores for both CDX2 and MUC1
Mafficini <i>et al.</i> ^[16] (MUC1, MUC2, CDX2, CK20)	Positive CK20 or CDX2 or MUC2, and negative MUC1	Positive MUC1 and negative CDX2, and negative MUC2 and any CK20	All other combinations	

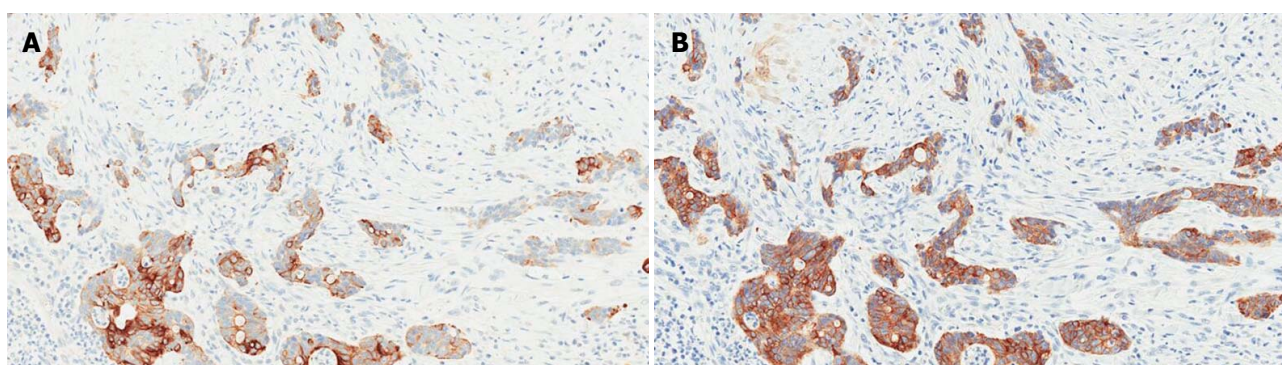


Figure 4 Immunohistochemical analysis of an ampullary adenocarcinoma of mixed subtype (original magnification 20 ×). A: Immunohistochemical analysis of an ampullary adenocarcinoma of mixed subtype, with cytokeratin 20 (CK20); B: Immunohistochemical analysis of an ampullary adenocarcinoma of mixed subtype, with cytokeratin 7 (CK7). This image highlights that, in the same area, some neoplastic glands may be positive not only for CK7 or for CK20, but for both markers even. The coexpression of an intestinal marker, such as CK20, and of a pancreatobiliary marker, such as CK7, supports the classification as mixed subtype.

suggested to overcome the difficulties in histological classification, also in order to stratify AVCs prognosis (Table 1)^[12,13,15-17]. A 4-marker panel including MUC1, CK20, CDX2 and MUC2 has been proposed by Ang *et al.*^[12]. This panel has shown improved capacities in defining intermediate/mixed cases, although its correlation with clinical outcomes has not been evaluated. Chang *et al.*^[13] proposed a 2-marker panel, composed of CDX2 and MUC1, showing that the PB phenotype was associated with a poor prognosis. However, more recent studies questioning the accuracy and reproducibility of this method failed in identifying direct or significant prognostic correlations with the immunohistochemical patterns^[15,16]. Notably, alterations in the “gastric” lineage marker MUC5AC have also been associated with poor outcome in AVCs, but further studies are needed to validate its prognostic role^[15].

The morphological heterogeneity that characterizes a significant proportion of AVCs and the lack of a

prognostic reliability of the histological classification, either individually or within immunohistochemical panels, led to the integration of molecular alterations into clinical practice in order to better define AVCs prognosis and treatment.

GENETIC LANDSCAPE

Although AVCs are usually sporadic neoplasms, they can also arise in the context of familial syndromes. Particularly, patients with familial adenomatous polyposis (FAP) frequently develop duodenal adenomas and have a 100- to 200-fold increased risk of developing AVCs^[7,30,31]. A previous seminal manuscript has indicated that sporadic AVCs differ from those occurring in FAP, according to frequency (17% vs 64%), as well as in the site of APC somatic mutations, suggesting a different molecular pathogenesis for the two conditions^[32]. The molecular basis for AVCs initially concentrated on chro-

mosomal alterations, indicating chromosome 5 loss as an early event in AVC carcinogenesis, and chromosome 17p loss as a poor prognostic moderator^[33,34].

Recent advances in sequencing technologies have permitted the in-depth characterization of the AVC molecular profile, providing important insights for the comprehension of the biology of this malignancy^[14,16,17]. Particularly, two different whole exome sequencing analyses for a total of 240 patients have refined the knowledge about the mutational landscape of AVCs^[14,17]. Both studies confirmed the presence of recurrent alterations in well-known AVC-related genes, including *TP53*, *KRAS* and those belonging to the Wnt-pathway, such as *APC*; at the same time, *ELF3* has been indicated as a novel AVC driver gene in this kind of tumor^[14].

The association between driver mutations and histological subtypes has been evaluated with conflicting results. The *APC* gene, an important actor of the Wnt-signaling pathway, is frequently mutated in the intestinal subtype (50%-65% of cases), similar to colorectal cancer^[35], while the pancreatobiliary type exhibits a higher prevalence of mutations in the pancreatic driver genes *KRAS*, *TP53* and *SMAD4*, with similar frequencies to pancreatic cancer^[14,17,36].

Although histological subtypes show differences in prevalence for some genes (Table 2), important drivers, including *KRAS*, *TP53* and *ELF3*, can be found mutated in all histotypes. The lack of a specific genetic signature for the histological types suggests the existence of common biological mechanisms in the development of ampullary carcinoma, highlighting the heterogeneity of AVCs from the morphological to the molecular levels. This further calls for a reconsideration of the utility of the histological classification, since the genetic landscape indicates the lack of a specific distinction corresponding to morphology^[16].

Both the recent whole-exome sequencing studies described inactivating mutations in the tumor-suppressor gene *ELF3*, in respectively 10% and 12% of cases^[14,17]. In particular, Yachida *et al.*^[14] demonstrated with functional analyses a role of such a gene as an AVC driver. *ELF3* encodes an ETS-domain transcription factor that is implicated in the regulation of epithelial differentiation. Using immortalized epithelial cell lines derived from the common bile duct and duodenal mucosa and knocked down for *ELF3* expression, they demonstrated *ELF3* to enhance proliferation, motility and invasion, associated with the concomitant up-regulation of markers of epithelial-to-mesenchymal transition, such as vimentin, matrix metalloproteinase-1 (MMP1) and MMP9^[14]. However, the exact functional role of *ELF3* as well as its potential role as a prognostic biomarker or target for therapy needs to be further investigated.

Interestingly, *ERBB2* amplification has been demonstrated in up 23% of cases^[16,37]. In a recent report, it was observed in 13% of AVCs regardless of histological subtype and was virtually mutually exclusive with downstream mutations in *KRAS/NRAS/BRAF*, that are responsible for resistance of therapies targeting *ERBB2*^[37].

Molecular profiling of AVCs has recently demonstrated a higher prognostic reliability than the histological subclassification. Indeed, analyzing a cohort of 80 AVCs, Mafficini *et al.*^[16] showed that *TP53* and *KRAS*, which were the most frequently mutated genes, were in respectively 41% and 35% of cases, were also independent prognostic predictors of survival regardless of histological subtypes. These data underline the importance of the mixed phenotype and the fact that the ampullary region is composed of various epithelia merging to form the complex epithelium of the ampulla. Common molecular alterations among different subtypes, such as *TP53* and *KRAS*, may indeed represent drivers of tumor progression at an early stage of disease. Whereas other genetic alterations, such as those belonging to the Wnt-pathway and those characterizing the pancreatobiliary type, such as *SMAD4* and *CDKN2A*, may occur at later stages of tumor growth^[14,17].

Current treatment approaches do not distinguish patients based on subtypes^[38,39], while molecular alterations may select patients that respond to different chemotherapeutic regimens, regardless of a clear histological differentiation^[17]. In particular, clinical testing for Wnt-signaling and microsatellite instability (MSI) could be used to subclassify tumors for target therapies since therapies targeting the Wnt-pathway are in development and MSI-positive tumors may respond to immunotherapeutic approaches^[17]. The detection of molecular alterations typical of late-stages may in the future support the choice of radical surgery with lymphadenectomy, rather than more conservative approaches. This highlights the importance of genetic analysis and the need of its future integration within the conventional pathology report.

TUMOR STAGING

The staging of AVCs is challenging due to the high complexity of this district and the three-dimensional spread pattern of tumors occurring in this region. In the new AJCC Cancer Staging System Manual, 8th edition^[21], the pathological tumor (pT) stages have been reclassified, taking into account the degree of extensions and therefore improving the clinical and prognostic relevance of each pT stage (Table 3). In particular, new subsets for pT1, pT2 and pT3 have been introduced according to survival analyses and suggesting further prognostic variability^[40]; the new pT4 stage comprises tumors involving peripancreatic arteries/axes, harmonizing with the exocrine pancreatic cancer staging system.

Metastatic lymph nodes are present in up to 60% of surgically resected AVCs^[41-43], with a higher rate for pancreatobiliary than intestinal type carcinomas (55% vs 18%)^[11]. The new staging system categorized the presence of nodal metastases in a three-tiered scale: N0 (no metastatic lymph node), N1 (one or two metastatic lymph nodes) and N2 (three or more metastatic lymph nodes); this subclassification has demonstrated a better predictive value in stratifying the prognosis than

Table 2 Frequency of significantly mutated ampulla of Vater carcinoma genes in different histotypes and compared to colorectal and pancreatic adenocarcinoma (adapted from Yachida *et al.*^[14])

Yachida <i>et al.</i> ^[14]			Gingras <i>et al.</i> ^[17]		Biankin <i>et al.</i> ^[36]	Colorectal Carcinoma (TCGA), % ^[35]
Intestinal type, %	Pancreato-biliary type, %	Mixed type, %	Pancreato-biliary type, %	Intestinal type, %	Pancreatic carcinoma, %	
APC (50)	KRAS (68)	KRAS (50)	TP53 (72)	TP53 (65)	KRAS (99)	APC (81)
TP53 (39)	TP53 (67)	APC (50)	KRAS (65)	KRAS (46)	TP53 (33)	TP53 (60)
KRAS (39)	SMAD4 (20)	TP53 (41)	SMAD4 (18)	APC (41)	SMAD4 (16)	KRAS (43)
CTNNB1 (26)	CTNNB1 (15)	SMARCA4 (27)	CDKN2A (16)	PIK3CA (26)	MLL3 (7)	TTN (31)
ARID2 (18)	ERBB3 (14)	PIK3CA (23)	PIK3CA (13)	SMAD4 (20)	ATM (5)	PIK3CA (18)
ERBB2 (14)	GNAS (12)	SMAD 4 (23)	ARID1A (13)	TGFBR2 (17)	NALCN (5)	FBXW7 (14)
ACVR2A (13)	CDH10 (12)	SOX 9 (23)	APC (11)	ARID2 (17)	ARID1A (4)	SMAD4 (10)
SMAD4 (13)	ELF3 (11)	CDKN2A (23)	ATM (10)	ELF3 (7)	SF3B1 (4)	NRAS (9)
GNAS (13)	CDKN2A (9)	ARID1A (18)	TGFBR2 (10)	CTNNB1 (17)	TGFBR2 (4)	TCF7L2 (9)
SOX9 (13)		TGFBR2 (14)	FBXW7 (8)	NF1 (15)	ARID2 (3)	FAM123B (7)

Table 3 Ampulla of Vater cancer staging AJCC 2017^[21]

Primary tumor (T)	
T category	T criteria
TX	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor limited to ampulla of Vater or sphincter of Oddi, or tumor invades beyond the sphincter of Oddi (perisphincteric invasion) and/or into the duodenal submucosa
T1a	Tumor limited to ampulla of Vater or sphincter of Oddi
T1b	Tumor invades beyond the sphincter of Oddi (perisphincteric invasion) and/or into the duodenal submucosa
T2	Tumor invades into the muscularis propria of the duodenum
T3	Tumor directly invades the pancreas (up to 0.5 cm) or tumor extends more than 0.5 cm into the pancreas, or extends into peripancreatic or periduodenal tissue or duodenal serosa without involvement of the celiac axis or superior mesenteric artery
T3a	Tumor directly invades pancreas, up to 0.5 cm
T3b	Tumor extends more than 0.5 cm into the pancreas, or extends into peripancreatic tissue or duodenal serosa without involvement of the celiac axis or superior mesenteric artery
T4	Tumor involves the celiac axis, superior mesenteric artery, and/or common hepatic artery, irrespective of size
Regional lymph nodes (N)	
N category	N criteria
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis to 1 to 3 regional lymph nodes
N2	Metastasis in > 3 regional lymph nodes

the previous dichotomous categories N0 (no metastatic lymph node) vs N1 (at least one metastatic lymph node). To reach a reliable value, the gross sampling of the surgical specimen should include a minimum of 12 lymph nodes^[44]. However, since pancreato-duodenal nodes are the most frequently involved and are usually resected within the specimen (pancreatico-duodenectomy), even if the minimum threshold of 12 is not met, pN0 should still be assigned. Notably, a preferential lymphatic spread from pancreato-duodenal nodes to lymph nodes around the superior mesenteric artery has been suggested, highlighting the importance of a systemic and radical lymphadenectomy in this area^[45].

The risk for lymph node metastases according to the T stage is clinically relevant since endoscopic ampullectomy has been proposed for early AVCs. Surgical series assessed a 8%-45% risk of lymph node metastases in tumors limited to ampulla of Vater and/or sphincter of Oddi (pT1a and pT1b, respectively, of the

new staging system)^[42,46-48]. The role of local excisions in surgically fit patients remains, being therefore controversial due to the relevant risk of lymph node metastases also in resected early cancers. Another pT-related issue regards the extra-nodal extension of nodal metastases, a histological feature indicating that the metastatic cells have reached the perinodal adipose tissue. In the new staging system, it has been not taken into account, whereas it has been demonstrated as an important prognostic factor in patients with AVC and other solid malignancies^[49-57].

Other prognostic factors not included in the staging system but with a potential prognostic role are included among the histologic grading and the perineural invasion.

CLINICAL ASPECTS

In the majority of cases, AVCs are present with obs-

tructive jaundice, resulting in a high resectability rate at diagnosis^[4,58,59]. Other symptoms, although less common, are upper gastrointestinal bleeding, pancreatitis and unspecific abdominal pain^[60-62]. Ampullary tumors can even be incidentally discovered during endoscopic procedures or at cross-sectional imaging performed for other reasons. Despite the potentially high resectability rate, only up to 40% of patients undergo surgical resection^[6], mostly due to the advanced age of presentation and the significant morbidity and mortality associated with pancreatic surgery.

The diagnostic work-up usually involves abdominal imaging using ultrasonography, computed tomography and/or magnetic resonance, aiming at excluding other causes of jaundice and at disease staging. Because of the anatomical location and the frequent small size of the tumor, an ampullary mass is often difficult to detect, but indirect signs such as pancreatic and/or bile duct obstruction/dilation can be observed^[62,63].

Endoscopy plays a major role in the differential diagnosis of an altered papilla (either bulging or ulcerated) as well as in the local staging of the disease. Endoscopic biopsies are characterized by high false negative rates for adenocarcinoma, often underestimating the actual pathology^[47,64], whereas endoscopic ultrasonography (EUS) guided-biopsies improve the diagnostic accuracy, assessing the correct pathology in almost 90% of the cases^[65]. In the local staging of ampullary masses, EUS plays a primary role thanks to its capacities of estimating the depth of tumor infiltration within the duodenal wall and in predicting the presence of local node metastases^[66-68], although their definitive demonstration is reserved for the histological examination.

ENDOSCOPIC VS SURGICAL TREATMENT

Radical resection represents, to date, the only established curative option for AVCs, while an endoscopic papillectomy is indicated for noninvasive tumors. A radical resection with an adequate lymphadenectomy is usually recommended for invasive tumors, even if very small, due to the nonnegligible risk of lymph node metastasization or of incomplete tumor resection. The correct local staging is essential to guide further treatment decisions.

Endoscopic papillectomy

Endoscopic papillectomy is the treatment of choice for benign or noninvasive ampullary lesions. When EUS shows a lesion confined within the mucosa, and there are not histological features of invasion or of high-grade dysplasia upon biopsy, endoscopic ampullectomy should be performed^[69,70]. The following histological examination of the endoscopic specimen must report the status of the resection margins and consider the potential presence of an invasive component^[66,71,72]. In the case of

high-grade dysplasia determined by endoscopic biopsy, an underlying adenocarcinoma on definitive pathology is present in 50%–100% of patients and usually in the context of voluminous intestinal-like villous adenomas, usually larger than tubular adenomas, and for which a radical endoscopic ampullectomy may be difficult^[66,73].

However, endoscopic ampullectomy should be considered part of the diagnostic process and potentially curative in cases of high-grade dysplasia and clear resection margins at the final pathological evaluation of the specimen. Considering the significant morbidity and mortality associated with pancreatic surgery, endoscopic papillectomy has also been suggested for early ampullary carcinoma, in particular for pT1 tumors^[46,48,68]. However, to date, this indication remains to date controversial, mainly due to the clinically relevant risk of lymph node metastases and the high rate of positive resection margins, reserving this procedure for patients unfit for surgical resection^[74]. Endoscopic ampullectomy is a safe procedure, characterized by a relatively low rate (about 10%) of postprocedural complications, the most common being acute pancreatitis, followed by papillary stenosis, cholangitis and bleeding^[75-78]. Most of these complications can be prevented by the placement of temporary pancreatic and biliary stents^[77,79,80].

Surgery

Surgical ampullectomy has been proposed as an alternative to pancreaticoduodenectomy for selected patients with ampullary neoplasms^[81]. This procedure is characterized by lower morbidity and mortality than major surgery, also allowing for performance of a partial lymphadenectomy (excluding the lymph nodes from the superior mesentery artery). However, its role in the treatment of AVCs is controversial, for the difficulties to obtain a radical resection^[47,71,82]. Surgical ampullectomy shares the same complications of the endoscopic ampullectomy, with the risk of duodenal dehiscence and intra-abdominal collections as well as additional complications^[71,83].

The current acceptable standard of care for resectable AVCs remains the pancreatoduodenectomy, either with conventional or pylorus-preserving approach^[42,46,47,84]. Surgery for AVCs is characterized by a high resectability rate, with close to 90% of cases undergoing laparotomy^[7,24,85], but also by a higher rate of significant complications than pancreatoduodenectomies performed for pancreatic cancer. Such complications include pancreatic fistula, pneumonia, intra-abdominal infection, anastomotic leak, and delayed gastric emptying^[86].

FUTURE PERSPECTIVES

The histological subtypes have revealed major issues on both interobserver reproducibility and its prognostic reliability. Since the ampulla of Vater is the crossroad of three distinct epithelia, the study of the tumors arising

in such a location represents a unique opportunity to better refine the knowledge about all periampullary cancers. The anatomical features of the ampulla of Vater may explain the histological heterogeneity of AVCs and the importance of also taking into account the mixed entity. Indeed, a significant part of this tumor type does not meet all the criteria for a definitive subclassification as intestinal or pancreaticobiliary-type. On the basis of such considerations, the integration of the molecular data appears as a fundamental step in understanding AVCs' biology, helping in better stratifying the prognosis, and highlighting potential targets for tailored therapy. Future therapeutic research studies should investigate, more in-depth, the AVCs histological and molecular features, which may represent the key to resolving intestinal-pancreaticobiliary heterogeneity.

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Laparoscopic and endoscopic cooperative surgery for gastric tumors: Perspective for actual practice and oncological benefits

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Abstract

Laparoscopic and endoscopic cooperative surgery (LECS) is a surgical technique that combines laparoscopic partial gastrectomy and endoscopic submucosal dissection. LECS requires close collaboration between skilled laparoscopic surgeons and experienced endoscopists. For successful LECS, experience alone is not sufficient. Instead, familiarity with the characteristics of both laparoscopic surgery and endoscopic intervention is necessary to overcome various technical problems. LECS was developed mainly as a treatment for gastric submucosal tumors without epithelial lesions, including gastrointestinal stromal tumors (GISTs). Local gastric wall dissection without lymphadenectomy is adequate for the treatment of gastric GISTs. Compared with conventional simple wedge resection with a linear stapler, LECS can provide both optimal surgical margins and oncological benefit that result in functional preservation of the residual stomach. As technical characteristics, however, classic LECS involves intentional opening of the gastric wall, resulting in a risk of tumor dissemination with contamination by gastric juice. Therefore, several modified LECS techniques have been developed to avoid even subtle tumor exposure. Furthermore, LECS for early gastric cancer has been attempted according to

the concept of sentinel lymph node dissection. LECS is a prospective treatment for GISTs and might become a future therapeutic option even for early gastric cancer. Interventional endoscopists and laparoscopic surgeons collaboratively explore curative resection. Simultaneous intraluminal approach with endoscopy allows surgeons to optimize the resection area. LECS, not simple wedge resection, achieves minimally invasive treatment and allows for oncologically precise resection. We herein present detailed tips and pitfalls of LECS and discuss various technical considerations.

Key words: Minimally invasive surgery; Laparoscopic and endoscopic cooperative surgery; Facility-based; Gastrointestinal stromal tumor; Early gastric cancer

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Core tip: Laparoscopic and endoscopic cooperative surgery (LECS) was first described as a treatment of gastric submucosal tumors in 2008, although a similar concept had been developed before that time. Thereafter, many researchers described LECS as a feasible technique for gastric resection, regardless of tumor location. LECS is a novel procedure that minimizes invasive damage to patients and preserves physiologic function of the residual stomach while securing oncological benefit. Currently, many physicians can fully utilize the advantages of LECS for gastric submucosal tumors located even at the esophagogastric junction by avoiding conventional total gastrectomy or proximal gastrectomy. This technique requires close cooperation between skilled surgeons and experienced endoscopists. Therefore, many tips and pitfalls should be discussed to accelerate this collaboration during LECS. We hope that the herein-described tips will benefit laparoscopic surgeons and interventional endoscopists who are interested in LECS.

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INTRODUCTION

Minimally invasive surgery is currently available for benign and borderline malignant tumors of the stomach^[1-3]. Resection is a curative treatment for submucosal tumors (SMTs) and early gastric cancer (EGC)^[4]. Many endoscopic physicians and general surgeons focus on the invention of novel tools and innovation of technical procedures^[3,5,6]. Various therapeutic options have become well developed^[2,3,5,7,8]. Interventional

endoscopists continue to search for techniques with curative resectability [e.g., endoscopic submucosal dissection (ESD)]^[9-11], and it was previously considered that endoscopic full-thickness resection is possible only by a surgical approach^[12,13]. Since laparoscopy-assisted gastrectomy was first reported in 1994^[14], a drastic evolution of laparoscopic surgery has occurred in parallel, and skilled laparoscopic surgeons now precisely perform minimally invasive segmental resection^[15-18]. A smooth postoperative course, good functional outcome, and rapid recovery after such procedures have been established^[15-17].

Each approach has its own strengths and limitations^[3,10]. Hence, a hybrid approach (*i.e.*, cooperation between endoscopic intervention and laparoscopic surgery) was developed^[3]. This technique aims to accumulate the strong points of intraluminal and intraperitoneal procedures and negate the technical limitations^[3]. This novel concept has been described using different names (*e.g.*, hybrid laparoscopic, combined laparoscopic and endoscopic, laparoscopic-endoscopic rendezvous, and cooperative laparoscopicendoscopic procedures)^[3,19-21]; however, use of these multiple terms might confuse endoscopic physicians and general surgeons. Despite the differing names, this hybrid concept focuses on a simultaneous approach via intraluminal and intraperitoneal pathways, subsequent precise resection with oncologic principles, and physiological closure of the defect^[3,22,23].

Optimal resection techniques for gastric SMTs and EGC have been established based on the oncologic behaviors of these lesions^[22,23]. Laparoscopic and endoscopic cooperative surgery (LECS), not simple wedge resection, achieves minimally invasive treatment and allows for precise resection of these tumors^[3]. We herein focus on LECS with a review of previous literature and describe the actual procedures, including technical tips and pitfalls. Moreover, this hybrid approach is discussed with respect to extended indications, oncological benefits, and technical developments.

HISTORY

From an oncological viewpoint, the clinical and pathological behaviors of EGC and SMTs, including gastrointestinal stromal tumors (GISTs), have been well investigated^[22,23]. Partial or segmental resection is considered acceptable based on oncologic principles^[3,22,23]. General surgeons have an interest in minimally invasive treatment by laparoscopic local resection for SMTs and EGC^[24-26]. Simple wedge resection is very easy to perform for most SMTs with extraluminal growth^[27]; however, a laparoscopic approach is often difficult with respect to accessing the posterior wall, and postoperative stenosis may occur near the esophagogastric junction (EGJ) or pyloric ring.

Gastric cancer originates from the mucosa, and

some SMTs are accompanied by intraluminal growth. A dilemma faced by interventional endoscopists is that endoscopic full-thickness resection is impossible without surgical assistance^[3,12,13]. In Japan, laparoscopic wedge resection using a lesion-lifting method was reported for treatment of SMTs with intraluminal growth and EGC^[28-31], and a stabbing tool with a T-shaped bar was developed for partial lifting of the target wall^[31,32]. However, this lesion-lifting method cannot minimize the resected area because the staple line cannot be determined by an intraluminal approach, and use of this method may increase the rate of positive surgical margins^[21].

LECS has long been attempted for treatment of EGC and SMTs^[21,33-35]. Interventional endoscopists and laparoscopic surgeons collaboratively explore the potential for curative resection (*i.e.*, a facility-based method) based on the abilities of the physicians at each individual institution^[36]. In laparoscopy-assisted endoscopic resection, laparoscopic surgeons assist in resolution of accidental perforation or control of blood loss^[37]. In endoscopic-assisted wedge resection, the target gastric wall is resected by linear staplers under intraluminal observation after laparoscopic mobilization of the stomach^[37,38]. This combined resection procedure is the most commonly performed because of its technical simplicity^[37,39]. Simple wedge resection and the lesion-lifting method are associated with difficulty in resection of tumors located in the posterior wall; thus, surgeons have also developed laparoscopic transluminal or intraluminal surgeries (*i.e.*, endoscope-assisted laparoscopic intraluminal surgery^[32,40,41], endoscope-assisted laparoscopic transluminal surgery^[42,43], and endoscope-assisted laparoscopic intragastric stapling^[44-46])^[3]. The resection line can be determined during transluminal or intraluminal surgeries, although these surgeries involve a gastric incision for creation of an intraluminal pathway and require advanced skills^[3,21,32].

Novel cooperative laparoscopic and endoscopic techniques for gastric tumors (EGC and SMTs) have been developed mainly in Asian regions^[34,47-50]. Procedures of both ESD and LECS originate in Japan, and this may be the reason why LECS is mainly developed in Asian countries so far. The term "LECS" was first reported in 2008^[50]; thereafter, this combined procedure was commonly referred to as LECS. Previously established procedures (*e.g.*, the lesion-lifting method^[31] and laparoscopy-assisted endoscopic resection^[37]) might retrospectively be included as types of LECS procedures. Many physicians have demonstrated that LECS for gastric tumors (mainly SMTs) is feasible and safe.

LECS as described above involves intentional opening of the gastric wall and thus has a risk of tumor dissemination via gastric juice and contamination of the peritoneal cavity by enterobacteria^[3,48,51]. LECS is therefore performed for gastric SMTs (mainly GIST), and the indications for LECS have been limited to

cases without epithelial lesions including depressed lesions and/or ulcers^[3,48]. To overcome this limitation and expand the indications for LECS, several modified LECS procedures have been developed (*e.g.*, inverted LECS^[47], laparoscopy-assisted endoscopic full-thickness resection^[52], nonexposed endoscopic wall inversion surgery^[53-57], clean non-exposure technique^[58], closed LECS^[51], and lift-and-cut method^[59]) and are currently applied to patients even with epithelial lesions. These novel LECS procedures are based on a clear concept of full-thickness resection without intentional perforation (*i.e.*, no exposure of gastric juice) for tumors accompanied by epithelial lesions.

SIMPLE WEDGE RESECTION BY A LINEAR STAPLER

Until LECS became well developed, simple wedge resection was generally conducted as a curative treatment for gastric SMTs. Wedge resection by a linear stapler has the advantage of avoiding the risk of intraoperative dissemination during laparoscopic surgery^[60]. Another advantage of wedge resection is its technical simplicity and lack of requirement for advanced skills^[3]. However, this simplicity easily results in rough resection and oncological inadequacy^[61]. The simple wedge resection technique is associated with both excessive and inadequate resection of the gastric wall, which may lead to postoperative gastric stenosis, gastric dysfunction, and local recurrence^[62,63]. Hence, simple wedge resection by a linear stapler is considered a technically easy but high-risk procedure^[3,62].

CLASSIC LECS

LECS is a surgical technique that combines laparoscopic partial gastrectomy and ESD (Figure 1A). This combined technique is used mainly for gastric SMTs, such as GISTs. The simultaneous intraluminal approach with endoscopy allows surgeons to resect the gastric wall according to the appropriate cutting line without excessive or inadequate margins^[63]. From an oncologic viewpoint, LECS optimizes the resection area by providing sufficient margins as a curative resection for gastric SMTs (Figure 1B). This is the most advantageous point of LECS compared with other approaches. Even if an SMT is located near the EGJ, optimal and precise resection by LECS may avoid the need for proximal gastrectomy.

As described above, modified LECS procedures using the concept of "no exposure" have been established for tumors accompanied by epithelial lesions^[47,51-58]. The first documented version of LECS^[50] has been categorized as "classic LECS" to distinguish it from other modified LECS procedures^[48].

Table 1 Clinical outcomes of laparoscopic endoscopic cooperative surgery

Author	Ref. ¹	Published year	Patient number (case)	Age ²	Gender (male/female)	BMI ² (kg/m ²)	Procedures	Diagnosis	Size ² (mm)	Conversion rate to gastrectomy or laparotomy (%)	Positive surgical margin (%)	Complications (treatment and case number)	Mortality	Recurrence rate (%)	Follow-up period ² (mo)
Hiki <i>et al</i>	[50]	2008	7	53 ± 6	0/7	22.0 ± 1	Classic LECS	SMT	46	0	0	None	0	-	-
Kikuchi <i>et al</i>	[51]	2017	10	62	5/5	-	Closed LECS	SMT	24.1	0	-	Intra-abdominal abscess (n = 1)	0	0	12
Mitsui <i>et al</i>	[56]	2014	6	60	4/2	-	NEWS	SMT	34	0	0	None	0	0	8
Inoue <i>et al</i>	[58]	2012	24	66.2	-	-	Clean-NET	EGC	-	-	-	Gastric deformity (Reoperation, n=1)	0	0	-
Okumura <i>et al</i>	[59]	2017	28	67.6	8/20	-	Lift-and-cut method	GIST	33	0	0	None	0	0	26.6
Matsuda <i>et al</i>	[71]	2016	100	59.8	47/53	22.7 ± 3.3	Classic LECS	SMT	30.9	5	0	Leakage (n = 1) Postoperative stenosis (n = 2) Postoperative bleeding (n = 1)	0	0	25.3
Tsujimoto <i>et al</i>	[72]	2012	20	59.3 ± 11.9	10/10	21.8 ± 2.7	NEWS	SMT	37.9 ± 11	0	0	None	0	0	20.7
Kawahira <i>et al</i>	[73]	2012	16	61	4/12	22.1	Classic LECS	SMT	27.5	0	0	Lymphorrhea (n = 1)	0	0	-
Hoteva <i>et al</i>	[74]	2014	25	60	10/15	-	LECS	SMT in EGJ	32.3	0	0	None	0	0	18

¹See the reference list; ²Data were given as mean ± SD, or the median. BMI: Body mass index; Clean-NET: Clean non-exposure technique; EGC: Early gastric cancer; EGJ: Esophagogastric junction; GIST: Gastrointestinal stromal tumor; LECS: Laparoscopic endoscopic cooperative surgery; NEWS: Non-exposed endoscopic wall-inversion surgery; SMT: Submucosal tumor.

INDICATIONS

The indications for LECS should be considered based on the patient's disease, institutional ability, and individual skills^[3,36]. Hence, the indications for LECS may be affected by both tumor- and facility-related factors^[36]. Indication and contraindication for LECS are mainly considered based on three factors (*i.e.*, the tumor's characteristics, institutional ability and individual skills). Other clinical factors (*e.g.*, age, gender, body mass index and comorbidity) never affect the indication for LECS, and these factors in previous documents are summarized in Table 1. In our institution, all patients with a suspicious diagnosis of a gastric GIST routinely undergo gastrointestinal endoscopy, an upper gastrointestinal series, endoscopic ultrasound, and enhanced computed tomography to identify the size and location of the tumor. Moreover, a preoperative pathological diagnosis is made by ultrasound-guided fine-needle aspiration because the therapeutic strategy will be affected by the pathological assessment. For example, although lymph node dissection is not required for surgical treatment of GISTs^[64], some SMTs (*e.g.*, carcinoid or submucosal carcinoma and submucosal adenocarcinoma) require lymph node dissection during surgery^[65]. In our institution, patients diagnosed with EGC are treated by robot-assisted gastrectomy with lymph node dissection^[66].

Classic LECS is mainly employed for gastric SMTs, and a GIST is a common target tumor. As described above, opening the gastric wall is associated with a risk of tumor dissemination via gastric juice^[3,48,51], and classic LECS has limitations in the treatment of epithelial lesions^[3,48]. From the viewpoint of tumor size, however, laparoscopic surgery for larger gastric GISTs is thought to carry a higher risk of tumor capsule injury^[67]. The National Comprehensive Cancer Network and European Society for Medical Oncology argue that there is no good evidence in support of laparoscopic surgery for GISTs of > 5 cm^[68], although skilled physicians emphasize that laparoscopic surgery

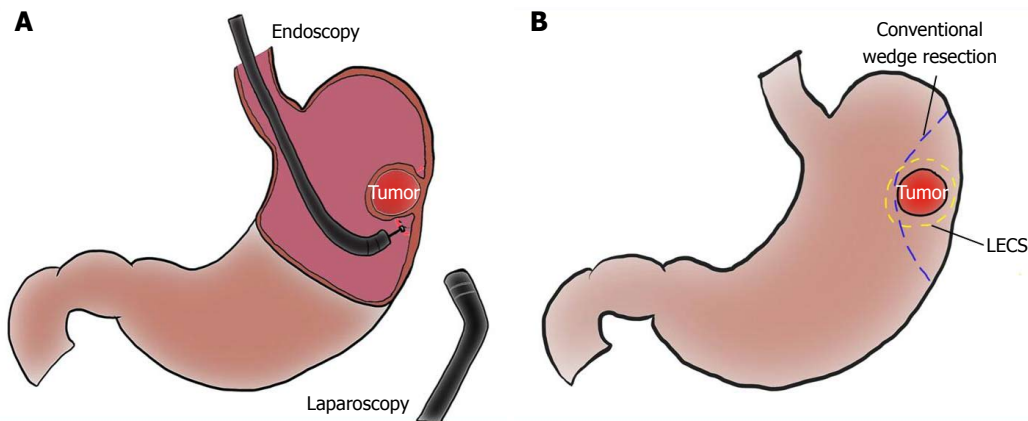


Figure 1 Schema of laparoscopic and endoscopic cooperative surgery, and comparison of resection line between laparoscopic and endoscopic cooperative surgery and conventional wedge resection. A: Laparoscopic and endoscopic cooperative surgery (LECS) is a combined procedure involving laparoscopy and endoscopy; B: The resection line of LECS minimizes the surgical margin, securing an adequate distance from the tumor. Conventional wedge resection is too close to the tumor and involves excessive wall dissection.

for gastric GISTs is safe and feasible regardless of tumor size^[69,70]. In our institution, we generally apply laparoscopic surgery to gastric GISTs of ≤ 5 cm in diameter, and we employ LECS only to intraluminal types without epithelial lesions. As a prerequisite, we routinely have detailed preoperative discussions with the patients and obtain adequate informed consent.

Skilled physicians have demonstrated that laparoscopic surgery can be applied to gastric GISTs of larger size and/or epithelial lesions if surgical and oncological safety (e.g., tumor location, layers involved/occupied, expected malignancy of the tumor, institutional ability, and individual skills) are guaranteed^[3,33,36,47,51-58,69,70]. Skilled physicians have also documented that LECS is feasible and safe for gastric SMTs in any location^[37,50,71-74]. LECS was recently applied to duodenal SMTs^[75]. However, application of LECS to SMTs near the EGJ should be carefully considered because laparoscopic suturing in this region requires advanced skill to avoid postoperative stenosis and leakage^[34,76-78]. In fact, when the tumor covers more than one-third of the whole circumference of the EGJ, patients have a high rate of conversion to open surgery or proximal gastrectomy^[71]. Tumor occupation of more than one-third of the whole circumference of the EGJ should be a contraindication for LECS. Although no definitive risk factors for anastomotic stenosis and postoperative leakage have been established, surgeons should not hesitate to convert to open surgery or proximal gastrectomy during laparoscopic surgery if surgical and oncological safety cannot be guaranteed.

INITIAL SET-UP FOR INTERVENTIONAL ENDOSCOPY AND LAPAROSCOPIC SURGERY

LECS is performed under general anesthesia in the leg-

open position. Both arms of the patient are fixed along the body to avoid interference with the procedures performed by the interventional endoscopists. The primary surgeon stands on the right side of the patient, and the assistant surgeon stands on the opposite side. The laparoscopist stands between the patient's legs. Both the interventional and assistant endoscopists stand beside the patient's head. The arrangement of various apparatuses and medical staff members in the operation room is shown in Figure 2A.

The patient is placed in the supine position with the head directed straight. The tracheal intubation tube has already been inserted through the mouth. Even if the patient's face can be slightly turned toward the left for endoscope insertion, the interventional endoscopists are repeatedly forced to handle the endoscope under unfamiliar situations (*i.e.*, supine body position, straight face direction, and competitive oral tube). Endoscopists must continuously perform very careful handling of the devices and patient, and placement of a flexible overtube (ST-SB1S; Olympus Medical Systems Corporation, Tokyo, Japan) is a solution for stress-free endoscopic maneuvers. Moreover, as described later, an overtube is a powerful tool for tumor removal *via* the mouth.

For the endoscopic intervention, an endoscopic system with fine vision and advanced apparatuses, including energy devices, is set up as for ESD. An insulation-tipped diathermic knife (ITknife2, KD-611L; Olympus Medical Systems Corporation) and soft coagulation system (VIO 300 D; Erbe, Tübingen, Germany) are prepared.

A camera port is placed on the umbilicus. Three additional ports (two 5-mm ports and one 12-mm port) are inserted into the left upper, left lower, and right upper quadrants, respectively, under pneumoperitoneum of 12 mmHg with a laparoscopic view. One additional 5-mm port in the right lower quadrant is acceptable, if

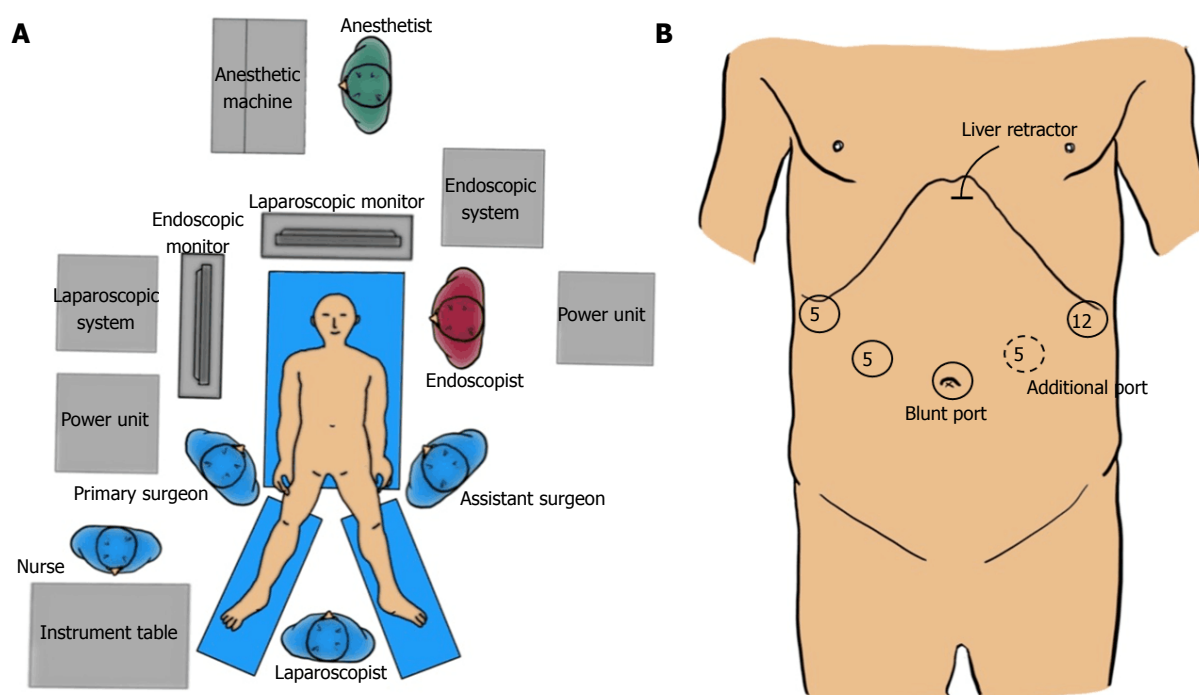


Figure 2 Set-up of staffs and devices in the operation theater and port placement. A: Apparatus position and staff placement in the operation room; B: Port placement.

necessary (Figure 2B).

During LECS, the laparoscopic surgeon should never forget that both the pneumoperitoneal pressure and light intensity are higher on the laparoscopic than endoscopic side. Under the conventional settings of usual laparoscopic surgery, interventional endoscopists cannot secure an adequate field because the stomach would collapse by pneumoperitoneal pressure and cannot obtain fine vision because the laparoscopic light would be too dazzling. The laparoscopic settings of these two factors should be optimally adjusted as necessary during LECS. In our institution, we adjust the light intensity manually as needed and downregulate the pneumoperitoneal pressure to 4 to 6 mm Hg while the interventional endoscope is being operated. However, the endoscopic setting is the same as or similar to that of usual ESD, according to the physician's preference.

ANATOMICAL RECOGNITION

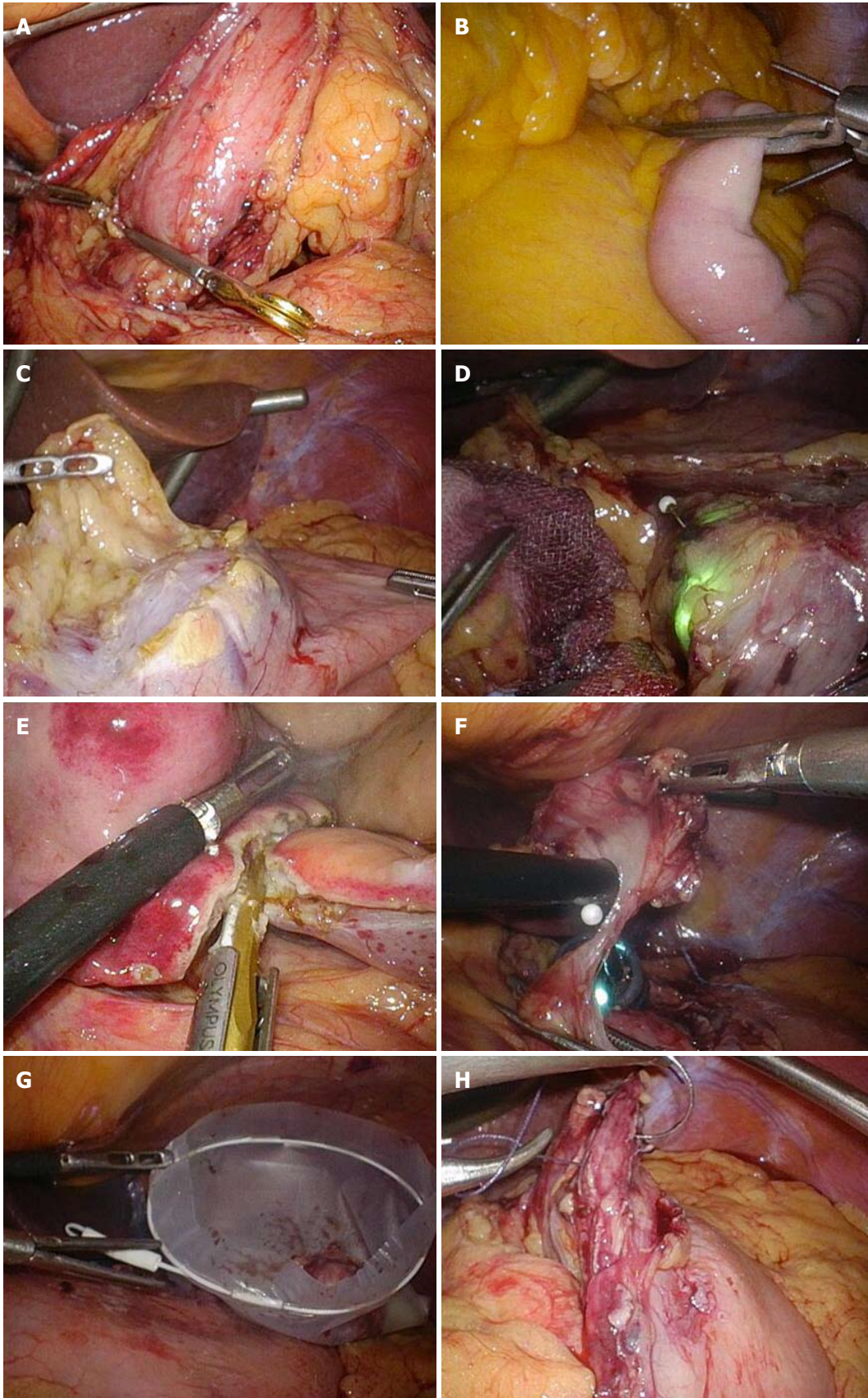
The stomach is fixed by ligaments and tendons that surround organs and structures such as the hepato-duodenal ligament, celiac axis, pancreatic capsule, crura of the diaphragm, and spleen. The target gastric wall should be mobilized ventrally with a free space made by carbon dioxide gas to ensure the safety of the interventional endoscopic procedure. Even subtle injury to the surrounding organs (*e.g.*, pancreas and aorta) during the endoscopic intervention should be avoided. Especially for SMTs at the posterior wall or EGJ,

adequate dissection of the posterior side is key to good mobilization of the target stomach wall. In patients with GISTs, the target gastric wall is directly exposed because of rare metastasis to the regional lymph nodes^[64].

PERITONEAL APPROACH BY A LAPAROSCOPIC VIEW

First, the tumor location is identified. Although gastric tumors are intraluminal, the tumor location can often be found from the extraluminal view because the gastric wall is slightly depressed or elevated. If the tumor location cannot be detected via the laparoscopic view, it should be confirmed by the endoscopic view. Excessive dilatation of the digestive tract by endoscopic insufflation of carbon dioxide should be prevented before the start of the intraluminal endoscopic investigation. Clamping of the antrum or jejunum should be performed using clamp forceps (PL541S; B. Braun Aesculap, Tokyo, Japan). Technically, placement of a jejunal clamp at about 10 cm on the anal side of the Treitz ligament is easier than placement of an antral clamp (Figure 3A and B), although an antral clamp provides a better surgical field by prevention of duodenal dilatation (Figure 3A). Notably, endoscopic insufflation into the intestines will remarkably disturb the laparoscopic field. In contrast, the stomach is well expanded by insufflation and clamping, providing an intraluminal working field for the endoscopic intervention.

The surrounding fat tissue and vessels of the gastric



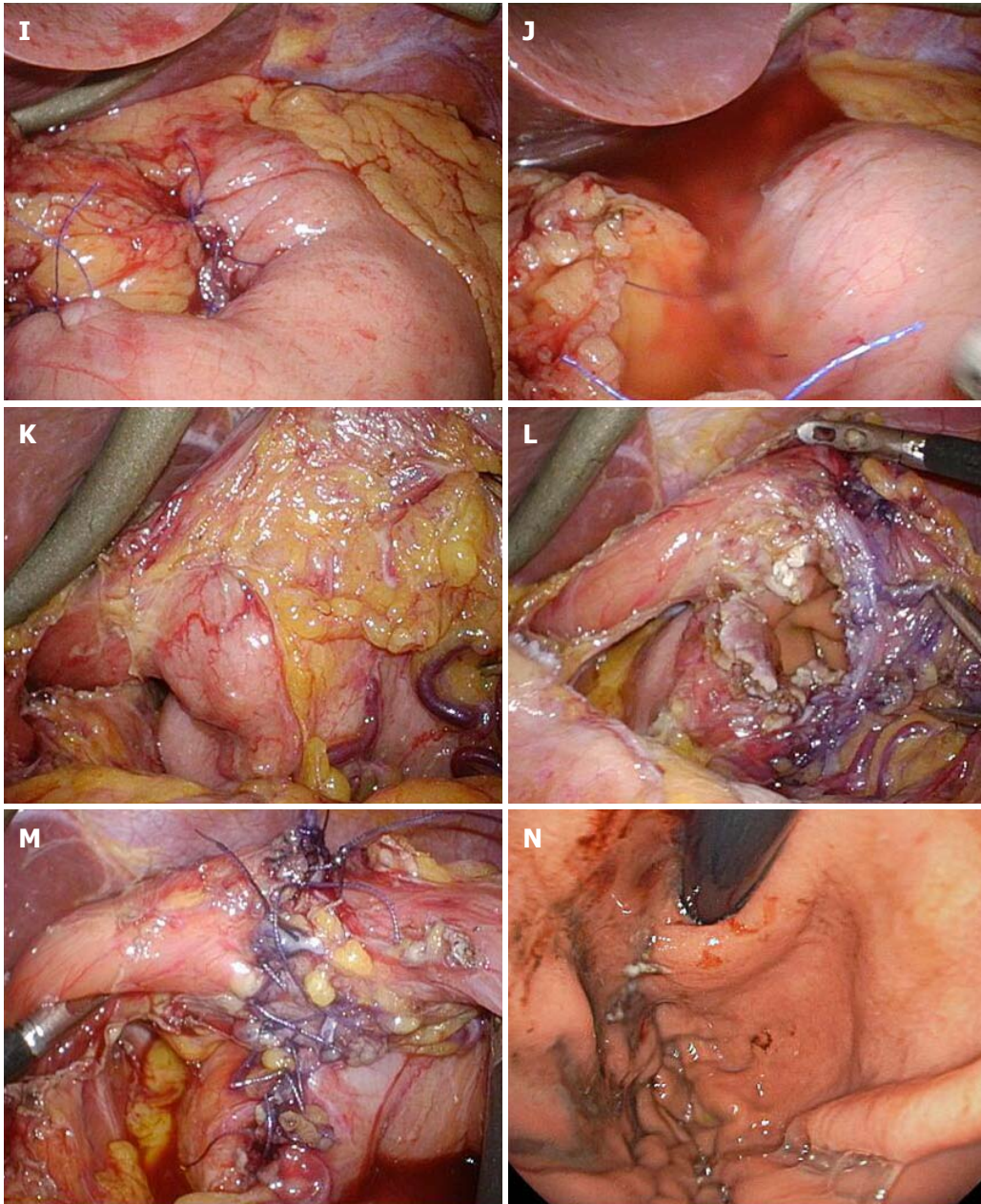


Figure 3 Intraoperative laparoscopic view of laparoscopic and endoscopic cooperative surgery. A and B: Clamping of the (A) antrum or (B) jejunum should be performed using clamp forceps. This allows for adequate gastric expansion that provides an intraluminal working field for the endoscopic intervention; C: The surrounding fat tissue and vessels of the gastric wall are dissected, and the target wall is then mobilized to the ventral side; D: The laparoscopic surgeon should mobilize the gastric wall and prevent it from touching any surrounding organs for a safe intraluminal intervention. The pneumoperitoneal pressure and light intensity of laparoscopy are decreased to avoid disturbing the endoscopist; E: The laparoscopic surgeon can dissect the proximal gastric wall on behalf of the interventional endoscopist, if necessary; F: The surgeon and the endoscopist cooperate to complete the operation while avoiding injury to the adjacent organs; G: The resected specimen is placed in a plastic bag and removed intraluminally using endoscopy; H: The mucosal layer is closed with a running 4-0 absorbable suture thread; I: The seromuscular layer is closed with interrupted 3-0 absorbable sutures; J: A leak test is performed after suturing. K: This image depicts a case involving a tumor located in the posterior wall near the EGJ; The target gastric wall is turned as much as possible with a marginal free space established by carbon dioxide gas. The right side of the EGJ has enough working space laparoscopically; L: The defect of the gastric wall tends to become larger than many physicians expect; M: The defect in the gastric wall is closed with the laparoscopic hand-sewn technique in a layer-to-layer fashion; N: Intraluminal view after suturing. The absence of stenosis and malformation is confirmed. EGJ: Esophagogastric junction.

wall are confirmed. To mobilize the stomach, omental fat tissue is cut while preserving the vessels coursing into the stomach (mainly gastroepiploic vessels). When excising the lesser omentum, the gastric branch of the vagus nerve should be maximally preserved to prevent postoperative gastroparesis. After the stomach mobilization, the stomach should be twisted until the

target wall faces the ventral side to ensure the safety of the gastric wall during the endoscopic intervention (Figure 3C). Briefly, the target gastric wall never touches any surrounding organs (e.g., pancreas and aorta) (Figure 3D). The ventrally mobilized target wall should then be exposed with a marginal free space established by carbon dioxide gas. Adequate dissection is performed

near the tumor and traced to the stomach, and the gastric wall around the tumor is exposed and mobilized to the ventral side. This process is very important to prevent unexpected injury to adjacent organs (e.g., pancreas, liver, aorta, and spleen). Laparoscopic surgeons can dissect the proximal gastric wall with the assistance of interventional endoscopists, if necessary (Figure 3E). The surgeon and the endoscopist cooperate to complete the operation without injuring the adjacent organs (Figure 3F).

Determination of the cutting line with optimal margins based on the endoscopic findings is an oncological benefit. Although the cutting line is set by the interventional endoscopist, resection of the seromuscular layers can be performed with either the interventional endoscopists' insulation-tipped diathermic knife or the laparoscopic surgeon's ultrasonic coagulation shears. The resected specimen is placed in a plastic bag and removed intraluminally using endoscopy (Figure 3G).

The defect in the gastric wall is closed with a layer-to-layer laparoscopic hand-sewn technique. The mucosal layer is closed with a running suture using 4-0 absorbable suture thread (4-0 VICRYL, SH-1; Ethicon, Cincinnati, OH, United States). To prevent laxity of the running suture, an assistant surgeon holds the end of the last suture with a needle forceps, which has a strong grip force without any slip. The seromuscular layer is then closed with interrupted sutures using 3-0 absorbable suture thread (3-0 VICRYL, SH-1; Ethicon) (Figure 3H and I). When suturing is completed, a leakage test should be performed. The absence of air leakage should be confirmed by excessively inflating the stomach with endoscopy under adequate saline accumulation using a laparoscopic irrigation device (Figure 3J). The clamp forceps must be removed when the laparoscopic surgery is finished.

The upper stomach is a common site of SMTs, especially GISTs^[4,79]. GISTs are frequently located at the fornix/fundus and/or near the EGJ^[76,79]. When tumors are located in the posterior wall near the EGJ or in the antrum near the pylorus, ventral mobilization of the stomach wall around the tumor is generally left incomplete. Two solutions are available in such cases. If the SMT has no epithelial lesion, one solution is utilization of the concept of transluminal and intraluminal surgeries, as described above. The gastric wall can be incised to approach the tumor in patients without a possibility of tumor dissemination. The other solution is endoscopic intervention performed under incomplete mobilization but secure surgical fixation of the stomach wall. Mobilization of the stomach is performed, and the target gastric wall is then turned as much as possible with a marginal free space created by carbon dioxide gas. The right side of the EGJ has enough laparoscopic working space^[17]. In our institution, the stomach wall around the tumor is securely fixed by laparoscopic forceps, with a marginal free space even if this space is not located ventrally (Figure 3K). When the incision

extends to the EGJ, the defect of the gastric wall tends to become larger than many physicians expect (Figure 3L). In such cases, closure of the larger defect should be started at the far side from the laparoscopic surgeons because the surgical field is unclear if the open defect remains on the far side (Figure 3H). Laparoscopic hand-sewn suturing is completed in a layer-to-layer fashion (Figure 3M). To avoid postoperative anastomotic stenosis, esophageal patency and gastric passage are endoscopically confirmed after suturing (Figure 3N). If the endoscope is set through the EGJ as a guide to prevent anastomotic stenosis, the EGJ caliber will be sustained during suturing. Notably, any damage or injury induced by the suture needles should be carefully avoided.

ORAL APPROACH BY ENDOSCOPIC VISUALIZATION

For an oral approach by endoscopic visualization, the location of the tumor is first confirmed (Figure 4A). The periphery of the tumor is then marked using argon plasma coagulation as close as possible to the tumor edge (Figure 4B). After injection of 10% glycerin mixed with indigo blue into the submucosal layer (Figure 4C), a small initial incision is made with a dual knife (Dual knife, KD-650L; Olympus Medical Systems Corporation), and the tip of an insulation-tipped diathermic knife is inserted into the submucosal layer. The whole circumference of the marked area is then cut using the insulation-tipped diathermic knife (Figure 4D). Finally, an intentional perforation is made (Figure 4E), and seromuscular dissection is circumferentially performed according to the determined line of the submucosal layer. The laparoscopic light is too dazzling for the endoscopic side (Figure 4F). The stomach rapidly collapses after gastric perforation, and thereafter, maintenance of an adequate intragastric field for endoscopic manipulation becomes difficult. Laparoscopic surgeons must help the endoscopist to appropriately perform these procedures, avoiding injury to the adjacent organs. According to determined cutting line with optimal margins, resection of the seromuscular layers can be performed by either the interventional endoscopist's insulation-tipped diathermic knife or the laparoscopic surgeon's ultrasonic coagulation shears. Especially when cutting the proximal side of the ventrally mobilized gastric wall, the interventional endoscopist may encounter some difficulties because of the reversed endoscopic image (Figure 5). Laparoscopic vision from the umbilicus may be a good solution to this problem. If necessary, the laparoscopic surgeon can dissect the proximal gastric wall on behalf of the interventional endoscopist. The absence of stenosis or malformation should be confirmed after suturing (Figure 4G).

The resected specimen is placed in a plastic bag (Rusch MemoBag; Teleflex, Tokyo, Japan) and removed

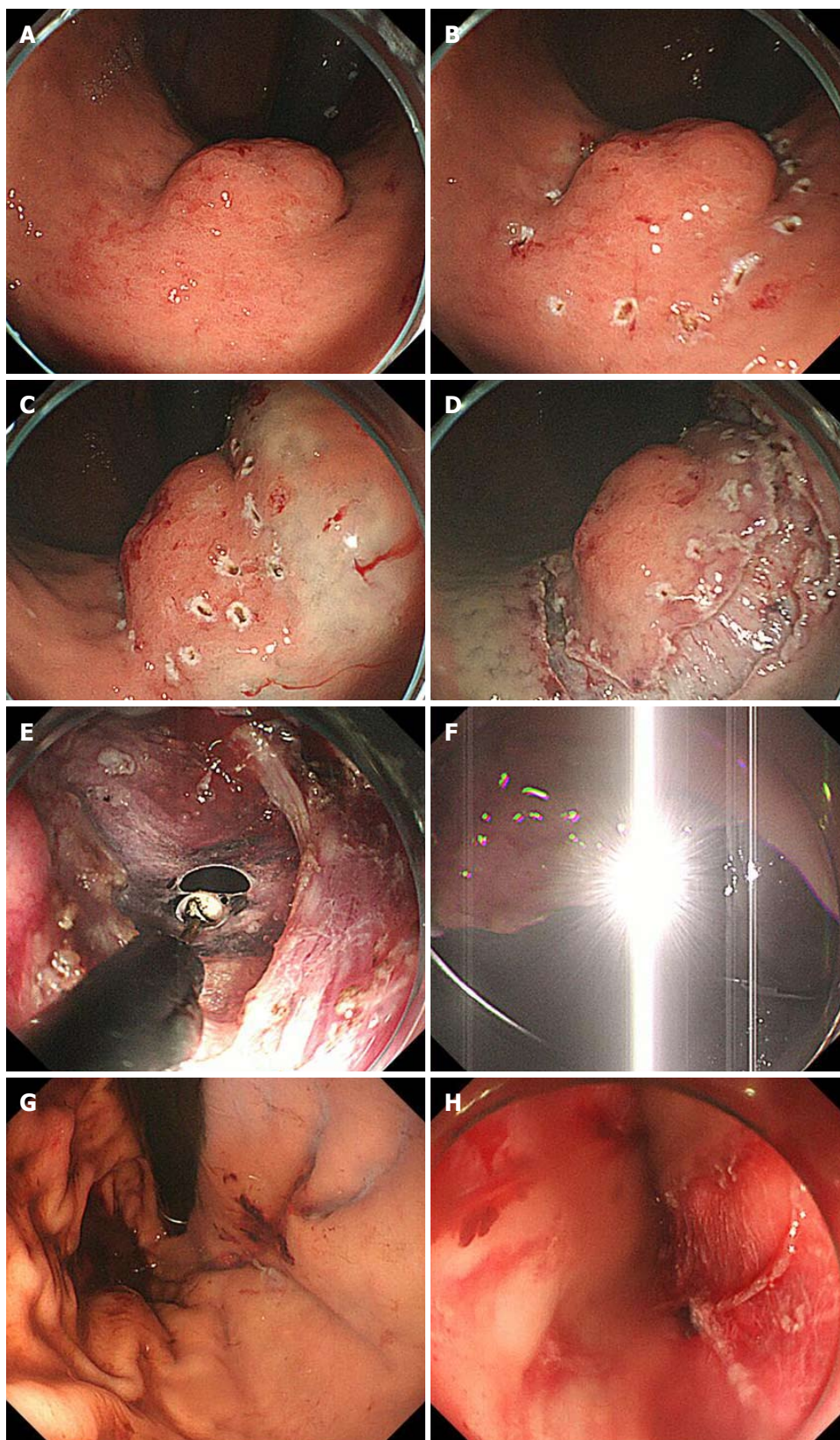


Figure 4 Intraoperative endoscopic view of laparoscopic and endoscopic cooperative surgery. A: First, the location of the tumor is confirmed; B: The periphery of the tumor is marked using argon plasma coagulation as close as possible to the tumor edge; C: Glycerin mixed with indigo blue is injected into the submucosal layer; D: The whole circumference of the marked area is cut using an insulation-tipped diathermic knife; E: An intentional perforation is made; F: The laparoscopic light is too dazzling for the endoscopic side; G: Intraluminal view after suturing. The absence of stenosis and malformation is confirmed; H: Esophageal mucosa injury by the plastic bag during specimen removal.

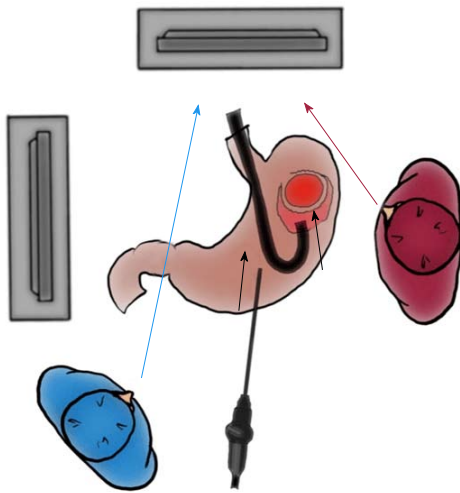


Figure 5 Importance of interventional endoscopist's line of vision while cutting the proximal side. The interventional endoscopist may experience some difficulties while cutting the proximal side of the gastric wall because of the reversed endoscopic image. If such difficulties are encountered, the endoscopist should turn his or her eyes to the laparoscopic monitor instead of the endoscopic monitor.

intraluminally using endoscopy if the size of the tumor is ≤ 5 cm^[20,80]. Larger tumors of > 5 cm are removed through the umbilicus with a plastic bag. The thread of the bag is ligated to the nasogastric tube (Figure 6A) or held by a strong grasper (Figure 6B). The stored tumor is then removed through the mouth with utilization of the overtube.

The endoscope is inserted through the overtube. The overtube is used to protect the mucosal wall during the procedure and specimen removal. Appropriate use of an overtube is essential for successful LECS. The stored tumor in the bag is conically set in the overtube (Figure 7), and the overtube is removed with the tumor bag. Hence, injury to the esophageal mucosa can be avoided during specimen removal (Figure 4H).

KEY POINTS AND TECHNICAL PITFALLS

Placement of an overtube has some advantages for repeated endoscopic insertion and tumor removal through the mouth. The cutting line is determined with an optimal circular margin according to the intraluminal findings. This is an oncological benefit of LECS. Laparoscopic pressure and light are stronger than those of endoscopy. Hence, laparoscopic surgeons must pay closer attention to avoid disturbances during endoscopic interventions. The stomach is dissected from related ligaments and omentum, and the target gastric wall is ventrally mobilized. The target gastric wall should be exposed with a marginal free space by carbon dioxide gas and should never touch any surrounding organs for safe intraluminal intervention. To cut the proximal side of the ventrally mobilized gastric wall, laparoscopic vision from the umbilicus may be

adequate for endoscopic maneuvers. The laparoscopic surgeon can dissect the proximal gastric wall on behalf of the interventional endoscopist if the interventional endoscopist experiences some difficulties. After tumor removal, the defect is closed in a layer-to-layer fashion. Because laxity of running suture results in leakage, an assistant surgeon holds the end of the last suture with a needle forceps, which has a strong grip force. A leak test can be performed with enough air pressure. To avoid excessive dilatation of the small intestine due to insufflation of carbon dioxide gas from endoscopy, clamp forceps are placed on the antrum or jejunum. This clamp should be removed at the end of surgery.

POSTOPERATIVE COURSE

Patients begin drinking on postoperative day 1 and eating on postoperative day 2. If the postoperative course is uneventful, the patients can be discharged around postoperative day 7. In previous studies, the postoperative hospital stay was 4.6 to 10.5 d^[37,71-74,81]. The postoperative hospital stay tends to be prolonged in patients with tumors involving the EGJ^[74], and postoperative obstruction due to stenosis is a major concern in patients with lesions near the cardia.

ONCOLOGICAL ADVANTAGES

In LECS, the tumor is resected with careful observation from both the intraluminal and extraluminal side. Consequently, the surgical margins from the tumor are guaranteed, and excessive gastric wall resection is minimized (Figure 8A)^[50,81]. Previous important studies reported no recurrent cases (Table 1). Conventional simple wedge resection with only an extraluminal approach results in excessive and unnecessary resection of the gastric wall (Figure 8B-D). It may also have a risk of unexpected crushing of the tumor with the stapler because it is an intraluminally blinded procedure.

LIMITATIONS OF LECS

Many researchers have reported that LECS is feasible and safe for the treatment of gastric SMTs^[37,71-74,81]. The main limitation of LECS is the possibility of tumor dissemination during opening of the gastric wall, and contamination with gastric juice into the abdominal cavity may occur. This is why LECS can only be applied to gastric SMTs without epithelial lesions. To overcome this weakness, several procedures based on the concept of "no exposure" have been developed, such as inverted LECS^[47], laparoscopy-assisted endoscopic full-thickness resection^[52], nonexposed endoscopic wallinversion surgery^[53-57], the clean non-exposure technique^[58], closed LECS^[51], and the lift-and-cut method^[59]. Closed LECS, endoscopic resection after plate statement under seromuscular layers, is an effective technique^[51].

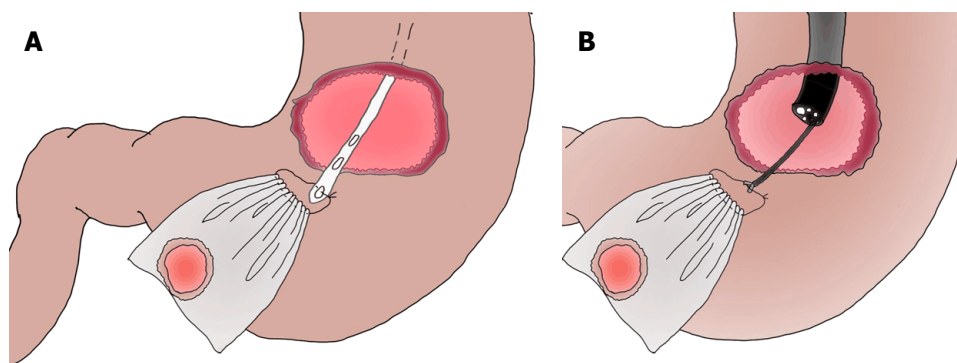


Figure 6 Options of specimen removal with plastic bag. A: Specimen removal with a nasogastric tube; B: Specimen removal with an endoscopic forcep.

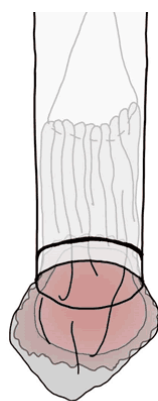


Figure 7 Effective use of an overtube when removing the specimen. The tumor encased in the bag should be sheathed as much as possible in the overtube and removed through the mouth along with the overtube. Hence, injury to the esophageal mucosa by the plastic bag during specimen removal can be avoided.

FACILITY-BASED PRIORITY BETWEEN SURGEONS AND PHYSICIANS

LECS is a combined procedure involving laparoscopic surgery and endoscopic intervention performed in an institution-based manner^[36]. However, the balance between the surgeons' technique and the endoscopists' skill will vary depending on each facility. Although close cooperation is essential, and collaboration of skilled surgeons and experienced endoscopists is ideal. Skills are set within each institution, and the best facility-based service should be considered on an individual basis^[36]. Whether the surgeons or endoscopists will take the initiative and proceed with the operation differs among individual facilities. This does not mean that if a skilled doctor is on one side, the other doctor can be unskilled. Of course, both must be skilled.

From a surgical viewpoint, experience alone is not enough for reliable laparoscopic surgery^[16]. Laparoscopic surgeries without reconstructive procedures (e.g., cholecystectomy and appendectomy) do not require advanced techniques, and these surgeries have

therefore rapidly spread worldwide. In contrast, complicated laparoscopic surgeries (e.g., gastrectomy and proctectomy) have not yet become typical procedures because of the need for skilled surgeons. LECS is not a markedly difficult procedure, although special skills of laparoscopic suturing are required. The laparoscopic closure is technically challenging. Minimally educated and poorly experienced surgeons who are not familiar with suturing in the abdominal cavity under laparoscopy and have no choice except to use staplers should not pursue this procedure. Ironically, simple wedge resection with linear staplers may accomplish the concept of "no exposure"^[60], and employment of a linear stapler itself is actually an effective option to avoid tumor dissemination^[60]. This is a critical issue; i.e., that the oncological benefits of LECS are ignored by misuse of simple wedge resection.

MORTALITY AND MORBIDITY

Clinical outcomes (e.g., oncological resectability, mortality, morbidity and follow-up term) in previous important documents were summarized in Table 1. LECS has demonstrated no mortality and a low incidence of postoperative complications^[48,81], and we speculate that strict performance of the leakage test may play an important role to avoid leakage.

Even subtle stenosis or obstruction of the upper digestive tract will easily result in refractory symptoms after surgery, and the risk factors for stenosis or obstruction remain undefined. There is no evidence of a lower frequency of postoperative stenosis or obstruction in LECS, conversions to proximal gastrectomy and open surgery have been reported, and a good operative course after double-flap technique anastomosis during proximal gastrectomy has been documented^[82].

FUTURE POTENTIAL OF LECS

Although LECS has a risk of tumor dissemination, its application for treatment of EGC has been reported

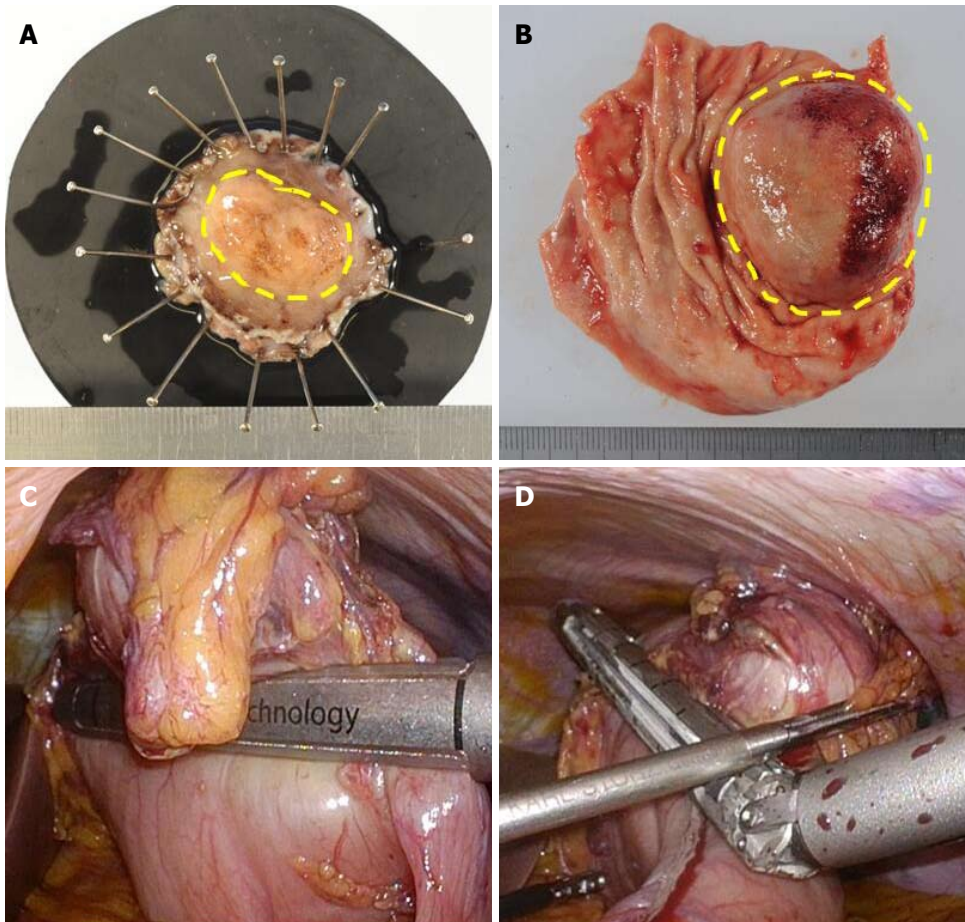


Figure 8 Comparison of surgical margins between laparoscopic and endoscopic cooperative surgery and conventional wedge resection. A: Specimen of Laparoscopic and endoscopic cooperative surgery (LECS). The surgical margin from the tumor is kept at the proper distance; B: Specimen of conventional wedge resection. Simple wedge resection causes both excessive and inadequate resection of the gastric wall, which may lead to postoperative gastric stenosis, gastric dysfunction, and local recurrence; C and D: Intraoperative view of conventional wedge resection with a linear stapler. The resection line is as shown in Figure 1B. The specimen has a portion too close to the tumor and a portion far from the tumor.

by some researchers^[47,57]. Laparoscopic-assisted endoscopic full-thickness resection is also an established procedure^[83]. LECS without lymph node dissection for EGC has been applied to limited cases involving technical difficulties when performing ESD such as severe ulcer-related scarring, an unfavorable tumor location, and a large tumor size. However, patients with lymph node metastasis have not been included. LECS for EGC has also been attempted according to the concept of sentinel lymph node dissection^[84]. Sentinel lymph node biopsy for EGC is reportedly useful when deciding whether to perform lymph node dissection^[85]. If the sentinel lymph node concept is established in the surgical treatment for gastric cancer, the indications for LECS for EGC could be expanded in the future, which could result in increasingly successful gastric cancer treatment. Gastrectomy with lymph node dissection for older patients with gastric cancer, especially those aged ≥ 85 years, has been highly associated with mortality during the postoperative course^[86]. To prevent postoperative morbidity and mortality, maintaining an appropriate balance in the surgical procedure and range of lymph node dissection is

very important based on the patient's general condition, comorbidities, and assumed risk. For selected patients, LECS may be useful as a palliative or symptom-alleviating measure.

ADVANCED TECHNIQUES AND COSMETIC ADVANTAGES

Stab and incisional wounds should be considered as distinct from each other^[16,87]. The tumor cased in the bag can be sheathed as much as possible in the overtube (Figure 7), and tumor removal through the mouth can omit the need for an incisional wound. To reduce the need for incisional wounds, natural orifice transluminal endoscopic surgery is currently challenged^[88,89].

Robot-assisted excision (da Vinci Surgical System; Intuitive Surgical, Inc., Sunnyvale, CA, United States) regardless of tumor size and location has been reported^[90]. Additionally, single-port robotic surgery (Single Port Robotic Surgical System, da Vinci Sp; Intuitive Surgical, Inc.) is currently available.

CONCLUSION

LECS can be safely introduced in a facility-based manner by either surgeons or endoscopists with advanced skills. LECS is a function-preserving surgery with oncological safety and is mainly indicated for gastric SMTs if educated, experienced, and skilled physicians are available. LECS has various possibilities for further developments.

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Conversion surgery for gastric cancer patients: A review

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Abstract

Gastric cancer (GC) is the third most common cancer-

related cause of death worldwide. In locally advanced tumors, neoadjuvant chemotherapy has recently been introduced in most international Western guidelines. For metastatic and unresectable disease, there is still debate regarding correct management and the role of surgery. The standard approach for stage IV GC is palliative chemotherapy. Over the last decade, an increasing number of M1 patients who responded to palliative regimens of induction chemotherapy have been subsequently undergone surgery with curative intent. The objective of the present review is to analyze the literature regarding this approach, known as "conversion surgery", which has become one of the most commonly adopted therapeutic options. It is defined as a treatment aiming at an R0 resection after chemotherapy in initially unresectable tumors. The 13 retrospective studies analyzed, with a total of 411 patients treated with conversion therapy, clearly show that even if standardization of unresectable and metastatic criteria, post-chemotherapy resectability evaluation and timing of surgery has not yet been established, an R0 surgery after induction chemotherapy with partial or complete response seems to offer superior survival results than chemotherapy alone. Additional larger sample-size randomized control trials are needed to identify subgroups of well-stratified patients who could benefit from this multimodal approach.

Key words: Metastatic gastric cancer; Gastric cancer; Conversion surgery; R0 resection; Stage IV gastric cancer; Palliative chemotherapy; Unresectable gastric cancer

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Core tip: Conversion surgery is defined as a surgical treatment with the goal of R0 resection in initially unresectable gastric cancer patients after response to chemotherapy. Although the heterogeneity of metastatic disease factors makes it difficult to identify true prognostic variables, a survival benefit has been

demonstrated in several reports. Further prospective large-scale studies seem to be necessary to improve patient selection and to validate this promising multimodal therapy.

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INTRODUCTION

Gastric cancer (GC) is known to be the third most common cancer-related cause of death worldwide^[1]. Surgical treatment with adequate extended lymphadenectomy is associated with good outcomes in early stages. However, in advanced GC, prognosis remains poor. Neoadjuvant chemotherapy (NAC) has been suggested for resectable, locally advanced GC based on well-known Randomized Controlled Trial (RCT)s^[2,3]. Despite many enrolled patients having lower esophagus or esophagogastric junction involvement and surgery not always including a standard extended lymphadenectomy, there was a survival advantage of NAC plus surgery compared to surgery alone. Therefore, NAC, or preferably preoperative chemotherapy, has been recently introduced as an option in most treatment guidelines^[4-9].

The SEER database shows that one third of Western patients with GC have unresectable disease, and different strategies have recently been adopted to manage advanced unresectable cancer^[10]. Generally, in these cases, surgery is upfront considered as a palliative treatment for obstruction or bleeding.

Palliative chemotherapy remains the main treatment strategy of IV stage GC patients^[11]. Although the median survival time (MST) of these patients has improved due to development of new chemotherapeutic agents, it is still unsatisfactory. Therefore, patients who demonstrated a response to chemotherapy have begun to be subsequently surgically treated with curative intent. This approach in stage IV patients, called "conversion surgery", is becoming one of the most common therapeutic options discussed in the literature over the last decades. The aim of the present review was to define the effective usefulness of this strategy, to identify its crucial aspects and to highlight critical issues and implications for future perspectives.

Literature search

We analyzed articles published in English from 1997 to 2017 using the following key words: Conversion surgery, conversion therapy, R0 resection stage IV GC, unresectable GC. We excluded case reports and case

series, ultimately obtaining 13 articles for 13 studies. We first analyzed stage IV factors singularly to define major current therapeutic strategies for any selected patient, and then, we considered oncological outcomes of palliative chemotherapy through experiences derived from several trials. Therefore, we focused on the emerging role of conversion therapy as a new treatment option for metastatic gastric cancer patients.

STAGE IV GC

Stage IV GC is a heterogeneous biological condition with a mixture of distant metastases, including hematologic, lymph nodal and/or peritoneal. To reduce this heterogeneity, the Japanese Gastric Cancer Association (JGCA) and the Union Internationale Contre le Cancer (UICC) minimized differences between their classifications and categorized similar groups^[12-16]. However, these systems do not seem sufficient to derive any significant clinical suggestions.

In the recent classification introduced by Yoshida *et al*^[17] with the proposal to identify objective principles for conversion surgery, stage IV patients were subdivided into 4 new categories (Figure 1). Initially, the presence of macroscopic peritoneal dissemination is considered as a different biological and prognostic finding compared with hematological metastases. Patients without peritoneal involvement belong to category 1 (potentially resectable metastases) and category 2 (marginally resectable metastases). Patients with macroscopic peritoneal metastases are stratified into category 3 (unresectable except certain situations) and category 4 (incurable metastases). Below we highlight different critical aspects in terms of staging, treatment and prognosis of different potential metastatic patterns in stage IV GC.

Peritoneal metastases

Synchronous peritoneal carcinomatosis (PC) is the most frequent site of metastasis in stage IV GC. PC occurs in 14%-43% of GC patients and represents 35% of all synchronous metastases^[18,19]. The prognosis of PC in GC is worse than that for other metastatic sites^[20,21]. Peritoneal dissemination of GC is a dynamic multistep process that involves several molecules acting in a coordinated way. As reported in a recent review by Kanda *et al*^[22], there are 4 steps in peritoneal dissemination: (1) migration to the abdominal cavity after detachment of cells from the tumor; (2) adaptation to the abdominal microenvironment; (3) adhesion to mesothelial cells and invasion of the baseline membrane; and (4) growth and angiogenesis of the tumor. These molecular mechanisms are very challenging because identification of a single pathway is not necessarily correlated with disease prognosis.

Survival of patients with PC is poor, despite the progress of chemotherapy. Hence, PC is often considered a determinant for a "real" curative treatment possibility,

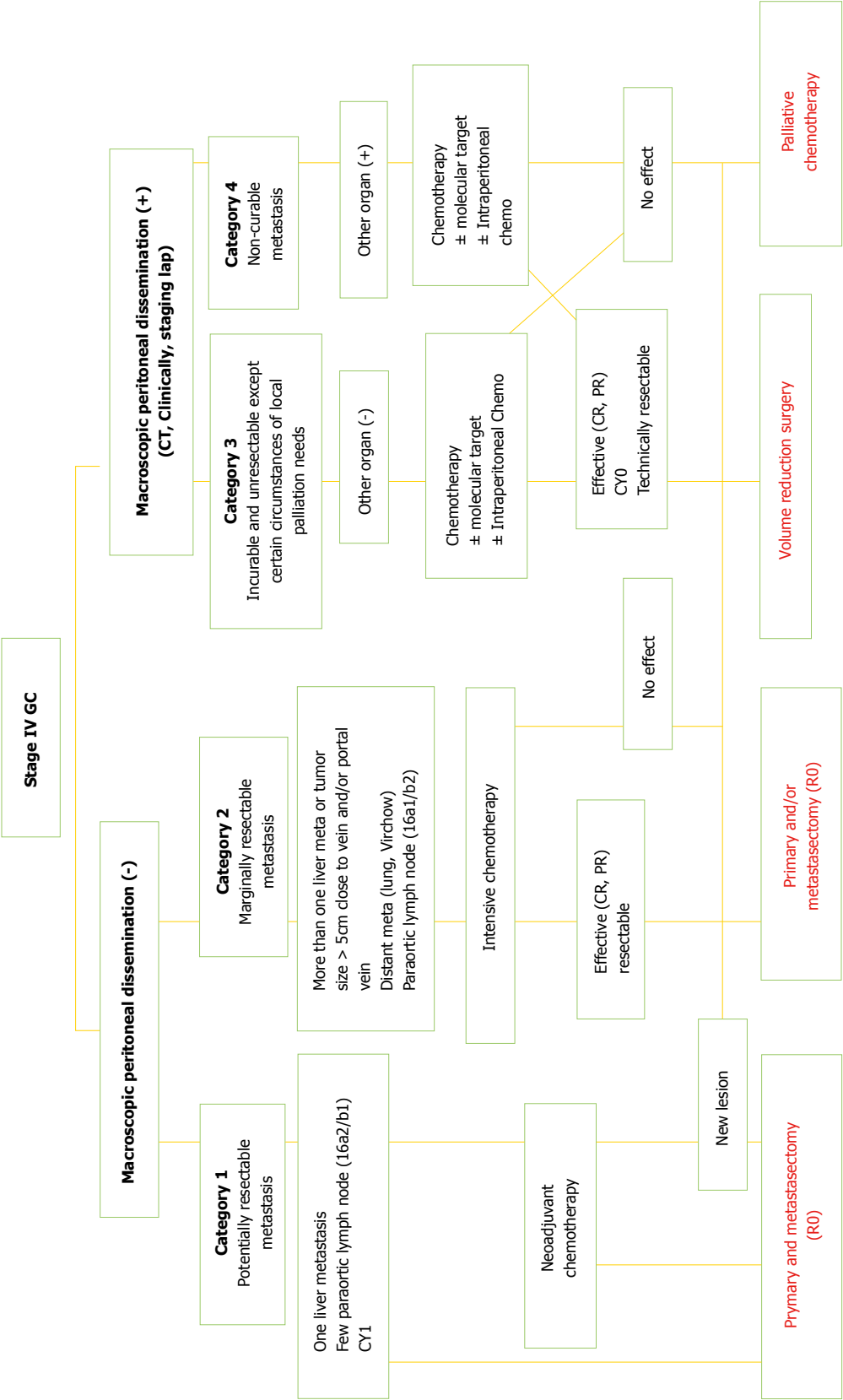


Figure 1 Biological categories proposed by Yoshida *et al*^[27], GC: Gastric cancer.

and several scoring systems on extension of PC have been validated to accurately discriminate treatment options, stratify patients prognosis, and, consequently, correct statistical analyses^[23-25]. Okabe *et al*^[26] noted that in curatively (R0) resected patients, after disappearance of limited peritoneal dissemination treated with induction therapy (S-1 plus cisplatin), MST was significantly longer (43.2 mo) than in patients who underwent non-curative resection (12.6 m), as well as in patients without surgery (10.3 m). To increase chemotherapy efficacy for PC, the literature suggests an additional benefit of hyperthermic intraperitoneal administration of drugs (hyperthermic intraperitoneal chemotherapy, HIPEC)^[27-31].

Recent advances in multimodal treatment for patients with peritoneal dissemination are highlighted by Ishigami *et al.*^[32] in the PHOENIX-GC trial that, although failing to show statistical superiority for intraperitoneal paclitaxel plus systemic chemotherapy, suggested possible clinical benefit for this treatment option. In a systematic review of 10 studies considering 441 patients treated with cytoreductive surgery plus HIPEC, a median overall survival of 15 mo after radical (R0) cytoreduction was shown by Gill *et al.*^[33]. Consistently, the phase III randomized trial by Yang *et al.*^[34] and the GYMSSA trial reported improved survival rates with surgery plus HIPEC compared with surgery alone^[35].

Distant metastasis

Many patients with stage IV GC have multiple metastatic sites. Usually, the first site of metastasis occurring through the hematogenous pathway is the liver. Systemic chemotherapy is a standard treatment approach for GC patients with liver metastases^[36], recommended by both the National Comprehensive Cancer Network (NCCN) Guidelines and the Japanese Guidelines^[37,38]. Surgical resection has been recently reported to prolong survival in highly selected patients^[39-41]. Li *et al.*^[42] reported a 100% response rate after chemotherapy with weekly DCF regimen before curative gastrectomy in 8 patients. A multidisciplinary approach, including surgery in selected GC patients when the liver is the only site of metastasis, is associated with interesting results^[43]. However, treatment of synchronous or metachronous hepatic metastases is not well standardized in GC patients. Once combined with gastrectomy and extended lymphadenectomy, there are no differences in 5-year survival after resection of synchronous and metachronous liver metastases^[44]. Considering metachronous metastases, patients submitted to surgery benefit from better selection and exhibit good survival over short and medium terms^[45]. Surgical treatment of the best subgroups of candidates can achieve good results that should encourage surgeons and medical oncologists^[41,46].

Lymph node metastases

A proper lymphadenectomy during surgical resection is a milestone for GC treatment. Patients with para-aortic lymph node (PAN) metastases, or bulky nodes around the hepatic, splenic, or celiac arteries are considered unresectable. Some retrospective studies demonstrated the presence of PAN metastases in greater than 20% of patients undergoing D2 + PAN dissection, and 5-year survival rates of patients with PAN metastases do not exceed 20%^[47,48]. Furthermore, a phase III trial JCOG9501 comparing D2 nodal dissection with or without PAN dissection for GC concluded that prophylactic PAN dissection does not improve survival rates^[49]. Interestingly, patients with macroscopic metastases in

these nodes were excluded from analysis, resulting in a low incidence of metastatic n° 16 nodes in patients receiving PAN dissection. This "selection bias" left open the issue of prognostic efficacy of removal of PAN station in PAN metastatic patients^[50]. On the other hand, since 2000, three phase II trials (JCOG0001, JCOG0405 and JCOG1002) have explored preoperative/induction chemotherapy and PAND gastrectomy for bulky N2/N3 gastric cancer^[51-54]. The JCOG0001 study reported a low 3-year survival rate (27%) after 2-3 cycles of irinotecan and cisplatin followed by surgery. Conversely, the JCOG0405 trial demonstrated an excellent response rate (up to 64.7%) with 3-year survival of 58.8% in patients who received 2-3 cycles of cisplatin and S-1 before surgery. Similarly, in the JCOG1002 study, among 52 eligible patients, 48 underwent surgery, 44 with R0 resection (84.6%), after 2-3 cycles of docetaxel, cisplatin and S-1 with a pathological response rate of 50%.

PALLIATIVE CHEMOTHERAPY

As specified above, according to current guidelines, palliative chemotherapy is the main strategy for treatment of stage IV GC patients. These cases have always represented the ideal setting for use of many new combinations of chemotherapeutic agents, both in Japan and in Western countries^[55-67]. The median overall survival observed in these studies varies between 3 and 17 mo. In the SPIRIT trial, an overall survival of 13 mo was reported using S-1 plus cisplatin, which is defined as the standard treatment for metastatic GC in Japan^[56]. In Western countries, the treatment most commonly used for metastatic GC is a combination of chemotherapy regimens, including fluoropyrimidine plus a platinum agent, though epirubicin or docetaxel can also be combined^[64,66]. Recent developments in chemotherapeutic and molecular targeted agents have added new clinical issues in the management of incurable GC. As reported in the ToGA trial, Trastuzumab plus chemotherapy in HER2-positive patients improved overall median survival from 11.1 to 13.8 mo^[60]. In addition, histological biomarkers have been identified to predict survival among GC patients^[68]. Recently, palliative chemotherapy seemed further validated compared with palliative surgery by results of the REGATTA trial. In fact, although some authors emphasized the beneficial role of palliative gastrectomy^[69,70], in this RCT, Fujitani *et al.*^[71] demonstrated no survival benefit for palliative gastrectomy prior to chemotherapy in advanced GC patients with a single non-curative factor. However, the methodological biases of the REGATTA trial negatively affect reliability of its results and weaken its potential clinical implications^[72]. Therefore, at the moment, for stage IV GC patients, we have no strong evidence to consider the results of palliative chemotherapy satisfactory. On the other hand, we also have no reliable data to suggest definitely abandoning surgery.

FROM SALVAGE SURGERY TO CONVERSION THERAPY

The heterogeneous presentation of stage IV GC characteristics makes it difficult to identify the best therapeutic strategy for these tumors due to their different biological behaviors. On the other hand, given the poor results achieved with chemotherapy alone, in order to further improve survival of these patients, new therapeutic approaches have been considered. Based on experiences of the multidisciplinary treatment of metastatic colorectal cancer, in the last 2 decades, many studies have been conducted to evaluate efficacy of the combination of chemotherapy and surgery for stage IV GC. Surgical resection for advanced tumors has historically been called "radical", "salvage", "adjuvant" or "secondary" gastrectomy. More specifically, the concept of conversion surgery has been recently treated by Yoshida^[17] to define a treatment aiming to R0 resection after chemotherapy in initially unresectable patients.

Tables 1 and 2 show patient characteristics and treatment options analyzed in the considered studies, as well as survival results. Below, we discuss in chronological order the main results of these studies, with particular focus on potential prognostic factors in conversion surgery strategy.

Examined studies

Probably, the first report of conversion surgery was in 1997 by Nakajima *et al.*^[73]. Thirty patients with incurable GC were treated with combined chemotherapy and radical surgery. Survival of patients with curative resection was 55.6% at 5 years. Long-term survivors were exclusively found among patients with distant metastatic lymph nodes. PC and extra-abdominal lesions did not respond to chemotherapy and, hence, did not reach surgery^[73].

Yano *et al.*^[74] analyzed 34 patients with inoperable GC who underwent NAC. Eight patients among 14 who received salvage surgery exhibited curative resection. Histological type, T4 as non-curative factors, clinical response, and salvage surgery were significant prognostic factors. T4 unresectable lesions and para-aortic node metastases showed high dissolution rates after chemotherapy, whereas peritoneal and distant metastases did not^[74]. A study on combined treatment with S-1 plus cisplatin followed by gastrectomy and post-operative S-1 for stage IV GC was conducted by Satoh *et al.*^[75]. Their results showed that 26 patients among 44 who received preoperative chemotherapy underwent R0 surgical resection. Interestingly, all 12 patients with pre-cy1 as a single pre-stage IV factor achieved R0 resection with a 2-year OS of 75%^[75].

In 2012, Kanda *et al.*^[76] reported a good response rate to S-1 chemotherapy in patients with incurable GC who were submitted to secondary surgery. Twenty-

six patients of 28 underwent R0 resection. The results showed that 1-, 3- and 5-year survival were 82.1, 45.9 and 34.4%, respectively. Multivariate analysis revealed histological lesion length to be the only significant prognostic factor^[76]. According to reports from Han *et al.*^[77], 22/34 M1 patients with one initial metastatic site who responded to induction chemotherapy exhibited good survival outcomes after R0 resection, with resection rates of 88% and 44% for one and two metastatic sites, respectively. MST of R0 was 22.9 mo, with a 3-year overall survival of 41.4%. Concerning gastric cancer patients with peritoneal seeding, Kim *et al.*^[78] published results of 18 conversion patients in which 10 received R0 resection after chemotherapy. MST and 3-year OS of R0 patients were 37 mo and 50%, respectively. Unexpectedly, 8 patients who received non-curative resection had longer survival rates than did other patients who continued chemotherapy^[78].

Fukuchi *et al.*^[79] reported a series of 40 out of 151 patients who underwent conversion surgery. In 32 of them, it was possible to perform R0 resection with a 5-year OS of 49% (MST: 62 mo). By multivariate analysis, the presence of just one non-curative factor and R0 resection were significant independent predictors for good OS^[79].

Kinoshita *et al.*^[80] analyzed the effects of conversion gastrectomy after docetaxel, cisplatin and S-1 (DCS) combined chemotherapy. Of 57 patients, 42 were categorized as unresectable, while 15 patients were potentially resectable cases, with a single incurable factor (16 a2-b1 metastases or < 3 peripheral liver metastases). The 3-year OS rate of potentially resectable cases was 92.9%, compared with 35.1% of unresectable cases^[80].

In a multi-institutional retrospective study, Sato *et al.*^[81] highlighted pathological response as a significant independent predictor for OS. He determined that 33/100 patients were able to undergo conversion therapy. Almost eighty-five of them received an R0 resection after DCS chemotherapy with a pathological response rate of 78.8%. Five-year OS in R0 patients was 48.6%^[81].

Ten patients with one incurable factor were retrospectively analyzed by Einama *et al.*^[82]. All cases were considered resectable after chemotherapy, achieving R0 resection. The authors reported a longer survival of surgical patients compared with those who received chemo alone (MST 29 mo). Non-invasive macroscopic type, higher differentiation, and absence of peritoneal dissemination were all favorable survival predictors^[82].

Another study concerning conversion surgery after combination chemotherapy of docetaxel, cisplatin, and S-1 from Mieno *et al.*^[83] reported that 74.2% of the study population (23/31) underwent R0 resection in patients with stage IV GC initially deemed unresectable. Fifty-eight point one percent of patients had extra regional

Table 1 Patient characteristics and onco-surgical treatments

Reference	Period	Population (conversion surgery)	Median - age	Unresectable criteria					Chemotherapy	Surgery	Lymphadenectomy (D2 or more)	Ro
				P1	H1	Cy1	PAN/N3	T4				
Nakajima <i>et al</i> ^[73] , 1997	1989-1995	30 (19)	53	9 (30%)	11 (37%)	23 (77%)	8 (27%)	3 (10%)	FLEP	NS	NS	9 (30%)
Yano <i>et al</i> ^[74] , 2002	May 1994-Dec 1999	34 (14)	54.4 (31-73)	26 (76%)	4 (12%)	10 (3.4%)	12 (35%)	1 (0.3%)	FEMTIXP or THP-FLEP	NS	NS	8 (24%)
Satoh <i>et al</i> ^[75] , 2012	May 2003-Mar 2008	51 (44)	63 (35-79)	24 (49%)	3 (6%)	12 (23%)	7 (14%)	5 (10%)	S1 + Cisplatin	TG (58%) DG (21.5%)	82%	26 (51%)
Kanda <i>et al</i> ^[76] , 2012	Apr 2000-Mar 2008	31(28)	65.5 (49-79)	7 (25%)	4 (14.3%)	15 (54%)	9 (32%)		S1 + Cisplatin or Paclitaxel or Irinotecan	TG (42.89%) DG (57.1%)	96.30%	26 (93%)
Han <i>et al</i> ^[77] , 2014	Jan 2000-Dec 2009	34 (34)	56 (28-71)	7 (14%)	5 (10%)	15 (29.4%)	7 (14%)	7 (14%)	5-FU + Platinum or 5-FU + Platinum + Taxane	NS	NS	26 (76.5%)
Kim <i>et al</i> ^[78] , 2014	Jan 2003-Dec 2012	43 (18)	52.8 (32-72)	43 (100%)					5-FU + Cisplatin or S1 + Cisplatin	TG (72.2%) DG (27.7%)	100%	10 (55%)
Fukuchi <i>et al</i> ^[79] , 2015	Feb 2003-Dec 2013	151 (40)	66 (31-79)	11 (28%)	5 (13%)	3 (8%)	6 (15%)	26 (65%)	S1 + Cisplatin or S1 + Paclitaxel	TG (72.5%) DG (27.5%)	NS	32 (80%)
Kinoshita <i>et al</i> ^[80] , 2015	Apr 2006-Mar 2012	57 (34)	65 (30-78)	15 (26%)	18 (32%)	23 (40%)	2 (3.5%)		DCS	TG (64.7%) DG (26.5%)	50%	27 (79%)
Sato <i>et al</i> ^[81] , 2017	Dec 2002-Apr 2014	100 (33)	63 (26-78)	33 (33%)	29 (29%)	61 (61%)	14 (14%)	11 (11%)	DCS I line, CPT-11 II line	TG (84.8%) DG (12.1%)	100%	28 (85%)
Einama <i>et al</i> ^[82] , 2017	Jan 2009-Dec 2015	10	70.5 (59-86)	3 (30%)	1 (10%)	4 (40%)	1 (10%)		S1 + CDDP or DOC	TG (40%) DG (30%)	100%	10 (100%)
Mieno <i>et al</i> ^[83] , 2017	Oct 2006-Dec 2012	31 (31)	63 (35-78)	25%	16%	58%	26%		DCS + DS (Docetaxel-S1) in responder patients	TG (74.2%) DG (22.6%)	77%	23 (74%)
Yamaguchi <i>et al</i> ^[84] , 2017	2001-2013	259 (84)	61.7 (21-78)	35 (41%)		37 (44%)	34 (40%)		DCS or S1 or S1 + Cisplatin or S1 + Taxane	TG (82.1%) DG (17.9%)	NS	43 (51%)
Morgagni <i>et al</i> ^[85] , 2018	Apr 2005-Aug 2016	73 (22)	69 (59-74)						Epirubicin + Cisplatin + 5-FU or Oxaliplatin + 5-FU or Docetaxel + Oxaliplatin + 5-FU or Other	TG (72.7%) DG (22.7%)	91.90%	22 (100%)

P1: Peritoneal carcinomatosis; H1: Hepatic metastases; Cy1: Positive cytology; PAN: Para-aortic node metastases; TG: Total gastrectomy; DG: Distal gastrectomy; FLEP: 5-FU + Leucovorin + Etoposide; CDDP: Cisplatin; DOC: Docetaxel; NS: Not specified.

lymph node as unresectable factor^[83].

In a study by Yamaguchi *et al*^[84], 84 patients among 259 with stage IV GC received conversion surgery after chemotherapy. Patients were classified into four categories

Table 2 Overall survival and median survival time

Reference	Years	OS (rate)			MST (mo)		
		CHT	CHT + surgery		CHT	CHT + surgery	
			R1/R2	R0		R1/R2	R0
Nakajima <i>et al</i> ^[73] , 1997	2/3-yr 5-yr			55.6	4.7	6.5	
Yano <i>et al</i> ^[74] , 2002 ²	2/3-yr 5-yr						
Satoh <i>et al</i> ^[75] , 2012	2/3-yr 5-yr		43	75 ¹			19.2
Kanda <i>et al</i> ^[76] , 2012	2/3-yr 5-yr		0	45.9 34.4			29
Han <i>et al</i> ^[77] , 2014	2/3-yr 5-yr			41.4		7.8	22.9
Kim <i>et al</i> ^[78] , 2014	2/3-yr 5-yr	0	0	50	8	18	37
Fukuchi <i>et al</i> ^[79] , 2015	2/3-yr 5-yr				14	30	62
Kinoshita <i>et al</i> ^[80] , 2015	2/3-yr 5-yr	0	16	63.5	9.6	29.9	
Sato <i>et al</i> ^[81] , 2017	2/3-yr 5-yr	18.7 0	0	48.6	15.7	21.7	47.9
Einama <i>et al</i> ^[82] , 2017	2/3-yr 5-yr						29
Mieno <i>et al</i> ^[83] , 2017	2/3-yr 5-yr	56.9		73.1		56.1	
Yamaguchi <i>et al</i> ^[84] , 2017	2/3-yr 5-yr				11.3	21.2	41.3
Morgagni <i>et al</i> ^[85] , 2018	2/3 yr 5 yr	0		39.4	14		38 ³

¹R0 in only pre-Cy1 patients; ²No data are specified but a *P* value < 0.0003 is shown between resected and not-resected 5-years OS rate; ³Patients who had cytoreductive surgery plus hyperthermic intraperitoneal chemotherapy had an MST of 50 mo. OS: Overall survival; MST: Median survival time; CHT: Chemotherapy.

previously published by the same authors^[17]. Survival results of this series rose from 24.7 to 31.0 of MST. Patients who underwent R0 resection had an MST of 41.3 mo^[84]. Recently, Morgagni *et al*^[85] reported a Western series of 22 patients among 73 unresectable subjects who underwent R0 resection after induction chemotherapy. Gastrectomy plus HIPEC was performed in 9 patients. The 1- and 3-year survival rates were 63.6% and 39.4%, respectively^[85].

DISCUSSION

Gastric cancer is known to be a heterogeneous disease. Dissemination may occur directly to the peritoneum, through the hematogenous and lymphatic systems. Moreover, the method whereby cancer cells enter into the portal circulation varies, resulting in significant variability of metastatic patients both for the site and the amount of tumor. Consequently, few metastatic patients are eligible for conversion surgery. Moreover, frequent coexistence of different factors of incurability make it difficult to identify true prognostic variables, as well as the rate of response to chemotherapeutic treatments. Despite progress in chemotherapy providing significant hope with new drug agents, the response rates of metastatic GC patients remain unsatisfactory

with non-optimal patient compliance. The definition of initial unresectable criteria and post-chemotherapy resectability has yet to be established. In many cases, the line between neoadjuvant and induction chemotherapy remains unclear. Therefore, analysis of experiences on conversion surgery in stage IV GC is very challenging due to the heterogeneity of series, makes it very difficult to compare results from different studies. Furthermore, the majority of analyzed studies have been performed in Eastern Asia (only one in Italy). As such, this could represent a potential bias for reliable evaluation independent of differences in chemotherapy schedules, quality of surgery, and patient biology, for example. Undoubtedly, the Regatta trial taught us that even a palliative gastrectomy increases patient morbidity compared with chemotherapy alone. Hence, a strict selection of patients who could potentially benefit from conversion surgery seems mandatory. Yoshida *et al*^[17] proposed a biological classification to stratify all stage IV GC patients to respond to this need (Figure 1). Probably, long-term survivors can be found mostly in the first three categories, though the small number of patients in the first category can be explained by this unusual condition. Actually, these patients are likely to benefit from NAC.

Although analyzed studies were retrospective and

limited with respect to number of patients enrolled, the possibility of curative resection seems a crucial aspect. The literature reports R0 resection rates ranging from 24%-100% (Table 1), and these numbers are closely correlated with prognosis (Table 2). Thus, the survival benefit derived from R0 resections might justify a predictable increase in morbidity compared with survival from medical therapy alone. Interestingly, even non-curative resection often results in superior survival compared to chemotherapy alone. Consistent with this suggestion from the literature, quality of life (QOL) after conversion (even if non curative) surgery remains an intriguing issue to be analyzed. In this regard, a meta-analysis conducted by Lasithiotakis *et al*^[86] underlined the relevant role of QOL outcomes after palliative gastrectomy.

Consistent with considerations by Yoshida *et al*^[17], the presence of only one-site of metastasis is one of the most important prognostic factors according to most analyzed studies. In this literature review, lymph node metastases and positive cytology on peritoneal washing as unresectable factors are also related to better prognoses after conversion surgery when partial or complete response to chemo was observed. In this regard, while the more reliable (and later) evaluation of pathological response was demonstrated to be correlated with survival after conversion therapy, we have no unquestionable prognostic data and no objective criteria for clinical response assessment. Indeed, another determining factor is the detection of the best timing to operate (or to decide to not operate). Generally, surgery occurs when the tumor decreases in sizes and before it develops any drug resistance. For this determinant decision making step, cooperation between oncologists and surgeons is mandatory for general management of patients (and not the tumor alone). Regarding type of surgery and extension of lymphadenectomy, total or distal gastrectomy (also with multivisceral approach) aiming at R0 resection was generally associated with D2 or more extended lymphadenectomy. We believe that a proper and standardized D2 lymphadenectomy could achieve optimal results with acceptable morbidity/mortality. Finally, whether chemotherapy is required after an R0 resection is an issue that needs clarification.

In conclusion, the survival efficacy of conversion surgery may dramatically improve when combined with targeted chemotherapy. Perhaps new cytotoxic and molecular targeted agents and progress in sensitive molecular biomarker development could shift treatment from standardized to personalized, leading to further improved outcomes. The promising results of this multimodal therapy are increasingly gaining the attention of medical and surgical oncologists in planning further studies. Although it seems hard to design a valuable trial due to the difficulty of enrolling patients, it appears mandatory to demonstrate the effectiveness of this strategy in stage IV GC patients, or at least in well-

selected and stratified stage IV patient subgroups. On the other hand, given that long-time survivors exist, we are convinced that the multidisciplinary discussion should always be recommended on a case-by-case basis. In conclusion, it is well known that some decades ago patients affected by unresectable GC represented a large population on whom medical oncologists applied new and promising therapies without great success. Today, the strategy of conversion surgery induces oncologists to consider that surgery could still have a role, even after almost "hopeless" systemic therapy.

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Retrospective Study

Prognostic significance of primary tumor localization in stage II and III colon cancer

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Abstract

AIM

To investigate the effects of tumor localization on disease free survival (DFS) and overall survival (OS) in patients with stage II - III colon cancer.

METHODS

This retrospective study included 942 patients with stage II and III colon cancer, which were followed up in our clinics between 1995 and 2017. The tumors from the caecum to splenic flexure were defined as right colon cancer (RCC) and those from splenic flexure to the sigmoid colon as left colon cancer (LCC).

RESULTS

The median age of the patients was 58 years (range: 19-94 years). Male patients constituted 54.2%. The rates of RCC and LCC were 48.4% ($n = 456$) and 51.6% ($n = 486$), respectively. During the median follow-up of 90 mo (range: 6-252 mo), 14.6% of patients developed recurrence and 9.1% of patients died. In patients with stage II and III disease with or without adjuvant therapy, DFS was similar in terms of primary tumor localization (stage II; $P = 0.547$ and $P = 0.481$, respectively; stage III; $P = 0.976$ and $P = 0.978$, respectively). In patients with stage II and III disease with or without adjuvant therapy, OS was not statistically significant with respect to primary tumor localization (stage II; $P = 0.381$ and $P = 0.947$, respectively; stage III; $P = 0.378$ and $P = 0.904$, respectively). The difference between median OS of recurrent RCC (26 ± 6.2 mo) and LCC (34 ± 4.9 mo) cases was eight months ($P = 0.092$).

CONCLUSION

Our study showed no association of tumor localization with either DFS or OS in patients with stage II or III colon cancer managed with or without adjuvant therapy. However, post-recurrence OS appeared to be worse in RCC patients.

Key words: Colon cancer; Tumor localization; Adjuvant treatment; Overall survival; Disease free survival

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Core tip: It is well known that metastatic right colon cancer is more aggressive than left colon cancer. However, the effects of tumor location on the decision of adjuvant therapy and survival are not clearly known in early stage disease. In this retrospective study, we investigated the effects of tumor location on disease free survival and overall survival in patients with and without adjuvant therapy for stage II-III colon cancer. There was no difference for disease free survival or overall survival between patients with right or left localized colon cancer, but we established that right localized tumors were more aggressive than left side after recurrence.

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INTRODUCTION

Colon cancer (CC) is a common and fatal disease. It is estimated that about 95520 CC cases are diagnosed

annually in the United States. CC is the third most common cancer in men and the second most common cancer in women. Despite a declining mortality since 1990, it ranked the third in women and the second in men in cancer-related deaths. From 1992 to 2012, the incidence of men and women under the age of 50 diagnosed with CC increased by 2.1% per year. These increases were primarily seen in left-sided cancers, and particularly in rectal cancer (3.9% per year). Approximately 39% of the cases are local, and 37% are locoregional at diagnosis. Seventy to 80% of patients with locoregional disease at diagnosis are suitable for curative surgery. While surgery is essential for curative treatment, some patients have recurrence even after curative surgery. The prognosis is worse after recurrence. For this reason, it is important to identify reliable factors for identification of patients at high risk of recurrence^[1,2].

The proximal and distal segments of the colon possess different embryological origins. The segment extending from the caecum to the proximal two-thirds of the transverse colon develops from the midgut. The part from the distal third of the transverse colon to the rectum develops from the hindgut. While the right colon consists of the caecum, ascending colon, hepatic flexure, and transverse colon, the left colon consists of the splenic flexure, descending colon, and sigmoid colon. Blood supply, innervation, and lymphatic drainage anatomically differ between the right and left colon. Considering these differences in anatomy and embryological origin, variation in clinical features may be identified for the same disease of the colon^[2].

It has been known for many years that right CC (RCC) and left CC (LCC) represent dissimilar tumors with differences in epidemiology, biology, pathology, and clinical outcomes. Recently, the relationship between tumor localization and prognosis in metastatic disease has been investigated. These studies, however, primarily focused on responses to chemo- or targeted therapy^[3,4]. For this reason, it is still not clear for patients and clinicians whether tumor localization is an important additional risk factor in locoregional disease.

In our study, we aimed to examine the association of tumor localization to disease free survival (DFS) and overall survival (OS) in patients who underwent curative surgery for stage II and III CC.

MATERIALS AND METHODS

Patients

This retrospective study included patients who were followed up in the oncology outpatient clinic of Okmeydanı Training and Research Hospital between 1995 and 2017. Clinical and pathological data were obtained from medical patient records. Those with rectal cancer, another malignancy distinct from CC, multiple primary tumors, metastatic disease, patients under 18 years and those without sufficient data were not included in the

study. A total of 942 patients with full medical records and a pathological diagnosis of stage II–III CC were identified. The study was approved by the institutional ethics committee.

Data collection

Data obtained from medical records included the age, gender, alcohol or tobacco use, type of surgery (emergent or elective), presence of diabetes mellitus (DM) or hypertension (HT), histological characteristic (adenocarcinoma, mucinous adenocarcinoma), grade, primary tumor localization, stage, pathological tumor stage (pT), pathological node stage (pN), lymph node status (≥ 12 or < 12), numbers of excised and involved lymph nodes, presence of perineural invasion (PNI) or lymphovascular invasion (LVI), surgical margin positivity, use of adjuvant therapy, adjuvant therapeutic regimen, recurrence, and most recent status (exitus-alive). Patients were re-staged according to the 8th tumor, node, and metastasis staging manual 2017 of the American Joint Committee on Cancer/Union for International Cancer Control. Patients were divided into two groups, right colon and left colon. Tumors extending from the caecum to the splenic flexure were classified as RCC, those from the splenic flexure to the sigmoid colon as LCC. Age was grouped as < 65 and ≥ 65 years. Grades were grouped as 1 + 2 and 3. pT was grouped as 1 + 2, 3 and 4. DFS was estimated as the time elapsed from diagnosis to local recurrence or systemic metastasis. OS was estimated as the time from diagnosis to death. OS2 was defined as the time from recurrence to death.

Statistical analysis

SPSS 15.0 for Windows software package was used for statistical analysis. Descriptive variables were expressed with mean, standard deviation, minimum, and maximum values for numerical parameters, and with number and percentage values for categorical parameters. Numeric variables in two independent groups were analyzed by a Student's *t*-test when the data were normally distributed and by Mann Whitney *U* test when the normal distribution condition was not met. Comparisons of rates in groups were made with chi-square. Monte Carlo simulation was applied when conditions were not met. The survival analyses were performed with Kaplan Meier. Determinants were analyzed by Cox regression. In univariate analysis, a forward stepwise model was used for values with $P < 0.250$. An overall 5% alpha error level was used to infer statistical significance.

RESULTS

The rates of RCC and LCC were 48.4% ($n = 456$) and 51.6% ($n = 486$), respectively. Male patients constituted 54.2%. The median patient age was 58 years (range: 19–94 years). Nearly one-third of patients

(32.5%) were equal to or above 65 years old (Table 1).

Twenty-six patients (2.8%) had a family history of CC in their first-degree relatives. The history of smoking and regular alcohol use was present in 45.8% ($n = 350$) and 5.2% ($n = 49$) of patients, respectively. Emergency surgery was performed in 151 patients (16%). DM and HT were present in 9.9% and 23.7% of the study population, respectively (Table 1).

Analysis of tumor histology showed mucinous adenocarcinoma in 17.3% of patients, grade III tumor in 6.7% of patients, and stage II disease in the majority of patients (60.2%). The rates of pT3 and pT4 were 79.8% and 6.1%, respectively. The mean number of lymph node dissections performed was 17.57 ± 10.8 , where lymph node involvement was 1.48 ± 4.0 . The rate of lymph node dissection below 12 was 31.4%. The number of patients with pN2 and pN1 were 102 (10.8%) and 273 (29%), respectively. PNI and LVI positivity was found in 21.7 and 32.2% of patients, respectively. Eight patients (0.8%) had positive surgical margins (Table 1).

Postoperative systemic therapy was initiated in 734 patients (77.9%), 67.2% ($n = 493$) of which received 5-FU-based (5-fluorouracil + leucovorin, capecitabine) and 32.8% ($n = 241$) received oxaliplatin-based (capecitabine + oxaliplatin, 5-fluorouracil + leucovorin + oxaliplatin) regimens. A total of 695 patients (94.7%) completed planned adjuvant chemotherapy regimens (Table 1).

During the median follow-up of 90 mo (range: 6–252 mo), 138 (14.6%) patients developed recurrence, and 40 (29.0%) of recurrences were locoregional and 98 (71.0%) were distant and 95 (9.1%) of patients died. Metastasectomy was performed for 48 of patients with recurrence (Table 1).

No statistical difference existed between RCC and LCC in terms of gender, smoking and alcohol use, history of DM and HT, tumor grade, stage, pT stage, pN stage, LVI and PNI positivity, positive surgical margins, adjuvant therapy use, the regimen used for adjuvant therapy, rates for recurrence (locoregional or distant), metastasectomy and death. Rate of mucinous adenocarcinoma histology, rate of LN number of ≥ 12 , and the mean number of LNs dissected were significantly higher in the RCC group ($P = 0.002$, $P < 0.001$, and $P < 0.001$, respectively) (Table 1).

At all stages, 1, 3, 5, 10, and 15-year DFS and OS rates were 97.9%, 89.8%, 87.0%, 84.4%, 82.7% and 99.8%, 96.7%, 92.4%, 86.7%, 86.6%, respectively. In stage II RCC and LCC, rates of DFS at 1, 3, 5, 10, and 15 years were 98.9%, 93.9%, 93.1%, 92.0%, 90.3% and 98.0%, 94.5%, 91.8%, 90.5%, 90.5%, respectively. In stage III RCC and LCC, rates of DFS at 1, 3, 5, 10, and 15 years were 96.2%, 83.6%, 79.4%, 75.0%, 73.2% and 96.8%, 81.9%, 78.2%, 74.4%, 72.2%, respectively (Table 2).

In stage II RCC and LCC, rates of OS at 1, 3, 5, 10, and 15 years were 99.3%, 96.2%, 94.5%, 92.7%, 92.7% and 99.7%, 99.3%, 97.0%, 93.8%, 92.1%,

Table 1 Comparison of clinical and pathological data according to tumor localization

		All patients (n = 942)		RCC (n = 456)		LCC (n = 486)		P
		n	%	n	%	n	%	
Age (yr)	< 65	636	67.5	304	66.7	332	68.3	0.590
	≥ 65	306	32.5	152	33.3	154	31.7	
Gender	Male	511	54.2	250	54.8	261	53.7	0.730
	Female	431	45.8	206	45.2	225	46.3	
Family history	No	916	97.2	439	96.3	477	98.1	0.790
	Yes	26	2.8	17	3.7	9	1.9	
Smoking status	No	592	62.8	277	60.7	315	64.8	0.192
	Yes	350	37.2	179	39.3	171	35.2	
Alcohol use status	No	893	94.8	434	95.2	459	94.4	0.614
	Yes	49	5.2	22	4.8	27	5.6	
Mode of surgery	Elective	791	84	400	87.7	391	80.5	0.002
	Emergent	151	16	56	12.3	95	19.5	
DM	No	845	89.7	407	89.3	438	90.1	0.527
	Yes	93	9.9	48	10.5	45	9.3	
HT	No	717	76.1	344	75.4	373	76.7	0.329
	Yes	223	23.7	112	24.6	111	22.8	
Histology	Adenocarcinoma	779	82.7	356	78.1	423	87	< 0.001
	Mucinous	163	17.3	100	21.9	63	13	
Tumor grade	adenocarcinoma							
	Well and moderately	879	93.3	420	92.1	459	94.4	0.151
	Poorly	63	6.7	36	7.9	27	5.6	
Tumor stage	II	567	60.2	271	59.4	296	60.9	0.644
	III	375	39.8	185	40.6	190	39.1	
pT stage	T1-2	133	14.1	57	12.5	76	15.6	0.267
	T3	752	79.8	374	82	378	77.8	
The number of removed lymph nodes	T4	57	6.1	25	5.5	32	6.6	< 0.001
	< 12	296	31.4	102	22.4	194	39.9	
pN	≥ 12	646	68.6	354	77.6	292	60.1	0.589
	N0	567	60.2	269	59	298	61.3	
PNI	N1	273	29	133	29.2	140	28.8	0.879
	N2	102	10.8	54	11.8	48	9.9	
LVI	Negative	728	78.3	354	78.5	374	78.1	0.777
	Positive	202	21.7	97	21.5	105	21.9	
Surgical margin	Negative	629	67.8	303	67.3	326	68.2	0.096
	Positive	299	32.2	147	32.7	152	31.8	
Adjuvant treatment	Negative	928	98.5	449	98.5	479	98.6	0.293
	Positive	8	0.8	6	1.3	2	0.4	
Adjuvant treatment regimen	No	208	22.1	94	20.6	114	23.5	0.978
	Yes	734	77.9	362	79.4	372	76.5	
Completion rate of adjuvant treatment	5-FU-based	493	67.2	243	67.1	250	67.2	0.685
	Oxaliplatin-based	241	32.8	119	32.9	122	32.8	
Tumor recurrence	No	695	94.7	344	95	351	94.4	0.971
	Yes	804	85.4	389	85.3	415	85.4	
Metastasesectomy	Locoregional recurrence	138	14.6	67	14.7	71	14.6	0.553
	Systemic recurrence	40	29	21	31.3	19	26.8	
Status	Exitus	98	71	46	68.7	52	73.2	0.804
	Alive	48	34.8	24	35.8	24	33.8	
Age (yr)	Median	58	19-94	57	19-89	58	21-94	0.141
	Min-Max	90	1-252	90	1-252	90	5-235	
Follow-up (mo)	mean	17.57	10.843	19.78	11.059	15.5	10.223	< 0.001
	SD	1.46	4.068	1.41	2.86	1.5	4.944	

DM: Diabetes mellitus; HT: Hypertension; Max: Maximum; Min: Minimum; LCC: Left colon cancer; LVI: Lymphovascular invasion; n: Number of patients; pN: Pathological lymph node stage; PNI: Perineural invasion; pT: Pathological tumor stage; RCC: Right colon cancer.

respectively. In stage III RCC and LCC, rates of OS at 1, 3, 5, 10, 15 years were 100.0%, 95.5%, 86.2%, 78.9%,

Table 2 Disease free survival and overall survival rates (%) at 12, 36, 60, 90, 120 and 180 mo according to tumor localization

DFS (mo)	All patients (%)	RCC (%)		LCC (%)	
		Stage II	Stage III	Stage II	Stage III
12	97.9	98.9	96.2	98.0	96.8
36	89.8	93.9	83.6	94.5	81.9
60	87.0	93.1	79.4	91.8	78.2
90	84.9	92.6	75.9	91.3	76.7
120	84.4	92.0	75.0	90.5	74.4
180	82.7	90.3	73.2	90.5	72.2
OS (mo)					
12	99.8	99.3	100.0	99.7	100.0
36	96.7	96.2	95.5	99.3	94.4
60	92.4	94.5	86.2	97.0	87.9
90	89.5	94.0	82.5	94.4	86.4
120	87.6	92.7	78.9	93.8	82.9
180	86.6	92.7	78.9	92.1	82.9

LCC: Left colon cancer; OS: Overall survival; RCC: Right colon cancer; DFS: Disease free survival.

78.9% and 100.0%, 94.4%, 87.9%, 82.9%, 82.9%, respectively (Table 2).

In patients with stage II and III disease with or without adjuvant therapy, DFS was similar in terms of primary tumor localization (stage II; log rank $P = 0.547$ and log rank $P = 0.481$, respectively; stage III; log rank $P = 0.976$ and log rank $P = 0.978$, respectively). In stage III disease, there was no statistically significant difference for DFS in patients receiving 5-FU-based or oxaliplatin-based regimens according to tumor location (log rank $P = 0.518$ and log rank $P = 0.638$, respectively) (Figure 1).

In patients with stage II and III disease with or without adjuvant therapy, OS was not statistically significant with respect to primary tumor localization (stage II; log rank $P = 0.381$ and log rank $P = 0.947$, respectively; stage III; log rank $P = 0.378$ and log rank $P = 0.904$, respectively). In stage III disease, there was no statistically significant difference for OS in patients receiving 5-FU-based or oxaliplatin-based regimens according to tumor location (log rank $P = 0.113$ and log rank $P = 0.806$, respectively) (Figure 2). No statistically significant difference was detected between median survival after recurrent/metastatic (OS2) RCC (26 ± 6.2 mo) and LCC (34 ± 4.9 mo) cases (log rank $P = 0.092$) (Figure 3).

Univariate analysis for DFS showed statistically significant factors as age ≥ 65 years, presentation with ileus, stage, pT stage, pN stage, dissected LN < 12 , PNI, LVI, surgical margin positivity, and adjuvant therapy ($P = 0.001$, $P = 0.003$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.008$, and $P = 0.041$, respectively). In multivariate analysis, age ≥ 65 years, presentation with ileus, stage, dissected LN < 12 , PNI, LVI, and adjuvant therapy were detected as statistically significant factors ($P = 0.001$, $P = 0.011$, $P < 0.001$, $P = 0.012$, $P < 0.001$, $P = 0.003$, and $P = 0.005$, respectively) (Table 3).

Univariate analysis for OS revealed statistically significant factors as age ≥ 65 years, HT, stage, pT stage,

pN stage, PNI, LVI, and adjuvant therapy ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, and $P = 0.017$, respectively). In multivariate analysis, age ≥ 65 years, stage, PNI, LVI, and adjuvant therapy were found to be statistically significant factors ($P < 0.001$, $P = 0.036$, $P = 0.001$, $P < 0.001$, and $P = 0.011$, respectively) (Table 4).

DISCUSSION

In this trial, we aimed to investigate whether tumor location had prognostic significance in patients who underwent curative surgery for stage II or III CC with or without adjuvant therapy. In our study, we found that primary tumor localization had no effect on DFS and OS. A number of studies have been conducted in different regions of the world to describe the differences between RCC and LCC^[5-10]. The data related to the prognosis of RCC and LCC are contradictory in recent studies^[5-9,11]. Most studies reported patients with RCC as likely to be older, often female, in advanced stages, and poorly differentiated^[6-12].

In their study of 1224 patients, Mik *et al*^[5] reported that RCC patients were older than LCC patients, with a median age of 67.8 years. LCC patients were likely to have operations for emergent indications. The number of dissected lymph nodes were reported to be higher in RCC (11.7 ± 6 vs 8.3 ± 5 , $P = 0.0001$)^[5]. In another study, the likelihood of RCC was associated with increased age. In addition, T4 tumor, poor differentiation rate, and presence of venous invasion were detected to be significantly higher in RCC^[6]. In our study, the median age was 58 years (range: 19-94 years). Similarly, in our study, LCC patients were more likely to have operations for emergent indications. Likewise, mucinous type was significantly more common in RCC. Unlike other studies, we did not detect significant differences between RCC and LCC in terms of age, gender, pT stage, stage, LVI, and PNI^[5-9,11-13].

Lim *et al*^[7] followed 414 patients with stage I - III

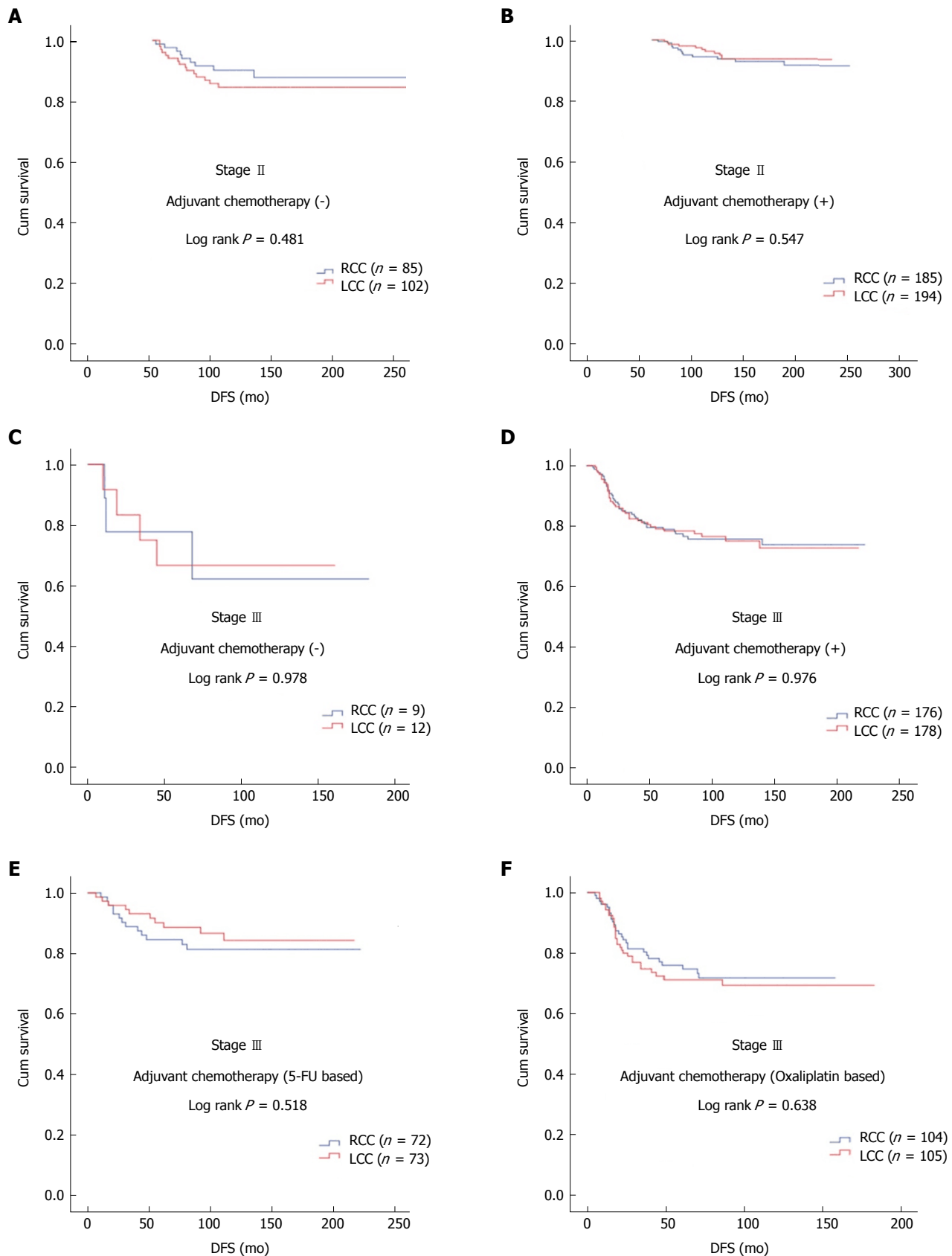


Figure 1 Disease free survival by primary tumor localization in Kaplan-Meier analysis. A: Stage II patients not receiving adjuvant therapy; B: Stage II patients receiving adjuvant therapy; C: Stage III patients not receiving adjuvant therapy; D: Stage III patients receiving adjuvant therapy; E: Stage III patients receiving adjuvant 5-fluorouracil based therapy; F: Stage III patients receiving adjuvant oxaliplatin based therapy. 5-FU: 5-Fluorouracil; DFS: Disease free survival; LCC: Left colon cancer; n : Number of patients; RCC: Right colon cancer.

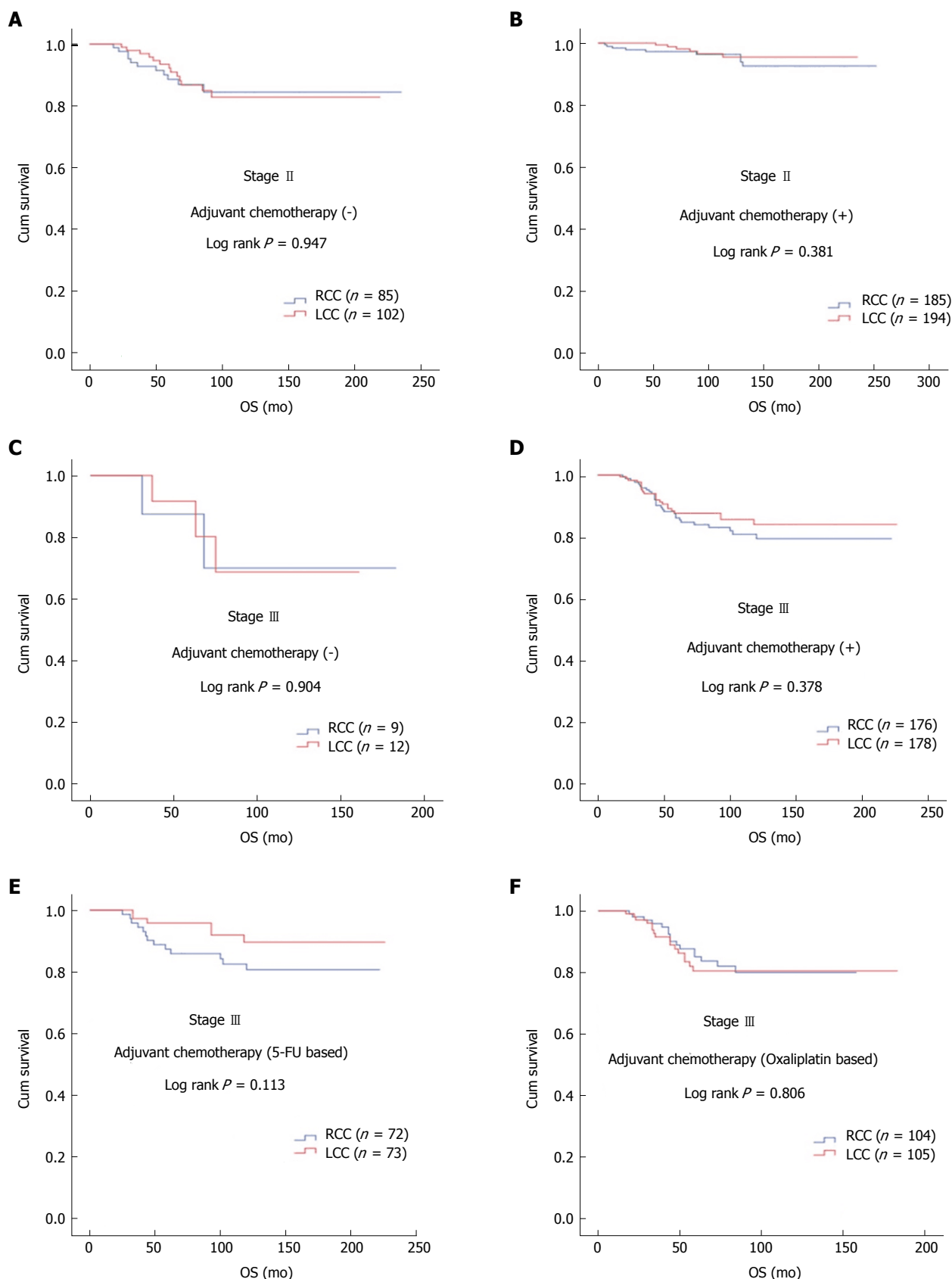


Figure 2 Overall survival by primary tumor localization in Kaplan-Meier analysis. A: Stage II patients not receiving adjuvant therapy; B: Stage II patients receiving adjuvant therapy; C: Stage III patients not receiving adjuvant therapy; D: Stage III patients receiving adjuvant therapy; E: Stage III patients receiving adjuvant 5-fluorouracil based therapy; F: Stage III patients receiving adjuvant oxaliplatin based therapy. 5-FU: 5-Fluorouracil; LCC: Left colon cancer; n : Number of patients; OS: Overall survival; RCC: Right colon cancer.

Table 3 Factors affecting disease free survival

		Univariate analysis				Multivariate analysis			
		HR	95%CI	P		HR	95%CI	P	
Age (yr)	< 65	1				1			
	≥ 65	1.779	1.268	2.496	0.001	1.88	1.305	2.708	0.001
Gender	Male	1							
	Female	0.96	0.686	1.343	0.812				
Family history	No	1							
	Yes	1.195	0.489	2.919	0.696				
Smoking status	No	1							
	Yes	0.908	0.641	1.287	0.587				
Alcohol using status	No	1							
	Yes	0.372	0.118	1.167	0.09				
Mode of surgery	Elective	1				1			
	Emergent	1.796	1.22	2.646	0.003	1.718	1.131	2.611	0.011
DM	No	1							
	Yes	0.973	0.549	1.724	0.925				
HT	No	1							
	Yes	1.541	0.967	2.224	0.067				
Histology	Adenocarcinoma	1							
	Mucinous adenocarcinoma	1.207	0.793	1.839	0.38				
Tumor grade	Well and moderately	1							
	Poorly	1.574	0.889	2.787	0.119				
Tumor location	RCC	1							
	LCC	0.997	0.714	1.392	0.984				
Tumor stage	II	1				1			
	III	2.99	2.109	4.238	< 0.001	2.281	1.485	3.505	< 0.001
pT stage	T1 + 2	1			< 0.001				
	T2	1.912	0.999	3.662	0.05				
	T4	9.308	4.478	19.348	< 0.001				
Number of removed lymph nodes	≥ 12	1				1			
	< 12	2.166	1.421	3.301	< 0.001	1.751	1.13	2.712	0.012
pN	N0	1			< 0.001				
	N1	2.779	1.908	4.047	< 0.001				
	N2	3.56	2.237	5.664	< 0.001				
PNI	Negative	1				1			
	Positive	3.953	2.801	5.578	< 0.001	2.277	1.549	3.347	< 0.001
LVI	Negative	1				1			
	Positive	3.372	2.382	4.774	< 0.001	1.825	1.221	2.728	0.003
Surgical margin	Negative	1							
	Positive	3.884	1.436	10.505	0.008				
Adjuvant treatment	No	1				1			
	Yes	0.591	0.346	0.954	0.041	0.514	0.323	0.82	0.005

DM: Diabetes mellitus; HT: Hypertension; Max: Maximum; Min: Minimum; LCC: Left colon cancer; LVI: Lymphovascular invasion; pN: Pathological lymph node stage; PNI: Perineural invasion; pT: Pathological tumor stage; RCC: Right colon cancer.

CC with a median duration of 66.7 mo, during which the 5-year DFS was significantly higher in LCC (88.3%) than in RCC (81.4%). In multivariate analysis, pT3-4, pN1-2, and histologic grades were reported to be prognostic factors for DFS^[7]. Moritani *et al.*^[8] recruited 820 stage I to III patients with a median follow-up of 55.8 ± 34.9 mo. No statistically significant difference was reported between RCC and LCC in five-year DFS (RCC 88.6%, LCC 89.4%, $P = 0.231$)^[8]. Another study had 4029 stage I to III patients, for which the median follow-up was five years. While three- and five-year DFS rates of patients with RCC were 79.8% and 76.7%, it was 82.0% and 77.6% for LCC, respectively, with no statistically significant difference ($P = 0.35$)^[9].

Five, ten, and 15-year DFS were 87.5%, 84.0%, and

82.1% for RCC and 86.7%, 84.2%, and 83.4% for LCC, respectively. In patients with stage II and III disease with or without adjuvant therapy, DFS was similar in terms of primary tumor localization. Independent risk factors for recurrence included age ≥ 65 years, presentation with ileus, advanced stage, dissected number of LNs < 12, and presence of PNI and LVI.

In the study by Aoyama *et al.*^[9], three and five-year median OS rates were 87.6% and 81.6% for RCC and 91.5% and 84.5% for LCC, where the difference was statistically significant ($P < 0.009$). Investigators have emphasized that this difference might originate from the fact that RCC patients were more likely to be older and to have poorly differentiated and mucinous histology^[9]. A Far East study performed with 4426 RCC,

Table 4 Factors affecting overall survival

		Univariate analysis				Multivariate analysis			
		HR	95%CI		P	HR	95%CI		P
Age (yr)	< 65	1				1			
	≥ 65	4.136	2.731	6.263	< 0.001	4.049	2.578	6.358	< 0.001
Gender	Male	1							
	Female	0.951	0.636	1.423	0.808				
Family history	No	1							
	Yes	0.306	0.043	2.196	0.239				
Smoking status	No	1							
	Yes	0.815	0.533	1.247	0.346				
Alcohol using status	No	1							
	Yes	0.348	0.086	1.411	0.139				
Mode of surgery	Elective	1							
	Emergent	1.342	0.812	2.219	0.252				
DM	No	1							
	Yes	1.683	0.953	2.972	0.073				
HT	No	1							
	Yes	3.067	2.035	4.623	< 0.001				
Histology	Adenocarcinoma	1							
	Mucinous adenocarcinoma	1.213	0.733	2.006	0.452				
Tumor grade	Well and moderately	1							
	Poorly	1.036	0.453	2.369	0.933				
Tumor location	RCC	1							
	LCC	0.807	0.539	1.208	0.297				
Tumor stage	II	1				1			
	III	2.363	1.57	3.557	< 0.001	1.723	1.037	2.863	0.036
pT stage	T1 + 2	1			< 0.001				
	T2	4.836	1.526	15.326	0.007				
Number of removed lymph nodes	T4	21.34	6.162	73.897	< 0.001				
	≥ 12	1							
pN	< 12	1.402	0.897	2.192	0.138				
	N0	1			< 0.001				
PNI	N1	2.122	1.353	3.327	0.001				
	N2	3.015	1.742	5.219	< 0.001				
LVI	Negative	1				1			
	Positive	3.653	2.4	5.562	< 0.001	2.198	1.374	3.517	0.001
Surgical margin	Negative	1				1			
	Positive	3.735	2.445	5.707	< 0.001	2.523	1.543	4.127	< 0.001
Adjuvant treatment	Negative	1			0.187				
	Positive	2.57	0.633	10.435					
	No	1				1			
	Yes	0.587	0.379	0.91	0.017	0.517	0.311	0.86	0.011

DM: Diabetes mellitus; HT: Hypertension; LCC: Left colon cancer; LVI: Lymphovascular invasion; pN: Pathological lymph node stage; PNI: Perineural invasion; pT: Pathological tumor stage; RCC: Right colon cancer.

LCC and rectal cancer patients in all stages reported significantly longer DFS and OS in LCC than those in RCC in univariate analysis, yet survival failed to show significant difference by localization in multivariate analysis. The authors concluded that primary tumor localization was not an independent prognostic factor in Chinese patients with stage I - III colorectal cancer (CRC)^[10]. Patel *et al*^[6] recruited stage II - III CRC patients, 40% of which were RCC and 31% of which had rectal cancer. Merely 45% of stage III CRC cases had received adjuvant therapy. No correlation was found between survival and tumor localization in patients receiving and not receiving adjuvant treatment^[6].

Weis *et al*^[12] reported no difference in 5-year mortality between RCC and LCC of any stage with stage

I to III. Analysis by stage indicated lower mortality at stage II of LCC than RCC and higher mortality at stage III of LCC than RCC^[12]. Warschkow *et al*^[13] reported 5-year OS rate for patients with RCC as 65.1% (95%CI: 64.6-65.6) and LCC as 72.1% (95%CI: 71.5-72.6). The prognosis of RCC in stages I and II was reported as better overall. RCC and LCC had a similar prognosis at stage III. In multivariate analysis, there was no difference between RCC and LCC in terms of 5-year OS^[13]. In another study by Huang *et al*^[14], with 1095 patients at all stages and at all sites including the rectum, only in stage 3 disease were right colon localized tumors worse for survival.

In our study, OS rates at five, ten, and 15 years were found as 91.2%, 87.1%, and 85.2% in RCC compared

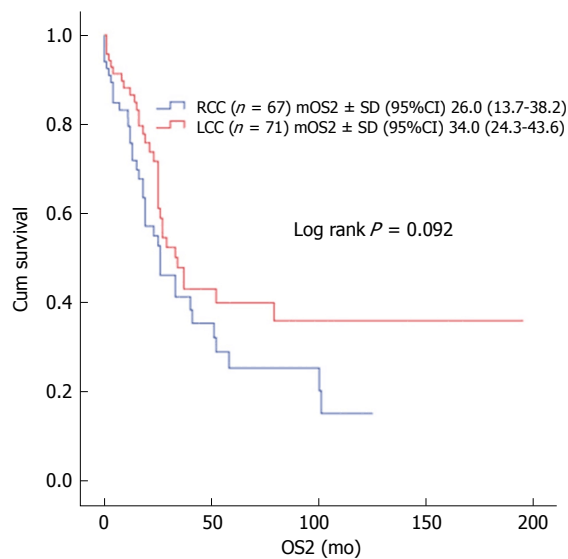


Figure 3 The overall survival effect of tumor localization after recurrence. LCC: Left colon cancer; OS2: Overall survival after recurrence; RCC: Right colon cancer; n: number of patients.

to 93.8%, 88.1%, 88.1% in LCC. There was no significant difference between stage 2 and stage 3 RCC and LCC patients without adjuvant treatment. Despite having a slightly higher mortality in RCC, especially in stage III patients receiving 5-FU-based regimens, but this difference did not reach statistical significance in terms of primary tumor localization in stage II and III patients. Age ≥ 65 years, advanced stage, PNI, and LVI were found to be the most statistically significant factors for mortality in multivariate analysis.

The relationship between tumor localization and prognosis in metastatic disease has been investigated, and studies reported worse prognosis of the right colon than the left colon^[3,4,15]. In a study of 1947 patients with metastatic disease, the median OS was 14 mo (95%CI: 12.7-15.3 mo) in RCC and 20.5 mo (95%CI: 18.5-22.5) in LCC, and this difference was statistically significant ($P < 0.001$)^[15]. In another study by Lee *et al.*^[16] using Australian CRC registry data, the post-recurrence survival in early stage patients was worse in right CC. In a study by Kerr *et al.*^[17], after recurrence, the median OS was 1.25 years and 2.25 years in RCC and LCC, respectively. In the subgroup analysis of 138 patients with recurrence in our study, median OS was 26 mo (95%CI: 13.7-38.2) in RCC and 34 mo (95%CI: 24.3-43.6) in LCC, where the difference did not reach statistical significance, possibly due to the small number of cases ($P = 0.092$).

It is known that in recent years, the incidence of CC at younger ages has increased^[1]. Surveillance, Epidemiology, and End Results (SEER) trials usually involve elderly patients, and data on comorbidities and family history are not available in the SEER database^[11,12]. It is not clear how much these parameters

may have affected the analyses. In our study, patients from all age groups (19-94 years) were included, and the median age was lower than that in other studies. In addition, the duration of median follow-up in our study was 90 mo (6-252 mo), which was longer than that in all other studies^[15-12,14-16]. Besides, our study only included stage II and III patients, unlike other studies^[4,5,8,15-18]. In our study, family history and comorbidities were added to the analysis, where those receiving and not receiving adjuvant therapies were assessed separately.

The causes of the inconsistent relationship between mortality and tumor localization are most likely related to tumor biology. Microsatellite instability (MSI) and BRAF mutations are more likely to be found in RCC than in LCC. BRAF mutations have been reported to be associated with poor prognosis^[13,18]. On the other hand, MSI was reported to have a positive effect on the prognosis of stage II CRC^[13]. Perhaps the most important limitation of our study is the absence of BRAF and MSI data of patients. It is not known how the MSI and BRAF situation affects the results of the study. In our study, the number of dissected LNs was lower than that in RCC, and the percentage of patients with < 12 dissected LN number were higher in LCC. This might have affected DFS and OS in LCC. In addition, our study did not analyze disease-specific survival; therefore, some of the mortal events might have occurred for non-cancer reasons during the long follow-up period.

In conclusion, tumor localization was not found to be associated with DFS or OS in stage II and III CC patients who were treated with or without adjuvant therapy. However, it was observed that OS was worse in RCC patients after recurrence. Further large and prospective studies also involving MSI and BRAF status are warranted.

ARTICLE HIGHLIGHTS

Research background

It is well known that metastatic right colon cancer (RCC) is more aggressive than left colon cancer (LCC). However, the effects of tumor location on the decision of adjuvant therapy and survival are not clearly known in early stage disease.

Research motivation

In recent trials, prognosis data of early stage RCC and LCC are conflicting. The uncertainty of whether tumor localization is functioning as an important additional risk factor for patients and clinicians in locoregional disease is still present.

Research objectives

In our study, we examined the effect of tumor localization on survival in patients who received or did not receive adjuvant therapy for stage II and III colon cancer. We also investigated the effects of chemotherapy regimens in stage III disease on survival in terms of tumor site.

Research methods

In the study, a total of 942 patients with stage II-III colon cancer, excluding rectal cancer, were included. Comorbidities (diabetes mellitus, hypertension),

family histories, adjuvant therapy status and chemotherapy regimens were added to the analysis. The tumors from the caecum to the splenic flexure were defined as RCC and those from the splenic flexure to the sigmoid colon as LCC.

Research results

There was no difference for age and gender in the groups. Mucinous adenocarcinoma rate and the number of removed lymph nodes was higher in the RCC group. Recurrence and mortality risk was lower in patients with adjuvant treatment for all stages. In patients with stage II and III disease with or without adjuvant therapy, disease free survival and overall survival were similar in terms of primary tumor localization. In stage III disease, there was no statistically significant difference for disease free survival and overall survival in patients receiving 5-Fluorouracil (commonly known as 5-FU)-based or oxaliplatin-based regimens according to tumor location. After recurrence, RCC was more aggressive.

Research conclusions

In conclusion, our study showed no association of tumor localization with either disease free survival or overall survival in patients with stage II or III colon cancer managed with or without adjuvant therapy. However, after recurrence, RCC was more aggressive.

Research perspectives

Further large and prospective studies also involving microsatellite instability and BRAF status are needed to determine the effectiveness of tumor location on decision of adjuvant therapy in patients with stage II-III colon cancer.

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Retrospective Study

Comparison of efficacy and safety between standard-dose and modified-dose FOLFIRINOX as a first-line treatment of pancreatic cancer

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Abstract

AIM

To directly compare the efficacy and toxicity of standard-dose FOLFIRINOX (sFOLFIRINOX) and modified-dose FOLFIRINOX (mFOLFIRINOX, 75% of standard-dose) for pancreatic cancer.

METHODS

One hundred and thirty pancreatic cancer patients who received sFOLFIRINOX ($n = 88$) or mFOLFIRINOX ($n = 42$) as their first-line chemotherapy from January 2013 to July 2017 were retrospectively reviewed. For efficacy analysis, the objective response rate (ORR),

disease control rate (DCR), progression-free survival (PFS), and overall survival (OS) were evaluated and compared using Pearson's chi-square test, Kaplan-Meier plot and log-rank test. The adverse events (AEs) were evaluated, and severe (\geq grade 3) AEs rates of the two groups were compared for toxicity analysis.

RESULTS

The mFOLFIRINOX group included more female patients (30.7% *vs* 57.1%; $P = 0.004$) and older patients [age (median), 57 *vs* 63.5; $P = 0.018$] than the sFOLFIRINOX group. In the efficacy analysis, the ORR and DCR were not significantly different between the two groups (ORR: 39.8% *vs* 35.7%; $P = 0.656$; DCR: 80.7% *vs* 83.3%; $P = 0.716$). The median PFS and OS were also not different between the groups (PFS: 8.7 mo *vs* 8.1 mo, $P = 0.272$; OS: 13.9 mo *vs* 13.7 mo, $P = 0.476$). In the safety analysis with severe AEs, the rates of neutropenia (83.0% *vs* 66.7%; $P = 0.044$), anorexia (48.9% *vs* 28.6%; $P = 0.029$) and diarrhea (13.6% *vs* 0.0%; $P = 0.009$) were markedly lower in the mFOLFIRINOX group.

CONCLUSION

mFOLFIRINOX showed comparable efficacy but better safety compared to sFOLFIRINOX. If clinically necessary, initiating FOLFIRINOX with 75% of the standard-dose can alleviate toxicity concerns without compromising efficacy.

Key words: Dose modification; Adverse event; Pancreatic cancer; Adenocarcinoma; FOLFIRINOX; Chemotherapy

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Core tip: Although the efficacy of FOLFIRINOX for pancreatic cancer has been well demonstrated, its relatively high toxicity rate is an important concern. We aimed to directly compare the efficacy and toxicity of standard-dose FOLFIRINOX and modified-dose FOLFIRINOX (mFOLFIRINOX, 75% of standard-dose) for pancreatic cancer. One hundred and thirty patients with pancreatic cancer (standard: 88 *vs* modified: 42) were reviewed retrospectively. Response rates, progression-free survival, and overall survival were not different between both groups. However, severe adverse events such as neutropenia, anorexia and diarrhea were significantly lower in the mFOLFIRINOX group. If clinically necessary, initiating FOLFIRINOX with 75% of the standard-dose can alleviate toxicity concerns without compromising efficacy.

Kang H, Jo JH, Lee HS, Chung MJ, Bang S, Park SW, Song SY, Park JY. Comparison of efficacy and safety between standard-dose and modified-dose FOLFIRINOX as a first-line treatment of pancreatic cancer. *World J Gastrointest Oncol* 2018; 10(11): 421-430 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i11/421.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i11.421>

INTRODUCTION

Pancreatic cancer (PC) is the fourth-most common cause of cancer deaths estimated in the United States^[1]. It is also reported as the fifth-most common cause of cancer-related deaths in South Korea^[2]. Despite the introduction of several novel regimens, the five-year survival rate for all stages of PC remains around ten percent^[1,2]. These statistics are based on the fact that < 20% of newly diagnosed PC cases are suitable candidates for surgical resection, while disseminated disease was noted in > 50% of new cases^[1].

Ever since the survival benefit of gemcitabine in patients with advanced PC was reported, gemcitabine-based regimens have been primarily used for > twenty years^[3-6]. Recently, a non-gemcitabine-based combination regimen comprising folinic acid (FA), 5-fluorouracil (5-FU), irinotecan, and oxaliplatin (FOLFIRINOX) was introduced for metastatic PC (MPC). In the PRODIGE4/ACCORD11 randomized phase III trial, FOLFIRINOX was associated with a significant survival benefit compared to gemcitabine monotherapy as the first-line therapy for patients with MPC^[7]. Thereafter, several studies were conducted to determine the role of FOLFIRINOX in locally advanced PC (LAPC) or borderline resectable PC (BRPC), and meta-analysis reports showed promising improvements in median survivals and resection rates^[8,9]. Consequently, FOLFIRINOX is recommended as a preferred front-line therapy for MPC in major up-to-date guidelines and on the list of options for BRPC or LAPC, although prospective randomized data are still lacking^[10-12].

However, the relatively high toxicity of FOLFIRINOX is still a concern. In the PRODIGE4/ACCORD11 trial, FOLFIRINOX showed higher severe toxicity rates than gemcitabine, particularly for grade three or four neutropenia in 45.7% of patients^[7]. The National Comprehensive Cancer Network guidelines for PC restrict FOLFIRINOX to patients with Eastern Cooperative Oncology Group performance status (ECOG-PS) 0 or 1^[12]. Owing to the high toxicity profile of FOLFIRINOX, several retrospective studies and phase II trials using modified-dose FOLFIRINOX (mFOLFIRINOX) were performed with variable modification strategies. This research showed improved safety profiles and comparable efficacy^[13-17]. Nevertheless, clinical feasibility or optimal strategy for dose-modification of FOLFIRINOX still remains unclear, since previous studies on mFOLFIRINOX indirectly compared their results to those of the PRODIGE4/ACCORD11 trial. Direct comparative study between standard-dose FOLFIRINOX (sFOLFIRINOX) and mFOLFIRINOX is still lacking. Therefore, in this study, we directly compared the therapeutic efficacy and safety of sFOLFIRINOX and mFOLFIRINOX as first-line

chemotherapies for PC.

MATERIALS AND METHODS

Patient selection

All patients diagnosed with PC who received FOLFIRINOX as their first-line chemotherapy in Severance Hospital from January 2013 to July 2017 were retrospectively reviewed. The inclusion criteria were as follows: (1) patients over 19 years of age; (2) histologically- or cytologically-proven pancreatic adenocarcinoma; and (3) at least one measurable lesion in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1^[18]. The exclusion criteria were as follows: (1) discontinued FOLFIRINOX for any reason before the first response evaluation; (2) dose adjustment in the first cycle other than 75% of the standard-dose; (3) did not start the first cycle of FOLFIRINOX in Severance Hospital; (4) diagnosed other active malignancy at the same time as PC diagnosis; (5) administered another agent in combination with FOLFIRINOX; and (6) regularly administered granulocyte colony stimulating factor (G-CSF) for primary prophylaxis. All patients who met the inclusion criteria and did not meet the exclusion criteria were identified. These patients were divided into sFOLFIRINOX and mFOLFIRINOX groups according to their starting dose of FOLFIRINOX.

Work-up and treatment

Pretreatment assessment was conducted for all patients. Appropriate imaging modalities were used for staging work-up, as needed. The specimen for histological or cytological confirmation of malignancy was obtained by endoscopic ultrasonography-guided fine needle aspiration, percutaneous biopsy, or exploratory laparotomy, as indicated. For each patient, the attending physician made a clinical decision on whether the first cycle should be initiated with sFOLFIRINOX or mFOLFIRINOX. sFOLFIRINOX comprised a 2 h intravenous infusion (IVF) of oxaliplatin 85 mg/m², followed by a 90 min IVF of irinotecan 180 mg/m². FA 400 mg/m² IVF was performed over 2 h after termination of irinotecan infusion. This was followed by a 5-FU 400 mg/m² bolus and 2400 mg/m² IVF for 46 h. Patients who received a standard dose at the first cycle were grouped as sFOLFIRINOX. Patients who started with a 75% of standard-dose based on the decision of the attending physician were grouped as mFOLFIRINOX. All patients were regularly administered 0.25 mg of palonosetron 30 min before oxaliplatin infusion for emesis prophylaxis. G-CSF was not used for primary prophylaxis of neutropenia, and was administered when grade three or four neutropenia or neutropenic fever occurred. FOLFIRINOX was repeated every 2 wk until evidence of progressive disease (PD), significant deterioration of patient condition, or patient unwillingness. Dose reduction or delay was at the treating physician's discretion and fully considered if the patient

did not appear to tolerate the dosage of the previous cycle.

Assessment of treatment efficacy

Primary endpoints of this study were objective response rate (ORR) and disease control rate (DCR). Secondary endpoints were progression-free survival (PFS) and overall survival (OS). Treatment response was evaluated after every four cycles using computed tomography or magnetic resonance image. All imaging modalities were conducted and reviewed in compliance with the institutional standard protocols. According to the RECIST, responses were reported by a professional radiologist, and the final assessment was independently made by each attending physician. The best treatment response of each patient was recorded. The ORR included the rate of complete response (CR) and partial response (PR), while DCR was defined as a sum of ORR and the rate of stable disease (SD). For survival analysis, the patient's survival status, date of death, and date of last follow-up were recorded. The cut-off date of both survival and follow-up data was February 6, 2018. PFS was defined from the date of initiation of FOLFIRINOX to PD or death. The patients who survived and remained without PD were censored at the date of the last follow-up. Patients who missed a follow-up without PD and with < a 6-mo follow-up period were censored at 6 mo from treatment initiation, even if deaths were confirmed after that. If a treatment switch occurred without PD, such as curative resection, irreversible electroporation, or another chemotherapeutic regimen, the date of switching treatment was considered as the censoring point. OS was always defined from the date of initiation of FOLFIRINOX to death. Patients whose deaths were not confirmed were censored at the date of the last follow-up.

Assessment of adverse events

Treatment-related AE was also included in the secondary endpoints of this study. During the period of chemotherapy, treatment-related adverse events (AEs) were monitored and recorded by the attending physicians at each visit. All of the patients' medical records on AEs were reviewed. The assessment of AEs was carried out in conformity with the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03^[19]. AEs leading to dose-reduction or dose-delay were recorded separately.

Statistical analysis

For comparing the variables of both groups, Mann-Whitney test was used for continuous variables and Pearson's χ^2 test or Fisher's exact test were used for categorical variables. For the analysis of survival data, the Kaplan-Meier method was used to estimate the median survival with a 95% confidence interval (CI) and the log-rank test was used for comparison. A

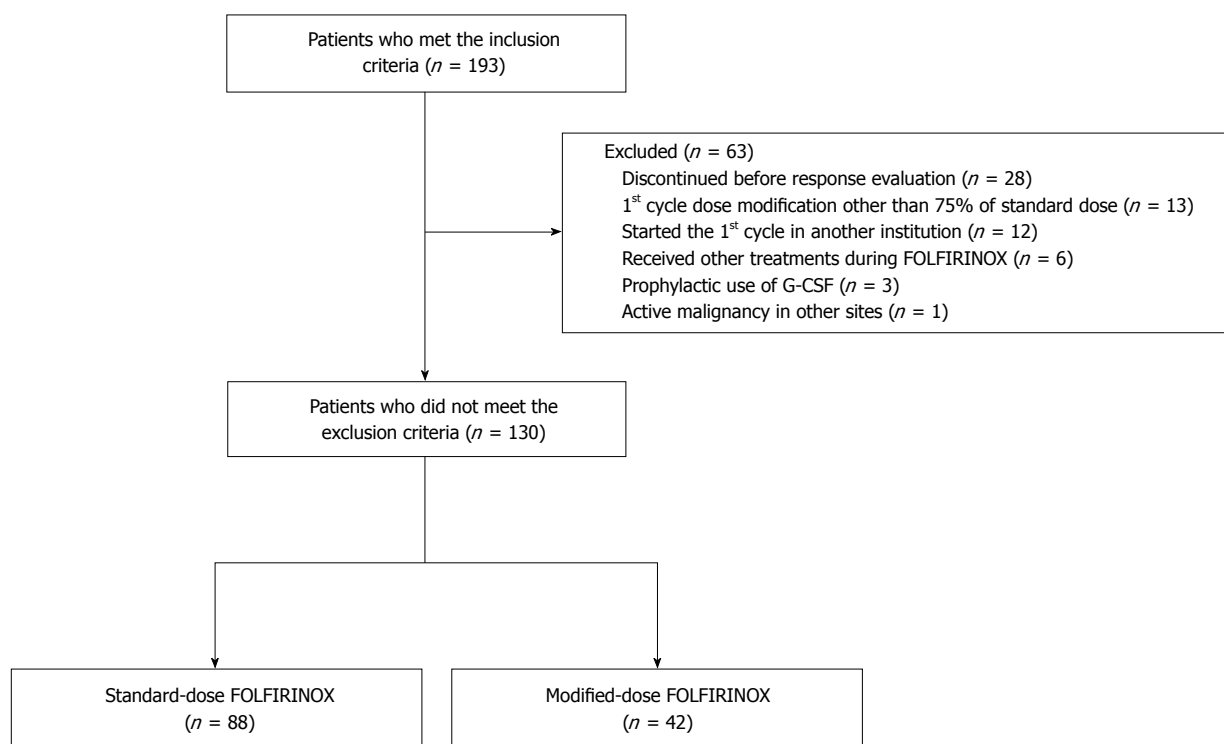


Figure 1 Flow chart of patient selection. G-CSF: granulocyte colony stimulating factor.

Cox proportional-hazards model was used to estimate the adjusted hazard ratios (HR). P -value < 0.05 was considered statistically significant. All statistical analyses were performed with IBM SPSS (version 23.0, IBM Corp., Armonk, NY, United States).

RESULTS

Patients and pretreatment characteristics

In total, 130 patients were included in the final analysis based on the inclusion and exclusion criteria. Of the 130 patients, 88 were assigned to the sFOLFIRINOX group and 42 patients were assigned to the mFOLFIRINOX group. The detailed flow chart of patient selection is shown in Figure 1. When comparing the pretreatment characteristics, the mFOLFIRINOX group included more female patients (30.7% vs 57.1%; $P = 0.004$) and older patients [age (median), 57 vs 63.5; $P = 0.018$] than the sFOLFIRINOX group (Table 1). Other characteristics did not differ between the two groups.

Treatment characteristics

The treatment characteristics are summarized in Table 2. The number of cycles administered and treatment duration were not different between the two groups. The median relative dose intensities (RDIs) of each of the four agents were significantly higher in the sFOLFIRINOX group than in the mFOLFIRINOX group. The proportion of patients who experienced dose-reduction after the first cycle was larger in the sFOLFIRINOX group than in the mFOLFIRINOX group

(70.5% vs 38.1%; $P < 0.001$); however, the rate of dose delay was not different between the two groups. Dose reduction due to neutropenia was higher in the sFOLFIRINOX group (60.2% vs 21.4%; $P < 0.001$), and, therefore, more patients were administered G-CSF (81.8% vs 64.3%; $P = 0.028$) and more G-CSF administrations were performed during the treatment period [3.5 times (range: 0-24) vs 2 times (range: 0-12); $P = 0.043$] than in the mFOLFIRINOX group.

Treatment responses and survivals

The ORR and DCR (primary end-points of this study) were not different between the two groups (Table 3). The median duration of follow-up was 10.3 mo in the sFOLFIRINOX group and 11.1 mo in the mFOLFIRINOX group ($P = 0.181$). The estimated median PFS of both groups were not different [sFOLFIRINOX: 8.7 mo (95%CI: 6.4-11.0) vs mFOLFIRINOX: 8.1 mo (95%CI: 6.7-9.6), $P = 0.272$] (Figure 2A). The estimated median OS of the sFOLFIRINOX group was 13.9 mo (95%CI: 11.5-16.4), and it was not different from that of the mFOLFIRINOX group [13.7 mo (95%CI: 9.5-17.9), $P = 0.476$] (Figure 2B). Additionally, age and sex-adjusted HRs of the mFOLFIRINOX group to the sFOLFIRINOX group were not statistically significant [HR for disease progression or death, 1.36 (95%CI: 0.81-2.26), $P = 0.242$; HR for death, 0.94 (95%CI: 0.55-1.60), $P = 0.813$].

Treatment-related AEs

Severe (grade three or higher) treatment-related AEs

Table 1 Pretreatment characteristics

	sFOLFIRINOX (<i>n</i> = 88)	mFOLFIRINOX (<i>n</i> = 42)	<i>P</i> value
Sex, <i>n</i> (%)			
Male	61 (69.3)	18 (42.9)	0.004 ¹
Female	27 (30.7)	24 (57.1)	
Age, yr			0.018 ¹
Median (range)	57 (31-79)	63.5 (41-77)	
ECOG-PS, <i>n</i> (%)			0.426
0	68 (77.3)	35 (83.3)	
1	20 (22.7)	7 (16.7)	
Laboratory test results, median (range)			
Absolute neutrophil count, / μ L	4200 (1610-11170)	4525 (2080-18930)	0.317
Hemoglobin, g/dL	12.3 (7.1-17.1)	12.1 (8.5-14.9)	0.36
Platelet count, $\times 10^3$ / μ L	218 (76-439)	245 (107-764)	0.247
Total bilirubin, mg/dL	0.7 (0.2-4.8)	0.5 (0.2-2.7)	0.144
Albumin, g/dL	3.9 (2.8-5.0)	3.9 (2.4-4.8)	0.797
Creatinine, mg/dL	0.67 (0.37-1.02)	0.70 (0.37-1.04)	0.516
Level of CA 19-9			
U/mL, median (range)	172.2 (0.6-20000.0)	455.5 (0.7-20000.0)	0.709
Normal, <i>n</i> (%)	17 (19.3)	11 (21.5)	0.274
Elevated, < 59 \times ULN, <i>n</i> (%)	53 (60.2)	19 (45.2)	
Elevated, $\geq 59 \times$ ULN, <i>n</i> (%)	18 (20.5)	12 (28.6)	
Biliary drainage, <i>n</i> (%)			0.435
Presence	29 (33.0)	11 (26.2)	
Tumor location in pancreas, <i>n</i> (%)			0.657
Head	40 (45.5)	16 (38.1)	
Body and tail	44 (50.0)	23 (54.8)	
Recurrent	4 (4.5)	3 (7.1)	
Tumor size, cm			0.313
Median (range)	3.6 (1.3-7.7)	4.0 (1.3-8.0)	
Disease extent, <i>n</i> (%)			0.243
Borderline resectable	17 (19.3)	6 (14.3)	
Locally advanced	26 (29.5)	8 (19.0)	
Metastatic	45 (51.1)	28 (66.7)	
Stage, <i>n</i> (%)			0.248
II	24 (27.3)	8 (19.0)	
III	19 (21.6)	6 (14.3)	
IV	45 (51.1)	28 (66.7)	
Prior treatment, <i>n</i> (%)			0.941
Naïve	75 (85.2)	33 (85.7)	
Curative resection	4 (4.5)	4 (9.5)	
CCRT	9 (10.2)	4 (9.5)	1.000

¹Values indicate statistical significance. mFOLFIRINOX: Modified FOLFIRINOX; sFOLFIRINOX: Standard FOLFIRINOX; ECOG-PS: Eastern Cooperative Oncology Group performance status; ULN: Upper limit of normal range; CA: Carbohydrate antigen; CCRT: Concurrent chemoradiotherapy.

in the two groups are listed and compared in Table 4. Of the hematologic AEs, the rate of severe neutropenia was significantly lower in the mFOLFIRINOX group than in the sFOLFIRINOX group (83.0% vs 66.7%; $P = 0.044$). Other hematologic AE rates, including febrile neutropenia, were not different. Severe anorexia and diarrhea occurred less frequently in the mFOLFIRINOX group than in the sFOLFIRINOX group (48.9% vs 28.6%; $P = 0.029$; 13.6% vs 0.0%; $P = 0.009$; respectively). All other non-hematologic severe AEs tended to occur less frequently in the mFOLFIRINOX group, with the exception of lung infection.

DISCUSSION

In this study, we aimed to retrospectively compare the therapeutic efficacy and safety of sFOLFIRINOX

and mFOLFIRINOX as first-line chemotherapies for PC. To the best of our knowledge, this is the first direct comparative study that evaluated the efficacy and safety of sFOLFIRINOX and mFOLFIRINOX within a single institution. We observed that the median cycle and median duration of FOLFIRINOX were not different in both groups. Although the median RDI of all four agents were significantly less in the mFOLFIRINOX group, the therapeutic parameters such as ORR, DCR, OS, and PFS were not different between the two groups. Regarding the treatment-related AE profiles, severe neutropenia, anorexia, and diarrhea were remarkably lower in the mFOLFIRINOX group than in the sFOLFIRINOX group. Therefore, our study supports dose modification from the initiation of treatment without compromising treatment efficacy, particularly in elderly and female patients, who tend to show more

Table 2 Treatment characteristics

	sFOLFIRINOX (<i>n</i> = 88)	mFOLFIRINOX (<i>n</i> = 42)	<i>P</i> value
Number of cycles administered, median (range)	9.5 (4-24)	12 (4-32)	0.421
Treatment duration, d, median (range)	126 (42-322)	154 (42-434)	0.595
RDI to sFOLFIRINOX, %, median (range)			
Oxaliplatin	85.3 (56.3-100)	75.0 (51.1-75.0)	< 0.001 ¹
Irinotecan	85.0 (56.3-100)	75.0 (51.1-75.0)	< 0.001 ¹
5-FU (bolus)	92.1 (21.4-100)	75.0 (51.1-75.0)	< 0.001 ¹
5-FU (infusion)	94.1 (56.3-100)	75.0 (51.1-75.0)	< 0.001 ¹
Patients with ≥ 1 dose reduction, <i>n</i> (%)	62 (70.5)	16 (38.1)	< 0.001 ¹
Cause of dose reduction (> 5%), <i>n</i> (%)			
Neutropenia	53 (60.2)	9 (21.4)	< 0.001 ¹
Febrile neutropenia	10 (11.4)	4 (9.5)	1.000
Patients with ≥ 1 dose delay, <i>n</i> (%)	55 (62.5)	22 (52.4)	0.272
Cause of dose delay (> 5%), <i>n</i> (%)			
Neutropenia	16 (18.2)	5 (11.9)	0.363
Febrile neutropenia	16 (18.2)	5 (11.9)	0.363
Fatigue	7 (8.0)	8 (19.0)	0.081
No. of G-CSF administered, median (range)	3.5 (0-24)	2 (0-12)	0.043 ¹
Patients received G-CSF, <i>n</i> (%)	72 (81.8)	27 (64.3)	0.028 ¹

¹Values indicate statistical significance. mFOLFIRINOX: Modified FOLFIRINOX; sFOLFIRINOX: Standard FOLFIRINOX; RDI: Relative dose intensity; 5-FU: 5-Fluorouracil; G-CSF: Granulocyte colony-stimulating factor.

Table 3 Response evaluation *n* (%)

	sFOLFIRINOX (<i>n</i> = 88)	mFOLFIRINOX (<i>n</i> = 42)	<i>P</i> value
CR	1 (1.1)	1 (2.4)	
PR	34 (38.6)	14 (33.3)	
SD	36 (40.9)	20 (47.6)	
PD	17 (19.3)	7 (16.7)	
Objective response ^a	35 (39.8)	15 (35.7)	0.656
Disease control ^b	71 (80.7)	35 (83.3)	0.716

^aObjective response includes CR and PR; ^bDisease control includes CR, PR, and SD. mFOLFIRINOX: Modified FOLFIRINOX; sFOLFIRINOX: Standard FOLFIRINOX; CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease.

concern about treatment-related toxicities.

Currently, FOLFIRINOX is a universally-used first-line treatment for MPC^[20,21], and it is also used for second-line or neoadjuvant treatment. Owing to its severe toxicities (grade ≥ 3 neutropenia in 45.7% of patients; grade ≥ 3 fatigue in 23.6% of patients) reported in the PRODIGE4/ACCORD11 trial^[7], treatment-related AE is a major concern when using FOLFIRINOX.

To reduce FOLFIRINOX-related toxicities, several groups have conducted studies focused on dose modification of FOLFIRINOX from the first cycle. Most of the FOLFIRINOX dose-modifying studies compared their results with the PRODIGE4/ACCORD11 trial. Retrospective research conducted in the UK using a reduced dose of irinotecan and omitting a 5-FU bolus reported a markedly lower rate of severe neutropenia than that in the historical trial, with similar rates of other severe AEs^[15]. In a US phase II trial using reduced doses of irinotecan and 5-FU bolus, the rates of severe neutropenia and vomiting were significantly lower than the rates in the historical trial; however, other severe AEs were similar^[17]. The toxicity of mFOLFIRINOX in this

study was less severe than sFOLFIRINOX. In addition, compared with that of the historical trial, the rate of severe diarrhea was lower, but the rates of severe neutropenia, febrile neutropenia, anemia, and vomiting were still higher in the mFOLFIRINOX.

Regarding neutropenia, 77.8% of patients experienced severe neutropenia in a Japanese phase II study of sFOLFIRINOX for chemotherapy-naïve MPC, which is similar to our study's findings^[22]. In addition, most studies conducted in Asian countries reported severe neutropenia in > 65% of patients^[23-26], which was more frequent than that in reports from western countries (11.0%-45.7%)^[7,27-29]. These results suggest that Asians may be prone to severe FOLFIRINOX-related neutropenia, and dose adjustment is an option that should be considered when treating patients belonging to the Asian population. Unlike the present study, prophylactic G-CSF was routinely administered at every cycle in the aforementioned studies focusing on dose modification of FOLFIRINOX^[13-17]. This distinction in therapeutic protocols should be considered when interpreting and comparing the rates of severe

Table 4 Adverse events (\geq Grade 3) *n* (%)

Event	sFOLFIRINOX (<i>n</i> = 88)	mFOLFIRINOX (<i>n</i> = 42)	<i>P</i> value
Hematologic			
Neutropenia	73 (83.0)	28 (66.7)	0.044 ¹
Febrile neutropenia	24 (27.3)	9 (21.4)	0.474
Anemia	19 (21.6)	11 (26.2)	0.561
Thrombocytopenia	8 (9.1)	2 (4.8)	0.499
Non-hematologic			
Fatigue	33 (37.5)	14 (33.3)	0.644
Anorexia	43 (48.9)	12 (28.6)	0.029 ¹
Nausea/Vomiting	53 (60.2)	19 (45.2)	0.108
Diarrhea	12 (13.6)	0 (0.0)	0.009 ¹
Peripheral sensory neuropathy	12 (13.6)	2 (4.8)	0.224
Sepsis	5 (5.7)	0 (0.0)	0.174
Lung infection	3 (3.4)	4 (9.5)	0.212
Biliary tract infection	6 (6.8)	0 (0.0)	0.176

¹Values indicate statistical significance. mFOLFIRINOX: Modified FOLFIRINOX; sFOLFIRINOX: Standard FOLFIRINOX.

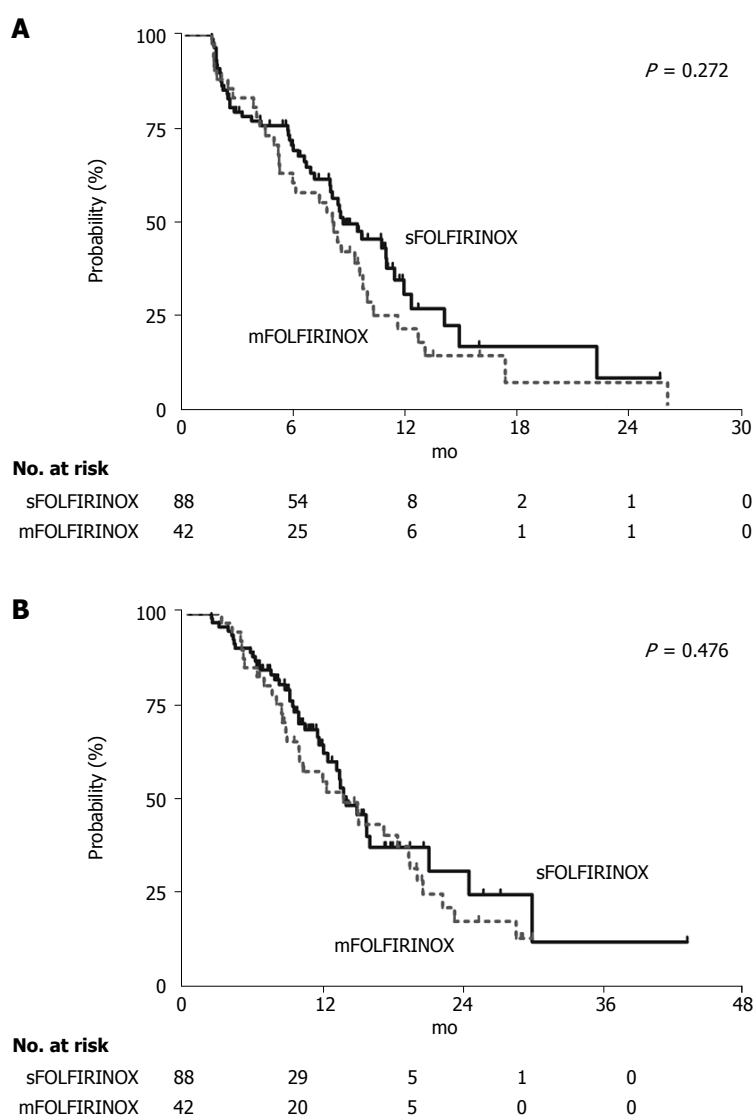


Figure 2 Survival analyses and comparisons. A: Progression-free survival; B: Overall survival, according to the treatment group. mFOLFIRINOX: Modified FOLFIRINOX; sFOLFIRINOX: Standard FOLFIRINOX.

neutropenia and neutropenic fever associated with mFOLFIRINOX in our study with those of prior research (67.9% vs 0%-12%; 26.4% vs 0%-5.6%; respectively).

Regarding efficacy, previous studies using a modified form of FOLFIRINOX showed 17.2%-46.7% of ORR and 80%-100% of DCR, which were similar to those of the PRODIGE4/ACCORD11 trial^[13,15,17]. Our modification of FOLFIRINOX with 75% of the standard-dose was able to markedly reduce toxicity, and the efficacy was comparable with that of sFOLFIRINOX or previous studies, including the PRODIGE4/ACCORD11 trial. This therefore suggests that, in our study population, dose modification to reduce toxicity is possible without compromising treatment efficacy.

There are certain limitations to this study. First, it has a retrospective study design. Although we selected patients based on strict exclusion criteria, the possibility of selection bias and information bias remains. Second, we included patients with BRPC and unresectable PC. When comparing the survival data with other trials, this characteristic of the patient population should be considered. Third, more females and older patients were included in the mFOLFIRINOX group. These differences may be attributed to the clinical characteristics of the patient, based on whether or not the attending physician decides to administer mFOLFIRINOX from the first cycle. These differences may affect the treatment outcome. A previous study reported that female gender could positively predict response to FOLFIRINOX in patients with advanced PC^[30]. However, the prognostic significance of gender in PC remains controversial and warrants further evaluation^[31]. Despite these limitations, this study is meaningful because it directly compares the two study groups, which underwent similar clinical practice within a single institution.

In conclusion, mFOLFIRINOX showed comparable efficacy to sFOLFIRINOX, with a better toxicity profile. Given the relatively high toxicity of sFOLFIRINOX, initiating FOLFIRINOX treatment, if clinically required, with 75% of the standard-dose can be an appropriate option to reduce toxicity concerns without compromising efficacy.

ARTICLE HIGHLIGHTS

Research background

Although FOLFIRINOX is one of the universally-used chemotherapies for pancreatic cancer, its relatively high rate of adverse events is still a major concern. Several studies suggest that dose-modified FOLFIRINOX (mFOLFIRINOX) can improve safety with comparable efficacy compared to the standard FOLFIRINOX (sFOLFIRINOX). However, clinical feasibility and the optimal strategy of mFOLFIRINOX remains unclear.

Research motivation

Previous studies on mFOLFIRINOX made conclusions based on comparing their results to the results of historical phase III trials of FOLFIRINOX. To date, direct comparative studies between sFOLFIRINOX and mFOLFIRINOX for pancreatic cancer is lacking.

Research objectives

We directly compared the safety and efficacy of sFOLFIRINOX and

mFOLFIRINOX in a single study. This could help clarify the clinical applicability of mFOLFIRINOX.

Research methods

The medical records of 130 pancreatic cancer patients [sFOLFIRINOX ($n = 88$), mFOLFIRINOX ($n = 42$)] were retrospectively reviewed. The objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), and overall survival (OS) were compared for efficacy analysis. Severe (\geq grade three) adverse event (AE) rates of the two groups were compared for toxicity analysis.

Research results

Although the median relative dose intensities of each of the drugs were significantly lower in the mFOLFIRINOX group, the response rates and survival were not different between the two groups (ORR: 39.8% vs 35.7%, $P = 0.656$; DCR: 80.7% vs 83.3%, $P = 0.716$; PFS: 8.7 mo vs 8.1 mo, $P = 0.272$; OS: 13.9 mo vs 13.7 mo, $P = 0.476$). Severe AE rates, including neutropenia (83.0% vs 66.7%; $P = 0.044$), anorexia (48.9% vs 28.6%; $P = 0.029$), and diarrhea (13.6% vs 0.0%; $P = 0.009$), were significantly lower in the mFOLFIRINOX group.

Research conclusions

In this direct comparative retrospective study, mFOLFIRINOX showed comparable efficacy to sFOLFIRINOX, with a better toxicity profile. Given the relatively high toxicity of sFOLFIRINOX, initiating FOLFIRINOX treatment, if clinically required, with 75% of the standard-dose could be an appropriate option to reduce toxicity concerns without compromising efficacy.

Research perspectives

In the future, prospective comparative studies need to be conducted to determine the optimal dose modification of FOLFIRINOX and who will benefit from this strategy.

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Retrospective Study

Effect of primary tumor side on survival outcomes in metastatic colorectal cancer patients after hepatic arterial infusion chemotherapy

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Abstract

AIM

To analyze the survival data between patients diagnosed with right-sided primary (RSP) tumors and patients diagnosed with left-sided primary (LSP) tumors after hepatic arterial infusion chemotherapy (HAIC) at our center.

METHODS

A retrospective analysis of pretreated metastatic colorectal cancer patients who received HAIC from May 2006 to August 2015 was conducted. A Cox proportional hazard regression analysis was used to assess the long-term survival outcomes. The mean and median age of patients was 61 years (range 27-85 years). There were 115 males and 53 females in our study.

RESULTS

One hundred sixty-eight patients were enrolled in this study. The overall response rate was 28.9% in LSP patients and 27.3% in RSP patients. The disease control rate was 76.3% in LSP patients and 69.7% in RSP patients. The median overall survival in response to HAIC was 16.3 mo in the LSP arm and 9.3 mo in the RSP arm ($P = 0.164$). The median progression-free survival was 5.7 mo in the LSP arm and 4.2 mo in the RSP arm ($P = 0.851$).

CONCLUSION

There was no significant difference in survival between LSP patients and RSP patients after HAIC. Further prospective studies are needed to confirm these findings.

Key words: Colorectal cancer; Hepatic arterial infusion chemotherapy; Primary tumor side; Local treatment; Hepatic metastasis

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Core tip: Our study shows that the prognosis of left-sided colorectal cancer liver metastasis patients is superior to that of right-sided patients, but no significant difference in survival was found between left-sided primary and right-sided primary patients in response to treatment with hepatic arterial infusion chemotherapy.

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INTRODUCTION

Colorectal cancer is the third leading cause of cancer death in both men and women in the Western world^[1]. In China, the incidence of colorectal cancer is gradually increasing and has become the fourth most frequent cancer in women and the fifth in men^[2]. Gene expression-based subtyping is now widely accepted as a predictive model of survival, including the mutually exclusive RAS and BRAF pathways, as well as the Wnt pathway^[3,4]. In addition, increasing evidence indicates that patients with a left-sided primary (LSP) tumor have a survival advantage compared to those with a right-sided primary (RSP) tumor, indicating that primary location could be a predictive factor^[5]. The distinguishing prognosis is ascribed to differences in biology, pathology, and epidemiology of colorectal cancer based on primary tumor location. LSP tumors arise from the hindgut at their embryological beginnings and are supplied by the inferior mesenteric artery, while RSP tumors arise from the midgut and are supplied by the superior mesenteric artery. There are also biological and molecular pathway variations between these two subtypes^[6-9].

Due to the dissimilar genotype and phenotype of LSP and RSP tumors, the location of primary tumor has turned out to be predictive of outcome^[10,11]. Subsequent studies have found that RSP patients have an inferior outcome in first-line chemotherapy^[12], and targeted agents, such as anti-epidermal growth factor receptor (EGFR) monoclonal antibody and anti-vascular EGFR monoclonal antibody, show differential efficacy in RSP and LSP patients^[5,13,14].

Metastasis occurs in approximately 50% of patients during disease^[15]. Without efficient treatment, metastatic colorectal cancer (mCRC) patients who fail to respond to systemic chemotherapy only survive approximately 3.5 mo^[16]. The survival benefit of third-line chemotherapies is 4.5-10.5 mo^[17]. However, interventional treatments are potential choices for mCRC patients. Transarterial chemoembolization and hepatic arterial infusion chemotherapy (HAIC) can achieve a higher local response rate than systemic chemotherapy and remain effective when patients have failed to respond to previous chemotherapy^[18,19]. Chemo-refractory patients treated with HAIC can survive 7.7-19 mo^[20-23]. However, no studies have reported the relationship between the efficacy of HAIC and the primary tumor side. We gathered survival information on mCRC patients after HAIC in our center to clarify this issue.

MATERIALS AND METHODS

Study design and patient population

This was a retrospective analysis of the survival and

efficacy of HAIC in mCRC patients. The primary criteria for inclusion were as follows: Pathological diagnosis of adenocarcinoma of the colon or rectum, inoperable liver metastases or contraindications for liver resection, systemic chemotherapy failure (experienced at least first-line chemotherapy previously), treated with HAIC in our center, and received tumor assessment after HAIC. Subject demographic variables examined included age, sex, and survival or censored data. Tumor variables examined included location, gene status, histologic grade (well, moderate, or poor), and extrahepatic metastasis. Treatment variables examined included previous treatment, combined liver radiotherapy or radiofrequency ablation, and combined molecular targeted drugs.

RSP patients have a tumor site in the cecum, ascending colon, hepatic flexure, or transverse colon, while LSP patients present tumors in the splenic flexure, descending colon, sigmoid colon, or rectum. Disease evaluation was repeated every two cycles using computed tomography scans, and the Response Evaluation Criteria in Solid Tumors 1.1 criteria was applied. The primary end-point of this study was the overall survival (OS) difference between RSP and LSP patients. Secondary end-points were progression-free survival (PFS) and efficacy of several different chemotherapy regimens. Our retrospective study was in accordance with the ethical standards of the Beijing Cancer Hospital Ethics Committee.

Statistical analysis

OS was defined from the first day of HAIC until death from any cause. PFS was defined from the first day of HAIC until the first objective observation of disease progression or death from any cause. The SPSS software program (version 19; SPSS, Chicago, IL, United States) was used for analyses. The Graph Pad Prism 6 program (Graph Pad Software, Inc., La Jolla, CA, United States) was used to create charts. A Student's *t*-test was used to analyze continuous variables, which are reported as mean \pm SD if normally distributed or as a median and range if skewed. A χ^2 test was used to analyze categorical variables, which are reported as a proportion (%) of the overall cohort. The Kaplan-Meier method was used to approximate PFS and OS, and the significance of survival differences between separate subgroups was assessed using the log-rank test. The Cox proportional hazards model was used to determine the univariate and multivariate hazards ratios for the study parameters. For all tests, a *P*-value < 0.05 was defined as statistically significant.

RESULTS

Patient characteristics

One hundred sixty-eight patients were included in this study between May 2006 and August 2015. The median age was 61 years (range 27-85 years), and

the last follow up day was July 5, 2016. Median follow-up time was 17 mo. Among all patients included in this study, 138 patients died, 14 patients were lost during the follow-up period, and 16 patients were still alive. There were 135 LSP patients and 33 RSP patients. Extrahepatic metastases accounted for more than half of all patients (94/168). There were 17 *KRAS* mutation patients and 48 *KRAS* wild type patients among LSP tumors. There were eight *KRAS* mutation patients and seven *KRAS* wild type patients among RSP tumors. The baseline information of patients, disease, and treatment characteristics by primary tumor location are shown in Table 1. Eighty-nine (65.9%) LSP patients were previously administered first-line systemic chemotherapy, and 46 (34.1%) patients were given second-line or subsequent therapies. Twenty-four (72.7%) RSP patients received first-line systemic chemotherapy, and nine (27.3%) patients received second-line or subsequent lines of chemotherapy.

Patients were injected with 20-40 mg epirubicin hydrochloride after routine arteriography by artery catheter, and iodipin was injected when obvious blood supply was found in the arteriography. Chemotherapy agents administered through the catheter after chemoembolization included oxaliplatin (85 mg/m²) or irinotecan (180 mg/m²) over 4 h, followed by fluorouracil (2000 mg/m²) administered over approximately 44 h and cisplatin/fluorouracil (200 mg/m²) over 2-4 h vs peripheral vein, combined with/without bevacizumab (7.5 mg/kg) or cetuximab (250 mg/m²). Treatments were repeated every three weeks. One hundred fifty-three patients received oxaliplatin-based chemotherapy, and only 15 patients received irinotecan-based chemotherapy. With respect to targeted therapy, 27 (20%) LSP patients were treated with bevacizumab; while another 13 (9.6%) were treated with cetuximab. In RSP patients, there were only two patients treated with bevacizumab and three with cetuximab.

No significant differences were found between RSP and LSP patients in terms of age, sex, tumor variables, or treatment variables (Table 1).

Efficacy of HAIC

The overall response rate was 28.9% in LSP patients and 27.3% in RSP patients. There were 0.7% complete response (*n* = 1), 28.9% partial response (*n* = 39), 47.4% stable disease (*n* = 64), and 23% progressive disease (*n* = 31) in LSP patients. There were 27.3% partial response (*n* = 9), 42.4% stable disease (*n* = 14), and 30.3% progressive disease (*n* = 10) in RSP patients. The disease control rate was 76.3% in LSP patients and 69.7% in RSP patients.

Progression-free survival time

Most of the patients (*n* = 84) who progressed did so due to liver metastasis, while a small number of patients (*n* = 45) progressed due to the progression

Table 1 Patient characteristics

Variable	Left side (<i>n</i> = 135)	Right side (<i>n</i> = 33)	<i>P</i> -value
Age, mean (range), years	60.5 (27-85)	63.8 (37-83)	0.392
Men, <i>n</i> (%)	95 (70.4)	20 (60.6)	0.279
Previous system treatment, <i>n</i> (%)			0.455
Only first line	89 (65.9)	24 (72.7)	
Second line or more	46 (34.1)	9 (27.3)	
Extrahepatic metastasis, <i>n</i> (%)	73 (54.1)	21 (63.6)	0.321
Primary tumor resected, <i>n</i> (%)			0.173
No surgery	22 (16.2)	10 (30.3)	
Palliative surgery	49 (36.3)	11 (33.3)	
Radical surgery	64 (47.4)	12 (36.4)	
Synchronous metastases, <i>n</i> (%)	103 (76.3)	26 (78.8)	0.761
Gene status, <i>n</i> (%)			0.127
<i>KRAS</i> mutation	17 (35.6)	8 (24.2)	
<i>KRAS</i> wild type	48 (12.6)	7 (21.2)	
Unknown	70 (51.9)	18 (54.5)	
Targeted therapy, <i>n</i> (%)			
Bevacizumab treated	27 (14.8)	2 (6.1)	0.21
Cetuximab treated	13 (9.6)	3 (9.1)	
Other local treatment, <i>n</i> (%)	31 (23)	4 (12.1)	0.169
Repeated times of HAIC, <i>n</i> (%)			0.554
2	29 (21.5)	10 (30.3)	
3-4	43 (21.9)	10 (30.3)	
> 6	63 (46.7)	13 (39.4)	

HAIC: Hepatic arterial infusion chemotherapy.

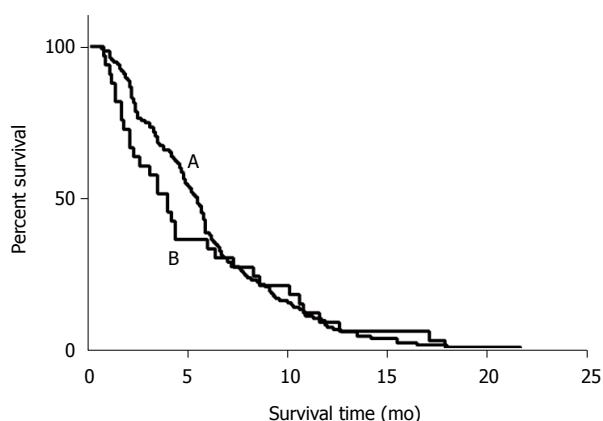


Figure 1 Overall survival data of patients who received hepatic arterial infusion chemotherapy treatment (*n* = 168). The median survival time of left-sided colorectal cancer liver metastasis patients was 16.3 mo (curve A). The median survival time of right-sided colorectal cancer liver metastasis patients was 9.3 mo (curve B).

of extrahepatic metastasis, and another 23 patients exhibited both liver and extrahepatic metastasis progression. Median PFS of all included patients was 5.5 mo (95%CI: 4.9-6.0 mo). The median PFS was 5.7 mo (95%CI: 5.3-6.1 mo) in LPS patients and 4.2 mo (95%CI: 3.2-5.1 mo) in RSP patients, and no significant difference was observed between these two groups ($P = 0.851$) (Table 2 and Figure 1).

The median PFS of LSP patients was 5.5 mo in liver progression ($n = 67$, 54%), 4.7 mo in extrahepatic progression ($n = 39$, 31%), and 6.7 mo in both liver and extrahepatic progression groups ($n = 18$, 15%)

($P = 0.155$). The median PFS of RSP patients was 4.0 mo in liver progression ($n = 16$, 57%), 4.4 mo in extrahepatic progression ($n = 7$, 25%), and 4.4 mo in both liver and extrahepatic progression groups ($n = 5$, 18%) ($P = 0.986$).

LSP patients who had only first-line systemic chemotherapy exhibited a median PFS of 5.9 mo, and those who received second or more lines of treatment exhibited a median PFS of 4.6 mo ($P = 0.001$). RSP patients who had only first-line systemic chemotherapy exhibited a median PFS of 4.4 mo, and those who received second or more lines of treatment exhibited a median PFS of 2.3 mo ($P = 0.018$).

OVERALL SURVIVAL TIME

There were 112 out of 135 LSP patients and 26 out of 33 RSP patients who died during the follow-up period. The median OS from the diagnosis of CRC was 31.4 mo in LSP patients and 22.2 mo in RSP patients ($P = 0.186$). The OS after HAIC was 16.3 mo in LSP patients and 9.3 mo in RSP patients ($P = 0.164$) (Figure 2).

The median OS after HAIC in patients treated with HAIC and bevacizumab was 22 mo, and patients treated with HAIC and cetuximab or HAIC only exhibited a median OS of 15.4 mo ($P = 0.162$). LSP patients treated with HAIC and bevacizumab had a median OS of 24.5 mo and 15.4 mo in the cetuximab arm ($P = 0.053$). No significant difference was observed between the bevacizumab and cetuximab arms. Only two RSP patients were treated with bevacizumab, and their OS was 9.3 mo and 13 mo. The three RSP patients treated

Table 2 Analyses of survival outcomes by primary tumor location

Subgroup	OS events <i>n</i> (%)	Median OS, mo (95%CI)		Hazard ratio (95%CI)	<i>P</i> -value	PFS events <i>n</i> (%)	Median PFS, mo (95%CI)		Hazard ratio (95%CI)	<i>P</i> -value
		Left-sided	Right-sided				Left-sided	Right-sided		
All eligible patients (<i>n</i> = 168)	138 (82.1)	16.3 (13.5-19.0)	9.3 (3.4-15.1)	0.74 (0.48-1.13)	0.164	151 (89.9)	5.7 (5.3-6.1)	4.2 (3.2-5.1)	0.96 (0.64-1.50)	0.851
KRAS wild type (<i>n</i> = 55)	44 (76.4)	17.6 (12.3-22.9)	15.4 (6.0-24.7)	0.85 (0.33-2.19)	0.74	51 (92.7)	5.1 (4.2-5.9)	4.0 (2.7-5.3)	0.76 (0.32-1.81)	0.529
KRAS mutation (<i>n</i> = 25)	18 (72)	10.9 (0-34.6)	9.0 (2.4-15.5)	0.77 (0.29-2.02)	0.6	22 (88)	4.8 (2.9-6.6)	2.1 (0-5.0)	0.97 (0.36-2.58)	0.956
KRAS unknown (<i>n</i> = 88)	78 (88.6)	16.1 (14.1-18.1)	9.3 (6.9-11.7)	0.69 (0.38-1.24)	0.218	78 (88.6)	6.2 (5.1-7.3)	6.0 (3.3-8.7)	0.75 (0.42-1.33)	0.324
Bevacizumab (<i>n</i> = 29)	21 (72.4)	24.5 (16.6-32.3)	9.3 (-)	0.30 (0.06-1.43)	0.11	27 (93.1)	6.2 (4.9-7.4)	4.0 (-)	0.45 (0.10-2.01)	0.285
Cetuximab (<i>n</i> = 16)	12 (75)	16.5 (9.0-23.9)	8.2 (-)	0.21 (0.03-1.29)	0.065	15 (93.8)	3.6 (0.89-6.3)	4.0 (-)	0.42 (0.08-2.06)	0.269

OS: Overall survival; PFS: Progression-free survival.

with cetuximab exhibited an OS of 2.6 mo, 3.8 mo, and 8.2 mo.

The median OS in KRAS wild type patients (*n* = 55) was 16.6 mo, 13 mo in patients with KRAS mutation (*n* = 25), and 15.6 mo in KRAS status unknown patients (*n* = 88). In KRAS wild type patients, ten were treated with cetuximab and six with bevacizumab. The median OS of these two group were 11.5 mo and 22 mo, respectively (*P* = 0.087) (Table 2). Among all 48 LSP KRAS wild type patients, nine were treated with bevacizumab and eleven with cetuximab. The median OS of these two different treatments was 28.1 mo and 21.1 mo, respectively (*P* = 0.444). There were only seven KRAS wild type patients in the RSP group.

LSP patients who progressed by liver metastases had a median OS of 18.8 mo, progression of extrahepatic metastasis was 14.6 mo, and progression of both liver and extrahepatic metastasis was 13.7 mo (*P* = 0.771). RSP patients who progressed by liver metastases exhibited a median OS of 8.6 mo, progression of extrahepatic metastasis was 10.1 mo, and progression of both liver and extrahepatic metastasis was 9.3 mo (*P* = 0.885). No significant difference was observed in survival between liver metastasis only and extrahepatic metastases patients (*P* = 0.493).

A prognostic factor analysis showed that different infusion agents resulted in differential survival. OXA-based infusion chemotherapy (*n* = 153) resulted in a median OS of 15.8 mo, while CPT-11-based chemotherapy (*n* = 15) reached 22.8 mo (*P* = 0.518). Neither LSP nor RSP patients experienced a significant difference in this treatment variable. Among all factors considered, primary tumor histology, radiofrequency ablation or liver radiotherapy, normal serum CA19-9 levels, and response to HAIC were protective factors associated with OS (Table 3).

DISCUSSION

Differences in survival resulting from differences in biological behavior were examined in LSP and RSP patients. In our study, we analyzed the survival data between patients with LSP tumors and those with RSP tumors after HAIC in mCRC in our center. When comparing PFS between LSP and RSP patients, no obvious advantages were found in LSP patients; however, a trend did exist. These results suggest that combined hepatic arterial infusion (HAI) does not change survival in patients with liver metastasis from either LSP or RSP colorectal cancer, which is inconsistent with the survival data for mCRC patients who undergo hepatic metastasis resection. Patients treated with hepatic metastasis surgery exhibit an OS similar to LSP and RSP patients after liver metastasis. However, this result was based on retrospective analysis, and patient selection bias was likely to have influenced the outcome. We cannot conclude that local treatment of liver metastasis reverses the worse prognosis in RSP patients.

In systemic chemotherapy, one of the most important prognostic factors is the use of molecular targeted drugs, especially with respect to differences between anti-EGFR and anti-vascular endothelial growth factor monoclonal antibodies. However, an interesting phenomenon was found in our study wherein the OS of LSP patients was significantly better in those treated with bevacizumab than in those treated with cetuximab, and the OS of RSP patients exhibited the same trend. This phenomenon is completely opposite to data concerning systemic chemotherapy in both LSP and RSP patients. Possible reasons for these discrepancies include the following: The optimal dose of bevacizumab and cetuximab in HAI treatment has not been clearly verified; only a few cases were treated with cetuximab; only KRAS genotyping was performed

Table 3 Univariate analysis of predictive factor of survival after first hepatic arterial infusion chemotherapy

Variable	MST (mo)	Univariate analysis		P-value
		HR	95%CI	
Primary tumor site (right/left)	9.3 vs 16.3	1.353	0.881-2.079	0.167
Age ($> 60 / < 60$ yr)	16 vs 15.5	1.026	0.731-1.440	0.88
Gender (male/female)	16.5 vs 13	0.744	0.520-1.063	0.104
Histology (poor/well to moderate)	10.3 vs 15.9	1.706	1.003-2.904	0.049*
Serum CA19-9 (≥ 37 U/mL/ < 37 U/mL)*	12.5 vs 21.2	2.108	1.444-3.076	$< 0.001^*$
Serum CA72-4 (≥ 6.7 U/mL/ < 6.7 U/mL)*	13 vs 20.8	1.605	1.114-2.311	0.011*
Serum CEA (≥ 5 U/mL/ < 5 U/mL)*	14.6 vs 21.1	1.428	0.867-2.351	0.162
Extrahepatic metastasis (present/absent)	15.8 vs 15.8	1.172	0.825-1.667	0.376
Time to liver metastasis (synchronous/ metachronous)	14.8 vs 16.5	1.125	0.802-1.580	0.495
Other local treatment (combined/uncombined)	21.1 vs 14.6	0.651	0.426-0.995	0.047*
Response to HAIC				$< 0.001^*$
PR	21.9	0.234	0.146-0.375	$< 0.001^*$
SD	16.1	0.285	0.185-0.439	$< 0.001^*$
PD	7.5	1	1	NA
Infusion agents (OXA/CPT-11)	15.8 vs 22.8	1.225	0.660-2.273	0.52

MST: Median survival time; HR: Hazard ratio; HAIC: Hepatic arterial infusion chemotherapy; PR: Partial response; SD: Stable disease; PD: Progressive disease.

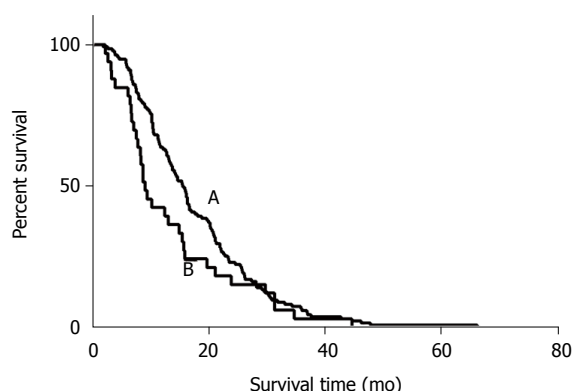


Figure 2 Progression-free survival data of patients who received hepatic arterial infusion chemotherapy treatment ($n = 168$). The median PFS of left sided colorectal cancer liver metastasis patients was 5.7 mo (curve A). The median PFS of right sided colorectal cancer liver metastasis patients was 4.2 mo (curve B).

instead of testing all RAS genes; and HAI treatment was not a first-line treatment in our study. Another study reported that RAS gene mutations might be influenced by previous treatment. However, in LSP patients, bevacizumab treatment showed an obvious advantage compared with cetuximab, and this advantage could even be observed in RAS wild-type patients. This demonstrates that in HAIC treatment, especially in left-sided colorectal cancer liver metastasis, bevacizumab is superior to cetuximab.

In comparison with cytotoxic agents, irinotecan seems superior to oxaliplatin in OS after HAI treatment. However, in first-line treatment of all patients, the vast majority received oxaliplatin-based systemic chemotherapy, so the data could support the conclusion

that irinotecan is superior to oxaliplatin in HAI treatment. However, it is worth noting that, as a second-line or subsequent treatment, HAIC obtained close to 30% objective remission rates in both LSP and RSP patients when most patients had previously received oxaliplatin. The overall response rate observed in this study was obviously superior to second-line systemic chemotherapy and was similar to systemic therapy treatment using FOLFOX and bevacizumab (E3200)^[24], suggesting that HAIC treatment might be superior to systemic cytotoxic chemotherapy in second-line conversion therapy for mCRC.

In conclusion, for HAIC treatment of mCRC, the survival of patients with left colon cancer remains better than that of right colon cancer patients. Subgroup analysis showed that bevacizumab might be superior to cetuximab, especially in left-sided colorectal cancer liver metastasis. However, further study is needed on the optimal dosage and mode of administration of molecular targeted drugs for HAIC treatment. Both oxaliplatin and irinotecan achieve considerable objective remission rates.

ARTICLE HIGHLIGHTS

Research background

Previous studies have shown that left-sided colorectal cancer has a better survival prognosis than right-sided colorectal cancer. However, whether this prognosis difference is also present in liver metastasis colorectal cancer (CRC) patients treated with hepatic arterial infusion chemotherapy (HAIC) is still unknown.

Research motivation

Our study attempted to analyze for the first time, whether there would be a difference in survival and overall response rate in liver metastasis CRC patients

treated with HAIC.

Research objectives

To analyze the overall survival and overall response rate difference of patients with liver metastasis of left-sided or right-sided colorectal cancer after HAIC.

Research methods

A retrospective analysis of liver metastasis CRC patients from May 2006 to August 2015 was conducted. Cox proportional hazard regression analysis was used to assess long-term survival outcomes.

Research results

Overall response rate was 28.9% in left-sided primary (LSP) patients, and 27.3% in right-sided primary (RSP) patients. Disease control rate was 76.3% in LSP patients and 69.7% in RSP patients. Median overall survival after HAIC was 16.3 mo in the LSP arm and 9.3 mo in the RSP arm ($P = 0.164$). Median progression-free survival was 5.7 mo in the LSP arm and 4.2 mo in the RSP arm ($P = 0.851$).

Research conclusions

The treatment response rate of HAIC in metastatic CRC patients is similar when compared by different primary tumor site. LSP patients seemed to have a superior survival compared to RSP patients when treated by HAIC but no significant difference was found.

Research perspectives

Further large sample size and multi-center prospective studies are needed to confirm the conclusion of this study.

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Prospective Study

Raman spectroscopy for the diagnosis of unlabeled and unstained histopathological tissue specimens

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Author contributions: Ikeda H and Ito H conceived and designed the study, collected clinical data, collected samples, performed the statistical analysis, and interpreted the data; Hikita M performed Raman spectroscopic analysis; Yamaguchi N, Uragami N, and Yokoyama N performed medical examinations and surgical operations; Hirota Y and Kushima M performed pathological examinations; Ajioka Y and Inoue H participated in the study design and coordination; all authors have read and approved the final paper.

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Abstract

AIM

To investigate the possibility of diagnosing gastric cancer from an unstained pathological tissue using Raman spectroscopy, and to compare the findings to those obtained with conventional histopathology.

METHODS

We produced two consecutive tissue specimens from areas with and without cancer lesions in the surgically resected stomach of a patient with gastric cancer. One of the two tissue specimens was stained with hematoxylin and eosin and used as a reference for laser irradiation positioning by the spectroscopic method. The other specimen was left unstained and used for Raman spectroscopy analysis.

RESULTS

A significant Raman scattering spectrum could be obtained at all measurement points. Raman scattering spectrum intensities of 725 cm^{-1} and 782 cm^{-1} , are associated with the nucleotides adenine and cytosine, respectively. The Raman scattering spectrum intensity ratios of $782\text{ cm}^{-1}/620\text{ cm}^{-1}$, $782\text{ cm}^{-1}/756\text{ cm}^{-1}$, $782\text{ cm}^{-1}/1250\text{ cm}^{-1}$, and $782\text{ cm}^{-1}/1263\text{ cm}^{-1}$ in the gastric adenocarcinoma tissue were significantly higher than those in the normal stomach tissue.

CONCLUSION

The results of this preliminary experiment suggest the feasibility of our spectroscopic method as a diagnostic tool for gastric cancer using unstained pathological specimens.

Key words: Label-free analysis; Raman spectroscopy; Histopathological examination; Gastric cancer

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Core tip: We investigated the possibility of diagnosing gastric cancer from an unstained pathological tissue using Raman spectroscopy, and the findings were compared to those obtained with conventional histopathology. We analyzed unstained gastric pathological specimens by Raman spectroscopy. The Raman scattering spectrum intensity ratios of $782\text{ cm}^{-1}/620\text{ cm}^{-1}$, $782\text{ cm}^{-1}/756\text{ cm}^{-1}$, $782\text{ cm}^{-1}/1250\text{ cm}^{-1}$, and $782\text{ cm}^{-1}/1263\text{ cm}^{-1}$ in the gastric adenocarcinoma tissue were significantly higher than those in the normal stomach tissue. The results of this preliminary experiment suggest the feasibility of our spectroscopic method as a diagnostic tool for gastric cancer using unstained pathological specimens.

Ikeda H, Ito H, Hikita M, Yamaguchi N, Urugami U, Yokoyama N, Hirota Y, Kushima M, Ajioka Y, Inoue H. Raman spectroscopy for the diagnosis of unlabeled and unstained histopathological tissue specimens. *World J Gastrointest Oncol* 2018; 10(11): 439-448 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i11/439.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i11.439>

INTRODUCTION

Histopathologic diagnosis represents the ultimate diagnostic method for many cancers^[1]. The histopathological diagnosis method involves microscopic observation of a formalin-fixed specimen for a morphological diagnosis. Although chemical tissue staining is generally performed, such as hematoxylin and eosin staining, immunohistochemical (IHC) tissue staining using an antigen-antibody reaction may also be performed on pathological tissue specimens to obtain more detailed information on the cells and tissues^[2,3]. Despite its advantage for improving diagnostic accuracy in carcinomas^[4,5], IHC is a longer process than general chemical tissue staining, and the antigen-antibody reaction requires precise conditions; thus, preparation of IHC specimens demands a relatively high level of professional skill.

Raman scattering spectroscopy is a non-destructive method for determining the types and components that make up a given substance^[6], allowing for qualitative evaluation without requiring direct contact with the substance through irradiation and subsequent evaluation of the reflected scattered light (*e.g.*, laser). The Raman scattering intensity is correlated with the target substance^[7], and this method can be used to evaluate substances in any state, *i.e.*, gas^[8], liquid^[9], or solid state^[10]. Besides its simplicity and minimally invasive non-destructive nature, Raman spectroscopy enables the evaluation of substances without staining or labeling for an antigen-antibody reaction, and thus has potential for use in unstained pathological tissue specimens. Moreover, since Raman scattering spectroscopy is also suitable for evaluation of living bodies^[11], evaluation of both the collected tissue as well as the living body might be possible with this approach^[12].

To date, Raman scattering spectroscopy has been used to analyze biological tissue specimens such as the brain^[13], thyroid gland^[14], mammary gland^[15], liver^[16], and kidney^[17]; however, its clinical significance has not yet been clarified.

As a preliminary examination of the potential of Raman scattering spectroscopy for diagnosis, we evaluated this method in an unstained stomach tissue specimen, and compared the findings with those of conventional histopathology.

MATERIALS AND METHODS

Patient and clinical sample

The Institutional Review Board of Showa University

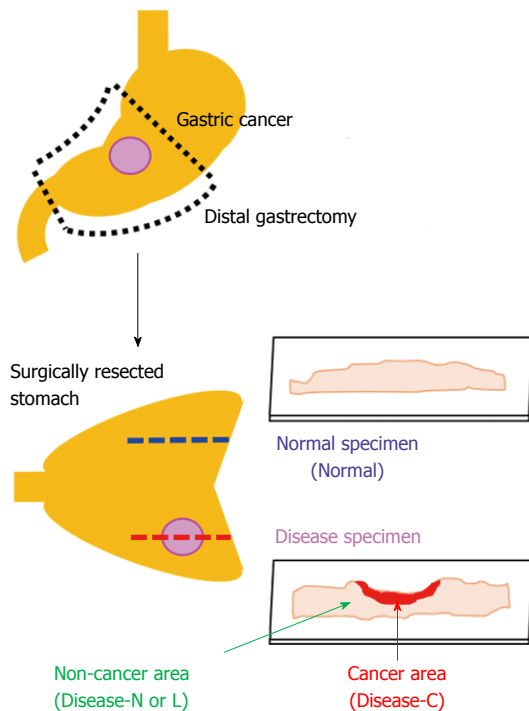


Figure 1 Two consecutive tissue specimens from areas with and without stomach cancer lesions. Each tissue specimen was sliced to a 3- μ m thickness with a microtome and attached to a 1-mm-thick low-autofluorescence slide (SUPER FROST, Matsunami Glass Ind., Ltd, Tokyo, Japan). A thin cover glass (NEO microscope cover glass, Matsunami Glass Ind., Ltd., Tokyo, Japan) was placed on the tissue. Sections were deparaffinized by sequential immersion in xylene, ethanol, and water. One of the two tissue specimens was stained with hematoxylin and eosin and used as a reference for laser irradiation positioning by the spectroscopic method. Another tissue specimen was left unstained and used for analysis by Raman spectroscopy. We acquired the Raman spectrum of the cancer area (Disease-C), non-cancerous lymphocytes infiltration area (Disease-L), non-cancerous normal area (Disease-N) in the stomach cancer specimen, and normal stomach tissue specimen (Normal).

approved the study. This study was registered with the University Hospital Medical Information Network in Japan, number UMIN000017045.

We used the surgically resected stomach of a patient who provided informed consent for its use for this study after explaining the study protocol. The patient was a 61-year-old man diagnosed with early-stage gastric cancer of the mid-stomach, who underwent laparoscopic distal gastrectomy at Showa University Koto Toyosu Hospital in April 2015. The resected stomach was processed using general histopathological specimen preparation procedures. First, it was immersed in 20% neutral buffered formalin solution for 3 d for fixation, and subsequently dehydrated by immersion in 70% ethanol, 90% ethanol, and then 100% ethanol for 100 min each. Finally, the specimen was immersed in xylene three times for 2 h each, and embedded in paraffin.

We produced two consecutive tissue specimens from areas with and without stomach cancer lesions. Each tissue specimen was sliced to a thickness of 3 μ m with a microtome and attached to a 1-mm-thick and low-autofluorescence slide (SUPER FROST, Matsunami

Glass Ind., Ltd., Osaka, Japan). A thin cover glass (NEO microscope cover glass, Matsunami Glass Ind., Ltd., Tokyo, Japan) was placed onto the tissue specimen.

The sections were deparaffinized by sequential immersion in xylene, ethanol, and water. One of the two tissue specimens was stained with hematoxylin and eosin and used as a reference for laser irradiation positioning by the spectroscopic method. Another tissue specimen was left unstained and used for Raman spectroscopy analysis. We acquired the Raman spectrum of the cancer area (Disease-C), non-cancerous lymphocytes infiltration area (Disease-L), and non-cancerous normal area (Disease-N) in the stomach cancer specimen and normal stomach tissue specimen (Normal) (Figure 1).

Histopathological diagnosis

Two specialized pathologists at Showa University Koto Toyosu Hospital performed the histopathological diagnosis, which was determined to be type 0-IIc, 30 mm \times 17 mm, well-differentiated adenocarcinoma, pT1bs (sm2), ly0, v0, pN0, Stage IA.

Spectroscopy

We used an inVia Raman microscope (Renishaw, Gloucestershire, United Kingdom), with a 100 \times objective lens and a laser light source with a wavelength of 532 nm. We irradiated the tissue specimen with minimum power, and then gradually raised the laser output until it became visible within the field of view. The minimum visible laser output was 0.0002 mW. We adjusted the focus so that the beam diameter was minimized, based on visual observation. Spectra were digitized using standard spectroscopy software (WiRE 4; Renishaw, Gloucestershire, United Kingdom).

Spectroscopic measurements

The conditions for laser output and laser irradiation time were established on a marginal part of an unstained tissue specimen that included both gastric cancer lesion and non-lesion areas. To prevent tissue degeneration, we reduced the laser power as much as possible while maintaining detection of the Raman spectrum. Optimal measurement conditions were determined to be a laser output of 1.7 mW and an irradiation time of 10 s.

We measured the tissue specimens at regular intervals from the mucous membrane to the submucosal layer. In principle, intersection points of straight lines every 100 μ m of both the length and width were used as the representative spectrum. We measured 121 points around one intersection point as far as a 10- μ m square, and defined the mean value as a spectrum of the intersection point. From each obtained spectrum, we removed a spectrum only for glass by data processing. Furthermore, we similarly removed the spectrum of auto-logous fluorescence by the fifth-polynomial expression^[18].

When a cell nucleus was observed, the field of view was fine-tuned to focus the laser on it. We measured 60

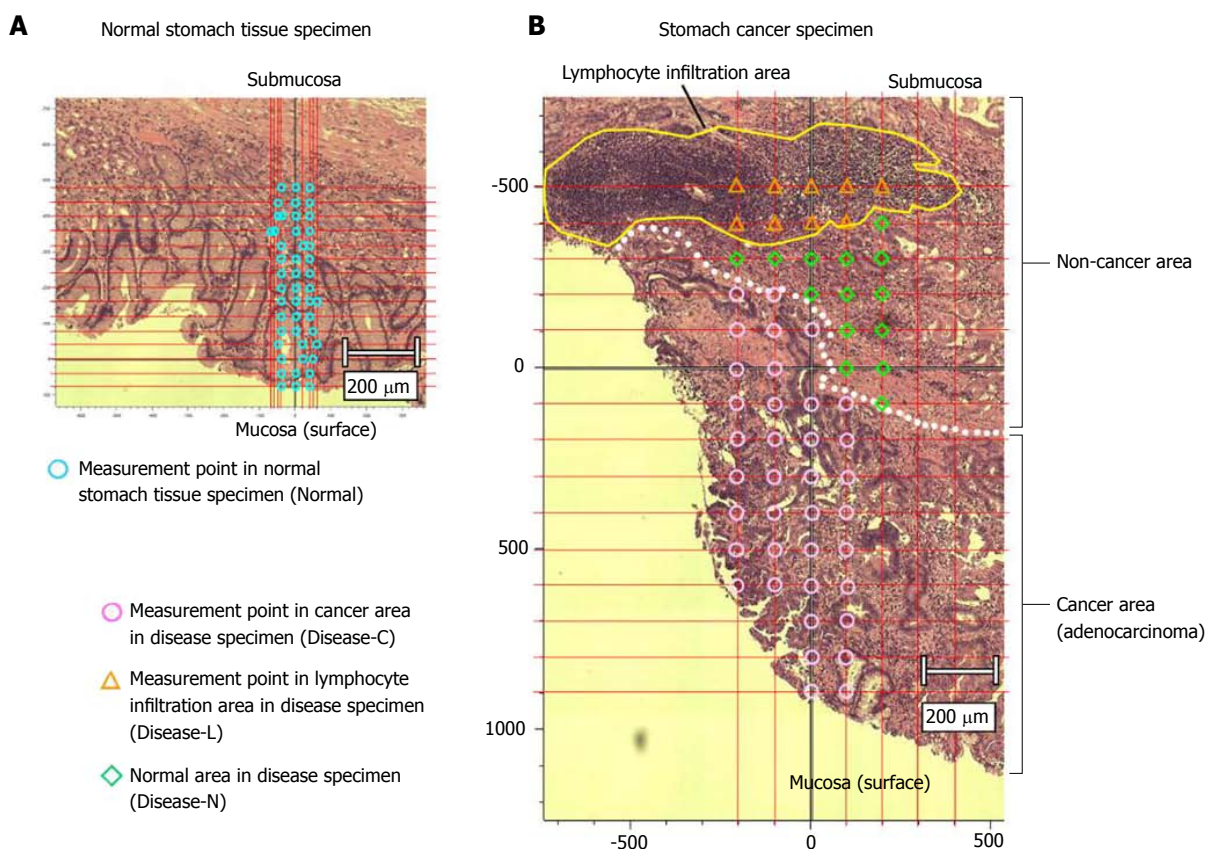


Figure 2 Measured points in the stomach cancer and normal tissue specimens. A: Normal stomach tissue specimen; B: Stomach cancer specimen. We established the conditions for laser output and laser irradiation time on a marginal part of an unstained tissue specimen that included both gastric cancer lesion and non-lesion areas. To prevent tissue degeneration, we reduced the laser power as much as possible, while maintaining detection of the Raman spectrum. Optimal measurement conditions were established as a laser output of 1.7 mW and an irradiation time of 10 s. We measured the tissue specimens at regular intervals from the mucous membrane to the submucosal layer.

and 48 points in the stomach cancer and normal tissue specimens, respectively. The 60 measured points in the stomach cancer specimen included 37 measured points in Disease-C and 23 measured points in the non-cancer area, nine of which were Disease-L and 14 were Disease-N (Figure 2).

Raman scattering spectrum intensity

We measured the Raman scattering spectrum intensities at 620 cm^{-1} (C-C twisting mode of phenylalanine)^[19], 725 cm^{-1} (adenine)^[19], 756 cm^{-1} (symmetric breathing of tryptophan)^[19], 782 cm^{-1} (cytosine)^[20], 1002 cm^{-1} (phenylalanine)^[20], 1250 cm^{-1} (amide III β -sheet)^[21], and 1263 cm^{-1} (amide III α -Helix)^[21], corresponding to the Raman scattering wavenumber of the organism constitution organic substance. We then calculated the ratio of the Raman scattering spectrum intensities of 725 cm^{-1} and 782 cm^{-1} , associated with the nucleotides, to those of the others.

Statistical analysis

Statistical analyses were performed using JMP Pro 13.2.1 software (SAS Institute Inc., Cary, NC, United States). We statistically compared spectral intensity

ratios among the four groups (Disease-C, Disease-N, Disease-L, and Normal) using a non-parametric Wilcoxon test. *P*-values less than 0.05 were considered statistically significant.

RESULTS

A significant Raman scattering spectrum could be obtained at all measurement points. Focusing on the intensity of the Raman scattering wavenumber 725 cm^{-1} derived from the nucleotide adenine, we found that all of the measured values for the ratios $725\text{ cm}^{-1}/620\text{ cm}^{-1}$, $725\text{ cm}^{-1}/756\text{ cm}^{-1}$, $725\text{ cm}^{-1}/1002\text{ cm}^{-1}$, $725\text{ cm}^{-1}/1250\text{ cm}^{-1}$, and $725\text{ cm}^{-1}/1263\text{ cm}^{-1}$ in the Disease-L tissue were significantly higher than those in the Disease-C, Disease-N, and Normal specimens, with no significant difference among these latter three groups (Figure 3). In the biaxial distribution, the distribution areas of the measured values of the Disease-C, Disease-N, and Normal specimens widely overlapped. Only the distribution area of the measurement value of Disease-L extended toward the higher value direction (Figure 4).

Similarly, focusing on the intensity of the Raman scattering wavenumber 782 cm^{-1} derived from the

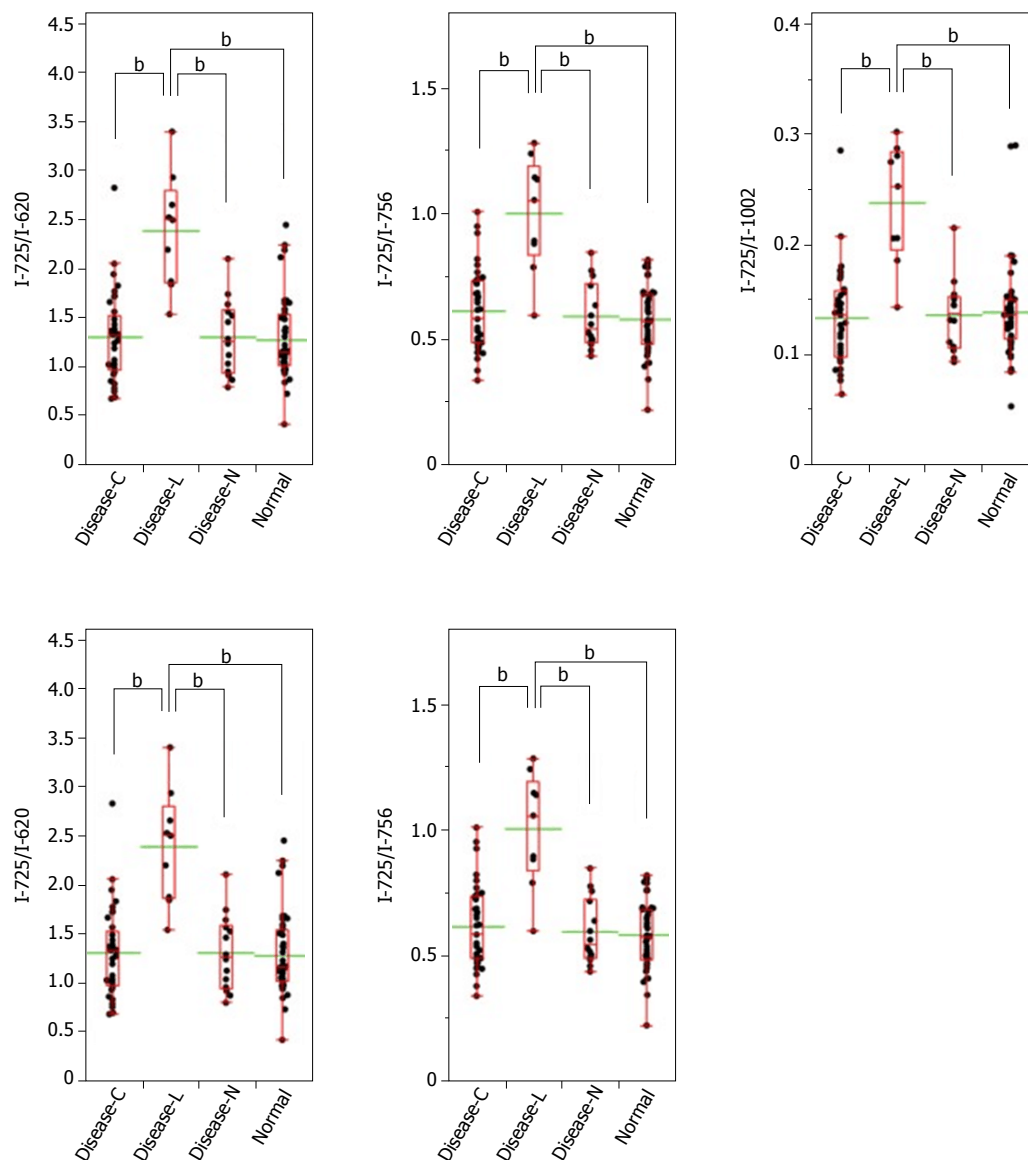


Figure 3 Raman scattering intensity ratio with intensity of wavenumber 725 cm^{-1} as the denominator. Dots indicate the ratio of Raman scattering intensity in each tissue specimen of the patient. The bottom and top of the red box represent the lower and upper quartiles, and the band across the box shows the median. The lower and upper bars at the ends of the whiskers show the lowest data point within the 1.5 interquartile range of the lower quartile, and the highest data point within the 1.5 interquartile range of the upper quartile, respectively. The green bar shows the average. ^a $P < 0.05$, ^b $P < 0.01$.

nucleotide cytosine, all of the measured values of $782\text{ cm}^{-1}/620\text{ cm}^{-1}$, $782\text{ cm}^{-1}/756\text{ cm}^{-1}$, $782\text{ cm}^{-1}/1002\text{ cm}^{-1}$, $782\text{ cm}^{-1}/1250\text{ cm}^{-1}$, and $782\text{ cm}^{-1}/1263\text{ cm}^{-1}$ in the Disease-L specimen were significantly higher than those of the other three groups. Moreover, the measured values of the $782\text{ cm}^{-1}/620\text{ cm}^{-1}$, $782\text{ cm}^{-1}/756\text{ cm}^{-1}$, $782\text{ cm}^{-1}/1250\text{ cm}^{-1}$, and $782\text{ cm}^{-1}/1263\text{ cm}^{-1}$ ratios in the Disease-C specimen were significantly higher than those in the Normal specimen. There was no significant difference of the measured values between the Disease-C and Disease-N specimens, and between the Disease-N and Normal specimens (Figure 5). In the biaxial distribution, the distribution areas of measured values of Disease-N and Normal specimens widely overlapped. The distribution area of the measurement

value of Disease-L extended toward the higher value direction, and the values for Disease-C were distributed in the middle of the range (Figure 6).

DISCUSSION

Gastrointestinal cancers such as esophageal cancer, stomach cancer, colon cancer, and rectal cancer are typically confirmed with an endoscope, and then tissues are collected for histopathological confirmation of the diagnosis, which requires histochemical or IHC staining. Although the procedure for general histochemical staining is relatively simple, the diagnostic capability is limited. By contrast, IHC can provide a more accurate histopathological diagnosis, but is relatively time-con-

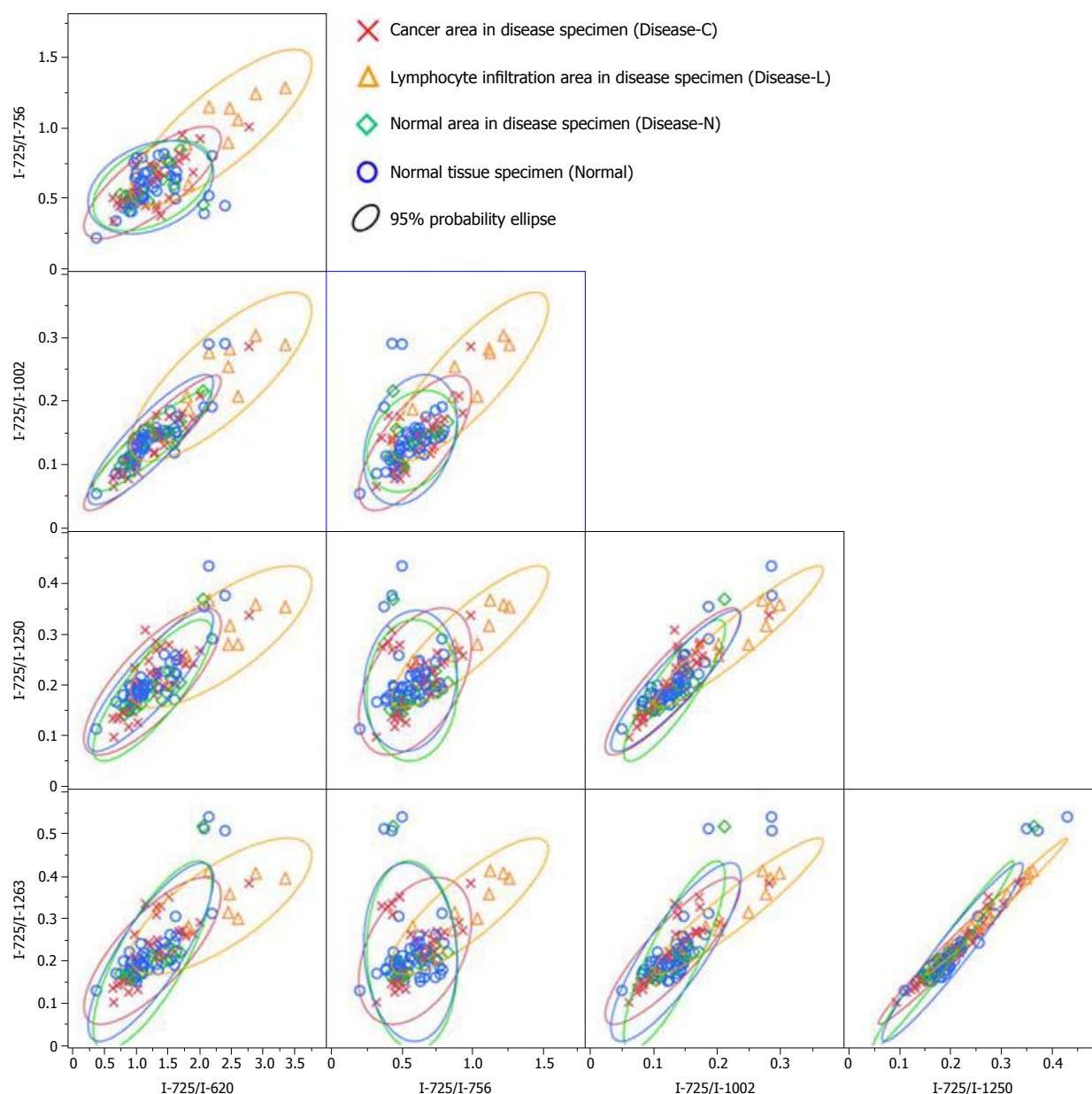


Figure 4 Biaxial distribution of the Raman scattering intensity ratio with the intensity of wavenumber 725 cm^{-1} as the denominator.

suming and requires specialized skills.

Raman scattering spectroscopy shows potential as a non-destructive method for live tissue evaluation, including the brain^[22] and lung^[23]; however, its potential utility for clinical *in vivo* evaluation has not yet been determined. Furthermore, although a few small-scale studies have been conducted on gastrointestinal tissue spectroscopy analysis^[24–26], standard spectroscopy evaluation methods for living organisms have not yet been established. Here, we demonstrated that Raman scattering spectroscopy could be used to qualitatively evaluate unstained pathological tissue specimens since the cancer lymphocyte infiltration area in the gastric cancer tissue specimen (Disease-N) showed the most characteristic measurement value, followed by the cancer portion in the stomach cancer tissue specimen

(Disease-C).

Based on comparison of the ratio of the Raman scattering spectrum intensities of 725 cm^{-1} and 782 cm^{-1} , associated with the nucleotides adenine and cytosine, respectively, to those of the others, our results suggested that cytosine is present in the Disease-C region at a relatively high concentration, and both adenine and cytosine exist in the Disease-L region at a relatively high concentration in the stomach tissue. In addition, both adenine and cytosine were presumed to be present at higher concentrations in the Disease-L specimen compared to the Disease-C specimen.

Adenine and cytosine are bases that make up DNA. In tumor cells, the nuclear DNA amount is often in aneuploidy; thus, the cytosine concentration is theoretically expected to be high in tumor cells^[27]. By

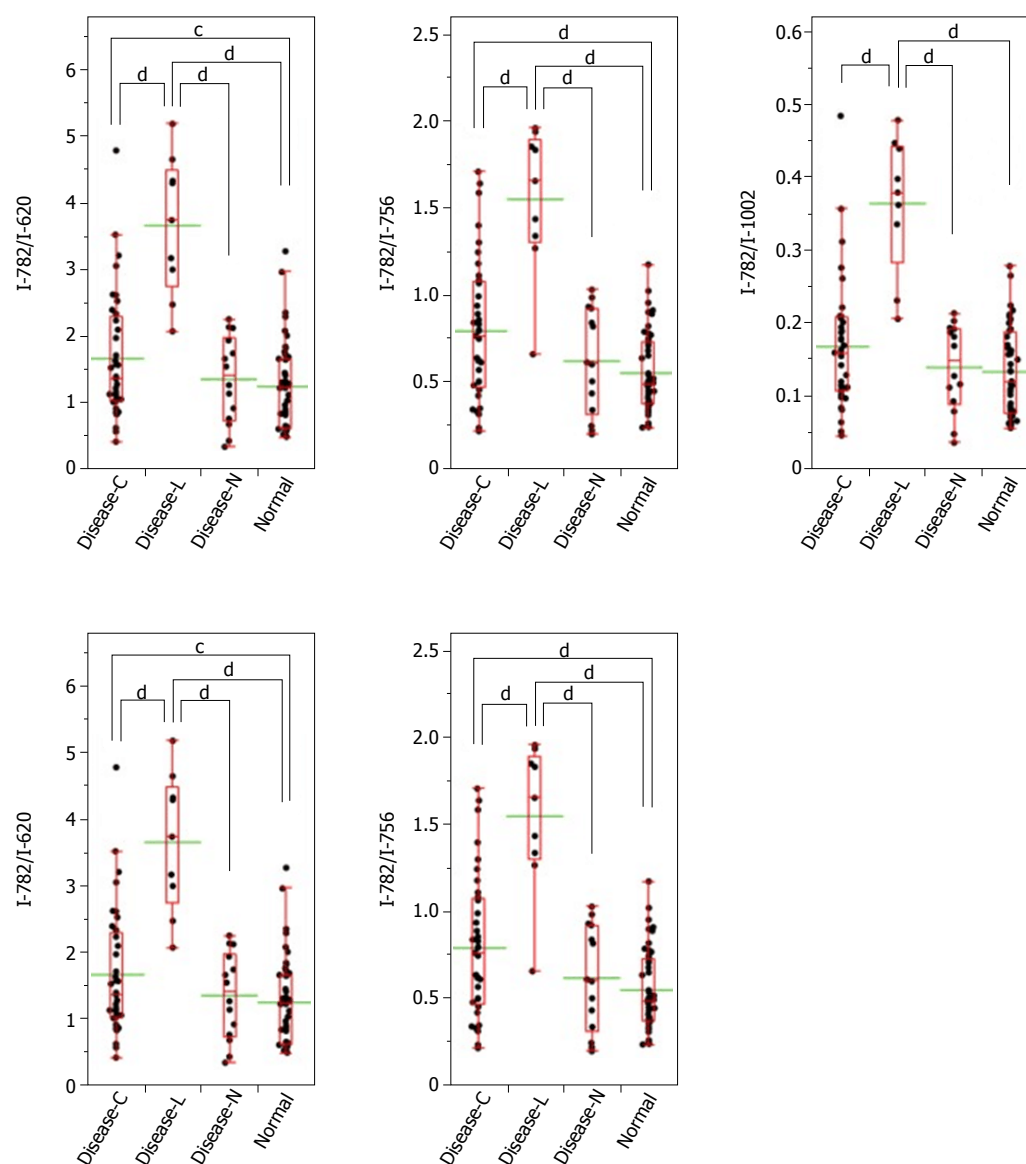


Figure 5 Raman scattering intensity ratio with the intensity of wavenumber 782 cm^{-1} as the denominator. Dots indicate the ratio of the Raman scattering intensity in each tissue specimen of the patient. The bottom and top of the red box represent the lower and upper quartiles, and the band across the box shows the median. The lower and upper bars at the ends of the whiskers show the lowest data point within the 1.5 interquartile range of the lower quartile, and the highest data point within the 1.5 interquartile range of the upper quartile, respectively. The green bar shows the average. ^c $P < 0.05$, ^d $P < 0.01$.

contrast, in lymphocytes, nuclear DNA is haploidal in many cases, and thus the amount of DNA in a given cell would not be expected to differ from that of a normal cell^[27]. The clustered lymphocytes observed in the stomach cancer tissue specimens used in this study had a nucleus size equivalent to that of normal cells albeit a smaller cell size. Therefore, in the Disease-L region, it is likely that the focal point of the laser struck the cell nucleus, so that the Raman scattering intensities of 725 cm^{-1} and 782 cm^{-1} , derived from adenine and cytosine, were more strongly measured. Lymphocyte infiltration in tissues suggests the presence of inflammation or an immune response. Given the significant relationship between malignancies and lymphocyte infiltration^[28,29], confirmation of lymphocyte infiltration may help to de-

tect any abnormalities, including malignant disease.

Limitations

Given the preliminary nature of the study, there are some limitations that should be mentioned. First, histopathological samples are intended for general histopathological diagnosis, but without staining, and they were not optimized for spectroscopy. For evaluation by spectroscopy, we need to consider conditions such as the thickness of the specimen and the material of the plate to which the specimen is attached. Second, the sample size was small, and we only focused on the stomach without assessment of other organs. Third, the data were obtained using a limited wavelength laser, and the focus position of the laser could not be precisely

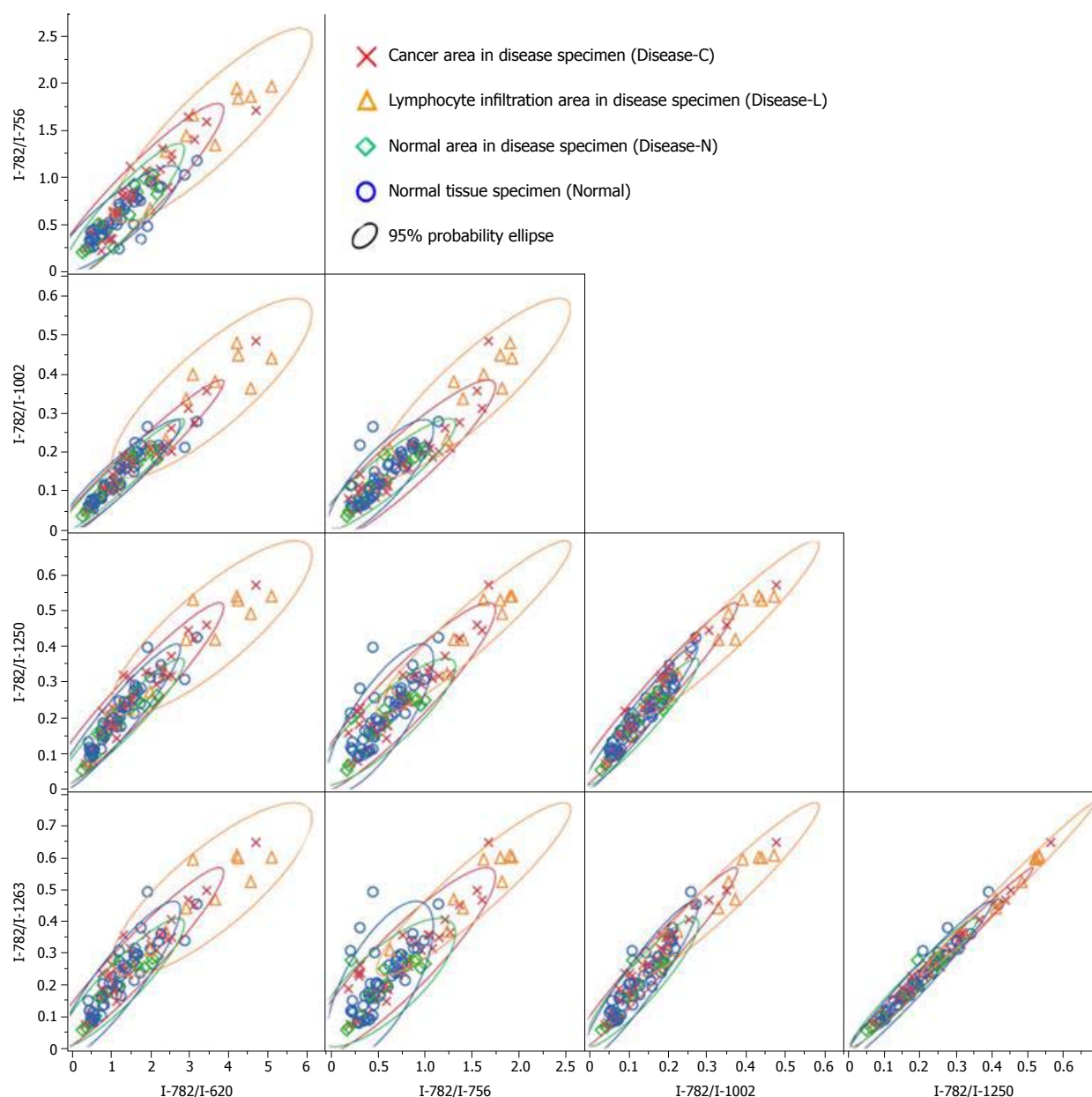


Figure 6 Biaxial distribution of the Raman scattering intensity ratio with intensity of wavenumber 782 cm^{-1} as the denominator.

controlled at a prescribed region of the cell. In particular, it has been suggested that lasers of longer wavelength such as 1064 nm are more suitable for analyzing samples with strong autofluorescence such as living tissue^[30]. Therefore, other laser light sources should be tested in future studies, including long-wavelength lasers.

Therefore, for future experiments, we will optimize the analytical sample for spectroscopy by examining the tissue specimen, material, and thickness of the slide glass, and conduct measurements under more precise regulation. Moreover, we plan to expand the experiments for testing the effects of different wavelengths and in different organs.

Finally, toward realizing the ultimate goal of more accurate cancer diagnosis, it will be important to com-

pare the results obtained from Raman scattering spectroscopy with the histopathological diagnosis as the present gold-standard, as well as with molecular biological findings obtained by next-generation sequencing and mass spectrometry (Figure 7).

Currently, Raman spectroscopy is an ancillary technique for adding qualitative information to histopathological morphological diagnosis. Further verification of our results and optimization of the technology as described above should help toward application of Raman spectroscopy as a diagnostic pathology technology without requiring staining or labeling. These advantages will help to more quickly and accurately diagnose cancer, and to realize early treatment initiation, with ultimate improvement of the treatment outcome. Moreover, such technology would allow for making a definitive diagnosis *in vivo* with-

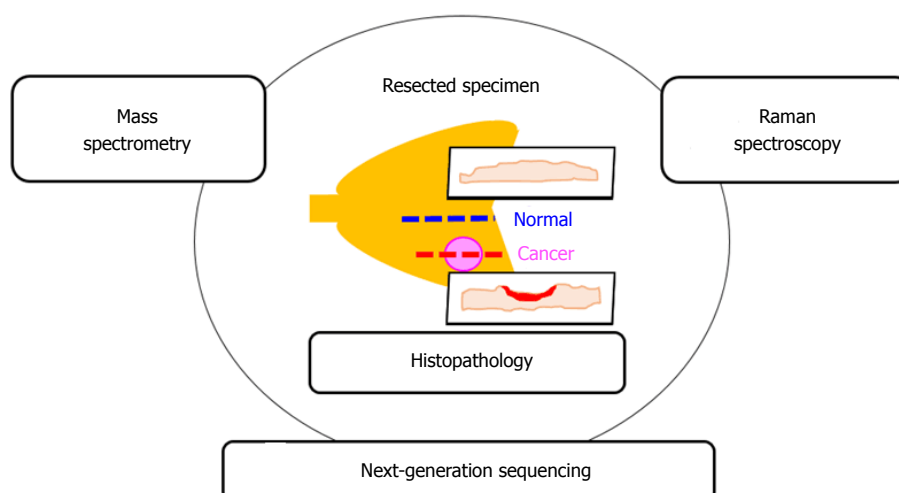


Figure 7 Schematic representation of potential histopathological diagnosis using Raman scattering spectroscopy, next-generation sequencing, and mass spectrometry for realizing a more accurate cancer diagnosis.

out invasive procedures of tissue collection and time-consuming histopathological diagnosis. Therefore, the biopsy step can be omitted to diagnose cancer quickly and less invasively.

ARTICLE HIGHLIGHTS

Research background

Histopathological evaluation is the gold-standard for cancer diagnosis. However, the diagnostic accuracy of histopathology staining is low, and the protocols for immunohistochemistry are complicated and time-consuming.

Research motivation

To achieve rapid, accurate and minimally invasive cancer diagnosis, a label-free and non-contact diagnostic technology is useful. Raman scattering spectroscopy has been used to analyze several types of biological tissue specimens; however, the clinical significance and diagnostic accuracy of this approach remain unclear. In addition, there are currently no standardized evaluation methods of gastrointestinal tissue spectroscopy analysis for living organisms.

Research objectives

We used the surgically resected stomach of a patient who underwent.

Research methods

The resected stomach was processed using general histopathological specimen preparation procedures. We produced two consecutive tissue specimens from areas with and without stomach cancer lesions. Each tissue specimen was sliced to a thickness of 3 μm and attached to a low-autofluorescence slide. One of the two tissue specimens was stained with hematoxylin and eosin and used as a reference for laser irradiation positioning by the spectroscopic method. Another tissue specimen was left unstained and used for Raman spectroscopy analysis by a laser light source with a wavelength of 532 nm.

Research results

Raman scattering spectrum intensities of 725 cm^{-1} and 782 cm^{-1} , are associated with the nucleotides adenine and cytosine, respectively. The Raman scattering spectrum intensity ratios of 782 cm^{-1} /620 cm^{-1} , 782 cm^{-1} /756 cm^{-1} , 782 cm^{-1} /1250 cm^{-1} , and 782 cm^{-1} /1263 cm^{-1} in the gastric adenocarcinoma tissue were significantly higher than those in the normal stomach tissue. In addition, both adenine and cytosine were presumed to be present at higher concentrations in the non-cancerous lymphocytes infiltration area surrounding cancer compared

to the cancer area in the gastric adenocarcinoma tissue specimen.

Research conclusions

This preliminary experiment suggests the feasibility of our spectroscopic method as a diagnostic tool for gastric cancer using unstained pathological specimens. The Molecular biological differences among cells in the resected stomach tissue can be detected by Raman spectroscopy. Adenine and cytosine may be influential substances for histopathological diagnosis by Raman spectroscopy. By focusing on adenine and cytosine, we were able to distinguish qualitative differences in the stomach tissue by Raman spectroscopy. Both adenine and cytosine were presumed to be present at higher concentration in the gastric adenocarcinoma tissue were significantly higher than those in the normal stomach tissue. We measured the Raman scattering spectrum intensities at 620 cm^{-1} (C-C twisting mode of phenylalanine), 725 cm^{-1} (adenine), 756 cm^{-1} (symmetric breathing of tryptophan), 782 cm^{-1} (cytosine), 1002 cm^{-1} (phenylalanine), 1250 cm^{-1} (amide III β -sheet), and 1263 cm^{-1} (amide III α -Helix), corresponding to the Raman scattering wavenumber of the organism constitution organic substance. We then calculated the ratio of the Raman scattering spectrum intensities of 725 cm^{-1} and 782 cm^{-1} , associated with the nucleotides, to those of the others. We compared the ratio of the Raman scattering spectrum intensities of 725 cm^{-1} and 782 cm^{-1} , associated with the nucleotides adenine and cytosine to qualitatively evaluate tissue. We found that Raman scattering spectrum intensities associated with the nucleotides adenine and cytosine were higher in adenocarcinoma than in normal tissue specimen of the stomach. In conclusion, we were able to distinguish qualitative differences in the stomach tissue by Raman spectroscopy.

Research perspectives

The Molecular biological differences among cells in the resected stomach tissue can be detected by Raman spectroscopy. In the future, we should raise the accuracy of estimation by Raman spectroscopy and to complete it as a technology that can obtain both high-precision morphological information and qualitative information.

ACKNOWLEDGMENTS

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Robotic total meso-rectal excision for rectal cancer: A systematic review following the publication of the ROLARR trial

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Abstract

AIM

To compare outcomes in patients undergoing rectal resection by robotic total meso-rectal excision (RTME) vs laparoscopic total meso-rectal excision (LTME).

METHODS

Standard medical electronic databases such as PubMed, MEDLINE, EMBASE and Scopus were searched to find relevant articles. The data retrieved from all types of included published comparative trials in patients undergoing RTME vs LTME was analysed using the principles of meta-analysis. The operative, post-operative and oncological outcomes were evaluated to assess the effectiveness of both techniques of TME. The summated outcome of continuous variables was expressed as standardized mean difference (SMD) and dichotomous data was presented in odds ratio (OR).

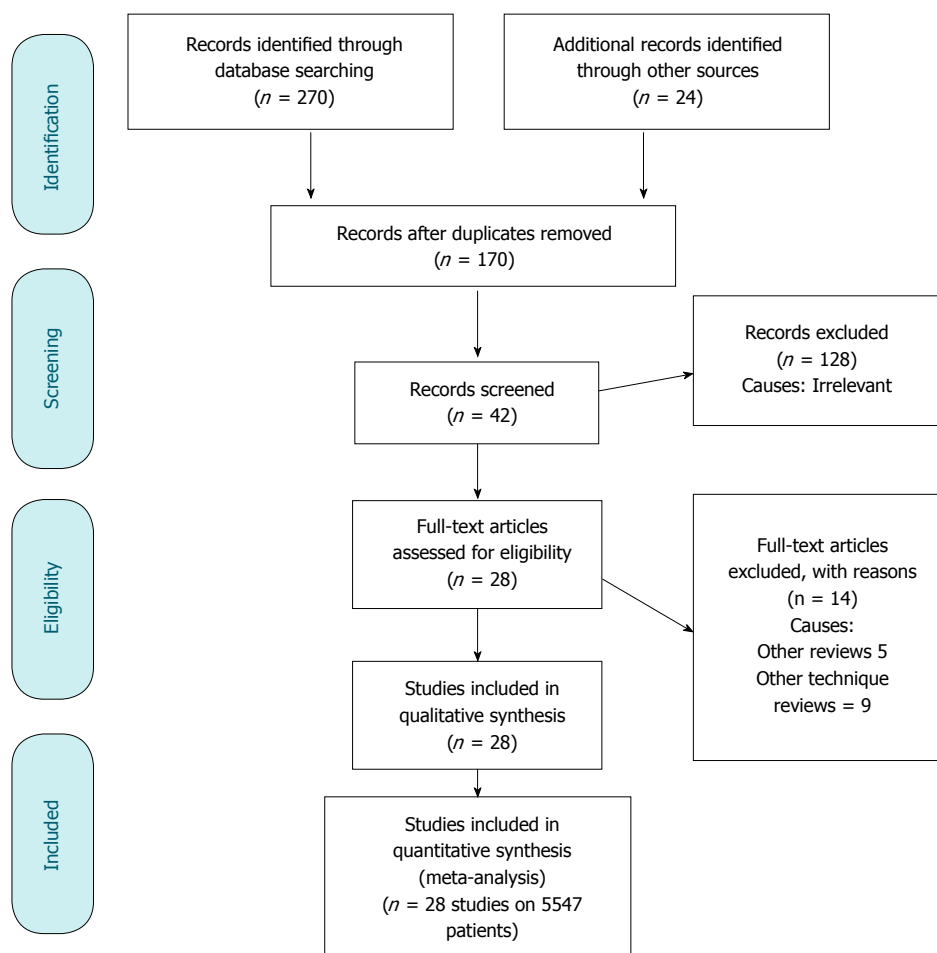


Figure 1 PRISMA flow diagram.

RESULTS

One RCT (ROLARR trial) and 27 other comparative studies reporting the non-oncological and oncological outcomes following RTME *vs* LTME were included in this review. In the random effects model analysis using the statistical software Review Manager 5.3, the RTME was associated with longer operation time (SMD, 0.46; 95%CI: 0.25, 0.67; $z = 4.33$; $P = 0.0001$), early passage of first flatus ($P = 0.002$), lower risk of conversion ($P = 0.00001$) and shorter hospitalization ($P = 0.01$). The statistical equivalence was seen between RTME and LTME for non-oncological variables like blood loss, morbidity, mortality and re-operation risk. The oncological variables such as recurrence ($P = 0.96$), number of harvested nodes ($P = 0.49$) and positive circumferential resection margin risk ($P = 0.53$) were also comparable in both groups. The length of distal resection margins was similar in both groups.

CONCLUSION

RTME is feasible and oncologically safe but failed to demonstrate any superiority over LTME for many surgical outcomes except early passage of flatus, lower

risk of conversion and shorter hospitalization.

Key words: Diverticular disease; Colorectal resections; Multi-incision laparoscopic surgery; Colorectal cancer; Single incision laparoscopic surgery

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Core tip: The findings of this meta-analysis of one RCT and 27 case control studies on 5547 patients are consistent with the recently published ROLARR trial validating the feasibility and oncological safety of robotic total meso-rectal excision (RTME). However, RTME failed to demonstrate any superiority over laparoscopic total meso-rectal excision except reduced conversion rate.

Jones K, Qassem MG, Sains P, Baig MK, Sajid MS. Robotic total meso-rectal excision for rectal cancer: A systematic review following the publication of the ROLARR trial. *World J Gastrointest Oncol* 2018; 10(11): 449-464 Available from: URL: <http://www.wjgnet.com/1948-5204/full/v10/i11/449.htm> DOI: <http://dx.doi.org/10.4251/wjgo.v10.i11.449>

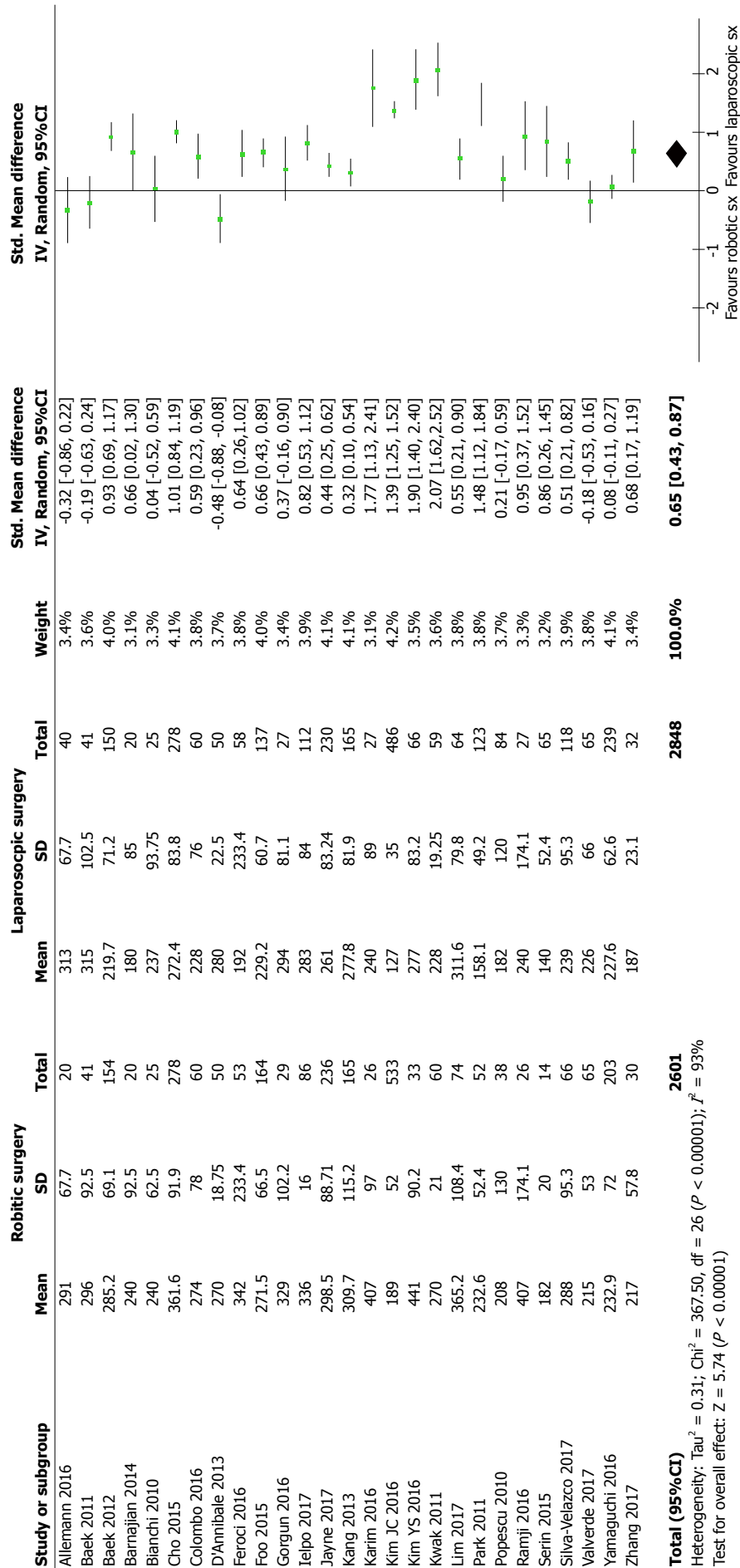


Figure 2 Forest plot for duration of operation following rectal resection by robotic surgery vs conventional laparoscopic surgery. Standardized mean difference is shown with 95% CIs.

INTRODUCTION

Colorectal cancer has higher prevalence rate in the developed world and almost one third of cancers are diagnosed in the rectum^[1-4]. Total mesorectal excision (TME) performed either by open or laparoscopic technique is an accepted gold standard treatment of rectal cancer worldwide. Laparoscopic TME (LTME) has apparent advantages and considered a preferred mode of surgery due to less tissue trauma^[5-11]. Due to precision in dissection, stable base unit and better visualization in difficult areas like lower pelvis, the robotic TME (RTME) is also considered a possible alternative to LTME^[12,13]. RTME may potentially offer same advantages which LTME offers as reported in some studies. There have been several previous systematic reviews^[14-21] on LTME vs RTME; however, none since the ROLARR trial, a multi-centre randomised controlled trial

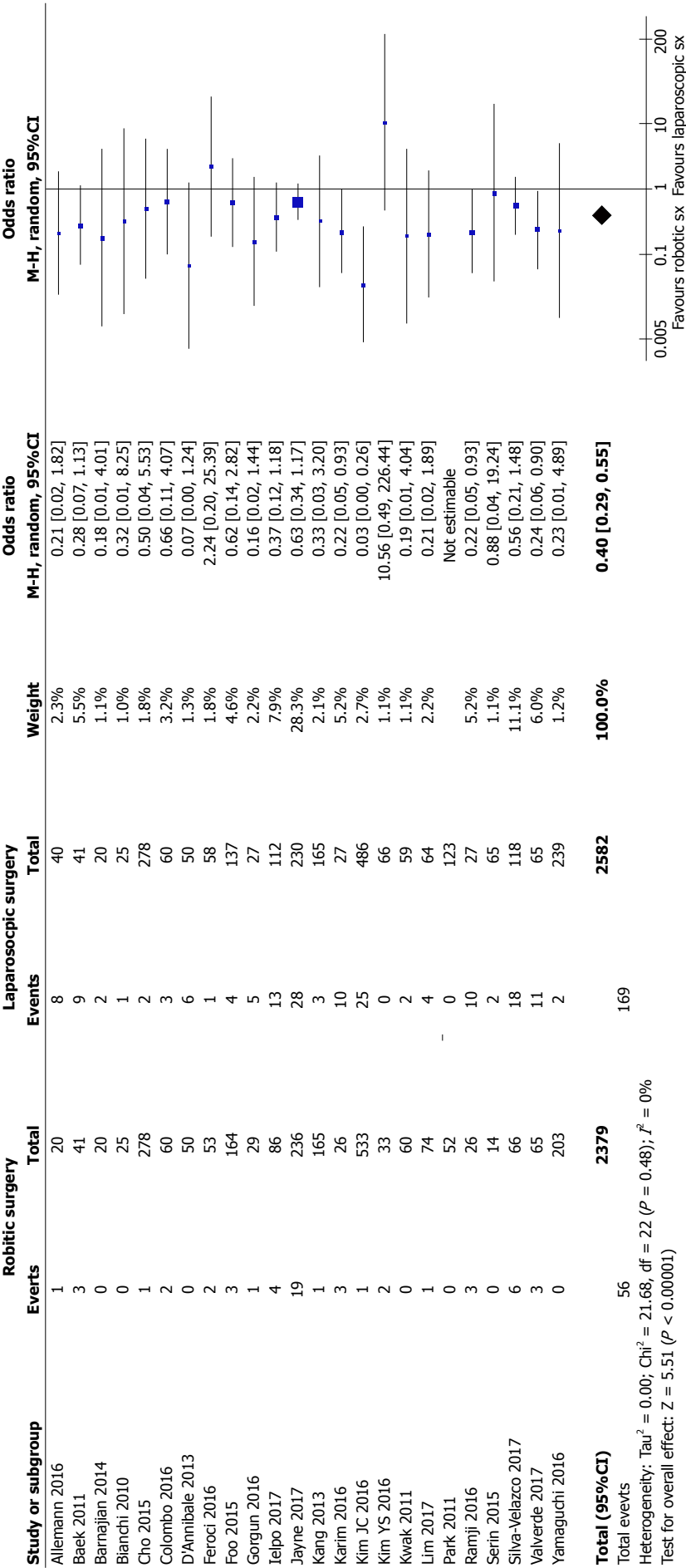


Figure 3 Forest plot for conversion following rectal resection by robotic surgery vs conventional laparoscopic surgery. Odds ratio is shown with 95%CIs.

comparing RTME to LTME, has published its results. In addition, several new comparative studies have also been reported which require combined statistical evaluation to generate up to date and better evidence.

Our study is an up to date meta-analysis of studies comparing RTME vs LTME including recently published ROLARR trial to be able to distinguish the potential advantages or disadvantages that robotics may play in the treatment of rectal cancer.

MATERIALS AND METHODS

Study suitability standards

Following are features of individual publications needed to qualify for inclusion in this systematic review as depicted in the following PICOS style: (1) Participants: Adult

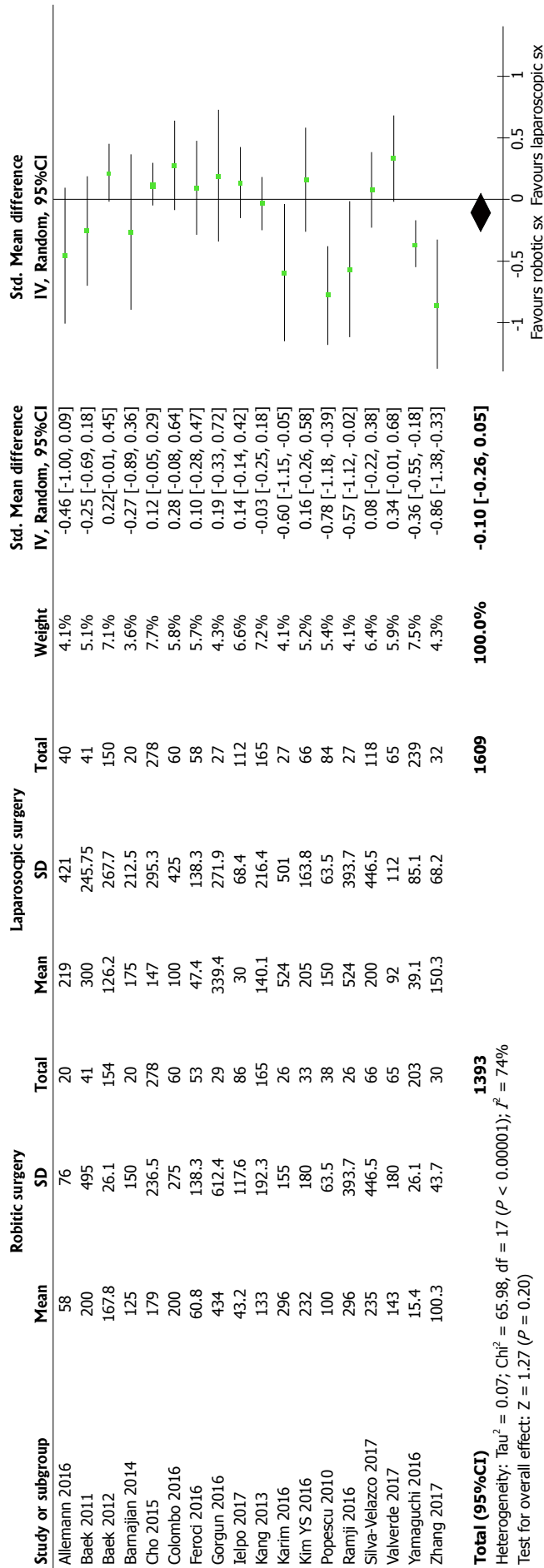


Figure 4 Forest plot for blood loss following rectal resection by robotic surgery vs conventional laparoscopic surgery. Standardized mean difference is shown with 95% CIs.

patients with histologically proven and MDT recommended resectable rectal cancer; (2) Intervention (Exposure): RTME; (3) Control: Patients with rectal cancer undergoing TME or rectal resection by laparoscopic approach; (4) Outcomes: Length of stay, operation time, blood loss, post-operative complications, mortality, positive circumferential resection margins, length of distal resection margins, lymph node harvesting, surgical site infections, time till first flatus, conversion rate, tumour recurrence and re-operation rate; (5) Study design: No restrictions were placed in study design. However, published studies should have reported comparison between two arms, those are RTME vs LTME. No studies were excluded based on the year of publication, publication centre, age or gender of the participants or publication language.

Electronic resources for studies selection

Four databases: PubMed, Ovid EMBASE, SCOPUS, and Cochrane Library were searched to find target studies. Four trial registries: ClinicalTrials.gov, European Clinical Trials Register, ISRCTN Register, and the International Clinical Trials Registry were examined. The literature exploration was performed until March 2018.

Search terms

For PubMed, the search was run by means of using MeSH words and by utilizing advanced search choice. The MeSH words "rectal cancer", AND ("laparoscopic resection OR minimal invasive surgery resection OR robotic surgery resection OR total meso-rectal excision") were used. Furthermore, an advanced study exploration was done using

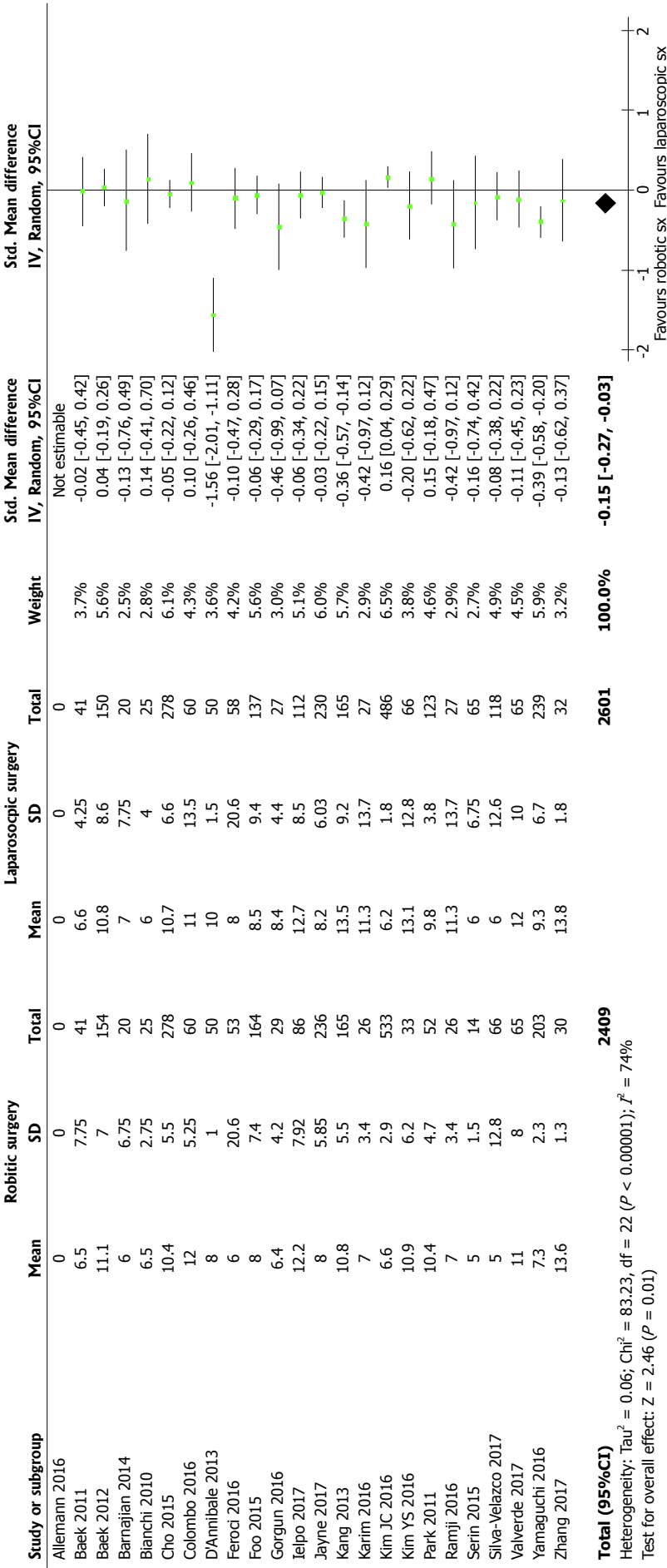


Figure 5 Forest plot for duration of hospital stay following rectal resection by robotic surgery vs conventional laparoscopic surgery. Standardized mean difference is shown with 95%CIs.

the terms “rectal resection”. Ovid, EMBASE and SCOPUS were searched with the same search terms as the PubMed advanced search. The Cochrane Library was searched using the term “rectal cancer”. Trial registries were all examined using the word “rectal cancer”.

Study selection

Two authors evaluated and studied initially titles and then abstracts, adding any curtained references into the Excel spreadsheet. The duplicate articles were excluded. Based upon the information given in the published abstracts, the initial decision of study inclusion or exclusion was made. The published article of the potentially includable studies was then evaluated. The conflicts about data, its accuracy and variability among extracting authors was sorted by agreement or by intercession of an experienced supervising consultant surgeon with had vast clinical and publication experience.

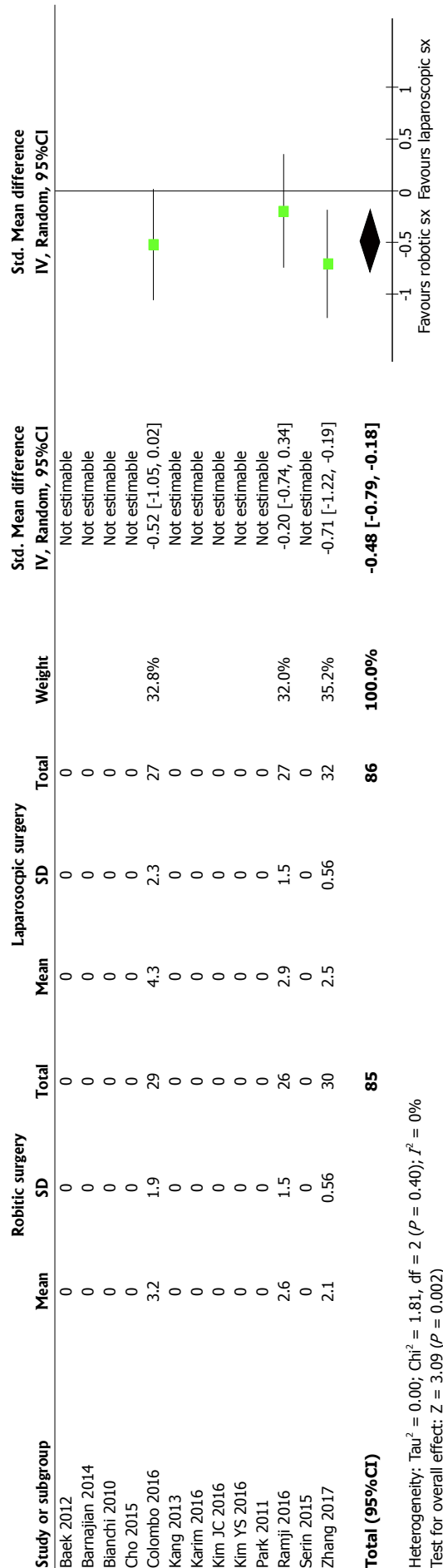


Figure 6 Forest plot for time to first flatus following rectal resection by robotic surgery vs conventional laparoscopic surgery. Standardized mean difference is shown with 95%CIs.

Data collection process

Data was independently collected by two reviewers into the electronic data collection forms of the Microsoft Excel spread sheet. The data items were agreed by the authors prior to commencing study selection. The data pertaining to the analysable outcomes was extracted in addition to the study citation, ethics committee approval, study registration and study quality indicators for all types of comparative studies. Once each author had completed data extraction, the data files were electronically compared and discrepancies in data entry were investigated and resolved.

Statistical analysis

The comparative efficacy of robotic surgery and conventional laparoscopic surgery for rectal resection was directly matched and pooled for each outcome of interest if there were at least two studies for each comparison. The odds ratio (OR) was estimated and pooled across studies using a random-effect model. Heterogeneity was assessed using Cochrane Q test and I^2 statistic. The statistical analysis of the data was conducted according to the guidelines provided by the Cochrane Collaboration including the use of RevMan 5.3® statistical software, and the use of forest plots for the graphical display of the combined outcomes^[22-28].

RESULTS

Characteristics of selected studies

A total of 294 studies were identified from Scopus and MEDLINE and other standard medical electronic databases. Among them, 1 RCT and 27 non-randomized comparative (both retrospective and prospective case control) studies^[29-56] published until March 2018, were eligible for inclusion. The inclusion and exclusion pathway is

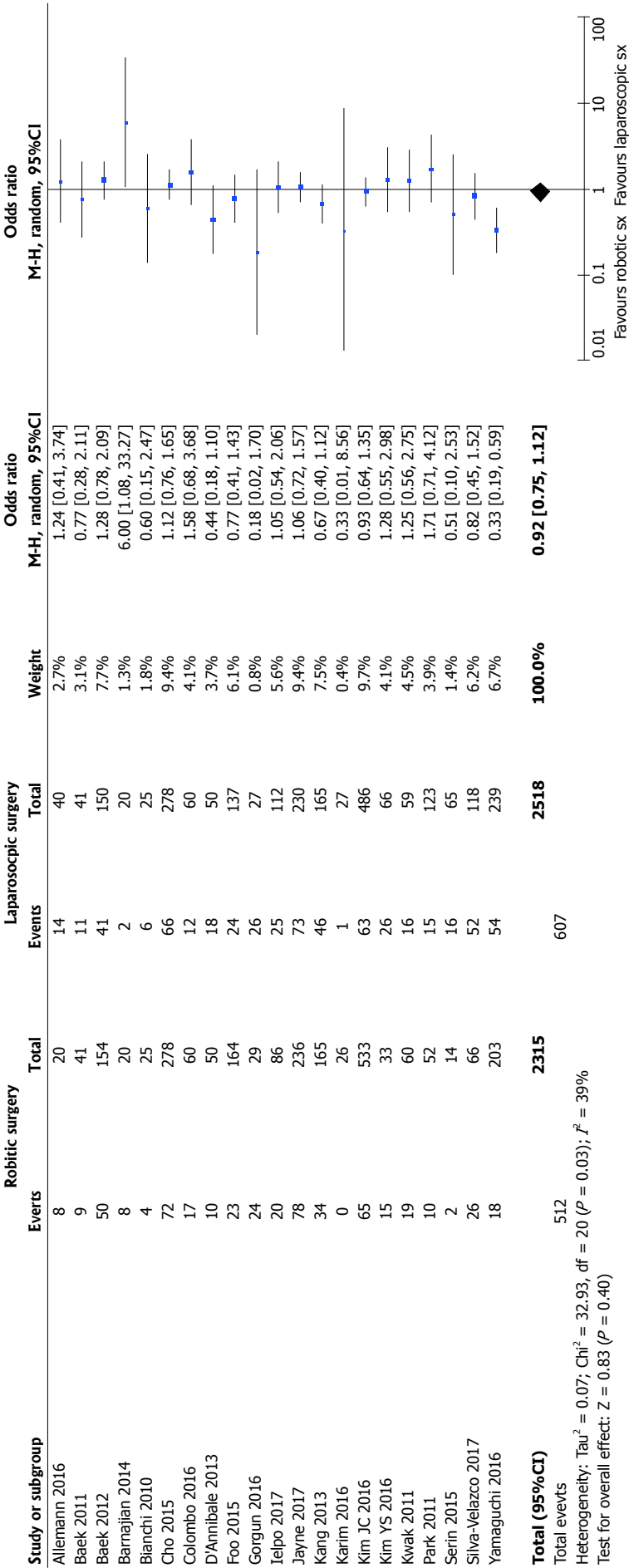


Figure 7 Forest plot for post-operative complications following rectal resection by robotic surgery vs conventional laparoscopic surgery. Odds ratio is shown with 95%CI.

described in PRISMA flow chart Figure 1.

Operative outcomes

All studies evaluated and reported the outcome of duration of operation. The duration of operation was longer (SMD, 0.20; 95%CI: -0.11, 0.52; $z = 1.28$; $P = 0.20$; Figure 2) in robotic surgery group, but with the clinical advantage of the reduced rate of conversion (OR, 0.40; 95%CI: 0.29, 0.55; $z = 5.51$; $P = 0.00001$; Figure 3) to open surgery. The reduced risk of conversion to laparotomy following RTME seems to be significantly advantageous in terms of the intensity of surgical trauma posed by laparotomy. There was similar risk of blood loss (SMD, 0.09; 95%CI: -0.14, 0.33; $z = 0.76$; $P = 0.45$; Figure 4) following both approaches of TME. Clinical and methodological heterogeneity [$\tau^2 = 0.31$, $\chi^2 = 367.50$, $df = 26$, ($P < 0.00001$); $I^2 = 93\%$] was noted in trials which was the basis for the random effects model analysis leading to the above outcomes.

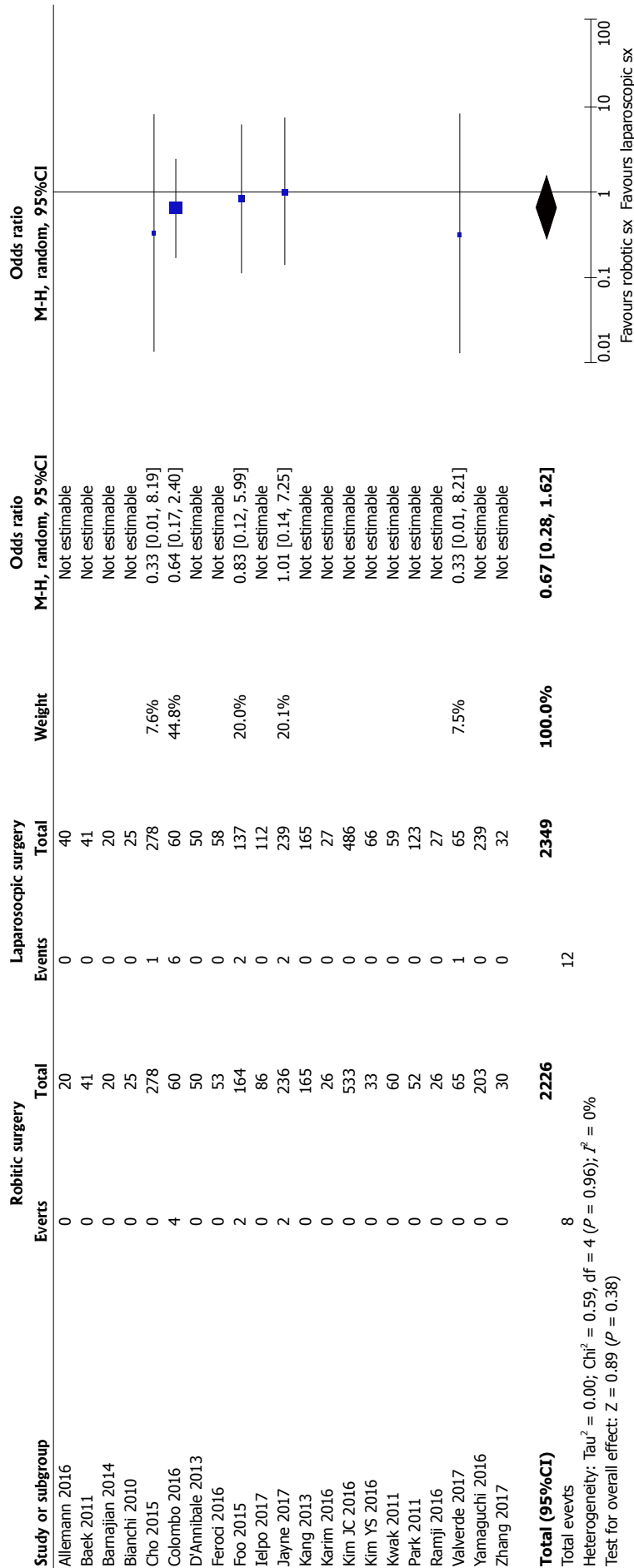


Figure 8 Forest plot for post-operative mortality following rectal resection by robotic surgery vs conventional laparoscopic surgery. Odds ratio is shown with 95%CIs.

Post-operative outcomes

The in-hospital stay (SMD, -0.15; 95%CI: -0.27, -0.03; $z = 2.46$; $P = 0.01$; Figure 5) and time to first flatus (SMD, -0.48; 95%CI: -0.79, -0.18; $z = 3.09$; $P = 0.002$; Figure 6) in patients undergoing RTME were shorter compared to LTME group. However, the post-operative morbidity (OR, 0.92; 95%CI: 0.75, 0.1.12; $z = 0.83$; $P = 0.40$; Figure 7), post-operative mortality (OR, 0.67; 95%CI: 0.28, 1.62; $z = 0.89$; $P = 0.38$; Figure 8) and re-operation rate (OR, 0.76; 95%CI: 0.50, 1.16; $z = 1.29$; $P = 0.20$; Figure 9) were statistically similar in both groups.

Oncological outcomes

Oncological safety is one of the most important surgical outcomes for any new surgical intervention because positive circumferential resection margins are directly associated with overall survival and disease free survival in rectal cancer patients. The risk of positive circumferential resection margins (OR, 0.91; 95%CI: 0.68, 1.22; $z = 0.62$; $P = 0.53$; Figure 10), length of distal resection margins (SMD, 0.00; 95%CI: -0.11, 0.11; $z = 0.04$; $P = 0.97$; Figure 11), and lymph node yield (SMD, 0.04; 95%CI:

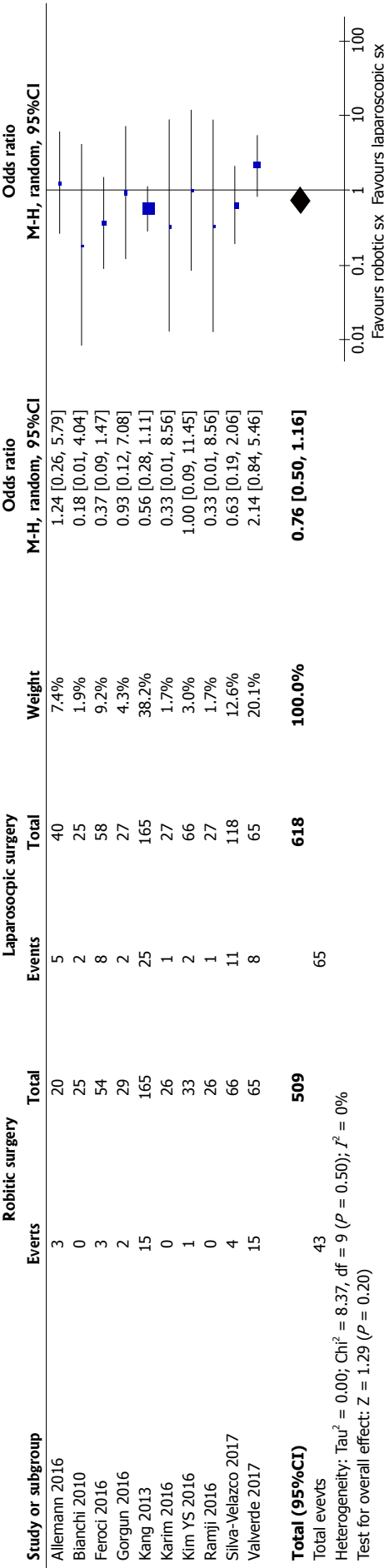


Figure 9 Forest plot for re-operation rate following rectal resection by robotic surgery vs conventional laparoscopic surgery. Odds ratio is shown with 95%CI's.

-0.07, 0.14; $z = 0.69$; $P = 0.49$; Figure 12) were statistically similar in both groups. Therefore, the risk of local and distant recurrence (OR, 1.10; 95%CI: 0.87, 1.39; $z = 0.79$; $P = 0.43$; Figure 13) was also found to be similar in both groups.

DISCUSSION

Colorectal cancer is the third most common cancer diagnosis internationally. The highest incidence of colorectal cancer is reported the Europe, North America and Australia^[1]. The highest incidence of colorectal cancer is in the anatomical area of rectum. In 2015 in the United Kingdom out of 41599 people diagnosed with colorectal cancer, 27% of these were located in the rectum. The incidence is slightly higher in men making up 32% of all colorectal cancer diagnoses compared to 23% for women^[2]. With the introduction of the total meso-rectal excision (TME), developed in 1989 by Professor Heald, survival rates and the rates of local recurrence have significantly improved^[3,4]. A TME is defined as an en bloc resection of the rectal tumour with endo-pelvic fascia to excise circumferential margins^[5]. The decision to undertake a TME is influenced by several factors including distance of the cancer from the anal verge, degree of invasion into the pelvic walls, presence of metastases to regional lymph nodes, the patient's co-morbidities and the ability to withstand trans-abdominal surgery^[6]. LTME has risen to become the gold standard for rectal cancer suitable for surgical resection^[7]. Many trials including the COLOR II trial established that it gave similar oncological outcomes compared to an open approach^[8,9] and further studies showed it resulted in shorter length of stay, less pain and quicker resumption of normal diet^[10,11]. The main reason behind these proven advantages of the laparoscopic TME is the reduced surgical trauma and tissue handling compared to open TME. As the use of robotics in surgery becomes more commonplace in gynaecological and urological procedures, the question arises on whether it has a place in colorectal surgery. Specifically, a TME demands precise dissection in an area that is difficult to visualise and access. These difficult aspects of a TME could be improved upon by robotics which offers a direct angle entry view, a stable retraction platform and more movement of instrument freedom^[12,13]. Some studies have shown that robotic TME (RTME) results in shorter length of stay, whilst other studies have shown that there is no difference

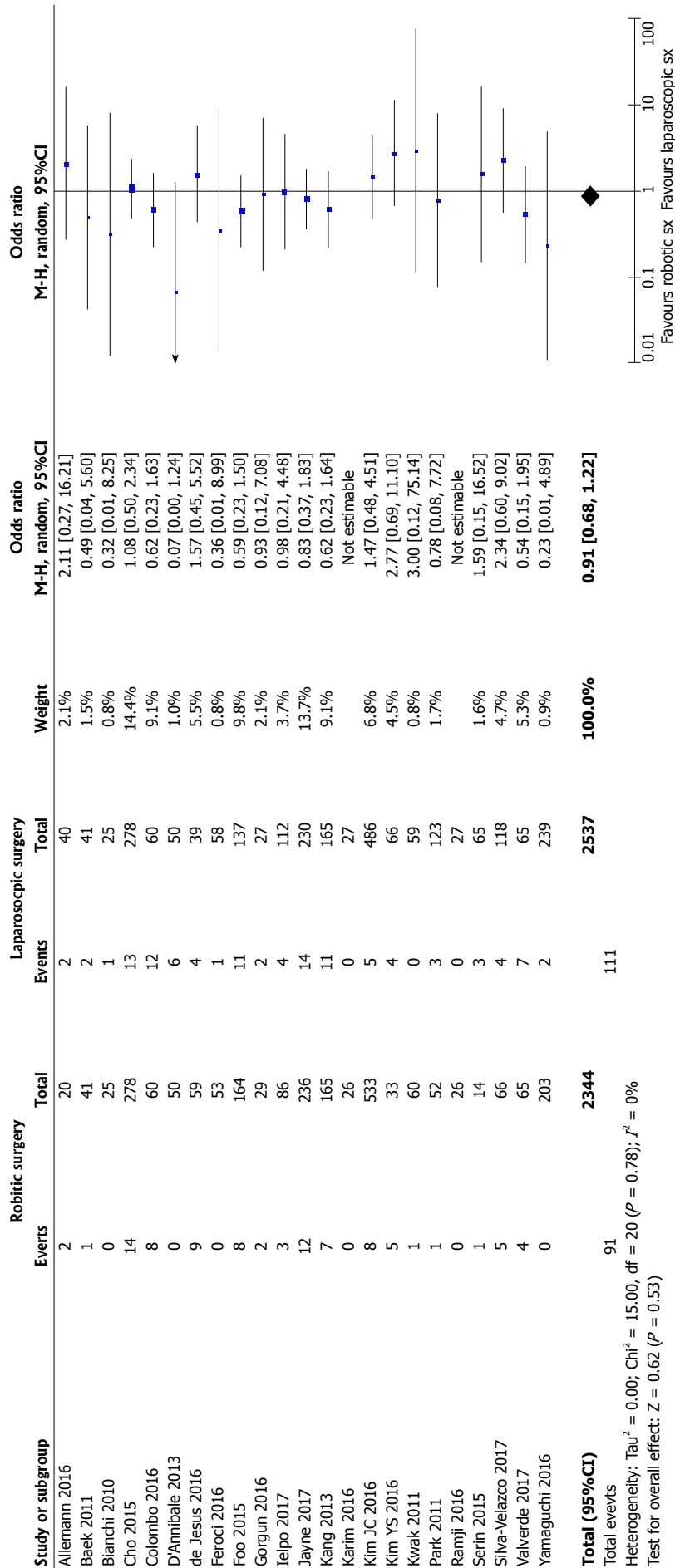
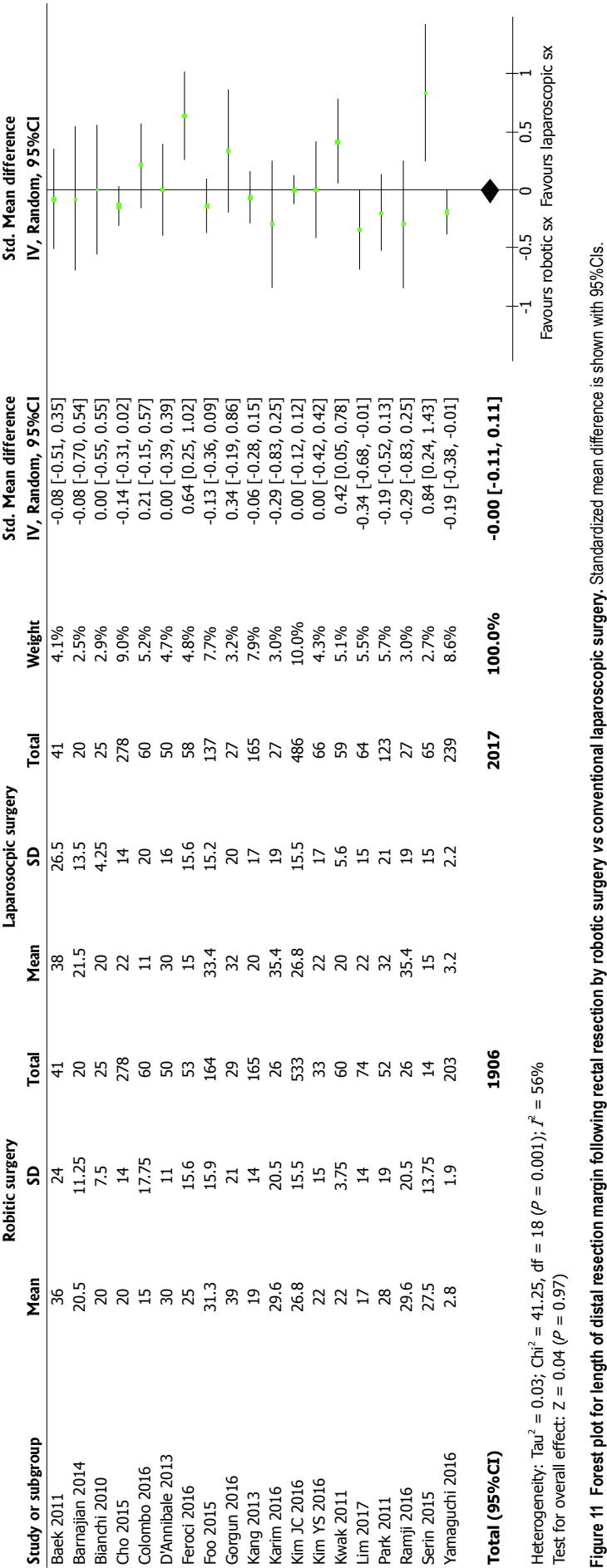


Figure 10 Forest plot for positive circumferential resection margins following rectal resection by robotic surgery vs conventional laparoscopic surgery. Odds ratio is shown with 95% CIs.

in hospital stay, oncological outcomes or rates of converting procedures to open. There is an argument that in terms of cost, the use of robotics is more expensive than laparoscopic instruments and the learning curve for surgeons is longer and requires more cases than for a LTME.

Based upon the findings of this largest ever series on the role of robotic surgery in rectal cancer resection, the RTME is certainly a feasible technique and oncologically safe surgical intervention but failed to demonstrate any superiority over LTME for many surgical outcomes. Mere advantage of robotic surgery was noted in only three post-operative outcomes, that is early passage of flatus, lower risk of conversion and shorter hospitalization. It seems like these advantages may not truly reflect into the routine use of RTME in rectal cancer surgery and therefore, the examination of current 28 studies to date did not designate a major value of RTME over LTME. Demonstration of this conclusion has already been reported in previously published meta-analyses^[14-21,57].

These findings needs further evaluation on the background of recently published ROLARR^[42] trial. Among operative outcomes, current study indicates similar blood loss and longer duration of operation and these both outcomes are concordant with the findings of ROLARR^[42] trial. The risk of conversion to open surgery was found to be lower in RTME arm of current study. Similarly the risk of conversion was lower in RTME group (8.1%) compared to LTME group (12.2%) in the ROLARR^[42] trial but statistically it



failed to demonstrate any significance. The length of hospital stay was similar between both groups in the ROLARR^[42] trial but current study shows significant reduction in the hospitalization time in patients undergoing RTME. Post-operative mortality, morbidity and re-operation rate were consistently similar in all included studies, current study and in the ROLARR^[42] trial. The oncological outcomes such as positive circumferential resection margins, length of distal resection margins, lymph node yield and recurrence rate were also not different. The publication of the ROLARR^[42] trials has answered several questions about the feasibility, safety and comparative equivalence of RTME and non-inferiority of LTME too. In addition, RTME seems to be relatively expensive^[29,42,58,59] and less cost-effective procedure and therefore routine use of this approach may not be justified for TME.

Authors frankly accept the major limitations of this study and the most apparent is the combined analysis of an RCT^[42] and 27 case control studies. Despite this limitation, the outcomes are almost matching with the conclusions of the only RCT^[42] published on this subject. Other confounding factors which can potentially be influencing the final outcome are diverse inclusion and exclusion criteria among included studies; variable post-operative follow up duration; lack of an agreed follow up screening pathways; presence and absence of the use of neoadjuvant chemoradiotherapy in the recruited population; use of variable diagnostic pathways in included studies and variable experience of the operating surgeons especially in the RTME group. More RCTs are needed to consolidate the findings of ROLARR trial^[42] and current study. Better outcomes and reduced cost may be anticipated in future trials due to the use of cost effective advanced technology and operating surgeons with extensive experience in robotic surgery. Until then the ROLARR trial and current study may provide the best possible evidence in this relatively innovative intervention for rectal cancer

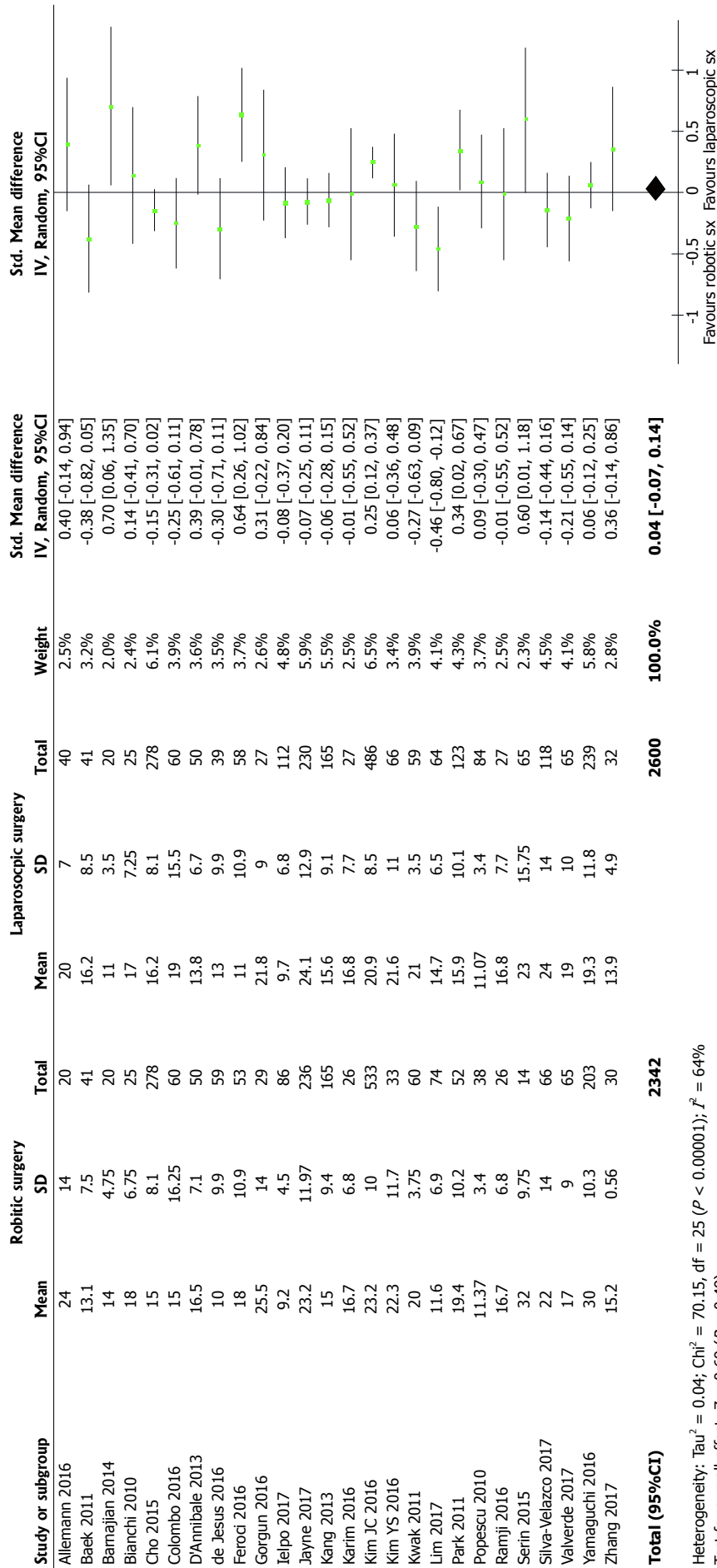


Figure 12 Forest plot for lymph node yield following rectal resection by robotic surgery vs conventional laparoscopic surgery. Standardized mean difference is shown with 95%CI.

management.

ARTICLE HIGHLIGHTS

Research background

Robotic total meso-rectal excision (TME) is used at least for a decade to treat rectal cancer and the only evidence in favour of robotic TME was based on case control studies. Recently first ever RCT evaluating feasibility of robotic TME was published as ROLARR trial. This aims of this study was to strengthen the existing evidence on this technique which is mainly based upon the meta-analysis of case control studies and compare it with the results of ROLARR trial.

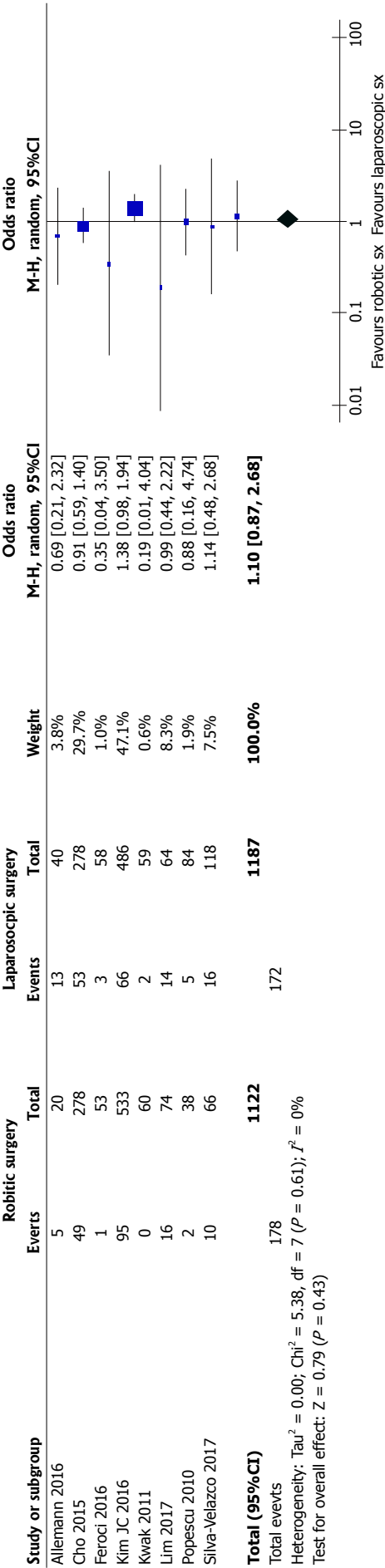


Figure 13 Forest plot for tumour recurrence following rectal resection by robotic surgery vs conventional laparoscopic surgery. Odds ratio is shown with 95%CI's.

Research motivation

Although robotic TME is being presented a way forward for rectal resection but its superiority over laparoscopic TME is not proven yet. Most of the evidence was based upon the systematic review of case-controlled studies, the publication of ROLARR trial is an attempt to answer this question. Comparison between the findings of ROLARR trial and systematic review of case-controlled trials can guide the surgeons in future about role of robotic TME.

Research objectives

The objective of this systematic review is to strengthen the existing evidence on the role of robotics for TME technique which is mainly based upon the meta-analysis of case control studies and compare it with the results of recently published ROLARR trial reporting robotic TME vs laparoscopic TME.

Research methods

Standard medical databases were searched. RCTs and all types of comparative studies reporting the effectiveness of robotic TME vs laparoscopic TME in the management of rectal cancer were retrieved and their data was extracted. The extracted data was analyzed using the principles of meta-analysis to generate higher level of evidence. RevMan 5.3 was used for statistical analysis and GradePro was used to generate summary of evidence.

Research results

One RCT (ROLARR trial) and 27 other comparative studies reporting the non-oncological and oncological outcomes following robotic TME vs laparoscopic TME were included in this review. In the random effects model analysis using the statistical software Review Manager 5.3, the RTME was associated with longer operation time (SMD, 0.46; 95%CI: 0.25, 0.67; $z = 4.33$; $P = 0.0001$), early passage of first flatus ($P = 0.002$), lower risk of conversion ($P = 0.00001$) and shorter hospitalization ($P = 0.01$). The statistical equivalence was seen between robotic TME and laparoscopic TME for non-oncological variables like blood loss, morbidity, mortality and re-operation risk. The oncological variables such as recurrence ($P = 0.96$), number of harvested nodes ($P = 0.49$) and positive circumferential resection margin risk ($P = 0.53$) were also comparable in both groups. The length of distal resection margins was similar in both groups.

Research conclusions

Robotic TME is feasible and oncologically safe but failed to demonstrate any superiority over laparoscopic TME for many surgical outcomes except early passage of flatus, lower risk of conversion, lower conversion to laparotomy rate and shorter hospitalization.

Research perspectives

Robotic TME failed to demonstrate superiority over laparoscopic TME. Laparoscopic TME may continuously be used to treat rectal cancer. More RCTs are needed to consolidate the findings of ROLARR trial^[42] and current study. Better outcomes and reduced cost may be anticipated in future trials due to the use of cost effective advanced technology and operating surgeons with extensive experience in robotic surgery. Until then the ROLARR trial and current study may provide the best possible evidence in this relatively innovative intervention for rectal cancer management.

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Current strategies for malignant pedunculated colorectal polyps

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Abstract

Despite significant advances in imaging techniques, the incidence of colorectal cancer has been increasing in recent years, with many cases still being diagnosed in advanced stages. Early detection and accurate staging remain the main factors that lead to a decrease in the cost and invasiveness of the curative techniques, significantly improving the outcome. However, the diagnosis of pedunculated early colorectal malignancy remains a current challenge. Data on the management of pedunculated cancer precursors, apart from data on nonpolypoid lesions, are still limited. An adequate technique for complete resection, which provides the best long-term outcome, is mandatory for curative intent. In this context, a discussion regarding the diagnosis of malignancy of pedunculated polyps, separate from non-pedunculated variants, is necessary. The purpose of this review is to provide a critical review of the most recent literature reporting the different features of malignant pedunculated colorectal polyps, including diagnosis and management strategies.

Key words: Pedunculated colorectal polyps; Malignant colorectal polyp; Early colorectal cancer; Polypoid early colon cancer; Advanced adenoma; Depth of invasion; Colorectal cancer; Polypectomy; Colorectal surgery; Early colorectal carcinoma

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Core tip: Colorectal cancer has the highest chance of curability as long as it is detected at an early stage, before lymph node metastasis, or as a premalignant lesion. However, few relevant studies address pedunculated polyps separately from nonpolypoid type lesions, often resulting in a source of bias. The objective of this paper is to offer an up-to-date overview, particularly on the management of malignant pedunculated polyps.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers worldwide. Of all gut malignancies, it has the highest chance of curability as long as it is detected at an early stage – either as a premalignant lesion or before lymph node metastasis. In European national screening programs, approximately 17% of cancers detected were pT1 CRCs, and the risk of developing advanced neoplasia following polypectomy was estimated at 0.6%^[1].

Most reports focus on sessile or flat lesions of the colorectum, while few studies discuss the management of pedunculated cancer precursor lesions. Studies often combine data for both sessile and pedunculated polyps. Moreover, submucosal invasion is presented in the literature as absolute depth, disregarding the presence of the stalk^[2], resulting in further bias. In particular, describing the macroscopic appearance of pedunculated lesions and the final histopathological diagnosis often remain challenging. At first sight, pedunculated lesions can easily be treated endoscopically; however, no large-scale reports exist to establish the real risk of lymph node metastasis stratified by depth of invasion. Additionally, an adequate technique for complete resection is mandatory for curative intent, providing the best long-term outcome. In this respect, a discussion regarding the diagnosis of malignancy inside pedunculated, separate from nonpedunculated, polyps is necessary. A clear distinction between head and stalk invasion of malignant cells is also required.

LITERATURE SEARCH

The aim of this article was to address strategies for diagnosis, staging, and risk stratification of patients with malignant pedunculated colorectal polyps (MPCP), as well as to provide a critical review of the literature regarding their management, to summarize their current state and to consider future perspectives. The literature search was conducted with PubMed and included full-text articles, up-to-date guidelines and recent abstracts with obvious conclusions as well as additional relevant publications by using the reference lists of the identified articles as a starting point. The following keywords were used: “pedunculated colorectal polyps”, “malignant colorectal polyp”, “early CRC”, “polypoid early colon cancer”, “early diagnosis”, “staging”, and “depth of invasion”, alone or in various combinations.

DEFINITIONS, CLASSIFICATIONS AND HISTOPATHOLOGICAL CHARACTERISTICS

By definition, a malignant polyp – either sessile or pedunculated, consists of cancer cells that invade the submucosa through the muscularis mucosae without crossing the submucosa, regardless of lymph node status and without distant metastasis (T1NxMo)^[3]. The term “early colorectal carcinoma” can also be used^[4].

An advanced adenoma is defined as a lesion of at least 10 mm with villous components or high-grade dysplasia^[5,6]. Currently, “high-grade dysplasia” is a term used for adenomas in which there is mucosal invasion without extension below the muscularis mucosae^[7]. According to the recommendations of the World Health Organization (WHO), this term is preferable to “intramucosal carcinoma”^[7,8]. The reason is that focal cancer that has not yet invaded through the muscularis mucosae is considered to have no risk of spreading to the lymph nodes because no lymphatic channels are located superficially to the muscularis mucosae^[7]. The patients in this situation are considered to be safe candidates for endoscopic resection.

Pedunculated polyps are recognized by their stalk of variable lengths that is attached to the colonic mucosa^[9]. They are described endoscopically in the Paris international classification as 0-Ip lesions. Although it has been reported to anticipate high-grade dysplasia and even invasive carcinoma, interobserver variability associated with the Paris classification has not been studied^[10]. Class 5 of Kudo’s pit pattern classification, characterized by an unstructured or excavated surface, demarcated depressed areas, loss of lobulation and stalk swelling, has been shown to correlate with the diagnosis of malignancy^[11,12]. A large multicenter cohort study emphasized the difficult diagnosis, as there has been a lack of agreement on the diagnosis of MPCP in a high percentage of cases^[13].

The level of invasion of the stalk further dictates management, from a minimally invasive endoscopy to an invasive surgical resection. MPCP should be discussed separately from nonpedunculated polyps to obtain accurate conclusions. If in the case of a sessile polyp, the cancer cells travel a short distance to become invasive and metastatic, should the stalk length be considered a favorable prognostic factor as a first barrier through the advanced cancer pathway?

Haggitt *et al.*^[14] classified the level of invasion in a pedunculated malignant polyp as follows: Level 1: invasive adenocarcinoma limited to the polyp head (invading through the muscularis mucosae); Level 2: neck involvement; Level 3: carcinoma cells in the stalk; and Level 4: carcinoma cells infiltrating the submucosa at the level of the adjacent bowel wall. The Haggitt line is the imaginary border drawn as the baseline to distinguish between head invasion and stalk invasion. A low risk of local recurrence or metastasis was deduced when the level of invasion was under 4. Although many studies^[15-17] reported a correlation between Haggitt level, lymph node invasion risk and outcome, there are currently no consensus guidelines to be included in the pathology report of a malignant polyp.

FACTORS PREDICTING LYMPH NODE STATUS IN MALIGNANT PEDUNCULATED COLORECTAL POLYPS

Even if pedunculated polyps are generally considered to have fewer lymph node metastases, variable morphology and length of the stalk can lead to problematic measurement of the depth of the submucosal invasion and to further controversies (Table 1).

In a recent systematic review and meta-analysis of histopathological factors influencing the risk of lymph node metastasis in early CRC^[2], a separate analysis of pedunculated polyps from sessile tumors was not possible because of insufficient data. They concluded that in early CRC, a depth of invasion of more than 1 mm in the submucosa by the primary tumor, poorly differentiated cancers, the presence of tumor budding and lymphovascular invasion were significantly associated with lymph node involvement.

Moreover, Kitajima *et al.*^[15] previously found a rate of lymph node metastasis of zero in head invasion cases (the deepest portion of invasion limited to above the baseline) and in stalk invasion cases with a depth of submucosal invasion < 3000 μ m (MPCP with the level 2 line according to Haggitt's classification used as the baseline and depth of submucosal invasion measured to the deepest portion in the submucosa).

In a large retrospective cohort study^[16], the authors concluded that MPCP diagnosed as head invasion by the pathologist can be safely treated by endoscopic polypectomy alone. They included 383 patients with

pathologically proven adenocarcinoma spread through the muscularis mucosae into the submucosa but without extension to the muscularis propria. The invasion depth was classified into two groups by using the upper limit of level 2 according to Haggitt's classification as the baseline for all lesions. When an endoscopy was suggestive of submucosal invasion into the polyp stalk, those patients were managed directly by surgery with lymph node dissection. Thus, they found a lymph node involvement rate and recurrence rate of 3.5% (8/230; 95%CI: 1.5%–6.7%) and 0.3% (1/340; 95%CI: 0.01%–1.6%), respectively. The incidence of metastasis to the lymph nodes and recurrence rate were 0% (0/101; 95%CI: 0.0%–3.6%) and 0%, respectively, (0/219; 95%CI: 0.0%–1.7%) for the lesions with head invasion, compared with 6.2% (8/129; 95%CI: 2.7%–11.9%) and 0.8% (1/121; 95%CI: 0.02%–4.50%), respectively, for stalk invasion. A total of 29% of lesions with head invasion were lymphovascular invasion positive, while 38% of stalk invasion lesions were lymphovascular invasion positive. Finally, the authors noted no significant difference in any other factors (such as tumor size, tumor differentiation grades, or even lymphovascular invasion) except for the depth of invasion (stalk invasion) between lymph node metastasis positive and negative groups.

In a previous study on 151 patients with colorectal polyps that included invasive carcinoma treated by resection, Nivatvongs *et al.*^[17] concluded that, unlike tumor size and grading, only the depth of invasion to the base of the stalk (Level 4) was associated with a high risk of lymph node metastasis (27%).

On the other hand, in another approach with patients who underwent systematic lymph node dissection, metastasis was observed in 14.6% of cases, and multivariate analysis showed that tumor budding was the only independent factor associated with lymph node metastasis^[18].

Interestingly, Kimura *et al.*^[19] recently suggested that head invasion is not a lymph node metastasis-free condition in a study on 76 pedunculated polyps with no significant differences in the lymph node metastasis rate between "head invasion" (4/30, 13.3%) and "stalk invasion" (5/46, 10.9%). They stated that even for MPCP with "head invasion", additional surgical resection with lymph node dissection should be taken into consideration if there are other risk factors.

Indeed, the detection of tumor buds has been reported as an indication for colorectal surgery because of the high risk for lymph node metastasis. Pathologically, tumor budding is defined as single tumor cells or small clusters of four or fewer tumor cells in the tumor stroma, at the invasive front and in malignant polyps^[20,21]. Widespread reporting of tumor budding has been limited in daily diagnostic practice due to a lack of consensus regarding guidelines on scoring methods^[20,21]. Although some authors^[8,22] consider it important that at least

Table 1 Histopathological factors predicting risk of lymph node metastases in malignant pedunculated colorectal polyps

Histopathological factors	Risk of LNM	Management
Depth of invasion in submucosa by the primary tumor of more than 1mm (Beaton <i>et al</i> ^[21])	High	Surgery with lymph node dissection
Poorly differentiated cancers (Beaton <i>et al</i> ^[21])		
Tumor budding (Beaton <i>et al</i> ^[21] , Sohn <i>et al</i> ^[18] , Geramizadeh <i>et al</i> ^[7] , Graham <i>et al</i> ^[22])		
Lymphovascular invasion (Beaton <i>et al</i> ^[21])		
Depth of invasion to the base of the stalk-Level 4 Haggitt (Nivatvongs <i>et al</i> ^[17] , Kimura <i>et al</i> ^[19])		
Submucosal invasion into the polyp stalk (Matsuda <i>et al</i> ^[16])		
Micropapillary component (Sonoo <i>et al</i> ^[26] , by Verdú <i>et al</i> ^[27] , Mukai <i>et al</i> ^[28])		
Head invasion (Kimura <i>et al</i> ^[19])		Surgical resection with lymph node dissection in case of additional pathological risk factors
Head invasion (Kitajima <i>et al</i> ^[15] , Matsuda <i>et al</i> ^[16])	Low	Endoscopic polypectomy
Depth of submucosal invasion/stalk invasion < 3000 µm (Kitajima <i>et al</i> ^[15])		
Tumor size (Nivatvongs <i>et al</i> ^[17])		
Grading (Nivatvongs <i>et al</i> ^[17])		
Pseudoinvasion (Backes <i>et al</i> ^[13])		Confirmation of t1 colorectal cancer by a second expert pathologist

LNM: Lymph node metastases.

high-grade tumor budding (more than 10 tumor buds in any microscopic field viewed at 25X) should be recorded in the pathology report as a prognostic factor.

Invasive micropapillary carcinoma is composed of small clusters of tumor cells lying within clear stromal spaces simulating vascular channels^[23,24] and is considered to be related to a high incidence of lymph node metastasis. However, its actual prevalence among early CRCs has not been reported^[25,26], as a limited number of cases are reported in the literature. Similar cases of pedunculated early sigmoid colon cancer with a micropapillary component and multiple lymph node metastases were reported by Sonoo *et al*^[26], Verdú *et al*^[27] and by Mukai *et al*^[28]. In another case of a sigmoid pedunculated polyp with a depressed surface without evidence of lymph node involvement or distant metastases on initial computed tomographic scans, the patient had local recurrence with lymph node metastases but also lung, liver, and spleen metastases at 6 months follow-up after the polypectomy^[29].

Therefore, even if the initial diagnosis is an MPCP, extensive surgical resection may still be taken into consideration for tumors with a micropapillary component due to the high risk for lymph node metastasis and poor outcome.

Beyond the conclusions of these studies, immunohistochemistry for the confirmation of the difficult-to-assess lymphovascular invasion is usually reserved for equivocal cases (*e.g.*, tumors with positive margins after resection)^[30].

Chicken-skin-like mucosa is an endoscopic finding described as pale yellow-speckled mucosa frequently surrounding pedunculated adenomas of the distal colon. Its clinical and pathophysiological significance have yet to be determined. Histopathologically, it represents fat accumulation in macrophages within the muscularis propria and, rarely, intestine-like microvilli. In two studies^[31,32], the prevalence of chicken-skin-like mucosa

was higher in carcinoma patients than in adenoma patients, and its role as a potential predictive marker of carcinogenetic progression was taken into consideration. However, it is a colonoscopic sign to search for a polyp in challenging locations. Additionally, it may serve as a potential marker of advanced pathology of colorectal adenoma in future research and might offer a better perspective on postpolypectomy management^[33].

Both endoscopists and histopathologists should also pay attention to possible pseudoinvasion. A histopathological pseudoinvasion (prolapse of the adenomatous epithelium into the polyp stalk), associated with ischemic changes when the polyp stalk is twisted, can be observed more often in large pedunculated polyps, which are typically located in the sigmoid colon and rarely in the rectum^[7]. Despite the lack of a gold standard diagnosis, invasive carcinoma could be distinguished from pseudoinvasion by the presence of stromal desmoplasia and high-grade dysplasia^[34]. However, the exact incidence of discordant diagnosis cannot be estimated; moreover, misplaced epithelium in pedunculated polyps has a lobular contour with a rim of lamina propria, along with hemorrhage, and/or hemosiderin^[35]. Biopsy-related misplacement can be even more difficult to recognize than typical pseudoinvasion in polyps with stalks^[36].

Thus, because misplaced epithelium can simulate early CRC in pedunculated polyps, British guidelines currently recommend diagnostic confirmation of T1 CRC by a second expert pathologist^[13].

CHALLENGES IN ENDOSCOPIC RESECTION TECHNIQUES

When we suspect a malignant pedunculated polyp, the snare should be placed as close as possible to the bowel wall to increase the chance of obtaining a cancer-free resection margin. Snare polypectomy is considered

curative when the histopathology report is favorable, but there is no consensus on the accurate assessment of negative margins. Most authors^[37,38] consider polypectomy technically satisfactory, with the lowest rate of local recurrence and metastases, if the margin from the invasive component to the diathermy burn is at least 2 mm. A new study^[39] reported a similar 5-year cumulative recurrence rate between surgical and endoscopic resection (8.2% and 2.4%, respectively) for patients with MPCP and a pathological margin ≥ 1 mm.

The site of resection should be inked with a tattoo to facilitate easy recognition if surgery is necessary; however, there is no guideline on the optimal placement of tattoos or metallic clips^[40].

Unlike sessile or flat polyps, in the case of pedunculated lesions, it is easier for the pathologist to avoid a diathermy artifact of the resected specimen and to better identify eventual invasive cancer cells at the polypectomy margin due to the distance of resection from the invasive component. Many studies^[16,41] have stated that pedunculated early polyp CRCs limited to the polyp head, without unfavorable histological features, could be managed by endoscopic resection alone with minimal risk of locoregional recurrence. However, in cases of unfavorable histological criteria (resection margins less than 1 mm, poor differentiation, lymphovascular invasion, invading the submucosa of the bowel wall below the stalk), endoscopy is not considered curative; therefore, surgery is recommended^[40].

Generally, giant pedunculated polyps (over 30 mm) have been managed surgically; further prospective studies are needed to establish if endoscopic resection of giant MPCP represents a feasible safe procedure^[42]. Recently, a prospective pilot study explored the safety and feasibility of insulated-tip knife endoscopic polypectomy for difficult giant polyps^[43]. Endoscopic submucosal dissection^[44] and the use of a dual knife procedure^[45] were reported to be options as well, but the patient number was too small to make definitive conclusions.

Pedunculated polyps have a higher risk of bleeding compared to sessile polyps^[46]. Postpolypectomy bleeding is the most common complication reported in the literature, and the rate varies between 24%^[47] and the more usual frequency of 3%–4%^[48]. When considering referral bias, the general frequency is thought to be lower, while other complications such as postcoagulation syndrome or perforation can rarely occur^[49]. The only polyp-related factor that has been constantly proven to increase the risk of delayed bleeding is the large size of the lesion^[50,51]. Therefore, pretreatment of stalks in large polyps may be necessary, and a variety of techniques are available. For polyps with a head ≥ 20 mm or a stalk ≥ 10 mm in diameter, recent European guidelines (ESGE) have recommend pretreatment of the stalk with injection of diluted adrenaline and/or mechanical hemostasis (moderate quality evidence,

strong recommendation)^[52].

Endoclips

Prophylactic clipping before or after polypectomy remains controversial, with conflicting results reported in different studies^[46].

Quintanilla *et al.*^[53] reported in a prospective randomized study of large pedunculated polyps that prophylactic clips (prior to polyp resection) did not decrease the risk of delayed bleeding after polypectomy. Technically, they suggested the use of hemoclips in the case of polyps with long and thin pedicles. However, this study was suspended early because of the high risk of morbidity in the clipping group, with higher rates of mucosal burns and perforation rather than bleeding.

Very thick and/or short stalks may be a challenge for clip placing, causing mucosal burns and risk of perforation due to the contact of the base of the polyp with the snare and the clip^[54].

Indeed, prophylactic clips applied before endoscopic removal for this type of polyps were actually associated with further risk of mucosal deep erosions and perforation^[55].

For MPCP resected by hot snaring, neither early nor delayed bleeding complications occurred for more than two decades during which clips were not used^[56].

On the other hand, Parikh *et al.*^[57] concluded that prophylactic placement of hemoclips after polypectomy was a cost-effective plan for patients on antiplatelet or anticoagulation therapy.

Endoloops

The use of the endoloop can also generate technical difficulties from looping large polyps and the endoloop removal^[53] to the transection by the loop of a thin stalk before the polypectomy or insufficient tightening of the loop^[58]. A prospective randomized multicenter study^[59] suggested that the application of a prophylactic hemoclip is as effective and safe as an endoloop in the prevention of postpolypectomy bleeding in large pedunculated colonic polyps.

Anchor clip technique

Mizukami *et al.*^[60] described the anchor clip device, which, placed before the resection of large polyps, constrains the base of the stalk after resection, avoiding immediate bleeding and mucosal burns.

Adrenaline injection

A prospective study on pedunculated polyps larger than 20 mm has shown that there are no differences between adrenaline injection and the use of endoloops or hemoclips in postpolypectomy bleeding prophylaxis^[48], although its addition to both techniques appeared to increase the efficiency in other studies^[61,62]. Recently, a prospective randomized study^[63] that compared the rates of bleeding after resection following single clipping alone

and a combined method (hemoclips plus epinephrine-saline injection) concluded that large pedunculated polyps can be successfully removed *via* hot snare by using the single prophylactic clipping method.

A recent meta-analysis of three randomized controlled studies^[64] that compared the efficacy of epinephrine injection and mechanical hemostasis in postpolypectomy bleeding in patients with pedunculated polyps over 20 mm demonstrated that prophylactic treatment with mechanical hemostasis is more effective than epinephrine injection for preventing overall postpolypectomy bleeding (2.2% vs 6.3%) and early postpolypectomy bleeding (1.1% vs 4.5%). The rate of delayed postpolypectomy bleeding was 1.9% in the epinephrine group and 1.1% in the mechanical group, and their implementation was not found to significantly affect the rate of delayed postpolypectomy bleeding (OR = 0.58, 95%CI: 0.13, 2.49; *P* = 0.46) without significant heterogeneity between the studies (*P* = 0.94, *I*² = 0%).

The impact of underlying comorbidities and other pedunculated polyp characteristics

The presence of comorbidity, beyond the size and location of the polyp, should also be taken into consideration when discussing further management.

Different risk factors for postpolypectomy complications, such as old age (older than 65 years of age), underlying diseases (cardiovascular or chronic renal disease), anticoagulant use, polyp size > 10 mm, a stalk size > 5 mm, polyps located on the right side of the colon, malignant polyps, use of cutting mode and low-volume endoscopists, have been described^[47,64-67].

A recently published review and meta-analysis^[68] identified cardiovascular disease, hypertension, polyp size over 10 mm, and polyp location as significant risk factors for delayed postpolypectomy bleeding, whereas pedunculated morphology, carcinoma histology, age, sex, alcohol use, smoking, diabetes and cerebrovascular disease were not.

Related to the polyp location, recent evidence^[50] suggests that right-sided polyps have a significantly higher risk of bleeding and perforation in comparison with left-sided polyps, for both sessile and pedunculated polyps.

In conclusion, the effectiveness of common preventive methods is variable, and no consensus has been reached to date on the strategy to avoid postpolypectomy bleeding. Large randomized controlled trials are necessary to confirm these observations, taking into consideration more potential risk factors such as pedunculated polyp characteristics (e.g., length of the pedicle) or other patient comorbidities (e.g., the bleeding risk from heparin - bridging therapy in patients with high thromboembolic risk^[69]). Interestingly, Shibuya *et al.*^[70] showed that the overall postpolypectomy bleeding rate under the new Japanese guidelines, which indicate that antithrombotic agents

are not to be discontinued in cases with a high-risk of thromboembolic incidents, was not significantly higher when compared with data from previous guidelines, without particularly addressing pedunculated polyps.

STRATEGIES FOR PATIENTS ON ANTIPLATELET THERAPY OR ANTICOAGULANTS

The risk of bleeding, as the most common adverse effect of polypectomy and particularly the higher risk of bleeding of pedunculated polyps, was already described in the section "Challenges in endoscopic resection techniques". Therefore, endoscopic polypectomy is considered to be a high-risk procedure based on the risk of bleeding, which is increased by the addition of antiplatelet or anticoagulant therapy. In this group of patients, the risk of hemorrhage should be balanced against the risk of thrombosis when antiplatelet or anticoagulant therapy is discontinued.

Patients with MPCP and indication of polypectomy should be managed as summarized in Table 2, according to the most recent British Society of Gastroenterology and ESGE general recommendations^[71].

ADEQUATE FOLLOW-UP AFTER RESECTION

Discussing surveillance after polypectomy can be challenging because the risks and outcomes are difficult to calculate. Generally, when the risk of the lesion seems to be low, interval surveillance is performed. For patients with a higher risk, further surgical resection is necessary, but there is no consensus on follow-up procedures and subsequent intervals for early cancer in pedunculated lesions. The management of an MPCP following endoscopic resection can generate anxiety for both the physician and patient because of possible residual cancerous cells and/or positive lymph nodes that are variable from one case to another^[72]. However, further management remains balanced between the general approach of postpolypectomy surveillance of patients with high-risk adenomas^[6,73,74] and the follow-up of a resected CRC with curative intent^[75-77]. However, it is also based on the experience and clinical sense of the physician.

The recent recommendations of the United States Multi-Society Task Force on Colorectal Cancer endorsed by the American Society for Gastrointestinal Endoscopy^[75] address only the use of colonoscopy in the follow-up of patients with resected CRC with curative intent and insist on the fact that the colorectum should be carefully cleared of synchronous neoplasia in the perioperative period, without any particular information on early cancer in pedunculated polyps.

Fortunately, pedunculated polyps are unusual

Table 2 Endoscopic polypectomy in patients on antiplatelet therapy or anticoagulants (British Society of Gastroenterology and European Society of Gastrointestinal Endoscopy Recommendations^[71])

Thrombosis risk factors		High thrombotic risk	Low thrombotic risk	Post-polypectomy
Discontinuation of warfarin concerning the requirement for heparin bridging	Discontinuation of clopidogrel, prasugrel or ticagrelor	Continuing aspirin and liaising with a cardiologist about the risk/benefit of discontinuing P2Y ₁₂ receptor antagonists (high quality evidence, strong recommendation)	Continuing aspirin in patients on dual antiplatelet therapy (low quality evidence, weak recommendation)	Antiplatelet or anticoagulant therapy should be suspended up to 48 h after the procedure depending on the perceived bleeding and thrombotic risks (moderate quality evidence, strong recommendation)
Prosthetic metal heart valve in mitral position	Drug-eluting coronary artery stents within 12 mo of placement	Warfarin should be temporarily stopped and substituted with LMWH (low quality evidence, strong recommendation)	Discontinuing P2Y ₁₂ receptor antagonists 5 d before the procedure (moderate quality evidence, strong recommendation)	
Prosthetic heart valve and atrial fibrillation	Bare metal coronary artery stents within 1 mo of placement	The last dose of DOAC should be taken at least 48 h before the procedure (very low quality evidence, strong recommendation)	Discontinuing warfarin 5 d before the procedure (high quality evidence, strong recommendation)	
Atrial fibrillation and mitral stenosis			Ensure the INR target < 1.5 prior to the procedure (low quality evidence, strong recommendation)	
< 3 mo after venous thromboembolism				

LMWH: Low molecular weight heparin; DOAC: Direct oral anticoagulants.

in the rectum. However, rectal cancer is generally associated with a higher risk of local recurrence than other segments of the colon, and additional considerations for surveillance^[77], such as endoscopic ultrasound for better detection of suspicious lymph nodes and recurrences^[75], are suggested. The utility of adjuvant chemoradiation or chemoradiation alone for high-risk early rectal carcinoma remains to be elucidated^[1].

In a long-term prospective study of 25 consecutive patients with MPCP treated with snare cautery polypectomy^[56], the author concluded that short-term outcomes after removal appeared to be similar to those with a nonmalignant polyp. He suggested that long-term surveillance should be considered in each patient, assuming reasonable life expectancy, because the risk of additional adenomas and metachronous colon cancer persists even after the initial five years of currently recommended surveillance. In addition to the small number of patients, the location of the lesions was limited to the sigmoid or descending colon, and both standard and high-definition colonoscopes were used without calculating the accuracy of polyp detection in separate subgroups. Personal or family history of intestinal neoplasia (such as previously resected adenomas) or underlying inflammatory bowel disease was excluded from the study.

A high carcinoembryonic antigen (CEA) value may be predictive of metastatic disease^[78-80]. There have been reported cases of MPCP with unfavorable histological criteria without initial local residual carcinoma or lymph node invasion but with distant metastasis even five years after surgery^[11,81], so close monitoring of such patients using CEA and imaging techniques seems prudent.

To our knowledge, to date, there are no particular guidelines including optimal treatment and surveillance of subgroups, such as synchronous CRCs, malignant pedunculated polyps, multiple malignant pedunculated polyps or malignant pedunculated polyps, associated with chronic inflammatory bowel disease.

UNRESOLVED ISSUES AND AREAS FOR FURTHER RESEARCH

There is a thin line between early cancer in pedunculated polyps and invasive cancer, due either to interobserver variation in detection rate by endoscopists or histologic interpretation by pathologists. Standard snare polypectomy is appropriate for pedunculated polyps with early cancer limited to the submucosa and favorable histology. The distance from the cancer to the margin of the resection excision is still under debate. These situations lead to a challenging evaluation of the natural history of the lesions.

Treatment plans and the best strategy to avoid postpolypectomy complications for colorectal malignant pedunculated polyps lack the evidence of randomized trials. Large randomized trials on this particular topic should be included in meta-analyses that develop further guidelines to provide relevant conclusions for patients' long-term

surveillance and outcomes. More long-term information focused on patients with endoscopically removed malignant polyps, including personal or family history of intestinal neoplasia, previously resected adenomas, or underlying inflammatory bowel disease^[82,83], would be valuable.

In addition to general unfavorable histological criteria, better stratification of patients with high-risk pedunculated polyps requiring surgery^[84], including those with high-grade tumor budding or invasive micropapillary components as reliable predictors of lympho-hematic metastases, is necessary. On the other hand, inadequate recognition of the pseudoinvasion pitfall as a benign condition can generate overdiagnosis and subsequent overtreatment of certain lesions. In this respect, a second histological opinion seems advisable for all cases of MPCP, especially when surgery is taken into consideration.

CONCLUSION

There are still unresolved issues requiring detailed recommendations according to the patient's and polyp's risk factors to avoid an overuse of surveillance procedures. Provided future novel imaging technologies and increased pathological recognition of high-risk markers for angiolymphatic invasion will be developed, it will be easier to decide on the optimal follow-up plan and therapy.

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Basic Study

Histological analysis of human pancreatic carcinoma following irreversible electroporation in a nude mouse model

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Abstract

AIM

To determine changes in the morphology and function of pancreatic cancer cells after irreversible electroporation (IRE) treatment, and to explore the clinical significance of IRE treatment for pancreatic cancer providing an experimental basis for the clinical application of IRE treatment.

METHODS

IRE was carried out in an athymic nude mouse model of pancreatic carcinoma generated with human pancreatic cancer cells 1. In therapy groups, IRE electrodes were inserted with 90 pulses per second at 800 V/cm applied to ablate the targeted tumor tissues. Histological assessment of the affected tissue was performed by hematoxylin and eosin staining (HE). Quantification of cell proliferation and apoptosis was performed by evaluating Ki67 and caspase-3 levels, respectively. Flow cytometry was used to assess cell apoptosis. Ultrasound

imaging was carried out to evaluate IRE treatment results. Pathological correlation studies showed IRE is effective for the targeted ablation of pancreatic tumors in an orthotopic mouse model.

RESULTS

IRE was efficacious in removing tumors in the orthotopic mouse model. The IRE-ablated zone displays characteristics of nude mouse models at different time-points as assessed by hematoxylin and eosin staining. Immunohistochemical analysis of samples from the pancreatic cancer models showed significantly enhanced caspase-3 cleavage and Ki67. Flow cytometry data corroborated the above findings that apoptosis in tumor cells was observed immediately on the first postoperative day, and with time the middle and late stages of apoptosis were observed. For ultrasound imaging studies, the IRE ablation zone became a hyperechoic area due to increasing inflammatory and immunologic cellular contents.

CONCLUSION

IRE is a promising new approach for pancreatic cancer, with many potential advantages over conventional ablation techniques.

Key words: Irreversible electroporation; Pancreatic carcinoma; Pathological evaluation; Transplantation model; Nude mouse

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Core tip: Patients with pancreatic cancer have a poor prognosis. It often quickly develops into locally advanced pancreatic cancer that is considered to be surgically unresectable. Irreversible electroporation represents a novel tumor ablation method that induces cell apoptosis with no thermal coagulation effects. This study aimed to assess the clinical significance of irreversible electroporation treatment in pancreatic cancer, and to provide an experimental basis for the clinical application of irreversible electroporation treatment.

Su JJ, Xu K, Wang PF, Zhang HY, Chen YL. Histological analysis of human pancreatic carcinoma following irreversible electroporation in a nude mouse model. *World J Gastrointest Oncol* 2018; 10(12): 476-486

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INTRODUCTION

Immunodeficient animals are obtained from genetic mutations or by artificial methods that cause one or more genes of the immune system to be defective.

Because such animals are immunodeficient, they are widely used in the fields of immunology, oncology, toxicology, and others. Currently, BALB/c mice are the most commonly used immunodeficient animals^[1]. A recent xenograft pancreatic cancer model employing human pancreatic cells was adopted^[2]. This constitutes a clinically relevant and reproducible animal model for assessing local and systemic treatments^[3]. Indeed, orthotopic models of pancreatic carcinoma mimic the main features of human disease, and are excellent tools for the biological characterization of this malignancy.

Pancreatic carcinoma is a malignant tumor with the characteristics of insidious onset, fast progression, high postoperative recurrence and overall 5-year survival rate below 5%^[4]. According to reports of the American Cancer Society, pancreatic carcinoma ranks fourth among deadliest cancers. Conventional therapeutic methods include surgical treatment and chemotherapy. However, the majority of cases cannot undergo surgery because they are diagnosed with advanced disease presenting distant metastasis; meanwhile, chemotherapeutics have low permeability and are limited by drug resistance^[5].

Considering the limited therapeutic options, irreversible electroporation (IRE) has been developed in recent years for the treatment of locally advanced pancreatic cancer^[6]. IRE represents a new modality that can be used independently for targeted tissue ablation, applying strong electrical fields instead of heat deposition or chemicals^[7]. Because IRE has a non-thermal mechanism of action, it can be used to target malignancies adjacent to vital structures (e.g., major vessels)^[8]. This study aimed to assess the efficacy of IRE in pancreatic cancer treatment using an orthotopic mouse model.

MATERIALS AND METHODS

Tumor cell line and culture

The human pancreatic cancer cells 1 (PANC-1) cell line was provided by American Type Culture Collection (Tumor Hospital of the Chinese Academy of Medical Sciences, China), and maintained in Dulbecco's modified Eagle's medium (DMEM; HyClone) with 10% FBS (Sigma), 100 U/mL penicillin, and 100 mg/mL streptomycin in a humid environment containing 5% CO₂. Cells were pelleted and re-suspended at 1×10^7 /mL in phosphate buffer solution (PBS, pH = 7.4). Prior to implantation, cell viability was assessed by Trypan blue staining (assessing the viability of the cultured cells for each tumor implantation procedure, > 95%). The tumor cells were kept on ice prior to injection into the pancreas.

Animal model

All animal experiments had approval from the Institutional Animal Care and Use Committee of Chinese

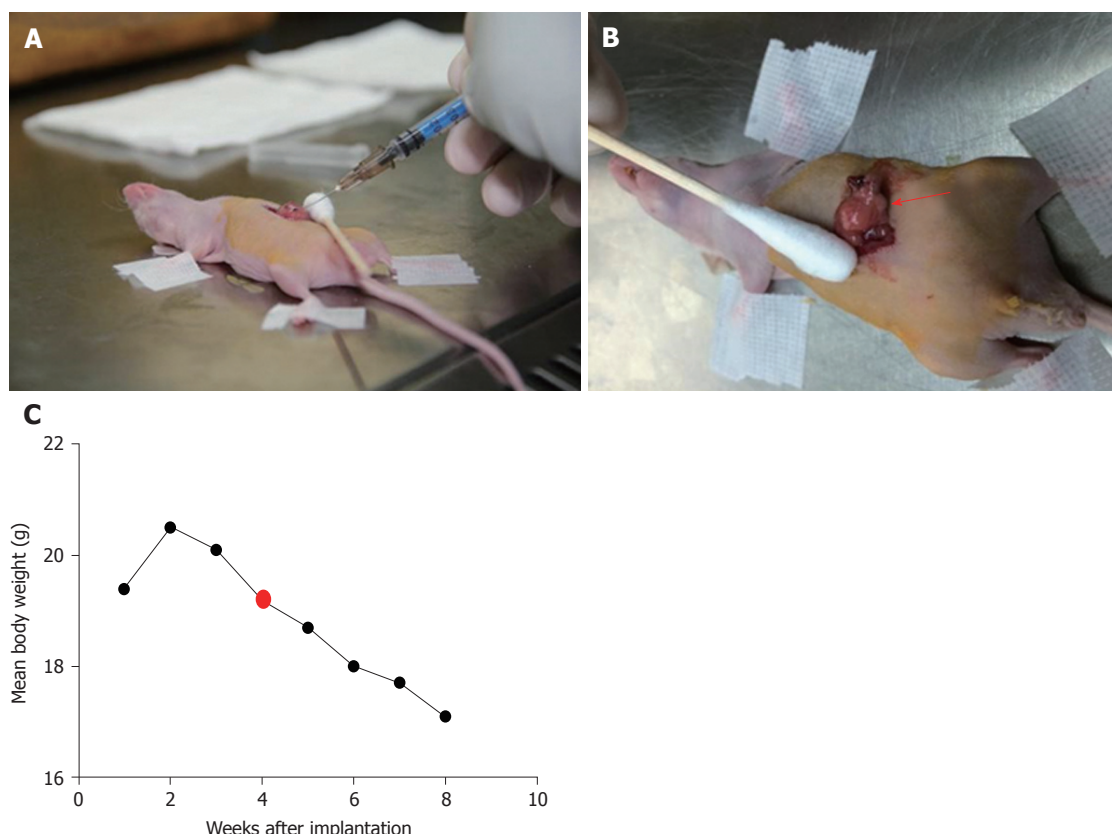


Figure 1 An orthotopic nude mouse with pancreatic cancer generated with human pancreatic cancer cells 1. A: The administered cells generated a bubble on the pancreatic surface; B: The median tumor area at the time of ablation approximated 1 cm²; C: Changes in body weights of tumor-bearing mice at different times after transplantation.

People's Liberation Army General Hospital. Fifty adult male BALB/c nude mice (Institute Of Medical Laboratory Animals, Chinese Academy Of Medical Sciences), initially weighing 18-20 g, were used in this study. The animals were housed in groups of five in facilities maintained at 22 °C ± 1 °C with 55 ± 10% relative humidity, under a 12 h – 12 h light/dark cycle for 1 wk before the experiments. Mice were anesthetized using inhaled 2%-3% isoflurane. Anesthesia depth was evaluated based on the lack of reflex to a toe pinch. The abdomen of each mouse was prepared with Anerdian skin disinfectant. Each mouse was turned on the side to raise the left side of the abdomen. Using a sterile scalpel, 1.5 cm skin incisions (about 1 cm left lateral from the midline) were made; 1.5 cm incisions in the underlying abdominal muscle were also made. Then, 50 µL of the cell suspension was injected into the pancreatic tail. These cells generated a bubble on the pancreatic surface (Figure 1A). The injection site was inspected to ensure that no leakage occurred. The abdominal musculature and the external skin in each mouse were separately closed with an absorbable braided suture using a continuous stitch. After wound healing (7 d), the mice were anesthetized, and the external sutures were removed. Following the initial implantation, approximately 5-10 d were required to allow sufficient

tumor growth for pretreatment (diameter < 1.5 cm). The animals were externally examined frequently; in addition, ultra-high frequency, high-definition ultrasound (US) (Philips, CX50, Epiq 7, Seattle, WA, United States) assessment was performed every 3 d from 30 d after tumor cell injection (Figure 1B).

IRE procedures

Forty-four nude mice from the initial 45 implanted animals produced pancreatic cancer (0.5 ± 1.5 cm in diameter) suitable for subsequent IRE treatment procedures. Forty-nine animals were assigned to three groups, including the normal (5 mice with no tumor cell implantation; Group 1), sham-operation (Group 2; *n* = 22), and IRE (Group 3; *n* = 22) groups. Mice in groups 2 and 3 were subsequently euthanized for histological examination at different time-points (1 d, 3 d, and 7 d) after the original baseline scan.

IRE tumor ablation was performed with an experimental IRE generator (Nanoknife; Angio Dynamics, Queensbury, NY, United States). IRE electrodes were positioned in parallel, with spacing set to 0.5 cm, and inserted into the diseased pancreas at a final depth of 0.5 cm. Ablation parameters included a voltage of 800 V/cm at 90 µs and a pulse length of 100 milliseconds. A total of 90 pulses were applied per minute, with an

ablation rate of 90 pulses per second. The abdominal wall in mice was closed after the procedure. The abdominal cavity was opened, exposing the pancreas after 4 wk of modeling. The gross morphologies of the pancreas in various groups were: (1) control group, no obvious mass or tissue adhesion; and (2) IRE group, average tumor diameter of 0.8 cm, with the tumors having a hard texture. With regard to gross appearance, the tumors were round or nodular, and the tumor tissue was usually grayish white. Some of the tumors had mild adhesion to the surrounding tissue, while others invaded adjacent organs such as the stomach, duodenum and peritoneum. Early tumors showed no ascites (Figure 1B).

Tissue collection and immunohistochemistry

Tumor samples were harvested 1 d, 3 d, and 7 d after IRE from anesthetized animals, fixed with 10% formalin, and paraffin embedded. The sections were then submitted to hematoxylin and eosin (HE) staining for histopathological assessment on an Olympus BX43 microscope (Olympus, Japan). Histology slides were blinded and reviewed by a pathologist specialized in gastrointestinal oncology (> 10 years of experience). For immunohistochemistry, four-micron sections were incubated with antibodies against Ki67 (550609, 1:300, BD) and cleaved caspase-3 (Asp175) (9661S, 1:200, Cell Signaling Technology, United States). Ki67 staining was used as a marker of active proliferation, while caspase-3 signals reflected active apoptosis^[9]. Tumor cell proliferation was assessed in five high power fields under a microscope in each slide; the Ki67 index was employed for quantitation. Immunohistochemistry (IHC) staining for Ki67 and cleaved caspase-3 was scored as the percentage of positive cells.

Statistical analysis

Statistical analysis was carried out with SPSS v.22 for Mac (SPSS, United States). Data were presented as mean \pm SD. Groups were compared with variance tests (comparisons between untreated and treated mice). Differences were considered statistically significant with a *P*-value < 0.05.

RESULTS

A total of 44 of the 45 mice implanted with PANC-1 cells developed pancreatic cancer. One mouse was euthanized prior to the beginning IRE procedures because of suture failure, and one animal died after the IRE from improper operation. No severe postoperative complications occurred in the treated mice (Groups 2 and 3).

HE staining

Histological examination of tumor tissues was performed by a pathologist. As shown in Figure 2A,

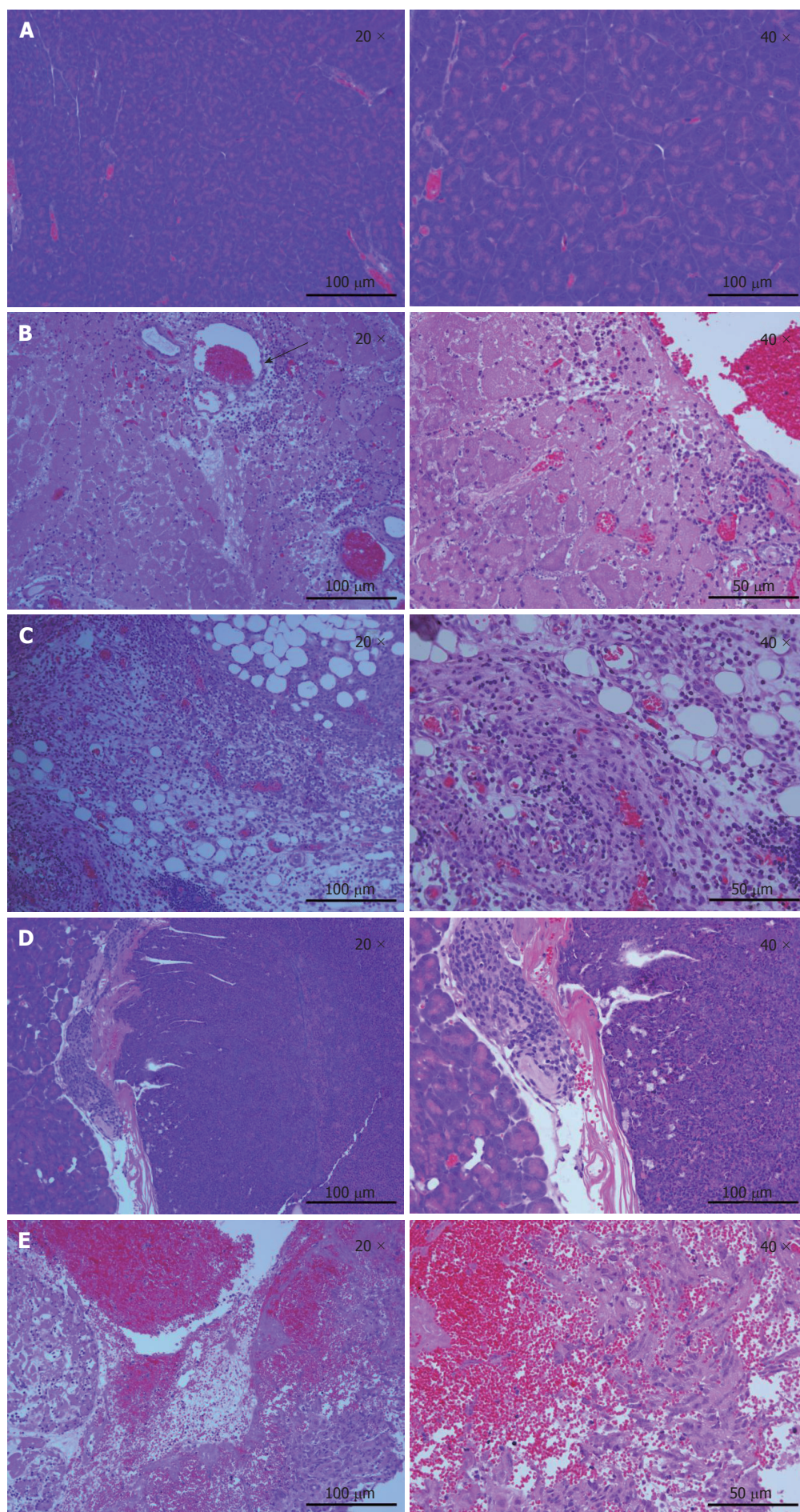
pancreas cells in the normal mouse had large nuclei surrounded by well-demarcated cytoplasm and well-defined cytoplasmic membrane. A total of 3 d post-IRE (Figure 2B), the ablation zone showed areas of acute, extensive, and severe pancreatic cell death. A seepage zone of erythrocytes was observed around the ablation zone. However, larger vessels in the ablated area appeared to be structurally well preserved. The normal pancreatic architecture was preserved. At 7 d after IRE ablation (Figure 2C), erythrocyte leakage continued to decrease. As shown in Figure 2E, most tumor cells were deformed and melded together. Large numbers of inflammatory cells began to permeate into the ablated area. At 3 d after IRE (Figure 2F), the ablation zone was characterized by edematous swelling of the interstitium and tumor tissue necrobiosis. Eosinophilia increased continually, with marked ablation zone inflammation. At 1 d post-IRE treatment (Figure 2G), complete cell death was achieved in the ablation zone, with a sharply delineated margin between ablated and non-ablated surrounding tissues. The majority of tumor cells were displaced by fibrosis, and mononuclear cells and chronic inflammation were observed.

Tumor IHC

To evaluate the effect of IRE on tumor tissues at different time points, *in vivo* IHC experiments were performed. Staining with antibodies targeting Ki67 and cleaved caspase-3 was performed to assess cell proliferation and apoptosis, respectively. Figure 3A is a representative IHC image in an untreated pancreatic parenchyma. In tumor tissues (Figure 3B), extensive caspase-3 activation was observed on the first postoperative day. IRE significantly increased cell proliferation (Ki67 staining) at 1 d post-treatment, but cell proliferation was decreased at 7 d post-treatment. Limited caspase-3 staining at 7 d post-IRE treatment was found in treated tumors, while most of the viable tumor tissues showed no caspase-3 activation (Figure 3C). Our results fully demonstrated overt tumor necrosis in the IRE group, especially the first day after treatment.

Flow cytometry

The propidium iodide staining assay was employed to assess cell cycle distribution. Tumor cells were fixed with 70% ethanol, washed, incubated in presence of 100 mg/mL RNase in PBS (30 min; 37 °C), and stained with 50 mg/mL propidium iodide in PBS. Cell cycle distribution was assessed on a Cell Lab Quanta SC flow cytometer (Beckman Coulter, United States). Meanwhile, Annexin V-fluorescein isothiocyanate Apoptosis Detection Kit (BioVision) was employed for apoptosis quantitation, as directed by the manufacturer. Spleens were extracted, weighed, and processed for analyses. For the cell cycle distribution assay, cells (5×10^5) were harvested, washed with PBS, and finally



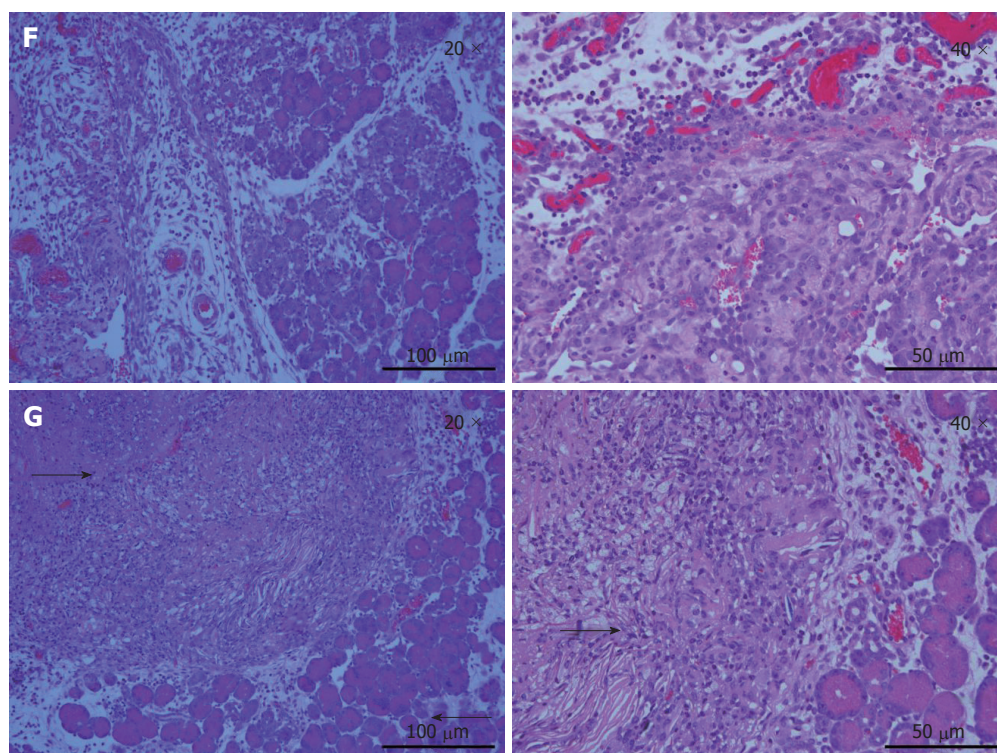


Figure 2 Hematoxylin and eosin staining of pancreatic cells in various groups. A: Histologically stained tissues of pancreatic parenchyma in untreated animals; B, C: Histology showed a normal pancreas after IRE, and a seepage area of erythrocytes was observed around the ablation zone (arrow). Additionally, larger amounts of erythrocytes were observed at 3 d post-IRE compared with 7 d post-IRE (black arrows represent the erythrocyte zone). The vascular structure was not damaged; D: Hematoxylin and eosin staining of tumor cells; E: Nuclear agglutination was observed 1 d post-IRE. The nucleus-to-cytoplasm ratio tended to increase; F: At 3 d post-therapy, a heterogeneous necrotizing tumor was present; G: Micrograph depicting the human pancreatic cancer cells 1 tumor xenograft 7 d post-IRE. A clear demarcation between the ablated (left side) and normal tumor (right side) tissues is depicted (arrows). Tumor cells were arranged more loosely in G compared with F ($\times 200$ or $\times 400$). IRE: Irreversible electroporation.

re-suspended in 500 mL binding buffer, followed by incubation with annexin V-fluorescein isothiocyanate (5 mL) and propidium iodide (5 mL) for 30 min at room temperature in the dark. After staining, cells were assessed on a flow cytometer. Flow cytometry data corroborated the above findings that apoptosis in tumor cells was observed immediately on the first postoperative day, and with time the middle and late stages of apoptosis were observed (Figure 4).

US imaging

For US studies, pre-ablation US (Philips, CX50, Epiq 7, Seattle, WA, United States) was performed to visualize the normal pancreatic anatomy, and US imaging was carried out to evaluate IRE treatment results. Image analysis was carried out by two radiologists with ≥ 10 years of experience in pancreatic US. Consensus was based on post-ablation discussion. In the normal group (Figure 5A and B), the position of the normal pancreatic parenchyma was accurately detected before and after the IRE treatment by US examination. In the tumor group (Figure 5C and 5D), tumor size was determined by US before and after IRE. Upon IRE treatment, US was repeated to acquire post-IRE images. These experiments successfully showed that IRE induced

rapid changes during ablation of the normal pancreatic tissue as well as tumor ablation, and these changes were apparent on US images^[10]. In the tumor tissue, the IRE ablation zone became a hyperechoic area due to increasing inflammatory and immunologic cellular contents.

DISCUSSION

Unresectable lesions amount to roughly 80% of pancreatic cancers at the time of diagnosis, and show a 5-year overall survival below 5%^[11]. This poor prognosis has historically reduced the enthusiasm for aggressive surgical resection^[12]. Recently, alternative tools for local therapy (e.g., radiation and various thermal and non-thermal ablation methods) have been assessed, but often produce discouraging outcomes^[13]. IRE represents a promising novel tool for tissue and tumor ablation^[6]. IRE destroys cells in the target region while preserving the collagen architecture of vascular, biliary, or neuronal structures^[14]. High electric voltage generating a large potential gradient to cause IRE has been assessed *in vitro* and *in vivo*^[8]. Such findings are interesting because IRE effectively causes cell death in the normal tissue as well as cancer cells^[15]. The main advantage of IRE is in the conservation of blood vessel and bowel wall

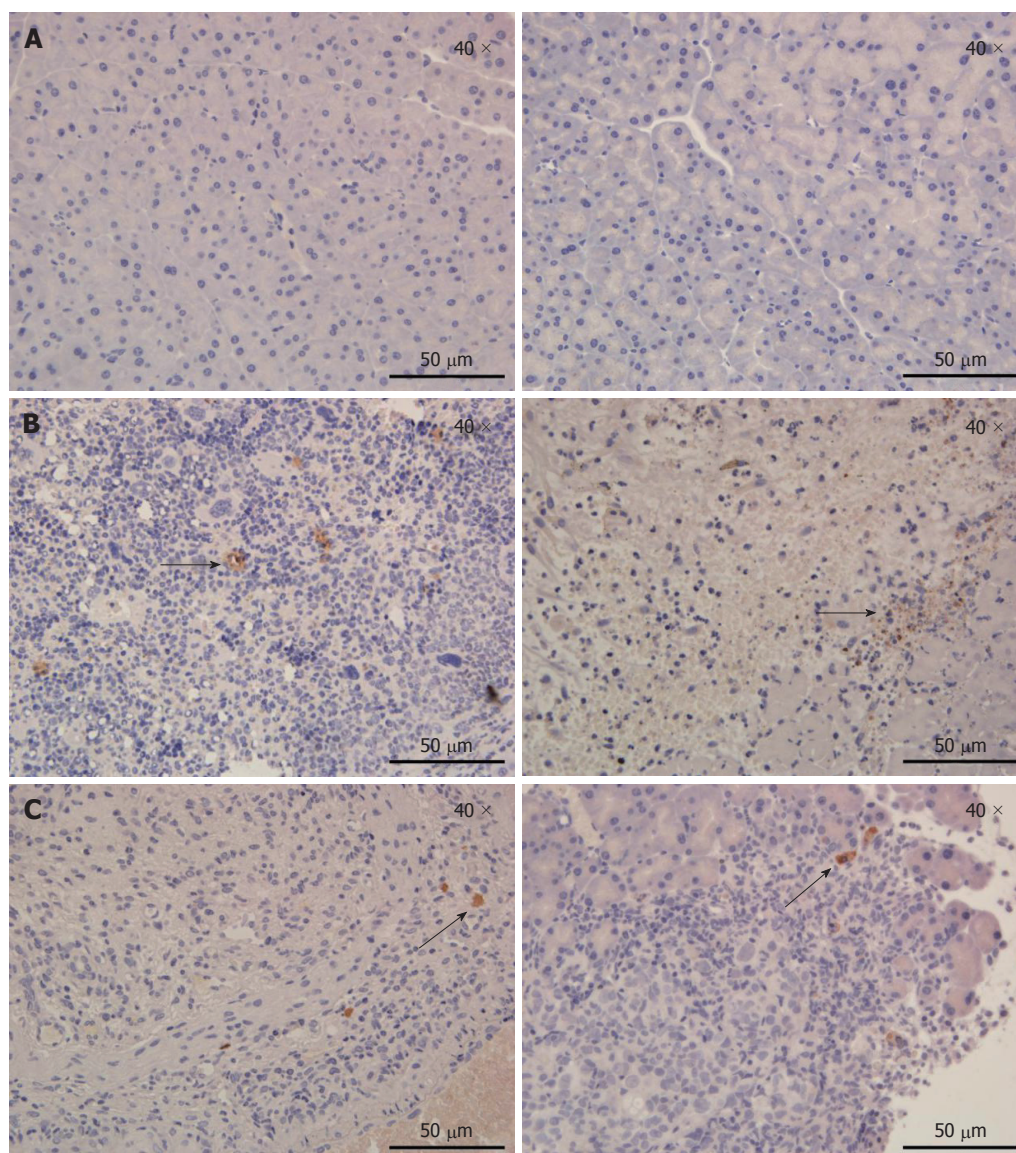


Figure 3 Tumor tissue sections at 1 d and 7 d after the irreversible electroporation treatment were stained for Ki67 and cleaved caspase-3. A: A representative immunohistochemistry image in an untreated pancreatic parenchyma; B: In tumor tissues, extensive caspase-3 activation was observed on the first postoperative day. Irreversible electroporation significantly increased cell proliferation (Ki67 staining) at 1 d post-treatment, but cell proliferation was decreased at 7 d post-treatment (arrows); C: Limited caspase-3 staining at 7 d post-irreversible electroporation treatment was found in treated tumors, while most of the viable tumor tissues showed no caspase-3 activation. The slides were imaged at 400 × by light microscopy.

integrity^[16]. The vascular structure in the ablation zone showed no damage and was only scarcely affected by the IRE treatment. In this study, we created a mouse model of orthotopic pancreatic carcinoma by treating BALB/c nude mice by transabdominal administration of PANC-1 cells. Orthotopic pancreatic cancer modeling was successfully achieved in forty-four nude mice. Studies on animal models demonstrated the efficacy of IRE for achieving anti-tumor effects in orthotopic mouse models of pancreatic cancer by HE staining, apoptosis-specific immunohistological analysis, flow cytometry, and US imaging.

HE staining (Figure 2) showed that the IRE-ablated zone (day 1) had an extensive necrotic area and the IRE ablation border zone had more infiltrated inflam-

matory cells compared with the ablation center zone. Large numbers of red blood cells were observed in the ablation area, which was probably due to IRE destroying microcirculation perfusion in the ablation area. Furthermore, there were large amounts of neutrophils with perivascular infiltration. Moreover, vessels in the ablation area showed an intact structure. We further demonstrated that extensive and severe cell death in the IRE ablation zone was completely different from that observed in the thermal ablation zone. Meanwhile, post-ablation inflammatory reactions were not the overall cause of necrosis resulting from the IRE treatment. At 7 d post-IRE, the tissue in the ablated area showed a uniform structure without cells. IRE caused an ablation of cells but left the cellular matrix intact, providing a

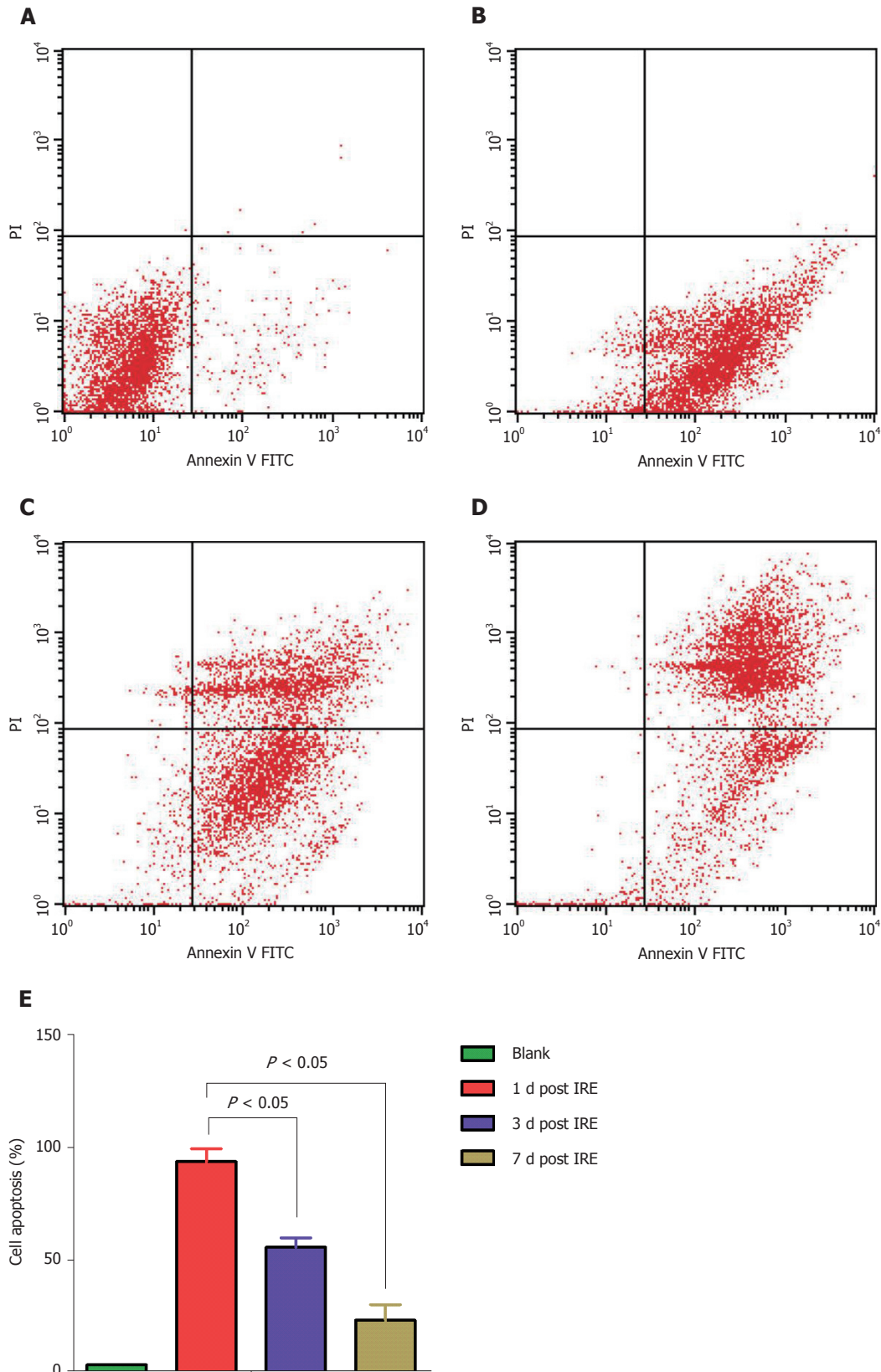


Figure 4 Apoptosis assay of mouse spleen cells before and after irreversible electroporation treatment using double-staining with annexin V-fluorescein isothiocyanate/propidium iodide. Apoptosis was quantified by flow cytometry. A: Control group; B: 1 d post-IRE; C: 3 d post-IRE; D: 7 d post-IRE; E: Percentages of apoptotic cells before and after the IRE intervention. Data are mean \pm SD ($n = 8$). IRE: irreversible electroporation.

good scaffold for new tissue formation^[17]. Due to tissue necrosis and cellulose formation in the ablated area,

the tissue structure was better visualized. We clearly observed that tissue dissolution and absorption occurred

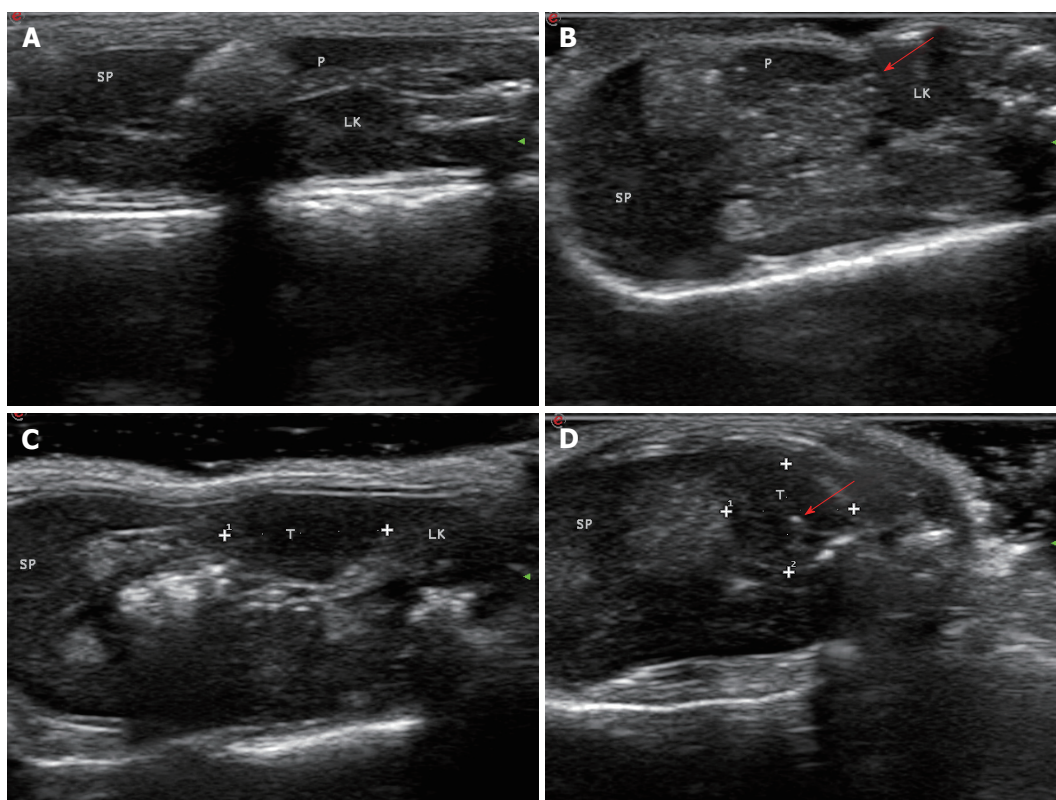


Figure 5 Evaluation of therapeutic effects of irreversible electroporation on tumors *in vivo* by ultrasound. A: A pre-irreversible electroporation ultrasound image showing the normal pancreatic parenchyma; B: The ablation zone showed hyperechoic signals with a comet tail sign in the normal pancreatic tissue (arrow); C: White dots indicate the region of the tumor; D: Ultrasound image showing that the irreversible electroporation ablation zone in the tumor tissue became hyperechoic (arrow). SP: Spleen; LK: Left kidney; P: Pancreas; T: Tumor.

between the necrotic area and the lacunae.

To further assess tissue cell proliferation and apoptosis occurring after the IRE treatment, we next performed IHC to evaluate the effect of IRE on tumor tissues. This study evaluated Ki67 staining and caspase-3 activity, which measure cell proliferation and apoptosis, respectively, in the region between the two electrodes; as shown above, these two measurements showed a positive correlation. The results showed that cell death in the IRE-ablation area was reflected by increased amounts of caspase-3 compared with the adjacent normal tissue on the first day after IRE ablation (Figure 3B). At 7 d postoperatively, very few apoptotic cells were stained, indicating that IRE induced cell death by apoptosis rather than coagulative thermal necrosis. Figure 3B shows blood vessel cells that were not stained, suggesting that large blood vessels are not affected by IRE in the ablation area. This may be an advantage for IRE to create an effective ablation of the undesirable tumor without damaging the underlying architecture of the healthy pancreatic parenchyma.

Furthermore, we used flow cytometry to examine cell cycle distribution and the apoptotic rate. To assess cell apoptosis efficiency, mouse spleen cells in the treatment group were analyzed by flow cytometry at different time-points (Figure 4). No overt cell apoptosis in the control group (Figure 4A) was observed. However,

cells treated by IRE (Figure 4B) had an apoptotic rate of 93.71%, which was markedly elevated compared with the control value (3.37%, $P < 0.05$), suggesting that the IRE treatment is highly effective. Cells in the middle and late stages of apoptosis increased with time (Figure 4C and 4D). As expected, cell apoptosis rates of the therapy groups were much higher than that of the control group. Meanwhile, significant differences were obtained in apoptotic rates at different time points (Figure 4E). These results indicated that the IRE treatment is effective for targeted ablation of pancreatic tumors in an orthotopic mouse model.

The above experiments successfully demonstrated that IRE induces changes detectable on US imaging. Pre-IRE US imaging showed the tumor appearing peripherally hyperechoic compared with the normal pancreatic parenchyma. This study showed that IRE ablation produced greater alterations to echogenicity in tumors compared with normal tissues. The above US findings demonstrated that ablated tissues in the normal pancreas and tumors became more hyperechoic. The US images obtained during IRE showed the hypoechoic ablation region as being mixed with the hyperechoic region in close proximity to the probes used. As shown in Figure 5B, both hyperechoic probe tips of the dual probe system in the ablated zone had a minimal amount of hyperechoic microbubbles. US

images showed that the area of hypoechogenicity became largely hyperechogenic due to increased inflammatory and immunologic cellular contents in the ablated zone. We also demonstrated that the treated areas correlated with pathological measurements. Such changes in echogenicity provide strong evidence that perioperative US is feasible in monitoring IRE.

The efficacy of IRE treatment was also evaluated by monitoring body weights in mice for 7 d (Figure 1C). The therapy groups displayed more pronounced body weights over time compared with control mice, which may be due to decreased tumor sizes in the treatment groups.

Another significant finding was that IRE had a safer and shorter operation procedure compared with traditional techniques, such as the radiofrequency and microwave ablation methods. This compares to routine thermal ablation methods requiring > 30-60 min for ablating a tumor of comparable size^[18]. A reduced operation time in IRE indicates less complications and improved safety for patients compared with conventional means.

In conclusion, this study systematically assessed the efficacy of IRE ablation, and demonstrated that the IRE-ablated zone displays characteristics of nude mouse models at different time-points as assessed by HE staining. IRE is a promising new approach for pancreatic cancer with many potential advantages over conventional ablation techniques. Follow-up US images demonstrated tumor size reduction suggesting that US may be used for ablation zone evaluation.

ARTICLE HIGHLIGHTS

Research background

Irreversible electroporation (IRE) is a medical technique that utilizes high voltage pulses to create permanent nanopores in the cell membrane, which in turn induces apoptosis of the targeted cells. Although there are benefits of IRE, many adverse events should be taken into consideration before its use. We aimed to assess the efficacy of IRE ablation in nude mouse models providing an experimental basis for the clinical application of IRE treatment.

Research motivation

Animal models of pancreatic cancer were successfully established and were successfully treated by IRE treatment. Tumor cell proliferation and apoptosis were detected by different methods, which proved that this treatment was effective.

Research objectives

The main objectives aimed to determine changes in the morphology and function of pancreatic cancer cells after IRE treatment providing an experimental basis for the clinical application of IRE treatment.

Research methods

Animal models of pancreatic cancer were successfully treated by IRE treatment. Histological assessment of the affected tissue was performed by hematoxylin and eosin staining. Quantification of cell proliferation and apoptosis was performed by evaluating Ki67 and caspase-3 levels, respectively. Flow cytometry was used to assess cell apoptosis. Ultrasound imaging was carried out to evaluate IRE treatment results. Pathological correlation studies showed

IRE is effective for the targeted ablation of pancreatic tumors in an orthotopic mouse model. Ultrasound imaging was repeatedly carried out to evaluate IRE treatment results.

Research results

This study systematically assessed the efficacy of IRE ablation and demonstrated that the main advantage of IRE is in the conservation of blood vessel and bowel wall integrity. Clinical data of patients after the application of IRE treatment is needed to prove that IRE treatment is effective in treating patients with pancreatic cancer.

Research conclusions

IRE ablation is safe and effective for treatment of pancreatic cancer in a mouse model. The implication of this study for future clinical practice is that advanced pancreatic cancer patients can use IRE ablation as an effective treatment.

Research perspectives

The future direction of research is the extensive safety application of IRE ablation in patients. The best method for future research is to study the practical application of IRE ablation in patients.

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Retrospective Study

Clutch Cutter knife efficacy in endoscopic submucosal dissection for early gastric neoplasms

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Informed consent statement: The patients had signed the informed consent.

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

Data sharing statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Abstract

AIM

To compare the outcomes of endoscopic submucosal dissection (ESD) for gastric neoplasms using Clutch Cutter (ESD-C) or other knives (ESD-O).

METHODS

This was a single-center retrospective study. Gastric neoplasms treated by ESD between April 2016 and October 2017 at Kitakyushu Municipal Medical Center were reviewed. Multivariate analyses and propensity score matching were used to reduce biases. Covariates included factors that might affect outcomes of ESD, including age, sex, underlying disease, anti-thrombotic drugs use, tumor location, tumor position, tumor size, tumor depth, tumor morphology, tumor histology, ulcer (scar), and operator skill. The treatment outcomes were compared among two groups. The primary outcome was ESD procedure time. Secondary outcomes were *en bloc*, complete, and curative resection rates, and adverse events rates including perforation and delayed bleeding.

RESULTS

A total of 155 patients were included in this study; 44 pairs were created by propensity score matching. Background characteristics were quite similar among two groups after matching. Procedure time was significantly shorter for ESD-C (median; 49 min) than for ESD-O (median; 88.5 min) ($P < 0.01$). However, there was no significant difference in treatment outcomes between ESD-C and ESD-O including *en bloc* resection rate (100% in both groups), complete resection rate (100% in both groups), curative resection rate (86.4% *vs* 88.6%, $P = 0.730$), delayed bleeding (2.3% *vs* 6.8%, $P = 0.62$) and perforation (0% in both groups).

CONCLUSION

ESD-C achieved shorter procedure time without an increase in complication risk. Therefore, ESD-C could become an effective ESD option for gastric neoplasms.

Key words: Endoscopic submucosal dissection; Clutch Cutter; Gastric neoplasm; Knife; Propensity score

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Core tip: Propensity score matching was performed to compare the outcomes of endoscopic submucosal

dissection (ESD) for gastric neoplasms using Clutch Cutter or other knives in this single-center retrospective study. Forty-four pairs were matched in this study. ESD using Clutch Cutter achieved shorter procedure time without an increase in complication risk (median procedure time; 49 min *vs* 88.5 min, $P < 0.01$). Therefore, ESD using Clutch Cutter could become an effective ESD option for gastric neoplasms.

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INTRODUCTION

Endoscopic submucosal dissection (ESD) is the standard treatment for gastrointestinal tract tumors including gastric neoplasms, achieving a higher rate of *en bloc* resection and low rates of local recurrence even for large and ulcerated lesions, as compared with endoscopic mucosal resection (EMR)^[1]. However, more advanced technical skills and greater experience are needed in ESD because of the longer procedure time and high risk of complications including bleeding and perforation^[1]. Although various types of endo-knife including needle-type knife and insulated-tip knife were invented and used in ESD, this remains a challenging procedure and there is no consensus on the best knife to be used^[2-5].

Clutch Cutter (DP2618DT, Fujifilm Medical, Tokyo, Japan; Figure 1) was invented as a scissor-type device for ESD, which allows for grasping of the targeted tissue and its subsequent cut with an electrosurgical unit^[6]. This procedure is similar to the technique of a standard bite biopsy, which is a common procedure during routine endoscopy. Furthermore, Clutch Cutter allows re-grasping of the tissue anytime during ESD, which may prevent miscutting and perforation. Therefore, Clutch Cutter may contribute to easier and safer ESD than other endo-knives. ESD with Clutch Cutter (ESD-C) may then become an option of endo-knife for ESD. Favorable outcomes of ESD-C have been reported, including in a large single-center study with single arm trial^[7-9]. However, few reports exist showing the comparison between scissors-type and non-scissors-type knives in the technical outcomes of ESD, which have been limited to non-experts in inclusion criteria^[10,11]. The advantage of scissor-type knife in ESD is controversial at present because comparative studies are still lacking.

We retrospectively compared the technical outcomes of ESD-C for gastric neoplasms with those of ESD



Figure 1 Clutch Cutter is a scissor-type device for endoscopic submucosal dissection.

with other knives (ESD-O) by using propensity score matching analysis, which compensated for differences in extraneous factors including baseline characteristics^[2]. We hypothesize that the outcomes using ESD-C will be superior to those of ESD-O.

MATERIALS AND METHODS

Study design and ethical approval

This was designed as a retrospective, observational cohort study, which was conducted based on the ESD databases at a single-center, Kitakyushu Municipal Medical Center (Fukuoka, Japan). These cases represented a consecutive and unselected cohort. The protocol of this study was developed in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Kitakyushu Municipal Medical Center on November 2017 (No. 201711050).

Patients

We enrolled 191 consecutive patients with gastric neoplasms treated by ESD between April 2016 and October 2017 at the Kitakyushu Municipal Medical Center. Three patients were excluded from analysis for previously having undergone gastric surgery. Furthermore, 33 patients were excluded because two or more lesions were simultaneously resected. Finally, 155 patients were analyzed in this study. We classified the patients into two groups: one group included patients treated by ESD-C and the other group included patients treated by ESD-O. Either IT Knife2 (KD-611L, Olympus, Tokyo, Japan) or Splash M-Knife (DN-D2718A; HOYA Corp., Pentax, Tokyo, Japan) was mainly used in the patients enrolled between April 2016 and March 2017, while Clutch Cutter was mainly used in the patients enrolled between April 2017 and October 2017. The flow chart of the patients enrolled in the present study is shown in Figure 2.

ESD procedure

All patients were admitted at Kitakyushu Municipal

Medical Center. All ESD procedures were carried out using a GIF-Q260J (Olympus, Tokyo, Japan) with a CO₂ insufflation system. VIO 300D (ERBE Elektromedizin, GmbH, Tübingen, Germany) was used as the electrical power source. The ESD procedure was described in detail in previous reports^[7,12,13]. In brief, marking dots were made 2 mm outside the lesion. A mixture of 4% hyaluronic acid and normal saline with a small amount of indigo carmine and epinephrine (0.001 mg/mL) was injected into the submucosa. After lifting the lesion, mucosal incision was conducted circumferentially using cutting and coagulation (Figure 3A). Once the circumferential mucosal incision was completed, submucosal dissection of the lesion was performed using cutting and coagulation (Figure 3B). Injection was added during dissection when needed. Prophylactic coagulation for visible vessels or hemostasis for active bleeding was conducted using endo-knives or hemostatic forceps (Figure 3C). When using Clutch Cutter, cutting was conducted by Endo Cut Mode (effect 1, duration 4, interval 1), while coagulation was conducted by Soft Coagulation Mode (80-100 W, effect; 5-6 in VIO300D) or Forced Coagulation Mode (30 W, effect 2). In this study, operators with an experience of performing at least 50 ESD procedures were defined as experts, while those who had performed less than 50 ESD procedures were defined as trainees. As a result, 4 operators were defined as experts and 5 as trainees in this study. All experts were familiar with using each device since they had used each device at least 10 times before this study.

Histology evaluation

ESD specimens were immediately stretched and fixed in 10% buffered formalin. The specimens were serially sectioned perpendicularly at 2-3 mm intervals. Then, histological type, depth of invasion, tumor size, lymphatic/vascular invasion, and resection margin were assessed. The pathological curability of the specimens was evaluated based on the Japanese Gastric Cancer Classification^[14].

Outcome

The primary outcome of this study was the procedure time during ESD, which was defined as the time from the start of marking to the completion of dissection. *En bloc* resection rate, complete resection rate, curative resection rate, and the rate of complications (delayed bleeding and perforation) were evaluated as secondary outcomes. *En bloc* resection was defined as resection in one piece. Complete resection was defined as *en bloc* resection with the lateral and vertical resection margins free of neoplasm. Curative resection was evaluated according to the guideline^[15]. Delayed bleeding was defined as clinical evidence of bleeding after ESD, requiring endoscopic hemostasis or blood transfusion. Perforation was diagnosed if mesenteric fat or the

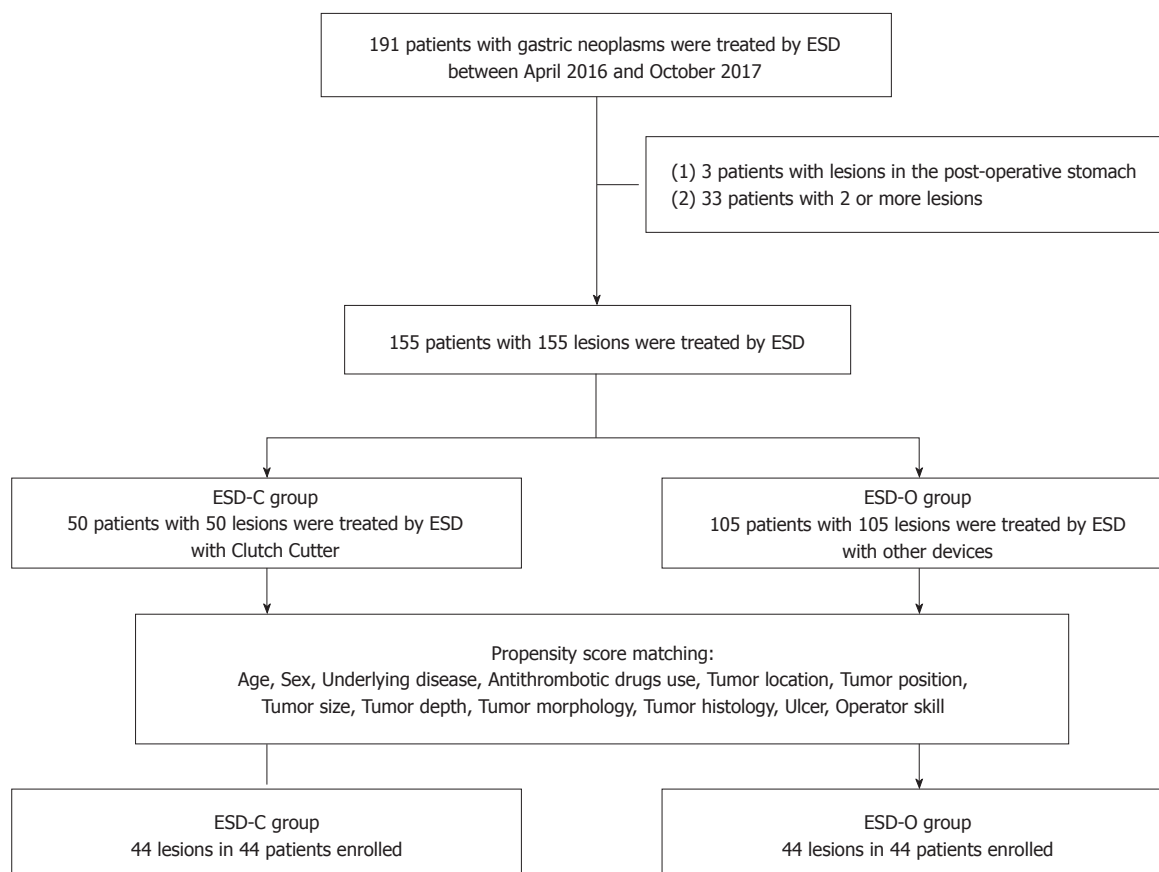


Figure 2 Flowchart of patients and lesions enrolled in this study. ESD: Endoscopic submucosal dissection; ESD-C: Endoscopic submucosal dissection using Clutch Cutter; ESD-O: Endoscopic submucosal dissection using other knives.

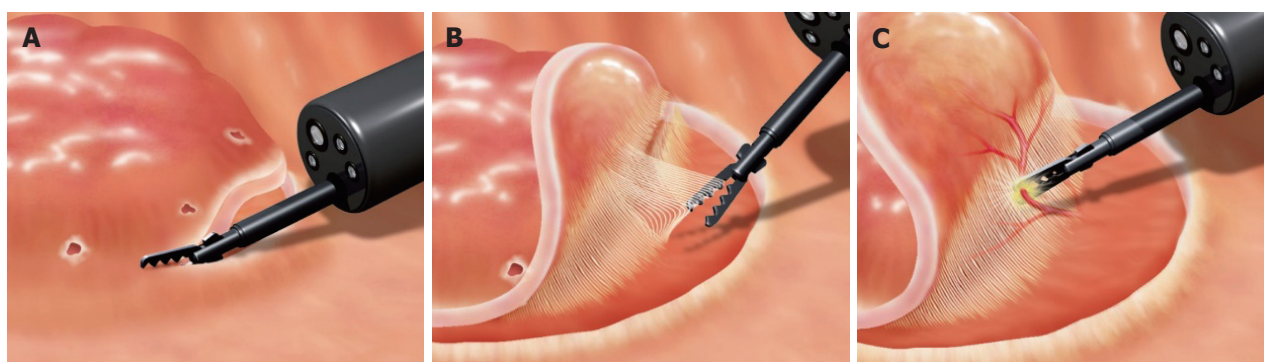


Figure 3 Procedures of endoscopic submucosal dissection. A: Mucosal incision using Clutch Cutter; B: Submucosal dissection using Clutch Cutter; C: Prophylactic coagulation for visible vessels using Clutch Cutter.

intra-abdominal space was observed during ESD procedure or free air was detected on chest and abdominal radiographs or computed tomography scans after ESD. All patients were given a proton pump inhibitor or potassium competitive acid blocker for a minimum of 4 wk.

Statistics

Background characteristics were not equal among two groups. Previous studies have reported some factors associated with the difficulty or complication of the

ESD procedure, which may affect outcomes of this study^[16-22]. Therefore, we adopted propensity score matching analysis to reduce bias. Logistic regression of the following factors with ESD device (Clutch Cutter vs other endo-knives) and calculation of propensity score were conducted: age (≥ 75 vs < 75 years old), sex (male vs female), underlying disease (presence vs none), anti-thrombotic drugs use (continuation vs not receiving or discontinuation), tumor location (upper third of the stomach vs middle or lower third), tumor position (lessor curvature of the stomach vs others),

tumor size (> 20 mm vs ≤ 20 mm), tumor depth (mucosa vs submucosa), tumor morphology (flat or depressed vs others), tumor histology (differentiated type vs undifferentiated type), ulcer (scar) (presence vs absence), and operator skill (expert vs trainee). Underlying disease included cardiomyopathy, liver cirrhosis, and chronic kidney disease. Nearest neighbor matching in a 1:1 ratio from the ESD-C and ESD-O groups was made in calipers (0.12) with a width equal to 0.25 of the standard deviation of the logit of the propensity score. Baseline characteristics and outcomes were analyzed using Fisher's exact test for categorical data, the Mann-Whitney U test for continuous data with non-normal distributions, and a *t* test for continuous data with normal distribution. $P < 0.05$ was considered statistically significant for all tests. All statistical analyses were performed using JMP Pro13.0 software (SAS Institute, Cary, NC, United States).

RESULTS

Propensity score matching

The area under the receiver operating characteristic curve, *i.e.*, C statistic, was estimated to be 0.681, which indicated good predictive power. Propensity score matching created 44 pairs in this study. We compared two groups by using the absolute standardized differences (ASD) before and after matching to assess the propensity score balance. After matching, all ASDs ranged within $1.96\sqrt{2/n}$, indicating that the characteristics were well-balanced^[23].

Characteristics before and after propensity score matching

The background characteristics of 155 patients enrolled in this study are shown in Table 1. Patients in the ESD-C group had a significantly higher rate of undifferentiated adenocarcinoma than those in the ESD-O group (10.0% vs 0.95%; $P = 0.014$). The median tumor size of patients in ESD-C group was significantly smaller than that of patients in the ESD-O group (13.5 mm vs 18.0 mm; $P = 0.027$).

Matching factors between two groups after propensity score matching are shown in Table 2. No significant differences were found in any matching factors.

Technical outcomes after propensity score matching

Treatment outcomes after matching are shown in Table 3. A significantly shorter procedure time was observed for ESD-C than for ESD-O in the adjusted comparison (49.0 min vs 88.5 min; $P < 0.001$). *En bloc* resection rates and complete resection rates were 100% in both groups. All ESDs were completed without perforation. Curative resection rates were similar between the two groups. The delayed bleeding rates of ESD-C tended to be lower than those of ESD-O, but these rates did not reach statistical significance (2.3% vs 6.8%, $P = 0.62$).

DISCUSSION

The present study is the first to show that the technical outcomes of ESD-C are superior to those of ESD-O for the endoscopic treatment of gastric neoplasms regardless of technical expertise, as shown by the propensity score matching analysis.

Currently, a wide variety of ESD devices is available. These devices are roughly classified into two types: scissor-type knives or non-scissor-type knives. The scissor-type knives commonly used in ESD include Clutch Cutter, SB knife, and SB knife Jr, while non-scissor-type knives mainly include IT Knife2, Dual knife, Flush knife, and Splash M-Knife. However, it is yet to be determined which type of knife is superior, scissor-type or non-scissor-type.

It has been reported that the scissor-type knives reduced the technical difficulty of gastrointestinal ESD for unexperienced as well as expert endoscopists^[24,25]. Rescue usage of the SB Jr knife has been reported to increase the self-completion rate of ESD of colorectal neoplasms using the Flush knife (63% in the SB Jr knife group vs 39% in the Flush knife only group; $P = 0.03$), without increasing the procedure time (59 min vs 51 min; $P = 0.14$)^[11]. In other studies, however, ESD-C was reported to be a time-consuming procedure compared with ESD with non-scissor-type knives, especially when performed by unexperienced endoscopists^[26,27]. Therefore, we carried out this study using a propensity score matching analysis to determine which was superior, ESD-C or ESD-O. We found that ESD-C achieved significantly shorter procedure time than ESD-O, indicating that ESD-C is a time-saving rather than a time-consuming procedure. Clutch Cutter might reduce the technical difficulties in gastric ESD similarly to those in colorectal ESD, which might have contributed to the reduction in procedure time. The scissors-type knives were invented several years after the invention of non-scissors-type knives^[4-6]. In general, ESD experts tended to use non-scissors-type knives rather than scissors-type knives. In previous studies on the usefulness of ESD-C, the ESD-C procedures were conducted mainly by trainees rather than experts. In this study, however, ESD procedures were performed by 4 experts and 5 trainees. After propensity score matching, up to 68.2% of ESD procedures were conducted by experts, which might explain the discrepancy in the outcomes between the present and previous studies. ESD with scissor-type knives is being widely used not only by trainees but also by experts.

Both delayed bleeding and perforation are major complications of ESD. In the present study, delayed bleeding occurred in only 1 case with ESD-C while in 3 cases with ESD-O after propensity score matching, although the results were not statistically significant. Moreover, the delayed bleeding rate was 2.0% in ESD-C before propensity score matching (data not shown),

Table 1 Characteristics of enrolled patients before propensity score matching

	ESD-C <i>n</i> = 50	ESD-O <i>n</i> = 105	<i>P</i> value	ASD
Age, yr				
Mean ± SD	73.1 ± 8.55	72.75 ± 8.04	0.806 ²	0.0422
Median (range)	74.0 (46–91)	73.0 (52–91)	0.580 ³	
Sex (<i>n</i>)				
Male	39	71	0.256 ¹	0.2350
Female	11	34		
Underlying disease, positive, <i>n</i> (%)	18 (36.0)	29 (27.6)	0.350 ¹	0.1810
Anti-thrombotic drugs (<i>n</i>)				
None or discontinuation	48	102	0.658 ¹	0.0623
Continuation	2	3		
Tumor location (<i>n</i>)				
Upper third	9	17	0.820 ¹	0.0481
Middle or lower third	41	88		
Tumor position (<i>n</i>)				
Lessor	21	57	0.731 ¹	0.2670
Others	29	48		
Morphology (<i>n</i>)				
Flat or depressed	29	63	0.862 ¹	0.0407
Others	21	42		
Histology (<i>n</i>)				
Undifferentiated	5	1	0.014 ^{1,4}	0.4060
Others	45	104		
Tumor size (mm)				
Mean ± SD	16.89 ± 12.65	20.90 ± 13.30	0.076 ²	0.3090
Median (range)	13.5 (3–67)	18.0 (3–82)	0.027 ^{3,4}	
Tumor depth (<i>n</i>)				
Mucosa	44	91	1.000 ¹	0.0401
Submucosa	6	14		
Ulceration positive, <i>n</i> (%)	7 (14.0)	22 (21.0)	0.381 ¹	0.1840
Operator skill				
Experts	34	73	0.854 ¹	0.0329
Trainees	16	32		

¹*P* value was calculated using Fisher's exact test; ²*P* value was calculated using a *t* test; ³*P* value was calculated using the Mann-Whitney *U* test; ⁴Significant value. ESD-C: Endoscopic submucosal dissection with Clutch Cutter; ESD-O: Endoscopic submucosal dissection with another end-knife; SD: Standard deviation; ASD: Absolute standardized difference.

Table 2 Matching factors between two groups after propensity score matching

	ESD-C <i>n</i> = 44	ESD-O <i>n</i> = 44	<i>P</i> value	ASD
Variable matching between groups				
Age, yr; mean ± SD	73.16 ± 8.59	71.11 ± 8.81	0.273 ²	0.2360
Sex: Male/female	8/36	6/38	0.772 ¹	0.1250
Underlying disease: No/yes	28/16	29/15	1 ¹	0.0476
Anti-thrombotic drugs: No/yes	2/42	3/41	1 ¹	0.0983
Tumor location: Upper third/others	7/37	2/42	0.250 ¹	0.3810
Tumor position: Lessor/others	19/25	21/23	0.831 ¹	0.0914
Morphology: Flat or depressed/others	25/19	28/16	0.663 ¹	0.1400
Histology: Undifferentiated/others	0/44	0/44	-	0
Tumor size, mm: mean ± SD	16.89 ± 12.65	20.90 ± 13.30	0.076 ²	0.3090
Tumor depth: Mucosa/submucosa	Jun-38	Jun-38	1 ¹	0
Ulceration, positive	6 (13.6%)	4 (9.1%)	0.739 ¹	0.1440
Operator skill: Expert/trainee	14/30	14/30	1 ¹	0

¹*P* value was calculated using Fisher's exact test; ²*P* value was calculated using a *t* test for continuous data. ESD-C: Endoscopic submucosal dissection with Clutch Cutter; ESD-O: Endoscopic submucosal dissection with another end-knife; SD: Standard deviation; ASD: Absolute standardized difference.

which was lower than previously reported delayed bleeding rates^[15]. Although it was unknown why delayed bleeding occurs, during ESD-C, hemostasis was conducted by grasping and coagulation, similar to the procedure by hemostatic forceps, which may contribute to the reduction in the delayed bleeding rate. On the

other hand, no perforation occurred in ESD-C before propensity score matching in this study. By contrast, perforation occurred in one case of ESD-O before matching (data not shown), although this case was excluded after propensity score matching. Although the number of patients undergoing ESD-C was smaller than

Table 3 Treatment outcomes between two groups after propensity score matching *n* (%)

	ESD-C <i>n</i> = 44	ESD-O <i>n</i> = 44	<i>P</i> value
Procedure time, min			< 0.001 ^{2,3}
Mean ± SD	63.1 ± 41.90	98.41 ± 51.77	
Median (range)	49 (9–190)	88.5 (26–290)	
<i>En bloc</i> resection	44 (100)	44 (100)	-
Complete resection	44 (100)	44 (100)	-
Curative resection	38 (86.4)	39 (88.6)	0.730 ¹
Perforation	0 (0)	0 (0)	-
Delayed bleeding	1 (2.3)	3 (6.8)	0.620 ¹

¹*P* value was calculated using Fisher's exact test; ²*P* value was calculated using the Mann-Whitney *U* test; ³Significant value. ESD-C: Endoscopic submucosal dissection with Clutch Cutter; ESD-O: Endoscopic submucosal dissection with another end-knife; SD: Standard deviation.

that of patients undergoing ESD-O, ESD-C might be a safer procedure than ESD-O. In ESD-C, tissue grasping and lifting were conducted before coagulation or cutting, which could reduce heat conduction to the muscular layer, contributing to the decreased risk of perforation. In terms of safety, it was reported that ESD-C could be preferred over ESD-O for elderly patients with some comorbidities^[28]. In the present study, over 90% (46/50 before matching, 41/44 after matching) of patients were aged 65 years or older; furthermore, over 40% (22/50 before matching, 20/44 after matching) of patients were aged 75 years or older. No patient experienced worsening of general condition or developed any severe complications. However, further studies are required to clarify whether ESD-C is safer than ESD-O. In addition, ESD-C has been recently used not only for ESD but also for other endoscopic procedures such as endoscopic treatment of Zenker's diverticulum and endoscopic necrosectomy for pancreatic necrosis^[29–31]. In the future, Clutch Cutter could be widely applied in additional endoscopic procedures.

This study had several limitations. First, this was a single-center retrospective study. Therefore, the sample size was relatively small. There might be a selection bias because lesions in the ESD-C group were significantly smaller than those in the ESD-O group and had significantly higher rate of undifferentiation in histology evaluation. Second, only 9 endoscopists conducted ESD. Therefore, a multicenter trial should be carried out to validate this outcome. Third, in some ESD procedures conducted by trainees, experts occasionally assisted in the procedure, which might affect the outcomes of this study. Fourth, we grouped other devices together, including needle-type and insulated-tip knives, for comparison with Clutch Cutter. Future studies are needed to compare each knife individually with Clutch Cutter. Fifth, there was a possibility of an institutional learning curve. We cannot compensate for this bias because the Clutch Cutter was used mainly in the latter phase of this study; other devices were used mainly in former phase of this study, which may also affect outcomes.

In conclusion, ESD-C achieved shorter procedure time than ESD-O without an increase in complication rates. Therefore, ESD-C could become one of the

best endoscopic procedure options in ESD for gastric neoplasms.

ARTICLE HIGHLIGHTS

Research background

Endoscopic submucosal dissection (ESD) is the standard treatment for early gastric neoplasms with negligible lymph node metastasis. However, it is a complex and difficult procedure. Many types of endo-knives have been invented and developed to improve the ESD procedure.

Research motivation

The Clutch Cutter is a novel scissor-type endo-knife, which may contribute to facilitating the ESD procedure. However, few studies have compared the technical outcomes of each knife.

Research objectives

The aim of this study was to compare the technical outcomes between ESD with the Clutch Cutter and ESD with other devices.

Research methods

Patients with early gastric neoplasms treated by ESD at Kitakyushu Municipal Medical Center between April 2016 and October 2017 were reviewed. ESD was performed using the Clutch Cutter (ESD-C group) or other devices (ESD-O group). Propensity score matching analysis was conducted to compensate for confounding differences between the two groups that may affect the outcomes. After matching, the technical outcomes of ESD were compared among the two groups.

Research results

A total of 155 patients were included and 44 pairs were matched. ESD with the Clutch Cutter achieved a significantly shorter procedure time (median, 49 min vs 88.5 min, *P* < 0.001). The other technical outcomes and complication rates were similar among the two groups.

Research conclusions

The Clutch Cutter contributed to shortening the ESD's procedure time. ESD with the Clutch Cutter could be an effective option in ESD with endo-knives for early gastric neoplasms.

Research perspectives

This was a single-center, retrospective study with a relatively small number of ESD cases. Therefore, further large-scale, randomized, prospective studies are needed.

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Retrospective Study

Stents combined with iodine-125 implantation to treat main portal vein tumor thrombus

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Abstract**AIM**

To evaluate the efficacy of main portal vein stents combined with iodine-125 (¹²⁵I) to treat main portal vein tumor thrombus.

METHODS

From January 1, 2010 to January 1, 2015, 111 patients were diagnosed with liver cancer combined with main portal vein tumor thrombus. They were non-randomly assigned to undergo treatment with transarterial chemoembolization (TACE)/transarterial embolization (TAE) + portal vein stents combined with ¹²⁵I implantation (Group A) and TACE/TAE + portal vein

stents only (Group B). After the operation, scheduled follow-up was performed at 6, 12 and 24 mo. The recorded information included clinical manifestations, survival rate, and stent restenosis rate. Kaplan–Meier curves, log-rank test and Cox regression were used for data analyses.

RESULTS

From January 1, 2010 to January 1, 2015, 54 and 57 patients were allocated to Groups A and B, respectively. The survival rates at 6, 12 and 24 mo were 85.2%, 42.6% and 22.2% in Group A and 50.9%, 10.5% and 0% in Group B. The differences were significant [log rank $P < 0.05$, hazard ratio (HR): 0.37, 95%CI: 0.24–0.56]. The rates of stent restenosis were 18.5%, 55.6% and 83.3% in Group A and 43.9%, 82.5% and 96.5% in Group B. The differences were significant (log rank $P < 0.05$, HR: 0.42, 95%CI: 0.27–0.63). Cox regression identified that treatment was the only factor affecting survival rate in this study.

CONCLUSION

Main portal vein stents combined with ^{125}I can significantly improve survival rate and reduce the rate of stent restenosis.

Key words: Iodine-125; Liver cancer; Stent; Main portal vein tumor thrombus; Transarterial chemoembolization/transarterial embolization

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Core tip: This study evaluated the efficacy of stents combined with iodine-125 (^{125}I) to treat main portal vein tumor thrombus and its complications. ^{125}I was placed between the stent and tumor thrombus, and not in the form of particle strands. In this way, the quantity and position of ^{125}I could be flexibly adjusted. Transarterial chemoembolization or transarterial embolization was used as basic treatment. Patients with liver cancer and main portal vein tumor thrombus were non-randomly assigned to undergo portal vein stents combined with ^{125}I implantation or portal vein stents only. Portal vein stent combined with ^{125}I significantly improved survival rate and reduced stent restenosis.

Wu YF, Wang T, Yue ZD, Zhao HW, Wang L, Fan ZH, He FL, Liu FQ. Stents combined with iodine-125 implantation to treat main portal vein tumor thrombus. *World J Gastrointest Oncol* 2018; 10(12): 496–504

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INTRODUCTION

Liver cancer is a common malignant tumor^[1], and it decreases patient quality of life^[2,3]. Tumor thrombus

in the main portal vein indicates late-stage disease. The treatment for portal vein tumor thrombus includes surgery and radiotherapy^[4,5]. However, the overall effect is limited. In recent years, radioactive iodine-125 (^{125}I) particles have been used to treat portal vein tumor thrombus to effectively decrease tumor thrombus volume and improve patient survival rates^[6]. However, ^{125}I was implanted in particle strands in those studies. This limits the amount of ^{125}I implanted and the ability to reposition the ^{125}I , which restrains the clinical use of ^{125}I . We studied the clinical effect of ^{125}I combined with main portal vein stents when the ^{125}I was placed between the tumor thrombus and the stents. This method avoids the above disadvantages and has never been previously reported.

MATERIALS AND METHODS

Study design

This was a nonrandomized controlled trial in which we compared transarterial chemoembolization (TACE)/transarterial embolization (TAE) + main portal vein stents combined with ^{125}I implantation and TACE/TAE + main portal vein stents only for the treatment of liver cancer with main portal vein tumor thrombus and portal hypertension.

Criteria

Inclusion criteria were as follows: (1) Liver cancer according to histological, cytological, or clinical diagnostic standards that conformed to the rules of diagnosis and treatment of primary hepatocellular carcinoma, 2011; (2) Main stem tumor thrombus of portal vein confirmed through biopsy (70%) or imaging, without tumor thrombus in the branches; (3) Clear indication of percutaneous liver puncture and main portal vein stent implantation; (4) Clear TACE or TAE treatment indication; (5) Age 18–70 years; and (6) No serious complications of portal hypertension, and only a small amount of ascites without bleeding or other complications. Exclusion criteria were: (1) Patients with serious disorders of the heart, lung, kidney, brain, or other important organs; (2) Active infection; (3) Women in pregnancy or lactation; (4) Life expectancy < 3 mo; and (5) Patients who could not cooperate with treatment and observation.

Patients

One hundred and eleven patients with main portal vein tumor thrombus were non-randomly assigned to undergo treatment with TACE/TAE + main portal vein stents combined with ^{125}I implantation or TACE/TAE + main portal vein stents alone from January 1, 2010 to January 1, 2015. There were 73 cases of hepatitis B cirrhosis, 21 cases of hepatitis C cirrhosis, seven cases of alcoholic cirrhosis, three cholestatic cirrhosis cases, three autoimmune liver cirrhosis cases and four cases of cirrhosis from other causes. Twenty-three patients

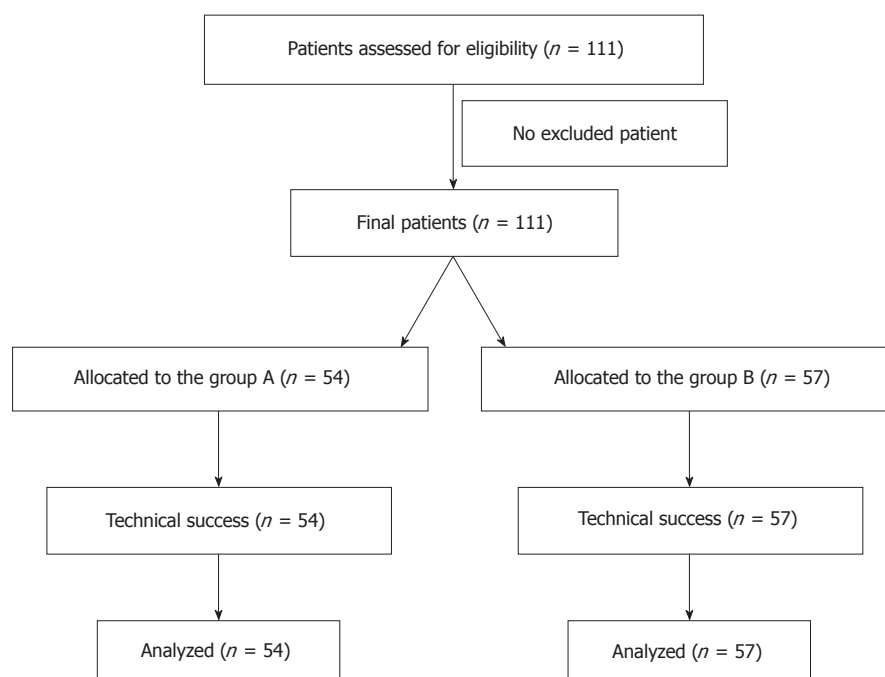


Figure 1 Study design and flow chart.

were diagnosed with primary carcinoma of the liver by percutaneous liver biopsy, whereas 88 patients were diagnosed by ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), serum α -fetoprotein levels and hepatic artery angiography. Imaging examination confirmed main portal vein tumor thrombus in all patients. According to the preoperative Child–Pugh classification, there were 49 cases of Class A, 62 cases of Class B, and zero class C cases. There were 20 patients with mild ascites and 91 without ascites. The flow chart is shown in Figure 1. Comparison between the two groups is shown in Table 1 and Figure 2.

Preoperative preparation

Before the operation, liver function tests, blood coagulation tests, routine blood tests, electrocardiography, CT, and/or MRI, color Doppler ultrasonography, and esophageal radiography were performed. In addition, gastroscopy was performed when necessary. Patients' coagulation function was corrected to the normal range. The operation-related concerns were explained to the patients and their family members, and they were asked to give signed informed consent. This study was approved by the Institutional Review Board of Beijing Shijitan Hospital and conducted in accordance with all current ethical guidelines.

Percutaneous transhepatic and portal vein stent implantation

Patients were assigned to receive percutaneous transhepatic and portal vein covered stents (Fluency, Bard, Tempe, AZ, United States) with local anesthesia at the puncture site. A puncture device (NPAS-100; Cook, Indianapolis, IN, United States), which included a

puncture needle, venous sheath and guide wire, was passed from the right hypochondriac region to the portal vein. After that, a pigtail catheter was advanced through the NPAS-100 to the distal end of the splenic vein or superior mesenteric vein to measure the portal vein pressure and conduct venography. The pigtail catheter was removed, and the stent was implanted through the vein sheath. A 10-mm covered stent was implanted. Portal vein pressure measurements before and after stent placement allowed assessment of the success of the procedure. The hepatic puncture passage was blocked during catheter removal to avoid intra-peritoneal or thoracic hemorrhage.

¹²⁵I implantation

In Group A, the patients received treatment with percutaneous transhepatic and portal vein covered stent implantation, like the patients in Group B. After measurement of portal vein pressure and conduction of venography, the pigtail catheter was removed, and a guide wire was inserted through the venous sheath of the NPAS-100. Because the NPAS-100 had one guide wire, there were two guide wires in the main portal vein. The venous sheath was drawn out and inserted into the portal vein again along one of the guide wires. Another guide wire was placed between the tumor thrombus and venous sheath. Stents were implanted through the venous sheath. A catheter was inserted through the guide wire that was between the tumor thrombus and venous sheath. The catheter between the stent and the tumor thrombus was linked to the particle release gun. The catheter was slowly retracted, and ¹²⁵I (Tong Fu, Beijing, China) was simultaneously

Table 1 Baseline characteristics, *n* (%)

	Group A (<i>n</i> = 54)	Group B (<i>n</i> = 57)	<i>P</i> value
Gender			0.693
Male	35 (64.8)	39 (68.4)	
Female	19 (35.2)	18 (31.6)	
Average age (yr)	43.6 ± 6.9	44.3 ± 5.2	0.697
Pathogenesis			0.788
Hepatitis B	35 (64.8)	38 (66.7)	
Hepatitis C	9 (16.7)	12 (21)	
Alcoholic	5 (9.3)	2 (3.5)	
Cholestasis	2 (3.7)	1 (1.8)	
Autoimmunity	1 (1.8)	2 (3.5)	
Others	2 (3.7)	2 (3.5)	
Child-Pugh classification			0.705
A	25 (46.3)	24 (42.1)	
B	29 (53.7)	33 (57.9)	
C	0 (0)	0 (0)	
Albumin (g/L)	34.5 ± 7.5	31.5 ± 11.5	0.880
Alanine aminotransferase (U/L)	62.5 ± 46.5	49.5 ± 37.5	0.396
Glutamyl transpeptidase (U/L)	73 ± 66	74 ± 62	0.647
Na ⁺	143.5 ± 8.5	140 ± 7	0.104
K ⁺	4.12 ± 1.08	4.75 ± 1.05	0.883
Direct bilirubin (μmol/L)	29.8 ± 25.2	24.5 ± 18.5	0.299
Aspartate aminotransferase (U/L)	39 ± 30	49 ± 39	0.349
MELD score	11.96 ± 1.68	12.76 ± 2.47	0.145
Ascites			0.624
Yes	11 (20.4)	9 (15.8)	
No	43 (79.6)	48 (84.2)	
Size of liver cancer (cm)			0.788
≤ 5	14 (25.9)	17 (29.8)	
5-8	31 (57.4)	29 (50.9)	
> 8	9 (16.7)	11 (19.3)	
No. of liver tumors			0.834
1	28 (51.9)	32 (56.1)	
2 or 3	19 (35.2)	17 (29.8)	
> 3	7 (12.9)	8 (14.1)	

MELD: Model for end-stage liver disease; Child-Pugh classification: Score for liver function.

released through the catheter up to the portal vein trunk and tumor thrombus. The radioactive particles were arranged as neatly as possible in all tumor thrombi. After implantation, portal vein pressure was measured and venography was conducted. These particles could emit characteristic electrons and photons through the recession of the electron capture surface. The electrons were absorbed by the titanium alloy wall of the sealed seeds of ¹²⁵I. The photons mainly emitted X rays of 27.4 and 31.4 keV as well as γ rays of 35.5 keV. The pictures taken during the operation are shown in Figure 3.

TACE/TAE

Patients in Group A were treated with TACE according to the position of the lesion and its blood supply. The embolization agent was 3–30 mL iodinated oil. The chemotherapeutics included 10–20 mg pirarubicin and 5–15 mg hydroxycamptothecine. Patients in Group B were treated with TAE to reduce damage to liver function. The embolization agent was 5–25 mL iodinated oil.

For the basic technical operation, a needle was passed from the right or left femoral artery to the hepatic artery followed by hepatic arteriography. A catheter was placed in the direct blood supply artery of the tumor

as close to the focus as possible, and embolization and infusion of chemotherapeutic drugs were performed. The interval and number of treatments depended on tumor size, arterial status and liver function status. The interval was usually once every 1–6 mo. In Group A, 25 and 29 patients were treated with TACE and TAE, respectively, while 24 and 33 patients in Group B were treated with TACE and TAE, respectively, with no significant difference in patient numbers (*P* = 0.705).

Patients with lesions ≤ 5 cm in size and with a rich blood supply underwent TACE or TAE first and radiofrequency ablation after 3–5 d. Patients whose lesion was > 5 cm underwent TACE or TAE once or several times and then radiofrequency ablation when the imaging showed that the lesions no longer had blood supply from the hepatic artery or when the catheter could not enter the artery supplying the lesion. Finally, all patients underwent radiofrequency ablation (WHK-IB; Weaver Electronics, Beijing, China).

Postoperative routine observation and treatment

Low molecular weight heparin (5000 IU, twice daily) was subcutaneously injected for 5 d, and then warfarin was administered for one year. Coagulation function of

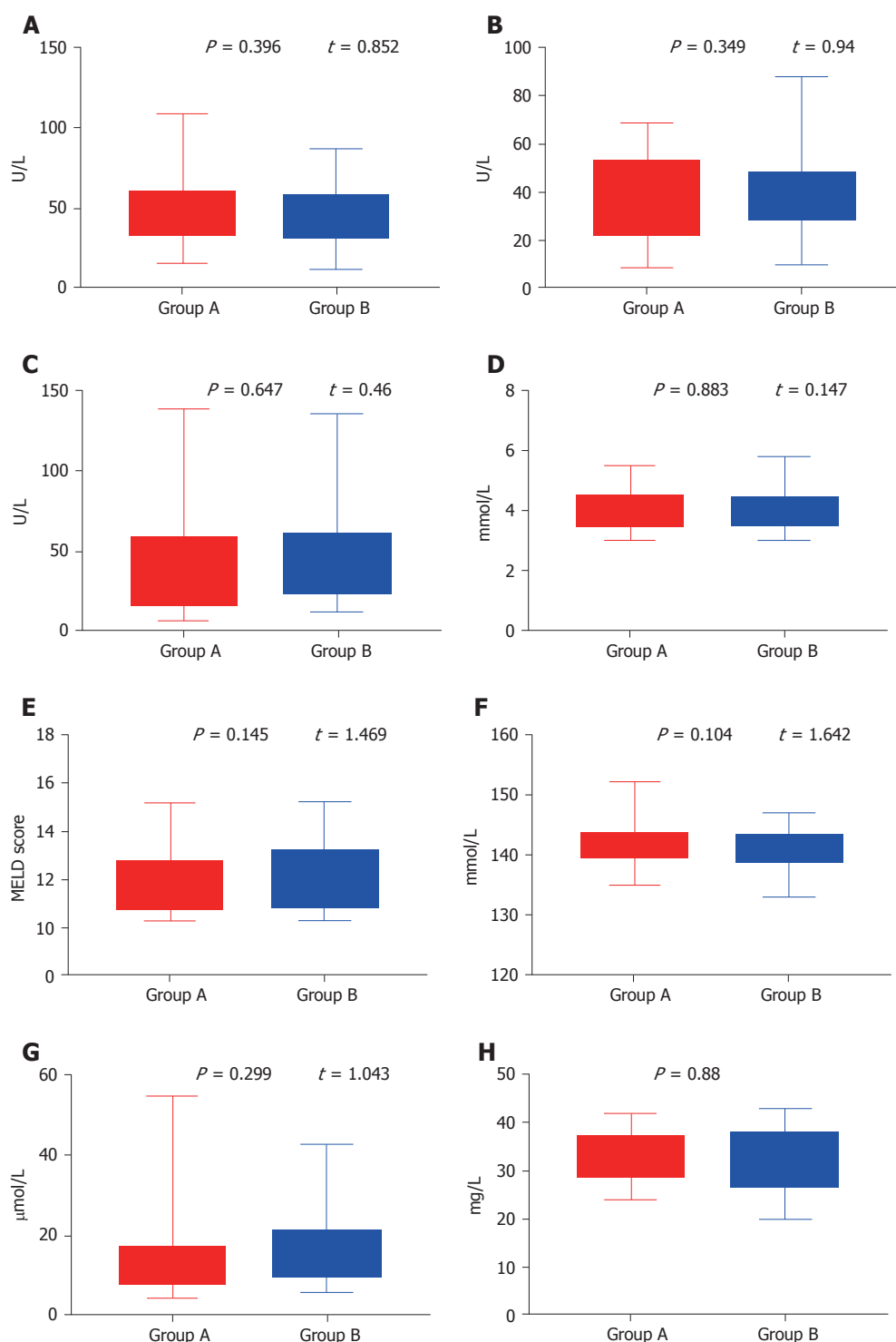


Figure 2 Boxplots of liver function. A: Alanine aminotransferase; B: Aspartate aminotransferase; C: Glutamyl transpeptidase; D: K⁺; E: Model for end-stage liver disease score; F: Na⁺; G: Direct bilirubin; H: Albumin (H) in Group A (red bars) and Group B (blue bars) were compared with Student's *t* test. MELD: Model for end-stage liver disease score.

each patient was examined every 15 d to ensure that the International Normalized Ratio ranged from 2 to 3.

Follow-up

Scheduled follow-up was performed at 6, 12 and 24 mo postoperatively. The recorded information included clinical manifestations, survival rate, physical exami-

nation, stent restenosis evaluation (through ultrasound and endoscopy) and laboratory tests. Telephone follow-up was performed to record patient conditions and details of relevant clinical events.

Statistical analysis

Continuous variables are presented as mean ± median

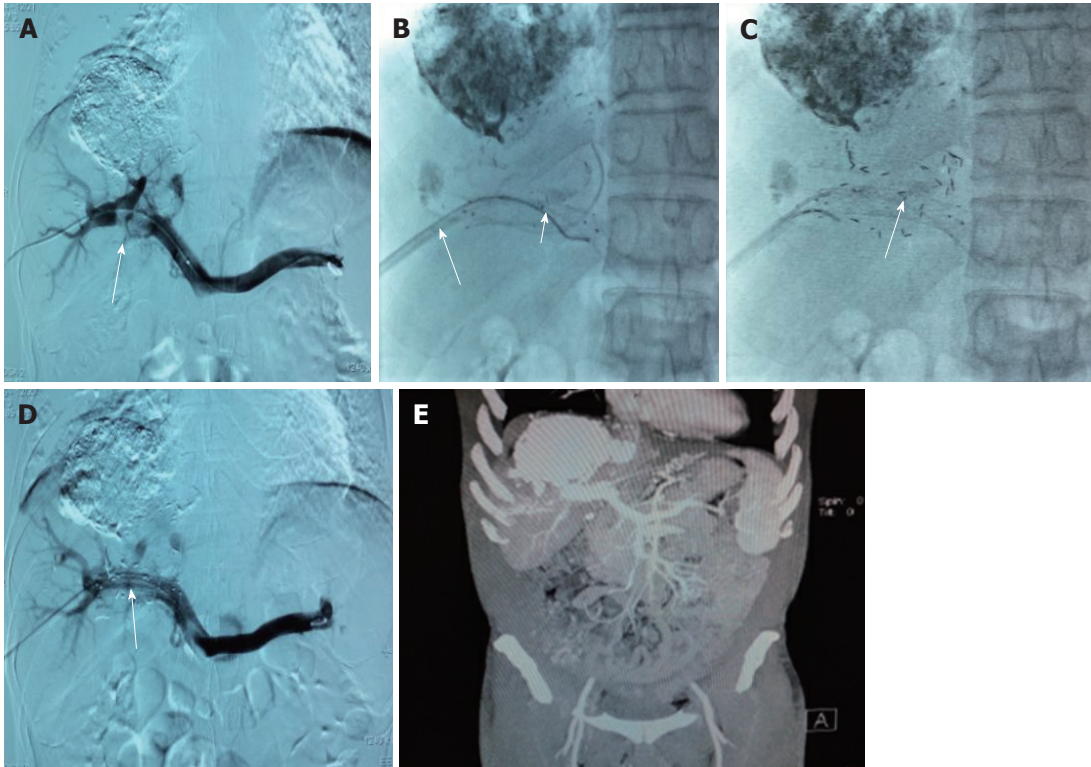


Figure 3 Pictures taken during operation of a 55-year-old male patient with hepatitis B cirrhosis and primary liver cancer with main portal vein tumor thrombus. The patient was treated with percutaneous liver puncture and ^{125}I implantation combined with portal vein stent implantation. A: Percutaneous transhepatic portal venography showing proximal and left branch of portal vein tumor thrombus (long white arrow); B: X-ray showing vein stent (short white arrow), catheter between the stent and tumor thrombus (long white arrow); C: X-ray showing ^{125}I between the stent and tumor thrombus (long white arrow); D: Postoperative portal vein angiography showing patency of stent blood flow (long white arrow); E: 18 mo after the operation, enhanced computed tomography showed that blood flow in the splenic vein, superior mesenteric vein, portal vein and stent was good.

and were compared by independent-sample or paired-sample t test. Categorical and ordinal variables are presented as frequencies or percentages and compared using χ^2 test. Time-to-event outcomes were evaluated with Kaplan–Meier curves and log-rank tests. Cox regression model was used to identify independent predictors. Unbalanced factors between groups were treated as covariates. Statistical analysis was performed using IBM SPSS Statistics version 22.0 (IBM, Chicago, IL, United States) and GraphPad Prism version 7.0 (GraphPad Software, La Jolla, CA, United States). Follow-up investigators and statisticians had access to all of the data and vouched for the integrity of the data analyses.

RESULTS

Portosystemic pressure gradient before and after operation

The portosystemic pressure gradient (PPG) in Group A decreased from 26.9 ± 6.22 to 13.6 ± 6.4 mmHg ($t = 18.11$, $P < 0.05$) after operation. The PPG before and after operation were significantly different. The PPG in Group B decreased from 26.77 ± 6.25 to 15.1 ± 7.2 mmHg ($t = 17.1$, $P < 0.05$). The PPG before and after operation were significantly different (Table 2). The pre-operative PPG was not significantly different

between the two groups ($t = 1.52$, $P = 0.132$). The post-operative PPG was also not significantly different between the two groups ($t = 1.20$, $P = 0.234$) (Figure 4).

Time-to-event outcomes

The rates of stent restenosis at 6, 12 and 24 mo were 18.5%, 55.6% and 83.3% in Group A and 43.9%, 82.5% and 96.5% in Group B, which differed significantly [log rank $P < 0.05$, hazard ratio (HR): 0.42, 95%CI: 0.27–0.63] (Figure 5A and Table 3). The rates of survival at 6, 12 and 24 mo were 85.2%, 42.6% and 22.2%, respectively in Group A and 50.9%, 10.5% and 0%, respectively in Group B, which differed significantly (log rank $P < 0.05$, HR: 0.37, 95%CI: 0.24–0.56) (Figure 5B and Table 3)

Cox regression

Cox regression showed that pathogenesis, tumor number and serum albumin had no significant effect on survival rate. Treatment was the only factor that affected survival rate (Table 4).

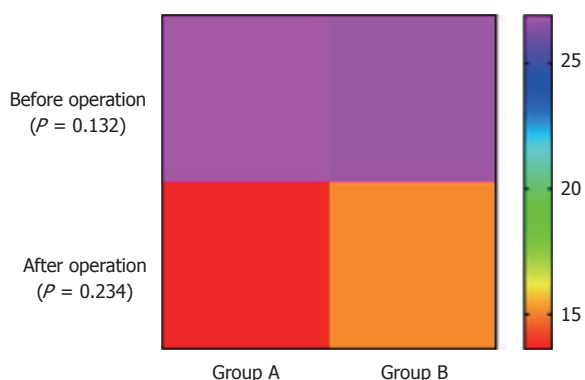
DISCUSSION

With a rapid increase in the number of patients with liver cancer, the incidence of portal vein tumor thrombus is gradually increasing. ^{125}I was reported to have a

Table 2 Differences of portosystemic pressure gradient in the two groups before and after operation

	PPG before operation (mmHg)	PPG after operation (mmHg)	<i>t</i>	df	<i>P</i> value
Group A	26.9 ± 6.22	13.6 ± 6.4	18.11	53	< 0.0001
Group B	26.77 ± 6.25	15.1 ± 7.2	17.10	56	< 0.0001

PPG: Portosystemic pressure gradient.

**Figure 4** Heat map of comparison of portosystemic pressure gradient measurements before and after operation between Groups A and B. Student's *t* test was used to compare portosystemic pressure gradient at each time point, and no difference was found between the two groups.

good therapeutic effect^[7]. However, ¹²⁵I implantation has been in the form of particle strands. This has some disadvantages, such as implantation of a limited number of particles. In addition, the position of ¹²⁵I cannot be adjusted. No one has studied the clinical effect of ¹²⁵I combined with main portal vein stents in which the ¹²⁵I is placed between the tumor thrombus and stents. This procedure could make it easier to adjust the position and amount of ¹²⁵I. Thus, in the present study, we compared TACE/TAE + main portal vein stents combined with ¹²⁵I implantation and TACE/TAE + main portal vein stents alone for the treatment of liver cancer patients with main portal vein tumor thrombus and portal hypertension. Overall, our study suggested a benign outcome: (1) ¹²⁵I combined with stents implanted in the main portal vein significantly improved survival rate and reduced stent restenosis rate; (2) stents implanted in the main portal vein reduced portal vein pressure and relieved clinical symptoms; and (3) TACE/TAE + main portal vein stents combined with ¹²⁵I implantation was safe and feasible.

The incidence of portal vein tumor thrombus is high, and its treatment includes surgical resection, chemotherapy, and stent^[8-10]. Stent implantation of the main portal vein can quickly reduce portal vein pressure, relieve clinical symptoms and improve quality of life^[11,12]. In recent years, portal vein stenting combined with ¹²⁵I implantation has achieved significant effects in treating main portal vein tumor thrombus. Sun *et al.*^[13] conducted a study to evaluate the effect of ¹²⁵I. In their study, the median survival was 147 d. The cumulative survival rates and stent patency rates at 90, 180, and 360 d were 94.1%, 61.8%, and 32.4% and 97.1% (33/34), 76.9% (24/34), and 29.4% (10/34), respectively^[13]. However,

in previous studies, ¹²⁵I particles were implanted in the form of particle strands, which has some drawbacks. It is important to find a better method. In our study, the survival rate in Group A was higher than in Group B, and the stent restenosis rate was lower in Group A than in Group B. Cox regression was used to evaluate the effects of various factors on survival and stent restenosis. It showed that pathogenesis, tumor number and serum albumin had no significant effect on survival rate. Treatment was the only factor influencing survival rate. TACE/TAE + main portal vein stents combined with ¹²⁵I implantation can improve patient survival rate and reduce stent restenosis rate.

Our study had several limitations. First, the radiation dose was not uniformly distributed. Second, the number of patients was small, which may have influenced the accuracy of the results.

In summary, TACE/TAE + main portal vein stents combined with ¹²⁵I implantation is effective in treating main portal vein tumor thrombus and its complications, improving quality of life and reducing mortality.

ARTICLE HIGHLIGHTS

Research background

Tumor thrombus in the main portal vein indicates late-stage disease. Treatment for portal vein tumor thrombus includes surgery, chemotherapy, radiotherapy, targeted therapy, and proton beam radiation. In recent years, radioactive iodine-125 (¹²⁵I) particles have been used to treat portal vein tumor thrombus. However, seed implantation in recent studies had some disadvantages. We carried out the present study to explore a new method of seed implantation.

Research motivation

Previously, ¹²⁵I was implanted in the form of particle strands. This limits the number of ¹²⁵I particles implanted, and their position cannot be adjusted. In this study, we performed ¹²⁵I seed implantation combined with stent implantation, placing the particles between the stent and tumor thrombus. The stent could hold the ¹²⁵I particles, and the method can be widely used in clinical application.

Research objectives

¹²⁵I has been shown to be effective in treating portal vein thrombosis. The main objective of this study was to determine the efficacy of stents combined with ¹²⁵I implantation in the treatment of liver cancer accompanied by main portal vein tumor thrombus, as well as the technical feasibility of this method of seed implantation.

Research methods

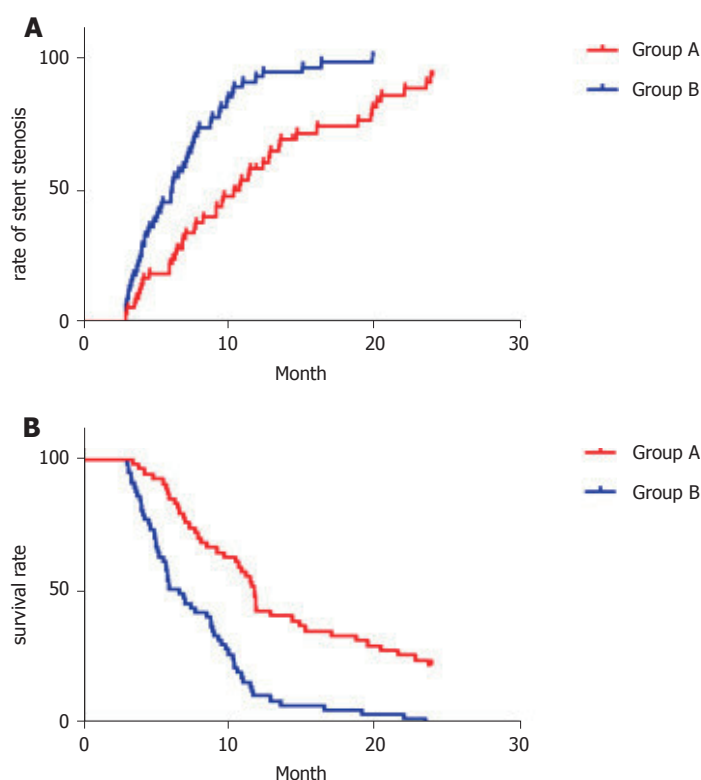
Patients were non-randomly assigned to undergo treatment with transarterial chemoembolization (TACE)/transarterial embolization (TAE) + portal vein stents combined with ¹²⁵I implantation (Group A) or TACE/TAE + portal vein stents only (Group B). It could show differences in treatment and outcomes between the two groups. After operation, scheduled follow-up was performed at 6, 12 and 24 mo. Follow-up included postoperative and preoperative portosystemic pressure gradient, postoperative stenting stenosis rate, and survival rate. Time-to-event outcomes were evaluated with Kaplan-Meier curves and log-rank test. Cox

Table 3 Time-to-event outcomes

	Group A (n = 54)	Group B (n = 57)	P value
Rate of stent stenosis			< 0.05
6 mo	18.5	43.9	
12 mo	55.6	82.5	
24 mo	83.3	96.5	
Survival rate			< 0.05
6 mo	85.2	50.9	
12 mo	42.6	10.5	
24 mo	22.2	0	

Table 4 Multivariate analysis of factors associated with postoperative outcomes

	Hazard ratio	95%CI	P value
Pathogenesis	1.227	0.773–1.948	0.385
Albumin (g/L)	1.266	0.829–1.932	0.275
Alanine aminotransferase (U/L)	1.222	0.798–1.872	0.357
Glutamy1 transpeptidase (U/L)	0.821	0.509–1.224	0.419
Direct bilirubin (μmol/L)	2.262	0.270–18.96	0.452
Aspartate aminotransferase (U/L)	1.270	0.800–2.017	0.311
No. of liver tumors	1.238	0.232–19.41	0.330

**Figure 5** Kaplan–Meier curves of postoperative stent restenosis (A) and survival (B).

regression model was used to identify independent predictors. Kaplan–Meier curves and log-rank test clearly demonstrated the differences in survival rate and restenosis rate between the two groups, as well as the efficacy of ^{125}I in the treatment of main portal vein tumor thrombus. Cox analysis could take various factors into account to make the results more convincing.

Research results

Compared with stents alone, stents combined with ^{125}I implantation had a good therapeutic effect in liver cancer with main portal vein tumor thrombus. This method reduced the restenosis rate and improved survival rate. Stents

combined with ^{125}I implantation were safe and reliable in clinical application. In this study, the ^{125}I was placed between the stent and tumor thrombus and the stent could hold the particles. Using this method of ^{125}I implantation, the number and position of the particles could be adjusted, which is more flexible in clinical application. However, as the size of the liver cancer shrinks, the particles may drift to other parts of the body via blood flow, and this needs further study.

Research conclusions

Stents combined with ^{125}I implantation have a good therapeutic effect in the treatment of liver cancer with main portal vein tumor thrombus. The ^{125}I was

placed between the stent and the tumor thrombus, and the stent could hold the particles. The new method can avoid the drawbacks of particle strands and can be widely used in the clinic. Stents combined with ^{125}I implantation have a good therapeutic effect in the treatment of liver cancer with main portal vein tumor thrombus. The method is technically safe and reliable. Tumor thrombus in the main portal vein indicates late-stage disease. ^{125}I is an effective treatment for main portal vein thrombosis. Compared with stents alone, stents combined with ^{125}I implantation can reduce restenosis rates and improve survival rate. It is technically safe and reliable. ^{125}I has made great achievements in the treatment of main portal vein tumor thrombus, but there are drawbacks in the method of ^{125}I implantation, and new methods should be explored.

Research perspectives

Liver cancer with portal vein thrombosis seriously affects patient quality of life and should be treated in a timely manner. Stents combined with ^{125}I implantation have a good therapeutic effect in liver cancer with main portal vein tumor thrombus. Appropriate patients were selected for seed implantation treatment according to the inclusion criteria. The particle drift rate of the patients was followed up at 6, 12 and 24 mo after the operation.

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Clinical Trials Study

Multicenter phase II trial of modified FOLFIRINOX in gemcitabine-refractory pancreatic cancer

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Abstract

AIM

To evaluate the efficacy and safety of modified FOLFIRINOX as a second-line treatment for gemcitabine (GEM)-refractory unresectable pancreatic cancer (PC).

METHODS

This study was a prospective, multicenter, one-arm, open-label, phase II trial. Patients with unresectable PC, who showed disease progression during GEM-based chemotherapy were enrolled. All patients were administered FOLFIRINOX with reduced irinotecan and oxaliplatin (RIO; irinotecan 120 mg/m² and oxaliplatin 60 mg/m²), which was set according to the phase I study of FOLFIRINOX. The objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), overall survival (OS), adverse events were evaluated. Additionally, changes in quality of life (QoL) were assessed using a questionnaire on QoL.

RESULTS

Between August 2015 and May 2016, a total of 48 patients were enrolled. The median follow-up time was 259 d with a median of 8.5 cycles. The ORR and DCR were 18.8% and 62.5%, respectively, including one patient who showed complete remission. The median PFS was 5.8 mo [95% confidence interval (CI): 3.7-7.9] and median OS was 9.0 mo (95%CI: 6.4-11.6). Neutropenia (64.6%) was the most common grade 3-4 adverse event, followed by febrile neutropenia (16.7%). Although 14.6% of patients experienced grade 3 fatigue, most non-hematologic AEs were under grade 2. In the QoL analysis, the global health status score before treatment was not different from the score at the last visit after treatment (45.43 ± 22.88 *vs* 48.66 ± 24.14, *P* = 0.548).

CONCLUSION

FOLFIRINOX with RIO showed acceptable toxicity and promising efficacy for GEM-refractory unresectable PC. However, this treatment requires careful observation of treatment-related hematologic toxicities.

Key words: Pancreatic cancer; FOLFIRINOX; Clinical Trial, Phase II; Chemotherapy; Gemcitabine refractory

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Core tip: For gemcitabine (GEM)-refractory unresectable pancreatic cancer (PC), there are limited options of second-line chemotherapy regimen. To find new treatment option for GEM-refractory unresectable PC, we conducted a multicenter phase II trial, which evaluated the efficacy and safety of uniquely modified FOLFIRINOX with reduced irinotecan and oxaliplatin. In our results, FOLFIRINOX with reduced irinotecan and oxaliplatin showed acceptable toxicity and promising efficacy. With careful observation of treatment-related hematologic toxicities, this chemotherapy regimen is a promising option for patients with GEM-refractory PC after first-line treatment failure.

Chung MJ, Kang H, Kim HG, Hyun JJ, Lee JK, Lee KH, Noh MH, Kang DH, Lee SH, Bang S, Pancreatobiliary Cancer Study Group of Korean Society of Gastrointestinal Cancer. Multicenter phase II trial of modified FOLFIRINOX in gemcitabine-refractory pancreatic cancer. *World J Gastrointest Oncol* 2018; 10(12): 505-515

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INTRODUCTION

Pancreatic cancer (PC) is among the major causes of cancer-related deaths in the United States^[1]. In South Korea, PC is the eighth highest-diagnosed cancer and the fifth most common cause of cancer-related death^[2]. Metastatic pancreatic cancer (MPC) accounts for 60% of all cases; the median survival of patients with MPC is 3-6 mo. Systemic chemotherapy is pivotal for treating such patients; however, effective regimens remain limited. Recently, two first-line combination regimens-FOLFIRINOX [a combination of oxaliplatin, folinic acid (FA), irinotecan, and 5-fluorouracil (5-FU)] and nanoparticle albumin-bound (nab) paclitaxel in combination with GEM-prolonged survival compared to gemcitabine (GEM) monotherapy and became standard treatments^[3,4]. However, the median progression-free survival (PFS) of these new treatment regimens was only 6.4 and 5.5 mo, respectively.

Proper second-line treatment can improve survival of patients with locally advanced pancreatic cancer (LAPC) or MPC who fail first-line treatment. Although some previous phase III trials showed survival improvement with their study regimens, the standard treatment remains unclear^[5-7].

Patients who received first-line FOLFIRINOX or nab-paclitaxel plus GEM may benefit from a novel second-line treatment, although toxicity should also be considered.

FOLFIRINOX, a standard first-line treatment, has been proposed as a second-line treatment for patients with good performance status who failed GEM-based chemotherapy. However, as the condition of many patients deteriorates after first-line chemotherapy, second-line therapy requires administration at attenuated doses and/or schedules, even in patients who maintain a preserved comorbidity profile.

Standard FOLFIRINOX has limited broad use as a second-line therapy because of toxicity; it includes irinotecan (180 mg/m²), oxaliplatin (85 mg/m²), 5-FU (400 mg/m² administered as a bolus followed by 2400 mg/m² administered as a 46-h continuous infusion), and leucovorin (400 mg/m²) every 2 wk^[3]. Several FOLFIRINOX trials have investigated reducing dosages while maintaining efficacy^[8-10]. However, studies focused on the efficacy and safety of a modified dose of FOLFIRINOX for patients with GEM-refractory PC are still rare. Therefore, we conducted a prospective, multicenter, one-arm, open-label, phase II trial using a modified FOLFIRINOX with reduced oxaliplatin and irinotecan (RIO) to minimize adverse events (AEs). Our aim was to evaluate the efficacy and safety of FOLFIRINOX with (RIO) in patients with unresectable PC who had earlier been treated with a GEM-based regimen until disease progression.

MATERIALS AND METHODS

Study population

This study was a prospective, multicenter, one-arm, open-label, phase II trial and conducted in eight Korean university hospitals. The inclusion criteria for this study were patients between 19 and 75 years old; Eastern Cooperative Oncology Group performance status ≤ 2 ; cytologically or histologically proven unresectable pancreatic adenocarcinoma that progressed after first-line GEM-based chemotherapy; adequate bone marrow function (white blood cell count $\geq 3500/\mu\text{L}$, absolute neutrophil count $\geq 1500/\mu\text{L}$, and platelet count $\geq 100000/\mu\text{L}$); adequate hepatic function (total bilirubin $\leq 1.5 \times$ the upper limit of the normal range [ULN], serum aspartate and alanine transaminase $\leq 3 \times$ ULN, and alkaline phosphatases $\leq 3 \times$ ULN or $\leq 5 \times$ ULN in case of liver metastasis); adequate renal function (serum creatinine ≤ 1.5 mg/dL); and adequate cardiopulmonary function. Patients were excluded if they had a concurrent malignancy other than PC; a serious, uncontrollable medical condition; or a psychiatric disorder. The study was performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. Written, informed consent was obtained from each participant after potential treatment complications had been fully explained. The institutional review boards at all participating institutions approved this study. This trial is registered with ClinicalTrials.gov, number NCT02440958.

Study endpoints

The primary endpoints were objective response rate [ORR; complete remission (CR) + partial response (PR)] and disease control rate (DCR; CR + PR + stable disease (SD)). The secondary endpoints were PFS, overall survival (OS), changes in quality of life (QoL), and safety. OS was calculated from the date of enrollment until death from any cause. In the absence of an event, data were censored on the last day of survival confirmation. PFS was calculated from the initiation of treatment until either imaging-confirmed disease progression or death from any cause; in their absence, data for such patients were censored on the day of their last imaging procedure.

Determination of study drug dose

In the phase I study of FOLFIRINOX, febrile neutropenia, prolonged (≥ 7 d) severe neutropenia, and severe non-hematologic AEs were not reported at a dose level of 120 mg/m² irinotecan and 60 mg/m² oxaliplatin^[11]. Based on these data, we set the study drug regimen – FOLFIRINOX with RIO – to 120 mg/m² irinotecan (66.6% of standard dose) and 60 mg/m² oxaliplatin (70.5% of standard dose) with standard dose of bolus and infusional 5-FU.

Treatment protocol and dose adjustments

Oxaliplatin was first administered as a 2-h intravenous infusion (IVF); 1 h later, irinotecan was administered as a 90-min IVF. Leucovorin (400 mg/m²) was administered as a 90-min IVF immediately after oxaliplatin and irinotecan. The 5-FU dose was a 400 mg/m² bolus followed by 2400 mg/m² administered over 46 h of IVF. Each cycle of FOLFIRINOX with RIO was administered every 2 wk and repeated until either evidence of progressive disease (PD), significant clinical deterioration, or withdrawal of patient consent. All patients routinely received palonosetron 30 min before the initiation of chemotherapy as a prophylactic anti-emetic agent. Atropine was administered to patients with irinotecan-caused cholinergic reactions. High-dose loperamide was administered for delayed diarrhea, followed by prophylactic oral fluoroquinolones if diarrhea continued for over 48 h. Granulocyte colony-stimulating factor (G-CSF) was administered for severe neutropenia. AEs were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03) before each cycle. In the event of predefined hematologic or non-hematologic AEs, protocol-specified treatment modifications or delays were performed to minimize additional treatment-related AEs.

For each patient, the study lasted up to 15 cycles with drugs donated by the pharmaceutical manufacturers; patients who completed these cycles without PD were admitted to a post-study phase and continued chemotherapy according to the study protocol at their

own expense. Treatment was discontinued if PD or intolerable toxicity was observed, if the patient withdrew from the study, or at the physician's discretion.

Data assessment

Pre-treatment evaluations included taking a complete medical history, physical examination, and laboratory tests. Evaluations were performed within 2 wk before, and every 2 wk during treatment. Tumor responses were assessed according to the Response Evaluation Criteria in Solid Tumors (version 1.1) based on high-resolution computed tomography scans every 8 wk. QoL was assessed every 8 wk using the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire Core 30 (QLQ-C30)^[12] and its supplement for patients with pancreatic cancer (QLQ-PAN26)^[13]. Additionally, changes in body weight and pain scale were checked every 2 wk. The Korean version of the questionnaire, officially translated and distributed by EORTC, was used. All patients filled-out and submitted the questionnaire by themselves on the day of visit. QoL changes between baseline and the last visit were analyzed, considering that the participation period varied among patients. Scores of all QoL scales range from 0 to 100; a higher score indicates a better functional status or a worse symptom.

Statistical analysis

When this clinical trial was being designed, the previously reported ORR of second-line chemotherapy for unresectable PC with GEM failure ranged from 0% to 11.4%^[5,14–17]. With this background, this trial was performed according to a Simon optimal two-stage design ($P_0 = 0.100$, $P_1 = 0.250$, $\alpha = 0.050$, and $\beta = 0.200$; P_0 and P_1 are the response proportions of a poor and good drug, respectively)^[18,19]. In the first stage, accepting a type I error of 10% and a power of 80%, 46 patients were planned for enrollment. If three or fewer of the 22 enrolled patients demonstrated an objective response, we would terminate the experiment at that stage based on the regimen's low efficacy. Otherwise, the regimen would be recommended for further testing and accrual would continue to 46 patients (assuming a 15% dropout rate).

All patients who received the study regimen at least once were included in the intention-to-treat (ITT) and toxicity analysis populations. All efficacy assessments were based on the ITT analyses. PFS and OS were estimated using Kaplan-Meier methods with 95% confidence interval (CIs). When comparing data (QoL questionnaire, weight, and pain scale) between baseline and last visit, the paired *t*-test was used for normally distributed data while the Wilcoxon signed rank test was used for non-normally distributed data. All statistical analyses were performed using IBM SPSS (version 23.0, IBM Corp., Armonk, NY, United States). A *P*-value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of patients

Between August 2015 and May 2016, 48 patients were enrolled. The median age at the time of enrollment was 63.5 years [interquartile range (IQR), 57.5–69.0 years]. All patients had cytologically or histologically confirmed adenocarcinoma according to the inclusion criteria. Also, all patients had LAPC or MPC including 38 patients (79.2%) with accompanying distant metastasis (Table 1). Close to 80% of the patients received GEM plus erlotinib as their first-line GEM-based treatment. Because GEM plus nab-paclitaxel became available in January 2016 in Korea, only one patient was administered this regimen prior to the study.

Treatment exposure

A flowchart of the 48 patients' treatments is shown in Supplementary Figure 1; at the time of analysis, 38 of these patients had died while two patients remained on FOLFIRINOX with RIO. Treatment was discontinued prior to completing 15 cycles in 33 patients, including 14 who showed PD, 10 who had treatment delays for unresolved infections ($n = 2$) or grade 3/4 toxicities ($n = 8$), five who declined further treatment, two who died after treatment (one of septic shock and the other of unknown reasons at another location), one who had acute cerebral infarction, and one who showed radiologic CR. All patients combined received a total of 493 cycles of chemotherapy. The median follow-up time was 259 d (IQR, 103.3–427.8 d), and the median number of chemotherapy cycles per patient was 8.5 (IQR, 3.0–16.5), with a median treatment duration of 145 d (IQR, 30.5–286.3 d). The relative dose intensity (proportion of the administered accumulated dose relative to the planned accumulated dose) of bolus 5-FU, infusional 5-FU, combined bolus plus infusional 5-FU, irinotecan, and oxaliplatin was $93.60\% \pm 15.86\%$, $93.60\% \pm 15.86\%$, $93.60\% \pm 15.86\%$, $95.65\% \pm 8.16\%$, and $95.65\% \pm 8.16\%$, respectively.

Tumor responses and survival

Tumor responses and survival analysis are shown in Table 2. The ORR and DCR of all patients were 18.8% and 62.5%, respectively. The DCR was 80% in ten LAPC patients, but no CR or PR was reported. Among 38 MPC patients (79.2% of all patients), the ORR and DCR were 23.7% and 57.9%, respectively. A sixty-year-old female patient, who progressed to multiple liver metastasis after GEM monotherapy, achieved radiologic CR after 12 cycle of FOLFIRINOX with RIO. After twelfth cycle, the patient had not experienced disease recurrence on serial radiologic studies without chemotherapy for a year, until peritoneal seeding and liver metastasis were confirmed.

The median PFS was 5.8 mo (95%CI: 3.7–7.9 mo) and the median OS was 9.0 mo (95%CI: 6.4–11.6 mo) for all patients (Figure 1). The PFS rates at 6, 12, and

Table 1 Baseline characteristics

Characteristics of the patients		<i>n</i> = 48	Percent
Age (yr)	median (IQR)	63.5 (57.5-69.0)	
	40-49	4	8.3
	50-59	10	20.8
	60-69	25	52.1
	70-79	9	18.8
Sex	Male	23	47.9
	Female	25	52.1
ECOG-PS	0	22	45.8
	1	24	50
	2	2	4.2
Duration since diagnosis (mo)	median (IQR)	7.0 (3.0-12.0)	
Location of pancreatic cancer	Head	18	37.5
	Body and tail	17	35.4
	Recurrence after resection	13	27.1
Number of metastatic site	0	10	20.8
	1	18	37.5
	2	14	29.2
	≥ 3	6	12.5
Metastatic sites (> 5%)	Liver	28	58.3
	Peritoneum	16	33.3
	Distant lymph node	8	16.7
	Lung	6	12.5
Level of CA 19-9	Normal	10	20.8
	> ULN	38	79.2
Prior GEM CTx	GEM monotherapy	6	12.5
	GEM + Erlotinib	38	79.2
	GEM + Capecitabine	2	4.2
	GEM + Cisplatin	1	2.1
	GEM + Nab-paclitaxel	1	2.1
Period of prior CTx (mo)	median (IQR)	4.1 (1.9-7.8)	
Prior treatment other than CTx	Operation	13	27.1
	CCRT	6	12.5

IQR: Interquartile range; ECOG-PS: Eastern Cooperative Oncology Group-performance status; CA 19-9: Carbohydrate antigen 19-9; GEM: Gemcitabine; ULN: Upper limit of the normal range; CCRT: Concurrent chemo-radiotherapy; CTx: Chemotherapy.

18 mo were 47.9%, 27.1%, and 6.3%, respectively, while the OS rates at 6, 12, and 18 mo were 60.4%, 37.5% and 10.4%, respectively. Eighteen patients (37.5%) survived more than 1 year. The estimated OS from the beginning of first-line treatment was 17.1 mo (95%CI: 10.6–23.6 mo). The median PFS and OS were respectively 5.4 and 8.4 mo for MPC patients, and 8.8 and 12.5 mo for LAPC patients. Analysis of changes in laboratory tests between before and after therapy showed that the median CA19-9 significantly decreased from 366.3 (1–16351) to 311.7 (2–16287) U/mL ($P = 0.041$).

Safety analysis

AEs that occurred in more than 5% of the 48 patients are listed in Table 3. Common AEs observed in more than 20% of patients were neutropenia (68.8%), fatigue (22.9%), nausea and vomiting (66.7%), diarrhea (35.4%), oral mucositis (31.3%), anorexia (20.8%), and fever (20.8%). Of a total of 511 AEs, 358 (70.1%) were considered related to therapy, and 163 (31.9%) were severe AEs (grade 3 or 4). The most common severe AE was neutropenia (64.6%), followed by febrile neutropenia and fatigue (16.7% for both). None of

the patients experienced severe nausea/vomiting or constipation. One patient died of septic shock related to grade 4 neutropenia after treatment.

Changes in QoL

The average body weight was 58.9 ± 9.81 kg at baseline and 59.0 ± 9.83 kg at the last visit. The average pain scale (Visual Analogue Scale) at baseline and the last visit were 2.12 ± 2.31 and 1.90 ± 2.15 , respectively. There were no significant changes in body weight and pain scale ($P = 0.93$ and $P = 0.71$, respectively). QoL questionnaires were available for 31 patients. The global health status scores of the EORTC QLQ-C30 did not worsen after treatment ($P = 0.548$). In general, most functional scores were not significantly decreased except role and cognitive functioning ($P = 0.044$ and $P = 0.015$, respectively) (Supplementary Table 1). Among symptom scores, fatigue and dyspnea were significantly worse than those in the pre-treatment period ($P = 0.021$ and $P = 0.038$, respectively). Among separate QLQ-PAN26 questions, worsening of dry mouth was observed ($P = 0.011$). In patients who achieved disease control ($n = 29$), the global health status score did not worsen after treatment,

Table 2 Tumor responses and survivals (intention-to-treat population)

	All (<i>n</i> = 48)	LAPC (<i>n</i> = 10)	MPC (<i>n</i> = 38)
Response, <i>n</i> (%)			
CR	1 (2.1)	0 (0.0)	1 (2.6)
PR	8 (16.7)	0 (0.0)	8 (21.1)
SD	21 (43.8)	8 (80.0)	13 (34.2)
PD	7 (14.6)	1 (10.0)	6 (15.8)
Could not be evaluated	11 (22.9)	1 (10.0)	10 (26.3)
ORR	9 (18.8)	0 (0.0)	9 (23.7)
DCR	30 (62.5)	8 (80.0)	22 (57.9)
Survival, mo (95%CI)			
Median PFS	5.8 (3.7-7.9)	8.8 (6.0-11.6)	5.4 (2.9-7.9)
Median OS (from 2 nd -line CTx)	9.0 (6.4-11.6)	12.5 (4.9-20.1)	8.4 (5.4-11.4)
Median OS (from 1 st -line CTx)	17.1 (10.6-23.6)	19.1 (13.8-24.4)	16.8 (8.8-24.8)

LAPC: Locally advanced pancreatic cancer; MPC: Metastatic pancreatic cancer; CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease; ORR: Objective response rate; DCR: Disease control rate; CI: Confidence interval; PFS: Progression-free survival; OS: Overall survival; CTx: Chemotherapy.

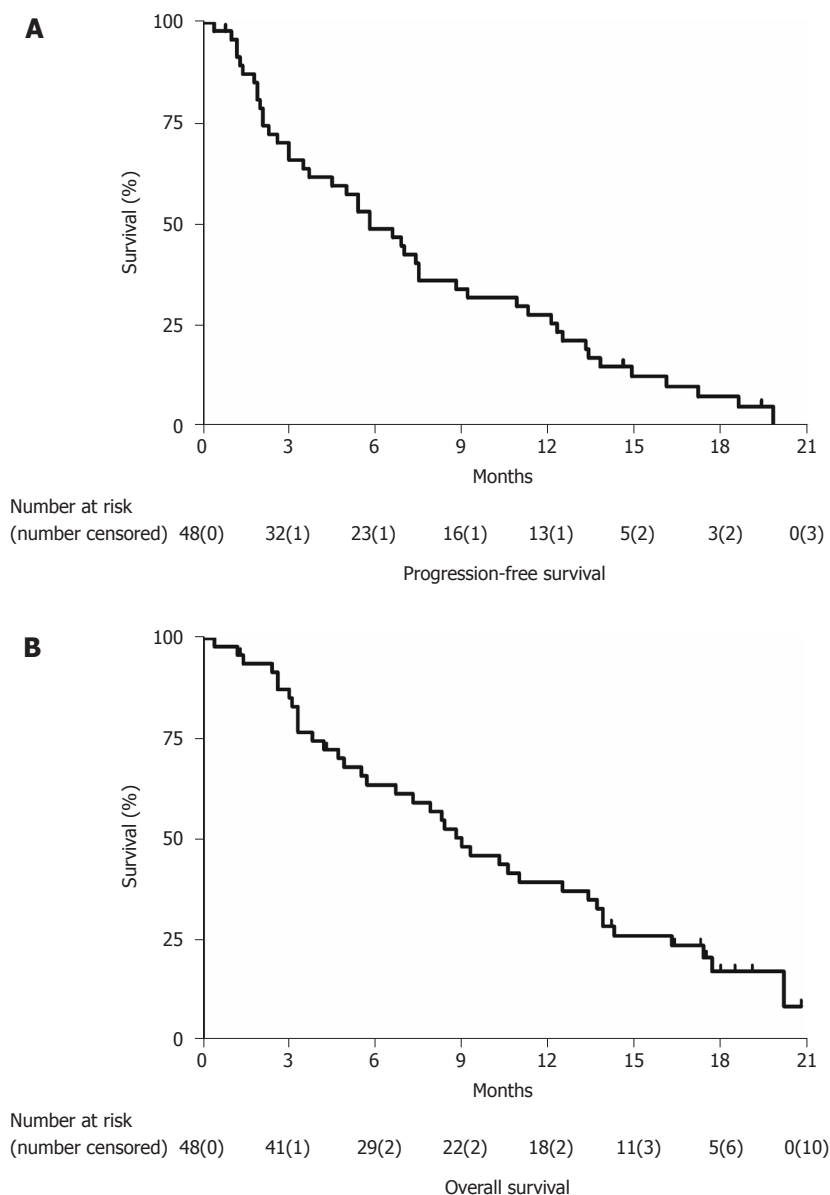


Figure 1 Kaplan-Meier analysis of survival data. A: The estimated median progression-free survival was 5.8 mo (95%CI: 3.7-7.9); B: The estimated median overall survival was 9.0 mo (95%CI: 6.4-11.6).

Table 3 Adverse events ($\geq 5\%$)

N = 48	n (%)	Intensity according to the NCI-CTCAE v4.03			
		Grade 1	Grade 2	Grade 3	Grade 4
Non-hematologic					
Fatigue	11 (22.9)	3 (6.3)	0	8 (16.7)	-
Nausea and vomiting	32 (66.7)	17 (35.4)	15 (31.3)	0	0
Diarrhea	17 (35.4)	7 (14.6)	9 (18.8)	1 (2.1)	0
Constipation	8 (16.7)	4 (8.3)	4 (8.3)	0	0
Oral mucositis	15 (31.3)	4 (8.3)	10 (20.8)	1 (2.1)	0
Anorexia	10 (20.8)	9 (18.8)	0	1 (2.1)	0
Peripheral neuropathy	7 (14.6)	6 (12.5)	0	1 (2.1)	0
Biliary tract infection	3 (6.3)	0	0	3 (6.1)	0
Fever	10 (20.8)	1 (2.1)	9 (18.8)	0	0
Hematologic					
Neutropenia	33 (68.8)	0	2 (4.2)	11 (22.9)	20 (41.7)
Thrombocytopenia	6 (12.5)	0	1 (2.1)	1 (2.1)	4 (8.3)
Febrile neutropenia	8 (16.7)	-	-	5 (10.4)	3 (6.3)

NCI: National Cancer Institute; CTCAE: Common Terminology Criteria for Adverse Events.

but some individual items including cognitive function, fatigue, digestive symptoms, and dry mouth were significantly worsened. Only one item (future worries) was significantly improved (Supplementary Table 2). In patients who completed 15 cycles, constipation and pancreatic pain were significantly improved by the end of treatment; only digestive symptoms were aggravated (Supplementary Table 3).

DISCUSSION

FOLFIRINOX with RIO showed an acceptable toxicity profile and promising efficacy as a second-line treatment for GEM-refractory unresectable PC. Although severe neutropenia occurred in almost 65% of participants, other severe AEs, particularly non-hematologic AEs, were infrequently reported. Moreover, the global health status scores of the EORTC QLQ-C30 were not changed significantly after treatment.

Second-line chemotherapy may be considered for many patients^[20]. At present, there is no recognized standard for patients with unresectable PC who experience PD after first-line chemotherapy, and PFS is consistently < 4 mo in patients receiving second-line chemotherapy. A meta-analysis showed that median OS was 6.0 mo with chemotherapy versus 2.8 mo with best supportive care^[21]. Patients whose cancers progress after first-line therapy have difficulty undergoing second-line chemotherapy since they are often older, unwell, and at risk of rapid deterioration^[22].

Only a few prospective trials have shown encouraging results with oxaliplatin plus 5-FU using various doses and schedules^[5,6,14,17,23]. Two phase III trials produced conflicting results. The CONKO-003 trial comparing 5-FU plus FA (FF) and oxaliplatin plus FF (OFF) showed survival benefits of second-line OFF in patients with unresectable GEM-refractory PC^[6]. In contrast, the PANCREOX trial evaluating the modified FOLFOX6 (mFOLFOX6) found no difference in PFS, while the OS

of mFOLFOX6 was inferior to that of FF^[24].

Research on irinotecan plus 5-FU as second-line chemotherapy for PC was also performed^[15,25]. The NAPOLI-1 phase III trial comparing nanoliposomal irinotecan (nal-IRI) alone or combined with FF showed that the combination of nal-IRI and FF was more effective than FF alone, but caused more frequent severe AEs^[7].

A recent comparative systematic review of four randomized trials evaluating oxaliplatin- or irinotecan-containing regimens as post-GEM therapies for patients with unresectable PC showed significant dissimilarity between them; therefore, it is unclear which regimen is best-suited for patients with unresectable PC previously treated with GEM^[26]. Table 4 summarizes clinical trials of second-line treatment for GEM-pre-treated unresectable PC. Our results using second-line FOLFIRINOX with RIO showed results that were superior to those in most previous trials.

Toxicities associated with standard FOLFIRINOX have prompted trials evaluating modifications of FOLFIRINOX^[8-10]. These previous studies of FOLFIRINOX modifications suggested that upfront dose attenuations of standard FOLFIRINOX can improve tolerability without reducing efficacy. A recent phase II trial showed that the efficacy of first-line FOLFIRINOX with reduced doses of the 5-FU bolus and irinotecan was comparable to that of the standard regimen; furthermore, neutropenia, vomiting, and fatigue were significantly reduced^[10]. However, only a few studies have evaluated FOLFIRINOX for patients with unresectable PC after failure of GEM-based chemotherapy; patients' performance statuses are likely to deteriorate after first-line treatment; necessitating second-line dose reductions as in our study. A Japanese phase II trial used modified FOLFIRINOX reducing only irinotecan for 18 MPC patients^[27]. Findings of that trial were consistent with our results except the PFS, which was longer in the present study (2.8 mo vs 5.8 mo).

FOLFIRINOX and nab-paclitaxel plus GEM are two

Table 4 Clinical trials of second-line treatment for gemcitabine pre-treated unresectable pancreatic cancer

Author (yr)	Type of study	Regimen	Patients, n	KPS ≥ 90 or ECOG ≤ 1, %	MPC, %	ORR, %	DCR, %	PFS/TTP, mo	OS, mo
Yoo <i>et al</i> ^[15] 2009	II	Modified FOLFOX	30	97	100	7	17	6.0 wk	14.9 wk
		Modified FOLFIRI3	31	100	100	0	23	8.3 wk	16.6 wk
Novarino <i>et al</i> ^[14] 2009	II	Oxaliplatin/5-FU/LV	23	73.9	69.6	0	23.5	11.6 wk ¹	17.1 wk
Pelzer <i>et al</i> ^[5] 2011	III	BSC	23	52.2	69.6	0	NA	NA	2.3
		Oxaliplatin/5-FU/LV (OFF)	23	47.8	73.9	0	NA	NA	4.8 (P = 0.008)
Chung <i>et al</i> ^[23] 2013	II	FOLFOX4	44	NA	100	11.4	40.9	9.9 wk ¹	31.1 wk
Oettle <i>et al</i> ^[6] 2014	III	5-FU/LV (FF)	84	47.6	88.1	NA	NA	2	3.3
		Oxaliplatin/5-FU/LV (OFF)	84	53.9	88.2	NA	NA	2.9 (P = 0.019)	5.9 (P = 0.01)
Zaanen <i>et al</i> ^[17] 2014	Prospective cohort	FOLFOX ²	27	44.4	100	0	36.4	1.7	4.3
Wang-Gillam <i>et al</i> ^[7] 2016	III	5-FU/LV	119	48	100	1	NA	1.5	4.2
		Nal-IRI/5-FU/LV	117	59	100	16	NA	3.1 (P < 0.001)	6.1 (P = 0.01)
		Nal-IRI	151	57	100	6	NA	2.7 (P = 0.1)	4.9 (P = 0.94)
Gill <i>et al</i> ^[24] 2016	III	Modified FOLFOX6	54	88.9	92.6	13.2	44.7	3.1	6.1
		Infusional 5FU/LV	54	94.3	94.4	8.5 (P = 0.36)	55.3	2.9 (P = 0.99)	9.9 (P = 0.02)
Present study	II	FOLFIRINOX with RIO	48 (MPC: 38)	95.8	79.2	18.8 (MPC: 23.7)	62.5 (MPC: 57.9)	5.8 (MPC: 5.4)	9 (MPC: 8.4)

¹Time to progression; First line therapy was GEM alone or FOLFIRI3 alternating with GEM. KPS: Karnofsky Performance Scale; ECOG: Eastern Cooperative Oncology Group performance status; MPC: Metastatic pancreatic cancer; ORR: Objective response rate; DCR: Disease control rate; PFS: Progression-free survival; TTP: Time to progression; OS: Overall survival; 5-FU: 5-fluorouracil; LV: Leucovorin; NA: Not available; II: Phase II study; III: Phase III study; BSC: Best supportive care; Nal-IRI: Nanoliposomal irinotecan; RIO: Reduced dosage of irinotecan and oxaliplatin.

Table 5 Comparison with previous studies focused on FOLFIRINOX as a second-line therapy

Study characteristics			Patients characteristics				Treatment outcomes					Grade ≥ 3 AE (%)				
Author (yr)	Type	Dose modification	Patients, n	Age, median (range)	ECOG	Cancer status (%)	ORR, %	DCR, %	PFS, mo	OS, mo	NP	Febrile	NP	Fatigue	Nausea	Diarrhoea
Assaf <i>et al</i> ^[29] 2011	Retro	Standard	27	63 (45-83)	1-3	MPC (100)	18.5	62.9	3	8.5	56	3.7		NA	11	11
Lee <i>et al</i> ^[30] 2013	Retro	Standard	18	57 (44-68)	0-1	MPC (88.9) LAPC (11.1)	27.8	55.6	2.8	8.4	38.9	11.1		NA	38.9	0
Kobayashi <i>et al</i> ^[27] 2017	II	Irinotecan 56% or 67%	18	63 (46-68)	0-1	MPC (100)	22.2	61.1	2.8	9.8	66.7	5.6		NA	0	0
Present study	II	Irinotecan 67% Oxaliplatin 71%	48	64 (40-79)	0-2	MPC (79.2) LAPC (20.8)	All: 18.8 MPC: 23.7 LAPC: 0.0	All: 62.5 MPC: 57.9 LAPC: 80.0	All: 5.8 MPC: 5.4 LAPC: 8.8	All: 9.0 MPC: 8.4 LAPC: 12.5	64.6	16.7	16.7	16.7	0	2.1

AE: Adverse event; ECOG: Eastern Cooperative Oncology Group performance status; ORR: Objective response rate; DCR: Disease control rate; PFS: Progression-free survival; OS: Overall survival; NP: Neutropenia; WHO-PS: World Health Organization performance status; II: Phase II study; Retro: Retrospective study; MPC: Metastatic pancreatic cancer; LAPC: Locally advanced pancreatic cancer; NA: Not available.

of the most effective first-line treatments for MPC, but the appropriate sequence of administration is unclear. A previous study in France concluded that nab-paclitaxel plus GEM appears to be effective, with a manageable toxicity profile, after FOLFIRINOX failure in patients with MPC^[28]. However, studies evaluating the reverse sequence of administration have not been performed to date. Only three studies have been performed evaluating second-line FOLFIRINOX following first-line GEM-based treatment (Table 5)^[27,29,30]. To our knowledge, this study is the first prospective multicenter phase II trial, which evaluated the efficacy and safety of FOLFIRINOX in GEM-refractory PC using

unique dose modification called “FOLFIRINOX with RIO”.

In our study, the relatively higher incidences of severe neutropenia may be related to the patients’ deteriorated physical status after first-line chemotherapy and the lack of prophylactic G-CSF support. For non-hematologic AEs, no grade 3 or 4 vomiting was observed, unlike in the PRODIGE 4/ACCORD 11 trial^[3]. This improved non-hematologic tolerability may be related to the routine administration of prophylactic palonosetron during every cycle of treatment. Peripheral neuropathy was also significantly reduced compared with the previous study, likely because of oxaliplatin dose reduction. In comparison with nal-IRI plus 5-FU regimen of the NAPOLI-1 trial, the preferred second-line therapy for MPC in current guidelines^[31,32], most severe non-hematologic AEs of the present study occurred at lower rates (diarrhea, 13% vs 2.1%; vomiting, 11% vs 0%; anorexia, 4% vs 2.1%)^[7]. However, the rate of severe neutropenia was much higher in the present study (27% vs 64.6%).

Considering QoL, some functional scales such as role and cognitive functioning were significantly reduced in our patients, and some symptom scales such as fatigue and dyspnea were significantly worsened. However, these changes were predictable given our patients’ ages and performance statuses. Global QoL indicators did not significantly deteriorate; moreover, the “physical functioning” QoL score (regarded as one of the strongest prognostic values^[33]) did not significantly worsen throughout treatment.

This study had several limitations. First, it was a non-randomized, single arm trial with a relatively small sample size. A prospective randomized trial including sufficient patients is warranted to provide the clinical recommendation about the treatment sequence for MPC. Second, we included patients with LAPC and MPC who were treated with various prior GEM-based regimens. This heterogeneity in patient population and first-line chemotherapy regimens needs to be improved in future research.

In conclusion, FOLFIRINOX with RIO showed encouraging results in terms of efficacy, with an acceptable safety profile. In addition to nal-IRI plus 5-FU regimen, FOLFIRINOX with RIO may be considered as a treatment option in patients with GEM-refractory unresectable PC. Because the condition of such patients can quickly deteriorate owing to rapid disease progression and treatment toxicity, this regimen may provide acceptable tolerability for patients in terms of patient QoL. However, the presence of hematologic toxicities should be carefully observed, nevertheless, and the routine use of G-CSF should be considered to minimize the risk of hematologic toxicities.

pancreatic cancer (PC) who fail first-line treatment with gemcitabine (GEM)-based regimen. Although some previous phase III trials showed survival improvement with their study regimens, the standard second-line treatment remains unclear.

Research motivation

FOLFIRINOX, a standard first-line treatment for PC, has been proposed as a second-line treatment regimen; however, concerns about relatively high toxicity limited broad use of FOLFIRINOX as a second-line therapy.

Research objectives

We evaluated the efficacy and safety of modified dose of FOLFIRINOX as a second-line treatment for GEM-refractory unresectable PC.

Research methods

In this prospective, multicenter, one-arm, open-label, phase II trial, unresectable PC patients, who showed disease progression during GEM-based therapy were enrolled. All patients were administered FOLFIRINOX with reduced irinotecan and oxaliplatin (RIO; irinotecan 120 mg/m² and oxaliplatin 60 mg/m²), which was set according to the previous phase I study of FOLFIRINOX, with the standard dose of 5-fluorouracil (5-FU). The objective response rate (ORR), disease control rate (DCR), progression-free survival (PFS), overall survival (OS), adverse events, and changes in quality of life (QoL) were evaluated.

Research results

A total of 48 patients were enrolled in eight Korean centers. The ORR and DCR were 18.8% and 62.5%, respectively, including one patient who showed complete remission. The median PFS was 5.8 mo [95% confidence interval (CI): 3.7-7.9] and median OS was 9.0 mo (95%CI: 6.4-11.6). Neutropenia (64.6%) was the most common grade 3-4 adverse event. Although 14.6% of patients experienced grade 3 fatigue, most non-hematologic AEs were under grade 2. In the QoL analysis, the global health status score before treatment was not different from the score at the last visit after treatment (45.43 ± 22.88 vs 48.66 ± 24.14, *P* = 0.548).

Research conclusions

FOLFIRINOX with RIO showed acceptable tolerability for patients in terms of patient QoL and may be considered as a treatment option in patients with GEM-refractory unresectable PC. However, the presence of hematologic toxicities should be carefully observed and the routine use of granulocyte colony-stimulating factor should be considered to minimize the risk of hematologic toxicities.

Research perspectives

Prospective study with larger population comparing the efficacy and safety between FOLFIRINOX with RIO and 5-FU plus leucovorin needs to be conducted.

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ARTICLE HIGHLIGHTS

Research background

Proper second-line treatment can improve survival of patients with unresectable

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Small intestinal hemangioma: Endoscopic or surgical intervention? A case report and review of literature

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Abstract

BACKGROUND

Hemangioma of the small intestine is a rare vascular malformation. Before the advent of capsule endoscopy (CE) and balloon-assisted enteroscopy (BAE), preoperative diagnosis of this disease was extremely difficult.

CASE SUMMARY

In this study, we report a 24-year-old female with a large transmural small bowel cavernous hemangioma, which was diagnosed with CE and BAE preoperatively and removed successfully using minimally invasive surgery. Meanwhile, we perform a literature review of the studies about intestinal hemangiomas published after 2000. Literature review revealed that 91.9% of the lesions were diagnosed preoperatively by CE and/or BAE and 45.9% of them were treated endoscopically, which is a marked improvement compared to before 2000. Therefore, CE and BAE are useful modalities for the preoperative diagnosis of hemangiomas in the small intestine.

CONCLUSION

Endoscopic treatment of intestinal hemangioma is

generally prudent and might be suitable for multiple, relatively small lesions.

Key words: Hemangioma; Capsule endoscopy; Balloon-assisted enteroscopy; Endoscopic intervention; Surgery; Case report

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Core tip: Hemangioma of the small intestine is a rare disease and mostly presents as gastrointestinal bleeding. With the advent of capsule endoscopy and balloon-assisted enteroscopy, the preoperative diagnosis of this disease has been considerably improved. Surgical resection is the conventional treatment modality. With the improvement of endoscopic therapeutic interventions, less invasive procedures are becoming possible. However, potential risks of endoscopic treatment include bleeding and intestinal perforation. Since intestinal hemangiomas originate from the submucosal layer and some of them are transmural, endoscopic treatment might sometimes result in uncontrolled bleeding or perforation.

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INTRODUCTION

Hemangioma of the small intestine is a rare disease, accounting for 7%-10% of all benign tumors of the small intestine^[1,2]. It may be solitary or multiple, with the jejunum being the most common site of involvement^[3]. The main presenting symptoms include hemorrhage, abdominal pain, obstruction, intussusceptions, or rarely, perforation^[4,5]. It originates from the submucosal vascular plexuses and may extend into the muscular layer or beyond^[6]. Histologically, hemangiomas are congenital benign vascular lesions that can be classified as capillary, cavernous, or mixed-type according to the size of the vascular channels^[2]. With the advent of capsule endoscopy (CE) and balloon-assisted enteroscopy (BAE), complete investigation of the small bowel is possible^[7]. The preoperative diagnosis of this disease has been considerably improved. Recent advances in endoscopic techniques have led to successful endoscopic intervention, but most large lesions have been treated surgically. Here, we present a case with solitary small bowel hemangioma, which was diagnosed preoperatively by CE and BAE and removed successfully using minimally invasive surgery.

CASE PRESENTATION

Chief complaints

A 24-year-old female suffered from recurrent melena and fatigue for 1 year.

History of present illness

Over the past year, the patient experienced repeated black stool, accompanied by fatigue, without hematemesis, hematochezia, abdominal pain or fever. The lowest level of hemoglobin was 42 g/L.

History of past illness

Past and family medical history was unremarkable.

Physical examination

Physical examination showed moderate anemia. Detailed dermatological evaluation did not show any cutaneous lesions.

Laboratory testing

Laboratory studies revealed moderated microcytic and hypochromic anemia (hemoglobin, 7.5 g/dL). Fecal occult blood test was positive.

Imaging examination

Gastroscopy and colonoscopy were normal. CE was performed, showing a prominent polypoid lesion in the ileum with no sign of active bleeding (Figure 1). Transanal double-balloon enteroscopy (DBE) revealed a reddish purple lesion in the ileum about 80 cm proximal to the ileocecal valve (Figure 2A). A titanium clip was used to mark the limit reached. Transoral DBE was performed to assess the remainder of small bowel, which revealed no additional lesions (Figure 2B).

MULTIDISCIPLINARY EXPERT CONSULTATION

Ping-Fang Hu, MD, Attending Doctor, Department of Gastroenterology

From the endoscopic appearance of the lesion, it was most likely a hemangioma. Considering that the lesion was large and diffuse, endoscopic interventions such as endoscopic mucosal resection (EMR) and endoscopic sclerotherapy might lead to uncontrolled bleeding or perforation. Therefore, laparoscopic surgery was deemed the best choice.

Bin Shi, MD, Professor, Department of Gastroenterology

The patient had repeated bleeding and a large amount of bleeding every time. Since the lesion was large and diffuse, surgery would be better for the patient.

Han Chen, MD, Attending Doctor, Department of Surgery

The patient suffered from recurrent melena in the past year. From the results of the CE and BAE, the cause

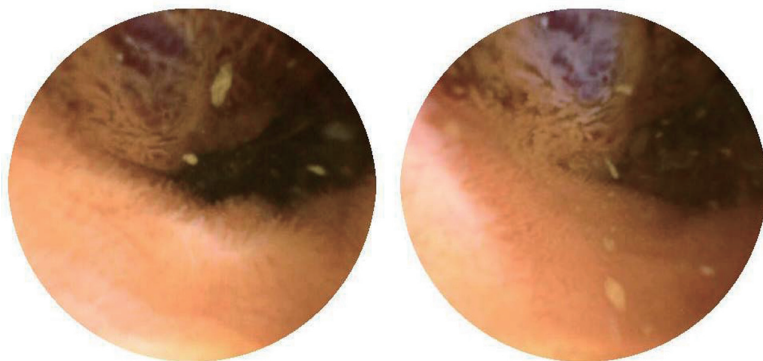


Figure 1 Capsule endoscopic appearance of the lesion. Capsule endoscopy showed a prominent polypoid lesion in the ileum.

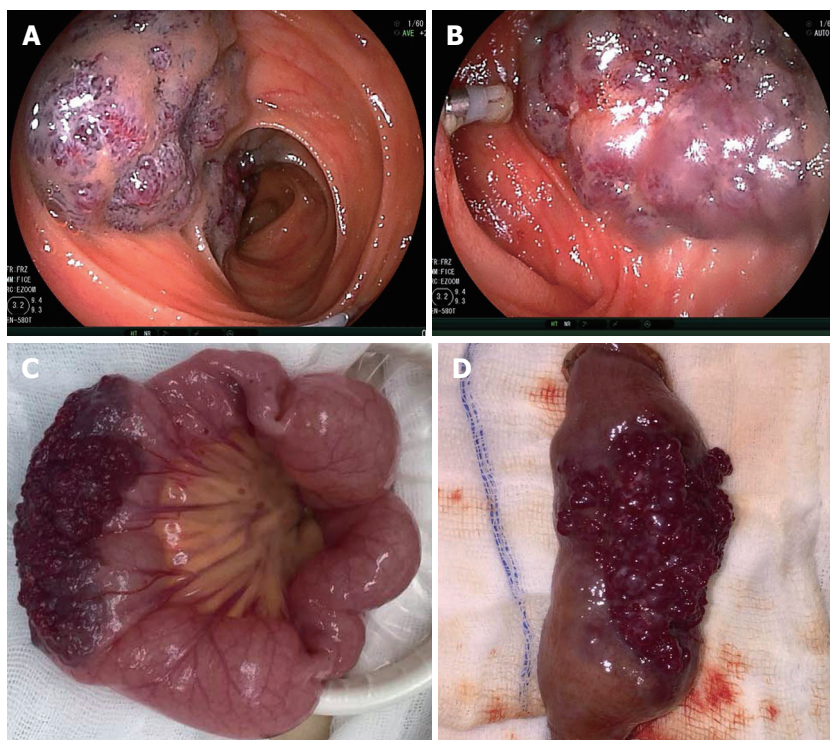


Figure 2 Endoscopic and gross appearance of the lesion. A: Transanal double-balloon enteroscopy revealed a reddish purple lesion in the ileum about 80 cm proximal to the ileocecal valve, and a titanium clip was used to mark the limit reached; B: Transoral double-balloon enteroscopy showed the same lesion and the marked titanium clip; C: Gross intraoperative appearance of the lesion; D: Gross appearance of the lesion after resection.

is likely the small intestinal hemangioma. The surgical indication was explicit.

Ning Su, MD, Attending Doctor, Department of Surgery

The diagnosis is relatively clear. Since biopsy might lead to uncontrolled bleeding, we could not verify the diagnosis preoperatively.

Wei-Jun Wang, Professor, Department of Surgery

Imaging examination including ultrasound and CT scan did not find any abnormalities. From the endoscopic appearance of the lesion, it was most likely a hemangioma. The patient was a young female with a good health status. We could consider resecting the

lesion laparoscopically.

FINAL DIAGNOSIS

Small bowel bleeding and small intestinal hemangioma.

TREATMENT

The patient was sent to laparoscopy, and a 5 cm × 3 cm × 3 cm purple-colored, raspberry-like lesion was found spreading diffusely along the serosal surface of the ileum (Figure 2C). The lesion was completely resected (Figure 2D). Hematoxylin-eosin staining (Figure 3A) and CD31 immunohistochemistry (Figure 3B) indicated

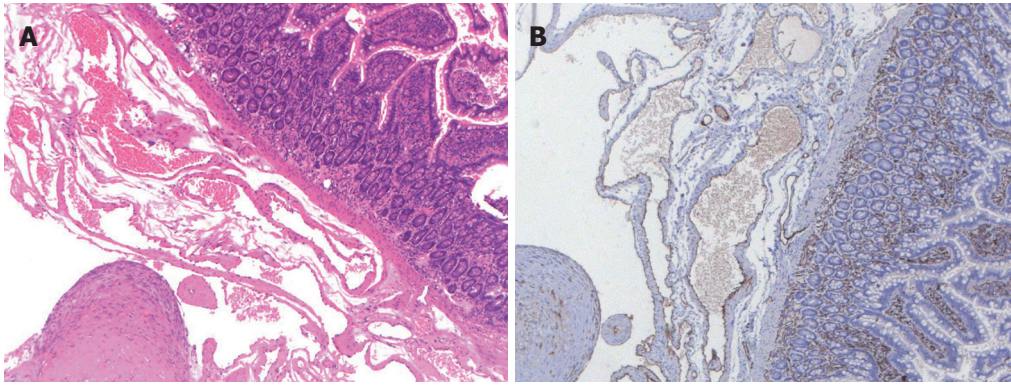


Figure 3 Histopathological examination of the lesion. A: Hematoxylin-eosin staining showed a blood-filled sinus-like space in the whole layer of the ileum ($\times 50$); B: Immunohistochemistry indicated the cells lined with the vascular spaces were CD31-positive ($\times 50$).

a transmural cavernous hemangioma.

OUTCOME AND FOLLOW-UP

The patient recovered quickly and had no further episodes of bleeding since the operation. The hemoglobin value increased to normal (12.4 g/dL) and was stable.

DISCUSSION

Hemangioma accounts for only 0.05% of all gastrointestinal (GI) neoplasms. They mostly present with occult GI bleeding and iron deficiency anemia. Because of its rarity, it is not considered a common cause of GI bleeding. Previously, the preoperative diagnosis of this disease was difficult, and almost all cases were diagnosed during or after the operation^[1]. With the introduction of CE and BAE over the past decades, the small intestine has now become an area that can be targeted^[8]. We searched the PubMed database for studies about intestinal hemangiomas published after 2000 utilizing the following search terms: "hemangioma", "vascular malformation", "small intestine" and "small bowel". A manual search was also performed using the references of eligible articles. The language was limited to English. A total of 37 cases (16 women, 21 men, mean age 39 years) were retrieved and reviewed (Table 1). The most common manifestation included GI bleeding and anemia. A total of 75.7% (28/37) of the cases were single, and the common location of the small intestine was the jejunum (60.9%). Thirty-four of the 37 lesions (91.9%) were diagnosed before operation by CE and/or BAE. Of these cases, 11 were detected with CE alone, and 22 were diagnosed with both CE and BAE. Compared with the cases reported before 2000, a markedly increased proportion of cases were preoperatively diagnosed^[1]. As in our case, CE was used to initially examine the GI tract, which was based on the algorithms for the diagnosis and treatment of obscure GI bleeding^[7]. Both transanal DBE and transoral DBE were then performed to complete total enteroscopy, which was useful to localize the lesion and rule out

other lesions.

Surgical resection, which is relatively more invasive, is the conventional treatment modality for intestinal hemangiomas. With the improvement of endoscopic therapeutic interventions, less invasive procedures are becoming more widely employed. Of the 37 cases of intestinal hemangiomas published after 2000 (Table 1), 17 cases (45.9%) were treated endoscopically. Among them, 3 cases were removed by EMR, one case was treated by argon plasma coagulation, and 13 cases were subjected to sclerotherapy. Most of these lesions were multiple (14/17, 82.4%), and the lesions were relatively small. As suggested by the guideline on the management of small bowel bleeding, the patient should be managed with endoscopic therapy if a source of bleeding is found. Surgical treatment is generally regarded as a last resort^[7]. Compared with surgery, endoscopic treatments including sclerotherapy and EMR are less invasive. However, they increase the potential risks of GI bleeding and intestinal perforation. Since intestinal hemangiomas originate from the submucosal layer, endoscopic treatment such as EMR is dangerous because of the risk of perforation. Endoscopic treatment might lead to perforation because some intestinal hemangiomas were transmural, as in our case. Considering that the hemangioma was large in the current case, uncontrolled bleeding would probably occur after endoscopic intervention. After discussion with a multidisciplinary team, which included gastroenterologists, endoscopists and surgeons, we decided to remove the lesion by laparoscopy. It turned out that a laparoscopic approach was likely the best choice for our case, as the lesion was relatively large and most importantly, transmural. Thus, endoscopic treatment of intestinal hemangioma should be prudent. It is likely suitable for multiple, relatively small lesions.

In conclusion, we present a case of small bowel hemangioma that was preoperatively diagnosed by CE and BAE and treated by laparoscopy. We believe it is important for both the endoscopist and surgeons to recognize this somewhat unusual lesion. It is recommended that careful consideration of the indications for

Table 1 Summary of hemangioma of small intestine reported after 2000

Ref.	Country	Case	Sex/age	Complaint	Diagnosis	Location	Single/multiple	Treatment	Pathology
Easler <i>et al</i> ^[9]	United States	1	M/71	Anemia, melena	BAE	Jejunum	Single	EMR	Cavernous
Ng <i>et al</i> ^[10]	China	1	F/20	Anemia	Small bowel enema	Terminal ileum	Multiple	APC	-
Wardi <i>et al</i> ^[11]	Israel	1	M/77	Anemia, melena	CE	Ileum	Single	Laparoscopy	Capillary
Ersoy <i>et al</i> ^[6]	Turkey	1	F/50	Melena, hematemesis	CE + BAE	Proximal jejunum	Single	Laparoscopy	Cavernous
Fernandes <i>et al</i> ^[12]	Portugal	1	F/56	Hematochezia, syncope	CE	Ileum	Single	Laparoscopy	Cavernous
Law ^[13]	China	1	F/31	Melena	CE + BAE	Jejunum	Single	Laparoscopy	Cavernous
Ning <i>et al</i> ^[4]	China	1	M/10	Melena	BAE	Jejunum/ileum	Multiple	Polidocanol injection	-
Elias <i>et al</i> ^[15]	United States	1	M/30	Anemia	CE + BAE	Jejunum	Multiple	Surgery	Cavernous
Shibuya <i>et al</i> ^[16]	Japan	1	M/74	Melena	CE + BAE	jejunum	Single	EMR	Capillary
Willert <i>et al</i> ^[6]	Australia	1	M/19	Anemia	CE + BAE	Jejunum/ileum	Multiple	EMR	Cavernous
Igawa <i>et al</i> ^[17]	Japan	12	6M/6F	Gastrointestinal bleeding	CE + BAE	Jejunum/ileum	7 single/5 multiple	Polidocanol injection	-
Takase <i>et al</i> ^[18]	Japan	2	F-62/M-52	Melena	CE + BAE	Jejunum/ileum	Single	Laparoscopy	Cavernous/capillary
Akazawa <i>et al</i> ^[19]	Japan	1	F/56	Melena	CE + BAE	Jejunum	Single	Laparoscopy	Cavernous
Chen <i>et al</i> ^[20]	United States	1	M/23	Fatigue	CE	Ileum	Single	Laparoscopy	Cavernous
Dhumane <i>et al</i> ^[21]	France	1	M/60	Anemia	CE + BAE	Jejunum	Single	Laparoscopy	Cavernous
Bae <i>et al</i> ^[22]	South Korea	1	M/13	Dizziness, fatigue	CE	Jejunum	Single	Laparoscopy	Cavernous
Huber <i>et al</i> ^[23]	Germany	1	M/23	Weakness, dizziness	CE + BAE	Jejunum	Single	Laparoscopy	Cavernous
Quentin <i>et al</i> ^[5]	France	1	F/32	Hematochezia	CE	Jejunum	Single	Laparoscopy	Cavernous
Khurana <i>et al</i> ^[24]	United States	1	M/62	Melena	BAE	Jejunum	Single	Surgery	Cavernous
Pera <i>et al</i> ^[25]	Spain	1	M/16	Fatigue	CE	Jejunum	Single	Laparoscopy	-
Pinho <i>et al</i> ^[26]	Portugal	1	F/9	Melena, anemia	CE	Ileum	Single	Surgery	Cavernous
Magnano <i>et al</i> ^[27]	Italy	1	M/13	Fatigue, malaise	CE	Ileum	Single	Laparoscopy	Cavernous
Kuo <i>et al</i> ^[28]	China	1	F/20	Abdominal pain	-	Jejunum	Single	Laparoscopy	Cavernous
Guardiola <i>et al</i> ^[29]	Spain	1	M/19	Anemia	CE	Ileum	Single	Laparoscopy	Cavernous
Purdy-Payne <i>et al</i> ^[30]	United States	1	F/20	Abdominal pain	-	Terminal ileum	Single	Laparoscopy	Cavernous

CE: Capsule endoscopy; BAE: Balloon assisted enteroscopy; EMR: Endoscopic mucosal resection; APC: Argon plasma coagulation.

endoscopic treatment. As in our case, hemangiomas may sometimes involve the entire wall of the intestine. Endoscopic intervention may lead to uncontrolled bleeding or perforation. For the large and diffuse lesions, a laparoscopic excision might be a better approach.

EXPERIENCES AND LESSONS

Hemangioma of the small intestine is a rare disease, which mostly presented as occult GI bleeding and iron deficiency anemia. With the advent of CE and BAE, the diagnosis of lesions in the small intestine has been considerably improved. Endoscopic treatment of intestinal hemangioma should be prudent, and it might be suitable for multiple and relatively small lesions.

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Experience in the diagnosis and treatment of mesenteric lymphangioma in adults: A case report and review of literature

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Abstract

BACKGROUND

Mesenteric lymphangioma (ML) in adults is a very rare disease. We report six hospitalized adult patients with ML in our hospital between January 2013 and July 2018 to investigate the characteristics and prognosis of ML in adults.

CASE SUMMARY

The male-to-female ratio was 3:3, and the median age at diagnosis was 55.2 years. Clinical manifestations varied; however, most were acute cases (5/6). No history of trauma was reported. None (0/6) of the patients were accurately diagnosed with ML in the emergency and outpatient departments. Mesenteric cysts were identified in four patients (66.7%) by abdominal ultrasound and in five patients (83.3%) by computed tomography. ML was postoperatively confirmed by pathology. Most MLs (4/6) were associated with infection of other systems. ML was located in the mesentery of the small intestine ($n = 4$), ileum ($n = 1$) and rectum ($n = 1$). Cyst fluid was clear ($n = 4$), chylous ($n = 1$) and bloody ($n = 1$). Surgical procedures included complete tumor removal and partial intestinal excision ($n = 6$). Recurrence and adhesive intestinal obstruction were not observed during the 3-12 mo follow-up period.

CONCLUSION

ML in adults is a rare benign acquired disease that can be cured by surgical treatment. Infection may be a cause of ML.

Key words: Mesenteric lymphangioma; Mesenteric cyst; Adults; Acute abdominal pain; Case report

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Core tip: Mesenteric lymphangioma (ML) is a rare congenital lymphangioma that predominantly affects children. We reported six cases of adult patients with ML and reviewed the literature. The report is helpful in comprehensively understanding the characteristics and prognosis of ML in adults and arousing the clinician's attention to this disease.

Chen J, Du L, Wang DR. Experience in the diagnosis and treatment of mesenteric lymphangioma in adults: A case report and review of literature. *World J Gastrointest Oncol* 2018; 10(12): 522-527

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INTRODUCTION

Mesenteric lymphangioma (ML) is a rare congenital lymphangioma of uncertain etiology that predominantly occurs in children. ML lacks specific clinical signs and symptoms, and patients are often admitted to the hospital due to complications, such as abdominal pain, abdominal distension, intestinal obstruction and other acute abdominal manifestations. In adults, it is often found coincidentally during auxiliary examinations or even exploratory abdominal laparotomy, leading to passive surgery or surgical preparation, sometimes missing the best surgical opportunity due to delayed diagnosis. Cases and misdiagnosed cases have been reported in previous studies^[1-3]. However, these studies mainly focused on imaging. Here, we retrospectively analyzed six adults with ML confirmed by pathological examination and admitted between January 2013 and July 2018. Our findings may improve our understanding of this disease and provide more clinical references for early and correct treatment.

CASE PRESENTATION**Chief complaints**

Case 1: A 45-year-old man with abdominal pain for 16 h.

Case 2: A 59-year-old man with severe abdominal pain, no defecation and exhaustion for 3 d.

Case 3: A 62-year-old man with abdominal pain, nausea and fever for 3 h.

Case 4: A 71-year-old woman with abdominal pain, fever, diarrhea, and gastrointestinal bleeding for 1 wk.

Case 5: A 42-year-old woman with abdominal distension for 2 years that had recurred for 1 d.

Case 6: A 52-year-old woman with mild abdominal pain and distension, nausea and fever for more than 1 d.

History of present illness

None of the patients had a significant history of trauma. Most MLs (4/6) were associated with infection of other systems. Case 1: cholecystitis; Case 2: no other systemic co-infection was found; Case 3: cholangitis; Case 4: urinary tract infection; Case 5: no other systemic co-infection was found; Case 6: Gastrointestinal tract infection, acute gastritis and colitis.

History of past illness

The history of symptoms ranged from 3 h to 2 yr. Case 3 had a medical history of cholangiolithiasis and endoscopic retrograde cholangiopancreatography, Case 5 had an untreated abdominal cyst 2 years previously and diabetes for more than 5 years and was admitted to our hospital due to acute abdominal distension. All other cases were admitted to the emergency department for acute abdominal pain without history of other chronic diseases.

Physical examination

Case 2: middle and upper abdominal tenderness and rebound tenderness, no muscle guarding; bowel sound was high pitched tinkling. Case 4: periumbilical tenderness, no rebound tenderness and muscle guarding, a palpable mass of about 10 cm in diameter was detected when abdominal pain occurred, but disappeared when abdominal pain was relieved. All other cases showed tenderness in different parts of the abdomen, no rebound tenderness and muscle guarding, and bowel sounds were normal.

Laboratory testing

Tumor markers were normal in all cases, and laboratory tests indicated increased white blood cell to different degrees. In addition, Case 2 and 3 showed a slight increase in alanine aminotransferase and γ -glutamyl transpeptidase. Case 4 and Case 6 indicated fecal occult blood (+), and fecal bacteria cultures were negative.

Imaging examination

All patients underwent abdominal ultrasound and abdominal computed tomography/magnetic resonance imaging (commonly known as CT/MRI) examination after admission (Figures 1 and 2), and some patients

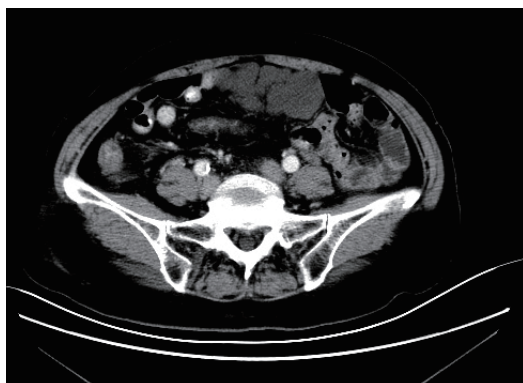


Figure 1 Abdominal computed tomography scan reveals lumpy low-density shadows around the upper middle intestine, 86 mm × 42 mm in size, and no enhancement was observed (Case 4).

had abdominal X-ray examination. In one patient with acute intussusception and one patient with diarrhea, abdominal X-rays showed fluid and an incomplete intestinal obstruction. In other patients with acute abdominal pain, abdominal X-ray showed no obvious abnormalities. Five patients were found to have an intraperitoneal cystic or solid cystic mass by CT/MRI. Abdominal ultrasonography failed to detect an abdominal cyst in Case 3 and Case 4. Preoperative ultrasound and CT failed to detect an abdominal cyst in Case 3.

FINAL DIAGNOSIS

All cysts were examined by pathology after operation, and all were ML (Figure 3).

TREATMENT

Case 6 of a suspected rectal tumor and Case 5 of an intra-abdominal benign cyst chose elective surgery, and the remaining four patients underwent emergency surgery within 48 h of hospital admission. Intraoperative ML was identified in the jejunum in four cases, the ileum in one case, and the rectum in one case. Four cases had clear cystic fluid, one case had chylous fluid and one case had bloody fluid. Tumor invasion was noted in the bile duct in one case, the duodenum in one case, the transverse colon in one case, and the rectum in one case. All six cases underwent complete removal of the tumor and partial intestinal excision, ranging from 6 to 50 cm.

OUTCOME AND FOLLOW-UP

Six patients were included in this study. Patient information and clinical manifestations are shown in Table 1. Of the six patients, three were male and three were female, aged 42–71 years, with an average age of 55.2 years. The clinical manifestations of ML included acute abdominal pain, acute intestinal obstruction (vomiting, abdominal distension, no defecation

and exhaustion), fever, diarrhea, and gastrointestinal bleeding. The initial diagnoses included: one case of acute intussusception, three cases of abdominal tumors (mesenteric lipoma, duodenal and rectum tumor), one case of acute cholangitis, and one case of acute hemorrhagic enteritis. All six patients were diagnosed with ML by pathology following surgery. The accuracy of initial diagnosis was zero (0/6). The diagnostic accuracy of ultrasound for mesenteric cyst was 66.7% (4/6). The diagnostic accuracy of CT/MRI for mesenteric cyst was 83.3% (5/6).

All of the patients had good postoperative recovery, the abdominal mass disappeared, appetite, defecation and urine output became normal. All patients were followed up for 3 mo by abdominal CT, and no recurrence or adhesive intestinal obstruction occurred. Five cases (83.3%) were followed for up to one year, and no recurrences were observed.

DISCUSSION

Lymphangiomas are uncommon congenital malformations of the lymphatic system. They can occur at any site in the body, but are most commonly found in the neck and head area as well as the abdominal wall, but rarely in the mesentery^[4]. It is reported that the incidence of ML is approximately 5%, and the male-female ratio is about 1.5–3:1^[5,6]. In addition, ML has been described in less than 1% of all lymphangiomas^[1,7,8].

The exact etiology of ML is unknown. It is likely to be a developmental anomaly of the lymphatic system, as 65% of MLs are present at birth and 90% of all patients are diagnosed before the age of 2^[7]. However, they can also develop due to an inflammatory process, lymphatic obstruction, surgery, radiation and abdominal trauma^[9,10]. ML is rarely seen in adults. Therefore, it is not clear what the incidence of ML is in adults. Only a few case reports of ML in adults are available in the published literature^[11]. In adults, lymphangiomas primarily occur on the body surface or in the abdominal cavity, and the incidence of ML is 1/100000^[2], mostly in the small intestine, followed by the omentum majus, mesentery and retroperitoneum. ML is a benign lesion, with a relatively asymptomatic onset, slow growth and a long disease course.

Most MLs are initially asymptomatic, and are therefore usually discovered incidentally. However, when the tumor is large, it can compress the surrounding viscera or block the intestine, producing corresponding symptoms. The clinical symptoms of ML vary depending on location. ML can manifest as abdominal pain, abdominal distension, diarrhea, hematochezia, constipation, hypoproteinemia, intussusception, and decreased physical quality^[12]. Due to the lack of specific clinical signs and symptoms, ML is easily missed and misdiagnosed. Abdominal ultrasonography or CT can be used to identify an early abdominal cystic mass, especially in patients with recurrent abdominal pain, abdominal distension, and stubborn constipation. Emer-

Table 1 Information and clinical manifestations in the six patients

Cases	1	2	3	4	5	6
Gender	M	M	M	F	F	F
Age, yr	45	59	62	71	42	52
Abdominal pain	+	+	+	+	-	+
Nausea	-	-	+	-	-	+
Vomiting	-	+	-	-	-	-
Diarrhea	-	-	-	+	-	-
GI bleeding	-	-	-	+	-	-
Fever	-	-	+	+	-	+
Concurrent infection	Cholecystitis	UK	Cholangitis	Urinary tract	UK	GI tract
Abdominal distension	-	-	+	+	+	+
Intestinal obstruction	-	+	-	-	-	-
Medical history	-	-	Cholangiolithiasis, ERCP	-	Diabetes	-
Trauma	-	-	-	-	-	-

UK: Unknown; ERCP: Endoscopic retrograde cholangiopancreatography; GI: Gastrointestinal tract.

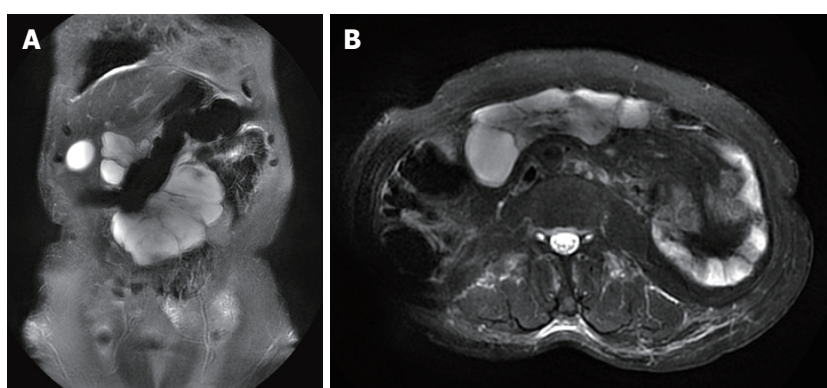


Figure 2 Abdominal magnetic resonance imaging shows cystic long T1 and long T2 signal masses in the anterior wall of the middle abdomen, 110 mm × 40 mm in size. No abnormal enhancement was observed (Case 4). A: Coronal position; B: Axial position.

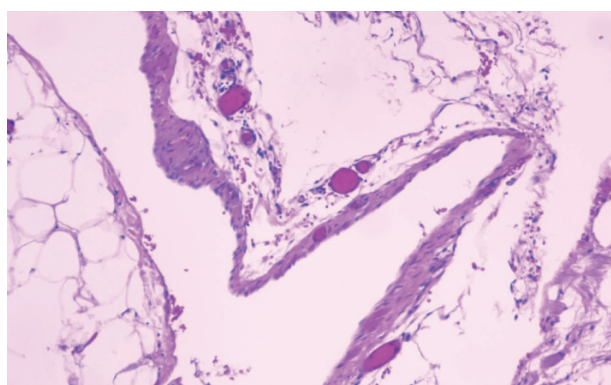


Figure 3 Histopathology shows that the cyst wall was composed of fibrous adipose tissue and a few lymphatic endothelial proliferations. Case 4, hematoxylin and eosin, 100 ×.

gency doctors should be vigilant, and early abdominal ultrasound or CT examination should be carried out to detect this disease as soon as possible.

Ultrasonography is of high diagnostic value in detecting the location, size, division of the cyst, cyst fluid, cyst wall and its relation to surrounding tissues^[13]. In small cysts, abdominal CT is more sensitive and helps to differentiate from other related abdominal

pelvic cysts, such as greater omentum cysts, intestinal repetitive malformations, ovarian cysts, common bile duct cysts, and kidney cysts. CT is useful for further understanding the relationship between the cyst and the surrounding tissues and organs, especially large blood vessels and the bowel, which ultimately aids treatment decisions and surgical approaches in these patients^[14]. MRI is more sensitive in patients with intracavitary hemorrhage. In the case of intrathecal hemorrhage, the imaging may show solid cystic signs^[15,16].

Pathological examination is the gold standard for the diagnosis of ML, and it provides strong evidence for postoperative identification of other types of cysts. Microscopy shows dilated lymphatics, and the thin wall lining epithelial cells in the lymphatic cavity gap and a small amount of smooth muscle tissue can be seen. During infection, the infiltration of lymphocytes, plasma cells, eosinophils and other inflammatory cells are also visible. MLs are classified as simple, cavernous and cystic. The simple type of ML is primarily situated superficially in the skin and is composed of small thin-walled lymphatic vessels. The cavernous type has dilated lymphatic vessels and has connections with normal adjacent lymphatics. Cystic lymphangioma is composed of large macroscopic lymphatic spaces surrounded

by collagen and smooth muscle and does not have connections with adjacent normal lymphatics (CD34 and CD31 positive)^[17].

ML needs to be differentiated from peritoneal abscess, hematoma, malignant tumor center liquefaction necrosis, malignant tumor cystic adenoma and some solid masses derived from mesenchymal tissue such as sarcomas. Simple abdominal ultrasonography may be difficult to distinguish, and abdominal CT/MRI can provide more information. However, the diagnosis of ML primarily depends on pathological diagnosis. When necessary, ultrasound-guided diagnostic puncture can further differentiate between cyst, abscess and hematoma. Ultrasound-guided needle aspiration cytology can identify whether the mass is benign or malignant, providing a basis for differential diagnosis.

To treat ML, most doctors recommend radical surgical excision, as ML can grow very large and invade adjacent structures, develop complications and the risk of sarcoma transformation upon irradiation^[9]. After excision of a mass that involves the whole mesentery, internal herniation is likely to occur due to the presence of skeletonized vessels. A biological collagen implant can be used to repair the mesenteric defect after excision of a large ML, and monitoring for recurrence during follow-up is necessary^[18]. However, opinions differ about the course of treatment.

A previous study^[19] showed that for asymptomatic or mild lymphangioma patients, conservative treatment and close follow-up are recommended. In a case report, colorectal lymphangioma spontaneously disappeared. We suggest that if the ML is relatively large, it is possible to infiltrate the surrounding organs and cause tissue ischemic necrosis, causing potentially life-threatening complications, such as traumatic rupture, anemia secondary to intraabdominal or intra-cavitary bleeding, intestinal gangrene secondary to volvulus and intermittent intestinal obstruction^[2,16]. It is better to remove it as soon as possible.

In the present study, the ratio of men to women was 3:3, with no significant gender difference. The rate of accurate initial diagnosis was zero. This indicated that clinicians lack awareness of ML in adults, and the average age at diagnosis in these patients was 55.2 years. All of these patients had received routine physical examination in the past few years, but no abdominal cysts were found. Five of these six cases had no previous history of abdominal cysts, but all patients had acute onset, mainly presenting with acute abdominal pain, incomplete intestinal obstruction, and mucinous bloody diarrhea. The remaining patient was found to have abdominal cysts two years previously, which were untreated. Acute abdominal distension was not treated with anti-inflammatory therapy and was diagnosed and cured after surgery.

Following symptom onset, the abdominal cysts were not identified by abdominal color Doppler ultrasound or CT. However, the diagnosis of abdominal cysts was

confirmed during laparotomy. It is speculated that adult ML may be an acquired disease and that infection may be a risk factor. Of these six cases, two had biliary tract infection, one had urinary tract infection, one had digestive tract infection, and two patients had no obvious infection. The pathogenesis of ML may include the formation of secondary cysts caused by lymphatic obstruction due to infection. Moreover, we found that the detection rate of abdominal cysts by ultrasound was lower than that of abdominal CT (66.7% vs 88.3%). This may have been due to the following possibilities: (1) due to the rarity of ML in adults, ultrasound clinicians lacked awareness of ML, resulting in misdiagnosis; (2) misdiagnosed cystic lesions such as ascites or dilated bowel may be caused by intestinal obstruction; and (3) ML may be an acquired disease.

As clinicians are accustomed to prescribing abdominal ultrasound first and then CT, an abdominal cyst may not yet have formed when ultrasonography is performed. Pathological examination is the gold standard in the diagnosis of ML. An accurate and thorough surgical technique is an effective method of treating ML. No recurrence was found during the follow-up period of 3-12 mo in the six patients included in this study. However, timely surgery is essential. In a patient with mucinous diarrhea, infiltration of the transverse colon was found during surgery, leading to transverse colonic ischemic necrosis, and partial transverse colectomy was performed.

In summary, ML in adults is an extremely rare benign, potentially acquired disease distinct from ML in children, which is due to congenital lymphatic dysplasia. Infection may be involved in the pathogenesis of ML in adults. However, the exact etiology should be confirmed in a large sample study. In some cases, ML can be fatal, as it may cause tissue ischemic necrosis due to infiltration of surrounding viscera, compression of the intestine or peritoneal vessels, resulting in serious complications. Timely and effective radical surgery is necessary, which requires increased awareness of the disease in clinicians to avoid misdiagnosis and missed diagnosis.

EXPERIENCES AND LESSONS

Although ML occurs more frequently in children, it also occurs in adults, but the exact etiology in adults requires further study. Although ML is benign, it can also lead to serious and fatal consequences. Timely and effective radical surgery can cure this disease. Clinicians should raise awareness of ML in adults.

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Considering FOLFOXIRI plus bevacizumab for metastatic colorectal cancer with left-sided tumors

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Abstract

A recent subgroup analysis of the TRIBE trial suggested that FOLFOXIRI plus bevacizumab may be a preferred option for the first-line treatment of only right-sided metastatic colorectal cancer (mCRC), regardless of RAS or *BRAF* status. Our subanalysis of a phase II trial of the FOLFOXIRI triplet regimen plus bevacizumab in patients with mCRC who had RAS mutant tumors showed that tumor shrinkage was better and the duration of treatment was longer in patients with left-sided tumors than in those with right-sided tumors, leading to a higher rate of conversion to surgery in mCRC patients with left-sided tumors. The early and deep responses to the triplet-regimen in patients with left-sided tumors might facilitate conversion treatment resulting in favorable survival. Our data suggest that the FOLFOXIRI plus bevacizumab might be a promising treatment for left-sided mCRC involving RAS mutant tumors.

Key words: Tumor sidedness; FOLFOXIRI; Bevacizumab; Colorectal cancer; RAS mutation

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Core tip: FOLFOXIRI plus bevacizumab regimen might be a preferred option for the first-line treatment of only right-sided metastatic colorectal cancer (mCRC) regardless of RAS or *BRAF* status. However, subanalysis of a phase II trial of the triplet plus bevacizumab in patients with RAS mutant mCRC demonstrated that more patients with left-sided tumors achieved good tumor shrinkage and long duration of treatment than did patients with right-sided tumors, leading to higher rate of conversion to surgery in mCRC patients with left-sided tumors. Our data suggest that FOLFOXIRI plus bevacizumab may be a promising treatment for left-sided mCRC associated with RAS mutant tumors.

Sunakawa Y, Satake H, Ichikawa W. Considering FOLFOXIRI plus bevacizumab for metastatic colorectal cancer with left-sided tumors. *World J Gastrointest Oncol* 2018; 10(12): 528-531
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TO THE EDITOR

A randomized study, the TRIBE trial, has demonstrated that FOLFOXIRI plus bevacizumab is more beneficial than FOLFIRI plus bevacizumab as first-line treatment in patients with metastatic colorectal cancer (mCRC). In addition, a number of clinical studies, including the STEAM and CHARTA trials, have shown similar clinical benefits of treatment with FOLFOXIRI plus bevacizumab^[1-3]. Therefore, the triplet-regimen is considered one of the standard first-line treatments for mCRC in the National Comprehensive Cancer Network and European Society for Medical Oncology consensus guidelines^[4,5]. Recently, a subgroup analysis of the TRIBE trial was performed to investigate the effect of upfront tumor sidedness on therapeutic effectiveness and whether this potentially heterogeneous effect differed according to RAS and *BRAF* mutational status in mCRC. The results indicated that patients who harbored right-sided tumors achieved an evident survival benefit from the triplet-regimen as backbone chemotherapy regardless of RAS and *BRAF* status. Namely, FOLFOXIRI plus bevacizumab can be considered to be a preferred treatment option in a first-line setting for only right-sided mCRC^[6].

The location of the primary tumor has an impact on clinical behavior and has prognostic value in mCRC. A recent meta-analysis suggested that tumor sidedness is a predictive marker of the response to anti-epidermal growth factor receptor (EGFR) therapy in patients with RAS wild-type mCRC. Patients with left-sided tumors were shown to derive a greater benefit from chemotherapy plus an anti-EGFR antibody than from chemotherapy plus bevacizumab, whereas right-sided tumors were associated with trends toward

detrimental effects of anti-EGFR therapy^[7]. Therefore, anti-EGFR therapy with cetuximab or panitumumab is recommended for only RAS wild-type and left-sided tumors. A subanalysis of the TRIBE trial according to tumor sidedness showed no higher survival benefit from a triplet-regimen as compared with a doublet-regimen in patients with RAS wild-type mCRC who had left-sided tumors, suggesting that a doublet-regimen plus an anti-EGFR antibody is the preferred treatment for patients with left-sided RAS wild-type tumors. On the other hand, in patients with RAS mutant tumors, which do no benefit from anti-EGFR antibodies, the question remains whether intensification of the triplet regimen plus bevacizumab should be limited to patients who have mCRC with right-sided tumors.

In the subgroup analysis of the TRIBE trial, the objective response rate (ORR) of the triplet-arm was 65.0% for left-side tumors and 62.5% for right-side tumors in patients with RAS mutant tumors. The median progression-free survival (PFS) was 12.5 mo for patients with left-side tumors and 11.0 mo for those with right-side tumors. The efficacy of intensive chemotherapy with bevacizumab did not appear to differ significantly between tumor sidedness in patients with RAS mutant mCRC. Moreover, in patients who had left-sided tumors with RAS mutation, the ORR and PFS were slightly but not significantly higher in the triplet-arm than in the doublet-arm.

We have conducted a phase II trial of first-line FOLFOXIRI plus bevacizumab for mCRC with RAS mutant tumors. The ORR was the primary endpoint, and the secondary endpoints included PFS, early tumor shrinkage (ETS), and depth of response (DpR). The ORR and ETS rates in enrolled patients were 75.8% and 73.8%, respectively. According to primary tumor side, the ORR and ETS were both much better in patients with left-sided tumors than in patients with right-sided tumors (82.2% vs 58.8%, 77.3% vs 64.7%, respectively)^[8]. Here, we performed an exploratory analysis of DpR using spider-plots in each tumor side. Interestingly, the spider-plots demonstrated that tumor shrinkage was better and the duration of treatment was longer in the patients with left-sided tumors than in those with right-sided tumors (Figure 1). One (6%) of 17 patients could undergo conversion surgery in the right-sided group, while 11 (28%) of 40 patients could receive conversion surgery in the left-sided group. The early and deep responses to the triplet regimen in the left-sided group may facilitate conversion treatment associated with favorable survival.

We showed an analysis of the evaluated radiographic responses to FOLFOXIRI plus bevacizumab for mCRC according to each tumor side using spider plots. Cremolini *et al.*^[9] has reported the association of ETS and DpR with clinical outcomes of triplet plus bevacizumab treatment using the TRIBE data; however, the results according to tumor sidedness have not been reported yet. Our phase II trial was a prospective

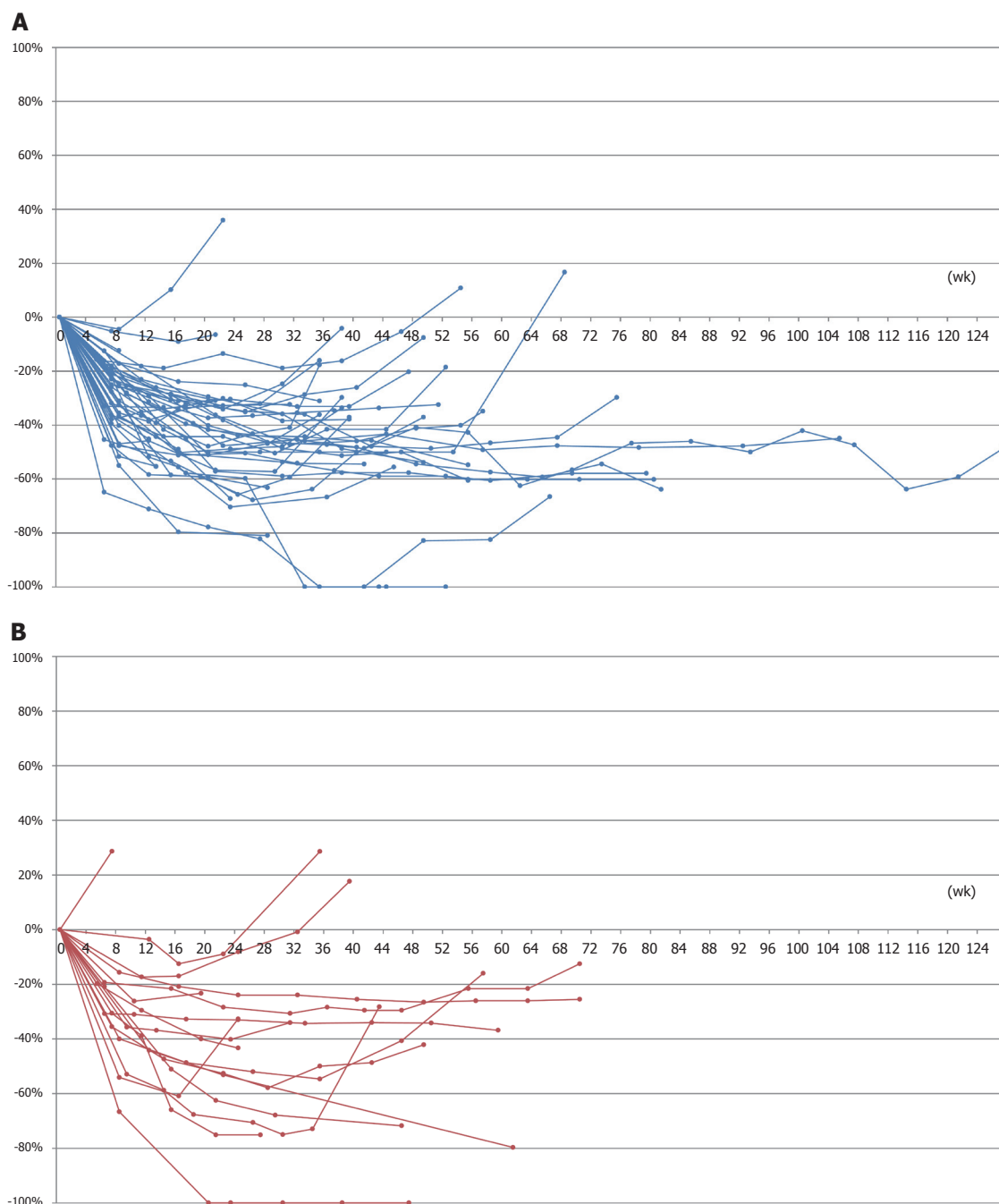


Figure 1 Spider plots of the response to 1st-line FOLFOXIRI plus bevacizumab in patients with RAS mutation (JACCRO CC-11). A: Spider plots of tumor burden changes in patients with left-sided tumors ($n = 44$); B: Spider plots of tumor burden changes in patients with right-sided tumors ($n = 17$).

study designed to evaluate the efficacy of FOLFOXIRI plus bevacizumab in molecular-selected patients with RAS mutation. Moreover, tumor response as a primary endpoint was evaluated prospectively by an external review board. Our findings for RAS mutant mCRC would be more reliable than the findings of the sub-analysis of the TRIBE trial. FOLFOXIRI plus bevacizumab was not beneficial in left-sided tumors in the TRIBE trial, while FOLFOXIRI plus bevacizumab appeared to be better compared to FOLFOX plus bevacizumab in left-sided tumors in other 2 trials^[2,3]. Types of backbone chemotherapy may affect the results of sub-analyses

by tumor sidedness. Although a recent study reported that FOLFOXIRI plus bevacizumab may be regarded as a preferred option for only right-sided mCRC^[6], our data suggest that the triplet-regimen may be a promising treatment for left-sided mCRC with RAS mutant tumors.

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