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Contents

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EDITORIAL

TOPIC HIGHLIGHT

CASE REPORT

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EDITORIAL

Targeted therapies for pancreatic adenocarcinoma: Where do we stand, how far can we go?

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Abstract

Pancreatic adenocarcinoma (usually referred to as pancreatic cancer) is a highly lethal and aggressive malignancy with a disease-related mortality almost equaling its incidence, and one of the most challenging cancers to treat. The notorious resistance of pancreatic cancer not only to conventional cytotoxic therapies but also to almost all targeted agents developed to date, continues to puzzle the oncological community and represents one of the biggest hurdles to reducing the death toll from this ominous disease. This editorial highlights the most important recent advances in preclinical and clinical research, with regards to targeted therapeutics for pancreatic cancer, outlines current challenges and provides an overview of potential future perspectives in this rapidly evolving field.

Key words: Clinical; Cytotoxic chemotherapy; Pancreatic cancer; Preclinical; Targeted agents

Core tip: Expansion of our knowledge regarding the molecular basis of pancreatic cancer has facilitated the development of a significant number of innovative targeted therapies for this lethal disease. Almost all these agents have, nevertheless, failed to produce statistically significant survival benefits when tested in clinical trial settings; therefore, successful clinical translation of preclinical advancements in pancreatic cancer research has yet to be materialized. Future treatment options might include multi-targeted and individualized molecular therapies, ideally guided by patient-specific genomic data, in combination with conventional cytotoxic or other regimens.

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INTRODUCTION

Despite recent advances in our understanding of the molecular mechanisms involved in the development and progression of pancreatic adenocarcinoma and an abundance of preclinical data suggesting the potential value of several targeted agents in treatment of this lethal disease, pancreatic cancer statistics remain grim and nearly the same as they were almost 30 years ago[1-3]. Pancreatic adenocarcinoma - usually referred to as "pancreatic cancer" - currently ranks as the fourth most frequent cause of cancer-related death among males and the fifth among females in the Western world, and is sadly expected to rise to the second leading position within the next decade[3,4]. Median survival is 4 to 6 mo following diagnosis while long term (5-year) survival rates do not exceed 4%-5%, for all stages combined[5]. The only treatment option with a curative potential is surgery, but less than 20% of patients are eligible for this approach, while the survival rates are poor (25%-30%) even among those with localized node-negative disease undergoing complete surgical resection and adjuvant chemotherapy[6].

This dismal clinical record inevitably leads to the following questions: Why have we failed thus far to reduce the death toll from this lethal disease? And, most importantly, what can we do to widen the range of available treatment options and improve their clinical effectiveness?

PRECLINICAL AND CLINICAL DATA: DISCREPANCY PREVAILS

In the preclinical arena of pancreatic cancer research the picture is much rosier; a significant and rather rapidly expanding number of different targeted agents have shown considerable efficacy in controlling growth of human pancreatic cancer cells, both *in vitro* and *in vivo*, and prolonging survival of pancreatic cancer models, as summarized in recent reviews on this topic[5-11]. This rather extensive armamentarium includes, among others, inhibitors of epidermal growth factor receptor (EGFR)[12,13], human epidermal growth factor receptor 2 (HER2)[14,15], vascular endothelial growth factor (VEGF) and VEGF receptors[16], insulin-like growth factor receptor[17-19], KRAS and its downstream effectors (mainly mitogen-activated protein kinase)[20,21], the developmental Wnt, Hedgehog and Notch signaling pathways[22-24], as well as reagents targeting the tumor extracellular matrix/stromal microenvironment or molecules overexpressed in the surface of pancreatic cancer cells (*i.e.,* mesothelin, carcinoembryonic antigen, epithelial cell adhesion molecule, MUC1)[25-29]. Dual-agent and multi-kinase molecular targeting represent additional exciting therapeutic possibilities and are gaining increasing research attention and popularity[30-34]. Alternative approaches, such as targeting the cellular process of autophagy - which plays a key role in the development and progression of malignancy or combined targeting of oncogene-driven signaling pathways and critical energy sources (such as mitochondrial respiration) of the subpopulation of dormant tumor cells surviving oncogene ablation, have also been studied as potential treatment options in pancreatic cancer, but are still in their infancy[7,35,36]. Interestingly, in accordance with increasing data suggesting potential preventive and therapeutic effects of aspirin and non-steroidal inflammatory drugs in gastrointestinal cancers, particularly colorectal cancer[37,38], aspirin is being explored as a targeted therapeutic agent for pancreatic cancer as well[39,40]. As shown in recent preclinical studies, aspirin, either alone or in combination with the antidiabetic drug metformin, may inhibit pancreatic cancer cell growth, counteract desmoplasia and cancer stem cell features and enhance the therapeutic efficacy of cytotoxic agents-such as gemcitabine- in pancreatic cancer by sensitizing pancreatic cancer cells to chemotherapy-mediated cytotoxicity[41-43].

Modified cytotoxic agents, mainly including nab-paclitaxel (paclitaxel conjugated with albumin nano-particles) or other nanovector-based anticancer drugs, such as cationic liposome encapsulated paclitaxel (EndoTAGTM-1) or liposomal doxorubicin, cisplatin and irinotecan, have been recently developed using sophisticated nanotechnology and tested in preclinical studies of pancreatic cancer, with some encouraging results[7,44-49]. These selective drug formulations offer the advantage of improved drug delivery to the tumor tissue and selective targeting *via* binding to tumor-associated receptors or macromolecules, thus positively modulating the pharmacokinetics and therapeutic index of cytotoxic chemotherapy[44]. Nab-paclitaxel, in particular, can bind to SPARC (secreted protein acid and rich in cysteine), an extracellular matrix protein which is frequently overexpressed in pancreatic adenocarcinomas[10,50,51], and, presumably, result in depletion of desmoplastic tumor stroma and an increase in vascularization, thus enhancing transvascular transport and delivery of cytotoxic agents to tumor cells[52].

The overwhelming majority of the abovementioned targeted therapies have, nevertheless, failed to demonstrate any statistically significant efficacy in clinical trials of pancreatic cancer patients; the EGFR and VEGF monoclonal antibodies cetuximab and bevacizumab, respectively, and the multikinase inhibitor sorafenib are representative examples of once-promising targeted agents who failed to produce a statistically significant improvement of survival when used in combination with gemcitabine *vs* gemcitabine alone in phase III randomized trials[53-55]. Hence, successful translation of our otherwise encouraging preclinical achievements into tangible clinical benefit remains an elusive goal. Two notable exceptions, though, leave some room for optimism. Erlotinib, an EGFR tyrosine kinase inhibitor which was United States Food and Drug Administration (FDA)-approved in 2007 for the treatment of advanced pancreatic cancer, is the first targeted agent which

succeeded in producing a significant-albeit modest-survival benefit when administered as an adjunct to gemcitabine, especially among patients experiencing erlotinib-induced skin rash[7,56]; still, given the marginal effect of erlotinib on survival and its unclear therapeutic value in localized, resectable disease this drug has yet to be widely adopted as standard of care in routine clinical practice[8,10]. Based on the results of the recent phase Ⅲ Metastatic Pancreatic Adenocarcinoma Clinical Trial[57] of nab-paclitaxel and gemcitabine combination *vs* gemcitabine alone in 861 patients with metastatic pancreatic cancer, showing a statistically significant survival benefit (as regards overall, progression-free and 1-year survival) in the combinatorial arm, nab-paclitaxel was also approved by the FDA in 2013 to be administered in combination with gemcitabine as first-line therapy for metastatic pancreatic cancer.

CONCLUSION

Considering all available evidence, as summarized above, we should first acknowledge that, although some revolutionary progress has indeed been achieved on the theoretical front, preclinical enthusiasm has been severely tempered by clinical disappointment. The reasons behind this discrepancy remain largely unknown and can only be speculated upon at this point. Resistance of pancreatic cancer to anticancer drugs, including both standard cytotoxic and novel targeted agents, is often attributed to the abundant, dense, fibroinflammatory stroma surrounding pancreatic tumor tissue, which is believed to function as a barrier to efficient delivery of drug formulations to their target tumor cells by restricting blood supply and limiting diffusion of large molecules[10,58,59]. The high genetic heterogeneity and complexity of pancreatic cancer may also explain why targeting a specific mutation in a tumor containing 63 genetic alterations on average -as shown by previous genomic studies[22,60] - or "randomly combining drugs in the hope of achieving a better outcome in an unselected patient population"[10], may be doomed to fail.

Hopefully, the results of ongoing clinical trials on current and emerging targeted therapeutics, including, among others, the anti-EGFR and anti-HER2/neu monoclonal antibodies nimotuzumab (NCT02395016) and trastuzumab (NCT01204372), respectively, the hedgehog inhibitors vismodegib (NCT01195415) and LDE225 (NCT01485744) and agents targeting the Notch pathway, such as the gamma-secretase inhibitor MK-0752 (NCT01098344), may help bridge the gap between preclinical and clinical outcomes. The increasing advances in structural and functional genomics are also expected to further elucidate the key molecular events underlying pancreatic tumorigenesis and identify additional targets for novel agents. Based on data derived from global genomic analyses of pancreatic tumors, previous authors have suggested that agents broadly targeting downstream mediators of critical physiologic functions (such as neo-angiogenesis or cell cycle alterations) may be preferable to agents targeting specific mutated genes[60]. Most importantly, personalized genomic medicine, utilizing patient-specific genomic data for guidance of treatment selection in each individual patient, may not only significantly enhance the clinical efficacy of molecular targeted therapy but also reduce the burden of unnecessary - and potentially harmful-drugs.

As previously commented by Kleger *et al*[7], in a recent review article critically discussing current and future targeted therapies for pancreatic cancer, "smart drugs need smart applications". Indeed, most experts concur that the latter applications should include multi-targeted and, ideally, individualized molecular therapies, in combination with conventional cytotoxic agents or other regimens (such as immunotherapy)[61], guided by reliable biomarkers of treatment response. Increased toxicity resulting from these combinatorial approaches as well as their cost-effectiveness and socioeconomic implications should, nevertheless, be carefully considered and may represent major limiting factors for their widespread use. In a disease as aggressive and lethal as pancreatic cancer, maintaining the highest possible quality of life for as long as possible is the most important target, and expectations should always be based on realistic goals.

REFERENCES

1 **Tanaka S**. Molecular Pathogenesis and Targeted Therapy of Pancreatic Cancer. *Ann Surg Oncol* 2015 Mar 7; Epub ahead of print [PMID: 25749932 DOI: 10.1245/s10434-015-4463-x]

2 **Vincent A**, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet* 2011; **378**: 607-620 [PMID: 21620466 DOI: 10.1016/S0140-6736(10)62307-0]

3 **Krejs GJ**. Pancreatic cancer: epidemiology and risk factors. *Dig Dis* 2010; **28**: 355-358 [PMID: 20814212 DOI: 10.1159/000319414]

4 **Cardin DB**, Berlin JD. Pancreas cancer on the rise: are we up to the challenge? *J Natl Cancer Inst* 2013; **105**: 1675-1676 [PMID: 24203986 DOI: 10.1093/jnci/djt316]

5 **Saif MW**. Pancreatic neoplasm in 2011: an update. *JOP* 2011; **12**: 316-321 [PMID: 21737886]

6 **Teague A**, Lim KH, Wang-Gillam A. Advanced pancreatic adenocarcinoma: a review of current treatment strategies and developing therapies. *Ther Adv Med Oncol* 2015; **7**: 68-84 [PMID: 25755680 DOI: 10.1177/1758834014564775]

7 **Kleger A**, Perkhofer L, Seufferlein T. Smarter drugs emerging in pancreatic cancer therapy. *Ann Oncol* 2014; **25**: 1260-1270 [PMID: 24631947 DOI: 10.1093/annonc/mdu013]

8 **Antoniou G**, Kountourakis P, Papadimitriou K, Vassiliou V, Papamichael D. Adjuvant therapy for resectable pancreatic adenocarcinoma: review of the current treatment approaches and future directions. *Cancer Treat Rev* 2014; **40**: 78-85 [PMID: 23810287 DOI: 10.1016/j.ctrv.2013.05.008]

9 **Huang ZQ**, Buchsbaum DJ. Monoclonal antibodies in the treatment of pancreatic cancer. *Immunotherapy* 2009; **1**: 223-229 [PMID: 20046965 DOI: 10.2217/1750743X.1.2.223]

10 **Oettle H**. Progress in the knowledge and treatment of advanced pancreatic cancer: from benchside to bedside. *Cancer Treat Rev* 2014; **40**: 1039-1047 [PMID: 25087471 DOI: 10.1016/j.ctrv.2014.07.003]

11 **Ozmen F**, Şahin TT, Ozmen MM. Current adjuvant therapeutic

approaches for pancreatic cancer. *Adv Ther* 2015; **32**: 42-56 [PMID: 25595483 DOI: 10.1007/s12325-015-0177-5]

12 **Huang ZQ**, Buchsbaum DJ, Raisch KP, Bonner JA, Bland KI, Vickers SM. Differential responses by pancreatic carcinoma cell lines to prolonged exposure to Erbitux (IMC-C225) anti-EGFR antibody. *J Surg Res* 2003; **111**: 274-283 [PMID: 12850474 DOI: 10.1016/S0022-4804(03)00076-3]

13 **Morgan MA**, Parsels LA, Kollar LE, Normolle DP, Maybaum J, Lawrence TS. The combination of epidermal growth factor receptor inhibitors with gemcitabine and radiation in pancreatic cancer. *Clin Cancer Res* 2008; **14**: 5142-5149 [PMID: 18698032 DOI: 10.1158/1078-0432.CCR-07-4072]

14 **Saeki H**, Yanoma S, Takemiya S, Sugimasa Y, Akaike M, Yukawa N, Rino Y, Imada T. Antitumor activity of a combination of trastuzumab (Herceptin) and oral fluoropyrimidine S-1 on human epidermal growth factor receptor 2-overexpressing pancreatic cancer. *Oncol Rep* 2007; **18**: 433-439 [PMID: 17611667]

15 **Kimura K**, Sawada T, Komatsu M, Inoue M, Muguruma K, Nishihara T, Yamashita Y, Yamada N, Ohira M, Hirakawa K. Antitumor effect of trastuzumab for pancreatic cancer with high HER-2 expression and enhancement of effect by combined therapy with gemcitabine. *Clin Cancer Res* 2006; **12**: 4925-4932 [PMID: 16914581 DOI: 10.1158/1078-0432.CCR-06-0544]

16 **Wilhelm SM**, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, Trail PA. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res* 2004; **64**: 7099-7109 [PMID: 15466206 DOI: 10.1158/0008-5472.CAN-04-1443]

17 **Neid M**, Datta K, Stephan S, Khanna I, Pal S, Shaw L, White M, Mukhopadhyay D. Role of insulin receptor substrates and protein kinase C-zeta in vascular permeability factor/vascular endothelial growth factor expression in pancreatic cancer cells. *J Biol Chem* 2004; **279**: 3941-3948 [PMID: 14604996 DOI: 10.1074/jbc.M303975200]

18 **Liu W**, Bloom DA, Cance WG, Kurenova EV, Golubovskaya VM, Hochwald SN. FAK and IGF-IR interact to provide survival signals in human pancreatic adenocarcinoma cells. *Carcinogenesis* 2008; **29**: 1096-1107 [PMID: 18263593 DOI: 10.1093/carcin/bgn026]

19 **Rowinsky EK**, Youssoufian H, Tonra JR, Solomon P, Burtrum D, Ludwig DL. IMC-A12, a human IgG1 monoclonal antibody to the insulin-like growth factor I receptor. *Clin Cancer Res* 2007; **13**: 5549s-5555s [PMID: 17875788 DOI: 10.1158/1078-0432.CCR-07-1109]

20 **End DW**, Smets G, Todd AV, Applegate TL, Fuery CJ, Angibaud P, Venet M, Sanz G, Poignet H, Skrzat S, Devine A, Wouters W, Bowden C. Characterization of the antitumor effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro. *Cancer Res* 2001; **61**: 131-137 [PMID: 11196150]

21 **Zimmermann G**, Papke B, Ismail S, Vartak N, Chandra A, Hoffmann M, Hahn SA, Triola G, Wittinghofer A, Bastiaens PI, Waldmann H. Small molecule inhibition of the KRAS-PDEδ interaction impairs oncogenic KRAS signalling. *Nature* 2013; **497**: 638-642 [PMID: 23698361 DOI: 10.1038/nature12205]

22 **Witkiewicz AK**, McMillan EA, Balaji U, Baek G, Lin WC, Mansour J, Mollaee M, Wagner KU, Koduru P, Yopp A, Choti MA, Yeo CJ, McCue P, White MA, Knudsen ES. Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. *Nat Commun* 2015; **6**: 6744 [PMID: 25855536 DOI: 10.1038/ncomms7744]

23 **Olive KP**, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Rückert F, Grützmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 2009; **324**: 1457-1461 [PMID: 19460966 DOI: 10.1126/science.1171362]

24 **Yen WC**, Fischer MM, Axelrod F, Bond C, Cain J, Cancilla B, Henner WR, Meisner R, Sato A, Shah J, Tang T, Wallace B, Wang M, Zhang C, Kapoun AM, Lewicki J, Gurney A, Hoey T. Targeting Notch signaling with a Notch2/Notch3 antagonist (tarextumab) inhibits tumor growth and decreases tumor-initiating cell frequency. *Clin Cancer Res* 2015; **21**: 2084-2095 [PMID: 25934888 DOI: 10.1158/1078-0432]

25 **Golfier S**, Kopitz C, Kahnert A, Heisler I, Schatz CA, Stelte-Ludwig B, Mayer-Bartschmid A, Unterschemmann K, Bruder S, Linden L, Harrenga A, Hauff P, Scholle FD, Müller-Tiemann B, Kreft B, Ziegelbauer K. Anetumab ravtansine: a novel mesothelin-targeting antibody-drug conjugate cures tumors with heterogeneous target expression favored by bystander effect. *Mol Cancer Ther* 2014; **13**: 1537-1548 [PMID: 24714131 DOI: 10.1158/1535-7163]

26 **Showalter SL**, Huang YH, Witkiewicz A, Costantino CL, Yeo CJ, Green JJ, Langer R, Anderson DG, Sawicki JA, Brody JR. Nanoparticulate delivery of diphtheria toxin DNA effectively kills Mesothelin expressing pancreatic cancer cells. *Cancer Biol Ther* 2008; **7**: 1584-1590 [PMID: 19039293 DOI: 10.4161/cbt.7.10.6562]

27 **Maawy AA**, Hiroshima Y, Zhang Y, Heim R, Makings L, Garcia-Guzman M, Luiken GA, Kobayashi H, Hoffman RM, Bouvet M. Near infra-red photoimmunotherapy with anti-CEA-IR700 results in extensive tumor lysis and a significant decrease in tumor burden in orthotopic mouse models of pancreatic cancer. *PLoS One* 2015; **10**: e0121989 [PMID: 25799218 DOI: 10.1371/journal.pone.0121989]

28 **Lund K**, Bostad M, Skarpen E, Braunagel M, Kiprijanov S, Krauss S, Duncan A, Høgset A, Selbo PK. The novel EpCAM-targeting monoclonal antibody 3-17I linked to saporin is highly cytotoxic after photochemical internalization in breast, pancreas and colon cancer cell lines. *MAbs* 2014; **6**: 1038-1050 [PMID: 24525727 DOI: 10.4161/mabs.28207]

29 **Tholey RM**, Lal S, Jimbo M, Burkhart RA, Blanco FF, Cozzitorto JA, Eisenberg JD, Jiang W, Iacobuzio-Donahue CA, Witkiewicz AK, Gibert M, Yeo CJ, Brody JR, Sawicki JA, Winter JM. MUC1 Promoter-Driven DTA as a Targeted Therapeutic Strategy against Pancreatic Cancer. *Mol Cancer Res* 2015; **13**: 439-448 [PMID: 25336517 DOI: 10.1158/1541-7786.MCR-14-0199]

30 **Larbouret C**, Robert B, Bascoul-Mollevi C, Penault-Llorca F, Ho-Pun-Cheung A, Morisseau S, Navarro-Teulon I, Mach JP, Pèlegrin A, Azria D. Combined cetuximab and trastuzumab are superior to gemcitabine in the treatment of human pancreatic carcinoma xenografts. *Ann Oncol* 2010; **21**: 98-103 [PMID: 19889608 DOI: 10.1093/annonc/mdp496]

31 **Larbouret C**, Gaborit N, Chardès T, Coelho M, Campigna E, Bascoul-Mollevi C, Mach JP, Azria D, Robert B, Pèlegrin A. In pancreatic carcinoma, dual EGFR/HER2 targeting with cetuximab/trastuzumab is more effective than treatment with trastuzumab/erlotinib or lapatinib alone: implication of receptors' down-regulation and dimers' disruption. *Neoplasia* 2012; **14**: 121-130 [PMID: 22431920 DOI: 10.1593/neo.111602]

32 **Bianco C**, Giovannetti E, Ciardiello F, Mey V, Nannizzi S, Tortora G, Troiani T, Pasqualetti F, Eckhardt G, de Liguoro M, Ricciardi S, Del Tacca M, Raben D, Cionini L, Danesi R. Synergistic antitumor activity of ZD6474, an inhibitor of vascular endothelial growth factor receptor and epidermal growth factor receptor signaling, with gemcitabine and ionizing radiation against pancreatic cancer. *Clin Cancer Res* 2006; **12**: 7099-7107 [PMID: 17145834 DOI: 10.1158/1078-0432.CCR-06-0833]

33 **Pan Y**, Zheng M, Zhong L, Yang J, Zhou S, Qin Y, Xiang R, Chen Y, Yang SY. A preclinical evaluation of SKLB261, a multikinase inhibitor of EGFR/Src/VEGFR2, as a therapeutic agent against pancreatic cancer. *Mol Cancer Ther* 2015; **14**: 407-418 [PMID: 25519702 DOI: 10.1158/1535-7163.MCT-14-0485]

34 **Ulivi P**, Arienti C, Zoli W, Scarsella M, Carloni S, Fabbri F, Tesei A, Chiadini E, Orlandi A, Passeri D, Zupi G, Milandri C, Silvestrini R, Amadori D, Leonetti C. In vitro and in vivo antitumor efficacy

of docetaxel and sorafenib combination in human pancreatic cancer cells. *Curr Cancer Drug Targets* 2010; **10**: 600-610 [PMID: 20491617 DOI: 10.2174/156800910791859489]

35 **Donadelli M**, Dando I, Zaniboni T, Costanzo C, Dalla Pozza E, Scupoli MT, Scarpa A, Zappavigna S, Marra M, Abbruzzese A, Bifulco M, Caraglia M, Palmieri M. Gemcitabine/cannabinoid combination triggers autophagy in pancreatic cancer cells through a ROS-mediated mechanism. *Cell Death Dis* 2011; **2**: e152 [PMID: 21525939 DOI: 10.1038/cddis.2011.36]

36 **Viale A**, Pettazzoni P, Lyssiotis CA, Ying H, Sánchez N, Marchesini M, Carugo A, Green T, Seth S, Giuliani V, Kost-Alimova M, Muller F, Colla S, Nezi L, Genovese G, Deem AK, Kapoor A, Yao W, Brunetto E, Kang Y, Yuan M, Asara JM, Wang YA, Heffernan TP, Kimmelman AC, Wang H, Fleming JB, Cantley LC, DePinho RA, Draetta GF. Oncogene ablation-resistant pancreatic cancer cells depend on mitochondrial function. *Nature* 2014; **514**: 628-632 [PMID: 25119024 DOI: 10.1038/nature13611]

37 **Liao X**, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, Imamura Y, Qian ZR, Baba Y, Shima K, Sun R, Nosho K, Meyerhardt JA, Giovannucci E, Fuchs CS, Chan AT, Ogino S. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med* 2012; **367**: 1596-1606 [PMID: 23094721 DOI: 10.1056/NEJMoa1207756]

38 **Domingo E**, Church DN, Sieber O, Ramamoorthy R, Yanagisawa Y, Johnstone E, Davidson B, Kerr DJ, Tomlinson IP, Midgley R. Evaluation of PIK3CA mutation as a predictor of benefit from nonsteroidal anti-inflammatory drug therapy in colorectal cancer. *J Clin Oncol* 2013; **31**: 4297-4305 [PMID: 24062397 DOI: 10.1200/JCO.2013.50.0322]

39 **Shen X**, Han L, Ma Z, Chen C, Duan W, Yu S, Li P, Zhang L, Li W, Xu Q, Ma Q. Aspirin: a potential therapeutic approach in pancreatic cancer. *Curr Med Chem* 2013; **20**: 4153-4162 [PMID: 23895681 DOI: 10.2174/09298673113209990196]

40 **Yue W**, Yang CS, DiPaola RS, Tan XL. Repurposing of metformin and aspirin by targeting AMPK-mTOR and inflammation for pancreatic cancer prevention and treatment. *Cancer Prev Res* (Phila) 2014; **7**: 388-397 [PMID: 24520038 DOI: 10.1158/1940-6207.CAPR-13-0337]

41 **Zhang Y**, Liu L, Fan P, Bauer N, Gladkich J, Ryschich E, Bazhin AV, Giese NA, Strobel O, Hackert T, Hinz U, Gross W, Fortunato F, Herr I. Aspirin counteracts cancer stem cell features, desmoplasia and gemcitabine resistance in pancreatic cancer. *Oncotarget* 2015; **6**: 9999-10015 [PMID: 25846752]

42 **Ou YQ**, Zhu Wb, Li Y, Qiu PX, Huang YJ, Xie J, He SM, Zheng XK, Leng TD, Xu D, Yan GM. Aspirin inhibits proliferation of gemcitabine-resistant human pancreatic cancer cells and augments gemcitabine-induced cytotoxicity. *Acta Pharmacol Sin* 2010; **31**: 73-80 [PMID: 19966835 DOI: 10.1038/aps.2009.172]

43 **Yue W**, Zheng X, Lin Y, Yang CS, Xu Q, Carpizo D, Huang H, DiPaola RS, Tan XL. Metformin combined with aspirin significantly inhibit pancreatic cancer cell growth in vitro and in vivo by suppressing anti-apoptotic proteins Mcl-1 and Bcl-2. *Oncotarget* 2015; **6**: 21208-21224 [PMID: 26056043]

44 **Tsai CS**, Park JW, Chen LT. Nanovector-based therapies in advanced pancreatic cancer. *J Gastrointest Oncol* 2011; **2**: 185-194 [PMID: 22811849 DOI: 10.3978/j.issn.2078-6891.2011.034]

45 **Neesse A**, Frese KK, Chan DS, Bapiro TE, Howat WJ, Richards FM, Ellenrieder V, Jodrell DI, Tuveson DA. SPARC independent drug delivery and antitumour effects of nab-paclitaxel in genetically engineered mice. *Gut* 2014; **63**: 974-983 [PMID: 24067278 DOI: 10.1136/gutjnl-2013-305559]

46 **Eichhorn ME**, Ischenko I, Luedemann S, Strieth S, Papyan A, Werner A, Bohnenkamp H, Guenzi E, Preissler G, Michaelis U, Jauch KW, Bruns CJ, Dellian M. Vascular targeting by EndoTAG-1 enhances therapeutic efficacy of conventional chemotherapy in lung and pancreatic cancer. *Int J Cancer* 2010; **126**: 1235-1245 [PMID: 19697323 DOI: 10.1002/ijc.24846]

47 **Mamidi RN**, Weng S, Stellar S, Wang C, Yu N, Huang T, Tonelli AP, Kelley MF, Angiuoli A, Fung MC. Pharmacokinetics, efficacy and toxicity of different pegylated liposomal doxorubicin formulations in preclinical models: is a conventional bioequivalence approach sufficient to ensure therapeutic equivalence of pegylated liposomal doxorubicin products? *Cancer Chemother Pharmacol* 2010; **66**: 1173-1184 [PMID: 20661737 DOI: 10.1007/s00280-010-1406-x]

48 **Yoshida M**, Takimoto R, Murase K, Sato Y, Hirakawa M, Tamura F, Sato T, Iyama S, Osuga T, Miyanishi K, Takada K, Hayashi T, Kobune M, Kato J. Targeting anticancer drug delivery to pancreatic cancer cells using a fucose-bound nanoparticle approach. *PLoS One* 2012; **7**: e39545 [PMID: 22808043 DOI: 10.1371/journal.pone.0039545]

49 **Pal A**, Khan S, Wang YF, Kamath N, Sarkar AK, Ahmad A, Sheikh S, Ali S, Carbonaro D, Zhang A, Ahmad I. Preclinical safety, pharmacokinetics and antitumor efficacy profile of liposome-entrapped SN-38 formulation. *Anticancer Res* 2005; **25**: 331-341 [PMID: 15816556]

50 **Neuzillet C**, Tijeras-Raballand A, Cros J, Faivre S, Hammel P, Raymond E. Stromal expression of SPARC in pancreatic adenocarcinoma. *Cancer Metastasis Rev* 2013; **32**: 585-602 [PMID: 23690170 DOI: 10.1007/s10555-013-9439-3]

51 **Sinn M**, Sinn BV, Striefler JK, Lindner JL, Stieler JM, Lohneis P, Bischoff S, Bläker H, Pelzer U, Bahra M, Dietel M, Dörken B, Oettle H, Riess H, Denkert C. SPARC expression in resected pancreatic cancer patients treated with gemcitabine: results from the CONKO-001 study. *Ann Oncol* 2014; **25**: 1025-1032 [PMID: 24562449 DOI: 10.1093/annonc/mdu084]

52 **Al-Batran SE**, Geissler M, Seufferlein T, Oettle H. Nab-paclitaxel for metastatic pancreatic cancer: clinical outcomes and potential mechanisms of action. *Oncol Res Treat* 2014; **37**: 128-134 [PMID: 24685917 DOI: 10.1159/000358890]

53 **Philip PA**, Benedetti J, Corless CL, Wong R, O'Reilly EM, Flynn PJ, Rowland KM, Atkins JN, Mirtsching BC, Rivkin SE, Khorana AA, Goldman B, Fenoglio-Preiser CM, Abbruzzese JL, Blanke CD. Phase III study comparing gemcitabine plus cetuximab versus gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group-directed intergroup trial S0205. *J Clin Oncol* 2010; **28**: 3605-3610 [PMID: 20606093 DOI: 10.1200/JCO.2009.25.7550]

54 **Van Cutsem E**, Vervenne WL, Bennouna J, Humblet Y, Gill S, Van Laethem JL, Verslype C, Scheithauer W, Shang A, Cosaert J, Moore MJ. Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol* 2009; **27**: 2231-2237 [PMID: 19307500 DOI: 10.1200/JCO.2008.20.0238]

55 **Kindler HL**, Wroblewski K, Wallace JA, Hall MJ, Locker G, Nattam S, Agamah E, Stadler WM, Vokes EE. Gemcitabine plus sorafenib in patients with advanced pancreatic cancer: a phase II trial of the University of Chicago Phase II Consortium. *Invest New Drugs* 2012; **30**: 382-386 [PMID: 20803052 DOI: 10.1007/s10637-010-9526-z]

56 **Moore MJ**, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960-1966 [PMID: 17452677]

57 **Tabernero J**, Chiorean EG, Infante JR, Hingorani SR, Ganju V, Weekes C, Scheithauer W, Ramanathan RK, Goldstein D, Penenberg DN, Romano A, Ferrara S, Von Hoff DD. Prognostic factors of survival in a randomized phase III trial (MPACT) of weekly nab-paclitaxel plus gemcitabine versus gemcitabine alone in patients with metastatic pancreatic cancer. *Oncologist* 2015; **20**: 143-150 [PMID: 25582141 DOI: 10.1634/theoncologist.2014-0394]

58 **Hidalgo M**. Pancreatic cancer. *N Engl J Med* 2010; **362**: 1605-1617 [PMID: 20427809 DOI: 10.1056/NEJMra0901557]

59 **Trédan O**, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. *J Natl Cancer Inst* 2007; **99**: 1441-1454 [PMID: 17895480]

60 **Jones S**, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin

MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; **321**: 1801-1806 [PMID: 18772397 DOI: 10.1126/science.1164368]

61 **Springett GM**. Novel pancreatic cancer vaccines could unleash the army within. *Cancer Control* 2014; **21**: 242-246 [PMID: 24955709]

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TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Fecal DNA testing for colorectal cancer screening: Molecular targets and perspectives

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Abstract

The early detection of colorectal cancer with effective screening is essential for reduction of cancer-specific mortality. The addition of fecal DNA testing in the armamentarium of screening methods already in clinical use launches a new era in the noninvasive part of colorectal cancer screening and emanates from a large number of previous and ongoing clinical investigations and technological advancements. In this review, we discuss the molecular rational and most important genetic alterations hallmarking the early colorectal carcinogenesis process. Also, representative DNA targets-markers and key aspects of their testing at the clinical level in comparison or/and association with other screening methods are described. Finally, a critical view of the strengths and limitations of fecal DNA tests is provided, along with anticipated barriers and suggestions for further exploitation of their use.

Key words: Colorectal cancer; Screening; Fecal DNA; Cologuard®; Adenoma

Core tip: The molecular DNA targets from genetic and epigenetic alterations hallmarking colorectal carcinogenesis are reviewed here in the context of fecal testing. Also, comparison with other screening methods in terms of limitations, advantages and future perspectives of fecal DNA tests are discussed.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in men and women and accounts for 8% of all cancer-related deaths[1]. The incidence of CRC varies within different geographic locations and racial/ethnic groups. These differences may be related with different dietary and environmental exposures in association with a different genotype-driven susceptibility[2]. Screening for CRC plays a key role in reduction of CRC-related mortality, and the observed decline in the incidence of CRC since the mid-1980s is a striking proof of this effect, along with changes in risk factors[1].

CRC screening may be divided into two main categories: (1) biological sample-based tests, including fecal, blood and urine tests, as well as (2) colon structure-based and image-based tests, including flexible sigmoidoscopy, total colonoscopy, CT colonography and double-contrast barium enema[3,4]. Stool-based tests, including guaiac-based fecal occult blood test (g-FOBT), and the newer ones, fecal immunochemical test (FIT) and stool DNA test are already included in the American Cancer Society recommendations for CRC screening[4].

MOLECULAR RATIONAL FOR FECAL DNA TESTING

The detection of altered DNA from cancerous and pre-cancerous lesions of the colonic mucosa is based on the natural exfoliation of these cells and is further facilitated by their high degree of "integrity" compared to DNA from stools of healthy patients. Accumulating data on key mutations occurring during the early stages of colon carcinogenesis including K-Ras, adenoma polyposis coli (APC), and p53, as well as epigenetic changes such as microsatellite instability (MSI), has guided the targeted development of clinically relevant detection tests[5].

The genetic heterogeneity of CRC is essentially the reason underlying the concept of targeting multiple DNA markers. K-Ras encodes a RAS family protein which is a GTPase involved in many downstream signal transduction pathways[6]. The mutation is found in 13%-95% of CRC patients and is one of the initial mutations in colon carcinogenesis[6]. APC is an important tumor suppressor gene product involved in the Wnt/β-catenin signaling pathway, which in turn is a transcription regulator of several growth-controlling genes, including the oncogene *MYC*[7]. Thus it is not surprising that mutation or inactivation of the APC protein is a driver of inherited (familial adenomatous polyposis) and sporadic forms of CRC, occuring in the early stages of transition from adenoma to carcinoma[7]. Another tumor suppressor gene, *p53* is found deleted or mutated in 30%-60% of CRC tumors[8]. Given its critical role in cell cycle control, apoptosis, and DNA damage response, p53 aberrations ultimately promote the development of increased genomic instability which facilitates transformation of colorectal adenomas to cancer[7].

MSI is a condition of genetic hypermutability within tandem repeats of short nucleotide sequences, the microsatellites, that results from impaired DNA mismatch repair (MMR) and is a frequent event in cancers, including 15% of all CRC[9]. The most common cause of sporadic MSI is epigenetic silencing of *MMR* genes, such as MLH1 due to promoter hypermethylation[7] and there are several MSI markers (BAT25, BAT26, D2S123, D5S346, and D17S2720) for detection of MSI with polymerase chain reaction. The clinical relevance of MSI lies in the fact that patients with MSI positive tumors have better prognosis and longer overall survival compared with non-MSI tumors[9].

Epigenetic methylation of gene promoters is a central mechanism that can promote carcinogenesis in the appropriate context and several preclinical studies have identified hypermethylated genes in stool samples from CRC patients, which are strikingly un-methylated in normal epithelial cells[9]. Characteristic examples include the genes secreted frizzled-related protein (SFRP), vimentin, MGMT, FBN1, and p16[7]. In addition, the panel of methylated genes varies depending on the different stages of carcinogenesis, involving (1) SLC5A8, SFRP1, SFRP2, CDH13, CRBP1, RUNX3, MINT1 and MINT31 from normal colon mucosa to aberrant crypt focus formation; (2) p14, HLTF, ITGA4, p16, CDH1, and ESR1 from aberrant crypt focus to adenoma formation; and (3) TIMP3, CXCL12, ID4, and IRF8 from adenoma to carcinoma formation and metastatic progression of CRC[7].

CLINICAL STUDIES OF FECAL DNA TESTS

An important limiting factor for developing a screening stool test with high sensitivity is the fact that only 0.01% of total fecal DNA is human and the tumor DNA is only a small percentage of the former[10].

K-RAS was the first gene tested for mutations in feces from CRC patients[11-13]. A comparative study assessed gFOBT and a fecal DNA test analyzing a panel of 21 gene mutations[14]. Imperiale *et al*[14] concluded that the multitarget fecal DNA test detected more invasive cancers plus adenomas with high-grade dysplasia than did gFOBT (40.8% *vs* 14.1%) without compromising specificity (94.4% *vs* 95.2%). In a blinded, multicenter, case-control study, with cases including CRC, advanced adenoma (AA), or sessile serrated adenoma $\geqslant$ 1 cm (SSA), an automated multitarget stool DNA assay was able to detect AA with high-grade dysplasia with 83% sensitivity[15]. Another blinded, multicenter, case-control study assessing a similar panel of DNA markers identified 85% of patients with CRC and 54% with AA,

Table 1 Fecal DNA markers for advanced adenoma and colorectal cancer *n* (%)

Ref.	Marker	Sensitivity		Specificity
		CRC	Adenoma > 1 cm	
[12]	Meth BMP3, hDNA, KRAS, APC	67 (91)	21 (78)	85 (85)
[13]	APC, KRAS, p53, long DNA	3 (25)	47 (8)	2246 (96)
[14]	APC, KRAS, p53, long DNA	16 (52)	84 (12)	1344 (94)
[15]	β-actin, KRAS, meth BMP3 and NDRG4, fecal hemoglobin	91 (98)	48 (57)	139 (90)
[16]	KRAS, a actina Meth NDRG4, BMP3, vimentin, TFPI2	214 (85)	72 (54)	264 (90)
[17]	KRAS, NDRG4, BMP3, β-actin, fecal hemoglobin	60 (92)	321 (42)	4457 (90)
[20]	Meth vimentin	9 (41)	9 (45)	63 (95)
[21]	Meth SFRP2	60 (87)	21 (62)	28 (93)
[22]	Meth TFPI2, long DNA	52 (87)	4 (44)	25 (83)
[23]	Meth SFRP2, HPPI, MGMT	50 (96)	15 (71)	23 (96)
[24]	Meth APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1	15 (75)	17 (68)	27 (90)
[25]	Meth vimentin, long DNA	68 (83)	6 (86)	298 (82)
[26]	Meth RASSF2 or SFRP2	63 (75)	25 (44)	101 (89)
[27]	Meth vimentin, MLH1, MGMT	45 (75)	31 (60)	32 (87)
[28]	Meth RARB2, p16INK4a, MGMT, APC	16 (62)	8 (40)	20 (100)

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without sensitivity differences based on location, but with tumor size affecting detection rates[16].

More recently, Imperiale *et al*[17] reported their results from comparison of fecal DNA to FIT in a huge patient population who had a complete screening colonoscopy (n = 9989). The sensitivity of fecal DNA test including evaluation of KRAS mutations, aberrant NDRG4 and BMP3 methylation, B-actin and a hemoglobin assay was superior to that of FIT (92.3% *vs* 73.8%). However, in addition to a lower specificity of fecal DNA and the lack of comparison with repeated FIT applications over time, a far higher number of patients (n = 689) were excluded due to problematic fecal DNA testing, compared to those who underwent FIT (n = 34)[18].

A systematic review of the literature for studies of biomarkers for early detection of colorectal cancer and polyps since 2007, disclosed overall sensitivities for colorectal cancer detection by fecal DNA markers ranging from 53% to 87%, with varying specificities above 76%[19]. The diversity and combinations of various fecal DNA markers with the corresponding sensitivities and specificities per study[12-17,20-28] are summarized in Table 1.

EVOLUTION OF FECAL DNA TESTING METHODOLOGY AND TECHNIQUES

Initially, the first fecal DNA tests were performed without stabilizing buffers, resulting in low sensitivities[13,14]. Upon incorporation of stabilizing buffers and introduction of more sensitive detection techniques such as the digital melt curve method and beads, emulsion, amplification, and magnetics (BEAMing), the initial detection threshold of 1% of mutated copies was decreased to less than 0.1%[10,12].

Furthermore, implementation of the allele-specific quantitative real-time target and signal amplification (QuARTS) technique led to detection of less frequent mutations, thus improving the sensitivity for AA[12]. Another technique termed fluorescent long DNA (FL-DNA), allows for identification of tumor DNA fragments longer than 150-200 base pairs, given that cancer cells evade apoptosis and subsequent DNA degradation. FL-DNA detects CRC with a sensitivity of 80%[29]. Other advances that have been introduced in different studies include neutralization of bacterial enzymes with EDTA[30], enrichment of the panel of DNA markers (*e.g.,* vimentin gene), and inclusion of hemoglobin detection in the same panel[16,31].

STRENGTHS AND LIMITATIONS OF FECAL DNA TESTS

A major advantage of fecal DNA tests as compared to either FOBT or colonoscopy is the fact that they are not affected by proximal location of tumors[32,33]. Another advantage is the lack of need for purging or dietary changes.

However, the sensitivity of fecal DNA tests appears to be lower for adenomas when compared to CRC detection (Table 1). In addition, although there is evidence of reductions in CRC incidence and mortality from randomized controlled trials of fecal occult blood test (FOBT) screening[34], similar data are lacking for fecal DNA tests.

Other technical difficulties may involve the burden of large volume stool collection and shipping for the patients undergoing screening[31]. In addition, the fact that in the latest study of Imperiale *et al*[17] the DNA tests had over twice as many abnormal results as FIT, with a higher rate of false-positive results implies that more colonoscopies would be needed to further evaluate for CRC in the former arm. Thus, the inevitably higher number of diagnostic testing would increase the costs and risks of screening. Only with the current screening method of gFOBT, 690011 colonoscopies for false positive screening tests result in an additional estimated annual cost of £800000000[19].

Cost-effectiveness *per se* seems to be a major disadvantage of fecal DNA tests as both older and newer studies, particularly based on a Markov model, have concluded that fecal DNA is cost-effective only when compared with no screening, but is essentially dominated by most of the other available screening options, including FOBT and colonoscopy[36,37]. This may necessitate the limitation of number of DNA markers to render their clinical use more reasonable[38].

CURRENT STATUS OF FECAL DNA TESTING (COLOGUARD®)

The United States Food and Drug Administration has recently approved Cologuard® (Exact Sciences Corporation, Madison, WI, United States), a multitarget stool DNA test in CRC screening[39]. The frequency of interval testing was determined to be every 3 years with adequate Medicare coverage[40]. Cologuard® incorporates molecular assays for aberrantly methylated *BMP3* and *NDRG4* gene promoter regions, mutant KRAS and β-actin as well as an immunochemical assay for human hemoglobin. It is based on the recent study of Imperiale *et al*[17] which showed a significantly better sensitivity for cancer detection compared to FIT. Further laboratory-based processing of the samples is necessary, entailing amplification and detection with the use of Quantitative Allele-specific Real-time Target and Signal Amplification (QuARTSTM) technology[41].

FUTURE PERSPECTIVES FOR FECAL DNA SCREENING TESTS

The combined use of screening tests would likely maximize the benefits of different biomarkers for early detection of CRC and adenomas. However, synchronous implementation of these tests in a mass screening program would not fulfill the cost-effectiveness requirement for clinical use.

Thus, there is a need for prospectively designed, systematic evaluations of the most promising fecal tests in a well-defined, large-scale screening population, with standardized sample collection, processing, and storage. This assessment should be combined with sigmoidoscopy or colonoscopy screening and ideally involve repeated testing and longitudinal monitoring of the screened population[19]. Another parameter that merits prospective evaluation is the clinical significance of fecal DNA-positive results in patients with negative colonoscopy results[40].

In the future, Imperiale and colleagues plan to "take this work forward by conducting a post-approval study, which will inform the important issue of test interval, that is, how often does the test need to be repeated". They will also conduct computer simulation studies that will inform comparative effectiveness and cost-effectiveness relative to other screening tests and strategies[42].

Given the high sensitivity for CRC that is unaffected by tumor location and its superior sensitivity over FIT for detection of SSA and AA with greatest risk of progression, Cologuard® may be a good candidate for interval testing after initial colonoscopy. For the same reason, in cases of poor preparation or incomplete colonoscopy, it might represent a convenient follow-up screening test alternative to repeat colonoscopy or other CT colonography, particularly for those patients who are either unable or unwilling to undergo repeat bowel preparation and invasive endoscopy[40].

In an expanding view, fecal DNA testing could be implemented as a screening in CRC predisposing conditions, such as inflammatory bowel disease, playing a role complementary to colonoscopy for early dysplasia detection and surveillance[40,43]. A relevant multicenter validation study has recently been initiated (Government-registered Trial: NCT01819766) and its results are eagerly awaited.

Finally, technological advancements in detection assays of small fragment DNA from stool may render the identification of altered DNA shed from upper GI pre-cancerous and malignant lesions feasible[44-46].

Discussion of screening tests involving non-DNA (*e.g.,* mRNA, miRNA) or non-fecal origin (*e.g.,* blood, urine) biomarkers was beyond the scope of this review. However, it is reasonable to assume that fecal shedding of tumor DNA is an earlier event compared to inner tissue and bloodstream invasion, and is also directly related to the natural, constant process of luminal colonic mucosa exfoliation; thus rendering fecal testing more timely sensitive for the purpose of screening.

Collectively, the accumulation of experience from clinical use of Cologuard® and the numerous ongoing studies on a plethora of biomarkers, as well as further technological advancement of colonoscopy with the full-spectrum endoscopy[47] are expected to further elucidate and expand the landscape of CRC screening research in the coming years, with the hope of further reducing CRC-specific mortality through earlier and accurate detection of pre-cancerous lesions.

REFERENCES

1 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; **65**: 5-29 [PMID: 25559415 DOI: 10.3322/caac.21254]
2 **Murphy G**, Devesa SS, Cross AJ, Inskip PD, McGlynn KA, Cook MB. Sex disparities in colorectal cancer incidence by anatomic subsite, race and age. *Int J Cancer* 2011; **128**: 1668-1675 [PMID: 20503269 DOI: 10.1002/ijc.25481]
3 **Stracci F**, Zorzi M, Grazzini G. Colorectal cancer screening: tests, strategies, and perspectives. *Front Public Health* 2014; **2**: 210 [PMID: 25386553 DOI: 10.3389/fpubh.2014.00210]
4 Colorectal Cancer Prevention and Early Detection. Available from: URL: http://www.cancer.org/acs/groups/cid/documents/webcontent/003170-pdf.pdf
5 **Vatandoost N**, Ghanbari J, Mojaver M, Avan A, Ghayour-Mobarhan M, Nedaeinia R, Salehi R. Early detection of colorectal cancer: from conventional methods to novel biomarkers. *J Cancer Res Clin Oncol* 2015 Feb 17; Epub ahead of print [PMID: 25687380]
6 **Tanaka T**, Tanaka M, Tanaka T, Ishigamori R. Biomarkers for colorectal cancer. *Int J Mol Sci* 2010; **11**: 3209-3225 [PMID: 20957089 DOI: 10.3390/ijms11093209]
7 **Coppedè F**, Lopomo A, Spisni R, Migliore L. Genetic and epigenetic biomarkers for diagnosis, prognosis and treatment of colorectal cancer. *World J Gastroenterol* 2014; **20**: 943-956 [PMID: 24574767 DOI: 10.3748/wjg.v20.i4.943]
8 **Kim HJ**, Yu MH, Kim H, Byun J, Lee C. Noninvasive molecular biomarkers for the detection of colorectal cancer. *BMB Rep* 2008; **41**: 685-692 [PMID: 18959813]
9 **Wang X**, Kuang YY, Hu XT. Advances in epigenetic biomarker research in colorectal cancer. *World J Gastroenterol* 2014; **20**: 4276-4287 [PMID: 24764665 DOI: 10.3748/wjg.v20.i15.4276]

10 **Diehl F**, Schmidt K, Durkee KH, Moore KJ, Goodman SN, Shuber AP, Kinzler KW, Vogelstein B. Analysis of mutations in DNA isolated from plasma and stool of colorectal cancer patients. *Gastroenterology* 2008; **135**: 489-498 [PMID: 18602395 DOI: 10.1053/j.gastro.2008.05.039]

11 **Sidransky D**, Tokino T, Hamilton SR, Kinzler KW, Levin B, Frost P, Vogelstein B. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. *Science* 1992; **256**: 102-105 [PMID: 1566048]

12 **Zou H**, Taylor WR, Harrington JJ, Hussain FT, Cao X, Loprinzi CL, Levine TR, Rex DK, Ahnen D, Knigge KL, Lance P, Jiang X, Smith DI, Ahlquist DA. High detection rates of colorectal neoplasia by stool DNA testing with a novel digital melt curve assay. *Gastroenterology* 2009; **136**: 459-470 [PMID: 19026650 DOI: 10.1053/j.gastro.2008.10.023]

13 **Ahlquist DA**, Sargent DJ, Loprinzi CL, Levin TR, Rex DK, Ahnen DJ, Knigge K, Lance MP, Burgart LJ, Hamilton SR, Allison JE, Lawson MJ, Devens ME, Harrington JJ, Hillman SL. Stool DNA and occult blood testing for screen detection of colorectal neoplasia. *Ann Intern Med* 2008; **149**: 441-450, W81 [PMID: 18838724]

14 **Imperiale TF**, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004; **351**: 2704-2714 [PMID: 15616205]

15 **Lidgard GP**, Domanico MJ, Bruinsma JJ, Light J, Gagrat ZD, Oldham-Haltom RL, Fourrier KD, Allawi H, Yab TC, Taylor WR, Simonson JA, Devens M, Heigh RI, Ahlquist DA, Berger BM. Clinical performance of an automated stool DNA assay for detection of colorectal neoplasia. *Clin Gastroenterol Hepatol* 2013; **11**: 1313-1318 [PMID: 23639600 DOI: 10.1016/j.cgh.2013.04.023]

16 **Ahlquist DA**, Zou H, Domanico M, Mahoney DW, Yab TC, Taylor WR, Butz ML, Thibodeau SN, Rabeneck L, Paszat LF, Kinzler KW, Vogelstein B, Bjerregaard NC, Laurberg S, Sørensen HT, Berger BM, Lidgard GP. Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. *Gastroenterology* 2012; **142**: 248-256; quiz e25-26 [PMID: 22062357 DOI: 10.1053/j.gastro.2011.10.031]

17 **Imperiale TF**, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, Ahlquist DA, Berger BM. Multitarget stool DNA testing for colorectal-cancer screening. *N Engl J Med* 2014; **370**: 1287-1297 [PMID: 24645800 DOI: 10.1056/NEJMoa1311194]

18 **Robertson DJ**, Dominitz JA. Stool DNA and colorectal-cancer screening. *N Engl J Med* 2014; **370**: 1350-1351 [PMID: 24645801 DOI: 10.1056/NEJMe1400092]

19 **Shah R**, Jones E, Vidart V, Kuppen PJ, Conti JA, Francis NK. Biomarkers for early detection of colorectal cancer and polyps: systematic review. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 1712-1728 [PMID: 25004920 DOI: 10.1158/1055-9965]

20 **Li M**, Chen WD, Papadopoulos N, Goodman SN, Bjerregaard NC, Laurberg S, Levin B, Juhl H, Arber N, Moinova H, Durkee K, Schmidt K, He Y, Diehl F, Velculescu VE, Zhou S, Diaz LA, Kinzler KW, Markowitz SD, Vogelstein B. Sensitive digital quantification of DNA methylation in clinical samples. *Nat Biotechnol* 2009; **27**: 858-863 [PMID: 19684580 DOI: 10.1038/nbt.1559]

21 **Wang DR**, Tang D. Hypermethylated SFRP2 gene in fecal DNA is a high potential biomarker for colorectal cancer noninvasive screening. *World J Gastroenterol* 2008; **14**: 524-531 [PMID: 18203283]

22 **Zhang J**, Yang S, Xie Y, Chen X, Zhao Y, He D, Li J. Detection of methylated tissue factor pathway inhibitor 2 and human long DNA in fecal samples of patients with colorectal cancer in China. *Cancer Epidemiol* 2012; **36**: 73-77 [PMID: 21621497 DOI: 10.1016/j.canep.2011.04.006]

23 **Huang Z**, Li L, Wang J. Hypermethylation of SFRP2 as a potential marker for stool-based detection of colorectal cancer and precancerous lesions. *Dig Dis Sci* 2007; **52**: 2287-2291 [PMID: 17410438]

24 **Leung WK**, To KF, Man EP, Chan MW, Hui AJ, Ng SS, Lau JY, Sung JJ. Detection of hypermethylated DNA or cyclooxygenase-2 messenger RNA in fecal samples of patients with colorectal cancer or polyps. *Am J Gastroenterol* 2007; **102**: 1070-1076 [PMID: 17378912]

25 **Itzkowitz SH**, Jandorf L, Brand R, Rabeneck L, Schroy PC, Sontag S, Johnson D, Skoletsky J, Durkee K, Markowitz S, Shuber A. Improved fecal DNA test for colorectal cancer screening. *Clin Gastroenterol Hepatol* 2007; **5**: 111-117 [PMID: 17161655]

26 **Nagasaka T**, Tanaka N, Cullings HM, Sun DS, Sasamoto H, Uchida T, Koi M, Nishida N, Naomoto Y, Boland CR, Matsubara N, Goel A. Analysis of fecal DNA methylation to detect gastrointestinal neoplasia. *J Natl Cancer Inst* 2009; **101**: 1244-1258 [PMID: 19700653 DOI: 10.1093/jnci/djp265]

27 **Baek YH**, Chang E, Kim YJ, Kim BK, Sohn JH, Park DI. Stool methylation-specific polymerase chain reaction assay for the detection of colorectal neoplasia in Korean patients. *Dis Colon Rectum* 2009; **52**: 1452-1459; discussion 1459-1463 [PMID: 19617759 DOI: 10.1007/DCR.0b013e3181a79533]

28 **Azuara D**, Rodriguez-Moranta F, de Oca J, Soriano-Izquierdo A, Mora J, Guardiola J, Biondo S, Blanco I, Peinado MA, Moreno V, Esteller M, Capellá G. Novel methylation panel for the early detection of colorectal tumors in stool DNA. *Clin Colorectal Cancer* 2010; **9**: 168-176 [PMID: 20643622 DOI: 10.3816/CCC.2010.n.023]

29 **Calistri D**, Rengucci C, Casadei Gardini A, Frassineti GL, Scarpi E, Zoli W, Falcini F, Silvestrini R, Amadori D. Fecal DNA for noninvasive diagnosis of colorectal cancer in immunochemical fecal occult blood test-positive individuals. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 2647-2654 [PMID: 20929882 DOI: 10.1158/1055-9965.EPI-10-0291]

30 **Olson J**, Whitney DH, Durkee K, Shuber AP. DNA stabilization is critical for maximizing performance of fecal DNA-based colorectal cancer tests. *Diagn Mol Pathol* 2005; **14**: 183-191 [PMID: 16106201]

31 **Anderson JC**, Shaw RD. Update on colon cancer screening: recent advances and observations in colorectal cancer screening. *Curr Gastroenterol Rep* 2014; **16**: 403 [PMID: 25108645 DOI: 10.1007/s11894-014-0403-3]

32 **Vilkin A**, Rozen P, Levi Z, Waked A, Maoz E, Birkenfeld S, Niv Y. Performance characteristics and evaluation of an automated-developed and quantitative, immunochemical, fecal occult blood screening test. *Am J Gastroenterol* 2005; **100**: 2519-2525 [PMID: 16279909]

33 **Brenner H**, Hoffmeister M, Arndt V, Stegmaier C, Altenhofen L, Haug U. Protection from right- and left-sided colorectal neoplasms after colonoscopy: population-based study. *J Natl Cancer Inst* 2010; **102**: 89-95 [PMID: 20042716 DOI: 10.1093/jnci/djp436]

34 **US Preventive Services Task Force**. Screening for colorectal cancer: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 2008; **149**: 627-637 [PMID: 18838716]

35 **Hoffman RM**. In persons at average risk, stool DNA tests had higher sensitivity than FIT for detecting colorectal cancer. *Ann Intern Med* 2014; **161**: JC10 [PMID: 25023264 DOI: 10.7326/0003-4819-161-2-201407150-02010]

36 **Song K**, Fendrick AM, Ladabaum U. Fecal DNA testing compared with conventional colorectal cancer screening methods: a decision analysis. *Gastroenterology* 2004; **126**: 1270-1279 [PMID: 15131787]

37 **Skally M**, Hanly P, Sharp L. Cost effectiveness of fecal DNA screening for colorectal cancer: a systematic review and quality appraisal of the literature. *Appl Health Econ Health Policy* 2013; **11**: 181-192 [PMID: 23549792 DOI: 10.1007/s40258-013-0010-8]

38 **Binefa G**, Rodríguez-Moranta F, Teule A, Medina-Hayas M. Colorectal cancer: from prevention to personalized medicine. *World J Gastroenterol* 2014; **20**: 6786-6808 [PMID: 24944469 DOI: 10.3748/wjg.v20.i22.6786]

39 A stool DNA test (Cologuard) for colorectal cancer screening. *JAMA* 2014; **312**: 2566 [PMID: 25514307 DOI: 10.1001/jama.2014.15746]

40 **Ahlquist DA**. Multi-target stool DNA test: a new high bar for noninvasive screening. *Dig Dis Sci* 2015; **60**: 623-633 [PMID: 25492503 DOI: 10.1007/s10620-014-3451-5]

41 **Huddy JR**, Ni MZ, Markar SR, Hanna GB. Point-of-care testing in the diagnosis of gastrointestinal cancers: current technology and future directions. *World J Gastroenterol* 2015; **21**: 4111-4120

[PMID: 25892860 DOI: 10.3748/wjg.v21.i14.4111]
42 **Hutchinson L**. Screening: Where does stool DNA testing FIT in the CRC screening menu? *Nat Rev Clin Oncol* 2014; **11**: 239 [PMID: 24732943 DOI: 10.1038/nrclinonc.2014.60]
43 **Kisiel JB**, Yab TC, Nazer Hussain FT, Taylor WR, Garrity-Park MM, Sandborn WJ, Loftus EV, Wolff BG, Smyrk TC, Itzkowitz SH, Rubin DT, Zou H, Mahoney DW, Ahlquist DA. Stool DNA testing for the detection of colorectal neoplasia in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2013; **37**: 546-554 [PMID: 23347191 DOI: 10.1111/apt.12218]
44 **Kisiel JB**, Yab TC, Taylor WR, Chari ST, Petersen GM, Mahoney DW, Ahlquist DA. Stool DNA testing for the detection of pancreatic cancer: assessment of methylation marker candidates. *Cancer* 2012; **118**: 2623-2631 [PMID: 22083596 DOI: 10.1002/cncr.26558]
45 **Strauss BB**, Yab TC, OConnor HM, Taylor WR, Mahoney DW, Simonson JA, Christensen JD, Chari ST, Ahlquist DA. Fecal recovery of ingested cellular DNA: implications for noninvasive detection of upper gastrointestinal neoplasms. *Gastroenterology* 2014; **146**: S-323-S-324 [DOI: 10.1016/S0016-5085(14)61168-9]
46 **Kisiel JB**, Taylor WR, Yab TC, Mahoney DW, Sun Z, Middha S, Zou H, Smyrk TC, Romero Y, Boardman L, Petersen GM, Ahlquist DA. Novel methylated DNA markers predict site of gastrointestinal cancer. *Gastroenterology* 2013; **144**: S-84 [DOI: 10.1016/S0016-5085(13)60313-3]
47 **Gralnek IM**, Siersema PD, Halpern Z, Segol O, Melhem A, Suissa A, Santo E, Sloyer A, Fenster J, Moons LM, Dik VK, D'Agostino RB, Rex DK. Standard forward-viewing colonoscopy versus full-spectrum endoscopy: an international, multicentre, randomised, tandem colonoscopy trial. *Lancet Oncol* 2014; **15**: 353-360 [PMID: 24560453 DOI: 10.1016/S1470-2045(14)70020-8]

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TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Role of retinoids in the prevention and treatment of colorectal cancer

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Abstract

Vitamin A and its derivatives, retinoids, have been widely studied for their use as cancer chemotherapeutic agents. With respect to colorectal cancer (CRC), several critical mutations dysregulate pathways implicated in progression and metastasis, resulting in aberrant Wnt/β-catenin signaling, gain-of-function mutations in K-ras and phosphatidylinositol-3-kinase/Akt, cyclooxygenase-2 over-expression, reduction of peroxisome proliferator-activated receptor γ activation, and loss of p53 function. Dysregulation leads to increased cellular proliferation and invasion and decreased cell-cell interaction and differentiation. Retinoids affect these pathways by various mechanisms, many involving retinoic acid receptors (RAR). RAR bind to *all-trans*-retinoic acid (ATRA) to induce the transcription of genes responsible for cellular differentiation. Although most research concerning the chemotherapeutic efficacy of retinoids focuses on the ability of ATRA to decrease cancer cell proliferation, increase differentiation, or promote apoptosis; as CRC progresses, RAR expression is often lost, rendering treatment of CRCs with ATRA ineffective. Our laboratory focuses on the ability of dietary vitamin A to decrease CRC cell proliferation and invasion *via* RAR-independent pathways. This review discusses our research and others concerning the ability of retinoids to ameliorate the defective signaling pathways listed above and decrease tumor cell proliferation and invasion through both RAR-dependent and RAR-independent mechanisms.

Key words: Colorectal cancer; Retinoid; Vitamin A; β-catenin; Phosphatidylinositol-3-kinase; K-ras; Cyclooxygenase-2; Peroxisome proliferator-activated receptor γ; P53; Phosphatase and tensin homolog deleted on chromosome 10

Core tip: Vitamin A and its derivatives, the retinoids, have been widely studied in many types of cancer for their ability to increase cell differentiation and decrease cell proliferation. This review focuses on the ability of retinoids to affect signaling pathways commonly disrupted in colorectal cancer. We discuss vitamin A metabolism and signaling, how this process becomes aberrant as colorectal cancer progresses, and how treatment with both dietary vitamin A and exogenous retinoids can alter these dysregulated signaling pathways to decrease colorectal cancer cell proliferation and invasion.

Applegate CC, Lane MA. Role of retinoids in the prevention and treatment of colorectal cancer. *World J Gastrointest Oncol* 2015; 7(10): 184-203 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i10/184.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i10.184

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and the second most commonly diagnosed cancer in women worldwide[1,2]. An estimated 1.2 million cases occurred worldwide in 2008, with the highest incidence rates occurring in developed countries including North America, Australia, New Zealand, Japan and Europe[1]. Global trends reflect an overall increase in the incidence of CRC, with the highest increases observed throughout Asia and Europe[1]. About 608700 deaths occurred as a result of CRC in 2008, accounting for 8% of all cancer-related deaths worldwide[1]. Approximately 50% of those patients diagnosed with CRC will experience metastasis to the liver, which is the primary site of CRC metastasis[3]. Risk factors for CRC are both genetic and environmental. A personal or family history of CRC and a personal history of chronic inflammatory bowel disease increase the risk for CRC[4]. Physical inactivity, obesity, smoking, and dietary patterns such as high red and processed meat consumption as well as moderate-to-heavy alcohol use also increase the risk for CRC[4]. Retinoids have long been studied for their effects on organismal development and cellular differentiation, particularly with respect to cancer. Retinoids are currently used as chemotherapies against cancers of epithelial origin, including basal and squamous cell carcinomas. Furthermore, retinoids (whose metabolism is shown in Figure 1) are known to affect signaling pathways frequently altered which result in the development and progression of CRC (Figure 2 and Table 1). CRC is highly influenced by diet, therefore it stands to reason that direct contact with retinoids from supplemented diets or exogenous retinoids administered as medication may have chemotherapeutic effects on CRC tumors.

VITAMIN A METABOLISM

Vitamin A (retinol) and its derivatives, the retinoids, are a group of fat-soluble compounds composed of a similar structure in which a hydrophobic β-ionone ring is joined to a hydrophilic polar moiety by a conjugated tetraene linear chain[5]. Retinol is also able to be synthesized from some types of fat-soluble, antioxidant carotenoids found in fruits and vegetables. While there are several different carotenoid molecules found in plants, only β-carotene, α-carotene, and β-cryptoxanthin have provitamin A activity[6,7]. In the diet, these carotenoids are consumed primarily through carrots, cantaloupes, sweet potatoes, and spinach[6]. Theoretically, cleaving the β-carotene molecule would yield two retinal molecules, each with a β-ionone ring, which can then be converted to two retinol molecules for cellular use[6]. However, this conversion occurs at a much lower rate *in vivo*, with the retinol activity equivalent of β-carotene being much lower than a 1:2 ratio of β-carotene: retinol[6]. Both α-carotene and β-cryptoxanthin only contain one β-ionone ring each and thus have about 50% of the provitamin A activity of β-carotene[6].

Retinol is derived from retinyl esters found in animal sources such as butter, eggs, and meats[8,9]. During digestion in the intestinal lumen, the long-chain fatty acids are cleaved from the retinyl esters *via* hydrolysis, yielding free retinol[10]. The free retinol is then absorbed into the mucosal cells where it is bound by cellular retinol binding protein-Ⅱ (CRBP-Ⅱ), which facilitates the re-esterification of retinol by lethicin retinol acyltransferase (LRAT)[10]. Once re-esterified with long-chain fatty acids such as palmitate, the resulting retinyl esters are incorporated into chylomicrons and secreted into the lymphatic circulation[10]. After draining into the general circulation and transferring their lipid contents into peripheral cells, the remaining chylomicron remnants containing the retinyl esters are taken up by hepatocytes[5]. Depending on bodily needs, the liver either stores the retinyl esters in stellate cells or hydrolyzes the retinyl esters to once again yield free retinol, which binds to retinol binding protein (RBP)[5]. The resulting RBP-retinol complex is released into circulation, where it binds to a small protein, transthyretin (TTR), which prevents the retinol from being excreted by the kidneys[5]. This RBP-retinol-TTR complex circulates in the plasma, until retinol dissociates from the protein complex to enter target cells[11]. The transport of retinol into the cell and its intracellular fate is shown in Figure 1. Because retinol is lipophilic, the molecule can freely diffuse through the plasma membrane of cells[11]. In some cells or during vitamin A deficiency, retinol may be taken up by cells through the RBP receptor, STRA6 (stimulated by retinoic acid 6')[5,11,12]. Cellular uptake of retinol *via* STRA6 is highly preserved in ocular cells, in which the loss of STRA6 leads to visual impairments[13]. However, in STRA6-*null* mice, retinoid homeostasis was only

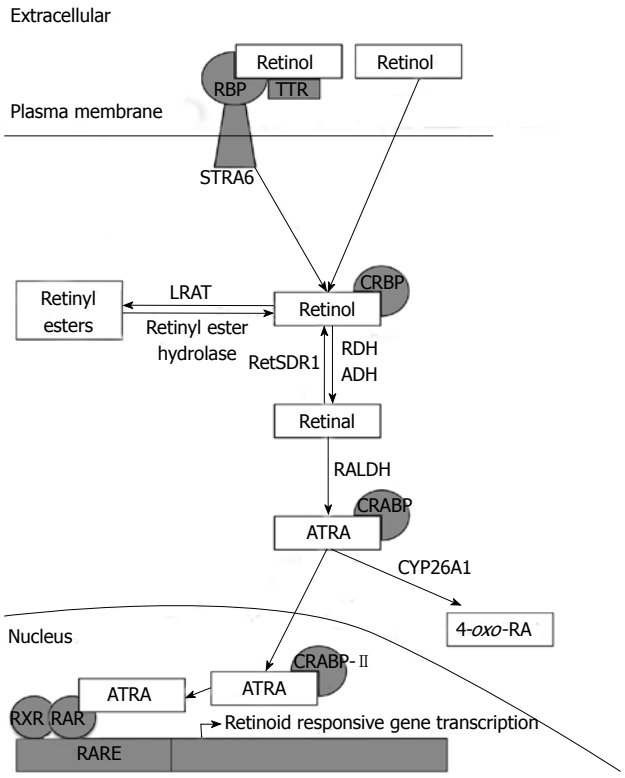

Figure 1 Retinoid metabolism. Vitamin A circulates as retinol bound to RBP and TTR. Retinol can be absorbed into cells *via* STRA6 or diffusion through the cell membrane. Intracellularly, retinol can be stored as retinyl esters or converted to ATRA. ATRA travels to the nucleus where it binds RAR to induce the transcription of retinoid-responsive genes. RBP: Retinol binding protein; TTR: Transthyretin; STRA6: Stimulated by retinoic acid 6; CRBP: Cellular retinol binding protein; LRAT: Lecithin retinol acyltransferase; RALDH: Retinaldehyde dehydrogenase; CRABP: Cellular retinoic acid binding protein; CYP26A1: Cytochrome P450 26A1; 4-*oxo*-RA: 4-*oxo*-retinoic acid; ATRA: *All-trans*-retinoic acid; RXR: Retinoid X receptor; RAR: Retinoic acid receptor; RARE: Retinoic acid response element.

moderately affected, with physiological functions that critically depend on *all-trans*-retinoic acid (ATRA) in both the adult and embryo remaining intact[14]. This indicates that while the receptor functions to assist cells in taking up retinol, STRA6 is not necessary to sustain normal function in cells other than those in the eyes. After diffusion into cells, the internalized free retinol is bound to CRBP or is oxidized to retinal by retinol dehydrogenases (RDH) or alcohol dehydrogenases (ADH) and then to ATRA by retinaldehyde dehydrogenases (RALDH)[5]. ATRA then binds to cellular retinoic acid binding proteins (CRABPs)[5]. CRABP-II shuttles ATRA to the nucleus of the cell, where ATRA serves as a ligand for retinoic acid receptors (RAR).

The RAR and retinoid X receptors (RXR) belong to the nuclear hormone receptor superfamily and are ligand-dependent transcription factors[15]. Each receptor occurs in three subtypes: RARα, -β, and -γ; and RXRα, -β, and -γ. Further, seven different splice variants of RARα (RARα1-7), four different splice variants of RARβ (RARβ1-4), and seven different splice variants of RARγ (RARγ1-7) have been identified[16]. Two different splice variants of each RXR subtype have also been identified that RXRα1 and 2, RXRβ1 and 2, and RXRγ1 and 2[17]. ATRA binds to and activates all subtypes of RAR with a high affinity[15,17]. While the only known retinoid ligand for RXR is 9-*cis*-RA, there has been a general inability to detect this retinoid isomer *in vivo*[18,19]. Recently, 9-*cis*-RA was detected in pancreatic tissue, but the ability of 9-*cis*-RA to act as a ligand for RXR in cells other than pancreatic cells remains controversial[20]. In the absence of ATRA, the RAR/RXR heterodimer binds to RA response elements (RARE) present on DNA promoter regions of ATRA-target genes[21]. The RAR/RXR complex recruits co-repressor proteins, which in turn recruit histone deacetylases (HDAC) to the DNA region[21]. HDAC remove acetyl groups from histone proteins, changing the chromatin structure and negatively regulating gene transcription[21]. By the binding of ATRA, RAR undergoes a conformational change to release inhibitory co-repressor proteins and recruit co-activator proteins, such as histone acetyl transferases, to enhance transcriptional activity[22]. The vast majority of research regarding the ability of retinoids to prevent cancer progression has focused on ATRA and RAR-mediated phenomena. However, as discussed below, cells become resistant to the effects of ATRA on cellular proliferation and differentiation as tumors progress[8,15]. To this end, our laboratory has shown that retinol has non-genomic effects, exclusive of ATRA, such as interference with pathways involving phosphatidylinositol 3-kinase (PI3K) and β-catenin, which play key roles in the progression of cancer[23-29].

ABBERANT VITAMIN A SIGNALING AND METABOLISM IN COLORECTAL CANCER

The luminal side of the colon is an epithelial layer of tissue which is composed of a single sheet of columnar epithelial cells which are folded into finger-like invaginations that are supported by the lamina propria to form a functional unit called a Lieberkuhn's crypt[30]. Different types of epithelial cells line the crypt, including epithelial colonocytes, goblet cells, and endocrine cells[31]. The cells at the bottom of the crypt are stem cells that differentiate into the various epithelial cell types as they move upward to the top of the crypt in a process known as "upward migration"[31]. As the cells migrate upwards, they become terminally differentiated and stop proliferating[31]. Once the cells reach the top of the crypt, they undergo apoptosis and are sloughed off into the lumen[31]. When these cells mutate to retain their proliferative capacity and avoid apoptosis once they reach the top of the crypt, they have the potential to form an adenomatous polyp[31]. These abnormalities may result as a process of inherited genetic mutations, replicative mistakes, or epigenetic changes. If undetected, these polyps may progress into a cancerous lesion[31].

The growth and differentiation of epithelial cells is strongly controlled by retinoid-activated genes. Genes

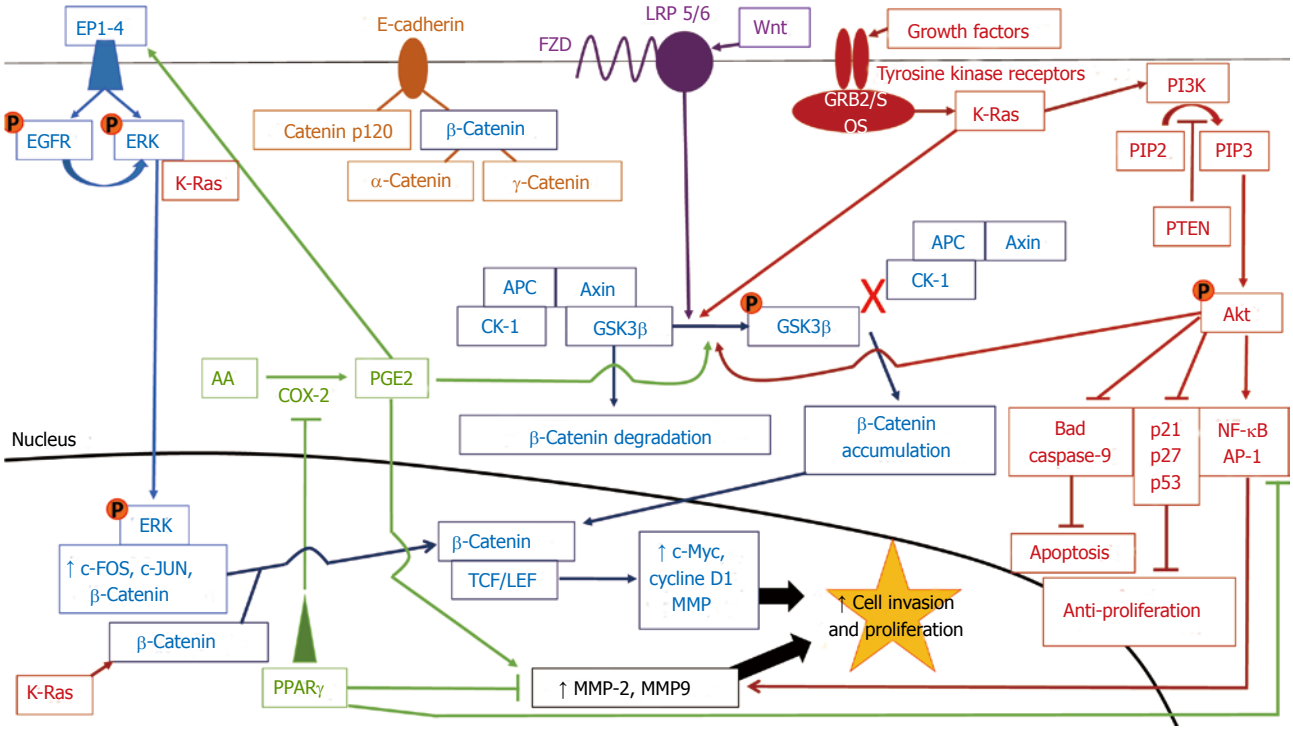

Figure 2 Crosstalk between signaling pathways that lead to colorectal cancer progression. Each pathway is indicated by a specific color. Orange circles represent phosphate groups. β-Catenin is found at the cell membrane, complexed with E-cadherin, in the cytosol, and in the nucleus. Cytosolic β-catenin can be targeted for proteosomal degradation by GSK3β when GSK3β is not phosphorylated and is complexed with APC, Axin, and CK-1. Nuclear β-catenin induces gene transcription when complexed with TCF/LEF transcription factors. Ultimately, all pathways increase the transcription of genes favoring cellular proliferation (c-Myc, cyclin D1) and invasion (MMPs), most *via* increasing β-catenin-mediated gene transcription. CRC: Colorectal cancer; EP1-4: E-prostanoid receptor types 1-4; EGFR: Epidermal growth factor receptor; ERK: Extracellular signal-regulated kinase; K-Ras: Kirsten rat sarcoma viral oncogene homolog; FZD: Frizzled; LRP: Lipoprotein related receptor proteins 5/6; GRB2/SOS: Growth factor receptor-bound protein 2/son of sevenless; PI3K: Phosphatidylinositol-3-kinase; PIP2: Phosphatidylinositol-4,5-bisphosphate; PIP3: Phosphatidylinositol-3,4,5-triphosphate; PTEN: Phosphatase and tensin homolog deleted on chromosome 10; APC: Adenomatous polyposis coli; CK-1: Casein kinase 1; GSK3β: Glycogen synthase kinase 3β; PGE2: Prostaglandin E2; COX2: Cyclooxygenase 2; AA: Arachidonic acid; PPARγ: Peroxisome proliferator-activated receptor γ; TCF/LEF: T-cell factor/lymphoid enhancer factor; MMP: Matrix metalloproteinase; NF-κB: Nuclear factor-kappa B; AP-1: Activator protein 1.

involved in transcription, cell signaling, and tumor suppression contain RAREs in their promoter regions, indicating the importance of ATRA in gene expression[18]. In many epithelial-derived adenomas and carcinomas, the expression of one or more RAR is lost and the cell loses its ability to regulate normal growth[17,32]. This phenomenon is termed "ATRA-resistance". The RARs themselves contain RAREs in their regulatory regions and are thus RA-inducible genes[21,33]. Treatment of patients with premalignant oral lesions with 13-*cis*-RA, a synthetic retinoid, increased the expression of RARβ, which correlated with clinical response, signifying the beneficial effects of retinoid treatment in increasing anti-tumor gene activity in cancers[33,34]. However, the loss of tumor-suppressive RARβ is common in premalignant and malignant tissues and cells, as reviewed in Xu[33]. Loss of RAR has been shown to be partly due to epigenetic changes such as histone modification and DNA methylation becoming aberrant during carcinogenesis, silencing *RAR* gene expression[33,35-38]. The loss of RARβ2 in the HCT-116 colon cancer cell line has been suggested to originate as a result of hypermethylation and the ensuing loss of RARα, which is an upstream regulator of RARβ2[39]. Restoration of RARα by a DNA methylation inhibitor resulted in the re-establishment of RARβ2 expression, indicating a potential role for the combined chemotherapeutic action of DNA methylation inhibitors and retinoids[39]. In contrast, Lee *et al*[32] demonstrated that treatment of RA-sensitive and RA-resistant human colon cancer cell lines with ATRA induced the expression of RARα in all cell lines while only increasing the expression of RARβ in colon cancer cell lines sensitive to RA. Over-expression of RARβ in the RA-resistant colon cancer cell line, DLD-1, resulted in the re-acquisition of RA-sensitivity, inducing growth inhibition and apoptosis in this cell line with ATRA treatment[32]. Over-expression of RARβ in LoVo cells, another RA-resistant human colon cancer cell line, showed similar results in which treatment with ATRA resulted in retinoid-mediated growth inhibition[40].

In addition to the loss of RAR expression and the consequential ATRA resistance, as CRC progresses, colorectal tumor cells appear to lose the ability to produce ATRA[26,41,42] while, at the same time, increasing ATRA degradation *via* the cytochrome P450 enzyme, CYP26A1[43]. Recently, Kropotova *et al*[41] found that all genes involved in ATRA synthesis were decreased in CRC tumors and colorectal cell lines. The researchers also found that ADH IB and IC, the most abundant retinol oxidizing enzymes, exhibited decreased gene

Table 1 Summary of pathways dsyregulated in colorectal cancer and the effect of retinoids on these pathways in both colorectal cancer and other tumor types

Protein	Mutation rate	Result of gene mutation	Response to retinoid treatment
APC	80%[57,65]	Loss of β-catenin degradation[58]; constitutive activation of the Wnt/β-catenin pathway[59]; decreased RDH levels inhibiting formation of ATRA[42]	Not determined
β-Catenin	5%[56]	Loss of β-catenin degradation[56]; constitutive activation of the Wnt/β-catenin pathway[56]; increased CYP26A1 levels resulting in increased degradation of ATRA	Increased degradation of β-catenin *via* RXR-mediated pathway[23,24]
PI3K	30%-50%[77,78]	Activation of Akt and loss of GSK3β function[80,82]; increased cancer metastasis[88], partially through NF-κB activation and increased expression of MMP-2 and -9[87,89,90]; positive cell cycle progression through cyclin D1[105]; loss of cell-cell adhesion by Snail accumulation to repress E-cadherin[106]	Decrease MMP-2 and MMP-9 activity[28]; increase TIMP-1 expression[28]; decrease the phosphorylation of GSK3β, decrease cellular proliferation, and increase the expression of pro-apoptotic proteins in human leiomyoma and myometrial cells[115]; CRBP-I inhibits PI3K/Akt activation in breast cancer cells[116]; inhibit PI3K activity to decrease CRC cell invasion *in vitro* and metastasis *in vivo*[25]
PTEN	20%-40%[80]	Loss of PI3K/Akt inhibition[80]; correlation with tumor aggressiveness and invasiveness[109-111]	Suppression of cellular proliferation and enhanced apoptosis by increasing PTEN expression in smooth muscle cells, neuroblastoma and glioblastoma cells, promyelocytes, leukemia cells, fibroblasts, and breast, endometrial, and hepatocellular carcinoma cells[119-128]
COX-2	80%-90%[134-136]	Increased PGE2 signaling[133,137,138], ERK activation[140], PI3K/Akt signaling through increased EGFR[133,140,141], β-catenin stabilization[142,143], and MMP-2 and MMP-9 expression to promote cellular proliferation[144,145]	Decrease COX-2 expression[146], PGE2, β-catenin levels, and MMP-9[135,144]; inhibition of cell growth[151]; increased apoptosis and RARβ expression[152]
PPARγ	8%[161]	Loss of inhibitory action of gene transcription of pro-survival and growth amplification genes[155,162-165]; increased expression of COX-2[154]	Suppress COX-2 and MMP-7 expression and induction of cell cycle arrest and apoptosis[171]; induce expression of RARβ mRNA in breast cancer cells[175]; increase apoptosis in glioblastoma cells[176]; stimulate PTEN expression in leukemia cells and fibroblasts[121,128]
p53	50%[177,178]	Loss of anti-growth and apoptotic activity; loss of p53/Siah-1-mediated β-catenin degradation[187]	Increase retinyl ester storage through transcription of retSDR1[54]; enhance p53-mediated cell cycle inhibition and apoptosis through activation of AP-2α and p21 in breast cancer cells[192], caspases in keratinocytes[188], Btg2 and CRABP-II in breast cancer cells[191]; STRA6 induction in ovarian cancer cells, fibroblasts, and CRC cells[193]

APC: Adenomatous polyposis coli; RDH: Retinol dehydrogenase; ATRA: *All-trans*-retinoic acid; CYP26A1: Cytochrome P450 26A1; RXR: Retinoid X receptor; PI3K: Phosphatidylinositol-3-kinase; GSK3β: Glycogen synthase kinase 3β; NF-κB: Nuclear factor-kappa B; MMP: Matrix metalloproteinase; TIMP-1: Tissue inhibitor of matrix metalloproteinase 1; CRBP: Cellular retinol binding protein; CRC: Colorectal cancer; PTEN: Phosphatase and tensin homolog deleted on chromosome 10; COX2: Cyclooxygenase 2; PGE2: Prostaglandin E2; ERK: Extracellular signal-regulated kinase; EGFR: Epidermal growth factor receptor; RARβ: Retinoic acid receptor β; PPARγ: Peroxisome proliferator-activated receptor γ; AP-2α: Activator protein 2α; Btg2: Beta cell translocation gene 2; CRABP-II: Cellular retinoic acid binding protein II; STRA6: Stimulated by retinoic acid 6.

expression when adenomas were compared to more advanced carcinomas. Similarly, mRNA levels for RDH-5 and L were decreased in colon tumors and CRC cell lines when compared to normal colon cells[42]. As a result, the CRC cell lines produced only small amounts of ATRA from retinol, a phenomenon our group also observed with the ATRA-resistant CRC cell lines HCT-116, SW620 and WiDR[26]. Loss of adenomatous polyposis coli (APC) function, as seen in the SW620 cell line[44], inhibits RDH expression, the enzyme which converts retinol to retinaldehyde[42]. Interestingly, transfection of APC into an APC-deficient cell line increased the expression of RDH-L and the formation of ATRA, indicating crosstalk between Wnt/β-catenin signaling and retinoid metabolism[42]. To elaborate, APC mediates the proteosomal degradation of C-terminal binding protein 1 (CtBP1). Loss of APC increases the levels of CtBP1. Increased CtBP1, in turn, decreases RDH levels, inhibiting the production of ATRA[45]. Loss of ATRA ultimately leads to less colonocyte differentiation, as ATRA is necessary for epithelial cell differentiation[46]. In fact, homozygous loss of APC causes failed intestinal cell differentiation independent of catenin-mediated gene transcription but dependent upon CtBP1, leading to the hypothetical two-step model of colon adenoma initiation and progression[47]. In this model, APC loss and the resulting increase in CtBP1 leads to adenoma initiation, successive K-ras activation, and the nuclear translocation of β-catenin causing progression to a carcinoma. An incongruity with this model is that administration of ATRA to Apc^{Min} mice, which are heterozygous for a dysfunctional APC mutation, did not prevent tumor formation[48]. Shelton *et al*[43] found that CYP26A1 was increased in tumors from APC^{Min} mice, spontaneous human CRC, and in tumors from patients with familial adenomatous polyposis coli (FAP). These researchers also showed that CYP26A1 expression was dependent upon β-catenin-induced gene expression[43]. Finally, retinoid storage may be altered in cancer. Lecithin retinol acyltransferase (LRAT)

esterifies retinol to retinyl esters, the storage form of vitamin A while retSDR1 converts retinal to retinol. The promoter of the *LRAT* gene is hypermethylated in CRC cell lines and tumors when compared to normal tissue[49]. This hypermethylation would decrease *LRAT* gene expression, potentially decreasing the availability of intracellular retinoids; however, the role of LRAT in cancer progression is controversial with some studies in non-CRC models showing that decreased LRAT levels are protective against carcinogens and correlate with better patient outcomes[50-52]. Proteins in the p53 family have also been shown to affect retinoid metabolism by modulating the expression of retinal short-chain dehydrogenase/reductase (retSDR1). The retSDR1 enzyme is important in regulating retinoid metabolism and storage in many different cell types[53]. Treatment of neuroblastoma cells with physiological concentrations of retinol leads to the accumulation and storage of retinyl esters through the induction of retSDR1 enzyme levels[53]. The overexpression of p53 in the colorectal adenocarcinoma cell line DLD-1 and the CRC cell line HCT-116 yielded a strong induction of both retSDR1 mRNA expression and protein level, even in cells with truncated reporters[54]. The binding of p53 to the retSDR1 promoter was further increased following DNA damage to the cells[54,55]. Importantly, retSDR1 mRNA was shown to be elevated in CRC tumor tissues when compared with healthy samples from the same individuals[54]. These results signify that one mechanism by which p53 acts as a tumor suppressor is by inducing retSDR1 expression in carcinomas to work against tumor progression by supporting retinoid metabolism in these cells[54].

In summary, colorectal tumors often (1) lack RAR, the receptors for ATRA; (2) lose the ability to synthesize ATRA, the RAR ligand, from vitamin A; (3) exhibit increased degradation of ATRA *via* CYP26A1 to 4-oxo-retinoic acid (4-oxo-RA) and (4) may have altered retinoid storage. The regulation of retinoid metabolism is controlled by proteins such as APC, β-catenin, and p53 that play crucial roles in the promotion and progression of CRC as we elaborate below.

THE WNT/β-CATENIN SIGNALING PATHWAY

The Wnt/β-catenin signaling pathway is an important process that regulates the proliferation, differentiation, and motility of cells in normal intestinal epithelium[3,56]. This pathway, and others affecting CRC progression, are shown in Figure 2. During normal intestinal functioning, the APC protein forms a cytoplasmic complex with Axin, another protein present in the cytosol. Both proteins contain binding sites for other members of their functional complex[57]. Together, the APC-Axin complex recruits other functional members, the serine and threonine kinases glycogen synthase kinase 3β (GSK3β) and casein kinase 1 (CK-1)[57]. Together, these proteins form what is known as the β-catenin "destruction complex"[57]. β-catenin, when present in the cytosol, is sequentially bound and phosphorylated by these kinases and thus earmarked for degradation through an ubiquitin-proteasome-mediated pathway[57].

β-catenin performs a dual function in the cell, where it acts as both a transcription factor in the nucleus and as a cell adhesion stabilizer at the cell membrane. When in the cytosol, β-catenin binds to E-cadherin, a transmembrane protein responsible for the formation and maintenance of intercellular adherens junctions formed when epithelial cells come into contact[58]. E-cadherin binds to catenin p120 and β-catenin, which then binds to α-catenin and γ-catenin to anchor E-cadherin to the actin cytoskeleton[58,59]. Together, these proteins form a functional unit termed the E-caderhin-catenin unit (ECCU), in which β-catenin plays the role of an intermediary protein connecting E-cadherin to the α- and γ-catenin proteins that bind to the actin cytoskeleton[58]. The loss of E-cadherin function is thought to occur late in carcinogenesis and leads to the destruction of the ECCU, which causes a loss of the adherens junction and subsequent increase in cell motility and migration[58]. While the function of APC results in the degradation of β-catenin and β-catenin is necessary to form the ECCU, APC and E-cadherin compete for binding of β-catenin and work together to maintain the equilibrium of β-catenin concentration in the cell[58]. Loss of APC function results in E-cadherin saturation and the consequent accumulation of cytosolic β-catenin, which then translocates to the nucleus to enhance the transcription of genes important in cell growth and motility[58,59]. Thus, loss of APC function leads to a disruption in the equilibrium of β-catenin concentration and increased Wnt signaling[58,59]. Similarly, truncation of APC may result in β-catenin binding but not degradation, making β-catenin unavailable for E-cadherin binding[58]. While the over-expression of β-catenin is an important step in early tumorigenesis, later stages of carcinogenesis and loss of tumor differentiation may lead to loss of both β-catenin and E-cadherin expression, leading to the loss of ECCU formation and increased ability to metastasize[58].

Because β-catenin is both degraded and sequestered to the cell membrane during normal APC and E-cadherin function, it is unable to accumulate in the cytosol and translocate to the nucleus, where it binds to proteins of the T-cell factor/lymphoid enhancer factor (TCF/LEF) families[56,57]. If allowed to form a complex with TCF/LEF proteins, β-catenin acts as a transcription co-factor to allow TCF/LEF transcription factors to bind to the regulatory regions of genes regulating cell differentiation, proliferation, and migration such as c-Myc, matrix metalloproteinase-7 (MMP-7), and cyclin D1[3,57,60,61]. Ligand-bound RARs have been shown to compete with TCF in breast cancer cells to decrease β-catenin-mediated gene transcription[62]. In contrast, others have shown that overexpression of RARγ in cholangiocarcinoma cells increases the

nuclear translocation of β-catenin[63], indicating that the effect of RARs on β-catenin varies with tumor type. In phosphorylating β-catenin and thus marking it for ubiquitin-mediated proteasomal degradation, APC and its protein complex constituents act as negative regulators of the Wnt/β-catenin signaling pathway and maintain the homeostasis of intestinal crypt cells and stem cells[3,57,60,64].

Due to its importance in negatively regulating the Wnt/β-catenin signaling pathway, mutations resulting in the loss of APC function are generally thought to be the earliest step in CRC tumorigenesis[56,57]. As a result, APC mutations are found in approximately 80% of human CRCs while mutations involving β-catenin are found in about 5% of all human CRCs[56,57,65]. This APC mutation can be due to an inherited mutation, as in the case of FAP, or due to environmentally-regulated hypermethylation or dysregulation of the *APC* gene[61,66]. In loss-of-function APC mutations, the ability to degrade β-catenin is lost, allowing the Wnt/β-catenin signaling pathway to become constitutively active and upregulate the transcription of oncogenes important in tumor cell proliferation and metastasis[56]. The mutation of the *APC* gene leads to the inability of the APC protein to be exported from the nucleus into the cytoplasm, where APC normally forms a complex with the other proteins involved in the β-catenin destruction complex[61]. The loss of APC results in the increased ability of Wnt proteins to bind to membrane-bound receptors in the Frizzled (FZD) and low density lipoprotein receptor-related families to activate kinases that phosphorylate GSK3β[60,61]. The phosphorylation of GSK3β causes the cytosolic β-catenin destruction complex to become de-stabilized, allowing for the accumulation of β-catenin in the cytosol and its subsequent translocation to the nucleus[60]. When Wnt[66] receptors are not engaged, CK-1 and GSK3β are available to phosphorylate β-catenin to mark it for degradation.

K-RAS MUTATIONS AND CROSSTALK WITH OTHER PATHWAYS

While the APC mutation is found in most colon tumors and is generally regarded to be the earliest step in carcinogenesis, doubt has been placed on its ability to single-handedly cause neoplastic formation. In 30%-50% of CRC tumors, mutation of the *K-ras* gene has also been found, implicating its co-involvement in tumorigenesis[3,60,65,67]. K-ras is responsible for the transduction of mitogenic signals from growth factor receptors on the cell surface to the nucleus[65]. K-ras acts as a molecular switch to regulate the extracellular signal-regulated kinase (ERK) and PI3K/Akt signaling pathways[3]. During K-ras activation, the binding of growth factors to receptor tyrosine kinases causes the recruitment of the growth factor receptor-bound protein 2/son of sevenless (GRB2/SOS) protein complex to the inner cell membrane[60]. This protein complex activates the G-protein Ras (rat sarcoma), resulting in the phosphorylated ERK translocation to the nucleus[60]. In the nucleus, ERK interacts with transcription factors to induce the transcription of target genes such as *c*-FOS and *c*-JUN, which regulate proliferation, differentiation, and apoptosis[60].

Additionally, K-ras activation results in the increased transcription of β-catenin, resulting in the increased accumulation of β-catenin in the cytosol[60]. Mutations of K-ras destroy the GTPase activity of K-ras and fix K-ras in its GTP-bound active forms to permanently activate K-ras and increase ERK signaling[3,60,65,67]. The K-ras mutation interacts with the Wnt/β-catenin signaling pathway by causing the phosphorylation of GSK3β through activation of PI3K[60]. As previously discussed, inactivation of GSK3β leads to de-stabilization of the destruction complex and the resultant stabilization and mobilization of cytosolic β-catenin to the nucleus[60]. Normal activity of GSK3β contributes to negative regulation of both the K-ras and Wnt/β-catenin signaling pathways by phosphorylating K-ras, contributing to its degradation[64]. Thus, GSK3β plays an important role in regulation of both the K-ras and Wnt/β-catenin signaling pathways by degrading key intermediates of each pathway and preventing the transcription of genes important in tumor promotion[64].

K-ras mutations develop after APC loss during progression and metastasis of CRCs, enhancing neoplastic growth[3]. This enhancement of neoplastic growth is achieved by enhanced activation of Wnt/β-catenin signaling[3]. In many cancers, simultaneous activation of K-ras- and β-catenin-dependent pathways are often seen[60]. In human CRC cells and CRC mouse models, gain-of-function K-ras mutations coupled with loss-of-function APC mutations were associated with increased nuclear β-catenin levels and increased size, number, and incidence of tumors when compared to cells or mice with K-ras or APC mutations alone[3]. The resulting tumors displayed an increased migration rate and invasive capability through the increased activity of cyclin D1, which promotes cell cycle progression[3,60]. This evidence results in the theory that carcinogenesis in colon cells requires APC loss with an additional K-ras mutation[3]. Administration of ATRA to mice treated with the carcinogen deoxycholic acid (DCA) decreased colon tumor incidence, but ATRA did not affect the rate of K-ras mutation due to DCA administration[68]. Although we are not aware of any additional research regarding the ability of retinoids to affect K-ras expression or function in CRC, our laboratory and others have shown that retinoids can decrease β-catenin levels and thereby β-catenin-dependent gene transcription as described below.

Table 1 summarizes the effect of retinoids on proteins that affect CRC progression. Although retinoids do not appear to directly alter APC or K-ras activity, they do directly affect β-catenin levels. β-catenin degradation has been shown to be mediated by the activity of three pathways: (1) the APC/GSK3β

pathway; (2) the p53/Siah-1 pathway; and (3) an RXRα-dependent pathway. The RXR-mediated pathway was discovered when Xiao *et al*[69] showed that RXR agonists caused the degradation of RXRα and reduced β-catenin-mediated activation of gene transcription and cell proliferation. Additional work has shown that there is a direct interaction between RXRα and β-catenin[70]. Specifically, in the RXRα-dependent pathway, RXRα binds to nuclear β-catenin and facilitates the transport of β-catenin back into the cytosol where β-catenin is ubiquitinated and degraded by the proteosome. Interestingly, RXRα expression is decreased in advanced CRC when compared to normal adjacent tissue and this decrease is associated with aberrant β-catenin expression[71]. Retinoids increase β-catenin degradation in a variety of tumor types. For example, N-(4 hydroxyphenyl)retinamide (fenretinide) induced the degradation of β-catenin in prostate cancer cells[72] and ATRA decreased β-catenin levels in head and neck cancer stem cells[73]. With respect to CRC, our laboratory has shown that retinol treatment increased β-catenin degradation in ATRA resistant CRC cell lines *via* a RXR-mediated pathway[23,24].

PHOSPHATIDYLINOSITOL 3-KINASE/AKT SIGNALING

The PI3K/protein kinase B (Akt) signaling pathway is another important pathway, the activation of which induces cellular transformation, proliferation, migration, and survival, all of which work together to promote tumor progression[74-76]. Mutations resulting in aberrant activation of this pathway have been implicated in 30%-50% of all human CRCs[77,78]. This dysregulation occurs *via* three mechanisms: (1) activating mutations in exons 9 and 20 on the *PIK3CA* gene; (2) overexpression of Akt itself or activating mutations in the Akt PH domain to increase signaling; and (3) loss of function or expression of the negative regulator phosphatase and tensin homolog deleted on chromosome 10 (PTEN)[79-81]. PI3K belongs to a family of lipid kinases, and is characterized by its ability to phosphorylate the inositol rings of phospholipids on the inner cell membrane[82]. PI3K is present on the cell membrane as a heterodimer, consisting of one of four catalytic p110 subunits and one of two regulatory subunits[80,82]. P110α (PIK3CA) and p110β (PIK3CB) are ubiquitously expressed, with PIK3CA commonly being the more abundant catalytic subunit[82]. PIK3CA and PIK3CB bind to one of two regulatory subunits: p85α or p85β[82]. Class I PI3K enzymes bind Akt *via* pleckstrin homology (PH) domain-containing proteins and are activated mainly by receptor tyrosine kinases, such as those belonging to the epidermal growth factor receptor (EGFR) family, which accept a variety of extracellular signals necessary to stimulate cellular proliferation[80,82]. Once activated, PI3K catalyzes the phosphorylation of membrane-bound phosphatidylinositol-4,5-bisphosphate (PIP2) to generate the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3)[82]. The generation of PIP3 allows for the recruitment of PH domain-containing proteins to the inner plasma membrane[80]. Most notably, the PH domains of 3-phosphoinositide-dependent protein kinase 1 (PDK1) and Akt are drawn together, and PDK1 mediates the phosphorylation of Akt at the threonine 308 site[80,83].

Activating mutations in the *Akt1* gene are rare, occurring in less than 2% of all CRCs[80]. Activating mutations in PDK1 are even rarer, occurring in less than 1% of all CRCs[80]; however, because these proteins are immediately downstream of PI3K, over-activation of PI3K due either to activating mutations of the *PI3K* gene or due to mutations of PTEN, the PI3K inhibitor, ultimately results in the over-activation of Akt. Akt occurs in three isoforms: Akt1, 2, and 3, with Akt1 being most broadly expressed[82]. Akt contains two phosphorylation sites, both of which are required to be phosphorylated for full Akt activation[84]. Phosphorylation of Akt at the threonine 308 site by PDK1 partially activates Akt, whereas full activation requires conjunctive phosphorylation of the serine 473 site by other kinases, such as the mammalian target of rapamycin (mTOR) complex 2 (mTORC2)[83,85]. Full activation of Akt enables Akt to modulate the activity of pathways and expression of genes involved in the regulation of cell survival and proliferation as well as metastasis[86]. As reviewed in Fresno Vara *et al*[82] and Danielsen *et al*[77], Akt prevents the anti-proliferative activities of tumor suppressor genes *p21*, *p27*, and *p53*. Akt also blocks apoptosis in cancer cells by inactivating signals produced by Bcl-2 associated-death promoter (Bad) and caspase-9 proteins, and activates nuclear factor-kappa B (NF-κB), a transcription factor involved in the transcription of genes important in maintaining cell survival and increasing cell invasion[77,82,87]. The mechanism by which Akt activation promotes metastasis is incompletely understood, but elevated Akt phosphorylation has been shown to be correlated with the invasiveness of cancer in human CRC tissues[88]. Specifically, increased levels of phosphorylated Akt are associated with venous invasion of colorectal carcinomas, tumor depth, and the presence of lymph node metastases[88].

One possible mechanism linking Akt activity to cell invasion relies on the activation of NF-κB. NF-κB upregulates the transcription of matrix metalloproteinases (MMPs), which are a class of zinc-dependent enzymes responsible for the degradation of the extracellular matrix[87,89,90]. Specifically, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) belong to a family of gelatinase enzymes that degrade the collagen component of the extracellular matrix[90,91]. Both MMP-2 and MMP-9 are overexpressed in many colon carcinomas when compared with non-cancerous tissue and are associated with increased invasiveness of cancers, advanced tumor stage, and poor survival[87,89,91,92]. Relevant to this review, MMP-9 and MMP-2 have been

shown to be overexpressed in colorectal carcinomas, but not adenomas, indicating their importance in tumor promotion and progression[93]. MMP-2 and -9 are present in the cytosol in inactive pro forms, and cleavage of MMP-2 and -9 by membrane-type matrix metalloproteinases (MT-MMP), such as MT1-MMP, convert inactive pro-MMP-2 and -9 to active MMP-2 and -9[94,95]. This cleavage is inhibited by tissue inhibitors of metalloproteinases (TIMPs), specifically TIMP-1 and -2, which interact with the intermediate (inactive) MMP-9 and -2, respectively, before the proteases are fully activated[94,96]. TIMP-1 expression is regulated by activator protein-1 (AP-1), a transcription factor regulated by the activation of the mitogen-activated protein kinase (MAPK) pathway[90]. Thus, it has been suggested that both PI3K/Akt and MAPK signaling activation must occur simultaneously to regulate MMP-2 and -9 activity and thereby cell invasion[90]. ATRA has been shown to decrease MMP-2 and -9 activity as well as protein and mRNA levels and increase TIMP-1 in a variety of cancers[97-101]. With respect to CRC, our laboratory has shown that treatment of the ATRA-resistant human CRC cancer cell lines HCT-116 and SW620 with retinol resulted in decreased MMP-9 mRNA levels[28]. MMP-2 mRNA levels were decreased in SW620 cells but not in HCT-116 cells[28]. Importantly, the reduction of MMP-2 and MMP-9 mRNA was matched by a reduction in MMP activity[28]. Retinol treatment of HCT-116 and SW620 cells also increased the expression of TIMP-1, potentiating the inhibition of MMP-9 activity in these cells[28].

While TIMP-1 and MMP-2 and 9 expression are regulated by AP-1 and AP-1 activity is in turn repressed by retinoids, this is not thought to be the mechanism by which retinoids affect TIMP-1 and MMP-2 and 9 expression. AP-1 is composed of the proto-oncogenes *c*-JUN and *c*-FOS and its activity is associated with cellular proliferation and invasion[102]. Suppression of AP-1 by 9-*cis*-RA led to the inhibition of cyclin D1 and MMP-2 and 9 in breast cancer cells, however this effect was not matched in SW480 CRC cells, which have low AP-1 activity[102]. Instead, the trans-repressive effects of the cyclin D1 promoter, which contains AP-1 and TCF sites, was independent of the AP-1 site in these CRC cells and required the involvement of a TCF binding element[103]. This data shows that while AP-1 activity is involved in cellular proliferation and invasion, retinoids appear to exert their repressive effects on MMP levels through their interaction with pathways that decrease β-catenin, as β-catenin forms a transactivation complex with TCF/LEF transcription factors. However, promising research involving novel synthetic retinoid derivatives may better target AP-1 for tumor suppression. Um *et al*[104] developed the synthetic retinoid 4-amino-2-(butyrylamino)phenyl-(2E,4E,6E,8E)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexenyl)-2,4,6,8-nonatetraenoate (ABPN), which greatly inhibited AP-1 activity in HCT-116 cells. ABPN suppressed *c*-JUN activity, which led to a decrease in MMP-2 expression, by directly affecting AP-1[104].

It is widely accepted that cross-talk between the PI3K/Akt pathway and the Wnt/β-catenin signaling pathway occurs with GSK3β. Activated Akt phosphorylates GSK3β, inactivating GSK3β and causing a loss of function[82]. Without GSK3β to phosphorylate cytosolic β-catenin and mark it for degradation, stabilized β-catenin can accumulate in the cytosol and eventually translocate to the nucleus to act as a co-factor for gene transcription, as discussed previously[82,86]. Additionally, it has been shown that GSK3β phosphorylation of cyclin D1 stimulates cyclin D1 degradation[105]. Therefore, in tumor cells with increased Akt signaling and loss of GSK3β activation, cyclin D1 remains stable and able to positively regulate cell cycle progression[105]. The loss of GSK3β functioning also results in the increased accumulation of Snail, a zinc-finger transcriptional repressor of E-cadherin[106]. Active, unphosphorylated GSK3β binds to Snail and activates its degradation[107]. Loss of GSK3β function by Akt hyperactivation permits Snail to act as a transcription factor to repress E-cadherin transcription, decreasing cell-cell adhesion through E-cadherin loss[106,107]. As discussed, Akt activation also increases NF-κB transcriptional activity, which in turn increases Snail expression in epithelial cells[106]. Alternatively, it has also been proposed that 3%-5% of total cellular GSK3β is stably bound to Axin to form a complex reserved specifically for Wnt signaling[108]. One study conducted in prostate and breast cancer cell lines and *C. elegans* has shown that inhibition of PI3K by the PI3K inhibitor, wortmannin, does not affect GSK3β phosphorylation[108]. Thus, Wnt signaling by PI3K inhibition remains unchanged, refuting the common theory that there is cross-talk between the two pathways[108]. Instead, this evidence suggests that CRC presents with activating mutations in both the Wnt/β-catenin pathway and the PI3K/Akt pathway simultaneously, creating the notion that cross-talk between the two pathways occurs with a common GSK3β protein[108].

PTEN functions as a negative regulator of PI3K signaling by dephosphorylating the second messenger PIP3 to convert PIP3 back to PIP2[109,110]. PTEN exists in the cell as a cytoplasmic protein in an inactive, phosphorylated state[110]. Phosphorylation of PTEN serine and threonine residues stabilizes the protein in a closed state[110]. Upon activation, dephosphorylated PTEN contains an active phosphatase domain[110]. However, this active site leaves PTEN in an unstable conformation susceptible to proteasomal degradation[110]. In this way, the normal negative feedback loop of PI3K signaling and PTEN inhibition can proceed[110]. When active, PTEN is recruited to the plasma membrane where it binds to PIP3 and dephosphorylates the second messenger, inhibiting the downstream Akt signaling[110]. The loss of PTEN expression results in the accumulation of PIP3 at the plasma membrane, resulting in increased recruitment of Akt to the plasma membrane and increased Akt activation[80]. Because of this negative

regulation of PI3K/Akt signaling, PTEN is associated with inhibition of cell cycle progression, induction of cell death, modulation of cell cycle arrest signals, and stimulation of angiogenesis[110].

PTEN mutations and loss of PTEN expression have been shown to occur in a high number of CRCs, with this loss correlating with tumor aggressiveness and invasiveness[109-111]. This correlation might be explained by the involvement of PTEN with maintaining normal cell polarity[109]. Loss of PTEN results in a loss of cell polarity, leading to increased epidermal-to-mesenchymal transition (EMT) of cancer cells and loss of tight junctions[109]. Similarly, reduced expression of PTEN and loss of PTEN are shown to indicate more advanced stages and metastasis of CRC[111]. Loss of PTEN occurs due to loss of chromosomal heterozygosity in CRC tumors with chromosomal instability and is estimated to occur in about 20%-40% of CRCs, while PTEN mutations in tumors without chromosomal instability occur much less frequently, in less than 5% of cases[80,81,110,111]. PTEN expression itself is regulated by peroxisome proliferator activated receptor γ (PPARγ) and p53 activity, both of which are implicated in CRC and will be discussed in further detail later in this review[110].

Due to PTEN interaction with the PI3K/Akt signaling pathway, it has been proposed that loss of PTEN expression and mutations in PIK3CA may work synergistically to increase the activity of both PI3K/Akt and Wnt/β-catenin signaling[79]. However, data obtained from the European Prospective Investigation of Cancer Norfolk Study showed that loss of PTEN expression and PIK3CA mutations occurred independently of one another in CRCs[81]. Further mechanistic studies involving CRC tumors supported these results and showed activating PIK3CA mutations to occur in about 30% of tumors, independent of PTEN loss[80].

As mentioned previously, there is cross-talk between the PI3K/Akt pathway and the Wnt/β-catenin pathway. Investigation into PIK3CA mutations in CRC revealed that in human CRC cells carrying APC mutations and showing constitutive Wnt pathway activation, PI3K inhibition led to no change in the subcellular localization of β-catenin[79]. Interestingly, although the nuclear localization of β-catenin was unaffected by PI3K inhibition, the concentration of β-catenin phosphorylated at the putative Akt serine 552 phosphorylation site was lower in cells in which PI3K activity was inhibited[79]. β-catenin/LEF/TCF-mediated gene transcription was also lower in the PI3K-inhibited cells, resulting in decreased expression of Wnt target genes *c-Myc*, *cyclin D1*, and *LEF-1*[79]. As a component of the β-catenin transcriptional complex, the decrease in LEF-1 expression indicates a further decrease in the transcriptional activity of β-catenin[79]. Taken together, these results demonstrate that the nuclear localization of β-catenin and its transcriptional activity are independent processes, but are linked by PI3K[79].

Interestingly, retinoid treatment in some cancer cell lines has been shown to upregulate the activity of the PI3K/Akt signaling pathway, increasing cell proliferation and invasion to promote tumor growth[112-114]. However, in other cancer cell lines, treatment with retinoids has been shown to inhibit PI3K/Akt signaling[115-118]. These retinoid effects have mostly been shown to be mediated through RAR-mediated pathways involving ATRA binding to receptors[115,116]. Specifically, ATRA has been shown to decrease the phosphorylation of GSK3β, decrease cellular proliferation, and increase the expression of pro-apoptotic proteins in human leiomyoma and myometrial cells[115]. In addition, CRBP-I inhibits PI3K/Akt activation in breast cancer cells through a RAR-mediated pathway by decreasing the heterodimerization of p85 and p110[116]. To our knowledge, our laboratory is the only laboratory to investigate retinoid inhibition of the PI3K/Akt signaling pathway in CRC. Furthermore, because retinoid receptor activity is often down-regulated in CRC, our laboratory studied the effects of retinol, the dietary form of vitamin A, on the PI3K/Akt signaling pathway in human CRC cells exhibiting ATRA-resistance[29]. We have shown that PI3K activity is inhibited by retinol in a dose-dependent manner independent of RAR signaling or inhibition of p85/p110 heterodimerization[29]. We recently showed that it is the ability of retinol to inhibit PI3K activity that confers the ability of vitamin A to decrease CRC cell invasion *in vitro* and metastasis *in vivo*[25]. Specifically, by comparing the effects of retinol treatment on parental HCT-116 cells, expressing one allele of constitutively active PI3K (caPI3K), to mutant HCT-116 cells expressing two alleles of caPI3K, we showed that retinol treatment decreased *in vitro* cell invasion in parental HCT-116 cells, but not in mutant HCT-116 cells[25]. Retinol treatment also decreased total MMP-9 protein levels and active MMP-9 levels in parental HCT-116 cells, while these levels remained unchanged in HCT-116 cells expressing two alleles of caPI3K[25]. Finally, dietary vitamin A supplementation tended to result in a lower incidence of hepatic metastases in mice intrasplenically injected with parental HCT-116 cells but not in mice intrasplenically injected with mutant HCT-116 cells.

More research is needed to determine the mechanism by which vitamin A inhibits PI3K activity in CRC, but one possible mechanism is by the up-regulation of PTEN. Although the effect of retinoids on PTEN activity has not been examined in CRC to our knowledge, retinoids have been shown to alter PTEN activity in smooth muscle cells, neuroblastoma and glioblastoma cells, promyelocytes, leukemia cells, fibroblasts, and breast, endometrial, and hepatocellular carcinoma cells[119-128]. In particular, ATRA treatment of breast cancer cells reduced the methylation of the *PTEN* gene promoter to activate PTEN transcription[122]. Suppression of growth factors by ATRA in hepatocellular carcinoma cells increases PTEN levels and synchronously decreases the presence of phosphorylated Akt[123]. Increases of PTEN and consequent decreases of Akt occur with retinoid treatment of neuroblastoma and glioblastoma cells and of smooth muscle cells as well[119,126,127]. By

increasing PTEN, cellular proliferation is suppressed and apoptosis is induced, perhaps partially through the inhibition of NF-κB transcriptional activity[126,127]. Concurrent activation of PPARγ with retinoid treatment may also be helpful in synergistically reducing carcinogenesis, which will be discussed further in the following section.

CYCLOOXYGENASE-2 AND PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR-γ

The use of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin reduces the incidence of CRC and other cancers of the gastrointestinal (GI) tract[129,130]. Chronic NSAID use has been shown to reduce the risk of CRC by as much as 40%-50%, as well as decrease the multiplicity and size of tumors presenting with APC loss[131,132]. These drugs mediate their effects through inhibition of cyclooxygenase (COX) enzymes. COX-2 is an inducible enzyme expressed in the presence of inflammatory cytokines, growth factors, and tumor promoters[133]. In the presence of these factors, COX-2 converts free arachidonic acid to prostaglandin H2 (PGH2), which is the precursor to other prostaglandins, specifically prostaglandin E2 (PGE2)[133,134]. COX-2 over-expression is associated with more aggressive tumors of the GI tract and increased levels of COX-2 mRNA are present in 80%-90% of CRCs[134-136]. This over-expression of COX-2 results in the increased levels of PGE2. Elevated PGE2 is present in high levels in cancer tissues and increases the carcinogenic process by stimulating cell proliferation, suppressing apoptosis, increasing cell motility, and promoting angiogenesis[133,137,138]. The biological effects of PGE2 are mediated by E-prostanoid (EP) G-protein coupled receptor subtypes 1-4 which are present in high levels in CRCs[133,139]. The loss of these EP receptors is associated with decreased PGE2 signaling and decreased cancer malignancy[139]. It should be noted that carcinoma cells that do not display increased COX-2 expression may still receive paracrine signals by PGE2 through EP receptors and thus still exhibit the growth stimulatory effects of PGE2 as well as increased cell motility and activation of ERK signaling[140]. PGE2 binding to EP receptors results in increased phosphorylation of EGFR and the downstream mediator ERK, which induces the expression of *c*-FOS, a gene involved in promoting cell proliferation[133,140,141].

While activation of EGFR contributes to increased PI3K/Akt signaling, COX-2 over-expression also results in the dissociation of GSK3β from the β-catenin destruction complex, leading to the stabilization of β-catenin for translocation to the nucleus[142,143]. PGE2 treatment in human CRC cells led to rapid phosphorylation of GSK3β on its serine 9 residue by Akt, inhibiting the kinase activity of GSK3β[143]. This action was, however, dependent on the loss of APC function in CRC because β-catenin stabilization by PGE2 occurs downstream of APC loss[143]. Inhibition of PGE2 in zebrafish embryos and human CRC cells demonstrating APC loss increased the degradation of β-catenin, with COX-2 knockdown reducing the levels of β-catenin[144]. ATRA treatment of zebrafish embryos and human CRC cells decreased the levels of β-catenin by a mechanism that requires the attenuation of COX-2 expression and subsequent decrease in PGE2 accumulation[144]. β-catenin reduction as a result of ATRA treatment also led to the decreased expression of MMP-9[144]. Furthermore, PGE2 led to the increased expression of TCF-4, a component of the β-catenin transactivation complex, resulting in increased transcription of genes downstream of β-catenin[142]. PGE2 thus leads to the expression of cyclin D1 and vascular endothelial growth factor (VEGF) *in vitro* and *in vivo*, which contribute to the increased formation of intestinal polyps[142]. This effect by PGE2 is synergistically perpetuated by mutated β-catenin[142].

COX-2 over-expression in CRC is also correlated with an increased expression of MMP-2 and MMP-9, both of which contribute to CRC motility and metastasis[145]. Suppression of COX-2 by selective inhibitors in mouse CRC cells decreased proliferation associated with cyclin D1 and inhibited cell migration and motility with an associated decrease in both MMP-2 and MMP-9[135]. This suppression of COX-2 also decreased tumor growth both *in vitro* and *in vivo*, while also slowing liver metastasis[135]. This process may be particularly important when considering metastasis of CRC, as COX-2 expression has been shown to be even higher in metastatic liver tumors[135]. Broad spectrum MMP inhibitors decreased the number of adenomas in mice lacking APC function by decreasing proliferation, inhibiting angiogenesis, and stimulating apoptosis, with a synergistic effect seen when combined with COX-2 inhibitors[145].

Moreover, the lack of a functional APC protein is correlated with the elevated expression of COX-2[146]. APC controls ATRA biosynthesis through the activity of RDH enzymes in human CRC, with this loss of RDH correlating with the increased expression of COX-2[146]. In zebrafish embryos and human CRC cells presenting with a functional loss of APC, this over-expression of COX-2 was attenuated by treatment with ATRA[146]. This attenuation of COX-2 expression was the result of a mechanism involving ATRA inhibition of the levels of CCAAT/enhancer-binding protein (C/EBP) *cis*-acting elements, which are present in the promoter region of the *COX-2* gene[146]. ATRA treatment decreased the expression of C/EBP-β, which leads to the decreased expression of COX-2[146].

The suppression of COX-2 by retinoids has been demonstrated in a variety of human epithelial carcinomas[147-150]. This suppression has been shown to be mediated by a multitude of factors, some of which have been described above, and which also includes a RARα-dependent pathway to limit the amount of CREB-binding protein (CBP)/p300 histone acetyltransferase activity available for AP-1 induction of COX-2[148]. In human CRC

cells, treatment with the retinoid analogue fenretinide decreased COX-2 mRNA and inhibited PGE2 expression, resulting in inhibition of cell growth[151]. Therapy with the selective COX-2 inhibitor celecoxib enhanced the growth inhibitory effects of ATRA in both COX-2-high-expressing HT-29 human CRC cells and COX-2-low-expressing SW480 human CRC cells, resulting in increased apoptosis and elevated RARβ expression through COX-2-independent mechanisms[152]. RARβ2 methylation was inversely associated with COX-2 expression, with increased methylation of RARβ2 in CRC tumors also presenting with high COX-2 expression[153]. These tumors correlated with a worse patient prognosis, proposing the importance of both COX-2 and RARβ2 expression in colorectal carcinogenesis[153]. Overall, COX-2 is over-expressed in CRC tumors, leading to elevated PGE2 and β-catenin and the resulting cellular proliferation and tumor metastasis. Treatment with retinoids inhibits this over-expression of COX-2, suppressing the tumor growth-inducing effects of COX-2.

COX-2 expression is regulated in part by PPARγ. Specifically, the activation of PPARγ decreases COX-2 expression by up to 90% and induces caspase-3-dependent apoptosis in human CRC cells[154]. The *COX-2* gene contains a peroxisome proliferator response element (PPRE) in its promoter, which allows the binding of PPARγ-RXRα heterodimers to inhibit *COX-2* gene transcription[155,156]. PPARγ belongs to the nuclear hormone receptor superfamily of ligand-dependent transcription factors[157]. Ligands existing for PPARγ include prostaglandins, polyunsaturated fatty acids (PUFAs), NSAIDs, and thiazolidinediones (TZDs)[158]. TZDs are a class of PPARγ agonist medications, used in diabetic patients to regulate lipid and glucose metabolism *via* PPARγ activation[158,159]. Upon ligand binding, PPARγ changes conformation to release corepressor proteins and recruit coactivator proteins, such as PPARγ-coactivator-1 (PGC-1)[160]. PPARγ then forms an obligate heterodimer with RXRα, and the resulting heterodimer binds to PPREs in the promoter regions of target genes to regulate expression[156]. In CRC, mutations of PPARγ occur in about 8% of cases, indicating its potential role as a tumor suppressor[161]. Many studies in CRC cell lines and animal models have demonstrated this effect, with PPARγ activation resulting in growth inhibition, apoptotic cell death, and decreased cell invasion[155,162-165]. However, the opposite effect has been observed in mice lacking APC function, with PPARγ activation resulting in tumor promotion[166,167]. In rats fed a high-fat diet, PPARγ and RARβ mRNA expression was suppressed, concomitant with an increase in COX-2 and β-catenin levels and in the number of aberrant crypt foci (ACF)[168]. Supplementing diets with retinyl esters or ATRA attenuated the increases in COX-2 and β-catenin expression and inhibited the formation of ACF[168]. This data indicates that dietary factors, such as lipids and retinoids, are strongly influential in protein expression and tumor formation.

The mechanisms by which PPARγ act on tumor formation are still unknown, yet the evidence presented thus far suggests the importance of PPARγ in tumor growth inhibition. PPRE-independent mechanisms may also be involved, as PPARγ activation has also been shown to interfere with NF-κB and AP-1 to inhibit the transcription of pro-survival and growth amplification genes[157,158,169]. As mentioned, the activation of PPARγ by ligand binding results in the suppression of COX-2 expression in human CRC cells with an ensuing decrease in PGE2 accumulation[156,170]. Additionally, PPARγ agonists lead to a decrease in both MMP-2 and MMP-9 and an increase in TIMP-1 and TIMP-2[156,159]. Treatment with ATRA and synthetic RXR ligands synergistically enhanced this effect, which ultimately led to a decrease in cell proliferation, invasion, and an increase in apoptosis[156,171]. Treatment of HCT-15 cells with ATRA and the TZD rosiglitazone synergistically suppressed COX-2 and MMP-7 expression and induced cell cycle arrest and apoptosis[171]. The growth suppressing effects of PPARγ in CRC have been shown to occur by modulating the transcription of genes regulating cell cycle progression. Treatment of human CRC cells with PPARγ agonists induced apoptosis in cells by halting cell cycling progression and inhibiting the expression of genes such as *cyclin D1* and *c-Myc*[157,158,172]. Adding synthetic RXR ligands to treatment with PPARγ agonists can augment cell growth inhibition and induce terminal differentiation by increasing the interaction of PPARγ and RXRα and their ability to form a heterodimer[169]. However, treatment of human CRC cells with RXR ligands alone does not cause PPARγ-RXRα heterodimer formation in the absence of PPARγ activation[156,172]. Therefore, dual treatment with synthetic rexinoid RXR ligands and PPARγ agonists may work together to inhibit the growth and metastasis of colonic tumors. As synthetic RXR ligands, rexinoids are not true retinoids. True retinoids bind RAR and are the focus of this review. Research regarding PPARγ and retinoids in CRC is lacking, as PPARγ only heterodimerizes with RXRα and not RAR. Yet, expression of RARβ mRNA can be induced by PPARγ activation in other cancers such as lung, breast, liver, and brain cancers[173-176]. ATRA alone and a combination of PPARγ and RXR ligands induced RARβ expression in ATRA-resistant breast cancer cells in the presence of HDAC inhibitors[175]. This induction of RARβ expression was reduced in the presence of a PPARγ antagonist, indicating the involvement of PPARγ/RXR heterodimer activity in RARβ transcription[175]. Treatment of breast and lung cancer cells with PPARγ and RXR ligands also induced apoptosis in these cells[175]. Apoptotic glioblastoma cells showed an increased level of RARβ expression when undergoing apoptosis, and PPARγ agonists induced RARβ mRNA in glioblastoma cells, suggesting that PPARγ activation may mediate apoptosis through RARβ activity[176]. Furthermore, treatment of leukemia cells with a combination of ATRA and the PPARγ agonist, ciglitazone, synergistically increased PTEN levels and

inhibited the growth and proliferation of these cells by inducing cell cycle arrest[121]. Both 9-*cis*-RA and PPARγ activation in fibroblasts stimulated PTEN expression, which led to a decrease in Akt phosphorylation[128]. Because PTEN expression is regulated in part by PPARγ activation, PPARγ ligands have been shown to decrease proliferation of endometrial cancer cells *via* PTEN induction and the inhibition of VEGF secretion[120]. Taken together, this research proposes that retinoid treatment in conjunction with PPARγ activation may be helpful in overcoming ATRA-resistance, inhibiting tumor growth, and promoting cancer cell death in CRC.

P53/SIAH-1 SIGNALING

Mutations of the tumor suppressor gene *p53* are the most common mutations found in human cancers, with p53 absence or mutations present in 50% of CRC cases[177,178]. As a tumor suppressor gene, *p53* is activated in response to genotoxic stimuli in healthy cells, to which p53 responds by arresting cell cycle progression and inducing apoptosis[179]. In healthy cells, p53 suppression is necessary for normal growth and is thus present at low concentrations, its expression is regulated through ubiquitin-dependent degradation most notably by the ubiquitin ligase, MDM2[179]. MDM2 is phosphorylated by kinases such as Akt, after which the activated MDM2 localizes to the nucleus and ubiquinates p53[179]. The ubiquitinated p53 is then exported from the nucleus, where it is degraded in the cytosol to maintain cell proliferative activity[179]. Up-regulation of MDM2 activity and transcription also occurs downstream of other oncogenic pathways to inhibit p53 activity, such as ERK and K-ras signaling[179]. Similarly, *MDM2* is a p53 target gene, creating a negative feedback loop to control p53 expression and activity[179]. In response to genotoxic damage, p53 is activated by kinases, which phosphorylate p53 in its MDM2 binding region, stabilizing p53 and allowing it to accumulate and bind to DNA to induce the transcription of genes such as cyclin kinase-dependent cell cycle inhibitor p21 and pro-apoptotic Bcl-2 associated x protein (BAX)[178-181]. P53 also directly inhibits anti-apoptotic proteins such as B-cell CLL/lymphoma-2 (Bcl-2) and Bcl-2 like isoform 1 (Bcl-xL), which inhibit the release of cytochrome c from the mitochondria to prevent the cell from initiating apoptosis[180]. Silencing of Bcl-2 in CRC cells leads to major p53-mediated apoptosis, demonstrating that Bcl-2 inhibits apoptosis in cells by also inhibiting p53 activity[180]. In CRC cells with mutant p53, transfection with wild-type p53 induces apoptosis and inhibits colony formation *in vitro* and inhibits tumor formation *in vivo*[182].

Missense mutations occur in 80% of all p53 mutations, resulting in a stable protein that accumulates inside the nucleus of tumor cells but lacks its specific DNA-binding activity and, therefore, lacks transcriptional activity[183]. As a result, an accumulation of p53 in the cell is generally thought to be mutagenic, although it is important to distinguish this mutant p53 accumulation in tumor cells from wild-type p53 expression[183]. The accumulation of mutant p53 in CRC patients is strongly correlated with increased metastasis and poor prognosis, further implicating the importance of p53 involvement in cell cycle regulation and stimulation of apoptosis in tumor cells[177]. Most p53 mutations occur in the later stages of adenoma-to-carcinoma progression, after which time many other pathways such as K-ras and the Wnt/β-catenin signaling pathway may already be dysregulated[184]. This point is particularly interesting to consider when looking at p53 involvement in β-catenin degradation. Siah-1 is a p53-inducible protein that binds ubiquitin-conjugating enzymes and targets proteins for degradation to ultimately result in tumor suppression[185]. Specifically, Siah-1 binds to the carboxyl terminus of APC and decreases β-catenin *via* a degradation pathway independent of GSK3β phosphorylation[185]. While Siah-1 does not affect APC levels, Siah-1 influence on β-catenin levels are dependent upon Siah-1 binding to APC[185]. In CRC cells with truncated APC, Siah-1 is unable to decrease β-catenin levels, making this process ineffective in cells expressing APC mutations[186]. Siah-1-mediated degradation of both mutant and wild-type β-catenin in CRC cells was supported by a decrease in TCF/LEF reporter activity and the consequent reduction of β-catenin target genes *cyclin D1* and *c-Myc* to result in cell cycle arrest[185-187]. Increased p53 expression in CRC cells resulted in increased degradation of β-catenin and a decrease in TCF/LEF activity only in the presence of Siah-1, indicating that p53 degradation of β-catenin is dependent on Siah-1 activity[185,187]. Because Siah-1 expression is regulated by p53, the loss of p53 transcriptional activity inhibits Siah-1 expression and activity, preventing the p53/Siah-1 pathway activity to cause β-catenin degradation[187].

In addition to affecting retinoid metabolism and storage, retinoid treatment in many different cell types induces p53 mRNA and protein expression to inhibit cell cycle progression and promote apoptosis[188-193]. ATRA treatment of keratinocytes led to an increase in p53 mRNA and protein levels and a corresponding increase in caspase-3, 6, 7, and 9 enzyme levels, which are responsible for mediating apoptosis[188]. Apoptosis and growth inhibition of mammary carcinoma cells is controlled by RA-induced p53 activity increase, which in turn upregulates the expression of the anti-proliferative B-cell translocation gene, member 2 (Btg2)[191]. Btg2 inhibits cell cycle progression by down-regulating the expression of cyclin D1, and this effect is further augmented by the over-expression of CRABP-Ⅱ, which transports RA to nuclear RAR, to induce the transcription of RA-responsive genes[191]. In murine embryonic stem cells, ATRA caused neural differentiation and apoptosis through increasing p53 mRNA and protein levels to instigate cell cycle arrest[189]. The up-regulation of p21 protein concentration is an important effect of p53 activation as shown in human mammary epithelial cells, of which treatment with 9-*cis*-RA, ATRA,

and fenretinide increases p21 expression and thus, cell growth, in a p53-dependent manner[190]. Furthermore, p21 expression in breast cancer cells and HCT-116 CRC cells is increased by p53 interaction with the tumor suppressor activating enhancer-binding protein-2 α (AP-2α), a RA-inducible gene that regulates apoptosis, cell growth, and differentiation[192]. AP-2α interaction with p53 resulted in enhanced binding to the promoter of p21, which led to cell cycle arrest in these cells[192]. The induction of STRA6, the RBP receptor, by p53 has also been shown to mediate apoptosis in ovarian cancer cells, normal human fibroblasts, and HCT-116 cells expressing wild type p53[193]. Transfection of these with STRA6 increased apoptosis, and inhibition of STRA6 severely compromised p53-induced apoptosis[193]. While the effects of retinoids on p53 expression and activity have not been widely studied with regard to CRC, the known results are summarized in Table 1. In general, retinoid treatment of CRC cells appears to enhance the expression and activity of p53 to further increase tumor suppressor p21 levels, ultimately leading to cell cycle arrest and the initiation of apoptosis.

CONCLUSION

Retinoids decrease signaling *via* the major pathways that promote CRC progression. Ultimately, each pathway is followed to its conclusion, retinoids decrease levels of MMPs, cyclin D1, and other factors that induce cellular invasion or proliferation. Often, β-catenin is an intermediate in these pathways, reflecting the central role of β-catenin in CRC progression. Overall pathway interactions are illustrated in Figure 2, and effects of mutations on CRC progression and the effects of retinoids on these mutated proteins are summarized in Table 1. Because retinoids inhibit critical pathways to decrease CRC progression, dietary vitamin A supplementation or retinoid chemotherapy, alone or in combination with other medications, may prove beneficial for the prevention of the progression and metastasis of CRC.

REFERENCES

1 **American Cancer Society**. Global Cancer Facts and Figures. 2nd ed. Atlanta: American Cancer Society, 2011

2 **International Agency for Research on Cancer**. Colorectal cancer statistics World Cancer Research Fund International. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Lyon: International Agency for Research on Cancer, 2014 [cited 2015 Jan 16]. Available from: URL: http://globocan.iarc.fr

3 **Moon BS**, Jeong WJ, Park J, Kim TI, Min do S, Choi KY. Role of oncogenic K-ras in cancer stem cell activation by aberrant Wnt/β-catenin signaling. *J Natl Cancer Inst* 2014; **106**: djt373 [PMID: 24491301 DOI: 10.1093/jnci/djt373]

4 **American Cancer Society**. Colorectal Cancer Facts and Figures 2014-2016. Atlanta: American Cancer Society, 2014

5 **Das BC**, Thapa P, Karki R, Das S, Mahapatra S, Liu TC, Torregroza I, Wallace DP, Kambhampati S, Van Veldhuizen P, Verma A, Ray SK, Evans T. Retinoic acid signaling pathways in development and diseases. *Bioorg Med Chem* 2014; **22**: 673-683 [PMID: 24393720 DOI: 10.1016/j.bmc.2013.11.025]

6 **Harrison EH**. Mechanisms of digestion and absorption of dietary vitamin A. *Annu Rev Nutr* 2005; **25**: 87-103 [PMID: 16011460 DOI: 10.1146/annurev.nutr.25.050304.092614]

7 **Reboul E**. Absorption of vitamin A and carotenoids by the enterocyte: focus on transport proteins. *Nutrients* 2013; **5**: 3563-3581 [PMID: 24036530 DOI: 10.3390/nu5093563]

8 **Bushue N**, Wan YJ. Retinoid pathway and cancer therapeutics. *Adv Drug Deliv Rev* 2010; **62**: 1285-1298 [PMID: 20654663 DOI: 10.1016/j.addr.2010.07.003]

9 **Alizadeh F**, Bolhassani A, Khavari A, Bathaie SZ, Naji T, Bidgoli SA. Retinoids and their biological effects against cancer. *Int Immunopharmacol* 2014; **18**: 43-49 [PMID: 24239628 DOI: 10.1016/j.intimp.2013.10.027]

10 **Harrison EH**. Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. *Biochim Biophys Acta* 2012; **1821**: 70-77 [DOI: 10.1016/j.bbalip.2011.06.002]

11 **Noy N**. Signaling by retinol and its serum binding protein. *Prostaglandins Leukot Essent Fatty Acids* 2015; **93**: 3-7 [PMID: 25481334 DOI: 10.1016/j.plefa.2014.10.004]

12 **Laursen KB**, Kashyap V, Scandura J, Gudas LJ. An alternative retinoic acid-responsive Stra6 promoter regulated in response to retinol deficiency. *J Biol Chem* 2015; **290**: 4356-4366 [PMID: 25544292 DOI: 10.1074/jbc.M114.613968]

13 **Amengual J**, Zhang N, Kemerer M, Maeda T, Palczewski K, Von Lintig J. STRA6 is critical for cellular vitamin A uptake and homeostasis. *Hum Mol Genet* 2014; **23**: 5402-5417 [PMID: 24852372 DOI: 10.1093/hmg/ddu258]

14 **Berry DC**, Jacobs H, Marwarha G, Gely-Pernot A, O'Byrne SM, DeSantis D, Klopfenstein M, Feret B, Dennefeld C, Blaner WS, Croniger CM, Mark M, Noy N, Ghyselinck NB. The STRA6 receptor is essential for retinol-binding protein-induced insulin resistance but not for maintaining vitamin A homeostasis in tissues other than the eye. *J Biol Chem* 2013; **288**: 24528-24539 [PMID: 23839944 DOI: 10.1074/jbc.M113.484014]

15 **Amann PM**, Eichmüller SB, Schmidt J, Bazhin AV. Regulation of gene expression by retinoids. *Curr Med Chem* 2011; **18**: 1405-1412 [PMID: 21366525 DOI: 10.2174/092986711795029618]

16 **Parrado A**, Despouy G, Kraïba R, Le Pogam C, Dupas S, Choquette M, Robledo M, Larghero J, Bui H, Le Gall I, Rochette-Egly C, Chomienne C, Padua RA. Retinoic acid receptor alpha1 variants, RARalpha1DeltaB and RARalpha1DeltaBC, define a new class of nuclear receptor isoforms. *Nucleic Acids Res* 2001; **29**: 4901-4908 [PMID: 11812818 DOI: 10.1093/nar/29.24.4901]

17 **di Masi A**, Leboffe L, De Marinis E, Pagano F, Cicconi L, Rochette-Egly C, Lo-Coco F, Ascenzi P, Nervi C. Retinoic acid receptors: from molecular mechanisms to cancer therapy. *Mol Aspects Med* 2015; **41**: 1-115 [PMID: 25543955 DOI: 10.1016/j.mam.2014.12.003]

18 **Al Tanoury Z**, Piskunov A, Rochette-Egly C. Vitamin A and retinoid signaling: genomic and nongenomic effects. *J Lipid Res* 2013; **54**: 1761-1775 [PMID: 23440512 DOI: 10.1194/jlr.R030833]

19 **Wolf G**. Is 9-cis-retinoic acid the endogenous ligand for the retinoic acid-X receptor? *Nutr Rev* 2006; **64**: 532-538 [PMID: 17274495 DOI: 10.1111/j.1753-4887.2006.tb00186.x]

20 **Kane MA**. Analysis, occurrence, and function of 9-cis-retinoic acid. *Biochim Biophys Acta* 2012; **1821**: 10-20 [PMID: 21983272 DOI: 10.1016/j.bbalip.2011.09.012]

21 **Soprano DR**, Qin P, Soprano KJ. Retinoic acid receptors and cancers. *Annu Rev Nutr* 2004; **24**: 201-221 [PMID: 15189119 DOI: 10.1146/annurev.nutr.24.012003.132407]

22 **le Maire A**, Bourguet W. Retinoic acid receptors: structural basis for coregulator interaction and exchange. *Subcell Biochem* 2014; **70**: 37-54 [PMID: 24962880 DOI: 10.1007/978-94-017-9050-5_3]

23 **Dillard AC**, Lane MA. Retinol decreases beta-catenin protein levels in retinoic acid-resistant colon cancer cell lines. *Mol Carcinog* 2007; **46**: 315-329 [PMID: 17219422 DOI: 10.1002/mc.20280]

24 **Dillard AC**, Lane MA. Retinol Increases beta-catenin-RXRalpha

binding leading to the increased proteasomal degradation of beta-catenin and RXRalpha. *Nutr Cancer* 2008; **60**: 97-108 [PMID: 18444141 DOI: 10.1080/01635580701586754]
25 **Lengyel JN**, Park EY, Brunson AR, Pinali D, Lane MA. Phosphatidylinositol 3-kinase mediates the ability of retinol to decrease colorectal cancer cell invasion. *Nutr Cancer* 2014; **66**: 1352-1361 [PMID: 25356626 DOI: 10.1080/01635581.2014.956258]
26 **Park EY**, Dillard A, Williams EA, Wilder ET, Pepper MR, Lane MA. Retinol inhibits the growth of all-trans-retinoic acid-sensitive and all-trans-retinoic acid-resistant colon cancer cells through a retinoic acid receptor-independent mechanism. *Cancer Res* 2005; **65**: 9923-9933 [PMID: 16267017 DOI: 10.1158/0008-5472.can-05-1604]
27 **Park EY**, Pinali D, Lindley K, Lane MA. Hepatic vitamin A preloading reduces colorectal cancer metastatic multiplicity in a mouse xenograft model. *Nutr Cancer* 2012; **64**: 732-740 [PMID: 22642873 DOI: 10.1080/01635581.2012.687425]
28 **Park EY**, Wilder ET, Chipuk JE, Lane MA. Retinol decreases phosphatidylinositol 3-kinase activity in colon cancer cells. *Mol Carcinog* 2008; **47**: 264-274 [PMID: 17918208 DOI: 10.1002/mc.20381]
29 **Park EY**, Wilder ET, Lane MA. Retinol inhibits the invasion of retinoic acid-resistant colon cancer cells in vitro and decreases matrix metalloproteinase mRNA, protein, and activity levels. *Nutr Cancer* 2007; **57**: 66-77 [PMID: 17516864]
30 **Ricci-Vitiani L**, Fabrizi E, Palio E, De Maria R. Colon cancer stem cells. *J Mol Med* (Berl) 2009; **87**: 1097-1104 [PMID: 19727638 DOI: 10.1007/s00109-009-0518-4]
31 **Fredericks E**. Colorectal carcinogenesis: Molecular aspects. *South African Gastroen Rev* 2013; **11**: 11-18
32 **Lee MO**, Han SY, Jiang S, Park JH, Kim SJ. Differential effects of retinoic acid on growth and apoptosis in human colon cancer cell lines associated with the induction of retinoic acid receptor beta. *Biochem Pharmacol* 2000; **59**: 485-496 [PMID: 10660115 DOI: 10.1016/S0006-2952(99)00355-X]
33 **Xu XC**. Tumor-suppressive activity of retinoic acid receptor-beta in cancer. *Cancer Lett* 2007; **253**: 14-24 [PMID: 17188427 DOI: 10.1016/j.canlet.2006.11.019]
34 **Lotan R**, Xu X-C, Lippman SM, Ro JY, Lee JS, Lee JJ, Hong WK. Suppression of retinoic acid receptor-beta in premalignant oral lesions and its up-regulation by isotretinoin. *N Engl J Med* 1995; **(21)**: 1405 [PMID: 7723796 DOI: 10.1056/NEJM199505253322103]
35 **Lai ZL**, Tsou YA, Fan SR, Tsai MH, Chen HL, Chang NW, Cheng JC, Chen CM. Methylation-associated gene silencing of RARB in areca carcinogens induced mouse oral squamous cell carcinoma. *Biomed Res Int* 2014; **2014**: 378358 [PMID: 25197641 DOI: 10.1155/2014/378358]
36 **Schenk T**, Stengel S, Zelent A. Unlocking the potential of retinoic acid in anticancer therapy. *Br J Cancer* 2014; **111**: 2039-2045 [PMID: 25412233 DOI: 10.1038/bjc.2014.412]
37 **Urvalek A**, Laursen KB, Gudas LJ. The roles of retinoic acid and retinoic acid receptors in inducing epigenetic changes. *Subcell Biochem* 2014; **70**: 129-149 [PMID: 24962884 DOI: 10.1007/978-94-017-9050-5_7]
38 **Mongan NP**, Gudas LJ. Diverse actions of retinoid receptors in cancer prevention and treatment. *Differentiation* 2007; **75**: 853-870 [PMID: 17634071 DOI: 10.1111/j.1432-0436.2007.00206.x]
39 **Moison C**, Senamaud-Beaufort C, Fourrière L, Champion C, Ceccaldi A, Lacomme S, Daunay A, Tost J, Arimondo PB, Guieysse-Peugeot AL. DNA methylation associated with polycomb repression in retinoic acid receptor β silencing. *FASEB J* 2013; **27**: 1468-1478 [PMID: 23299856 DOI: 10.1096/fj.12-210971]
40 **Nicke B**, Riecken EO, Rosewicz S. Induction of retinoic acid receptor beta mediates growth inhibition in retinoid resistant human colon carcinoma cells. *Gut* 1999; **45**: 51-57 [PMID: 10369704 DOI: 10.1136/gut.45.1.51]
41 **Kropotova ES**, Zinovieva OL, Zyryanova AF, Dybovaya VI, Prasolov VS, Beresten SF, Oparina NY, Mashkova TD. Altered expression of multiple genes involved in retinoic acid biosynthesis in human colorectal cancer. *Pathol Oncol Res* 2014; **20**: 707-717 [PMID: 24599561 DOI: 10.1007/s12253-014-9751-4]
42 **Jette C**, Peterson PW, Sandoval IT, Manos EJ, Hadley E, Ireland CM, Jones DA. The tumor suppressor adenomatous polyposis coli and caudal related homeodomain protein regulate expression of retinol dehydrogenase L. *J Biol Chem* 2004; **279**: 34397-34405 [PMID: 15190067 DOI: 10.1074/jbc.M314021200]
43 **Shelton DN**, Sandoval IT, Eisinger A, Chidester S, Ratnayake A, Ireland CM, Jones DA. Up-regulation of CYP26A1 in adenomatous polyposis coli-deficient vertebrates via a WNT-dependent mechanism: implications for intestinal cell differentiation and colon tumor development. *Cancer Res* 2006; **66**: 7571-7577 [PMID: 16885356 DOI: 10.1158/0008-5472.can-06-1067]
44 **Bordonaro M**, Mariadason JM, Aslam F, Heerdt BG, Augenlicht LH. Butyrate-induced apoptotic cascade in colonic carcinoma cells: modulation of the beta-catenin-Tcf pathway and concordance with effects of sulindac and trichostatin A but not curcumin. *Cell Growth Differ* 1999; **10**: 713-720 [PMID: 10547075]
45 **Nadauld LD**, Chidester S, Shelton DN, Rai K, Broadbent T, Sandoval IT, Peterson PW, Manos EJ, Ireland CM, Yost HJ, Jones DA. Dual roles for adenomatous polyposis coli in regulating retinoic acid biosynthesis and Wnt during ocular development. *Proc Natl Acad Sci USA* 2006; **103**: 13409-13414 [PMID: 16938888 DOI: 10.1073/pnas.0601634103]
46 **Baltes S**, Nau H, Lampen A. All-trans retinoic acid enhances differentiation and influences permeability of intestinal Caco-2 cells under serum-free conditions. *Dev Growth Differ* 2004; **46**: 503-514 [PMID: 15610140 DOI: 10.1111/j.1440-169x.2004.00765.x]
47 **Phelps RA**, Chidester S, Dehghanizadeh S, Phelps J, Sandoval IT, Rai K, Broadbent T, Sarkar S, Burt RW, Jones DA. A two-step model for colon adenoma initiation and progression caused by APC loss. *Cell* 2009; **137**: 623-634 [PMID: 19450512 DOI: 10.1016/j.cell.2009.02.037]
48 **Møllersen L**, Paulsen JE, Olstørn HB, Knutsen HK, Alexander J. Dietary retinoic acid supplementation stimulates intestinal tumour formation and growth in multiple intestinal neoplasia (Min)/+ mice. *Carcinogenesis* 2004; **25**: 149-153 [PMID: 14514656 DOI: 10.1093/carcin/bgg176]
49 **Cheng YW**, Pincas H, Huang J, Zachariah E, Zeng Z, Notterman DA, Paty P, Barany F. High incidence of LRAT promoter hypermethylation in colorectal cancer correlates with tumor stage. *Med Oncol* 2014; **31**: 254 [PMID: 25260806 DOI: 10.1007/s12032-014-0254-7]
50 **Hassel JC**, Amann PM, Schadendorf D, Eichmüller SB, Nagler M, Bazhin AV. Lecithin retinol acyltransferase as a potential prognostic marker for malignant melanoma. *Exp Dermatol* 2013; **22**: 757-759 [PMID: 24433184 DOI: 10.1111/exd.12236]
51 **Amann PM**, Luo C, Owen RW, Hofmann C, Freudenberger M, Schadendorf D, Eichmüller SB, Bazhin AV. Vitamin A metabolism in benign and malignant melanocytic skin cells: importance of lecithin/retinol acyltransferase and RPE65. *J Cell Physiol* 2012; **227**: 718-728 [PMID: 21465477 DOI: 10.1002/jcp.22779]
52 **Shirakami Y**, Gottesman ME, Blaner WS. Diethylnitrosamine-induced hepatocarcinogenesis is suppressed in lecithin: retinol acyltransferase-deficient mice primarily through retinoid actions immediately after carcinogen administration. *Carcinogenesis* 2012; **33**: 268-274 [PMID: 22116467 DOI: 10.1093/carcin/bgr275]
53 **Cerignoli F**, Guo X, Cardinali B, Rinaldi C, Casaletto J, Frati L, Screpanti I, Gudas LJ, Gulino A, Thiele CJ, Giannini G. retSDR1, a short-chain retinol dehydrogenase/reductase, is retinoic acid-inducible and frequently deleted in human neuroblastoma cell lines. *Cancer Res* 2002; **62**: 1196-1204 [PMID: 11861404]
54 **Kirschner RD**, Rother K, Müller GA, Engeland K. The retinal dehydrogenase/reductase retSDR1/DHRS3 gene is activated by p53 and p63 but not by mutants derived from tumors or EEC/ADULT malformation syndromes. *Cell Cycle* 2010; **9**: 2177-2188 [PMID: 20543567 DOI: 10.4161/cc.9.11.11844]
55 **Zhou H**, van Bokhoven H. Regulation of vitamin metabolism by p53 and p63 in development and cancer. *Cell Cycle* 2010; **9**: 2709 [PMID: 20676025 DOI: 10.4161/cc.9.14.12591]
56 **Voloshanenko O**, Erdmann G, Dubash TD, Augustin I, Metzig M,

Moffa G, Hundsrucker C, Kerr G, Sandmann T, Anchang B, Demir K, Boehm C, Leible S, Ball CR, Glimm H, Spang R, Boutros M. Wnt secretion is required to maintain high levels of Wnt activity in colon cancer cells. *Nat Commun* 2013; **4**: 2610 [PMID: 24162018 DOI: 10.1038/ncomms3610]

57 **Burgess AW**, Faux MC, Layton MJ, Ramsay RG. Wnt signaling and colon tumorigenesis--a view from the periphery. *Exp Cell Res* 2011; **317**: 2748-2758 [PMID: 21884696 DOI: 10.1016/j.yexcr.2011.08.010]

58 **Ilyas M**, Tomlinson IP. The interactions of APC, E-cadherin and beta-catenin in tumour development and progression. *J Pathol* 1997; **182**: 128-137 [PMID: 9274521]

59 **Pellón-Cárdenas O**, Schweitzer J, D'Souza-Schorey C. Endocytic trafficking and Wnt/β-catenin signaling. *Curr Drug Targets* 2011; **12**: 1216-1222 [PMID: 21561414 DOI: 10.2174/138945011795906552]

60 **Zeller E**, Hammer K, Kirschnick M, Braeuning A. Mechanisms of RAS/β-catenin interactions. *Arch Toxicol* 2013; **87**: 611-632 [PMID: 23483189 DOI: 10.1007/s00204-013-1035-3]

61 **Wu WKK**, Wang XJ, Cheng ASL, Luo MXM, Ng SSM, To KF, Chan FKL, Cho CH, Sung JJY, Yu J. Dysregulation and crosstalk of cellular signaling pathways in colon carcinogenesis. *Crit Rev Oncol Hematol* 2013; **86**: 251-277 [DOI: 10.1016/j.critrevonc.2012.11.009]

62 **Easwaran V**, Pishvaian M, Byers S, Byers S. Cross-regulation of B-catenin-LEF/TCF and retinoid signaling pathways. *Curr Biol* 1999; **9**: 1415-1418 [DOI: 10.1016/S0960-9822(00)80088-3]

63 **Huang GL**, Luo Q, Rui G, Zhang W, Zhang QY, Chen QX, Shen DY. Oncogenic activity of retinoic acid receptor γ is exhibited through activation of the Akt/NF-κB and Wnt/β-catenin pathways in cholangiocarcinoma. *Mol Cell Biol* 2013; **33**: 3416-3425 [PMID: 23798555 DOI: 10.1128/mcb.00384-13]

64 **Jeong WJ**, Yoon J, Park JC, Lee SH, Lee SH, Kaduwal S, Kim H, Yoon JB, Choi KY. Ras stabilization through aberrant activation of Wnt/β-catenin signaling promotes intestinal tumorigenesis. *Sci Signal* 2012; **5**: ra30 [PMID: 22494971 DOI: 10.1126/scisignal.2002242]

65 **Raskov H**, Pommergaard HC, Burcharth J, Rosenberg J. Colorectal carcinogenesis--update and perspectives. *World J Gastroenterol* 2014; **20**: 18151-18164 [PMID: 25561783 DOI: 10.3748/wjg.v20.i48.18151]

66 **Sancho E**, Batlle E, Clevers H. Signaling pathways in intestinal development and cancer. *Annu Rev Cell Dev Biol* 2004; **20**: 695-723 [PMID: 15473857 DOI: 10.1146/annurev.cellbio.20.010403.092805]

67 **Goel S**, Huang J, Klampfer L. K-ras, intestinal homeostasis and colon cancer. *Curr Clin Pharmacol* 2015; **10**: 73-81 [PMID: 24219000]

68 **Narahara H**, Tatsuta M, Iishi H, Baba M, Uedo N, Sakai N, Yano H, Ishiguro S. K-ras point mutation is associated with enhancement by deoxycholic acid of colon carcinogenesis induced by azoxymethane, but not with its attenuation by all-trans-retinoic acid. *Int J Cancer* 2000; **88**: 157-161 [PMID: 11004662 DOI: 10.1002/1097-0215(20001015)88:2<157::AID-IJC2>3.0.CO;2-B]

69 **Xiao JH**, Ghosn C, Hinchman C, Forbes C, Wang J, Snider N, Cordrey A, Zhao Y, Chandraratna RA. Adenomatous polyposis coli (APC)-independent regulation of beta-catenin degradation via a retinoid X receptor-mediated pathway. *J Biol Chem* 2003; **278**: 29954-29962 [PMID: 12771132 DOI: 10.1074/jbc.M304761200]

70 **Han A**, Tong C, Hu D, Bi X, Yang W. A direct protein-protein interaction is involved in the suppression of beta-catenin transcription by retinoid X receptor alpha in colorectal cancer cells. *Cancer Biol Ther* 2008; **7**: 454-459 [PMID: 18196974 DOI: 10.4161/cbt.7.3.5455]

71 **Zhang F**, Meng F, Li H, Dong Y, Yang W, Han A. Suppression of retinoid X receptor alpha and aberrant β-catenin expression significantly associates with progression of colorectal carcinoma. *Eur J Cancer* 2011; **47**: 2060-2067 [PMID: 21561764 DOI: 10.1016/j.ejca.2011.04.010]

72 **Benelli R**, Monteghirfo S, Venè R, Tosetti F, Ferrari N. The chemopreventive retinoid 4HPR impairs prostate cancer cell migration and invasion by interfering with FAK/AKT/GSK3beta pathway and beta-catenin stability. *Mol Cancer* 2010; **9**: 142 [PMID: 20537156 DOI: 10.1186/1476-4598-9-142]

73 **Lim YC**, Kang HJ, Kim YS, Choi EC. All-trans-retinoic acid inhibits growth of head and neck cancer stem cells by suppression of Wnt/β-catenin pathway. *Eur J Cancer* 2012; **48**: 3310-3318 [PMID: 22640830 DOI: 10.1016/j.ejca.2012.04.013]

74 **Xu L**, Zhang Y, Wang H, Zhang G, Ding Y, Zhao L. Tumor suppressor miR-1 restrains epithelial-mesenchymal transition and metastasis of colorectal carcinoma via the MAPK and PI3K/AKT pathway. *J Transl Med* 2014; **12**: 244 [PMID: 25196260 DOI: 10.1186/s12967-014-0244-8]

75 **Kobayashi M**, Nagata S, Iwasaki T, Yanagihara K, Saitoh I, Karouji Y, Ihara S, Fukui Y. Dedifferentiation of adenocarcinomas by activation of phosphatidylinositol 3-kinase. *Proc Natl Acad Sci USA* 1999; **96**: 4874-4879 [PMID: 10220386 DOI: 10.1073/pnas.96.9.4874]

76 **Chen J**, Shao R, Li L, Xu ZP, Gu W. Effective inhibition of colon cancer cell growth with MgAl-layered double hydroxide (LDH) loaded 5-FU and PI3K/mTOR dual inhibitor BEZ-235 through apoptotic pathways. *Int J Nanomedicine* 2014; **9**: 3403-3411 [PMID: 25075187 DOI: 10.2147/IJN.S61633]

77 **Danielsen SA**, Eide PW, Nesbakken A, Guren T, Leithe E, Lothe RA. Portrait of the PI3K/AKT pathway in colorectal cancer. *Biochim Biophys Acta* 2015; **1855**: 104-121 [PMID: 25450577 DOI: 10.1016/j.bbcan.2014.09.008]

78 **Bauer TM**, Patel MR, Infante JR. Targeting PI3 kinase in cancer. *Pharmacol Ther* 2015; **146**: 53-60 [PMID: 25240910 DOI: 10.1016/j.pharmthera.2014.09.006]

79 **Ormanns S**, Neumann J, Horst D, Kirchner T, Jung A. WNT signaling and distant metastasis in colon cancer through transcriptional activity of nuclear β-catenin depend on active PI3K signaling. *Oncotarget* 2014; **5**: 2999-3011 [PMID: 24930890]

80 **Ihle NT**, Powis G, Kopetz S. PI-3-Kinase inhibitors in colorectal cancer. *Curr Cancer Drug Targets* 2011; **11**: 190-198 [PMID: 21158718 DOI: 10.2174/156800911794328448]

81 **Naguib A**, Cooke JC, Happerfield L, Kerr L, Gay LJ, Luben RN, Ball RY, Mitrou PN, McTaggart A, Arends MJ. Alterations in PTEN and PIK3CA in colorectal cancers in the EPIC Norfolk study: associations with clinicopathological and dietary factors. *BMC Cancer* 2011; **11**: 123 [PMID: 21473780 DOI: 10.1186/1471-2407-11-123]

82 **Fresno Vara JA**, Casado E, de Castro J, Cejas P, Belda-Iniesta C, González-Barón M. PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev* 2004; **30**: 193-204 [PMID: 15023437 DOI: 10.1016/j.ctrv.2003.07.007]

83 **Hemmings BA**, Restuccia DF. PI3K-PKB/Akt pathway. *Cold Spring Harb Perspect Biol* 2012; **4**: a011189 [PMID: 22952397 DOI: 10.1101/cshperspect.a011189]

84 **Saji M**, Ringel MD. The PI3K-Akt-mTOR pathway in initiation and progression of thyroid tumors. *Mol Cell Endocrinol* 2010; **321**: 20-28 [PMID: 19897009 DOI: 10.1016/j.mce.2009.10.016]

85 **Sarbassov DD**, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005; **307**: 1098-1101 [PMID: 15718470 DOI: 10.1126/science.1106148]

86 **Setia S**, Nehru B, Sanyal SN. The PI3K/Akt pathway in colitis associated colon cancer and its chemoprevention with celecoxib, a Cox-2 selective inhibitor. *Biomed Pharmacother* 2014; **68**: 721-727 [PMID: 25107843 DOI: 10.1016/j.biopha.2014.07.006]

87 **Kim D**, Kim S, Koh H, Yoon SO, Chung AS, Cho KS, Chung J. Akt/PKB promotes cancer cell invasion via increased motility and metalloproteinase production. *FASEB J* 2001; **15**: 1953-1962 [PMID: 11532975 DOI: 10.1096/fj.01-0198com]

88 **Itoh N**, Semba S, Ito M, Takeda H, Kawata S, Yamakawa M. Phosphorylation of Akt/PKB is required for suppression of cancer cell apoptosis and tumor progression in human colorectal carcinoma. *Cancer* 2002; **94**: 3127-3134 [PMID: 12115344 DOI: 10.1002/cncr.10591]

89 **Cheng JC**, Chou CH, Kuo ML, Hsieh CY. Radiation-enhanced hepatocellular carcinoma cell invasion with MMP-9 expression

through PI3K/Akt/NF-kappaB signal transduction pathway. *Oncogene* 2006; **25**: 7009-7018 [PMID: 16732316 DOI: 10.1038/sj.onc.1209706]
90 **Qiu Q**, Yang M, Tsang BK, Gruslin A. EGF-induced trophoblast secretion of MMP-9 and TIMP-1 involves activation of both PI3K and MAPK signalling pathways. *Reproduction* 2004; **128**: 355-363 [PMID: 15333786 DOI: 10.1530/rep.1.00234]
91 **Wilson CL**, Heppner KJ, Labosky PA, Hogan BL, Matrisian LM. Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. *Proc Natl Acad Sci USA* 1997; **94**: 1402-1407 [PMID: 9037065 DOI: 10.1073/pnas.94.4.1402]
92 **Chen JS**, Wang Q, Fu XH, Huang XH, Chen XL, Cao LQ, Chen LZ, Tan HX, Li W, Bi J, Zhang LJ. Involvement of PI3K/PTEN/AKT/mTOR pathway in invasion and metastasis in hepatocellular carcinoma: Association with MMP-9. *Hepatol Res* 2009; **39**: 177-186 [PMID: 19208038 DOI: 10.1111/j.1872-034X.2008.00449.x]
93 **Heslin MJ**, Yan J, Johnson MR, Weiss H, Diasio RB, Urist MM. Role of matrix metalloproteinases in colorectal carcinogenesis. *Ann Surg* 2001; **233**: 786-792 [PMID: 11371737]
94 **Visse R**, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res* 2003; **92**: 827-839 [PMID: 12730128 DOI: 10.1161/01.RES.0000070112.80711.3D]
95 **Hwang YP**, Yun HJ, Kim HG, Han EH, Lee GW, Jeong HG. Suppression of PMA-induced tumor cell invasion by dihydroartemisinin via inhibition of PKCalpha/Raf/MAPKs and NF-kappaB/AP-1-dependent mechanisms. *Biochem Pharmacol* 2010; **79**: 1714-1726 [PMID: 20152819 DOI: 10.1016/j.bcp.2010.02.003]
96 **Hornebeck W**, Lambert E, Petitfrère E, Bernard P. Beneficial and detrimental influences of tissue inhibitor of metalloproteinase-1 (TIMP-1) in tumor progression. *Biochimie* 2005; **87**: 377-383 [PMID: 15781325 DOI: 10.1016/j.biochi.2004.09.022]
97 **Liu H**, Zang C, Fenner MH, Possinger K, Elstner E. PPARgamma ligands and ATRA inhibit the invasion of human breast cancer cells in vitro. *Breast Cancer Res Treat* 2003; **79**: 63-74 [PMID: 12779083 DOI: 10.1023/A:1023366117157]
98 **Lateef H**, Stevens MJ, Varani J. All-trans-retinoic acid suppresses matrix metalloproteinase activity and increases collagen synthesis in diabetic human skin in organ culture. *Am J Pathol* 2004; **165**: 167-174 [PMID: 15215172 DOI: 10.1016/S0002-9440(10)63285-3]
99 **Benbow U**, Schoenermark MP, Mitchell TI, Rutter JL, Shimokawa K, Nagase H, Brinckerhoff CE. A novel host/tumor cell interaction activates matrix metalloproteinase 1 and mediates invasion through type I collagen. *J Biol Chem* 1999; **274**: 25371-25378 [PMID: 10464264 DOI: 10.1074/jbc.274.36.25371]
100 **Nwankwo JO**. Anti-metastatic activities of all-trans retinoic acid, indole-3-carbinol and (+)-catechin in Dunning rat invasive prostate adenocarcinoma cells. *Anticancer Res* 2002; **22**: 4129-4135 [PMID: 12553043]
101 **Andela VB**, Rosier RN. The proteosome inhibitor MG132 attenuates retinoic acid receptor trans-activation and enhances trans-repression of nuclear factor kappaB. Potential relevance to chemo-preventive interventions with retinoids. *Mol Cancer* 2004; **3**: 8 [PMID: 15035668 DOI: 10.1186/1476-4598-3-8]
102 **Shah S**, Pishvaian MJ, Easwaran V, Brown PH, Byers SW. The role of cadherin, beta-catenin, and AP-1 in retinoid-regulated carcinoma cell differentiation and proliferation. *J Biol Chem* 2002; **277**: 25313-25322 [PMID: 12000762 DOI: 10.1074/jbc.M203158200]
103 **Shah S**, Hecht A, Pestell R, Byers SW. Trans-repression of beta-catenin activity by nuclear receptors. *J Biol Chem* 2003; **278**: 48137-48145 [PMID: 12972427 DOI: 10.1074/jbc.M307154200]
104 **Um SJ**, Han HS, Kwon YJ, Park SH, Rho YS, Sin HS, Park JS. Novel retinoic acid derivative ABPN has potent inhibitory activity on cell growth and apoptosis in cancer cells. *Int J Cancer* 2003; **107**: 1038-1046 [PMID: 14601067 DOI: 10.1002/ijc.11489]
105 **Diehl JA**, Cheng M, Roussel MF, Sherr CJ. Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev* 1998; **12**: 3499-3511 [PMID: 9832503 DOI: 10.1101/gad.12.22.3499]
106 **Bachelder RE**, Yoon SO, Franci C, de Herreros AG, Mercurio AM. Glycogen synthase kinase-3 is an endogenous inhibitor of Snail transcription: implications for the epithelial-mesenchymal transition. *J Cell Biol* 2005; **168**: 29-33 [PMID: 15631989 DOI: 10.1083/jcb.200409067]
107 **Qiao M**, Sheng S, Pardee AB. Metastasis and AKT activation. *Cell Cycle* 2008; **7**: 2991-2996 [PMID: 18818526 DOI: 10.4161/cc.7.19.6784]
108 **Ng SS**, Mahmoudi T, Danenberg E, Bejaoui I, de Lau W, Korswagen HC, Schutte M, Clevers H. Phosphatidylinositol 3-kinase signaling does not activate the wnt cascade. *J Biol Chem* 2009; **284**: 35308-35313 [PMID: 19850932 DOI: 10.1074/jbc.M109.078261]
109 **Langlois MJ**, Bergeron S, Bernatchez G, Boudreau F, Saucier C, Perreault N, Carrier JC, Rivard N. The PTEN phosphatase controls intestinal epithelial cell polarity and barrier function: role in colorectal cancer progression. *PLoS One* 2010; **5**: e15742 [PMID: 21203412 DOI: 10.1371/journal.pone.0015742]
110 **Molinari F**, Frattini M. Functions and Regulation of the PTEN Gene in Colorectal Cancer. *Front Oncol* 2013; **3**: 326 [PMID: 24475377 DOI: 10.3389/fonc.2013.00326]
111 **Waniczek D**, Śnietura M, Młynarczyk-Liszka J, Pigłowski W, Kopeć A, Lange D, Rudzki M, Arendt J. PTEN expression profiles in colorectal adenocarcinoma and its precancerous lesions. *Pol J Pathol* 2013; **64**: 15-20 [PMID: 23625595 DOI: 10.5114/pjp.2013.34598]
112 **García-Regalado A**, Vargas M, García-Carrancá A, Aréchaga-Ocampo E, González-De la Rosa CH. Activation of Akt pathway by transcription-independent mechanisms of retinoic acid promotes survival and invasion in lung cancer cells. *Mol Cancer* 2013; **12**: 44 [PMID: 23693014 DOI: 10.1186/1476-4598-12-44]
113 **Uruno A**, Sugawara A, Kanatsuka H, Kagechika H, Saito A, Sato K, Kudo M, Takeuchi K, Ito S. Upregulation of nitric oxide production in vascular endothelial cells by all-trans retinoic acid through the phosphoinositide 3-kinase/Akt pathway. *Circulation* 2005; **112**: 727-736 [PMID: 16043647 DOI: 10.1161/CIRCULATIONAHA.104.500959]
114 **López-Carballo G**, Moreno L, Masiá S, Pérez P, Barettino D. Activation of the phosphatidylinositol 3-kinase/Akt signaling pathway by retinoic acid is required for neural differentiation of SH-SY5Y human neuroblastoma cells. *J Biol Chem* 2002; **277**: 25297-25304 [PMID: 12000752 DOI: 10.1074/jbc.M201869200]
115 **Ben-Sasson H**, Ben-Meir A, Shushan A, Karra L, Rojansky N, Klein BY, Levitzki R, Ben-Bassat H. All-trans-retinoic acid mediates changes in PI3K and retinoic acid signaling proteins of leiomyomas. *Fertil Steril* 2011; **95**: 2080-2086 [PMID: 21354561 DOI: 10.1016/j.fertnstert.2011.01.155]
116 **Farias EF**, Marzan C, Mira-y-Lopez R. Cellular retinol-binding protein-I inhibits PI3K/Akt signaling through a retinoic acid receptor-dependent mechanism that regulates p85-p110 heterodimerization. *Oncogene* 2005; **24**: 1598-1606 [PMID: 15608670 DOI: 10.1038/sj.onc.1208347]
117 **So PL**, Wang GY, Wang K, Chuang M, Chiueh VC, Kenny PA, Epstein EH. PI3K-AKT signaling is a downstream effector of retinoid prevention of murine basal cell carcinogenesis. *Cancer Prev Res* (Phila) 2014; **7**: 407-417 [PMID: 24449057 DOI: 10.1158/1940-6207.CAPR-13-0304]
118 **Baba A**, Shimizu M, Ohno T, Shirakami Y, Kubota M, Kochi T, Terakura D, Tsurumi H, Moriwaki H. Synergistic growth inhibition by acyclic retinoid and phosphatidylinositol 3-kinase inhibitor in human hepatoma cells. *BMC Cancer* 2013; **13**: 465 [PMID: 24103747 DOI: 10.1186/1471-2407-13-465]
119 **Tran-Lundmark K**, Tannenberg P, Rauch BH, Ekstrand J, Tran PK, Hedin U, Kinsella MG. Perlecan Heparan Sulfate Is Required for the Inhibition of Smooth Muscle Cell Proliferation by All-trans-Retinoic Acid. *J Cell Physiol* 2015; **230**: 482-487 [PMID: 25078760 DOI: 10.1002/jcp.24731]
120 **Nickkho-Amiry M**, McVey R, Holland C. Peroxisome proliferator-activated receptors modulate proliferation and angiogenesis in human endometrial carcinoma. *Mol Cancer Res* 2012; **10**: 441-453 [PMID: 22205725 DOI: 10.1158/1541-7786.MCR-11-0233]

121 **Lee YR**, Yu HN, Noh EM, Kim JS, Song EK, Han MK, Kim BS, Lee SH, Park J. Peroxisome proliferator-activated receptor gamma and retinoic acid receptor synergistically up-regulate the tumor suppressor PTEN in human promyeloid leukemia cells. *Int J Hematol* 2007; **85**: 231-237 [PMID: 17483060 DOI: 10.1532/IJH97.A30615]

122 **Stefanska B**, Salamé P, Bednarek A, Fabianowska-Majewska K. Comparative effects of retinoic acid, vitamin D and resveratrol alone and in combination with adenosine analogues on methylation and expression of phosphatase and tensin homologue tumour suppressor gene in breast cancer cells. *Br J Nutr* 2012; **107**: 781-790 [PMID: 21801466 DOI: 10.1017/S0007114511003631]

123 **Li M**, Li H, Li C, Wang S, Jiang W, Liu Z, Zhou S, Liu X, McNutt MA, Li G. Alpha-fetoprotein: a new member of intracellular signal molecules in regulation of the PI3K/AKT signaling in human hepatoma cell lines. *Int J Cancer* 2011; **128**: 524-532 [PMID: 20473866 DOI: 10.1002/ijc.25373]

124 **Janardhanan R**, Banik NL, Ray SK. N-Myc down regulation induced differentiation, early cell cycle exit, and apoptosis in human malignant neuroblastoma cells having wild type or mutant p53. *Biochem Pharmacol* 2009; **78**: 1105-1114 [PMID: 19540207 DOI: 10.1016/j.bcp.2009.06.009]

125 **Song MS**, Salmena L, Carracedo A, Egia A, Lo-Coco F, Teruya-Feldstein J, Pandolfi PP. The deubiquitinylation and localization of PTEN are regulated by a HAUSP-PML network. *Nature* 2008; **455**: 813-817 [PMID: 18716620 DOI: 10.1038/nature07290]

126 **Zhang R**, Banik NL, Ray SK. Combination of all-trans retinoic acid and interferon-gamma upregulated p27(kip1) and down regulated CDK2 to cause cell cycle arrest leading to differentiation and apoptosis in human glioblastoma LN18 (PTEN-proficient) and U87MG (PTEN-deficient) cells. *Cancer Chemother Pharmacol* 2008; **62**: 407-416 [PMID: 17960384 DOI: 10.1007/s00280-007-0619-0]

127 **Zhang R**, Banik NL, Ray SK. Combination of all-trans retinoic acid and interferon-gamma suppressed PI3K/Akt survival pathway in glioblastoma T98G cells whereas NF-kappaB survival signaling in glioblastoma U87MG cells for induction of apoptosis. *Neurochem Res* 2007; **32**: 2194-2202 [PMID: 17616812 DOI: 10.1007/s11064-007-9417-7]

128 **Lee SJ**, Yang EK, Kim SG. Peroxisome proliferator-activated receptor-gamma and retinoic acid X receptor alpha represses the TGFbeta1 gene via PTEN-mediated p70 ribosomal S6 kinase-1 inhibition: role for Zf9 dephosphorylation. *Mol Pharmacol* 2006; **70**: 415-425 [PMID: 16611854]

129 **Sandler RS**, Halabi S, Baron JA. Daily Aspirin Use Was Associated with a Reduced Incidence of Colorectal Adenomas. *Annals of Internal Medicine* 2004; **141**: 378-379

130 **Baron JA**, Cole BF, Sandler RS, Haile RW, Ahnen D, Bresalier R, McKeown-Eyssen G, Summers RW, Rothstein R, Burke CA, Snover DC, Church TR, Allen JI, Beach M, Beck GJ, Bond JH, Byers T, Greenberg ER, Mandel JS, Marcon N, Mott LA, Pearson L, Saibil F, van Stolk RU. A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med* 2003; **348**: 891-899 [PMID: 12621133 DOI: 10.1056/NEJMoa021735]

131 **Peek RM**. Prevention of colorectal cancer through the use of COX-2 selective inhibitors. *Cancer Chemother Pharmacol* 2004; **54** Suppl 1: S50-S56 [PMID: 15309515 DOI: 10.1007/s00280-004-0887-x]

132 **Jacoby RF**, Seibert K, Cole CE, Kelloff G, Lubet RA. The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. *Cancer Res* 2000; **60**: 5040-5044 [PMID: 11016626]

133 **Wu WK**, Sung JJ, Lee CW, Yu J, Cho CH. Cyclooxygenase-2 in tumorigenesis of gastrointestinal cancers: an update on the molecular mechanisms. *Cancer Lett* 2010; **295**: 7-16 [PMID: 20381235 DOI: 10.1016/j.canlet.2010.03.015]

134 **Roelofs HM**, Te Morsche RH, van Heumen BW, Nagengast FM, Peters WH. Over-expression of COX-2 mRNA in colorectal cancer. *BMC Gastroenterol* 2014; **14**: 1 [PMID: 24383454 DOI: 10.1186/1471-230X-14-1]

135 **Yao M**, Lam EC, Kelly CR, Zhou W, Wolfe MM. Cyclooxygenase-2 selective inhibition with NS-398 suppresses proliferation and invasiveness and delays liver metastasis in colorectal cancer. *Br J Cancer* 2004; **90**: 712-719 [PMID: 14760389 DOI: 10.1038/sj.bjc.6601489]

136 **Chen WS**, Wei SJ, Liu JM, Hsiao M, Kou-Lin J, Yang WK. Tumor invasiveness and liver metastasis of colon cancer cells correlated with cyclooxygenase-2 (COX-2) expression and inhibited by a COX-2-selective inhibitor, etodolac. *Int J Cancer* 2001; **91**: 894-899 [PMID: 11275997]

137 **Wang D**, Dubois RN. Prostaglandins and cancer. *Gut* 2006; **55**: 115-122 [PMID: 16118353 DOI: 10.1136/gut.2004.047100]

138 **Brown JR**, DuBois RN. COX-2: a molecular target for colorectal cancer prevention. *J Clin Oncol* 2005; **23**: 2840-2855 [PMID: 15837998]

139 **Wu CH**, Shih YW, Chang CH, Ou TT, Huang CC, Hsu JD, Wang CJ. EP4 upregulation of Ras signaling and feedback regulation of Ras in human colon tissues and cancer cells. *Arch Toxicol* 2010; **84**: 731-740 [PMID: 20571779 DOI: 10.1007/s00204-010-0562-4]

140 **Sheng H**, Shao J, Washington MK, DuBois RN. Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. *J Biol Chem* 2001; **276**: 18075-18081 [PMID: 11278548]

141 **Fujimura T**, Ohta T, Oyama K, Miyashita T, Miwa K. Role of cyclooxygenase-2 in the carcinogenesis of gastrointestinal tract cancers: a review and report of personal experience. *World J Gastroenterol* 2006; **12**: 1336-1345 [PMID: 16552798]

142 **Shao J**, Jung C, Liu C, Sheng H. Prostaglandin E2 Stimulates the beta-catenin/T cell factor-dependent transcription in colon cancer. *J Biol Chem* 2005; **280**: 26565-26572 [PMID: 15899904 DOI: 10.1074/jbc.M413056200]

143 **Castellone MD**, Teramoto H, Williams BO, Druey KM, Gutkind JS. Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. *Science* 2005; **310**: 1504-1510 [PMID: 16293724 DOI: 10.1126/science.1116221]

144 **Eisinger AL**, Nadauld LD, Shelton DN, Prescott SM, Stafforini DM, Jones DA. Retinoic acid inhibits beta-catenin through suppression of Cox-2: a role for truncated adenomatous polyposis coli. *J Biol Chem* 2007; **282**: 29394-29400 [PMID: 17673467 DOI: 10.1074/jbc.M609768200]

145 **Wagenaar-Miller RA**, Hanley G, Shattuck-Brandt R, DuBois RN, Bell RL, Matrisian LM, Morgan DW. Cooperative effects of matrix metalloproteinase and cyclooxygenase-2 inhibition on intestinal adenoma reduction. *Br J Cancer* 2003; **88**: 1445-1452 [PMID: 12778076 DOI: 10.1038/sj.bjc.6600867]

146 **Eisinger AL**, Nadauld LD, Shelton DN, Peterson PW, Phelps RA, Chidester S, Stafforini DM, Prescott SM, Jones DA. The adenomatous polyposis coli tumor suppressor gene regulates expression of cyclooxygenase-2 by a mechanism that involves retinoic acid. *J Biol Chem* 2006; **281**: 20474-20482 [PMID: 16699180 DOI: 10.1074/jbc.M602859200]

147 **Mestre JR**, Subbaramaiah K, Sacks PG, Schantz SP, Tanabe T, Inoue H, Dannenberg AJ. Retinoids suppress phorbol ester-mediated induction of cyclooxygenase-2. *Cancer Res* 1997; **57**: 1081-1085 [PMID: 9067275]

148 **Subbaramaiah K**, Cole PA, Dannenberg AJ. Retinoids and carnosol suppress cyclooxygenase-2 transcription by CREB-binding protein/p300-dependent and -independent mechanisms. *Cancer Res* 2002; **62**: 2522-2530 [PMID: 11980644]

149 **Kanekura T**, Higashi Y, Kanzaki T. Inhibitory effects of 9-cis-retinoic acid and pyrrolidinedithiocarbamate on cyclooxygenase (COX)-2 expression and cell growth in human skin squamous carcinoma cells. *Cancer Letters* 2000; **161**: 177-183 [DOI: 10.1016/S0304-3835(00)00604-2]

150 **Li M**, Song S, Lippman SM, Zhang XK, Liu X, Lotan R, Xu XC. Induction of retinoic acid receptor-beta suppresses cyclooxygenase-2 expression in esophageal cancer cells. *Oncogene* 2002; **21**: 411-418 [PMID: 11821953 DOI: 10.1038/sj.onc.1205106]

151 **Merritt G**, Aliprandis ET, Prada F, Rigas B, Kashfi K. The retinoid fenretinide inhibits proliferation and downregulates cyclooxygenase-2 gene expression in human colon adenocarcinoma cell lines. *Cancer Lett* 2001; **164**: 15-23 [PMID: 11166911]

152 **Liu JP**, Wei HB, Zheng ZH, Guo WP, Fang JF. Celecoxib increases retinoid sensitivity in human colon cancer cell lines. *Cell Mol Biol Lett* 2010; **15**: 440-450 [PMID: 20496179 DOI: 10.2478/s11658-010-0016-2]

153 **Miladi-Abdennadher I**, Abdelmaksoud-Damak R, Ayadi L, Khabir A, Frikha F, Kallel L, Amouri A, Frikha M, Sellami-Boudawara T, Gargouri A, Mokdad-Gargouri R. Hypermethylation of RARβ2 correlates with high COX-2 expression and poor prognosis in patients with colorectal carcinoma. *Tumour Biol* 2010; **31**: 503-511 [PMID: 20571967 DOI: 10.1007/s13277-010-0063-3]

154 **Yang WL**, Frucht H. Activation of pparR induces apoptosis and inhibit COX-2 in human colon cancer cells. *Gastroenterology* 2000; **118** (4, Part 1): A682 [DOI: 10.1016/S0016-5085(00)84863-5]

155 **Allred CD**, Talbert DR, Southard RC, Wang X, Kilgore MW. PPARgamma1 as a molecular target of eicosapentaenoic acid in human colon cancer (HT-29) cells. *J Nutr* 2008; **138**: 250-256 [PMID: 18203887]

156 **Papi A**, Rocchi P, Ferreri AM, Orlandi M. RXRγ and PPARγ ligands in combination to inhibit proliferation and invasiveness in colon cancer cells. *Cancer Letters* 2010; **297**: 65-74 [DOI: 10.1016/j.canlet.2010.04.026]

157 **Ban JO**, Kwak DH, Oh JH, Park EJ, Cho MC, Song HS, Song MJ, Han SB, Moon DC, Kang KW, Hong JT. Suppression of NF-kappaB and GSK-3beta is involved in colon cancer cell growth inhibition by the PPAR agonist troglitazone. *Chem Biol Interact* 2010; **188**: 75-85 [PMID: 20540935 DOI: 10.1016/j.cbi.2010.06.001]

158 **Theocharis S**, Giaginis C, Parasi A, Margeli A, Kakisis J, Agapitos E, Kouraklis G. Expression of peroxisome proliferator-activated receptor-gamma in colon cancer: correlation with histopathological parameters, cell cycle-related molecules, and patients' survival. *Dig Dis Sci* 2007; **52**: 2305-2311 [PMID: 17393321 DOI: 10.1007/s10620-007-9794-4]

159 **Shen D**, Deng C, Zhang M. Peroxisome proliferator-activated receptor gamma agonists inhibit the proliferation and invasion of human colon cancer cells. *Postgrad Med J* 2007; **83**: 414-419 [PMID: 17551074]

160 **Feilchenfeldt J**, Bründler MA, Soravia C, Tötsch M, Meier CA. Peroxisome proliferator-activated receptors (PPARs) and associated transcription factors in colon cancer: reduced expression of PPARγ-coactivator 1 (PGC-1). *Cancer Letters* 2004; **203**: 25-33 [DOI: 10.1016/j.canlet.2003.08.024]

161 **Sarraf P**, Mueller E, Smith WM, Wright HM, Kum JB, Aaltonen LA, de la Chapelle A, Spiegelman BM, Eng C. Loss-of-function mutations in PPAR gamma associated with human colon cancer. *Mol Cell* 1999; **3**: 799-804 [PMID: 10394368 DOI: 10.1016/S1097-2765(01)80012-5]

162 **Au-Yeung KK**, Liu PL, Chan C, Wu WY, Lee SS, Ko JK. Herbal isoprenols induce apoptosis in human colon cancer cells through transcriptional activation of PPARgamma. *Cancer Invest* 2008; **26**: 708-717 [PMID: 18608213 DOI: 10.1080/07357900801898656]

163 **Schwab M**, Reynders V, Loitsch S, Shastri YM, Steinhilber D, Schröder O, Stein J. PPARgamma is involved in mesalazine-mediated induction of apoptosis and inhibition of cell growth in colon cancer cells. *Carcinogenesis* 2008; **29**: 1407-1414 [PMID: 18544567 DOI: 10.1093/carcin/bgn118]

164 **Toaldo C**, Pizzimenti S, Cerbone A, Pettazzoni P, Menegatti E, Daniela B, Minelli R, Giglioni B, Dianzani MU, Ferretti C, Barrera G. PPARgamma ligands inhibit telomerase activity and hTERT expression through modulation of the Myc/Mad/Max network in colon cancer cells. *J Cell Mol Med* 2010; **14**: 1347-1357 [PMID: 19912441 DOI: 10.1111/j.1582-4934.2009.00966.x]

165 **Aires V**, Brassart B, Carlier A, Scagliarini A, Mandard S, Limagne E, Solary E, Martiny L, Tarpin M, Delmas D. A role for peroxisome proliferator-activated receptor gamma in resveratrol-induced colon cancer cell apoptosis. *Mol Nutr Food Res* 2014; **58**: 1785-1794 [PMID: 24975132 DOI: 10.1002/mnfr.201300962]

166 **Tsukahara T**, Hanazawa S, Kobayashi T, Iwamoto Y, Murakami-Murofushi K. Cyclic phosphatidic acid decreases proliferation and survival of colon cancer cells by inhibiting peroxisome proliferator-activated receptor γ. *Prostaglandins Other Lipid Mediat* 2010; **93**: 126-133 [PMID: 20932931 DOI: 10.1016/j.prostaglandins.2010.09.002]

167 **Choi IK**, Kim YH, Kim JS, Seo JH. PPAR-gamma ligand promotes the growth of APC-mutated HT-29 human colon cancer cells in vitro and in vivo. *Invest New Drugs* 2008; **26**: 283-288 [PMID: 18161004 DOI: 10.1007/s10637-007-9108-x]

168 **Delage B**, Bairras C, Buaud B, Pallet V, Cassand P. A high-fat diet generates alterations in nuclear receptor expression: prevention by vitamin A and links with cyclooxygenase-2 and beta-catenin. *Int J Cancer* 2005; **116**: 839-846 [PMID: 15856452 DOI: 10.1002/ijc.21108]

169 **Yamazaki K**, Shimizu M, Okuno M, Matsushima-Nishiwaki R, Kanemura N, Araki H, Tsurumi H, Kojima S, Weinstein IB, Moriwaki H. Synergistic effects of RXR alpha and PPAR gamma ligands to inhibit growth in human colon cancer cells--phosphorylated RXR alpha is a critical target for colon cancer management. *Gut* 2007; **56**: 1557-1563 [PMID: 17604322 DOI: 10.1136/gut.2007.129858]

170 **Cesario RM**, Stone J, Yen WC, Bissonnette RP, Lamph WW. Differentiation and growth inhibition mediated via the RXR: PPARgamma heterodimer in colon cancer. *Cancer Lett* 2006; **240**: 225-233 [PMID: 16271436 DOI: 10.1016/j.canlet.2005.09.010]

171 **Miao R**, Xu T, Liu L, Wang M, Jiang Y, Li J, Guo R. Rosiglitazone and retinoic acid inhibit proliferation and induce apoptosis in the HCT-15 human colorectal cancer cell line. *Exp Ther Med* 2011; **2**: 413-417 [PMID: 22977519]

172 **Shimada T**, Kojima K, Yoshiura K, Hiraishi H, Terano A. Characteristics of the peroxisome proliferator activated receptor gamma (PPARgamma) ligand induced apoptosis in colon cancer cells. *Gut* 2002; **50**: 658-664 [PMID: 11950812 DOI: 10.1136/gut.50.5.658]

173 **Wan YJ**, Cai Y, Magee TR. Retinoic acid differentially regulates retinoic acid receptor-mediated pathways in the Hep3B cell line. *Exp Cell Res* 1998; **238**: 241-247 [PMID: 9457077 DOI: 10.1006/excr.1997.3851]

174 **Han S**, Wada RK, Sidell N. Differentiation of human neuroblastoma by phenylacetate is mediated by peroxisome proliferator-activated receptor gamma. *Cancer Res* 2001; **61**: 3998-4002 [PMID: 11358817]

175 **James SY**, Lin F, Kolluri SK, Dawson MI, Zhang XK. Regulation of retinoic acid receptor beta expression by peroxisome proliferator-activated receptor gamma ligands in cancer cells. *Cancer Res* 2003; **63**: 3531-3538 [PMID: 12839938]

176 **Morosetti R**, Servidei T, Mirabella M, Rutella S, Mangiola A, Maira G, Mastrangelo R, Koeffler HP. The PPARgamma ligands PGJ2 and rosiglitazone show a differential ability to inhibit proliferation and to induce apoptosis and differentiation of human glioblastoma cell lines. *Int J Oncol* 2004; **25**: 493-502 [PMID: 15254749]

177 **Pancione M**, Forte N, Fucci A, Sabatino L, Febbraro A, Di Blasi A, Daniele B, Parente D, Colantuoni V. Prognostic role of beta-catenin and p53 expression in the metastatic progression of sporadic colorectal cancer. *Hum Pathol* 2010; **41**: 867-876 [PMID: 20129645 DOI: 10.1016/j.humpath.2009.09.019]

178 **Zeestraten EC**, Benard A, Reimers MS, Schouten PC, Liefers GJ, van de Velde CJ, Kuppen PJ. The prognostic value of the apoptosis pathway in colorectal cancer: a review of the literature on biomarkers identified by immunohistochemistry. *Biomark Cancer* 2013; **5**: 13-29 [PMID: 24179395 DOI: 10.4137/BIC.S11475]

179 **Vousden KH**. Review: Activation of the p53 tumor suppressor protein. *BBA - Reviews on Cancer* 2002; **1602**: 47-59 [PMID: 11960694 DOI: 10.1016/S0304-419X(02)00035-5]

180 **Jiang M**, Milner J. Bcl-2 constitutively suppresses p53-dependent apoptosis in colorectal cancer cells. *Genes Dev* 2003; **17**: 832-837 [PMID: 12670866]

181 **Huerta S**, Goulet EJ, Livingston EH. Colon cancer and apoptosis. *Am J Surg* 2006; **191**: 517-526 [PMID: 16531147 DOI: 10.1016/j.amjsurg.2005.11.009]

182 **Shaw P**, Bovey R, Tardy S, Sahli R, Sordat B, Costa J. Induction of apoptosis by wild-type p53 in a human colon tumor-derived

cell line. *Proc Natl Acad Sci USA* 1992; **89**: 4495-4499 [PMID: 1584781 DOI: 10.1073/pnas.89.10.4495]

183 **Soussi T**. p53 alterations in human cancer: more questions than answers. *Oncogene* 2007; **26**: 2145-2156 [PMID: 17401423 DOI: 10.1038/sj.onc.1210280]

184 **Fazeli A**, Steen RG, Dickinson SL, Bautista D, Dietrich WF, Bronson RT, Bresalier RS, Lander ES, Costa J, Weinberg RA. Effects of p53 mutations on apoptosis in mouse intestinal and human colonic adenomas. *Proc Natl Acad Sci USA* 1997; **94**: 10199-10204 [PMID: 9294187 DOI: 10.1073/pnas.94.19.10199]

185 **Liu J**, Stevens J, Rote CA, Yost HJ, Hu Y, Neufeld KL, White RL, Matsunami N. Siah-1 mediates a novel beta-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein. *Mol Cell* 2001; **7**: 927-936 [PMID: 11389840 DOI: 10.1016/S1097-2765(01)00241-6]

186 **Gwak J**, Song T, Song JY, Yun YS, Choi IW, Jeong Y, Shin JG, Oh S. Isoreserpine promotes beta-catenin degradation via Siah-1 up-regulation in HCT116 colon cancer cells. *Biochem Biophys Res Commun* 2009; **387**: 444-449 [PMID: 19607803 DOI: 10.1016/j.bbrc.2009.07.027]

187 **Matsuzawa SI**, Reed JC. Siah-1, SIP, and Ebi collaborate in a novel pathway for beta-catenin degradation linked to p53 responses. *Mol Cell* 2001; **7**: 915-926 [PMID: 11389839 DOI: 10.1016/S1097-2765(01)00242-8]

188 **Mrass P**, Rendl M, Mildner M, Gruber F, Lengauer B, Ballaun C, Eckhart L, Tschachler E. Retinoic acid increases the expression of p53 and proapoptotic caspases and sensitizes keratinocytes to apoptosis: a possible explanation for tumor preventive action of retinoids. *Cancer Res* 2004; **64**: 6542-6548 [PMID: 15374966 DOI: 10.1158/0008-5472.CAN-04-1129]

189 **Sarkar SA**, Sharma RP. All-trans-retinoic acid-mediated modulation of p53 during neural differentiation in murine embryonic stem cells. *Cell Biol Toxicol* 2002; **18**: 243-257 [PMID: 12206137 DOI: 10.1023/A:1016003027850]

190 **Zhang J**, Tu Y, Smith-Schneider S. Activation of p53, inhibition of telomerase activity and induction of estrogen receptor beta are associated with the anti-growth effects of combination of ovarian hormones and retinoids in immortalized human mammary epithelial cells. *Cancer Cell Int* 2005; **5**: 6 [PMID: 15755327 DOI: 10.1186/1475-2867-5-6]

191 **Donato LJ**, Suh JH, Noy N. Suppression of mammary carcinoma cell growth by retinoic acid: the cell cycle control gene Btg2 is a direct target for retinoic acid receptor signaling. *Cancer Res* 2007; **67**: 609-615 [PMID: 17234770 DOI: 10.1158/0008-5472.CAN-06-0989]

192 **McPherson LA**, Loktev AV, Weigel RJ. Tumor suppressor activity of AP2alpha mediated through a direct interaction with p53. *J Biol Chem* 2002; **277**: 45028-45033 [PMID: 12226108 DOI: 10.1074/jbc.M208924200]

193 **Carrera S**, Cuadrado-Castano S, Samuel J, Jones GD, Villar E, Lee SW, Macip S. Stra6, a retinoic acid-responsive gene, participates in p53-induced apoptosis after DNA damage. *Cell Death Differ* 2013; **20**: 910-919 [PMID: 23449393 DOI: 10.1038/cdd.2013.14]

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TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Treatment of colorectal cancer in the elderly

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Abstract

Colorectal cancer has a high incidence, and approximately 60% of colorectal cancer patients are older than 70, with this incidence likely increasing in the near future. Elderly patients (> 70-75 years of age) are a very heterogeneous group, ranging from the very fit to the very frail. Traditionally, these patients have often been under-treated and recruited less frequently to clinical trials than younger patients, and thus are under-represented in publications about cancer treatment. Recent studies suggest that fit elderly patients can be treated in the same way as their younger counterparts, but the treatment of frail patients with comorbidities is still a matter of controversy. Many factors should be taken into account, including fitness for treatment, the wishes of the patient and family, and quality of life. This review will focus on the existing evidence for surgical, oncologic, and palliative treatment in patients over 70 years old with colorectal cancer. Careful patient assessment is necessary in order to individualize treatment approach, and this should rely on a multidisciplinary process. More well-designed controlled trials are needed in this patient population.

Key words: Colorectal cancer; Surgery; Chemotherapy; Radiotherapy; Elderly; Palliative care

Core tip: With the rise in the incidence of colorectal cancer and in the population > 70 years of age, the need to decide what type of treatment is most appropriate for patients > 70 with colorectal cancer will become more frequent. Age in itself should not be an exclusion criterion for radical treatment, but there will be many elderly patients that will not tolerate or respond well to standard therapies. These patients need to be properly assessed before proposing treatment, and a tailored, individualized approach should be offered in a multidisciplinary setting.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers worldwide, and its incidence is increasing[1]. The choice of treatment is based on several factors, including stage at presentation, location, and the conditions of the patient. Current treatment in general for CRC includes surgery for CRC stage Ⅰ or Ⅱ; surgery followed by adjuvant chemotherapy for stage Ⅲ colon cancer; and in cases of metastatic CRC (mCRC), systemic chemotherapy alone or in combination with targeted biologics. mCRC requires multidisciplinary management, where surgical resection of metastatic disease is considered wherever possible. The treatment of rectal cancer includes surgery alone in stage Ⅰ or short-course radiotherapy or chemoradiotherapy with surgical resection followed by adjuvant chemotherapy in selected stage Ⅱ and Ⅲ patients[2].

Approximately 60% of CRC patients are > 70 years of age at the time of diagnosis, and 43% are > 75[1]. These proportions will likely continue to increase in the near future. Many of these older patients will have problems of frailty and comorbidity that demand careful patient assessment, and, if necessary, individualized treatment approaches[3].

Aging may be defined as a progressive decline in the functional reserve of multiple organ systems. This process is highly individualized, and poorly reflected in chronological age. The treatment of cancer should be based on the assessment of the physiological age, the patient's life expectancy, and tolerance to treatment[4]. Older patients risk being undertreated, and, therefore, presenting a worse oncologic outcome. If they are over treated, however, there is an increased risk of morbidity and mortality[5].

The challenge in this group of patients comes from the physiological heterogeneity of the older patient population, with frequent discrepancies between physiological and chronological age, coupled with the additional complications of coexisting medical conditions and potential psychological and social care issues[6].

The treatment of those at the upper extreme of life often presents significant clinical dilemmas. A critical appraisal is needed of the costs and benefits of treatment, and a better selection of patients who can benefit from available therapies is warranted. There is a paucity of controlled trials including this group of patients, and, therefore, evidence-based decision-making is difficult. Many elderly patients will benefit from radical treatment approaches, but others will not, and in some cases, non-operative "palliative" management should be offered, even though the cancer is "curable". This review aims to focus on the existing evidence to aid in the decision-making process for treatment of CRC in elderly patients.

GERIATRIC ASSESSMENT

The patient's biological age should ideally be established through a comprehensive geriatric assessment in order to aid therapeutic decisions.

There is a paucity of clinical trial data in these patients who, in many cases, have poor functional reserves, major comorbidities, and frailty. In older patients, functional levels vary widely- from robust and able to tolerate cancer treatments to frail and unable to tolerate even minor interventions without life-threatening consequences. At either end of this spectrum, treatment decisions are clear, but the identification of individuals at risk for functional decline and frailty, where interventions or treatment modifications are needed, is where geriatrics could have the biggest impact on oncology[7].

By distinguishing the fit from the vulnerable older patients, treatment can be adjusted to maximize its effectiveness, avoid complications, and better meet the individual requirements of the older patient. When choosing between various treatment options, quality of life and function may be at least as important for the elderly as the cancer-specific or surgical outcome[6].

The main difficulty for individualizing treatment in elderly patients is the capacity to evaluate vulnerability to treatment. Several aspects should be taken into account[8], which include: (1) an estimation of life-expectancy based on functional evaluation and co-morbidities; (2) an estimation of the risk of cancer-related morbidity: a: Tumor stage at diagnosis; b: Risk of recurrence and tumor progression; and c: Tumor aggressiveness; (3) an evaluation of the conditions that could interfere in the cancer treatment and tolerance; a Comprehensive Geriatric Assessment[7] (CGA), which includes: a: undernutrition (recent loss of > 5% weight/body mass index < 19); b: polypharmacy (more than 10 medications); c: social isolation; d: depression; e: cognitive disorder; f: risk of falls; g: side effects of neoplasia: sensory deterioration, urinary incontinence, sexual dysfunction; h: comorbidities (number and severity of co-existing illnesses); and (4) an evaluation of the goals of the patient (what the patient expects from treatment). An important aspect of this evaluation is quality of life (subjective evaluation of life as a whole). The instruments that can be used to measure quality of life include, at least three of the following 10 aspects[9,10]: Pain and other somatic symptoms, functional capacity, social and family well-being, emotional well-being, spirituality, satisfaction with care, future hopes and wishes, sexuality, body image, and social and work-related function.

Elements of the CGA, especially comorbidity, functional status, cognitive dysfunction, and frailty, are consistently associated with adverse treatment outcomes in relation to both toxicity and mortality[11-13].

A complete CGA is time-consuming. For now, it might be beneficial for all elderly patients with cancer

to receive a complete geriatric assessment[14], although recent publications show promise in the use of frailty screening methods to select which patients will benefit from a complete CGA or further assessment: (1) test Timed Up and Go: Patients who require more than 10 s to perform the exercise, need to use their arms to get up, or perform an erroneous trajectory will need a full CGA[15,16]; (2) seven-item physical performance: this test takes 10 min to perform. If the total result is less than 20, a CGA would be beneficial. It has been demonstrated to be more sensitive than the Karnofsky Performance Status in recognising patients with a higher risk of functional decline[16]; and (3) the Vulnerable Elderly Survey 13 (VES-13)[17]: when the scores are equal or above 3 it indicates a higher risk of functional deterioration, and a 4-fold increased probability of death in the next 2 years, and, therefore, a complete CGA is indicated[18-21].

In 2012[22], an algorithm was proposed to evaluate an elderly cancer patient that uses the frailty criteria, the VES-13 scale and the CGA. All patients diagnosed with cancer would be tested using VES-13. If the score is < 3 the patient can receive the standard treatment recommended for adult patients according to tumor stage. If the score is > 3, a full CGA is recommended, and further recommendations can be made according to the possibilities of treatment of the patient's comorbidities or functional dependence; palliative or standard treatment could be recommended.

The concept of frailty is still under construction and has many common aspects with the definition of aging. Fried *et al*[23] criteria include an assessment of weight loss, physical exhaustion, physical activity level, grip strength, and walking speed. Any degree of frailty measured by the Hopkins Frailty Score[24] has been linked to a worse postoperative outcome after surgery for CRC. Core features of frailty include impairments in multiple, interrelated systems, resulting in a reduced ability to tolerate stressors. This is associated with an increase in vulnerability to severe complications with cancer treatment, which translates into an increase in global mortality[25,26].

The CGA should include the following determinations[27]: (1) functional status: Evaluation of dependency in daily activities using scales such as Barthel and Lawron, the TITAN scale, and Karnofsky index. Functional decline in elderly patients is a predictor of short- and medium-term mortality, independent of the disease process[28]; (2) coexisting illness (Comorbiditiy): The Charlson comorbidity index[29] predicts 1-year mortality in patients with comorbidities. Sarcopenia (skeletal muscle depletion) in older patients is related to infection, requirements for rehabilitation following surgery, and length of hospital stay[30]; (3) socio-economic evaluation: the elderly population is at a greater risk of social deprivation[28]. The social situation of the elderly patient should always be evaluated, and the detection of social isolation should lead to the application of the necessary social resources; (4) nutritional status: Mini Nutritional Assessment[31]. An albumin < 2.5 g/dL + CT < 156 mg/dL + weight loss of 10% indicates terminal illness; (5) cognitive status: Mental Status Questionnaire-Pfeiffer and Mini Mental State Examination. The impact of depression and dementia on oncologic treatment is not well known[32,33], but it has been identified as one of the determinant factors in receiving inadequate treatment[34,35]; (6) geriatric syndromes: sleep disturbances, incontinence, risk of falls, *etc*. The presence of geriatric syndromes is an indicator of frailty. An assessment of the cognitive and emotional state is especially important in older cancer patients. Polypharmacy is common in older patients, and the possibility of drug interactions and the delicate clinical situation in a geriatric cancer patient should be considered; (7) surgical risk: The American Society of Anesthesiologists (ASA) classification continues to be one of the most reliable predictors of postoperative morbidity and mortality[34,35]. Multiple studies have shown that the presence of comorbidities increases the risk of postoperative complications, and this is more evident in patients over 70 years of age[35]; and (8) An evaluation of the patient's views on the goals of treatment (what does the patient expect and want?). Optimal treatment of the older adult patient who has cancer starts with a careful delineation of goals through conversation. There is a general tendency to think that geriatric patients do not want to be informed about the diagnosis and prognosis of their disease; however, several studies refute this hypothesis[36,37]. In reality, there does not seem to be any difference with respect to age regarding the wish of cancer patients to receive information[38].

Multidisciplinary cooperation involving oncologists, gastroenterologists, radiotherapists, anesthetists, radiologists, pathologists, and surgeons has become essential in elderly patients. Geriatricians are not typically members of MDTs, but there is clear evidence that older CRC patients should be treated in centers where the expertise is available to provide the most favorable surgical and oncologic treatment and care[21,39].

Balducci[40] studied the role of CGA in the selection of oncologic treatment and divided patients into three groups depending on the severity of frailty symptoms and signs: Type Ⅰ: Functionally independent patient without important comorbidities: these patients would be candidates to receive onco-specific treatment in standard conditions; Type Ⅱ: Functionally dependent patient with two or less comorbidities: these patients could benefit from a modified onco-specific treatment with standard intention; and Type Ⅲ: Partially dependent patient with three or more comorbidities or the presence of a geriatric syndrome: these patients would be candidates for symptom treatment exclusively (palliative care).

SURGERY

There is no consensus about the optimal surgical

management of elderly people, who are a heterogeneous group of patients, ranging from very fit to very frail individuals. This population is undertreated compared with younger patients, with a lower percentage of patients operated on; a lower rate of curative surgery, and more emergency surgery. Elderly patients are generally recruited to clinical trials less often than younger patients and are under-represented in publications about cancer treatment[41].

A comprehensive geriatric assessment is a major consideration when assessing operative risk, treatment decision making, and adapting perioperative care, if surgery is undertaken.

Surgical risk stratification remains one of the most important aspects of management in elderly patients[42]. Age is associated with increased mortality following elective colorectal resection, up to 15.6% in patients > 80 years of age. Elderly patients with higher levels of comorbidity might be expected to have significantly higher rates of complications, longer hospital stays, and higher mortality[43].

Elderly patients deemed to be optimized for surgery through traditional clinical and biochemical markers may still have poor outcomes. The concept of frailty can be used to identify a group of patients for further investigation before surgery[23]. Patients who were positive for frailty had 4 times higher risk of developing major complications (OR = 4.083; 95%CI: 1.433-11.638)[43]. Decreased survival in older (> 75 years) patients post-surgery has mainly been attributed to differences in early mortality[44-48]. The rate of cardiovascular complications increases significantly with age. Pulmonary complications are also twice as common. Postoperative complications are more severe in elderly patients[49-52]. The occurrence of a complication was associated with a significantly increased risk of 6 mo mortality. Overall, 6 mo mortality was 4 times higher in elderly patients than in younger patients (14% *vs* 3.3%; $P < 0.0001$) as was the 1-year mortality rate (20.1% *vs* 5.1%)[53]. Progressive loss of stress tolerance with aging exacerbates the consequences in case of postoperative complications[54]. However, older patients with CRC who survived the first year after surgery had the same overall cancer-related survival as younger patients[53].

Therefore, the focus should be on survival and minimizing postoperative complications during the first postoperative year. Pre-habilitation programs could be of great importance in elderly patients: Correction of malnutrition, optimization of cardiovascular and pulmonary comorbidities, and medication use have been shown to reduce complications after elective surgery in elderly patients and are a promising area of future research[54].

Emergency surgery should be avoided if possible. The presence of obstruction or perforation increases the perioperative mortality rate in older patients. Several studies show the correlation between advanced age, mortality, and emergent surgery. Kurian *et al*[55] reported a postoperative 30 d mortality rate of 28% in emergent surgery compared to only 5% in elective surgery. Morse *et al*[56] found similar outcomes in 39 patients older than 80 in open colectomy for colon cancer. In the same way, Louis *et al*[57] observed the close correlation between advanced age, advanced ASA grade, and emergent surgery, and other authors found that no patients with an ASA grade of 3 or more survived more than 6 mo[58]. Modini *et al*[59] reported a 6 fold higher 30 d postoperative mortality in elderly patients > 80 years of age with respect to others. They noted that although morbidity and mortality rates in elderly patients could be similar to that of younger patients, it would rise up to 9 fold higher in cases of emergent surgery[60,61]. Patients over 70 years of age after emergency surgery have been shown to have a higher rate of postoperative myocardial infarction, and this complication is associated with a 6 times higher rate of mortality in the postoperative period[62]. Other common complications are pulmonary failure, acute renal failure, and sepsis; anastomotic leakage also occurred more frequently in elderly patients after emergency colorectal surgery and presented a significant association with postoperative mortality[63].

A feasible alternative management to emergency surgery for colonic obstruction could be the endoscopic placement of stents, especially in acute left-sided colonic obstruction. Use of these self-expanding metallic stents would provide "extra time" to better study the patient's clinical situation and the tumor-stage, improve the nutritional status, optimize comorbidities, and, in some cases, allow a subsequent elective surgery. Consequently, it is an appealing option either for palliation or as a "bridge" to definitive surgery in the management of left-sided colonic obstruction for elderly patients. Nevertheless, the current data are controversial and the advantages in terms of early morbidity and mortality compared to emergency surgery are not as clear as originally described[64].

Laparoscopic surgery has been shown to reduce postoperative pain, allowing a decreased use of narcotics and opioids, reduced postoperative ileus, and a reduced hospital stay[65]. Furthermore, elderly patients benefit from laparoscopic surgery because it reduces the risk of cardiovascular and pulmonary complications, reduces intraoperative blood loss, and seems to accelerate gastrointestinal recovery. Stocchi *et al*[66] found that the preoperative functional status of patients was more frequently maintained at the time of discharge in elderly patients operated on by laparoscopy. In a randomized trial including 553 patients, Frasson *et al*[65] similarly concluded that laparoscopy should be the first choice in elderly patients operated on for CRC because it increases preservation of functional status, allowing a higher rate of independence during the postoperative period and discharge and a faster postoperative recovery.

However, most trial protocols of laparoscopic surgery for CRC have been biased to exclude or under-

represent the elderly. Decision-making for such patients is, therefore, still based on inadequate evidence[67-69]. Clinical trials on laparoscopic surgery in the older population are lacking: 44% of trial protocols excluded elderly patients. Nevertheless, since a higher systemic inflammatory response to the surgical aggression and lower physiological reserve appear to be the origin of the high postoperative mortality in the elderly patient[70-73], laparoscopic surgery could be beneficial due to its decrease in inflammatory response and lower surgical stress[74-79].

The literature suggests that elderly patients benefit from multimodal rehabilitation programs or enhanced recovery programs after surgery (ERAS) in the same way as younger patients[80]. Initial studies by Senagore *et al*[75] and more recent studies by Keller *et al*[81] and Wang *et al*[82] showed better results in terms of length of stay, readmission rate, and reoperation rates for elderly people using ERAS programs. Elderly patients benefit from the avoidance of bowel preparation, opioid restriction, and early mobilization. There does not seem to be an increased risk of aspiration pneumonitis in elderly patients following early resumption of oral feeding, although overall complications are higher in elderly patients[80].

Delays in discharge of elderly patients can be attributable to inadequate levels of social support or resources in the community, even when the postoperative course has been uneventful. Liaison with elderly care physicians may minimize avoidable hospital stay by optimizing the management of geriatric syndromes and by pre-emptively addressing the psychosocial needs of older patients. Specialized, organized, and coordinated geriatric care in the hospital setting improves outcomes, such as survival and in their own home up to 1 year after surgery[83-85].

In spite of all of the above, the fact still remains that some elderly patients will do very well after curative surgery, and others will not[86,87]. It is quite clear from the literature that the risks and benefits of surgery for CRC in the elderly have not been clearly reviewed[86]. There is, therefore, still no common consensus on how actively we should treat the elderly and when not to push them into unnecessary surgery, which could lead to severe functional impairment and diminished quality of life. Over 74% of patients interviewed in a recent study stated that they would refuse, or be reluctant, to receive treatment leading to severe functional impairment[87]. Life-expectancy, higher rates of 60 d mortality, higher likelihood of impairment of physical and mental function, and the possibility of never returning home and needing permanent residential care, should ideally be considered and discussed with the patient and family before deciding on surgical treatment[88].

RECTAL CANCER

Older patients with rectal cancer undergoing surgery should receive the same treatment as their younger counterparts, but with an adjustment of treatment strategy in the case of comorbidity, limited physiologic reserves, and emergency situations. Complete mesorectal excision is considered the "gold-standard" surgical treatment for rectal cancer, but we continue to look for alternatives to avoid the high rates of postoperative morbidity[89]. Elderly patients are less frequently treated with neoadjuvant radiotherapy or chemotherapy, and non-restorative procedures are more frequently used. Anterior resection is performed less often in elderly patients, although tumor location and stage does not differ[90-92].

Population-based studies clearly show that older patients with rectal cancer are treated less often with RT[90-92]. Fewer older patients are likely to receive preoperative RT with proportionately more receiving palliative RT as an alternative[93]. Older patients with stage II or III rectal cancer who are fit enough for surgery are generally fit enough for preoperative neoadjuvant radiation therapy. Tolerability and response rates are similar to those seen in younger patients. However, Stockholm I and II Trials have shown the distinct negative effects of neoadjuvant radiotherapy in older patients (> 80 years). The incidence of venous thromboembolism, femoral neck and pelvic fractures, intestinal obstruction, and postoperative fistulas was significantly increased after preoperative radiotherapy in this group of patients[90,94].

The aim of rectal cancer surgery in older patients should be not only to avoid local recurrence but also to maintain health and function with a view to optimizing their chances of coping with their treatment. Older patients are keen to avoid a permanent stoma and may accept a higher risk of local recurrence to achieve this. The impact of cancer surgery on quality of life is very important in elderly people. Sphincter function, assessed clinically and if necessary after manometry, is an essential element to consider in the preoperative assessment and the decision-making procedure. The delay of surgery following short-course radiotherapy has also been associated with a decrease in postoperative morbidity.

Rather than age itself, the frailty of patients and preoperative sphincter function determine the operative indication and type of surgery[94,95]. Sphincter preservation in the elderly could give poor functional results with a higher risk of anal incontinence, and the potential effect of a permanent stoma on quality of life should be considered. Age was found as a significant risk factor associated with a decreased likelihood of stoma reversal[95].

Proctectomy in nursing-home residents has been associated with a 1 year postoperative mortality of 51% in patients with a permanent colostomy. Substantial postoperative mortality occurred in the first 6 mo after proctectomy and was significantly higher in elderly populations[96,97].

It has been observed that with neoadjuvant treatment there is a percentage of patients who present a

complete pathological response (pCR), up to 44%[98,99]. There is an increasing interest in a more conservative treatment for these patients. Several authors have proposed a "watch and wait" policy for patients when no residual tumor can be found. In a study published in 2010[100], the authors proposed an analytical decision model comparing the results between empirical radical surgery and observation alone in patients with pCR, and concluded that observation is better than surgery in cases where the ability to detect patients with pCR is higher than 58%, when patients will not have a good quality of life after surgery, or when the risk of recurrence was less than 43% when compared to observation. This study only included patients < 65 years of age, and excluded elderly patients with comorbidity[100].

Following the same working model, Smith *et al*[101] published a study in 2015 evaluating the differences between radical surgery and observation after neo-adjuvant treatment in cases of pCR and divided patients into three groups: Healthy 60-year-old patients, healthy 80-year-old patients, and 80-year-old patients with associated comorbidity. The study concluded that elderly patients, because of their higher surgical risk, obtained the greatest benefit from the "watch and wait" policy and showed an improved survival at 1 year after treatment.

The groups of patients that present a significant tumor regression with neoadjuvant chemoradiation, and especially those with lymph node regression (ypN0), could be candidates for alternative treatments for rectal cancer without needing total mesorectal excision (TME). Transanal endoscopic surgery could be an interesting option in these patients[102,103]. Recent studies have attempted to detect the subgroups of patients with a good response to neoadjuvant treatment where transanal endoscopic surgery could reduce the recurrence rate[104-106]. Habr-Gama *et al*[107] pioneered the decision not to operate on patients with rectal cancer who presented a complete clinical response after chemoradiation. This same group has published a series of "watch and wait" in 70 patients with cT2-4cN1-2 treated with chemoradiation, and of the 47 patients with a complete clinical response, eight (17%) presented an early recurrence and four a late recurrence. All had subsequent radical R0 surgery and were disease-free 56 mo later. This could be an option for patients who are not considered fit for surgery; the difference would be that it does not have to be considered a palliative treatment but a possible standard treatment with a 50% probability of cure in frail elderly patients.

No prospective randomized trials comparing the results of neoadjuvant chemoradiation and local excision include elderly patients, but the results in the general population can be taken into consideration in these patients. A study by Bhangu *et al*[108] analyzed the results of local excision in elderly patients and concluded that local excision achieved the same results as radical surgery in patients with pT1 tumors, the same as in the general population, but decreased survival in pT2. The difference with the general population could be due to the amount of comorbidities present in this group of patients; they would not be candidates for the same type of chemoradiation treatment, and, therefore, the results would not be comparable with those published up to the present time.

However, transanal endoscopic surgery can also be considered as a palliative treatment in patients with comorbidities who are not fit for radical surgery or who refuse a stoma, after carefully considering all options[109].

BIOLOGICAL FEATURES OF CRC IN THE ELDERLY

CRC is related to age, but there are few available data on the genetic differences and alterations in the carcinogenesis process between younger and older patients.

In many studies, younger patients are more likely to have mucinous, poorly differentiated and signet ring tumors, but there are mixed results in terms of prognosis. Several studies have suggested that younger age was a poor prognostic factor[110-112], but others suggested the opposite when adjusting for confounding variables, such as tumor, treatment, and patient factors[113-118].

The most frequently observed somatic mutations in CRC were found in the *APC*, *TP53*, *KRAS,* and *PIK3CA* genes.

A model has been proposed for the carcinogenic process in sporadic CRC, in which normal colonic mucosa would transform into invasive carcinoma. This model, named chromosomal instability pathway (CIN), implicates somatic mutations in a multi-step process, with alterations in different genes in chronological order [APC, Kirsten rat sarcoma (KRAS), Smad2/4, and tumor protein 53 (TP53)]. In a minority of cases of sporadic CRC, approximately 15%, the pathway responsible for the transformation of the colon epithelium is through an inappropriate mismatch repair system (MMR). The system cannot repair the mismatches, resulting in a length variability of DNA microsatellites, called microsatellite instability (MSI). Another proposed pathway responsible for the carcinogenic process is DNA hypermethylation [CpG island methylator phenotype (CIMP)][119,120].

Patients with the same stage of disease have a different natural history and a different prognosis, as a result of the heterogeneity of the process. Some conditions give a more favorable prognosis (MSI, BRAF not mutated) or a worse prognosis (hypermethylation and not MSI). Currently, the only marker applicable to clinical practice is the *RAS* mutation.

In an analysis of 181 patients with CRC, patients were divided into different groups: Those under 50 years of age, from 51 to 70, and over 70. In the

group of patients over 70 years of age, the MSI and BRAF mutations were correlated, but there was no correlation in the group under 50. Mutations in the *KRAS* and *BRAF* genes were more common with age, but no phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) mutations were found. TP53 mutations were more common in older patients. There were no differences in the frequency of phosphatase and tensin (*PTEN*) gene mutations. The conclusions were that older patients had a greater index of genetic mutations, and the incidence of BRAF mutations was higher. CIMP tumors are more common in the older population, who also have a higher rate of KRAS and BRAF mutations. These mutations have treatment implications[120]. TP53 mutation is associated with more advanced stages and vascular and lymphatic involvement[121]. *KRAS* gene mutation is a predictor of resistance to treatment with monoclonal antibody receptor endothelial growth factor (EGFR)[122-124]. BRAF V600E mutation confers worse prognosis[125,126]. A deficiency of the MMR system appears to be a favorable prognostic factor associated with adjuvant treatment in stage Ⅱ CRC[127,128].

CHEMOTHERAPY

The aging process involves an organic functional impairment, with decreased liver and kidney function, decreased bone marrow reserve, increased risk of cardiovascular events, cognitive impairment, other comorbidities, or use of polypharmacy. These conditions favor a greater toxicity with chemotherapy, which results in a diminished quality of life and adherence to treatment. The most commonly used scales to evaluate functional status, such as the Karnofsky performance status or the Eastern Cooperative Oncology Group (ECOG), should be used in the context of a comprehensive geriatric assessment in order to classify the elderly as fit or frail, the latter being more exposed to higher toxicity with chemotherapy, hospitalization, and death.

There is a consensus that frail patients with ECOG PS 3 or 4 or IK less than 60 are not eligible for chemotherapy due to poor benefits and high toxicity; the consensus seems also clear about being more aggressive in fit patients. The challenge is to decide the best treatment for those who are neither fit nor frail[129,130].

Adjuvant treatment

The benefit of adjuvant chemotherapy for stage Ⅲ (node positive) CRC is well established, representing approximately a 30% reduction in the risk of recurrence and a 22%-32% reduction in the risk of death compared with observation alone. Elderly patients are referred to the oncologist less frequently than younger patients, especially those with comorbidities, and when referred they are less likely to be treated with chemotherapy. An update of SEER - Medicare analysis data and three population-based data sets conducted by Sanoff *et al*[131] showed that only 44% of the 5941 patients evaluated received adjuvant chemotherapy within 3 mo of surgical resection for stage Ⅲ CRC.

Since 2001, intravenous 5-fluorouracil modulated with leucovorin (FU/LV) in the adjuvant setting has shown better outcomes than observation, even in elderly patients. A pooled analysis of 3351 patients from seven randomized phase Ⅲ adjuvant chemotherapy trials comparing chemotherapy *vs* surgery alone for stage Ⅱ or Ⅲ colon cancer showed a 29% reduction in the risk of death at 5 years[132]. The benefit was independent of age, and no differences in toxicity were seen with respect to younger patients. Only one study showed a greater proportion of grade 3 or 4 neutropenia (8% *vs* 4%) without increased neurological toxicity, diarrhea, infection, nausea, or vomiting.

Capecitabine (an oral fluoropyrimidine) also proved to be as effective as FU/LV in adjuvant treatment in a subgroup analysis of patients equal to or greater than 70 years of age, with no differences in toxicity by age, although it was more toxic than FU/LV[133,134].

These results are supported by other studies with patients of 80 years of age or more, where there was a higher incidence of grade 3 or 4 toxicity, especially diarrhea (31% *vs* 13%) and hand-foot syndrome[135]. With the MOSAIC trial, oxaliplatin was established as a new adjuvant standard in combination with 5FU/LV plus infusional 5FU short-term and leucovorin (FOLFOX) as compared with 5FU and leucovorin alone in resected stage Ⅲ colon cancer, with a 20% reduction in the risk of recurrence and a 16% reduction in risk of death at 6 years. But the analysis of 315 patients over 70-75 years of age revealed that although there was a survival benefit with fluoropyrimidines, there was no benefit in disease-free survival (DFS), overall survival (OS), or time to recurrence (TTR) by adding oxaliplatin [OS hazard ratio (HR) 1.10, 95%CI: 0.73-1.65] or in patients with stage Ⅱ tumours[136].

The National Surgical Adjuvant Breast and Bowel Project (NSABP) C-07 trial analyzed 2409 patients in stage Ⅱ or Ⅲ treated with weekly bolus of FU and leucovorin with or without oxaliplatin. The results showed that the addition of oxaliplatin to 5FU/LV gave no survival benefit in patients equal to or greater than 70 years of age in stage Ⅱ or Ⅲ colon cancer (n = 396), but a higher grade 4 toxicity (20% *vs* 13%) was found. The benefit in OS was only observed in patients under 70 years of age[137]. In contrast, the N016968 trial, which randomized capecitabine *vs* bolus 5FU and oxaliplatin in stage Ⅲ exclusively, showed an increase in DFS in both populations under or over 65 years of age with an HR 0.8[138].

The Adjuvant CC End Points (ACCENT) database (including seven randomized trials such as MOSAIC, NSABP C-07, and N016968) included 14528 patients in stage Ⅱ or Ⅲ treated with a 5FU combination with oxaliplatin or irinotecan *vs* 5FU alone. The results of the 2575 patients greater than or equal to 70 years of age did not show a benefit in DFS or OS by

adding oxaliplatin to adjuvant treatment (DFS: HR = 0.94; 95%CI: 0.78-1.13; OS: HR = 1.04; 95%CI: 0.85-1.27). They did not consider death from other causes or change in efficacy due to reductions or delays of doses[139]. In contrast to these data, the analysis of Sanoff *et al*[131] with 4060 patients in stage Ⅲ CRC including five cohorts, the largest cohort of the SEER-Medicare database, saw a marginal benefit with no statistically significant difference when adding oxaliplatin. Also, there were more adverse events with oxaliplatin compared with fluoropyrimidine. Among patients older than 75 years of age, more neutropenia (OR = 17.3, 95%CI: 9.8-30.42) and nausea or vomiting were found (OR = 2.14, 95%CI: 1.73-2.65) without differences in diarrhea or hydration[140]. In summary, it seems that the benefit and toxicity of 5FU/LV in the adjuvant setting is similar between young and elderly patients.

Although adjuvant treatment is offered to patients in stage Ⅱ CRC with risk factors (T4, perforation, lymphovascular or perineural invasion, poorly differentiated histology), the benefit of adjuvant chemotherapy for stage Ⅱ is more controversial, and there are no data to ensure which patients are most likely to benefit from adjuvant treatment.

In an attempt to identify the subgroup of patients with stage Ⅱ CRC who may benefit from adjuvant therapy, there have been efforts to find prognostic biomarkers. The deficiency of the MMR system or MSI seems a promising marker. Several studies have found an association between high microsatellite instability (MSI-H) and better prognosis but resistance to treatment with fluorouracil[141].

It seems reasonable to analyze the MMR deficiency in patients with T3 stage Ⅱ to select those who could benefit from treatment with 5FU. Its application has not been validated in clinical practice, and, therefore, clinical decisions to administer chemotherapy should not be based on this analysis. It is not a common occurrence in the metastatic context and does not seem to play a role in the prognostic stratification.

Data from the SEER-Medicare database indicate that adjuvant treatment does not increase the OS in patients over 65 years of age with stage Ⅱ CRC with or without risk factors[142]. In stage Ⅱ patients with risk factors, the chemotherapy options are FU/LV or capecitabine if the patient is capable of adhering to the medication, although no differences were found in the Quasar study. This study showed a marginal benefit in OS of 3.6% in patients greater than or equal to 70 years of age with stage Ⅱ CRC[143]. The lack of benefit in stage Ⅱ does not justify the use of oxaliplatin. The benefit of adding oxaliplatin in patients > 70 years of age in stage Ⅲ CRC is doubtful and is not supported by data from the results of clinical trials, such as MOSAIC and NSABP, even though the elderly population included was very small. It is difficult to establish whether 70 years old is a reasonable cut-off age to safely extrapolate these results or if the decision should depend on the physical and functional status of the patient, not only on the chronological age. In fit elderly patients with stage Ⅲ CRC with a life expectancy of at least 5 years, the benefit of adding oxaliplatin must be discussed. The modified FOLFOX 6 scheme (due to less hematologic toxicity, without bolus if necessary), or XELOX with capecitabine at 1000 mg/m^2, should be considered. If the patient has no serious comorbidity, the full dose should be given. In patients neither fit nor frail with some comorbidity, dose reduction should be considered.

Frail patients with Eastern Cooperative Oncology Group Performance Status 3 or 4 are not candidates for chemotherapy treatment. Therapy with targeted agents is not indicated in adjuvant treatment because of lack of benefit[144].

Treatment in metastatic patients

The goal of palliative chemotherapy in the elderly should be the same as in young patients but with special attention to treatment toxicity. It has been demonstrated in several studies and a meta-analysis that chemotherapy improves the overall survival and time to progression compared to observation. An analysis by Folprecht *et al*[145] of 22 trials showed benefits in OS, progression free survival (PFS), and TTR similar to younger patients (in 629 patients over 70 years of age).

Exposure to the drugs currently available is able to increase the OS, time to response , and the rate of metastatic resection with an average of approximately 24 mo of OS. Even with this data and probably due to toxicity concerns, elderly patients are less likely to be treated with these agents. A population-based study by Ho *et al*[146] reported that less than 50% of elderly patients with mCRC received palliative systemic chemotherapy.

Fluoropyrimidines are the mainstay of treatment and can also benefit elderly patients. Depending on the administration schedule, the toxicity profile is different; diarrhea and leukopenia are more frequent when administered in bolus (24% *vs* 14% and 24% *vs* 10% respectively)[147]. Treatment with capecitabine, because it is administered orally, is perceived to be innocuous, but although it is well tolerated in fit elderly patients, it is still more toxic than 5FU in combination therapy[148-154]. The MRC Focus 2 trial of elderly and frail patients confirmed the higher rate of gastrointestinal toxicity, such as diarrhea, vomiting, and anorexia, with no differences in efficacy[155].

The question is whether a more aggressive regimen is better. There are conflicting data: three phase Ⅲ studies did not observe a survival benefit with combination chemotherapy *vs* 5 FU/LV alone[155-157]. The MRC FOCUS 2 trial included 459 patients who were deemed not fit or too frail for full doses. They were randomized to 5 FU/LV with or without oxaliplatin, or capecitabine with or without oxaliplatin. Approximately 43% were older than 75 years of age, 13% older than 80%, and 29% with a Performance Status of 2. The addition of oxaliplatin improved response rate but not

DFS or OS, and the rate of grade 3 or 4 toxicity was not increased in the oxaliplatin arm, perhaps due to a lower administered dose. Capecitabine and 5FU were equivalent in terms of benefit on PFS (HR = 0.99, 95%CI: 0.82-1.2, *P* = 0.93) or OS (HR = 0.96, 95%CI: 0.79-1.17, *P* = 0.71); however, higher toxicity was observed with capecitabine and, as a consequence, also a lower quality of life.

The combination of irinotecan and 5FU provides the same benefits in the elderly as it does in younger patients, as seen in phase Ⅱ and Ⅲ trials, albeit at the expense of an increased gastrointestinal and hematologic toxicity[158,159]. The tri-weekly administration of irinotecan requires dose reduction in patients over 70 years of age because of an increase in the rates of neutropenia and diarrhea[160].

A phase Ⅲ French study FFCD 2001-02 randomized 282 patients older than 75 with mCRC treated by a first line of palliative chemotherapy with 5FU with or without irinotecan. A geriatric assessment was obtained in 123 (44%). Greater toxicity grades 3-4 (61% *vs* 39%) were observed in the combination arm, and these patients required more hospitalizations or dose reduction. There is no OS data available to justify the increase in toxicity. The study was not designed with sufficient statistical power, so more studies are still needed. IADL dependence and cognitive impairment were established as predictors of greater toxicity[154]. The combination of oxaliplatin and capecitabine (denominated Xelox) is well tolerated, although more toxic as seen in the MRC FOCUS 2 trial[152]. The combination of capecitabine with irinotecan (XELIRI) is more toxic with a high rate of dehydration and asthenia, and it is infrequently used in elderly patients[154-158].

The benefit of the new molecular targets has also been reported in the elderly population[159]. Specifically, bevacizumab (the vascular endothelial growth factor VEGF) increases both PFS and OS, as was observed in a retrospective subgroup analysis and pooled analysis of randomized trials, along with observational cohort studies. A pooled analysis of two randomized trials by Kabbinavar *et al*[160] with 439 patients older than 65 and 276 > 70 years of age, showed an improvement with bevacizumab in PFS of 9.2 mo *vs* 6.2 mo; HR = 0.52: *P* < 0.0001, and OS of 19.3 mo *vs* 14.3 mo, which is statistically significant (HR = 0.7). Another analysis by Cassidy *et al*[161], which included two more phase Ⅲ trials with 712 patients equal to or > 70 years of age and 1142 > 65, confirmed the benefit in OS and PFS with bevacizumab, even though an increased incidence of thrombotic events in patients over 65 years of age was seen (5.7% *vs* 2.5% patients > 65 years, and 6.7% *vs* 3.2% in those > 70 years of age).

The BRITE observational study, which included 896 patients > 65 years of age, also showed better PFS, despite a greater toxicity profile with regard to the incidence of thromboembolic events, that increased with age[162].

The AVEX study, designed to assess the efficacy and tolerability of capecitabine plus bevacizumab *vs* capecitabine alone, included 280 frail patients equal to or greater than 70 years of age. The results showed an increase in PFS (9.1 mo *vs* 5.1 mo) and relative risk (RR) (19.3% *vs* 10%) with no statistically significant difference in OS (21 ms *vs* 17 ms) but more toxic events in the bevacizumab arm (40% *vs* 22%) at the expense of hypertension, hand-foot syndrome, bleeding, and thromboembolic events[163].

In elderly patients, the combination of capecitabine and bevacizumab is effective, but the risk *vs* benefit must be discussed, especially in patients with vascular disease, myocardial infarction, thrombotic events, or severe uncontrolled hypertension in the 6-12 mo prior to the start of treatment.

Aflibercept, another angiogenesis-targeting agent, has demonstrated efficacy in treating mCRC in a recent randomized Phase Ⅲ trial (VELOUR). As a result, it has been approved in combination with FOLFIRI in the second line treatment for metastatic mCRC, supported by an improvement in OS of 13.5 mo *vs* 12.1 mo. The efficacy was similar in the elderly population studied. However, there is no more data available in this population[164]. The most frequently reported adverse events with aflibercept compared with the placebo arm were hemorrhage (2.9% *vs* 1.7%), arterial and venous thromboembolic events (9.7% *vs* 6.8%), grade 3 hypertension (19.1% *vs* 1.5%), and grade 3 or 4 proteinuria (7.9% *vs* 1.2%). Other adverse effects associated with chemotherapy were higher in the aflibercept arm: diarrhea, asthenia, stomatitis, infections (12.3% *vs* 6.9%), palmar-plantar erythrodysesthesia (2.8% *vs* 0.5%), neutropenia (36.7% *vs* 29.5%), and thrombocytopenia (3.3% *vs* 1.7%).

The data on the anti-EGFRs cetuximab and panitumumab in the elderly population are limited. They have been investigated in several trials either in combination or monotherapy in mCRC, with a manageable toxicity profile. Patients with mutations in codon 12 or 13 of the *KRAS* gene should not be treated with anti-EGFR antibody due to lack of benefit. The main adverse effect of these drugs is skin toxicity. The correlation between development and severity of rash with treatment response is unclear. An analysis of EGFR polymorphisms observed that carriers of D994D polymorphism have lower dermatological toxicity than other genotypes, with no difference in PFS or OS and age[165-169]. Mutations in RAS, BRAF, and PIK3CA have also been shown to be associated with resistance to anti-EGFR[170].

Several prospective and retrospective studies have shown no differences in toxicity compared to younger patients and the same clinical benefit. Therefore, these agents should be considered in fit elderly patients[163-169].

The latest drug approved for the treatment of mCRC, the multikinase inhibitor regorafenib, adds a modest increase in PFS without increasing OS. Median overall survival was 6.4 mo with regorafenib *vs* 5.0 mo with placebo (HR = 0.77; 95%CI: 0.64-0.94; one-sided *P* = 0.0052). Adverse events due to treatment

occurred in 465 (93%) patients with regorafenib and in 154 (61%) of those assigned to placebo. The most common adverse events of grade 3 or higher related to regorafenib were hand-foot skin reaction (17%), fatigue (10%), diarrhea (7%), hypertension (7%), and rash or desquamation (6%). There were no differences in toxicity between patients older or younger than 65 years of age in the subgroup analyzed, but there are no available data on efficacy or toxicity in the elderly or frail population[168]. Ramucirumab is a human IgG-1 monoclonal antibody that targets the extracellular domain of VEGF receptor 2. Ramucirumab in combination with FOLFIRI has recently been approved as a second line treatment, after progression with bevacizumab, oxaliplatin, and a fluoropyrimidine. Median overall survival was 13.3 mo for patients in the ramucirumab group *vs* 11.7 mo for the placebo with FOLFIRI group (HR = 0.844, P = 0.0219). The most frequently observed adverse effects grade 3 or worse were neutropenia (38% *vs* 23%), hypertension (11% *vs* 3%), diarrhea (11% *vs* 10%), and fatigue (12% *vs* 8%). The median patient age was 62, and, therefore, there is still not enough data in the elderly or frail population. One of the latest drugs, pending Food and Drug Administration approval, for the treatment of CRC is TAS-102. TAS-102 is an antitumor agent composed of a combination of trifluorothymidine (FTD), a nucleoside that incorporates into DNA and inhibits a variety of genetic functions required for the proliferation of cancer cells, and tipiracil hydrochloride, an inhibitor of thymidine phosphorylase (which degrades FTD) that maintains an effective blood concentration of FTD. Tipiracil protects trifluridine from being broken down when taken orally.

In a Phase 3 study, 800 patients with advanced CRC in refractory to oxaliplatin, irinotecan, fluorouracil, bevacizumab, regorafenib, and anti-EGFR (RAS wild type) were randomized to TAS-102 *vs* placebo. An increase of median overall survival was observed, from 5.3 mo with placebo to 7.1 mo with TAS-102 (HR of death 0.68, P < 0.001). The main grade 3 or higher toxicity was neutropenia (38%) and patients in the TAS-102 group were also more likely than those in the placebo group to have nausea of grade 3 or higher (2% *vs* 1%), vomiting (2% *vs* < 1%), and diarrhea (3% *vs* < 1%). The median patient age was 63. The benefit was seen in patients younger than and older than 65, but data are lacking in elderly or frail patients[171].

In summary, an elderly fit patient may be treated with FOLFIRI and FOLFOX (or XELOX) with or without antibodies, given the high response rate, especially if the treatment is given with neoadjuvant intention prior to surgery for metastases (M1), with certain precautions due to different toxicity profiles. Age by itself should not be a contraindication for M1 surgery. There are more data available for hepatic resections than pulmonary resections[172-176]. Surgical series that include all patients have a median OS of 40% at 5 years after liver resection, with a general perioperative mortality lower than 5%. Fit elderly patients with little comorbidity should be offered chemotherapy with the newer agents that increase the response rate and therefore resectability before surgery.

Two retrospective series of neoadjuvant chemotherapy prior to surgery based on oxaliplatin showed higher response rates as expected. Those who were operated had better recurrence-free survival[176,177].

For those patients unfit or with low IK or PS 2, the treatment may be of benefit if deterioration is related to the oncologic disease, although the benefit is lower and the toxicity higher. The risks or benefit should be evaluated and discussed individually in these patients. Fluoropyrimidine monotherapy or supportive care is probably the best choice in frail patients.

PALLIATIVE CARE

The "frail elderly" may be good candidates for palliative treatment, which can provide a better quality of remaining life. When to begin palliative care is a troublesome question for patients, but when frailty is severe, delivery of palliative care focused on relief of discomfort and enhancement of quality of life is highly appropriate. In addition to symptom management, preservation of functional independence is a major goal of treatment in the elderly. The application of multidisciplinary, team-based palliative approaches is beneficial for treating these patients because of the complexity of their coexisting social, psychological, and medical needs. Although death occurs far more commonly in older people than in any other age group, the evidence base for palliative care in older adults is scarce[178].

CONCLUSION

Older patients with colon or rectal cancer are less likely to receive guideline-recommended therapies. Decisions about cancer treatment in the elderly may be influenced by a number of factors, including pre-existing health problems (comorbidities) and other conditions that might cause the potential risks of surgery, chemotherapy, and radiotherapy to outweigh the benefits of treatment. Risk stratification based on comorbidities and biochemical and physiological markers could help to decide whether to perform surgery, what type of surgery, and the timing of surgery. Physiological rather than chronological age should determine the management of cancer in each individual[5].

Optimal treatment of the older adult patient who has cancer starts with a careful delineation of goals through conversation. Most elderly patients with cancer will have priorities besides simply prolonging their lives. Surveys have found that their top concerns include avoiding suffering, strengthening relationships with family and friends, being mentally aware, not being a burden on others, and achieving a sense that their life is complete[179]. The treatment plan should be comprehensive: cancer-specific treatment, symptom-

specific treatment, supportive treatment modalities, and end-of-life care[180].

The careful assessment of the patient, taking into consideration their functional status, level of frailty, life-expectancy, and wishes, should become an essential and central issue in their management, and choosing the appropriate therapy for each patient within a multidisciplinary process should be the future in the treatment of elderly patients with CRC.

REFERENCES

1 National Cancer Intelligence Network. Available from: URL: http://www.ncin.org.uk/cancer_information_tools/ukcis

2 **Schmoll HJ**, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K, Nordlinger B, van de Velde CJ, Balmana J, Regula J, Nagtegaal ID, Beets-Tan RG, Arnold D, Ciardiello F, Hoff P, Kerr D, Köhne CH, Labianca R, Price T, Scheithauer W, Sobrero A, Tabernero J, Aderka D, Barroso S, Bodoky G, Douillard JY, El Ghazaly H, Gallardo J, Garin A, Glynne-Jones R, Jordan K, Meshcheryakov A, Papamichail D, Pfeiffer P, Souglakos I, Turhal S, Cervantes A. ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making. *Ann Oncol* 2012; **23**: 2479-2516 [PMID: 23012255 DOI: 10.1093/annonc/mds236]

3 **Boya-Cristià MJ**. Reciben un tratamiento adecuado los pacientes con cancer. *Rev Esp Geriatria Gerontologia* 2005; **40**: 371-377 [DOI: 10.1016/S0211-139X(05)74886-1]

4 **Robinson TN**, Wu DS, Stiegmann GV, Moss M. Frailty predicts increased hospital and six-month healthcare cost following colorectal surgery in older adults. *Am J Surg* 2011; **202**: 511-514 [PMID: 21890098 DOI: 10.1016/j.amjsurg.2011.06.017]

5 **Sheridan J**, Walsh P, Kevans D, Cooney T, O'Hanlon S, Nolan B, White A, McDermott E, Sheahan K, O'Shea D, Hyland J, O' Donoghue D, O'Sullivan J, Mulcahy H, Doherty G. Determinants of short- and long-term survival from colorectal cancer in very elderly patients. *J Geriatr Oncol* 2014; **5**: 376-383 [PMID: 24845215 DOI: 10.1016/j.jgo.2014.04.005]

6 **Balducci L**. Recomendaciones para el tratamiento del cancer en el anciano: Implicaciones para la calidad de vida. *Rev Esp Geriatr Gerontol* 2004; **39**: 270-276 [DOI: 10.1016/S0211-139X(04)74969-0]

7 **Ellis G**, Whitehead MA, O'Neill D, Langhorne P, Robinson D. Comprehensive geriatric assessment for older adults admitted to hospital. *Cochrane Database Syst Rev* 2011; **(7)**: CD006211 [PMID: 21735403 DOI: 10.1002/14651858.cd006211.pub2]

8 NCCN Guidelines Version 2.2014. Senior Adult Oncology. National Comprehensive Cancer Network. Available from: URL: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp

9 **Extermann M**, Hurria A. Comprehensive geriatric assessment for older patients with cancer. *J Clin Oncol* 2007; **25**: 1824-1831 [PMID: 17488980 DOI: 10.1200/JCO.2007.10.6559]

10 **Gonzalez Barón M**, Feliu Batlle J, Gonzalez Montalvo J. I. Cáncer en el anciano, 1ª ed. Barcelona: Masson SA, 2001: 236-265

11 **Naeim A**, Reuben D. Geriatric syndromes and assessment in older cancer patients. *Oncology* (Williston Park) 2001; **15**: 1567-1577, 1580; discussion 1581, 1586, 1591 [PMID: 11780701]

12 **Rubenstein LZ**, Rubenstein LV. Multidimensional Geriatric Assessment. In: Howard M, Rockwood K, Woodhouse K, editors. Brocklehurst's Textbook of Geriatric Medicine and Gerontology. 7th ed. Philadelphia: Saunders Elsevier, 2010: 211-217 [DOI: 10.1016/b978-1-4160-6231-8.10035-2]

13 **Ingram SS**, Seo PH, Martell RE, Clipp EC, Doyle ME, Montana GS, Cohen HJ. Comprehensive assessment of the elderly cancer patient: the feasibility of self-report methodology. *J Clin Oncol* 2002; **20**: 770-775 [PMID: 11821460 DOI: 10.1200/JCO.20.3.770]

14 **Hamaker ME**, Jonker JM, de Rooij SE, Vos AG, Smorenburg CH, van Munster BC. Frailty screening methods for predicting outcome of a comprehensive geriatric assessment in elderly patients with cancer: a systematic review. *Lancet Oncol* 2012; **13**: e437-e444 [PMID: 23026829 DOI: 10.1016/S1470-2045(12)70259-0]

15 **Gill TM**, Baker DI, Gottschalk M, Peduzzi PN, Allore H, Byers A. A program to prevent functional decline in physically frail, elderly persons who live at home. *N Engl J Med* 2002; **347**: 1068-1074 [PMID: 12362007 DOI: 10.1056/NEJMoa020423]

16 **Terret C**, Zulian G, Droz JP. Statements on the interdependence between the oncologist and the geriatrician in geriatric oncology. *Crit Rev Oncol Hematol* 2004; **52**: 127-133 [PMID: 15501077 DOI: 10.1016/S1040-8428(04)00138-6]

17 **Rockwood K**, Mitnitski A. Frailty defined by deficit accumulation and geriatric medicine defined by frailty. *Clin Geriatr Med* 2011; **27**: 17-26 [PMID: 21093719 DOI: 10.1016/j.cger.2010.08.008]

18 **Monfardini S**, Gridelli C, Pasetto LM, Soubeyran P, Droz JP, Basso U. Vulnerable and frail elderly: an approach to the management of the main tumour types. *Eur J Cancer* 2008; **44**: 488-493 [PMID: 18242078 DOI: 10.1016/j.ejca.2008.01.002]

19 **Luciani A**, Ascione G, Bertuzzi C, Marussi D, Codecà C, Di Maria G, Caldiera SE, Floriani I, Zonato S, Ferrari D, Foa P. Detecting disabilities in older patients with cancer: comparison between comprehensive geriatric assessment and vulnerable elders survey-13. *J Clin Oncol* 2010; **28**: 2046-2050 [PMID: 20308657 DOI: 10.1200/JCO.2009.25.9978]

20 **Monfardini A**, Basso U, Fiduccia P, Brunello A, Baretta Z, Soldà C. Can the short screening test Vulnerable Elders Survey 13 (VES-13) substitute for the time-consuming comprehensive geriatric assessment (CGA) to identify vulnerable/frail elderly breast cancer patients? *J Clin Oncol* 2010; **28** (suppl 9114): 15s

21 **Monfardini S**, Giordano G, Sandri R, Gnocchi PL, Galetti G. Bringing geriatrics into oncology or also oncology into geriatrics? *Ann Oncol* 2012; **23**: 801 [PMID: 22219017 DOI: 10.1093/annonc/mdr597]

22 **Cadena MO**, Lopez JH, Insuasty JS, Santacruz JG, Becerra H. Importancia de la valoracion geriátrica integral en el manejo de pacientes con cáncer. Medicas UIS. *Geriatria* 2012; **25**: 121-127

23 **Fried LP**, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 2001; **56**: M146-M156 [PMID: 11253156 DOI: 10.1093/gerona/56.3.M146]

24 **Revenig LM**, Canter DJ, Taylor MD, Tai C, Sweeney JF, Sarmiento JM, Kooby DA, Maithel SK, Master VA, Ogan K. Too frail for surgery? Initial results of a large multidisciplinary prospective study examining preoperative variables predictive of poor surgical outcomes. *J Am Coll Surg* 2013; **217**: 665-670.e1 [PMID: 24054409 DOI: 10.1016/j.jamcollsurg.2013.06.012]

25 **Balducci L**. New paradigms for treating elderly patients with cancer: the comprehensive geriatric assessment and guidelines for supportive care. *J Support Oncol* 2003; **1**: 30-37 [PMID: 15346998]

26 **Basso U**, Tonti S, Bassi C, Brunello A, Pasetto LM, Scaglione D, Falci C, Beda M, Aversa SM, Stefani M, Castegnaro E, Tamellini F, Monfardini S. Management of Frail and Not-Frail elderly cancer patients in a hospital-based geriatric oncology program. *Crit Rev Oncol Hematol* 2008; **66**: 163-170 [PMID: 18243726 DOI: 10.1016/j.critrevonc.2007.12.006]

27 **Wieland D**, Hirth V. Comprehensive geriatric assessment. *Cancer Control* 2003; **10**: 454-462 [PMID: 14652521]

28 **Alarcón T**, Bárcena A, González-Montalvo JI, Penãlosa C, Salgado A. Factors predictive of outcome on admission to an acute geriatric ward. *Age Ageing* 1999; **28**: 429-432 [PMID: 10529035 DOI: 10.1093/ageing/28.5.429]

29 **Charlson ME**, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; **40**: 373-383 [PMID: 3558716 DOI: 10.1016/0021-9681(87)90171-8]

30 **Lieffers JR**, Bathe OF, Fassbender K, Winget M, Baracos VE. Sarcopenia is associated with postoperative infection and delayed recovery from colorectal cancer resection surgery. *Br J Cancer*

2012; **107**: 931-936 [PMID: 22871883 DOI: 10.1038/bjc.2012.350]
31 **Guigoz Y**. The Mini Nutritional Assessment (MNA) review of the literature--What does it tell us? *J Nutr Health Aging* 2006; **10**: 466-485; discussion 485-487 [PMID: 17183419]
32 **Monfardini S**. What do we know on variables influencing clinical decision-making in elderly cancer patients? *Eur J Cancer* 1996; **32A**: 12-14 [PMID: 8695218 DOI: 10.1016/0959-8049(95)00580-3]
33 **Mark R**, Katlic MR. Principles of geriatric surgery. In: Rosenthal RA, Zenilman ME, Katlic MR, editors. Principles and practice of geriatric surgery. New York: Springer-Verlag, 2001: 92-104
34 **Darlene Gabea U**, Ronnie A. Preoperative evaluation of the elderly surgical patient. In: Rosenthal RA, Zenilman ME, Katlic MR, editors. Principles and practice of geriatric surgery. New York: Springer-Verlag, 2001: 126-143
35 **Kemeny MM**, Busch-Devereaux E, Merriam LT, O'Hea BJ. Cancer surgery in the elderly. *Hematol Oncol Clin North Am* 2000; **14**: 169-192 [PMID: 10680077 DOI: 10.1016/S0889-8588(05)70283-5]
36 **Ajaj A**, Singh MP, Abdulla AJ. Should elderly patients be told they have cancer? Questionnaire survey of older people. *BMJ* 2001; **323**: 1160 [PMID: 11711408 DOI: 10.1136/bmj.323.7322.1160]
37 **Reig L**, Fernandez M, Garcia M, Martin-Baranera M. Factores que influyen sobre las preferencias de reanimación cardiovascular y de información médica en una poblacion geriátrica. *Med Clin* (Barc) 2002; **118**: 94-96 [DOI: 10.1016/S0025-7753(02)72296-3]
38 **Yun YH**, Lee CG, Kim SY, Lee SW, Heo DS, Kim JS, Lee KS, Hong YS, Lee JS, You CH. The attitudes of cancer patients and their families toward the disclosure of terminal illness. *J Clin Oncol* 2004; **22**: 307-314 [PMID: 14722040 DOI: 10.1200/jco.2004.07.053]
39 **Terret C**, Zulian GB, Naiem A, Albrand G. Multidisciplinary approach to the geriatric oncology patient. *J Clin Oncol* 2007; **25**: 1876-1881 [PMID: 17488986 DOI: 10.1200/JCO.2006.10.3291]
40 **Balducci L**. Management of cancer in the elderly. *Oncology* (Williston Park) 2006; **20**: 135-143; discussion 144, 146, 151-152 [PMID: 16562648]
41 **Townsley CA**, Selby R, Siu LL. Systematic review of barriers to the recruitment of older patients with cancer onto clinical trials. *J Clin Oncol* 2005; **23**: 3112-3124 [PMID: 15860871 DOI: 10.1200/JCO.2005.00.141]
42 **Tan KY**, Kawamura YJ, Tokomitsu A, Tang T. Assessment for frailty is useful for predicting morbidity in elderly patients undergoing colorectal cancer resection whose comorbidities are already optimized. *Am J Surg* 2012; **204**: 139-143 [PMID: 22178483 DOI: 10.1016/j.amjsurg.2011.08.012]
43 **Faiz O**, Haji A, Bottle A, Clark SK, Darzi AW, Aylin P. Elective colonic surgery for cancer in the elderly: an investigation into postoperative mortality in English NHS hospitals between 1996 and 2007. *Colorectal Dis* 2011; **13**: 779-785 [PMID: 20412094 DOI: 10.1111/j.1463-1318.2010.02290.x]
44 **van Leeuwen BL**, Påhlman L, Gunnarsson U, Sjövall A, Martling A. The effect of age and gender on outcome after treatment for colon carcinoma. A population-based study in the Uppsala and Stockholm region. *Crit Rev Oncol Hematol* 2008; **67**: 229-236 [PMID: 18440820 DOI: 10.1016/j.critrevonc.2008.03.005]
45 **Hill AL**, Russell MM. The special needs of elderly patients. *Seminars in Colon and Rectal Surg* 2013; **24**: 200-208 [DOI: 10.1053/j.scrs.2013.08.007]
46 **Mäkelä JT**, Kiviniemi H, Laitinen S. Survival after operations for colorectal cancer in patients aged 75 years or over. *Eur J Surg* 2000; **166**: 473-479 [PMID: 10890544 DOI: 10.1080/11024150750008790]
47 **Ong ES**, Alassas M, Dunn KB, Rajput A. Colorectal cancer surgery in the elderly: acceptable morbidity? *Am J Surg* 2008; **195**: 344-348; discussion 348 [PMID: 18222410 DOI: 10.1016/j.amjsurg.2007.12.022]
48 **Bouassida M**, Charrada H, Chtourou MF, Hamzaoui L, Mighri MM, Sassi S, Azzouz MM, Touinsi H. Surgery for Colorectal Cancer in Elderly Patients: How Could We Improve Early Outcomes? *J Clin Diagn Res* 2015; **9**: PC04-PC08 [PMID: 26155516 DOI: 10.7860/JCDR/2015/12213.5973]
49 **Sanoff HK**, Bleiberg H, Goldberg RM. Managing older patients with colorectal cancer. *J Clin Oncol* 2007; **25**: 1891-1897 [PMID: 17488988 DOI: 10.1200/JCO.2006.10.1220]
50 **Doat S**, Thiébaut A, Samson S, Ricordeau P, Guillemot D, Mitry E. Elderly patients with colorectal cancer: treatment modalities and survival in France. National data from the ThInDiT cohort study. *Eur J Cancer* 2014; **50**: 1276-1283 [PMID: 24447833 DOI: 10.1016/j.ejca.2013.12.026]
51 **Bergman H**, Ferrucci L, Guralnik J, Hogan DB, Hummel S, Karunananthan S, Wolfson C. Frailty: an emerging research and clinical paradigm--issues and controversies. *J Gerontol A Biol Sci Med Sci* 2007; **62**: 731-737 [PMID: 17634320 DOI: 10.1093/gerona/62.7.731]
52 **Scandrett KG**, Zuckerbraun BS, Peitzman AB. Operative risk stratification in the older adult. *Surg Clin North Am* 2015; **95**: 149-172 [PMID: 25459549 DOI: 10.1016/j.suc.2014.09.014]
53 **Dekker JW**, Gooiker GA, Bastiaannet E, van den Broek CB, van der Geest LG, van de Velde CJ, Tollenaar RA, Liefers GJ. Cause of death the first year after curative colorectal cancer surgery; a prolonged impact of the surgery in elderly colorectal cancer patients. *Eur J Surg Oncol* 2014; **40**: 1481-1487 [PMID: 24985723 DOI: 10.1016/j.ejso.2014.05.010]
54 **Ugolini G**, Ghignone F, Zattoni D, Veronese G, Montroni I. Personalized surgical management of colorectal cancer in elderly population. *World J Gastroenterol* 2014; **20**: 3762-3777 [PMID: 24833841 DOI: 10.3748/wjg.v20.i14.3762]
55 **Kurian A**, Suryadevara S, Ramaraju D, Gallagher S, Hofmann M, Kim S, Zebley M, Fassler S. In-hospital and 6-month mortality rates after open elective vs open emergent colectomy in patients older than 80 years. *Dis Colon Rectum* 2011; **54**: 467-471 [PMID: 21383568 DOI: 10.1007/DCR.0b013e3182060904]
56 **Morse BC**, Cobb WS, Valentine JD, Cass AL, Roettger RH. Emergent and elective colon surgery in the extreme elderly: do the results warrant the operation? *Am Surg* 2008; **74**: 614-618; discussion 618-619 [PMID: 18646479]
57 **Louis DJ**, Hsu A, Brand MI, Saclarides TJ. Morbidity and mortality in octogenarians and older undergoing major intestinal surgery. *Dis Colon Rectum* 2009; **52**: 59-63 [PMID: 19273957 DOI: 10.1007/DCR.0b013e31819754d4]
58 **Zerbib P**, Kulick JF, Lebuffe G, Khoury-Helou A, Plenier I, Chambon JP. Emergency major abdominal surgery in patients over 85 years of age. *World J Surg* 2005; **29**: 820-825 [PMID: 15951923 DOI: 10.1007/s00268-005-7855-4]
59 **Modini C**, Romagnoli F, De Milito R, Romeo V, Petroni R, La Torre F, Catani M. Octogenarians: an increasing challenge for acute care and colorectal surgeons. An outcomes analysis of emergency colorectal surgery in the elderly. *Colorectal Dis* 2012; **14**: e312-e318 [PMID: 22230094 DOI: 10.1111/j.1463-1318.2012.02934.x]
60 **McGillicuddy EA**, Schuster KM, Davis KA, Longo WE. Factors predicting morbidity and mortality in emergency colorectal procedures in elderly patients. *Arch Surg* 2009; **144**: 1157-1162 [PMID: 20026835 DOI: 10.1001/archsurg.2009.203]
61 **Basili G**, Lorenzetti L, Biondi G, Preziuso E, Angrisano C, Carnesecchi P, Roberto E, Goletti O. Colorectal cancer in the elderly. Is there a role for safe and curative surgery? *ANZ J Surg* 2008; **78**: 466-470 [PMID: 18522567 DOI: 10.1111/j.1445-2197.2008.04536.x]
62 **Moghadamyeghaneh Z**, Mills SD, Carmichael JC, Pigazzi A, Stamos MJ. Risk factors of postoperative myocardial infarction after colorectal surgeries. *Am Surg* 2015; **81**: 358-364 [PMID: 25831181]
63 **Bakker IS**, Grossmann I, Henneman D, Havenga K, Wiggers T. Risk factors for anastomotic leakage and leak-related mortality after colonic cancer surgery in a nationwide audit. *Br J Surg* 2014; **101**: 424-432; discussion 432 [PMID: 24536013 DOI: 10.1002/bjs.9395]
64 **Guo MG**, Feng Y, Zheng Q, Di JZ, Wang Y, Fan YB, Huang XY. Comparison of self-expanding metal stents and urgent surgery for left-sided malignant colonic obstruction in elderly patients. *Dig Dis Sci* 2011; **56**: 2706-2710 [PMID: 21442324 DOI: 10.1007/s10620-011-1648-4]

65 **Frasson M**, Braga M, Vignali A, Zuliani W, Di Carlo V. Benefits of laparoscopic colorectal resection are more pronounced in elderly patients. *Dis Colon Rectum* 2008; **51**: 296-300 [PMID: 18197453 DOI: 10.1007/s10350-007-9124-0]

66 **Stocchi L**, Nelson H, Young-Fadok TM, Larson DR, Ilstrup DM. Safety and advantages of laparoscopic vs. open colectomy in the elderly: matched-control study. *Dis Colon Rectum* 2000; **43**: 326-332 [PMID: 10733113 DOI: 10.1007/BF02258297]

67 **Gooiker GA**, Dekker JW, Bastiaannet E, van der Geest LG, Merkus JW, van de Velde CJ, Tollenaar RA, Liefers GJ. Risk factors for excess mortality in the first year after curative surgery for colorectal cancer. *Ann Surg Oncol* 2012; **19**: 2428-2434 [PMID: 22396000 DOI: 10.1245/s10434-012-2294-6]

68 **Schiphorst AH**, Pronk A, Borel Rinkes IH, Hamaker ME. Representation of the elderly in trials of laparoscopic surgery for colorectal cancer. *Colorectal Dis* 2014; **16**: 976-983 [PMID: 25331635 DOI: 10.1111/codi.12806]

69 **Biondo S**. Commentary on Schiphorst et al, 'Representation of the elderly in trial on laparoscopic surgery for colorectal cancer'. *Colorectal Dis* 2014; **16**: 984-985 [PMID: 25421116 DOI: 10.1111/codi.12783]

70 **Makary MA**, Segev DL, Pronovost PJ, Syin D, Bandeen-Roche K, Patel P, Takenaga R, Devgan L, Holzmueller CG, Tian J, Fried LP. Frailty as a predictor of surgical outcomes in older patients. *J Am Coll Surg* 2010; **210**: 901-908 [PMID: 20510798 DOI: 10.1016/j.jamcollsurg.2010.01.028]

71 **Golfinopoulos V**, Pentheroudakis G, Pavlidis N. Treatment of colorectal cancer in the elderly: a review of the literature. *Cancer Treat Rev* 2006; **32**: 1-8 [PMID: 16337087 DOI: 10.1016/j.ctrv.2005.10.002]

72 **Papamichael D**, Audisio R, Horiot JC, Glimelius B, Sastre J, Mitry E, Van Cutsem E, Gosney M, Köhne CH, Aapro M. Treatment of the elderly colorectal cancer patient: SIOG expert recommendations. *Ann Oncol* 2009; **20**: 5-16 [PMID: 18922882 DOI: 10.1093/annonc/mdn532]

73 **Devon KM**, Vergara-Fernandez O, Victor JC, McLeod RS. Colorectal cancer surgery in elderly patients: presentation, treatment, and outcomes. *Dis Colon Rectum* 2009; **52**: 1272-1277 [PMID: 19571704 DOI: 10.1007/DCR.0b013e3181a74d2e]

74 **Vallribera Valls F**, Landi F, Espín Basany E, Sánchez García JL, Jiménez Gómez LM, Martí Gallostra M, Salgado Cruz L, Armengol Carrasco M. Laparoscopy-assisted versus open colectomy for treatment of colon cancer in the elderly: morbidity and mortality outcomes in 545 patients. *Surg Endosc* 2014; **28**: 3373-3378 [PMID: 24928231 DOI: 10.1007/s00464-014-3597-4]

75 **Senagore AJ**, Madbouly KM, Fazio VW, Duepree HJ, Brady KM, Delaney CP. Advantages of laparoscopic colectomy in older patients. *Arch Surg* 2003; **138**: 252-256 [PMID: 12611568 DOI: 10.1001/archsurg.138.3.252]

76 **Chautard J**, Alves A, Zalinski S, Bretagnol F, Valleur P, Panis Y. Laparoscopic colorectal surgery in elderly patients: a matched case-control study in 178 patients. *J Am Coll Surg* 2008; **206**: 255-260 [PMID: 18222377 DOI: 10.1016/j.jamcollsurg.2007.06.316]

77 **Lian L**, Kalady M, Geisler D, Kiran RP. Laparoscopic colectomy is safe and leads to a significantly shorter hospital stay for octogenarians. *Surg Endosc* 2010; **24**: 2039-2043 [PMID: 20174947 DOI: 10.1007/s00464-010-0900-x]

78 **Mathis KL**, Nelson H. Controversies in laparoscopy for colon and rectal cancer. *Surg Oncol Clin N Am* 2014; **23**: 35-47 [PMID: 24267164 DOI: 10.1016/j.soc.2013.09.006]

79 **Tomimaru Y**, Ide Y, Murata K. Outcome of laparoscopic surgery for colon cancer in elderly patients. *Asian J Endosc Surg* 2011; **4**: 1-6 [PMID: 22776166 DOI: 10.1111/j.1758-5910.2010.00061.x]

80 **Bagnall NM**, Malietzis G, Kennedy RH, Athanasiou T, Faiz O, Darzi A. A systematic review of enhanced recovery care after colorectal surgery in elderly patients. *Colorectal Dis* 2014; **16**: 947-956 [PMID: 25039965 DOI: 10.1111/codi.12718]

81 **Keller DS**, Lawrence JK, Nobel T, Delaney CP. Optimizing cost and short-term outcomes for elderly patients in laparoscopic colonic surgery. *Surg Endosc* 2013; **27**: 4463-4468 [PMID: 23877762 DOI: 10.1007/s00464-013-3088-z]

82 **Wang Q**, Suo J, Jiang J, Wang C, Zhao YQ, Cao X. Effectiveness of fast-track rehabilitation vs conventional care in laparoscopic colorectal resection for elderly patients: a randomized trial. *Colorectal Dis* 2012; **14**: 1009-1013 [PMID: 21985126 DOI: 10.1111/j.1463-1318.2011.02855.x]

83 **Dhesi J**. Improving outcomes in older people undergoing elective surgery. *J R Coll Physicians Edinb* 2010; **40**: 348-353 [PMID: 21254711 DOI: 10.4997/JRCPE.2010.416]

84 **Harari D**, Martin FC, Buttery A, O'Neill S, Hopper A. The older persons' assessment and liaison team 'OPAL': evaluation of comprehensive geriatric assessment in acute medical inpatients. *Age Ageing* 2007; **36**: 670-675 [PMID: 17656421 DOI: 10.1093/ageing/afm089]

85 **Tan KY**, Tan P, Tan L. A collaborative transdisciplinary "geriatric surgery service" ensures consistent successful outcomes in elderly colorectal surgery patients. *World J Surg* 2011; **35**: 1608-1614 [PMID: 21523500 DOI: 10.1007/s00268-011-1112-9]

86 **Neuman HB**, O'Connor ES, Weiss J, Loconte NK, Greenblatt DY, Greenberg CC, Smith MA. Surgical treatment of colon cancer in patients aged 80 years and older: analysis of 31,574 patients in the SEER-Medicare database. *Cancer* 2013; **119**: 639-647 [PMID: 22893570 DOI: 10.1002/cncr.27765]

87 **Ahmed S**, Howel D, Debrah S. The influence of age on the outcome of treatment of elderly patients with colorectal cancer. *J Geriatr Oncol* 2014; **5**: 133-140 [PMID: 24495704 DOI: 10.1016/j.jgo.2013.12.005]

88 **Bethune R**, Arulampalam T. What happens when we don't operate? *Colorectal Dis* 2015; **17**: 279-280 [PMID: 25800071 DOI: 10.1111/codi.12828]

89 **Manceau G**, Karoui M, Werner A, Mortensen NJ, Hannoun L. Comparative outcomes of rectal cancer surgery between elderly and non-elderly patients: a systematic review. *Lancet Oncol* 2012; **13**: e525-e536 [PMID: 23182193 DOI: 10.1016/S1470-2045(12)70378-9]

90 **Jung B**, Påhlman L, Johansson R, Nilsson E. Rectal cancer treatment and outcome in the elderly: an audit based on the Swedish Rectal Cancer Registry 1995-2004. *BMC Cancer* 2009; **9**: 68 [PMID: 19245701 DOI: 10.1186/1471-2407-9-68]

91 **Ayanian JZ**, Zaslavsky AM, Fuchs CS, Guadagnoli E, Creech CM, Cress RD, O'Connor LC, West DW, Allen ME, Wolf RE, Wright WE. Use of adjuvant chemotherapy and radiation therapy for colorectal cancer in a population-based cohort. *J Clin Oncol* 2003; **21**: 1293-1300 [PMID: 12663717 DOI: 10.1200/JCO.2003.06.178]

92 **Chang GJ**, Skibber JM, Feig BW, Rodriguez-Bigas M. Are we undertreating rectal cancer in the elderly? An epidemiologic study. *Ann Surg* 2007; **246**: 215-221 [PMID: 17667499 DOI: 10.1097/SLA.0b013e318070838f]

93 **Martijn H**, Vulto JC. Should radiotherapy be avoided or delivered differently in elderly patients with rectal cancer? *Eur J Cancer* 2007; **43**: 2301-2306 [PMID: 17714937 DOI: 10.1016/j.ejca.2007.06.014]

94 **De Caluwé L**, Van Nieuwenhove Y, Ceelen WP. Preoperative chemoradiation versus radiation alone for stage II and III resectable rectal cancer. *Cochrane Database Syst Rev* 2013; **2**: CD006041 [PMID: 23450565 DOI: 10.1002/14651858.cd006041.pub3]

95 **Serra-Rexach JA**, Jimenez AB, García-Alhambra MA, Pla R, Vidán M, Rodríguez P, Ortiz J, García-Alfonso P, Martín M. Differences in the therapeutic approach to colorectal cancer in young and elderly patients. *Oncologist* 2012; **17**: 1277-1285 [PMID: 22923453 DOI: 10.1634/theoncologist.2012-0060]

96 **Finlayson E**, Zhao S, Varma MG. Outcomes after rectal cancer surgery in elderly nursing home residents. *Dis Colon Rectum* 2012; **55**: 1229-1235 [PMID: 23135580 DOI: 10.1097/DCR.0b013e318267bfe3]

97 **Rutten H**, den Dulk M, Lemmens V, Nieuwenhuijzen G, Krijnen P, Jansen-Landheer M, van de Poll Franse L, Coebergh JW, Martijn H, Marijnen C, van de Velde C. Survival of elderly rectal cancer patients not improved: analysis of population based data on the impact of TME surgery. *Eur J Cancer* 2007; **43**: 2295-2300 [PMID: 17709242 DOI: 10.1016/j.ejca.2007.07.009]

98 **Garcia-Aguilar J**, Shi Q, Thomas CR, Chan E, Cataldo P, Marcet

J, Medich D, Pigazzi A, Oommen S, Posner MC. A phase II trial of neoadjuvant chemoradiation and local excision for T2N0 rectal cancer: preliminary results of the ACOSOG Z6041 trial. *Ann Surg Oncol* 2012; **19**: 384-391 [PMID: 21755378 DOI: 10.1245/s10434-011-1933-7]

99 **Smith FM**, Waldron D, Winter DC. Rectum-conserving surgery in the era of chemoradiotherapy. *Br J Surg* 2010; **97**: 1752-1764 [PMID: 20845400 DOI: 10.1002/bjs.7251]

100 **Neuman HB**, Elkin EB, Guillem JG, Paty PB, Weiser MR, Wong WD, Temple LK. Treatment for patients with rectal cancer and a clinical complete response to neoadjuvant therapy: a decision analysis. *Dis Colon Rectum* 2009; **52**: 863-871 [PMID: 19502849 DOI: 10.1007/DCR.0b013e31819eefba]

101 **Smith FM**, Rao C, Oliva Perez R, Bujko K, Athanasiou T, Habr-Gama A, Faiz O. Avoiding radical surgery improves early survival in elderly patients with rectal cancer, demonstrating complete clinical response after neoadjuvant therapy: results of a decision-analytic model. *Dis Colon Rectum* 2015; **58**: 159-171 [PMID: 25585073 DOI: 10.1097/DCR.0000000000000281]

102 **Engelen SM**, Beets-Tan RG, Lahaye MJ, Lammering G, Jansen RL, van Dam RM, Konsten J, Leijtens JW, van de Velde CJ, Beets GL. MRI after chemoradiotherapy of rectal cancer: a useful tool to select patients for local excision. *Dis Colon Rectum* 2010; **53**: 979-986 [PMID: 20551748 DOI: 10.1007/DCR.0b013e3181dc64dc]

103 **Perez RO**, Habr-Gama A, Lynn PB, São Julião GP, Bianchi R, Proscurshim I, Gama-Rodrigues J. Transanal endoscopic microsurgery for residual rectal cancer (ypT0-2) following neoadjuvant chemoradiation therapy: another word of caution. *Dis Colon Rectum* 2013; **56**: 6-13 [PMID: 23222274 DOI: 10.1097/DCR.0b013e318273f56f]

104 **Kim CJ**, Yeatman TJ, Coppola D, Trotti A, Williams B, Barthel JS, Dinwoodie W, Karl RC, Marcet J. Local excision of T2 and T3 rectal cancers after downstaging chemoradiation. *Ann Surg* 2001; **234**: 352-358; discussion 358-359 [PMID: 11524588 DOI: 10.1097/00000658-200109000-00009]

105 **Lezoche G**, Baldarelli M, Guerrieri M, Paganini AM, De Sanctis A, Bartolacci S, Lezoche E. A prospective randomized study with a 5-year minimum follow-up evaluation of transanal endoscopic microsurgery versus laparoscopic total mesorectal excision after neoadjuvant therapy. *Surg Endosc* 2008; **22**: 352-358 [PMID: 17943364 DOI: 10.1007/s00464-007-9714-x]

106 **Borschitz T**, Wachtlin D, Möhler M, Schmidberger H, Junginger T. Neoadjuvant chemoradiation and local excision for T2-3 rectal cancer. *Ann Surg Oncol* 2008; **15**: 712-720 [PMID: 18163173 DOI: 10.1245/s10434-007-9732-x]

107 **Habr-Gama A**, Perez RO, Nadalin W, Sabbaga J, Ribeiro U, Silva e Sousa AH, Campos FG, Kiss DR, Gama-Rodrigues J. Operative versus nonoperative treatment for stage 0 distal rectal cancer following chemoradiation therapy: long-term results. *Ann Surg* 2004; **240**: 711-717; discussion 717-718 [PMID: 15383798 DOI: 10.1097/01.sla.0000141194.27992.32]

108 **Bhangu A**, Kiran RP, Audisio R, Tekkis P. Survival outcome of operated and non-operated elderly patients with rectal cancer: A Surveillance, Epidemiology, and End Results analysis. *Eur J Surg Oncol* 2014; **40**: 1510-1516 [PMID: 24704032 DOI: 10.1016/j.ejso.2014.02.239]

109 **Garcia-Aguilar J**. Transanal endoscopic microsurgery following neoadjuvant chemoradiation therapy in rectal cancer: a word of caution about patient selection? *Dis Colon Rectum* 2013; **56**: 1-3 [PMID: 23222272 DOI: 10.1097/DCR.0b013e318273f58c]

110 **Lin JT**, Wang WS, Yen CC, Liu JH, Yang MH, Chao TC, Chen PM, Chiou TJ. Outcome of colorectal carcinoma in patients under 40 years of age. *J Gastroenterol Hepatol* 2005; **20**: 900-905 [PMID: 15946138]

111 **Taylor MC**, Pounder D, Ali-Ridha NH, Bodurtha A, MacMullin EC. Prognostic factors in colorectal carcinoma of young adults. *Can J Surg* 1988; **31**: 150-153 [PMID: 3365608]

112 **Palmer ML**, Herrera L, Petrelli NJ. Colorectal adenocarcinoma in patients less than 40 years of age. *Dis Colon Rectum* 1991; **34**: 343-346 [PMID: 1706654]

113 **Marble K**, Banerjee S, Greenwald L. Colorectal carcinoma in young patients. *J Surg Oncol* 1992; **51**: 179-182 [PMID: 1434643]

114 **Adloff M**, Arnaud JP, Schloegel M, Thibaud D, Bergamaschi R. Colorectal cancer in patients under 40 years of age. *Dis Colon Rectum* 1986; **29**: 322-325 [PMID: 3009108]

115 **Beckman EN**, Gathright JB, Ray JE. A potentially brighter prognosis for colon carcinoma in the third and fourth decades. *Cancer* 1984; **54**: 1478-1481 [PMID: 6467172]

116 **Heys SD**, Sherif A, Bagley JS, Brittenden J, Smart C, Eremin O. Prognostic factors and survival of patients aged less than 45 years with colorectal cancer. *Br J Surg* 1994; **81**: 685-688 [PMID: 8044547]

117 **Lee PY**, Fletcher WS, Sullivan ES, Vetto JT. Colorectal cancer in young patients: characteristics and outcome. *Am Surg* 1994; **60**: 607-612 [PMID: 8030817]

118 **McKay A**, Donaleshen J, Helewa RM, Park J, Wirtzfeld D, Hochman D, Singh H, Turner D. Does young age influence the prognosis of colorectal cancer: a population-based analysis. *World J Surg Oncol* 2014; **12**: 370 [PMID: 25466394 DOI: 10.1186/1477-7819-12-370]

119 **Wood LD**, Parsons DW, Jones S, Lin J, Sjöblom T, Leary RJ, Shen D, Boca SM, Barber T, Ptak J, Silliman N, Szabo S, Dezso Z, Ustyanksky V, Nikolskaya T, Nikolsky Y, Karchin R, Wilson PA, Kaminker JS, Zhang Z, Croshaw R, Willis J, Dawson D, Shipitsin M, Willson JK, Sukumar S, Polyak K, Park BH, Pethiyagoda CL, Pant PV, Ballinger DG, Sparks AB, Hartigan J, Smith DR, Suh E, Papadopoulos N, Buckhaults P, Markowitz SD, Parmigiani G, Kinzler KW, Velculescu VE, Vogelstein B. The genomic landscapes of human breast and colorectal cancers. *Science* 2007; **318**: 1108-1113 [PMID: 17932254]

120 **Berg M**, Danielsen SA, Ahlquist T, Merok MA, Ågesen TH, Vatn MH, Mala T, Sjo OH, Bakka A, Moberg I, Fetveit T, Mathisen Ø, Husby A, Sandvik O, Nesbakken A, Thiis-Evensen E, Lothe RA. DNA sequence profiles of the colorectal cancer critical gene set KRAS-BRAF-PIK3CA-PTEN-TP53 related to age at disease onset. *PLoS One* 2010; **5**: e13978 [PMID: 21103049 DOI: 10.1371/journal.pone.0013978]

121 **Diep CB**, Thorstensen L, Meling GI, Skovlund E, Rognum TO, Lothe RA. Genetic tumor markers with prognostic impact in Dukes' stages B and C colorectal cancer patients. *J Clin Oncol* 2003; **21**: 820-829 [PMID: 12610180]

122 **Bokemeyer C**, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de Braud F, Donea S, Ludwig H, Schuch G, Stroh C, Loos AH, Zubel A, Koralewski P. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009; **27**: 663-671 [PMID: 19114683]

123 **Douillard JY**, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Oliner KS, Wolf M, Gansert J. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 2010; **28**: 4697-4705 [PMID: 20921465 DOI: 10.1200/JCO.2009.27.4860]

124 **Van Cutsem E**, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 1408-1417 [PMID: 19339720 DOI: 10.1056/NEJMoa0805019]

125 **Di Nicolantonio F**, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 5705-5712 [PMID: 19001320 DOI: 10.1200/JCO.2008.18.0786]

126 **Souglakos J**, Philips J, Wang R, Marwah S, Silver M, Tzardi M, Silver J, Ogino S, Hooshmand S, Kwak E, Freed E, Meyerhardt

JA, Saridaki Z, Georgoulias V, Finkelstein D, Fuchs CS, Kulke MH, Shivdasani RA. Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. *Br J Cancer* 2009; **101**: 465-472 [PMID: 19603024 DOI: 10.1038/sj.bjc.6605164]

127 **Hemminki A**, Mecklin JP, Järvinen H, Aaltonen LA, Joensuu H. Microsatellite instability is a favorable prognostic indicator in patients with colorectal cancer receiving chemotherapy. *Gastroenterology* 2000; **119**: 921-928 [PMID: 11040179]

128 **Popat S**, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005; **23**: 609-618 [PMID: 15659508]

129 **Dotan E**, Browner I, Hurria A, Denlinger C. Challenges in the management of older patients with colon cancer. *J Natl Compr Canc Netw* 2012; **10**: 213-224; quiz 225 [PMID: 22308516]

130 **McCleary NJ**, Dotan E, Browner I. Refining the chemotherapy approach for older patients with colon cancer. *J Clin Oncol* 2014; **32**: 2570-2580 [PMID: 25071118]

131 **Sanoff HK**, Carpenter WR, Stürmer T, Goldberg RM, Martin CF, Fine JP, McCleary NJ, Meyerhardt JA, Niland J, Kahn KL, Schymura MJ, Schrag D. Effect of adjuvant chemotherapy on survival of patients with stage III colon cancer diagnosed after age 75 years. *J Clin Oncol* 2012; **30**: 2624-2634 [PMID: 22665536 DOI: 10.1200/JCO.2011.41.1140]

132 **Sargent DJ**, Goldberg RM, Jacobson SD, Macdonald JS, Labianca R, Haller DG, Shepherd LE, Seitz JF, Francini G. A pooled analysis of adjuvant chemotherapy for resected colon cancer in elderly patients. *N Engl J Med* 2001; **345**: 1091-1097 [PMID: 11596588 DOI: 10.1056/NEJMoa010957]

133 **Twelves C**, Wong A, Nowacki MP, Abt M, Burris H, Carrato A, Cassidy J, Cervantes A, Fagerberg J, Georgoulias V, Husseini F, Jodrell D, Koralewski P, Kröning H, Maroun J, Marschner N, McKendrick J, Pawlicki M, Rosso R, Schüller J, Seitz JF, Stabuc B, Tujakowski J, Van Hazel G, Zaluski J, Scheithauer W. Capecitabine as adjuvant treatment for stage III colon cancer. *N Engl J Med* 2005; **352**: 2696-2704 [PMID: 15987918 DOI: 10.1056/NEJMoa043116]

134 **Scheithauer W**, McKendrick J, Begbie S, Borner M, Burns WI, Burris HA, Cassidy J, Jodrell D, Koralewski P, Levine EL, Marschner N, Maroun J, Garcia-Alfonso P, Tujakowski J, Van Hazel G, Wong A, Zaluski J, Twelves C. Oral capecitabine as an alternative to i.v. 5-fluorouracil-based adjuvant therapy for colon cancer: safety results of a randomized, phase III trial. *Ann Oncol* 2003; **14**: 1735-1743 [PMID: 14630678 DOI: 10.1093/annonc/mdg500]

135 **Cassidy J**, Twelves C, Van Cutsem E, Hoff P, Bajetta E, Boyer M, Bugat R, Burger U, Garin A, Graeven U, McKendric J, Maroun J, Marshall J, Osterwalder B, Pérez-Manga G, Rosso R, Rougier P, Schilsky RL. First-line oral capecitabine therapy in metastatic colorectal cancer: a favorable safety profile compared with intravenous 5-fluorouracil/leucovorin. *Ann Oncol* 2002; **13**: 566-575 [PMID: 12056707 DOI: 10.1093/annonc/mdf089]

136 **André T**, Boni C, Navarro M, Tabernero J, Hickish T, Topham C, Bonetti A, Clingan P, Bridgewater J, Rivera F, de Gramont A. Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. *J Clin Oncol* 2009; **27**: 3109-3116 [PMID: 19451431 DOI: 10.1200/JCO.2008.20.6771]

137 **Tournigand C**, André T, Bonnetain F, Chibaudel B, Lledo G, Hickish T, Tabernero J, Boni C, Bachet JB, Teixeira L, de Gramont A. Adjuvant therapy with fluorouracil and oxaliplatin in stage II and elderly patients (between ages 70 and 75 years) with colon cancer: subgroup analyses of the Multicenter International Study of Oxaliplatin, Fluorouracil, and Leucovorin in the Adjuvant Treatment of Colon Cancer trial. *J Clin Oncol* 2012; **30**: 3353-3360 [PMID: 22915656 DOI: 10.1200/JCO.2012.42.5645]

138 **Haller DG**, Tabernero J, Maroun J, de Braud F, Price T, Van Cutsem E, Hill M, Gilberg F, Rittweger K, Schmoll HJ. Capecitabine plus oxaliplatin compared with fluorouracil and folinic acid as adjuvant therapy for stage III colon cancer. *J Clin Oncol* 2011; **29**: 1465-1471 [PMID: 21383294 DOI: 10.1200/JCO.2010.33.6297]

139 **McCleary NJ**, Meyerhardt JA, Green E, Yothers G, de Gramont A, Van Cutsem E, O'Connell M, Twelves CJ, Saltz LB, Haller DG, Sargent DJ. Impact of age on the efficacy of newer adjuvant therapies in patients with stage II/III colon cancer: findings from the ACCENT database. *J Clin Oncol* 2013; **31**: 2600-2606 [PMID: 23733765 DOI: 10.1200/JCO.2013.49.6638]

140 **O'Connor ES**, Greenblatt DY, LoConte NK, Gangnon RE, Liou JI, Heise CP, Smith MA. Adjuvant chemotherapy for stage II colon cancer with poor prognostic features. *J Clin Oncol* 2011; **29**: 3381-3388 [PMID: 21788561 DOI: 10.1200/JCO.2010.34.3426]

141 **Sinicrope FA**, Foster NR, Thibodeau SN, Marsoni S, Monges G, Labianca R, Kim GP, Yothers G, Allegra C, Moore MJ, Gallinger S, Sargent DJ. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. *J Natl Cancer Inst* 2011; **103**: 863-875 [PMID: 21597022 DOI: 10.1093/jnci/djr153]

142 **Gray R**, Barnwell J, McConkey C, Hills RK, Williams NS, Kerr DJ. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. *Lancet* 2007; **370**: 2020-2029 [PMID: 18083404 DOI: 10.1016/S0140-6736(07)61866-2]

143 **Simmonds PC**. Palliative chemotherapy for advanced colorectal cancer: systematic review and meta-analysis. Colorectal Cancer Collaborative Group. *BMJ* 2000; **321**: 531-535 [PMID: 10968812 DOI: 10.1136/bmj.321.7260.531]

144 **Chibaudel B**, Tournigand C, André T, Larsen AK, de Gramont A. Targeted therapies as adjuvant treatment for early-stage colorectal cancer: first impressions and clinical questions. *Clin Colorectal Cancer* 2010; **9**: 269-273 [PMID: 21208840 DOI: 10.3816/CCC.2010.n.039]

145 **Folprecht G**, Cunningham D, Ross P, Glimelius B, Di Costanzo F, Wils J, Scheithauer W, Rougier P, Aranda E, Hecker H, Köhne CH. Efficacy of 5-fluorouracil-based chemotherapy in elderly patients with metastatic colorectal cancer: a pooled analysis of clinical trials. *Ann Oncol* 2004; **15**: 1330-1338 [PMID: 15319237 DOI: 10.1093/annonc/mdh344]

146 **Ho C**, Ng K, O'Reilly S, Gill S. Outcomes in elderly patients with advanced colorectal cancer treated with capecitabine: a population-based analysis. *Clin Colorectal Cancer* 2005; **5**: 279-282 [PMID: 16356306 DOI: 10.3816/CCC.2005.n.040]

147 **Stein BN**, Petrelli NJ, Douglass HO, Driscoll DL, Arcangeli G, Meropol NJ. Age and sex are independent predictors of 5-fluorouracil toxicity. Analysis of a large scale phase III trial. *Cancer* 1995; **75**: 11-17 [PMID: 7804963 DOI: 10.1002/1097-0142(19950101)75]

148 **Ershler WB**. Capecitabine use in geriatric oncology: an analysis of current safety, efficacy, and quality of life data. *Crit Rev Oncol Hematol* 2006; **58**: 68-78 [PMID: 16473520 DOI: 10.1016/j.critrevonc.2005.08.006]

149 **Petrioli R**, Pascucci A, Francini E, Marsili S, Fiaschi AI, Civitelli S, Tanzini G, Battistelli S, Lorenzi M, Roviello F, Francini G. Continuous oral capecitabine at fixed dose in patients older than 75 years with metastatic colorectal and gastric cancer: a study of the Multidisciplinary Oncology Group on Gastrointestinal Tumors. *Anticancer Drugs* 2008; **19**: 91-96 [PMID: 18043134 DOI: 10.1097/CAD.0b013e3282f21363]

150 **Pasetto LM**, Monfardini S. The role of capecitabine in the treatment of colorectal cancer in the elderly. *Anticancer Res* 2006; **26**: 2381-2386 [PMID: 16821620]

151 **Feliu J**, Escudero P, Llosa F, Bolaños M, Vicent JM, Yubero A, Sanz-Lacalle JJ, Lopez R, Lopez-Gómez L, Casado E, Gómez-Reina MJ, González-Baron M. Capecitabine as first-line treatment for patients older than 70 years with metastatic colorectal cancer: an oncopaz cooperative group study. *J Clin Oncol* 2005; **23**: 3104-3111 [PMID: 15860870 DOI: 10.1200/JCO.2005.06.035]

152 **Seymour MT**, Thompson LC, Wasan HS, Middleton G, Brewster AE, Shepherd SF, O'Mahony MS, Maughan TS, Parmar M, Langley RE. Chemotherapy options in elderly and frail patients with metastatic colorectal cancer (MRC FOCUS2): an open-label,

randomised factorial trial. *Lancet* 2011; **377**: 1749-1759 [PMID: 21570111 DOI: 10.1016/S0140-6736(11)60399-1]

153 **Koopman M**, Antonini NF, Douma J, Wals J, Honkoop AH, Erdkamp FL, de Jong RS, Rodenburg CJ, Vreugdenhil G, Loosveld OJ, van Bochove A, Sinnige HA, Creemers GJ, Tesselaar ME, Slee PH, Werter MJ, Mol L, Dalesio O, Punt CJ. Sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer (CAIRO): a phase III randomised controlled trial. *Lancet* 2007; **370**: 135-142 [PMID: 17630036 DOI: 10.1016/S0140-6736(07)61086-1]

154 **Ducreux M**, Malka D, Mendiboure J, Etienne PL, Texereau P, Auby D, Rougier P, Gasmi M, Castaing M, Abbas M, Michel P, Gargot D, Azzedine A, Lombard-Bohas C, Geoffroy P, Denis B, Pignon JP, Bedenne L, Bouché O. Sequential versus combination chemotherapy for the treatment of advanced colorectal cancer (FFCD 2000-05): an open-label, randomised, phase 3 trial. *Lancet Oncol* 2011; **12**: 1032-1044 [PMID: 21903473 DOI: 10.1016/S1470-2045(11)70199-1]

155 **Folprecht G**, Seymour MT, Saltz L, Douillard JY, Hecker H, Stephens RJ, Maughan TS, Van Cutsem E, Rougier P, Mitry E, Schubert U, Köhne CH. Irinotecan/fluorouracil combination in first-line therapy of older and younger patients with metastatic colorectal cancer: combined analysis of 2,691 patients in randomized controlled trials. *J Clin Oncol* 2008; **26**: 1443-1451 [PMID: 18349394 DOI: 10.1200/JCO.2007.14.0509]

156 **Souglakos J**, Androulakis N, Syrigos K, Polyzos A, Ziras N, Athanasiadis A, Kakolyris S, Tsousis S, Kouroussis Ch, Vamvakas L, Kalykaki A, Samonis G, Mavroudis D, Georgoulias V. FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) vs FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) as first-line treatment in metastatic colorectal cancer (MCC): a multicentre randomised phase III trial from the Hellenic Oncology Research Group (HORG). *Br J Cancer* 2006; **94**: 798-805 [PMID: 16508637 DOI: 10.1038/sj.bjc.6603011]

157 **Fuchs CS**, Moore MR, Harker G, Villa L, Rinaldi D, Hecht JR. Phase III comparison of two irinotecan dosing regimens in second-line therapy of metastatic colorectal cancer. *J Clin Oncol* 2003; **21**: 807-814 [PMID: 12610178 DOI: 10.1200/JCO.2003.08.058]

158 **Aparicio T**, Jouve JL, Teillet L, Gargot D, Subtil F, Le Brun-Ly V, Cretin J, Locher C, Bouché O, Breysacher G, Charneau J, Seitz JF, Gasmi M, Stefani L, Ramdani M, Lecomte T, Mitry E. Geriatric factors predict chemotherapy feasibility: ancillary results of FFCD 2001-02 phase III study in first-line chemotherapy for metastatic colorectal cancer in elderly patients. *J Clin Oncol* 2013; **31**: 1464-1470 [PMID: 23460711 DOI: 10.1200/JCO.2012.42.9894]

159 **Rosati G**, Cordio S, Bordonaro R, Caputo G, Novello G, Reggiardo G, Manzione L. Capecitabine in combination with oxaliplatin or irinotecan in elderly patients with advanced colorectal cancer: results of a randomized phase II study. *Ann Oncol* 2010; **21**: 781-786 [PMID: 19713248 DOI: 10.1093/annonc/mdp359]

160 **Kabbinavar FF**, Hurwitz HI, Yi J, Sarkar S, Rosen O. Addition of bevacizumab to fluorouracil-based first-line treatment of metastatic colorectal cancer: pooled analysis of cohorts of older patients from two randomized clinical trials. *J Clin Oncol* 2009; **27**: 199-205 [PMID: 19064978 DOI: 10.1200/JCO.2008.17.7931]

161 **Cassidy J**, Saltz LB, Giantonio BJ, Kabbinavar FF, Hurwitz HI, Rohr UP. Effect of bevacizumab in older patients with metastatic colorectal cancer: pooled analysis of four randomized studies. *J Cancer Res Clin Oncol* 2010; **136**: 737-743 [PMID: 19904559 DOI: 10.1007/s00432-009-0712-3]

162 **Kozloff M**, Yood MU, Berlin J, Flynn PJ, Kabbinavar FF, Purdie DM, Ashby MA, Dong W, Sugrue MM, Grothey A. Clinical outcomes associated with bevacizumab-containing treatment of metastatic colorectal cancer: the BRiTE observational cohort study. *Oncologist* 2009; **14**: 862-870 [PMID: 19726453 DOI: 10.1634/theoncologist.2009-0071]

163 **Cunningham D**, Lang I, Marcuello E, Lorusso V, Ocvirk J, Shin DB, Jonker D, Osborne S, Andre N, Waterkamp D, Saunders MP. Bevacizumab plus capecitabine versus capecitabine alone in elderly patients with previously untreated metastatic colorectal cancer (AVEX): an open-label, randomised phase 3 trial. *Lancet Oncol* 2013; **14**: 1077-1085 [PMID: 24028813 DOI: 10.1016/S1470-2045(13)70154-2]

164 **Tabernero J**, Van Cutsem E, Lakomý R, Prausová J, Ruff P, van Hazel GA, Moiseyenko VM, Ferry DR, McKendrick JJ, Soussan-Lazard K, Chevalier S, Allegra CJ. Aflibercept versus placebo in combination with fluorouracil, leucovorin and irinotecan in the treatment of previously treated metastatic colorectal cancer: prespecified subgroup analyses from the VELOUR trial. *Eur J Cancer* 2014; **50**: 320-331 [PMID: 24140268 DOI: 10.1016/j.ejca.2013.09.013]

165 **Jehn CF**, Böning L, Kröning H, Possinger K, Lüftner D. Cetuximab-based therapy in elderly comorbid patients with metastatic colorectal cancer. *Br J Cancer* 2012; **106**: 274-278 [PMID: 22215062 DOI: 10.1038/bjc.2011.554]

166 **Douillard JY**, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Smakal M, Canon JL, Rother M, Oliner KS, Tian Y, Xu F, Sidhu R. Final results from PRIME: randomized phase III study of panitumumab with FOLFOX4 for first-line treatment of metastatic colorectal cancer. *Ann Oncol* 2014; **25**: 1346-1355 [PMID: 24718886 DOI: 10.1093/annonc/mdu141]

167 **Sastre J**, Grávalos C, Rivera F, Massuti B, Valladares-Ayerbes M, Marcuello E, Manzano JL, Benavides M, Hidalgo M, Díaz-Rubio E, Aranda E. First-line cetuximab plus capecitabine in elderly patients with advanced colorectal cancer: clinical outcome and subgroup analysis according to KRAS status from a Spanish TTD Group Study. *Oncologist* 2012; **17**: 339-345 [PMID: 22363067 DOI: 10.1634/theoncologist.2011-0406]

168 **Grothey A**, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, Humblet Y, Bouché O, Mineur L, Barone C, Adenis A, Tabernero J, Yoshino T, Lenz HJ, Goldberg RM, Sargent DJ, Cihon F, Cupit L, Wagner A, Laurent D. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013; **381**: 303-312 [PMID: 23177514 DOI: 10.1016/S0140-6736(12)61900-X]

169 **Saito R**, Suzuki H, Yamada T, Endo S, Moriwaki T, Ueno T, Hirose M, Hirai S, Yamato K, Mizokami Y, Hyodo I. Predicting skin toxicity according to EGFR polymorphisms in patients with colorectal cancer receiving antibody against EGFR. *Anticancer Res* 2013; **33**: 4995-4998 [PMID: 24222141]

170 **Vakiani E**, Solit DB. KRAS and BRAF: drug targets and predictive biomarkers. *J Pathol* 2011; **223**: 219-229 [PMID: 21125676 DOI: 10.1002/path.2796]

171 **Tabernero J**, Yoshino T, Cohn AL, Obermannova R, Bodoky G, Garcia-Carbonero R, Ciuleanu TE, Portnoy DC, Van Cutsem E, Grothey A, Prausová J, Garcia-Alfonso P, Yamazaki K, Clingan PR, Lonardi S, Kim TW, Simms L, Chang SC, Nasroulah F. Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): a randomised, double-blind, multicentre, phase 3 study. *Lancet Oncol* 2015; **16**: 499-508 [PMID: 25877855 DOI: 10.1016/S1470-2045(15)70127-0]

172 **Menon KV**, Al-Mukhtar A, Aldouri A, Prasad RK, Lodge PA, Toogood GJ. Outcomes after major hepatectomy in elderly patients. *J Am Coll Surg* 2006; **203**: 677-683 [PMID: 17084329 DOI: 10.1016/j.jamcollsurg.2006.07.025]

173 **Adam R**, Frilling A, Elias D, Laurent C, Ramos E, Capussotti L, Poston GJ, Wicherts DA, de Haas RJ. Liver resection of colorectal metastases in elderly patients. *Br J Surg* 2010; **97**: 366-376 [PMID: 20101645 DOI: 10.1002/bjs.6889]

174 **Tamandl D**, Gruenberger B, Herberger B, Kaczirek K, Gruenberger T. Surgery after neoadjuvant chemotherapy for colorectal liver metastases is safe and feasible in elderly patients. *J Surg Oncol* 2009; **100**: 364-371 [PMID: 19235181 DOI: 10.1002/jso.21259]

175 **de Liguori Carino N**, van Leeuwen BL, Ghaneh P, Wu A, Audisio RA, Poston GJ. Liver resection for colorectal liver metastases in older patients. *Crit Rev Oncol Hematol* 2008; **67**: 273-278 [PMID:

18595728 DOI: 10.1016/j.critrevonc.2008.05.003]
176 **Lee L**, Jannapureddy M, Albo D, Awad SS, Farrow B, Bellows CC, Berger DH. Outcomes of Veterans Affairs patients older than age 80 after surgical procedures for colon malignancies. *Am J Surg* 2007; **194**: 646-651 [PMID: 17936428 DOI: 10.1016/j.amjsurg.2007.08.003]
177 **Schiffmann L**, Ozcan S, Schwarz F, Lange J, Prall F, Klar E. Colorectal cancer in the elderly: surgical treatment and long-term survival. *Int J Colorectal Dis* 2008; **23**: 601-610 [PMID: 18343931 DOI: 10.1007/s00384-008-0457-5]
178 **Brighi N**, Balducci L, Biasco G. Cancer in the elderly: is it time for palliative care in geriatric oncology? *J Geriatr Oncol* 2014; **5**: 197-203 [PMID: 24560041 DOI: 10.1016/j.jgo.2014.01.007]
179 **Gawande A**. Being Mortal. London: Wellcome Collection, 2014: 155
180 **Audisio RA**, Bozzetti F, Gennari R, Jaklitsch MT, Koperna T, Longo WE, Wiggers T, Zbar AP. The surgical management of elderly cancer patients; recommendations of the SIOG surgical task force. *Eur J Cancer* 2004; **40**: 926-938 [PMID: 15093567 DOI: 10.1016/j.ejca.2004.01.016]

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TOPIC HIGHLIGHT

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Immune cell interplay in colorectal cancer prognosis

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Abstract

The immune response to colorectal cancer has proven to be a reliable measure of patient outcome in several studies. However, the complexity of the immune response in this disease is not well understood, particularly the interactions between tumour-associated cells and cells of the innate and adaptive immune system. This review will discuss the relationship between cancer associated fibroblasts and macrophages, as well as between macrophages and T cells, and demonstrate how each population may support or prevent tumour growth in a different immune environment.

Key words: Colorectal cancer neoplasms; Fibroblasts; Immune system processes; Macrophages; T lymphocytes

Core tip: The outcome of patients with colorectal cancer is influenced by the complex local immune system. Understanding how multiple relationships between immune cells may affect tumour growth or elimination will be key in designing new therapies to treat this disease.

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PERSPECTIVE

Colorectal cancer (CRC) is the second and third most common cancer in women and men, respectively, worldwide[1]. In most cases, the disease occurs sporadically, but can also be caused by genetic predisposition or prior intestinal inflammation. While resection is often curative, approximately 45% of patients still die from the disease.

The recent introduction of successful immunotherapies against cancer, specifically checkpoint blockade antibodies, has increased attention on the immune response to tumours. These new treatments have provided opportunities for the development of new

immune-based therapies for less responsive tumours, such as CRC.

The complexity of the anti-tumour immune response is vast - not only are there multiple cells, these cells interact with each other, and are plastic so can change phenotype and function in response to inflammatory or suppressive signals from the tumour and tumour associated cells[2]. Understanding the relationships between cancer cells and immune cells is critical to understanding and, ultimately, manipulating the tumour immune microenvironment.

The importance of local immunity is particularly true in CRC where the immune response in the gut has been "trained" to ignore commensal microflora, and yet retain the ability to induce an attack against a pathogen. The ability of the gut to do this relies on a series of signals and interactions between bacteria, epithelial cells, and innate cells such as dendritic cells, monocytes and gut resident macrophages. In CRC, there are local adaptive immune cells such as effector T cells likely to have an antitumor effect, and regulatory or inflammatory T cells predicted to have a pro-tumour effect[3].

Recent study of the immune response in CRC has resulted in the development of the Immunoscore, a means of measuring T cell infiltrate into CRCs[4]. The Immunoscore thus far has shown to be predictive of outcome and also superior to other methods for staging patients. Innate immune responses, particularly those involving tumour associated macrophages (TAMs), have been studied and data show that the frequency of these cells infiltrating the tumour can be associated with poor patient outcome, although this is controversial[5].

Immune responses against colorectal tumours can be detected in early stage cancers, indicating that the immune system is capable of recognizing a tumour[6]. However, the tumour produces molecules that inhibit immune cell infiltration, that reduce activity of immune cells, or that change the phenotype of immune cells to a less effective anti-tumour function, ultimately allowing tumour outgrowth[7].

The inflammatory immune environment underlying tumour initiation and progression in CRC has been reviewed extensively[8], although much of the supporting data relies on animal models of colitis-induced cancer[9]. However, colitis-associated cancer accounts for only a small percentage (1%-4%) of CRC cases in humans[10]. The influence of inflammation mediated by immune cells in established familial or sporadic human CRC has been much less studied. In addition, new data demonstrate an impressive complexity of innate and adaptive immune cells[11], suggesting that some associations with cancer progression may have been too simplistic in their interpretation.

This review will concentrate on the networks of innate and adaptive immune cells, and tumour-associated immune cells in established CRC, and how these interactions can influence subsequent patient outcome (Figure 1). Despite recent interest in the immunology of CRC, there are limited experimental data studying the complexity of the immune response and the interactions between cancer cells and immune cells, particularly in humans. We will discuss (1) the interplay between the tumour stromal cells [particularly cancer-associated fibroblasts (CAFs)] and the macrophages infiltrating the tumour; and (2) the interactions between macrophages and T cells and how T cell populations may influence each other. We will attempt to describe the complexity and plasticity of these immune populations and discuss how they can be used to better understand the disease and to predict patient outcomes.

CANCER ASSOCIATED FIBROBLASTS AND TUMOUR ASSOCIATED MACROPHAGES - INNATE CELLS AND TUMOUR PROMOTION

CAFs in CRC

Fibroblasts are a key component of the connective tissue and are found embedded in the extracellular matrix (ECM). Fibroblasts have important roles in tissue homeostasis and remodelling. They produce multiple cytokines and can therefore modulate the immune microenvironment. Fibroblasts found in tumour stroma are referred to as CAFs.

The exact origin of CAFs is not clear. It has been proposed that they are cancer cells that have undergone an epithelial-mesenchymal transition[12]. Other research suggests that fibroblasts mature from fibrocytes that, in turn, have differentiated from monocytes[13] and thus have a similar haematopoietic lineage to macrophages. It is then not surprising that there is significant phenotypic overlap between CAFs and macrophages. CAFs do not express the immune cell marker CD45, however they can express CD68, a marker commonly used to differentiate macrophages[14]. Madar *et al*[15] hypothesised that CAFs were the result of convergent differentiation from any one of multiple pathways within the tumour microenvironment, and that CAF is a description of a functional state rather than a defined lineage.

CAFS may have a direct role in promoting CRC cell growth. Primary CAFs cultured from human colorectal tumours developed into distinct populations, some inducing a pro-migratory effect on CRC cells[16]. These pro-tumour CAFS had a distinct genetic signature with significant prognostic value. In addition, CAFs have been shown to promote metastases in CRC[17].

CAF interactions promoting tumour growth

Because of their role in in tissue homeostasis, CAFs are able to promote tumour growth *via* similar pathways, including *via* inflammatory mediators consistent with the wound healing process. These pathways were reviewed recently[12], so we will discuss the role of CAFs briefly, and focus on their influence on innate immune

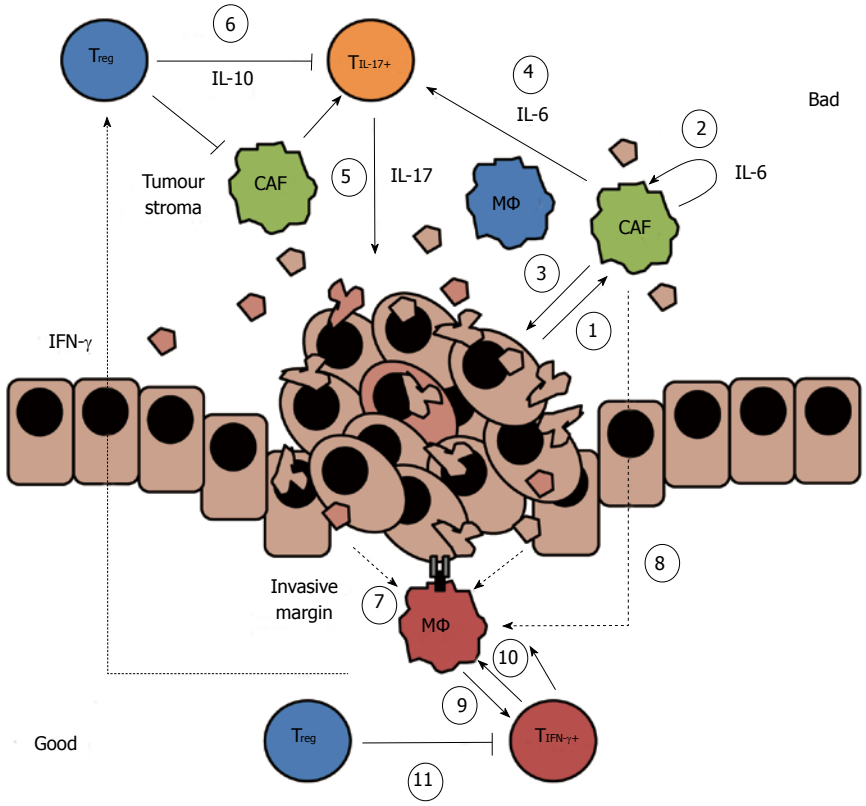

Figure 1 Immune cell interplay in established colorectal cancer. CAFs and macrophages play an important role in promoting tumour progression in the stroma, mediated by IL-6 ("Bad"). Conversely, immune responses at the invasive margin, including macrophage and T cell compartments inhibit tumour growth ("Good"). (1): Unknown factors from colorectal tumours promote IL-6 production from CAFs; (2) IL-6 promotes further IL-6 production from CAFs as well as initiation of VEGF production; (3) IL-6, IL-17, VEGF and ECM modulators produced by CAFs promote growth, angiogenesis and invasion of colorectal tumours; (4) IL-6 produced by CAFs or stromal macrophages promotes T cell differentiation towards an inflammatory IL-17 producing phenotype; (5) IL-17 producing T cells promote colorectal tumour progression and are associated with poorer patient prognosis; (6) Tregs suppress the inflammatory IL-17 response; (7) Macrophages at the invasive margin are associated with improved prognosis; (8) IL-6 produced in the stroma enhances the anti-tumour phenotype; (9) Invasive margin macrophages are primed to induce good effector T cell responses; (10) IFN-γ+ effector T cells are associated with improved prognosis in CRC; (11) Tregs can inhibit effector anti-tumour T cell responses. CAFs: Cancer-associated fibroblasts; IL: Interleukin; VEGF: Vascular endothelial growth factor; ECM: Extracellular matrix.

cells. CAF-derived inflammatory mediators can both promote tumour growth and tumour invasion (Figure 1). An important inflammatory cytokine produced by CAFs in the regulation of wound healing, interleukin (IL)-6, is also associated with disease progression in CRC.

IL-6 in patient serum has been associated with poor patient prognosis in many cancers, including CRC[18]. IL-6 promotes cell survival and supports the production of vascular endothelial growth factor (VEGF) from both tumour and immune cells. VEGF was associated with enhanced tumour progression and poor patient prognosis in CRC[19], likely through its role in angiogenesis[20]. CAFs produced more IL-6 than cancer cells, and CAF-derived IL-6 was increased in the presence of CRC cell lines[21]. In response to greater IL-6 production, CAFs up-regulated production of VEGF, leading to the proposal that the indirect effect of IL-6 on tumour growth *via* CAFs was more important that the direct effect of IL-6 on tumour cells[21].

Other inflammatory mediators produced by CAFs also increase IL-6 production, including IL-1β and TNFα[21]. In patients, high plasma levels of the TNFα receptor, TNFR-2, were associated with an increased relative risk of CRC[22]. Expression of both VEGF[23] and FSTL-1[24] (which enhances inflammatory cytokine and chemokine expression) was increased in CRC-associated CAFs. Chemotherapy, known to cause inflammation as cancer cells are killed[25], resulted in increased numbers of active CAFs in a cohort of CRC patients[26], and enhanced tumour growth in *in vitro* assays.

CAF recruitment of inflammatory cells

Fibroblasts both recruit, and are recruited by, monocytes/macrophages[12]. CAFs have been shown to recruit monocytes to the tumour microenvironment and thus may directly affect the local macrophage compartment. Indeed, Schellerer *et al*[27] showed there were more Intracellular Adhesion Molecule-1$^+$ fibroblasts in tumour tissue than healthy bowel tissue from CRC patients, implying that cancer-associated cells have a higher affinity for monocytic cells. In an *in vitro* human breast cancer model, CAFs produced high levels of the chemokines CCL2 and CCL5 that attracted monocytes[28,29]. The production of these chemokines required IL-6, in a suggested IL-6-CCL2 auto-regulatory cycle[29]. CCL2 and CCL5 were also produced by tumour

cells as well as the recruited monocyte/macrophages, creating a positive feedback loop and generating an inflammatory tumour microenvironment[28].

TAMs in CRC

The prognostic significance of TAMs is controversial, particularly in CRC[30]. Macrophages are myeloid derived cells of the innate immune system. They are potent phagocytes and are involved in clearance of pathogens and cellular debris. They also initiate the adaptive response by functioning as antigen presenting cells (APCs). Macrophages reside in all tissues where they also maintain tissue integrity (reviewed in[31]). The phenotype and ontogeny of tissue resident macrophages varies between tissues. Some are freshly recruited bone marrow-monocyte derived macrophages, whereas others derive from the embryonic yolk sac (reviewed in[32]). In most adult tissue, however, resident macrophages are fetal liver derived. Both the ontogeny and microenvironment of resident macrophages influence their phenotype. As such, resident macrophage populations are often heterogeneous.

The phenotypic diversity of macrophages makes analysis of subpopulations challenging. A great deal of work has been undertaken assessing macrophage subsets using only one or two surface markers to determine function. However, a recent opinion suggests this approach to be misleading, due to the many causes of diversity[33]. Instead, multiple markers must be used to estimate the function of macrophage populations, or, where possible, primary functional data. It has been proposed that minimum reporting standards be introduced to allow better meta-analysis of macrophage data between research groups. This type of approach is paramount when assessing highly plastic macrophages, for example, human macrophages were shown to switch from anti-inflammatory to pro-inflammatory cytokine production within 24 h in response to IFNγ, Granulocyte-Monocyte Colony Stimulating Factor and lipopolysaccharide *in vitro*[34].

The link between macrophage infiltration and prognosis in CRC is still poorly understood. While some studies have shown a positive correlation between macrophage infiltration and patient prognosis, others have shown the opposite[30]. For example, Forssell *et al*[35] demonstrated that a dense macrophage infiltration at the tumour invasive margin was associated with improved patient prognosis, and that macrophage inhibition of tumour spread and growth required direct cell-to-cell contact in an *in vitro* CRC model. In contrast, Kang *et al*[36] demonstrated that intra-tumoural TAM count correlated with parameters of worse disease progression (depth of invasion, lymph node metastasis and stage). Using an *in vitro* co-culture macrophage and CRC cell lines these researchers also demonstrated that macrophages increased cancer cell invasiveness and migration. It may be that the conflicting data relating to the role of macrophages in CRC prognosis is due to inaccuracies of reporting culture conditions or a lack of detailed phenotype[33].

Gut resident macrophages and CRC

Regular interaction between immune cells and microbes in the gut creates an immune environment that must be tightly regulated. Gut resident macrophages provide an important role in regulating this commensal barrier. These particular macrophages have an anergic phenotype; they destroy any bacteria that breach the epithelial barrier but do not initiate an immune reaction against them under homeostatic conditions[37,38].

Unlike most tissue resident macrophage populations, gut resident macrophages are bone marrow derived[32,37]. Newly recruited monocytes undergo a conditioning process, mediated by the gut epithelia, that matures them into the resident anergic phenotype. However, upon acute inflammatory insult, such as that seen in inflammatory bowel disorders, this conditioning process becomes dysregulated, resulting in a mature macrophage population that acquires and maintains migratory and inflammatory characteristics[37,39].

In the context of CRC, monocyte conditioning is unlikely to be modulated only by inflammation, but also factors actively produced by the tumour[40], hypoxic conditions[41] and glucose starvation[28]. As a result, unique macrophage populations will exist depending strongly on the context of the local microenvironment. Hence, describing a homogeneous macrophage population in CRC can be misleading.

TAMs promote an inflammatory pro-tumour environment

It is well documented that TAMs can promote tumour growth, both directly on tumour cells, and indirectly *via* cells in the tumour microenvironment (reviewed in[42]). The human monocytic cell line, THP-1, produced IL-6 in the presence of a colorectal cell line[43], and macrophage-derived IL-6 induced expression of IL-6 by the HT29 CRC cell line[44]. TAMs also upregulated the expression of metalloproteinase (MMP)-2 and MMP-9 on cancer cells, molecules associated with lymph node metastasis[42,45]. TAM-derived IL-6 promoted STAT-3 mediated IL-10 production in CRC cells, a cytokine that has also been associated with poor patient prognosis[46]. In fact, p-STAT3 overexpression in the tumours of CRC patients is significantly correlated with tumour specific mortality[47]. Together, these studies demonstrate that TAMs and CAFs promote an environment to support tumour progression in CRC.

Macrophages have been shown to preferentially migrate to hypoxic regions of tumours[48]. In a mouse model of colitis-associated CRC, repression of hypoxia inducible factor 1 led to decreased macrophage infiltration in tumours[49]. Interestingly, under hypoxic conditions, macrophages can acquire a phenotype similar to that seen in macrophages involved in wound-healing role - a phenotype likely to promote tumour growth. More specifically, human macrophages in hypoxic conditions (0.5% oxygen) up-regulated expression of both VEGF and glucose transporter (GLUT)-1 compared

to normoxia[50]. GLUT-1 is the primary rate limiting glucose transporter in inflammatory macrophages[51]. Using transgenic RAW264.7 macrophages that stably overexpressed GLUT-1, it was shown that high glucose trafficking *via* GLUT-1 promoted a pro-inflammatory macrophage phenotype[51]. It is then possible to hypothesise that under hypoxic conditions such as those in a tumour, macrophages up-regulate GLUT-1 in an attempt to scavenge more glucose in a low glucose environment.

Beyond the production of inflammatory modulators, colorectal tumours also cause barrier defects, which allow for contact between immune cells and microbial products. Myeloid cells showed an increase in production of the inflammatory cytokine IL-23 under inflammatory conditions compared with homeostatic conditions in the APC^{min} mouse model of CRC[52]. IL-23 stimulates and maintains IL-17 production from both tumour cells and T cells. In a mouse model of colitis associated CRC, IL-23- and IL-17-mediated inflammation disrupted the commensal microflora, and created a population of microbes that promoted tumour progression[53]. Furthermore, confocal microscopy of human CRC patient samples revealed that IL-17 production was not limited to T cells, but was also co-expressed with the myeloid cell marker, CD68[54]. These findings indicate that myeloid cells such as macrophages may be capable of producing IL-17 in CRC *in vivo*.

Location of TAMs and influence on CRC prognosis

A high infiltrate of macrophages at the invasive margin of colorectal tumours has been associated with improved patient prognosis[35], and macrophages at the invasive margin of patients with CRC displayed characteristics of an anti-tumour phenotype[55]. These cells expressed the co-stimulatory molecules CD80 and CD86, and apoptotic signalling molecule FasL at greater levels than stromal macrophages. Moreover, macrophages have been closely associated with apoptotic cancer cells along the invasive margin[56] and, using cell lines, CRC TAMs have been observed to be highly phagocytic[57]. In an *in vitro* model of macrophage differentiation, with either human peripheral blood mononuclear cells or murine bone marrow derived macrophages, IL-6 promoted maintenance of the established macrophage phenotype, even when the original cytokine stimuli were removed[58]. Because macrophages themselves also produce IL-6, as well as respond to CAF-produced IL-6, they are especially sensitive to the conditioning signals in their immediate environment. For example, macrophages pre-exposed to IL-4/13, acquired a phenotype characterised by increased IL-10 production in response to IL-6. However, macrophages pre-exposed to IFNγ, acquired a phenotype characterised by production of IL-1β and TNFα in the presence of IL-6. We propose that, in CRC, IL-6 both promotes and inhibits tumour growth *via* uniquely located macrophage populations (Figure 1).

T cells and the anti-tumour immune response

While considerable evidence on the role of T cells in preventing tumour growth in animal models has been acquired over decades, it was not until 2005 that a definitive role for T cells in CRC outcome was shown in patients[59]. Galon *et al*[60] demonstrated, in 2006, that a high infiltrate of $CD3^+$ $CD8^+$ $CD45RO^+$ T cells at the invasive margin and the centre of the tumour was predictive of improved Overall Survival and Disease-Free Survival in a large cohort of people with CRC. Since then, these data have been confirmed by other groups, and have led to the introduction of the Immunoscore to quantify infiltrating T cells in clinical practice[61].

The Immunoscore uses immunohistochemistry techniques to quantify the $CD3^+$ $CD8^+$ T cell infiltrate cell analysis at the centre of the tumour and at the invasive margin in people with CRC[4]. To date, the Immunoscore has proven to provide an accurate staging diagnosis as well as to predict patient outcome[62]. Although the Immunoscore is an improvement on the current staging methods for CRC, its efficacy may be hindered by the interference of T cell subsets that are not associated with good prognosis.

Although it remains clear that the infiltrate of $CD3^+$ $CD8^+$ $CD45RO^+$ T cells is associated with good patient prognosis in CRC, some T cell subsets have been associated with poor prognosis. Specifically, inflammatory $CD4^+$ T cells (Th17 cells), usually measured *via* production of the cytokine IL-17; and regulatory $CD4^+$ T cells (Tregs), often quantified by expression of the transcription factor, FoxP3; have been associated with both good and bad outcomes (reviewed in[63]). In addition, a low ratio of $CD4^+$ to $CD8^+$ T cells is associated with improved outcome[64]. Interestingly, Väyrynen *et al*[65] measured infiltrates of innate cells and adaptive cells in 117 CRC patients and found three parameters associated with Disease Free Survival at 24 mo: High infiltration of $CD3^+$ cells at the invasive margin and high infiltration of $FoxP3^+$ cells at the invasive margin and at the tumour stroma. Taken together, these findings indicate that that $CD8^+$ T cells may be more effective than $CD4^+$ T cells in an anti-tumour immune response, or that beneficial $CD4^+$ T cell subsets are masked by subsets associated with poor outcome[64]. The phenotype of T cells resident in the tumour is controlled by the local cytokine environment, particularly APCs such as macrophages. The efficacy of the T cell response against the tumour is therefore dependent on interactions with other cells (Figure 1).

Effective anti-tumour T cell responses

T cells respond to specific antigens expressed by pathogens or tumours. These antigens are presented by a subset of immune cells, APCs, including dendritic cells and macrophages, but also non-immune cells such as epithelial cells or tumour cells. The T cell infiltrate in CRC is likely to be maximally effective if those cells are specific for tumour antigens.

Nagorsen *et al*[66] used HLA tetramer analysis to show that tumour specific $CD8^+$ T cells in the blood were not correlated with improved clinical outcome in people with CRC or breast cancer, highlighting the need to study the tumour microenvironment. In a separate study, tumour-associated-antigen specific T cells were detected in 30%-40% of patients with CRC[67]. This study also showed that only a small subpopulation of infiltrating T cells could respond to tumour-associated antigens, indicating that not all infiltrating T cells were tumour-specific. Recently, Reissfelder *et al*[68] proposed that a subpopulation of tumour antigen-specific T cells infiltrating the tumours of people with CRC was responsible for the prognostic impact of T cells shown by other studies.

Multiple studies in animals have shown that cytotoxic T cells, *via* IFNγ, perforin and granzymes, can destroy established tumours. Gene cluster analysis of a large cohort of 602 patients with early stage CRC revealed that those patients with high $CD8^+$ and $CD45RO^+$ T cell infiltrates into the tumour also had increased expression of genes associated with anti-tumour responses compared with those patients with low $CD8^+$ and $CD45RO^+$ T cell infiltrates into the tumour[69]. The up-regulated anti-tumour gene signature included genes encoding for granzymes and perforin, as well as effector molecules such as IFNγ and the related transcription factor T-bet. The expression of Granzyme B protein in tumours from CRC patients was also associated with improved survival[70]. These, and many other data, support a role for $CD8^+$ T cells and T cells producing the effector molecules IFNγ and granzymes in eliminating CRC.

Effective T cells must become activated by interactions with APCs presenting antigen in the context of an appropriate cytokine milieu. TAMs were shown to express higher levels of the co-stimulatory molecule, CD80, than tumour stromal cells, indicating that these cells could activate T cells within the tumour[55]. In addition, using a multi-cellular tumour spheroid model, Ong *et al*[71] showed that TAMs up-regulated the expression of CD25 and IFNγ in T cells better than *in vitro* macrophages did. They also showed that the frequency of TAMs in human CRC tumours correlated with the frequency of infiltrating IFNγ-producing T cells *in vivo*. These data indicate that TAMs may be able to promote effector T cell responses within the tumour microenvironment (Figure 1). We propose that effective anti-tumour immunity is determined by TAM-T cell interactions occurring at the invasive margin in CRC.

Th17 cells, inflammation and cancer

Inflammatory T cells [defined here as IL-17-producing (or Th17) cells] are important in antimicrobial responses in the gut (reviewed in[72]). The acquisition of an IL-17-producing phenotype occurs when naïve T cells are activated in the presence of IL-6, IL-1β, TGFβ and IL-23; the maintenance of the phenotype is regulated by these same cytokines. Inflammatory IL-17 responses involve production of cytokines (especially IL-17) that recruit monocytes and neutrophils to sites of inflammation[73]. These innate cells in turn produce the same cytokines to promote ongoing Th17 responses[74].

IL-17 production in CRC has been associated with low Disease-Free Survival and Overall Survival[75] but the exact role of Th17 cells in CRC is not understood. Liu *et al*[54] showed that Th17 induced production of VEGF in CRC cell lines *in vitro*, which decreased T cell production of IFNγ and Granzyme B. This study also showed that in human CRC tumours, high expression of IL-17 correlated with high VEGF expression. VEGF expression has been inversely correlated with $CD8^+$ $CD45RO^+$ T cell infiltrate in tumours of CRC patients[69].

Th17 cells indirectly affect tumour growth via CAFs

CAFs may be activated *via* microbial products that cross the compromised epithelial barrier and promote IL-23 secretion[52], further supporting Th17 responses. Using a mouse model of CRC, Numasaki *et al*[76] showed that tumour cells engineered to express IL-17 led to increased production of angiogenic factors, including VEGF, not only by tumour cells, but also by CAFs. Th17 responses may therefore directly aid in the inflammatory responses of innate cells in CRC.

Th17 cells directly promote tumour growth

Liu *et al*[54] showed that IL-17 was increased in tumour tissue compared to healthy bowel tissue in a cohort of CRC patients, and that it was strongly correlated with overall survival. IL-17 added to human CRC cells *ex vivo* stimulated glucose metabolism by the tumour cells[77]. IL-17 promoted tumour growth through a STAT3-mediated pathway in CRC patients[78]; this result has also been shown in other models of cancer[79]. Together, these data indicate that the presence of intra-tumoural IL-17 may support tumour angiogenesis *via* VEGF and IL-6, and directly promote tumour cell proliferation (Figure 1).

Tregs and IL-10 controlling immunity

Regulatory T cells (Tregs) suppress inflammatory responses in the healthy gut and regulate normal immune responses by inhibiting proliferation and activity of effector T cells. Induced Tregs acquire a suppressive phenotype in the presence of cytokines such as TGFβ; the regulatory phenotype is characterised by up-regulation of the transcription factor FoxP3 and the production of IL-10, amongst other cytokines (reviewed in[80]). Dysregulated immune responses of the gut, for example inflammatory bowel diseases, are often typified by a high infiltrate of Tregs. In the presence of excess inflammatory cytokines from innate and adaptive immune cells, particularly IL-6, Tregs can convert into IL-17 inflammatory cells, or maintain their regulatory function while co-producing IL-17 (reviewed in[81]). Conversely, Treg differentiation can also inhibit the generation of Th17 cells.

In many human cancers an accumulation of Tregs is associated with poor patient outcome, presumably

by suppressing effector T cell responses against the tumour[63]. Controversially, in CRC, Tregs have been associated with both good and poor outcomes for patients[82]. It is possible that because Tregs suppress other T cells, they could impair the function of anti-tumour effector cells as well as pro-tumour inflammatory Th17 cells.

Using a complex library of tumour associated antigen-polypeptides, tumour-antigen specific Tregs were identified in the blood of CRC patients[83] providing evidence that these cells have the potential to inhibit specific anti-tumour immune responses. Therefore, the nature of the tumour immune microenvironment may influence the action of infiltrating Tregs.

Tregs suppress anti-tumour immune responses

Tumour-specific Tregs isolated from ovarian tumours suppressed effector $CD8^+$ T cell production of IFNγ *in vitro* after stimulation with tumour antigen[84]. The infiltrate of Tregs correlated with poor patient prognosis. In CRC patients with recurrent disease, specific T cell responses to the tumour antigens CEA and 5T4 were also suppressed[85]. In the same study, tumour specific Tregs and effector T cells were required to have the same specificity in order for Tregs to suppress the T cell response. Indeed, in an independent study, while tumour-antigen specific Tregs were identified in the tumours of CRC patients, the specificity of the majority of these cells was distinct from that of the effector and memory T cells in the same patients[83]. By depleting Tregs *ex vivo* in culture, only the effector anti-tumour T cells with the same specificity as the Tregs were increased.

The mechanism of Treg mediated suppression in tumour environments is not clear. In a mouse model of transplantable CRC using CMT93 cells, TAMs were able to recruit $CCR6^+$ Tregs to the tumour *via* production of the chemokine CCL20[86]. The infiltrate of Treg cells was associated with tumour development. Similarly, in breast cancer patients, the infiltrate of $CCR6^+$ Tregs into the tumour was inversely correlated with IFNγ production from tumour infiltrating $CD8^+$ T cells[87]. Using flow cytometry, the authors showed that $CCR6^+$ Tregs, but not $CCR6^-$ Tregs were associated with poor survival in breast cancer patients. This leads us to hypothesise that, in CRC, tumour-antigen specific Treg populations are actively recruited to the tumour by TAMs and inhibit the anti-tumour immune response, leading to poor prognosis of patients.

Tregs suppress pro-tumour T cells

Tregs recovered from blood of CRC patients were shown to inhibit the proliferation of Th17 cells sorted from blood and to suppress IL-17 production[88]. It is possible, therefore, that an accumulation of Tregs in the tumour of some CRC patients suppresses the inflammatory Th17 cell response rather than the anti-tumour effector response, leading to improved patient outcome.

Role for IL-10 in regulating tumour immune responses

Tregs are characterised by production of IL-10, a multifunctional cytokine generally believed to support anti-inflammatory immune responses. CRC patients had elevated levels of serum IL-10, and IL-10 remained high in those patients who had recurrent disease following tumour resection[89]. However, it has become clear that treatment of cancer with IL-10 could lead to improved anti-tumour responses (reviewed in[90]). In human CRC, the amount of IL-17 was inversely correlated with the amount of IL-10 produced[91]. Interestingly, it has been shown that IL-10 mediated suppression of IL-17 responses was dependent on type-I IFN signalling[92]. Further, Mumm *et al*[93] showed that IL-10 production induced the production of IFNγ and granzymes from human effector $CD8^+$ T cells *in vitro*. Together these data suggest that IL-10 production from Tregs may, in fact, inhibit pro-tumour inflammatory responses as well as promote anti-tumour immune responses. Phase 1 clinical trials have now begun in advanced solid tumours using recombinant human IL-10 as a therapy (https://clinicaltrials.gov/show/NCT02009449).

CLINICAL RELEVANCE

Experimental limitations

Studying the immune response to CRC is difficult because of the complexity of both the gut immune response and the tumour microenvironment. As with most human studies, much of what has been studied has been observational and compounded by individual patient variation and individual tumour variation. The vast majority of CRC cases in humans are sporadic and the mutations that lead to tumour initiation and progression, and therefore immune responses, differ from person to person. Further, while animal models of CRC have provided useful information, their ability to truly mimic human disease is limited (reviewed in[94]). The two most commonly used models represent colitis-associated CRC (1%-4% of human CRC) or APC^{min} mice representing familial CRC (about 20% of human CRC)[95]. We (and others[96,97]) have developed orthotopic surgical murine models of CRC that result in a tumour immune microenvironment more similar to that seen in sporadic human CRC than other mouse models. It is possible these models may be used to test new immune-based interventions.

Checkpoint blockade in CRC

Two new immune-based drugs have recently been introduced in the treatment of cancer - anti-CTLA-4 (ipilimumab) and anti-PD-L1/anti-PD-1 (nivolumab or pembrolizumab). Both types of drugs act to prevent the tumour-mediated suppression of effector T cell responses, and have been successful in melanoma (reviewed in[98]). However, both checkpoint blockade drugs have shown much less success in CRC[99-102]. The reasons behind this are unclear but it has been

shown that many colorectal tumours do not express PD-L1, the ligand for PD-1. Therefore, if the suppressive effect of PD-L1 on anti-tumour T cells is absent, then therapy targeting the PD-1 pathway is unlikely to be successful[101]. However, it has recently been shown that microsatellite instability (MSI) high CRC tumours (15% of CRC tumours that have mutations in mismatch repair genes and are more immunogenic) expressed more PDL1 than MSI low tumours, indicating that checkpoint blockade may be more successful in the MSI high subset of CRC patients[103]. Clinical trials using anti-PD1 therapy in such a subset of patients are now underway to exploit this possibility.

Adoptive T cell therapy in CRC

Adoptive cell therapy (ACT) has been trialled in CRC to some success. Karlsson *et al*[104] used *ex vivo* T cells (recovered from tumour-draining lymph nodes) of CRC patients as a therapy. No side effects were observed and complete responses were seen in 4 out of 9 patients with metastatic disease. A Phase II trial is currently being undertaken to further test ACT in patients with metastatic CRC (https://clinicaltrials.gov/ct2/show/NCT01174121). The use of genetically engineered tumour-antigen specific T cells has been less successful in CRC. T cells genetically engineered to target carcinogenic embryonic antigen (CEA) caused a measurable decrease in serum CEA levels in 4/4 CRC patients treated but also induced severe colitis in all patients[105], consistent with studies in other cancers. Targeting neo-antigens in tumours and individualising therapy may be the way forward in ACT of CRC.

CONCLUSION

Recent technological breakthroughs have allowed the analysis of single cells, providing enormous amounts of data on the immune system (reviewed in[11]). These data provide novel insights into the function and complex connectivity of immune cells. This new network approach to studying immunology is likely to transform our understanding of the immune microenvironment of individuals with CRC. The immune response to CRC in humans is complex and involves a panoply of cells interacting with each other and the tumour. Patient outcome is unlikely to be accurately predicted by measuring one immune parameter independently. Moreover, any new immune-based therapies will need to take into account the pro- as well as anti-tumour activities of specific innate and adaptive immune cells.

REFERENCES

1 **Schreuders EH**, Ruco A, Rabeneck L, Schoen RE, Sung JJ, Young GP, Kuipers EJ. Colorectal cancer screening: a global overview of existing programmes. *Gut* 2015; **64**: 1637-1649 [PMID: 26041752 DOI: 10.1136/gutjnl-2014-309086]

2 **Hölzel M**, Bovier A, Tüting T. Plasticity of tumour and immune cells: a source of heterogeneity and a cause for therapy resistance? *Nat Rev Cancer* 2013; **13**: 365-376 [PMID: 23535846 DOI: 10.1038/nrc3498]

3 **Hooper LV**, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science* 2012; **336**: 1268-1273 [PMID: 22674334 DOI: 10.1126/science.1223490]

4 **Galon J**, Pagès F, Marincola FM, Angell HK, Thurin M, Lugli A, Zlobec I, Berger A, Bifulco C, Botti G, Tatangelo F, Britten CM, Kreiter S, Chouchane L, Delrio P, Arndt H, Asslaber M, Maio M, Masucci GV, Mihm M, Vidal-Vanaclocha F, Allison JP, Gnjatic S, Hakansson L, Huber C, Singh-Jasuja H, Ottensmeier C, Zwierzina H, Laghi L, Grizzi F, Ohashi PS, Shaw PA, Clarke BA, Wouters BG, Kawakami Y, Hazama S, Okuno K, Wang E, O'Donnell-Tormey J, Lagorce C, Pawelec G, Nishimura MI, Hawkins R, Lapointe R, Lundqvist A, Khleif SN, Ogino S, Gibbs P, Waring P, Sato N, Torigoe T, Itoh K, Patel PS, Shukla SN, Palmqvist R, Nagtegaal ID, Wang Y, D'Arrigo C, Kopetz S, Sinicrope FA, Trinchieri G, Gajewski TF, Ascierto PA, Fox BA. Cancer classification using the Immunoscore: a worldwide task force. *J Transl Med* 2012; **10**: 205 [PMID: 23034130 DOI: 10.1186/1479-5876-10-205]

5 **Biswas SK**, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* 2010; **11**: 889-896 [PMID: 20856220 DOI: 10.1038/ni.1937]

6 **Di Caro G**, Bergomas F, Grizzi F, Doni A, Bianchi P, Malesci A, Laghi L, Allavena P, Mantovani A, Marchesi F. Occurrence of tertiary lymphoid tissue is associated with T-cell infiltration and predicts better prognosis in early-stage colorectal cancers. *Clin Cancer Res* 2014; **20**: 2147-2158 [PMID: 24523438 DOI: 10.1158/1078-0432.CCR-13-2590]

7 **Devaud C**, John LB, Westwood JA, Darcy PK, Kershaw MH. Immune modulation of the tumor microenvironment for enhancing cancer immunotherapy. *Oncoimmunology* 2013; **2**: e25961 [PMID: 24083084 DOI: 10.4161/onci.25961]

8 **Djaldetti M**, Bessler H. Modulators affecting the immune dialogue between human immune and colon cancer cells. *World J Gastrointest Oncol* 2014; **6**: 129-138 [PMID: 24834143 DOI: 10.4251/wjgo.v6.i5.129]

9 **Antoniou E**, Margonis GA, Angelou A, Zografos GC, Pikoulis E. Cytokine networks in animal models of colitis-associated cancer. *Anticancer Res* 2015; **35**: 19-24 [PMID: 25550530]

10 **Mattar MC**, Lough D, Pishvaian MJ, Charabaty A. Current management of inflammatory bowel disease and colorectal cancer. *Gastrointest Cancer Res* 2011; **4**: 53-61 [PMID: 21673876]

11 **Chattopadhyay PK**, Gierahn TM, Roederer M, Love JC. Single-cell technologies for monitoring immune systems. *Nat Immunol* 2014; **15**: 128-135 [PMID: 24448570 DOI: 10.1038/ni.2796]

12 **Öhlund D**, Elyada E, Tuveson D. Fibroblast heterogeneity in the cancer wound. *J Exp Med* 2014; **211**: 1503-1523 [PMID: 25071162 DOI: 10.1084/jem.20140692]

13 **Mishra PJ**, Mishra PJ, Humeniuk R, Medina DJ, Alexe G, Mesirov JP, Ganesan S, Glod JW, Banerjee D. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. *Cancer Res* 2008; **68**: 4331-4339 [PMID: 18519693 DOI: 10.1158/0008-5472.CAN-08-0943]

14 **Pilling D**, Fan T, Huang D, Kaul B, Gomer RH. Identification of markers that distinguish monocyte-derived fibrocytes from monocytes, macrophages, and fibroblasts. *PLoS One* 2009; **4**: e7475 [PMID: 19834619 DOI: 10.1371/journal.pone.0007475]

15 **Madar S**, Goldstein I, Rotter V. 'Cancer associated fibroblasts'--more than meets the eye. *Trends Mol Med* 2013; **19**: 447-453 [PMID: 23769623 DOI: 10.1016/j.molmed.2013.05.004]

16 **Herrera M**, Islam AB, Herrera A, Martín P, García V, Silva J, Garcia JM, Salas C, Casal I, de Herreros AG, Bonilla F, Peña C. Functional heterogeneity of cancer-associated fibroblasts from human colon tumors shows specific prognostic gene expression signature. *Clin Cancer Res* 2013; **19**: 5914-5926 [PMID: 24052018 DOI: 10.1158/1078-0432.CCR-13-0694]

17 **Tommelein J**, Verset L, Boterberg T, Demetter P, Bracke M, De Wever O. Cancer-associated fibroblasts connect metastasis-promoting communication in colorectal cancer. *Front Oncol* 2015; **5**: 63 [PMID: 25853091 DOI: 10.3389/fonc.2015.00063]

18 **Knüpfer H**, Preiss R. Serum interleukin-6 levels in colorectal cancer patients--a summary of published results. *Int J Colorectal*

Dis 2010; **25**: 135-140 [PMID: 19898853 DOI: 10.1007/s00384-009-0818-8]
19 **Khorana AA**, Ryan CK, Cox C, Eberly S, Sahasrabudhe DM. Vascular endothelial growth factor, CD68, and epidermal growth factor receptor expression and survival in patients with Stage II and Stage III colon carcinoma: a role for the host response in prognosis. *Cancer* 2003; **97**: 960-968 [PMID: 12569594 DOI: 10.1002/cncr.11152]
20 **Barbera-Guillem E**, Nyhus JK, Wolford CC, Friece CR, Sampsel JW. Vascular endothelial growth factor secretion by tumor-infiltrating macrophages essentially supports tumor angiogenesis, and IgG immune complexes potentiate the process. *Cancer Res* 2002; **62**: 7042-7049 [PMID: 12460925]
21 **Nagasaki T**, Hara M, Nakanishi H, Takahashi H, Sato M, Takeyama H. Interleukin-6 released by colon cancer-associated fibroblasts is critical for tumour angiogenesis: anti-interleukin-6 receptor antibody suppressed angiogenesis and inhibited tumour-stroma interaction. *Br J Cancer* 2014; **110**: 469-478 [PMID: 24346288 DOI: 10.1038/bjc.2013.748]
22 **Chan AT**, Ogino S, Giovannucci EL, Fuchs CS. Inflammatory markers are associated with risk of colorectal cancer and chemopreventive response to anti-inflammatory drugs. *Gastroenterology* 2011; **140**: 799-808, quiz e11 [PMID: 21115010 DOI: 10.1053/j.gastro.2010.11.041]
23 **Koshida Y**, Kuranami M, Watanabe M. Interaction between stromal fibroblasts and colorectal cancer cells in the expression of vascular endothelial growth factor. *J Surg Res* 2006; **134**: 270-277 [PMID: 16600304 DOI: 10.1016/j.jss.2006.02.025]
24 **Torres S**, Bartolomé RA, Mendes M, Barderas R, Fernandez-Aceñero MJ, Peláez-García A, Peña C, Lopez-Lucendo M, Villar-Vázquez R, de Herreros AG, Bonilla F, Casal JI. Proteome profiling of cancer-associated fibroblasts identifies novel proinflammatory signatures and prognostic markers for colorectal cancer. *Clin Cancer Res* 2013; **19**: 6006-6019 [PMID: 24025712 DOI: 10.1158/1078-0432.CCR-13-1130]
25 **Grivennikov SI**, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010; **140**: 883-899 [PMID: 20303878 DOI: 10.1016/j.cell.2010.01.025]
26 **Lotti F**, Jarrar AM, Pai RK, Hitomi M, Lathia J, Mace A, Gantt GA, Sukhdeo K, DeVecchio J, Vasanji A, Leahy P, Hjelmeland AB, Kalady MF, Rich JN. Chemotherapy activates cancer-associated fibroblasts to maintain colorectal cancer-initiating cells by IL-17A. *J Exp Med* 2013; **210**: 2851-2872 [PMID: 24323355 DOI: 10.1084/jem.20131195]
27 **Schellerer VS**, Langheinrich M, Hohenberger W, Croner RS, Merkel S, Rau TT, Stürzl M, Naschberger E. Tumor-associated fibroblasts isolated from colorectal cancer tissues exhibit increased ICAM-1 expression and affinity for monocytes. *Oncol Rep* 2014; **31**: 255-261 [PMID: 24253852 DOI: 10.3892/or.2013.2860]
28 **Murdoch C**, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood* 2004; **104**: 2224-2234 [PMID: 15231578 DOI: 10.1182/blood-2004-03-1109]
29 **Silzle T**, Kreutz M, Dobler MA, Brockhoff G, Knuechel R, Kunz-Schughart LA. Tumor-associated fibroblasts recruit blood monocytes into tumor tissue. *Eur J Immunol* 2003; **33**: 1311-1320 [PMID: 12731056 DOI: 10.1002/eji.200323057]
30 **Zhang QW**, Liu L, Gong CY, Shi HS, Zeng YH, Wang XZ, Zhao YW, Wei YQ. Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. *PLoS One* 2012; **7**: e50946 [PMID: 23284651 DOI: 10.1371/journal.pone.0050946]
31 **Davies LC**, Jenkins SJ, Allen JE, Taylor PR. Tissue-resident macrophages. *Nat Immunol* 2013; **14**: 986-995 [PMID: 24048120 DOI: 10.1038/ni.2705]
32 **Ginhoux F**, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. *Nat Rev Immunol* 2014; **14**: 392-404 [PMID: 24854589 DOI: 10.1038/nri3671]
33 **Murray PJ**, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, Locati M, Mantovani A, Martinez FO, Mege JL, Mosser DM, Natoli G, Saeij JP, Schultze JL, Shirey KA, Sica A, Suttles J, Udalova I, van Ginderachter JA, Vogel SN, Wynn TA. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 2014; **41**: 14-20 [PMID: 25035950 DOI: 10.1016/j.immuni.2014.06.008]
34 **Krausgruber T**, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, Hussell T, Feldmann M, Udalova IA. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat Immunol* 2011; **12**: 231-238 [PMID: 21240265 DOI: 10.1038/ni.1990]
35 **Forssell J**, Oberg A, Henriksson ML, Stenling R, Jung A, Palmqvist R. High macrophage infiltration along the tumor front correlates with improved survival in colon cancer. *Clin Cancer Res* 2007; **13**: 1472-1479 [PMID: 17332291 DOI: 10.1158/1078-0432.CCR-06-2073]
36 **Kang JC**, Chen JS, Lee CH, Chang JJ, Shieh YS. Intratumoral macrophage counts correlate with tumor progression in colorectal cancer. *J Surg Oncol* 2010; **102**: 242-248 [PMID: 20740582 DOI: 10.1002/jso.21617]
37 **Bain CC**, Scott CL, Uronen-Hansson H, Gudjonsson S, Jansson O, Grip O, Guilliams M, Malissen B, Agace WW, Mowat AM. Resident and pro-inflammatory macrophages in the colon represent alternative context-dependent fates of the same Ly6Chi monocyte precursors. *Mucosal Immunol* 2013; **6**: 498-510 [PMID: 22990622 DOI: 10.1038/mi.2012.89]
38 **Smith PD**, Smythies LE, Shen R, Greenwell-Wild T, Gliozzi M, Wahl SM. Intestinal macrophages and response to microbial encroachment. *Mucosal Immunol* 2011; **4**: 31-42 [PMID: 20962772 DOI: 10.1038/mi.2010.66]
39 **Zigmond E**, Jung S. Intestinal macrophages: well educated exceptions from the rule. *Trends Immunol* 2013; **34**: 162-168 [PMID: 23477922 DOI: 10.1016/j.it.2013.02.001]
40 **Bingle L**, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J Pathol* 2002; **196**: 254-265 [PMID: 11857487 DOI: 10.1002/path.1027]
41 **Obeid E**, Nanda R, Fu YX, Olopade OI. The role of tumor-associated macrophages in breast cancer progression (review). *Int J Oncol* 2013; **43**: 5-12 [PMID: 23673510 DOI: 10.3892/ijo.2013.1938]
42 **Erreni M**, Mantovani A, Allavena P. Tumor-associated Macrophages (TAM) and Inflammation in Colorectal Cancer. *Cancer Microenviron* 2011; **4**: 141-154 [PMID: 21909876 DOI: 10.1007/s12307-010-0052-5]
43 **Xu H**, Lai W, Zhang Y, Liu L, Luo X, Zeng Y, Wu H, Lan Q, Chu Z. Tumor-associated macrophage-derived IL-6 and IL-8 enhance invasive activity of LoVo cells induced by PRL-3 in a KCNN4 channel-dependent manner. *BMC Cancer* 2014; **14**: 330 [PMID: 24885636 DOI: 10.1186/1471-2407-14-330]
44 **Li YY**, Hsieh LL, Tang RP, Liao SK, Yeh KY. Interleukin-6 (IL-6) released by macrophages induces IL-6 secretion in the human colon cancer HT-29 cell line. *Hum Immunol* 2009; **70**: 151-158 [PMID: 19272324 DOI: 10.1016/j.humimm.2009.01.004]
45 **Illemann M**, Bird N, Majeed A, Sehested M, Laerum OD, Lund LR, Danø K, Nielsen BS. MMP-9 is differentially expressed in primary human colorectal adenocarcinomas and their metastases. *Mol Cancer Res* 2006; **4**: 293-302 [PMID: 16687484 DOI: 10.1158/1541-7786.MCR-06-0003]
46 **Herbeuval JP**, Lelievre E, Lambert C, Dy M, Genin C. Recruitment of STAT3 for production of IL-10 by colon carcinoma cells induced by macrophage-derived IL-6. *J Immunol* 2004; **172**: 4630-4636 [PMID: 15034082]
47 **Morikawa T**, Baba Y, Yamauchi M, Kuchiba A, Nosho K, Shima K, Tanaka N, Huttenhower C, Frank DA, Fuchs CS, Ogino S. STAT3 expression, molecular features, inflammation patterns, and prognosis in a database of 724 colorectal cancers. *Clin Cancer Res* 2011; **17**: 1452-1462 [PMID: 21310826 DOI: 10.1158/1078-0432.CCR-10-2694]
48 **Tripathi C**, Tewari BN, Kanchan RK, Baghel KS, Nautiyal N, Shrivastava R, Kaur H, Bhatt ML, Bhadauria S. Macrophages are

recruited to hypoxic tumor areas and acquire a pro-angiogenic M2-polarized phenotype via hypoxic cancer cell derived cytokines Oncostatin M and Eotaxin. *Oncotarget* 2014; **5**: 5350-5368 [PMID: 25051364]

49 **Shay JE**, Imtiyaz HZ, Sivanand S, Durham AC, Skuli N, Hsu S, Mucaj V, Eisinger-Mathason TS, Krock BL, Giannoukos DN, Simon MC. Inhibition of hypoxia-inducible factors limits tumor progression in a mouse model of colorectal cancer. *Carcinogenesis* 2014; **35**: 1067-1077 [PMID: 24408928 DOI: 10.1093/carcin/bgu004]

50 **Burke B**, Giannoudis A, Corke KP, Gill D, Wells M, Ziegler-Heitbrock L, Lewis CE. Hypoxia-induced gene expression in human macrophages: implications for ischemic tissues and hypoxia-regulated gene therapy. *Am J Pathol* 2003; **163**: 1233-1243 [PMID: 14507633 DOI: 10.1016/S0002-9440(10)63483-9]

51 **Freemerman AJ**, Johnson AR, Sacks GN, Milner JJ, Kirk EL, Troester MA, Macintyre AN, Goraksha-Hicks P, Rathmell JC, Makowski L. Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. *J Biol Chem* 2014; **289**: 7884-7896 [PMID: 24492615 DOI: 10.1074/jbc.M113.522037]

52 **Grivennikov SI**, Wang K, Mucida D, Stewart CA, Schnabl B, Jauch D, Taniguchi K, Yu GY, Osterreicher CH, Hung KE, Datz C, Feng Y, Fearon ER, Oukka M, Tessarollo L, Coppola V, Yarovinsky F, Cheroutre H, Eckmann L, Trinchieri G, Karin M. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. *Nature* 2012; **491**: 254-258 [PMID: 23034650 DOI: 10.1038/nature11465]

53 **Arthur JC**, Perez-Chanona E, Mühlbauer M, Tomkovich S, Uronis JM, Fan TJ, Campbell BJ, Abujamel T, Dogan B, Rogers AB, Rhodes JM, Stintzi A, Simpson KW, Hansen JJ, Keku TO, Fodor AA, Jobin C. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 2012; **338**: 120-123 [PMID: 22903521 DOI: 10.1126/science.1224820]

54 **Liu J**, Duan Y, Cheng X, Chen X, Xie W, Long H, Lin Z, Zhu B. IL-17 is associated with poor prognosis and promotes angiogenesis via stimulating VEGF production of cancer cells in colorectal carcinoma. *Biochem Biophys Res Commun* 2011; **407**: 348-354 [PMID: 21396350 DOI: 10.1016/j.bbrc.2011.03.021]

55 **Ohtani H**, Naito Y, Saito K, Nagura H. Expression of costimulatory molecules B7-1 and B7-2 by macrophages along invasive margin of colon cancer: a possible antitumor immunity? *Lab Invest* 1997; **77**: 231-241 [PMID: 9314947]

56 **Sugita J**, Ohtani H, Mizoi T, Saito K, Shiiba K, Sasaki I, Matsuno S, Yagita H, Miyazawa M, Nagura H. Close association between Fas ligand (FasL; CD95L)-positive tumor-associated macrophages and apoptotic cancer cells along invasive margin of colorectal carcinoma: a proposal on tumor-host interactions. *Jpn J Cancer Res* 2002; **93**: 320-328 [PMID: 11927015]

57 **Edin S**, Wikberg ML, Rutegård J, Oldenborg PA, Palmqvist R. Phenotypic skewing of macrophages in vitro by secreted factors from colorectal cancer cells. *PLoS One* 2013; **8**: e74982 [PMID: 24058644 DOI: 10.1371/journal.pone.0074982]

58 **Fernando MR**, Reyes JL, Iannuzzi J, Leung G, McKay DM. The pro-inflammatory cytokine, interleukin-6, enhances the polarization of alternatively activated macrophages. *PLoS One* 2014; **9**: e94188 [PMID: 24736635 DOI: 10.1371/journal.pone.0094188]

59 **Pagès F**, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Galon J. Effector memory T cells, early metastasis, and survival in colorectal cancer. *N Engl J Med* 2005; **353**: 2654-2666 [PMID: 16371631 DOI: 10.1056/NEJMoa051424]

60 **Galon J**, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006; **313**: 1960-1964 [PMID: 17008531 DOI: 10.1126/science.1129139]

61 **Galon J**, Pagès F, Marincola FM, Thurin M, Trinchieri G, Fox BA, Gajewski TF, Ascierto PA. The immune score as a new possible approach for the classification of cancer. *J Transl Med* 2012; **10**: 1 [PMID: 22214470 DOI: 10.1186/1479-5876-10-1]

62 **Angell H**, Galon J. From the immune contexture to the Immunoscore: the role of prognostic and predictive immune markers in cancer. *Curr Opin Immunol* 2013; **25**: 261-267 [PMID: 23579076 DOI: 10.1016/j.coi.2013.03.004]

63 **Fridman WH**, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer* 2012; **12**: 298-306 [PMID: 22419253 DOI: 10.1038/nrc3245]

64 **Diederichsen AC**, Hjelmborg Jv, Christensen PB, Zeuthen J, Fenger C. Prognostic value of the CD4+/CD8+ ratio of tumour infiltrating lymphocytes in colorectal cancer and HLA-DR expression on tumour cells. *Cancer Immunol Immunother* 2003; **52**: 423-428 [PMID: 12695859 DOI: 10.1007/s00262-003-0388-5]

65 **Väyrynen JP**, Tuomisto A, Klintrup K, Mäkelä J, Karttunen TJ, Mäkinen MJ. Detailed analysis of inflammatory cell infiltration in colorectal cancer. *Br J Cancer* 2013; **109**: 1839-1847 [PMID: 24008661 DOI: 10.1038/bjc.2013.508]

66 **Nagorsen D**, Scheibenbogen C, Schaller G, Leigh B, Schmittel A, Letsch A, Thiel E, Keilholz U. Differences in T-cell immunity toward tumor-associated antigens in colorectal cancer and breast cancer patients. *Int J Cancer* 2003; **105**: 221-225 [PMID: 12673683 DOI: 10.1002/ijc.11052]

67 **Koch M**, Beckhove P, Op den Winkel J, Autenrieth D, Wagner P, Nummer D, Specht S, Antolovic D, Galindo L, Schmitz-Winnenthal FH, Schirrmacher V, Büchler MW, Weitz J. Tumor infiltrating T lymphocytes in colorectal cancer: Tumor-selective activation and cytotoxic activity in situ. *Ann Surg* 2006; **244**: 986-992; discussion 992-993 [PMID: 17122624 DOI: 10.1097/01.sla.0000247058.43243.7b]

68 **Reissfelder C**, Stamova S, Gossmann C, Braun M, Bonertz A, Walliczek U, Grimm M, Rahbari NN, Koch M, Saadati M, Benner A, Büchler MW, Jäger D, Halama N, Khazaie K, Weitz J, Beckhove P. Tumor-specific cytotoxic T lymphocyte activity determines colorectal cancer patient prognosis. *J Clin Invest* 2015; **125**: 739-751 [PMID: 25562322 DOI: 10.1172/JCI74894]

69 **Pagès F**, Kirilovsky A, Mlecnik B, Asslaber M, Tosolini M, Bindea G, Lagorce C, Wind P, Marliot F, Bruneval P, Zatloukal K, Trajanoski Z, Berger A, Fridman WH, Galon J. In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. *J Clin Oncol* 2009; **27**: 5944-5951 [PMID: 19858404 DOI: 10.1200/JCO.2008.19.6147]

70 **Salama P**, Phillips M, Platell C, Iacopetta B. Low expression of Granzyme B in colorectal cancer is associated with signs of early metastastic invasion. *Histopathology* 2011; **59**: 207-215 [PMID: 21884199 DOI: 10.1111/j.1365-2559.2011.03915.x]

71 **Ong SM**, Tan YC, Beretta O, Jiang D, Yeap WH, Tai JJ, Wong WC, Yang H, Schwarz H, Lim KH, Koh PK, Ling KL, Wong SC. Macrophages in human colorectal cancer are pro-inflammatory and prime T cells towards an anti-tumour type-1 inflammatory response. *Eur J Immunol* 2012; **42**: 89-100 [PMID: 22009685 DOI: 10.1002/eji.201141825]

72 **Blaschitz C**, Raffatellu M. Th17 cytokines and the gut mucosal barrier. *J Clin Immunol* 2010; **30**: 196-203 [PMID: 20127275 DOI: 10.1007/s10875-010-9368-7]

73 **Iwakura Y**, Ishigame H, Saijo S, Nakae S. Functional specialization of interleukin-17 family members. *Immunity* 2011; **34**: 149-162 [PMID: 21349428 DOI: 10.1016/j.immuni.2011.02.012]

74 **Reynolds JM**, Angkasekwinai P, Dong C. IL-17 family member cytokines: regulation and function in innate immunity. *Cytokine Growth Factor Rev* 2010; **21**: 413-423 [PMID: 21074482 DOI: 10.1016/j.cytogfr.2010.10.002]

75 **Tosolini M**, Kirilovsky A, Mlecnik B, Fredriksen T, Mauger S, Bindea G, Berger A, Bruneval P, Fridman WH, Pagès F, Galon J. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, th2, treg, th17) in patients with colorectal cancer. *Cancer Res* 2011; **71**: 1263-1271 [PMID: 21303976 DOI: 10.1158/0008-5472.CAN-10-2907]

76 **Numasaki M**, Fukushi J, Ono M, Narula SK, Zavodny PJ, Kudo

T, Robbins PD, Tahara H, Lotze MT. Interleukin-17 promotes angiogenesis and tumor growth. *Blood* 2003; **101**: 2620-2627 [PMID: 12411307 DOI: 10.1182/blood-2002-05-1461]

77 **Straus DS**. TNFα and IL-17 cooperatively stimulate glucose metabolism and growth factor production in human colorectal cancer cells. *Mol Cancer* 2013; **12**: 78 [PMID: 23866118 DOI: 10.1186/1476-4598-12-78]

78 **De Simone V**, Franzè E, Ronchetti G, Colantoni A, Fantini MC, Di Fusco D, Sica GS, Sileri P, MacDonald TT, Pallone F, Monteleone G, Stolfi C. Th17-type cytokines, IL-6 and TNF-α synergistically activate STAT3 and NF-kB to promote colorectal cancer cell growth. *Oncogene* 2015; **34**: 3493-3503 [PMID: 25174402 DOI: 10.1038/onc.2014.286]

79 **Chang Q**, Bournazou E, Sansone P, Berishaj M, Gao SP, Daly L, Wels J, Theilen T, Granitto S, Zhang X, Cotari J, Alpaugh ML, de Stanchina E, Manova K, Li M, Bonafe M, Ceccarelli C, Taffurelli M, Santini D, Altan-Bonnet G, Kaplan R, Norton L, Nishimoto N, Huszar D, Lyden D, Bromberg J. The IL-6/JAK/Stat3 feed-forward loop drives tumorigenesis and metastasis. *Neoplasia* 2013; **15**: 848-862 [PMID: 23814496]

80 **Shevach EM**, Thornton AM. tTregs, pTregs, and iTregs: similarities and differences. *Immunol Rev* 2014; **259**: 88-102 [PMID: 24712461 DOI: 10.1111/imr.12160]

81 **Kleinewietfeld M**, Hafler DA. The plasticity of human Treg and Th17 cells and its role in autoimmunity. *Semin Immunol* 2013; **25**: 305-312 [PMID: 24211039 DOI: 10.1016/j.smim.2013.10.009]

82 **Ladoire S**, Martin F, Ghiringhelli F. Prognostic role of FOXP3+ regulatory T cells infiltrating human carcinomas: the paradox of colorectal cancer. *Cancer Immunol Immunother* 2011; **60**: 909-918 [PMID: 21644034 DOI: 10.1007/s00262-011-1046-y]

83 **Bonertz A**, Weitz J, Pietsch DH, Rahbari NN, Schlude C, Ge Y, Juenger S, Vlodavsky I, Khazaie K, Jaeger D, Reissfelder C, Antolovic D, Aigner M, Koch M, Beckhove P. Antigen-specific Tregs control T cell responses against a limited repertoire of tumor antigens in patients with colorectal carcinoma. *J Clin Invest* 2009; **119**: 3311-3321 [PMID: 19809157 DOI: 10.1172/JCI39608]

84 **Curiel TJ**, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004; **10**: 942-949 [PMID: 15322536 DOI: 10.1038/nm1093]

85 **Betts G**, Jones E, Junaid S, El-Shanawany T, Scurr M, Mizen P, Kumar M, Jones S, Rees B, Williams G, Gallimore A, Godkin A. Suppression of tumour-specific $CD4^+$ T cells by regulatory T cells is associated with progression of human colorectal cancer. *Gut* 2012; **61**: 1163-1171 [PMID: 22207629 DOI: 10.1136/gutjnl-2011-300970]

86 **Liu J**, Zhang N, Li Q, Zhang W, Ke F, Leng Q, Wang H, Chen J, Wang H. Tumor-associated macrophages recruit CCR6+ regulatory T cells and promote the development of colorectal cancer via enhancing CCL20 production in mice. *PLoS One* 2011; **6**: e19495 [PMID: 21559338 DOI: 10.1371/journal.pone.0019495]

87 **Xu L**, Xu W, Qiu S, Xiong S. Enrichment of CCR6+Foxp3+ regulatory T cells in the tumor mass correlates with impaired CD8+ T cell function and poor prognosis of breast cancer. *Clin Immunol* 2010; **135**: 466-475 [PMID: 20181533 DOI: 10.1016/j.clim.2010.01.014]

88 **Crome SQ**, Clive B, Wang AY, Kang CY, Chow V, Yu J, Lai A, Ghahary A, Broady R, Levings MK. Inflammatory effects of ex vivo human Th17 cells are suppressed by regulatory T cells. *J Immunol* 2010; **185**: 3199-3208 [PMID: 20720207 DOI: 10.4049/jimmunol.1000557]

89 **Galizia G**, Orditura M, Romano C, Lieto E, Castellano P, Pelosio L, Imperatore V, Catalano G, Pignatelli C, De Vita F. Prognostic significance of circulating IL-10 and IL-6 serum levels in colon cancer patients undergoing surgery. *Clin Immunol* 2002; **102**: 169-178 [PMID: 11846459 DOI: 10.1006/clim.2001.5163]

90 **Mumm JB**, Oft M. Pegylated IL-10 induces cancer immunity: the surprising role of IL-10 as a potent inducer of IFN-γ-mediated CD8(+) T cell cytotoxicity. *Bioessays* 2013; **35**: 623-631 [PMID: 23666891 DOI: 10.1002/bies.201300004]

91 **Blatner NR**, Bonertz A, Beckhove P, Cheon EC, Krantz SB, Strouch M, Weitz J, Koch M, Halverson AL, Bentrem DJ, Khazaie K. In colorectal cancer mast cells contribute to systemic regulatory T-cell dysfunction. *Proc Natl Acad Sci USA* 2010; **107**: 6430-6435 [PMID: 20308560 DOI: 10.1073/pnas.0913683107]

92 **Stewart CA**, Metheny H, Iida N, Smith L, Hanson M, Steinhagen F, Leighty RM, Roers A, Karp CL, Müller W, Trinchieri G. Interferon-dependent IL-10 production by Tregs limits tumor Th17 inflammation. *J Clin Invest* 2013; **123**: 4859-4874 [PMID: 24216477 DOI: 10.1172/JCI65180]

93 **Mumm JB**, Emmerich J, Zhang X, Chan I, Wu L, Mauze S, Blaisdell S, Basham B, Dai J, Grein J, Sheppard C, Hong K, Cutler C, Turner S, LaFace D, Kleinschek M, Judo M, Ayanoglu G, Langowski J, Gu D, Paporello B, Murphy E, Sriram V, Naravula S, Desai B, Medicherla S, Seghezzi W, McClanahan T, Cannon-Carlson S, Beebe AM, Oft M. IL-10 elicits IFNγ-dependent tumor immune surveillance. *Cancer Cell* 2011; **20**: 781-796 [PMID: 22172723 DOI: 10.1016/j.ccr.2011.11.003]

94 **Karim BO**, Huso DL. Mouse models for colorectal cancer. *Am J Cancer Res* 2013; **3**: 240-250 [PMID: 23841024]

95 **Munkholm P**. Review article: the incidence and prevalence of colorectal cancer in inflammatory bowel disease. *Aliment Pharmacol Ther* 2003; **18** Suppl 2: 1-5 [PMID: 12950413]

96 **Tseng W**, Leong X, Engleman E. Orthotopic mouse model of colorectal cancer. *J Vis Exp* 2007; **(10)**: 484 [PMID: 18989400 DOI: 10.3791/484]

97 **Terracina KP**, Aoyagi T, Huang WC, Nagahashi M, Yamada A, Aoki K, Takabe K. Development of a metastatic murine colon cancer model. *J Surg Res* 2015 Apr 15; Epub ahead of print [PMID: 26009494 DOI: 10.1016/j.jss.2015.04.030]

98 **Naidoo J**, Page DB, Wolchok JD. Immune modulation for cancer therapy. *Br J Cancer* 2014; **111**: 2214-2219 [PMID: 25211661 DOI: 10.1038/bjc.2014.348]

99 **Brahmer JR**, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med* 2012; **366**: 2455-2465 [PMID: 22658128 DOI: 10.1056/NEJMoa1200694]

100 **Callahan MK**, Wolchok JD. At the bedside: CTLA-4- and PD-1-blocking antibodies in cancer immunotherapy. *J Leukoc Biol* 2013; **94**: 41-53 [PMID: 23667165 DOI: 10.1189/jlb.1212631]

101 **Topalian SL**, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012; **366**: 2443-2454 [PMID: 22658127 DOI: 10.1056/NEJMoa1200690]

102 **Topalian SL**, Sznol M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, Brahmer JR, Lawrence DP, Atkins MB, Powderly JD, Leming PD, Lipson EJ, Puzanov I, Smith DC, Taube JM, Wigginton JM, Kollia GD, Gupta A, Pardoll DM, Sosman JA, Hodi FS. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *J Clin Oncol* 2014; **32**: 1020-1030 [PMID: 24590637 DOI: 10.1200/JCO.2013.53.0105]

103 **Llosa NJ**, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, Blosser RL, Fan H, Wang H, Luber BS, Zhang M, Papadopoulos N, Kinzler KW, Vogelstein B, Sears CL, Anders RA, Pardoll DM, Housseau F. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov* 2015; **5**: 43-51

[PMID: 25358689 DOI: 10.1158/2159-8290.CD-14-0863]
104 **Karlsson M**, Marits P, Dahl K, Dagöö T, Enerbäck S, Thörn M, Winqvist O. Pilot study of sentinel-node-based adoptive immunotherapy in advanced colorectal cancer. *Ann Surg Oncol* 2010; **17**: 1747-1757 [PMID: 20119674 DOI: 10.1245/s10434-010-0920-8]
105 **Parkhurst MR**, Yang JC, Langan RC, Dudley ME, Nathan DA, Feldman SA, Davis JL, Morgan RA, Merino MJ, Sherry RM, Hughes MS, Kammula US, Phan GQ, Lim RM, Wank SA, Restifo NP, Robbins PF, Laurencot CM, Rosenberg SA. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. *Mol Ther* 2011; **19**: 620-626 [PMID: 21157437 DOI: 10.1038/mt.2010.272]

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TOPIC HIGHLIGHT

2015 Advances in Colorectal Cancer

Relationship between intestinal microbiota and colorectal cancer

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Abstract

The human gastrointestinal tract hosts a complex and vast microbial community with up to 10^{11}-10^{12} microorganisms colonizing the colon. The gut microbiota has a serious effect on homeostasis and pathogenesis through a number of mechanisms. In recent years, the relationship between the intestinal microbiota and sporadic colorectal cancer has attracted much scientific interest. Mechanisms underlying colonic carcinogenesis include the conversion of procarcinogenic diet-related factors to carcinogens and the stimulation of procarcinogenic signaling pathways in luminal epithelial cells. Understanding each of these mechanisms will facilitate future studies, leading to the development of novel strategies for the diagnosis, treatment, and prevention of colorectal cancer. In this review, we discuss the relationship between colorectal cancer and the intestinal microbiota.

Key words: Sporadic; Colorectal; Cancer; Intestinal; Microbiota

Core tip: Microbiota's role in providing intestinal homeostasis is not as an audience, but it is active. Both the composition of microbiota and its metabolic activity impact the sensitivity of the host and can cause many pathologies including colorectal cancer.

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INTRODUCTION

Colorectal cancer is the third commonest cancer type

worldwide and causes 600000 deaths every year[1]. Because colorectal cancer patients are frequently asymptomatic in the early phase of the disease, diagnosis at this stage presents a significant clinical challenge. Detection of early stage cancers (stages 1-2) allows curative surgery with a 5-year survival rate of 80%. However, survival rates decrease to approximately 10% for metastatic and late stage tumors[2]. Although there are currently methods for the early diagnosis methods, including computed tomography, colonoscopy, and blood tests, it is expected that evaluation of the intestinal microbiota will prove to be a valuable method allowing earlier diagnosis of colorectal cancer.

In humans, a relationship between cancer and microorganisms has been demonstrated in a number of organs, with the most well-known example being the relationship between *Helicobacter pylori* and gastric cancer and mucosa-associated lymphoid tissue lymphoma[3].

In adults, while the bacterial population in the stomach and small intestine is smaller (10^3-10^4 CFU/g contents), increased concentrations of microorganisms are found in the colon (10^{11}-10^{12} CFU/g contents) compared with the upper gastrointestinal tract. The majority of these microorganisms exist in a favorable symbiotic relationship with humans[3,4]. The intestinal microbiota develops specific to individual variation and environmental conditions beginning at birth[5].

Recently, etiology of colorectal cancer has been shown to be related to genetic mutations, diet, inflammatory processes, lifestyle, and the gut microbiota, with up to 95% of colorectal cancer thought to sporadically develop in individuals with no genetic predisposition[6].

The colonic microbiota is thought to contribute to the development of colorectal cancer by controlling the epithelial cell proliferation and differentiation, synthesizing essential nutrients and bioactive products, preventing the reproduction of pathogenic organisms, and stimulating the immune system[7]. In this review, studies investigating the role of the intestinal microbiota in the development of colorectal cancer development are discussed.

MICROBIOTA OF THE HUMAN INTESTINE

There are 100 billion bacteria in the human intestine with an approximate weight equivalent to 1.5-2 kg. Bacteroidetes and Firmicutes are the major species of the adult intestinal microbiota with the next most frequent species being Actinobacteria, Proteobacteria, and Verrucomicrobia[8].

Normally, colonic bacteria exist in a mutually beneficial symbiotic relationship with humans without adverse effects on the host cells. In situations where this balance is deregulated because of a number of possible causes, the numbers and species of harmful bacteria increase, providing a basis for the development of inflammatory and chronic disease. Changes in the intestinal microbiota have been shown to be associated with obesity, fatty liver, type 1 and 2 diabetes, kidney disease, arthritis, inflammatory bowel disease, and colorectal cancer[9-13]. However, the precise relationship between changes in the microbiota and colorectal cancer has yet to be fully elucidated.

FACTORS INFLUENCING GASTROINTESTINAL MICROBIOTA

The intestinal microbiota is affected by a number of factors, such as antibiotics, diet, and inflammation[4-18]. A number of studies have reported a high degree of similarity in the intestinal microbiota between members of the same family but a low degree of similarity between heterozygous mice despite being housed in the same cage[9,14,19].

The intestinal microbiota of mice fed standard low-in-fat nutrients has been shown to change within a few weeks with particularly great changes in the composition of Bacteroidetes and Firmicutes species. After mice returned to a low-fat diet, a particularly significant reduction in Mollicutes, a species of Firmicutes, was observed[9,20]. Similar changes have observed with diets high in fat, particularly in obese people, genetically obese mice, and obesity-resistant mice[9,14,21]. Transfer of colon microbiota from mice fed a high-fat diet to mice fed a low-in-fat diet has been shown to accelerate tumor growth suggesting diet-induced changes in the colon microbiota may have a synergistic effect with genetic factors on tumor development[22]. Diet-related changes in intestinal microbiota have also been shown to be associated with colorectal cancer[23].

MICROBIAL INFLUENCE ON COLORECTAL CANCER

The relationship between the intestinal microbiota and disease has drawn increased attention in recent years. In particular, recent studies have demonstrated strong associations between the development of colorectal cancer and intestinal bacteria. In these studies, DNA damage caused by superoxide radicals, genotoxin formation, increased T-cell proliferation, and activation of procarcinogenic pathways through a number of receptors have all been shown to contribute to cancer development[24-27].

The enzymatic activation or detoxification of carcinogens, and therefore modulation of their tumorigenic activity, has been shown to be influenced by the intestinal microbiota[24,28-35]. In the 1960s, it was observed that germ-free rats exposed to the glycoside, cyasin, did not develop intestinal tumors. Conversely, germ-free rats directly exposed to methylazoximethanol, a sub-active metabolite of cyasin, did develop intestinal tumors[36]. As the formation of methylazoximethanol depends on bacterial β-glucosidase enzyme activity[36], this study was a potent demonstration of the effect

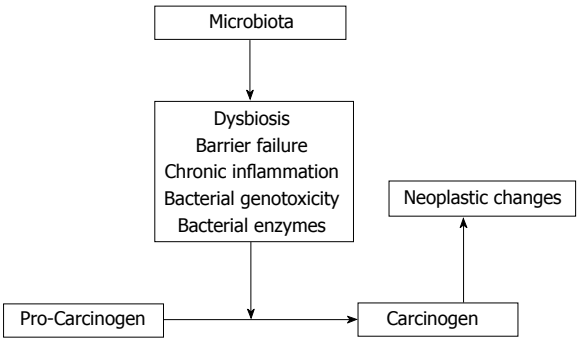

Figure 1 The factors releated to intestinal microbiota promotes neoplasia in the gastrointestinal tract.

of the intestinal microbiota on bioactive carcinogenic compounds. Subsequent research has revealed that the intestinal microbiota converts latent carcinogens to bioactive forms through a number of enzymes, including β-glucuronidase, β-glucosidase, azoreductase, and nitroreductase[37]. Azoxymethane (AOM) is the most frequently used experimental colon carcinogen. AOM is first hydrolyzed in the liver to methylazoximethanol and conjugated to glucuronic acid before bilious excretion into the intestine where it is converted into a highly reactive methyl carbon ion by bacterial β-glucuronidase[34,37,38]. Interestingly, it has been reported that inhibition of β-glucuronidase activity significantly decreases the tumor-inducing potential of AOM in rats[39]. Furthermore, probiotic bacteria, such as Lactobacillus and Bifidobacterium species, have been shown to have anti-carcinogenic effects through the inactivation of microbial enzymes involved in procarcinogenic activation[40]. For example, Lactobacillales, such as *L. Casei* and *L. Acidophilus* suppress β-glucuronidase, azoreductase, and nitroreductase activity[41,42]. This balance between the activation and detoxification of potential carcinogens underlies the activation of host oncogenes and tumor suppressors (Figure 1).

In the study by Boleij *et al*[43] investigating the expression of the Bacteroides fragilis gene (*BFT*) in colonoscopic samples from 49 healthy individuals and 49 colorectal cancer patients, *BFT* gene expression was detected more frequently in samples from colorectal cancer patients. When comparing early and late stage cancer patients, *BFT* gene expression was more frequently detected in late stage cancer patients.

DNA damage and chromosomal instability are early genetic events in the development of colorectal cancer. As with aneuploidy, chromosomal instability is associated with long-term inflammatory bowel disease (IBD) and frequently a precedent event in the subsequent development of colorectal cancer[44-46]. *Enterococcus faecalis* (*E. faecalis*), an intestinal bacteria, has been repeatedly found to induce aneuploidy in colonic epithelial cells in monoassociated interleukin (IL)-10 -/- rats and cause aggressive colitis[47,48]. Inhibitors of reactive oxygen and nitrogen species can prevent aneuploidy induced by *E. faecalis*[49]. These findings demonstrate that intestinal microbiota (particularly specific species) can induce RONS and lead to carcinogenesis.

In intestinal hemostasis, the protective role of the microbiota is thought to be through an effect on epithelial cell proliferation and apoptosis. The main mechanism underlying this effect has been proposed as the conversion of dietary fiber into short chain fatty acids (SCFA), such as acetate, propionate, and butyrate, through microbial fermentation. These SCFAs, particularly butyrate, are readily absorbed easily by the colon and are used as a primary energy source. In addition to significant anti-inflammatory effects[50,51], SCFAs stimulate cell proliferation and differentiation in non-neoplastic normal colon, promote intestinal hemostasis, and the resolution of intestinal injury[51,52]. In addition, SCFAs demonstrate a trans-effect on cancer cells. In particular, butyrate induces apoptosis in colorectal cancer cell lines through a number of mechanisms but predominantly *via* inhibition of histone deacetylase and activation of intrinsic/mitochondrial apoptosis[53-57].

However, SLC5A and GPR109A, the two major receptors of butyrate, provide protection in the early phases of tumorigenesis as they are frequently inactivated in human cancers[58-60]. It is believed that regulation of microbiota species responsible for the production of butyrate will have efficacy in the treatment of gastrointestinal diseases[61,62]. Therefore, probiotics and in-absorbable food are thought to alter the intestinal microbiota leading to a beneficial increase in the production of short chain fatty acids[63].

Although the development of colorectal cancer has not been attributed to any specific microorganism, a number of cancer-promoting bacteria have been identified (Table 1).

In rats, Helicobacter hepaticus increases the development of colorectal cancer related to experimental colitis and spontaneous colorectal cancer[65,67]. Bacteroides fragilis is a widespread intestinal bacteria and a potential cause of spontaneous colon tumorigenesis in rats as an enterotoxigenic variant[26].

Exclusion of opportunist pathogens by colonic bacteria may represent a natural defense against colorectal cancer. Similarly, food containing species of *Lactobacillus* and *Bifidobacteria*, used as probiotics, provide a number of protective benefits against inflammatory bowel diseases[93-95]. Upon colonizing the host and on the condition of the formation of an additional biofilm, probiotic bacteria have been shown to prevent the adhesion and invasion of pathogen types, maintain host tight junction protein structure, decrease host cytokine production, modulate inflammation and immunity, and neutralize carcinogens and toxins[96-100].

Intestinal microbiota have been shown to cause the release of host antibacterial lectins, stimulate antimicrobial host epithelial responses, and deplete subsets of potentially pathogenic bacteria providing a protective role against abnormal immune responses.

In a study by Sobhani *et al*[81] of 179 individuals

Table 1 The relationship between bacterial types and colorectal cancer

Bacteria	Subject of study	Evidence	Ref.
Helicobacter hepaticus	Animal	Augments azoxymethane induced, and spontaneous colorectal cancer in mice	[64-69]
H. hepaticus + *H.bilis*	Animal	Dual infection induces colorectal cancer in mice	[70,71]
H. typhlonius + *H. rodentium*	Animal	Dual infection in neonates induces colorectal cancer in mice	[72,73]
Streptococcus bovis	Human	*S.bovis* bacteremia and endocarditis associated with human colorectal cancer	[74-77]
	Animal	Augments azoxymethane induced colorectal cancer in rats	[78]
	Human	Increased humoral immune response to *S.bovis* antigenRpL7/L12, sassociated with increased risk for colorectal cancer	[79]
Bacteroides fragilis	Animal	Enterotoxigenic *B.fragilis* augments spontaneous colorectal cancer in mice	[26]
	Human	Increased prevalence of enterotoxigenic *B.fragilis* in human colorectal cancer	[80]
	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis	[81]
	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis	[43]
B. vulgatus	Animal	Induces azoxymethane induced, colorectal cancer in mice	[82]
Escherichia coli	Human	Increased mucosa-associated *Escherichia coli* in human colorectal cancer	[83]
Citrobacter rodentium and C. freundii	Animal	Etiologic agent of transmissible murine colonic hyperplasia	[84]
	Animal	Augments spontaneous and 1,2 dimethylhydrazine induced colorectal cancer in mice	[85,86]
Fusobacterium nucleatum	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis	[87]
	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis and 16S ribosomal RNA Gene V3 pyrosequencing analysis	[88]
	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis	[89]
	Animal	16S ribosomal RNA Gene V3 pyrosequencing analysis	[90]
Enterococcus faecalis	Human	Increased in the feces of colorectal cancer patients by quantative PCR analysis	[91]
Furmicutes	Animal	16S ribosomal RNA Gene V3 pyrosequencing analysis	[90]
Akkermansia muciniphila	Human	16S ribosomal RNA Gene V4 pyrosequencing analysis and Gas Chromatography-Mass Spectrometry	[92]
Methanobrevibacterium	Human	Increased prevalence in tumor *vs* normal colonic tissue by quantative PCR analysis and 16S ribosomal RNA Gene V3 pyrosequencing analysis in fecal samples	[89]

PCR: Polymerase chain reaction; RNA: Ribonucleic acid; *H. Hepaticus*: *Helicobacter hepaticus*; *H. bilis*: *Helicobacter bilis*; *H. typhlonius*: *Helicobacter typhlonius*; *H. Rodentium*: *Helicobacter rodentium*; *B. vulgatus*: *Bacteroides vulgatus*; *C. freundii*: *Citrobacter freundii*.

undergoing colonoscopy (60 colorectal cancer, 119 normal), significantly greater levels of Bacteroides/Prevotella bacterial DNA were found in patients with colorectal cancer. Further, it was shown that a greater proportion of IL-17 immunomodulatory cells were isolated from patients with colorectal cancer.

In a study by Gao *et al*[88] in 2015 examining colon samples from 30 healthy and 31 cancer patients, distal and proximal colon microbiota from both healthy individuals and cancer patients were evaluated using the 16S RNA V3 sequence. No significant difference was observed between proximal and distal colon microbiota; however, in patients with colorectal cancer, Firmicutes and Fusobacteria were over-represented and Proteobacteria were under-represented. Further, Lactococcus and Fusobacterium were identified more often, and Pseudomonas and Escherichia–Shigella less often, in tissues from patients with colorectal cancer compared to those without cancer[88].

In a study by Zhu *et al*[90] using the 1,2-dimethylhydrazine cancer model, V3 sequences of 16S ribosomal RNA isolated from intestinal microbiota samples from rats with cancer and healthy rats were determined. While Firmucutesin was more frequently observed in rats with colorectal cancer, Bacteroidetes and Spirochetes were less commonly observed. There was no significant difference in the Proteobacteria types between the two groups; however, Prevotella, Lactobacillus, and Treponema were more frequently detected in healthy rats. Furthermore, while Fusobacterium was not observed in healthy rats, it could be identified specifically in cancer rats[90]. In a study of feces samples from healthy individuals and colorectal cancer patients, Akkermansia muciniphila was identified 4 times as often in colorectal cancer patients than healthy individuals[92].

As emphasized in many studies discussed above, intestinal microbiota have a substantial impact on intestinal health through controlling the immune and inflammatory response to individual species of intestinal microbiota, the activation or detoxification of carcinogens, the stimulation of DNA damage and chromosomal instability, dysregulation of the balance between proliferation and apoptosis, and prevention of invasion by pathogens.

CONCLUSION

Although colorectal cancer development is a complex process, recent studies have shown that the microbiota is actively involved.

Recently, we have developed a greater under-

standing of the effect of the microbiota on bowel health and diseases, including esophagitis/Barrett's esophagus, stomach cancer, IBD, and colorectal cancer. However, while a strong relationship between gastrointestinal diseases and the microbiota content is evident, many questions remain unanswered. One of the most clinically challenging issues is to understand how a change in intestinal microbiota will likely impact on the course of disease. Knowledge obtained from dysbiotic microbiota research in germ-free animals and clinical studies involving a variety of intestinal diseases will help provide answers to these important questions. Further, there is currently a lack of data regarding which microorganisms in the microbiota cause disease and are protective.

Continuous improvements in the development of increasingly cost-effective research methods, gene sequencing technology, and high productivity techniques are expected to provide substantial information regarding the healthy and dysbiotic microbiota composition. This information will facilitate functional experiments utilizing cause and effect animal models.

Understanding the relationship between pathology and the microbiota is important; however, the role of microbiota in pathogenesis has yet to be fully elucidated. Therapeutic microbial transplantation has been trialed in metabolic syndrome and also has utility in the treatment of colorectal cancer; however, this technique has many limitations including infection and the promotion of autoimmune disease. Despite this, there is hope that treatments targeting the human microbiota may provide therapies for the prevention and treatment of colorectal cancer in the future.

In summary, the microbiota plays an active role in intestinal homeostasis. Both the composition of microbiota and its metabolic activity have an impact on the host susceptibility to disease and can directly contribute to a number of varied pathologies, including colorectal cancer.

REFERENCES

1 **Bonnet M**, Buc E, Sauvanet P, Darcha C, Dubois D, Pereira B, Déchelotte P, Bonnet R, Pezet D, Darfeuille-Michaud A. Colonization of the human gut by E. coli and colorectal cancer risk. *Clin Cancer Res* 2014; **20**: 859-867 [PMID: 24334760 DOI: 10.1158/1078-0432.CCR-13-1343]

2 **O'Connell JB**, Maggard MA, Liu JH, Etzioni DA, Ko CY. Are survival rates different for young and older patients with rectal cancer? *Dis Colon Rectum* 2004; **47**: 2064-2069 [PMID: 15657655 DOI: 10.1007/s10350-004-0738-1]

3 **Gueimonde M**, Ouwehand A, Huhtinen H, Salminen E, Salminen S. Qualitative and quantitative analyses of the bifidobacterial microbiota in the colonic mucosa of patients with colorectal cancer, diverticulitis and inflammatory bowel disease. *World J Gastroenterol* 2007; **13**: 3985-3989 [PMID: 17663515 DOI: 10.3748/wjg.v13.i29.3985]

4 **Savage DC**. Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol* 1977; **31**: 107-133 [PMID: 334036 DOI: 10.1146/annurev.mi.31.100177.000543]

5 **Salminen S**, Isolauri E. Intestinal colonization, microbiota and probiotics. *J Pediatr* 2006; **149**: 115-120 [DOI: 10.1016/j.jpeds.2006.06.062]

6 **Watson AJ**, Collins PD. Colon cancer: a civilization disorder. *Dig Dis* 2011; **29**: 222-228 [PMID: 21734388 DOI: 10.1159/000323926]

7 **Tappenden KA**, Deutsch AS. The physiological relevance of the intestinal microbiota--contributions to human health. *J Am Coll Nutr* 2007; **26**: 679S-683S [PMID: 18187433 DOI: 10.1080/07315724.2007.10719647]

8 **Bäckhed F**, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. *Science* 2005; **307**: 1915-1920 [PMID: 15790844 DOI: 10.1126/science.1104816]

9 **Larsen N**, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sørensen SJ, Hansen LH, Jakobsen M. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010; **5**: e9085 [PMID: 20140211 DOI: 10.1371/journal.pone.0009085]

10 **Abdollahi-Roodsaz S**, Joosten LA, Koenders MI, Devesa I, Roelofs MF, Radstake TR, Heuvelmans-Jacobs M, Akira S, Nicklin MJ, Ribeiro-Dias F, van den Berg WB. Stimulation of TLR2 and TLR4 differentially skews the balance of T cells in a mouse model of arthritis. *J Clin Invest* 2008; **118**: 205-216 [PMID: 18060042 DOI: 10.1172/JCI32639]

11 **Ley RE**, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**: 1022-1023 [PMID: 17183309 DOI: 10.1038/4441022a]

12 **Sidhu H**, Allison MJ, Chow JM, Clark A, Peck AB. Rapid reversal of hyperoxaluria in a rat model after probiotic administration of Oxalobacter formigenes. *J Urol* 2001; **166**: 1487-1491 [PMID: 11547118 DOI: 10.1016/S0022-5347(05)65817-X]

13 **Tannock GW**. Molecular analysis of the intestinal microflora in IBD. *Mucosal Immunol* 2008; **1** Suppl 1: S15-S18 [PMID: 19079221 DOI: 10.1038/mi.2008.54]

14 **Turnbaugh PJ**, Bäckhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 2008; **3**: 213-223 [PMID: 18407065 DOI: 10.1016/j.chom.2008.02.015]

15 **Hoffmann C**, Hill DA, Minkah N, Kirn T, Troy A, Artis D, Bushman F. Community-wide response of the gut microbiota to enteropathogenic Citrobacter rodentium infection revealed by deep sequencing. *Infect Immun* 2009; **77**: 4668-4678 [PMID: 19635824 DOI: 10.1128/IAI.00493-09]

16 **Preidis GA**, Versalovic J. Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. *Gastroenterology* 2009; **136**: 2015-2031 [PMID: 19462507 DOI: 10.1053/j.gastro.2009.01.072]

17 **Wlodarska M**, Finlay BB. Host immune response to antibiotic perturbation of the microbiota. *Mucosal Immunol* 2010; **3**: 100-103 [PMID: 20016473 DOI: 10.1038/mi.2009.135]

18 **Lupp C**, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, Finlay BB. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. *Cell Host Microbe* 2007; **2**: 204 [PMID: 18030708 DOI: 10.1016/j.chom.2007.08.002]

19 **Zhang C**, Zhang M, Wang S, Han R, Cao Y, Hua W, Mao Y, Zhang X, Pang X, Wei C, Zhao G, Chen Y, Zhao L. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *ISME J* 2010; **4**: 232-241 [PMID: 19865183 DOI: 10.1038/ismej.2009.112]

20 **Wen L**, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, Hu C, Wong FS, Szot GL, Bluestone JA, Gordon JI, Chervonsky AV. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* 2008; **455**: 1109-1113 [PMID: 18806780 DOI: 10.1038/nature07336]

21 **Turnbaugh PJ**, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480-484 [PMID: 19043404 DOI: 10.1038/nature07540]

22 **Schulz MD**, Atay C, Heringer J, Romrig FK, Schwitalla S, Aydin B, Ziegler PK, Varga J, Reindl W, Pommerenke C, Salinas-Riester G, Böck A, Alpert C, Blaut M, Polson SC, Brandl L, Kirchner T, Greten FR, Polson SW, Arkan MC. High-fat-diet-mediated

dysbiosis promotes intestinal carcinogenesis independently of obesity. *Nature* 2014; **514**: 508-512 [PMID: 25174708 DOI: 10.1038/nature13398]
23 **Bingham S**, Riboli E. Diet and cancer--the European Prospective Investigation into Cancer and Nutrition. *Nat Rev Cancer* 2004; **4**: 206-215 [PMID: 14993902 DOI: 10.1038/nrc1298]
24 **Toprak NU**, Yagci A, Gulluoglu BM, Akin ML, Demirkalem P, Celenk T, Soyletir G. A possible role of Bacteroides fragilis enterotoxin in the aetiology of colorectal cancer. *Clin Microbiol Infect* 2006; **12**: 782-786 [PMID: 16842574 DOI: 10.1111/j.1469-0691.2006.01494.x]
25 **Wang X**, Allen TD, May RJ, Lightfoot S, Houchen CW, Huycke MM. Enterococcus faecalis induces aneuploidy and tetraploidy in colonic epithelial cells through a bystander effect. *Cancer Res* 2008; **68**: 9909-9917 [PMID: 19047172 DOI: 10.1158/0008-5472.CAN-08-1551]
26 **Wu S**, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, Huso DL, Brancati FL, Wick E, McAllister F, Housseau F, Pardoll DM, Sears CL. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat Med* 2009; **15**: 1016-1022 [PMID: 19701202 DOI: 10.1038/nm.2015]
27 **Cuevas-Ramos G**, Petit CR, Marcq I, Boury M, Oswald E, Nougayrède JP. Escherichia coli induces DNA damage in vivo and triggers genomic instability in mammalian cells. *Proc Natl Acad Sci USA* 2010; **107**: 11537-11542 [PMID: 20534522 DOI: 10.1073/pnas.1001261107]
28 **Ge Z**, Rogers AB, Feng Y, Lee A, Xu S, Taylor NS, Fox JG. Bacterial cytolethal distending toxin promotes the development of dysplasia in a model of microbially induced hepatocarcinogenesis. *Cell Microbiol* 2007; **9**: 2070-2080 [PMID: 17441986 DOI: 10.1111/j.1462-5822.2007.00939.x]
29 **Kim DH**, Jin YH. Intestinal bacterial beta-glucuronidase activity of patients with colon cancer. *Arch Pharm Res* 2001; **24**: 564-567 [PMID: 11794536 DOI: 10.1007/BF02975166]
30 **Jubelin G**, Chavez CV, Taieb F, Banfield MJ, Samba-Louaka A, Nobe R, Nougayrède JP, Zumbihl R, Givaudan A, Escoubas JM, Oswald E. Cycle inhibiting factors (CIFs) are a growing family of functional cyclomodulins present in invertebrate and mammal bacterial pathogens. *PLoS One* 2009; **4**: e4855 [PMID: 19308257 DOI: 10.1371/journal.pone.0004855]
31 **Bagnoli F**, Buti L, Tompkins L, Covacci A, Amieva MR. Helicobacter pylori CagA induces a transition from polarized to invasive phenotypes in MDCK cells. *Proc Natl Acad Sci USA* 2005; **102**: 16339-16344 [PMID: 16258069 DOI: 10.1073/pnas.0502598102]
32 **Knasmüller S**, Steinkellner H, Hirschl AM, Rabot S, Nobis EC, Kassie F. Impact of bacteria in dairy products and of the intestinal microflora on the genotoxic and carcinogenic effects of heterocyclic aromatic amines. *Mutat Res* 2001; **480-481**: 129-138 [PMID: 11506806 DOI: 10.1016/S0027-5107(01)00176-2]
33 **Femia AP**, Dolara P, Giannini A, Salvadori M, Biggeri A, Caderni G. Frequent mutation of Apc gene in rat colon tumors and mucin-depleted foci, preneoplastic lesions in experimental colon carcinogenesis. *Cancer Res* 2007; **67**: 445-449 [PMID: 17234750 DOI: 10.1158/0008-5472.CAN-06-3861]
34 **Fiala ES**. Investigations into the metabolism and mode of action of the colon carcinogens 1,2-dimethylhydrazine and azoxymethane. *Cancer* 1977; **40**: 2436-2445 [PMID: 200341 DOI: 10.1002/1097-0142(197711)40]
35 **Rowland IR**, Rumney CJ, Coutts JT, Lievense LC. Effect of Bifidobacterium longum and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. *Carcinogenesis* 1998; **19**: 281-285 [PMID: 9498277 DOI: 10.1093/carcin/19.2.281]
36 **Laqueur GL**, McDaniel EG, Matsumoto H. Tumor induction in germfree rats with methylazoxymethanol (MAM) and synthetic MAM acetate. *J Natl Cancer Inst* 1967; **39**: 355-371 [PMID: 18623950]
37 **Rowland IR**. The role of the gastrointestinal microbiota in colorectal cancer. *Curr Pharm Des* 2009; **15**: 1524-1527 [PMID: 19442169 DOI: 10.2174/138161209788168191]
38 **Neufert C**, Becker C, Neurath MF. An inducible mouse model of colon carcinogenesis for the analysis of sporadic and inflammation-driven tumor progression. *Nat Protoc* 2007; **2**: 1998-2004 [PMID: 17703211 DOI: 10.1038/nprot.2007.279]
39 **Takada H**, Hirooka T, Hiramatsu Y, Yamamoto M. Effect of beta-glucuronidase inhibitor on azoxymethane-induced colonic carcinogenesis in rats. *Cancer Res* 1982; **42**: 331-334 [PMID: 7053860]
40 **Geier MS**, Butler RN, Howarth GS. Probiotics, prebiotics and synbiotics: a role in chemoprevention for colorectal cancer? *Cancer Biol Ther* 2006; **5**: 1265-1269 [PMID: 16969130 DOI: 10.4161/cbt.5.10.3296]
41 **Goldin BR**, Gorbach SL. Alterations of the intestinal microflora by diet, oral antibiotics, and Lactobacillus: decreased production of free amines from aromatic nitro compounds, azo dyes, and glucuronides. *J Natl Cancer Inst* 1984; **73**: 689-695 [PMID: 6433097]
42 **Goldin BR**, Swenson L, Dwyer J, Sexton M, Gorbach SL. Effect of diet and Lactobacillus acidophilus supplements on human fecal bacterial enzymes. *J Natl Cancer Inst* 1980; **64**: 255-261 [PMID: 6766508]
43 **Boleij A**, Hechenbleikner EM, Goodwin AC, Badani R, Stein EM, Lazarev MG, Ellis B, Carroll KC, Albesiano E, Wick EC, Platz EA, Pardoll DM, Sears CL. The Bacteroides fragilis toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clin Infect Dis* 2015; **60**: 208-215 [PMID: 25305284 DOI: 10.1093/cid/ciu787]
44 **Rubin CE**, Haggitt RC, Burmer GC, Brentnall TA, Stevens AC, Levine DS, Dean PJ, Kimmey M, Perera DR, Rabinovitch PS. DNA aneuploidy in colonic biopsies predicts future development of dysplasia in ulcerative colitis. *Gastroenterology* 1992; **103**: 1611-1620 [PMID: 1426881]
45 **Porschen R**, Robin U, Schumacher A, Schauseil S, Borchard F, Hengels KJ, Strohmeyer G. DNA aneuploidy in Crohn's disease and ulcerative colitis: results of a comparative flow cytometric study. *Gut* 1992; **33**: 663-667 [PMID: 1612484 DOI: 10.1136/gut.33.5.663]
46 **Sjöqvist U**, Befrits R, Söderlund S, Ost A, Karlén P, Tribukait B, Rubio C, Rutgeerts P, Geboes K, Löfberg R. Colorectal cancer in colonic Crohn's disease--high frequency of DNA-aneuploidy. *Anticancer Res* 2005; **25**: 4393-4397 [PMID: 16334114]
47 **Balish E**, Warner T. Enterococcus faecalis induces inflammatory bowel disease in interleukin-10 knockout mice. *Am J Pathol* 2002; **160**: 2253-2257 [PMID: 12057927 DOI: 10.1016/S0002-9440(10)61172-8]
48 **Kim SC**, Tonkonogy SL, Albright CA, Tsang J, Balish EJ, Braun J, Huycke MM, Sartor RB. Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. *Gastroenterology* 2005; **128**: 891-906 [PMID: 15825073 DOI: 10.1053/j.gastro.2005.02.009]
49 **Wang X**, Huycke MM. Extracellular superoxide production by Enterococcus faecalis promotes chromosomal instability in mammalian cells. *Gastroenterology* 2007; **132**: 551-561 [PMID: 17258726 DOI: 10.1053/j.gastro.2006.11.040]
50 **Segain JP**, Raingeard de la Blétière D, Bourreille A, Leray V, Gervois N, Rosales C, Ferrier L, Bonnet C, Blottière HM, Galmiche JP. Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. *Gut* 2000; **47**: 397-403 [PMID: 10940278 DOI: 10.1136/gut.47.3.397]
51 **Maslowski KM**, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM, Mackay CR. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009; **461**: 1282-1286 [PMID: 19865172 DOI: 10.1038/nature08530]
52 **Scheppach W**. Effects of short chain fatty acids on gut morphology and function. *Gut* 1994; **35**: S35-S38 [PMID: 8125387 DOI: 10.1136/gut.35.1_Suppl.S35]
53 **Bolden JE**, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov* 2006; **5**: 769-784 [PMID: 16955068 DOI: 10.1038/nrd2133]
54 **Heerdt BG**, Houston MA, Augenlicht LH. Short-chain fatty acid-initiated cell cycle arrest and apoptosis of colonic epithelial cells is linked to mitochondrial function. *Cell Growth Differ* 1997; **8**:

523-532 [PMID: 9149903]
55 **Bonnotte B**, Favre N, Reveneau S, Micheau O, Droin N, Garrido C, Fontana A, Chauffert B, Solary E, Martin F. Cancer cell sensitization to fas-mediated apoptosis by sodium butyrate. *Cell Death Differ* 1998; **5**: 480-487 [PMID: 10200499 DOI: 10.1038/sj.cdd.4400371]
56 **Hague A**, Elder DJ, Hicks DJ, Paraskeva C. Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. *Int J Cancer* 1995; **60**: 400-406 [PMID: 7829251 DOI: 10.1002/ijc.2910600322]
57 **Ruemmele FM**, Schwartz S, Seidman EG, Dionne S, Levy E, Lentze MJ. Butyrate induced Caco-2 cell apoptosis is mediated via the mitochondrial pathway. *Gut* 2003; **52**: 94-100 [PMID: 12477768 DOI: 10.1136/gut.52.1.94]
58 **Thangaraju M**, Cresci GA, Liu K, Ananth S, Gnanaprakasam JP, Browning DD, Mellinger JD, Smith SB, Digby GJ, Lambert NA, Prasad PD, Ganapathy V. GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon. *Cancer Res* 2009; **69**: 2826-2832 [PMID: 19276343 DOI: 10.1158/0008-5472.CAN-08-4466]
59 **Park JY**, Helm JF, Zheng W, Ly QP, Hodul PJ, Centeno BA, Malafa MP. Silencing of the candidate tumor suppressor gene solute carrier family 5 member 8 (SLC5A8) in human pancreatic cancer. *Pancreas* 2008; **36**: e32-e39 [PMID: 18437076 DOI: 10.1097/MPA.0b013e3181630ffe]
60 **Whitman SP**, Hackanson B, Liyanarachchi S, Liu S, Rush LJ, Maharry K, Margeson D, Davuluri R, Wen J, Witte T, Yu L, Liu C, Bloomfield CD, Marcucci G, Plass C, Caligiuri MA. DNA hypermethylation and epigenetic silencing of the tumor suppressor gene, SLC5A8, in acute myeloid leukemia with the MLL partial tandem duplication. *Blood* 2008; **112**: 2013-2016 [PMID: 18566324 DOI: 10.1182/blood-2008-01-128595]
61 **Donohoe DR**, Collins LB, Wali A, Bigler R, Sun W, Bultman SJ. The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. *Mol Cell* 2012; **48**: 612-626 [PMID: 23063526 DOI: 10.1016/j.molcel.2012.08.033]
62 **Belcheva A**, Irrazabal T, Martin A. Gut microbial metabolism and colon cancer: can manipulations of the microbiota be useful in the management of gastrointestinal health? *Bioessays* 2015; **37**: 403-412 [PMID: 25601287 DOI: 10.1002/bies.201400204]
63 **Yang T**, Owen JL, Lightfoot YL, Kladde MP, Mohamadzadeh M. Microbiota impact on the epigenetic regulation of colorectal cancer. *Trends Mol Med* 2013; **19**: 714-725 [PMID: 24051204 DOI: 10.1016/j.molmed.2013.08.005]
64 **Erdman SE**, Poutahidis T, Tomczak M, Rogers AB, Cormier K, Plank B, Horwitz BH, Fox JG. CD4+ CD25+ regulatory T lymphocytes inhibit microbially induced colon cancer in Rag2-deficient mice. *Am J Pathol* 2003; **162**: 691-702 [PMID: 12547727 DOI: 10.1016/S0002-9440(10)63863-1]
65 **Nagamine CM**, Rogers AB, Fox JG, Schauer DB. Helicobacter hepaticus promotes azoxymethane-initiated colon tumorigenesis in BALB/c-IL10-deficient mice. *Int J Cancer* 2008; **122**: 832-838 [PMID: 17957786 DOI: 10.1002/ijc.23175]
66 **Maggio-Price L**, Treuting P, Zeng W, Tsang M, Bielefeldt-Ohmann H, Iritani BM. Helicobacter infection is required for inflammation and colon cancer in SMAD3-deficient mice. *Cancer Res* 2006; **66**: 828-838 [PMID: 16424015 DOI: 10.1158/0008-5472.CAN-05-2448]
67 **Nagamine CM**, Sohn JJ, Rickman BH, Rogers AB, Fox JG, Schauer DB. Helicobacter hepaticus infection promotes colon tumorigenesis in the BALB/c-Rag2(-/-) Apc(Min/+) mouse. *Infect Immun* 2008; **76**: 2758-2766 [PMID: 18411292 DOI: 10.1128/IAI.01604-07]
68 **Rao VP**, Poutahidis T, Ge Z, Nambiar PR, Boussahmain C, Wang YY, Horwitz BH, Fox JG, Erdman SE. Innate immune inflammatory response against enteric bacteria Helicobacter hepaticus induces mammary adenocarcinoma in mice. *Cancer Res* 2006; **66**: 7395-7400 [PMID: 16885333 DOI: 10.1158/0008-5472.CAN-06-0558]
69 **Erdman SE**, Rao VP, Poutahidis T, Ihrig MM, Ge Z, Feng Y, Tomczak M, Rogers AB, Horwitz BH, Fox JG. CD4(+)CD25(+) regulatory lymphocytes require interleukin 10 to interrupt colon carcinogenesis in mice. *Cancer Res* 2003; **63**: 6042-6050 [PMID: 14522933]
70 **Maggio-Price L**, Bielefeldt-Ohmann H, Treuting P, Iritani BM, Zeng W, Nicks A, Tsang M, Shows D, Morrissey P, Viney JL. Dual infection with Helicobacter bilis and Helicobacter hepaticus in p-glycoprotein-deficient mdr1a-/- mice results in colitis that progresses to dysplasia. *Am J Pathol* 2005; **166**: 1793-1806 [PMID: 15920164 DOI: 10.1016/S0002-9440(10)62489-3]
71 **Maggio-Price L**, Shows D, Waggie K, Burich A, Zeng W, Escobar S, Morrissey P, Viney JL. Helicobacter bilis infection accelerates and H. hepaticus infection delays the development of colitis in multiple drug resistance-deficient (mdr1a-/-) mice. *Am J Pathol* 2002; **160**: 739-751 [PMID: 11839595 DOI: 10.1016/S0002-9440(10)64894-8]
72 **Chichlowski M**, Sharp JM, Vanderford DA, Myles MH, Hale LP. Helicobacter typhlonius and Helicobacter rodentium differentially affect the severity of colon inflammation and inflammation-associated neoplasia in IL10-deficient mice. *Comp Med* 2008; **58**: 534-541 [PMID: 19149410]
73 **Hale LP**, Perera D, Gottfried MR, Maggio-Price L, Srinivasan S, Marchuk D. Neonatal co-infection with helicobacter species markedly accelerates the development of inflammation-associated colonic neoplasia in IL-10(-/-) mice. *Helicobacter* 2007; **12**: 598-604 [PMID: 18001399 DOI: 10.1111/j.1523-5378.2007.00552.x]
74 **Zarkin BA**, Lillemoe KD, Cameron JL, Effron PN, Magnuson TH, Pitt HA. The triad of Streptococcus bovis bacteremia, colonic pathology, and liver disease. *Ann Surg* 1990; **211**: 786-791; discussion 791-792 [PMID: 2357141]
75 **Gold JS**, Bayar S, Salem RR. Association of Streptococcus bovis bacteremia with colonic neoplasia and extracolonic malignancy. *Arch Surg* 2004; **139**: 760-765 [PMID: 15249410 DOI: 10.1001/archsurg.139.7.760]
76 **Klein RS**, Recco RA, Catalano MT, Edberg SC, Casey JI, Steigbigel NH. Association of Streptococcus bovis with carcinoma of the colon. *N Engl J Med* 1977; **297**: 800-802 [PMID: 408687 DOI: 10.1056/NEJM197710132971503]
77 **Ruoff KL**, Miller SI, Garner CV, Ferraro MJ, Calderwood SB. Bacteremia with Streptococcus bovis and Streptococcus salivarius: clinical correlates of more accurate identification of isolates. *J Clin Microbiol* 1989; **27**: 305-308 [PMID: 2915024]
78 **Ellmerich S**, Schöller M, Duranton B, Gossé F, Galluser M, Klein JP, Raul F. Promotion of intestinal carcinogenesis by Streptococcus bovis. *Carcinogenesis* 2000; **21**: 753-756 [PMID: 10753212 DOI: 10.1093/carcin/21.4.753]
79 **Boleij A**, Roelofs R, Schaeps RM, Schülin T, Glaser P, Swinkels DW, Kato I, Tjalsma H. Increased exposure to bacterial antigen RpL7/L12 in early stage colorectal cancer patients. *Cancer* 2010; **116**: 4014-4022 [PMID: 20564125 DOI: 10.1002/cncr.25212]
80 **Huang JQ**, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology* 2003; **125**: 1636-1644 [PMID: 14724815 DOI: 10.1053/j.gastro.2003.08.033]
81 **Sobhani I**, Tap J, Roudot-Thoraval F, Roperch JP, Letulle S, Langella P, Corthier G, Tran Van Nhieu J, Furet JP. Microbial dysbiosis in colorectal cancer (CRC) patients. *PLoS One* 2011; **6**: e16393 [PMID: 21297998 DOI: 10.1371/journal.pone.0016393]
82 **Uronis JM**, Mühlbauer M, Herfarth HH, Rubinas TC, Jones GS, Jobin C. Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. *PLoS One* 2009; **4**: e6026 [PMID: 19551144 DOI: 10.1371/journal.pone.0006026]
83 **Martin HM**, Campbell BJ, Hart CA, Mpofu C, Nayar M, Singh R, Englyst H, Williams HF, Rhodes JM. Enhanced Escherichia coli adherence and invasion in Crohn's disease and colon cancer. *Gastroenterology* 2004; **127**: 80-93 [PMID: 15236175 DOI: 10.1053/j.gastro.2004.03.054]
84 **Luperchio SA**, Schauer DB. Molecular pathogenesis of Citrobacter rodentium and transmissible murine colonic hyperplasia.

Microbes Infect 2001; **3**: 333-340 [PMID: 11334751 DOI: 10.1016/S1286-4579(01)01387-9]

85 **Barthold SW**, Jonas AM. Morphogenesis of early 1, 2-dimethylhydrazine-induced lesions and latent period reduction of colon carcinogenesis in mice by a variant of Citrobacter freundii. *Cancer Res* 1977; **37**: 4352-4360 [PMID: 922726]

86 **Newman JV**, Kosaka T, Sheppard BJ, Fox JG, Schauer DB. Bacterial infection promotes colon tumorigenesis in Apc(Min/+) mice. *J Infect Dis* 2001; **184**: 227-230 [PMID: 11424022 DOI: 10.1086/321998]

87 **Castellarin M**, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, Barnes R, Watson P, Allen-Vercoe E, Moore RA, Holt RA. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. *Genome Res* 2012; **22**: 299-306 [PMID: 22009989 DOI: 10.1101/gr.126516.111]

88 **Gao Z**, Guo B, Gao R, Zhu Q, Qin H. Microbiota disbiosis is associated with colorectal cancer. *Front Microbiol* 2015; **6**: 20 [PMID: 25699023 DOI: 10.3389/fmicb.2015.00020]

89 **Mira-Pascual L**, Cabrera-Rubio R, Ocon S, Costales P, Parra A, Suarez A, Moris F, Rodrigo L, Mira A, Collado MC. Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaeal biomarkers. *J Gastroenterol* 2015; **50**: 167-179 [PMID: 24811328 DOI: 10.1007/s00535-014-0963-x]

90 **Zhu Q**, Jin Z, Wu W, Gao R, Guo B, Gao Z, Yang Y, Qin H. Analysis of the intestinal lumen microbiota in an animal model of colorectal cancer. *PLoS One* 2014; **9**: e90849 [PMID: 24603888 DOI: 10.1371/journal.pone.0090849]

91 **Balamurugan R**, Rajendiran E, George S, Samuel GV, Ramakrishna BS. Real-time polymerase chain reaction quantification of specific butyrate-producing bacteria, Desulfovibrio and Enterococcus faecalis in the feces of patients with colorectal cancer. *J Gastroenterol Hepatol* 2008; **23**: 1298-1303 [PMID: 18624900 DOI: 10.1111/j.1440-1746.2008.05490.x]

92 **Weir TL**, Manter DK, Sheflin AM, Barnett BA, Heuberger AL, Ryan EP. Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults. *PLoS One* 2013; **8**: e70803 [PMID: 23940645 DOI: 10.1371/journal.pone.0070803]

93 **Lightfoot YL**, Yang T, Sahay B, Mohamadzadeh M. Targeting aberrant colon cancer-specific DNA methylation with lipoteichoic acid-deficient Lactobacillus acidophilus. *Gut Microbes* 2013; **4**: 84-88 [PMID: 23137966 DOI: 10.4161/gmic.22822]

94 **Mohamadzadeh M**, Pfeiler EA, Brown JB, Zadeh M, Gramarossa M, Managlia E, Bere P, Sarraj B, Khan MW, Pakanati KC, Ansari MJ, O'Flaherty S, Barrett T, Klaenhammer TR. Regulation of induced colonic inflammation by Lactobacillus acidophilus deficient in lipoteichoic acid. *Proc Natl Acad Sci USA* 2011; **108** Suppl 1: 4623-4630 [PMID: 21282652 DOI: 10.1073/pnas.1005066107]

95 **Licciardi PV**, Wong SS, Tang ML, Karagiannis TC. Epigenome targeting by probiotic metabolites. *Gut Pathog* 2010; **2**: 24 [PMID: 21172038 DOI: 10.1186/1757-4749-2-24]

96 **Jones SE**, Versalovic J. Probiotic Lactobacillus reuteri biofilms produce antimicrobial and anti-inflammatory factors. *BMC Microbiol* 2009; **9**: 35 [PMID: 19210794 DOI: 10.1186/1471-2180-9-35]

97 **Resta-Lenert S**, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). *Gut* 2003; **52**: 988-997 [PMID: 12801956 DOI: 10.1136/gut.52.7.988]

98 **Qin H**, Zhang Z, Hang X, Jiang Y. L. plantarum prevents enteroinvasive Escherichia coli-induced tight junction proteins changes in intestinal epithelial cells. *BMC Microbiol* 2009; **9**: 63 [PMID: 19331693 DOI: 10.1186/1471-2180-9-63]

99 **Pagnini C**, Saeed R, Bamias G, Arseneau KO, Pizarro TT, Cominelli F. Probiotics promote gut health through stimulation of epithelial innate immunity. *Proc Natl Acad Sci USA* 2010; **107**: 454-459 [PMID: 20018654 DOI: 10.1073/pnas.0910307107]

100 **Liévin-Le Moal V**, Amsellem R, Servin AL, Coconnier MH. Lactobacillus acidophilus (strain LB) from the resident adult human gastrointestinal microflora exerts activity against brush border damage promoted by a diarrhoeagenic Escherichia coli in human enterocyte-like cells. *Gut* 2002; **50**: 803-811 [PMID: 12010882 DOI: 10.1136/gut.50.6.803]

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TOPIC HIGHLIGHT

2015 Advances in Pancreatic Cancer

Management of borderline resectable pancreatic cancer

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Abstract

Pancreatic cancer is the fourth most common cause of cancer death in the United States. Surgery remains the only curative option; however only 20% of the patients have resectable disease at the time of initial presentation. The definition of borderline resectable pancreatic cancer is not uniform but generally denotes to regional vessel involvement that makes it unlikely to have negative surgical margins. The accurate staging of pancreatic cancer requires triple phase computed tomography or magnetic resonance imaging of the pancreas. Management of patients with borderline resectable pancreatic cancer remains unclear. The data for treatment of these patients is primarily derived from retrospective single institution experience. The prospective trials have been plagued by small numbers and poor accrual. Neoadjuvant therapy is recommended and typically consists of chemotherapy and radiation therapy. The chemotherapeutic regimens continue to evolve along with type and dose of radiation therapy. Gemcitabine or 5-fluorouracil based chemotherapeutic combinations are administered. The type and dose of radiation vary among different institutions. With neoadjuvant treatment, approximately 50% of the patients are able to undergo surgical resections with negative margins obtained in greater than 80% of the patients. Newer trials are attempting to standardize the definition of borderline resectable pancreatic cancer and treatment regimens. In this review, we outline the definition, imaging requirements and management of patients with borderline resectable pancreatic cancer.

Key words: Pancreatic cancer; Surgery; Chemotherapy; Radiation; Borderline

Core tip: The diagnosis and treatment of borderline resectable pancreatic cancer (BRPC) remains unclear. The definition of BRPC is not uniform and generally refers to regional blood vessel involvement by the tumor. Recent attempts have been made to standardize the definition of BRPC. Neoadjuvant therapy is recommended in the hopes of obtaining negative surgical margins and consists of chemotherapy and radiation therapy. Data for therapeutic approaches is primarily

derived from single institution retrospective series. In this article, we review the definition, imaging modalities for diagnosis and treatment of patients with BRPC.

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INTRODUCTION

Pancreatic cancer is the fourth most common cause of cancer death in the United States with 48960 incident cases and 40560 deaths estimated in 2015[1]. Despite the recent advances in therapeutic interventions, the 5-year relative survival rate remains approximately 6%. At initial presentation, approximately 50%-55% of the patients are found to have metastatic disease, 20%-25% have locally advanced disease and only 20% have resectable disease[2]. Surgery provides the only curative option with long term survivors. Modern advances in surgical techniques have substantially decreased post-operative mortality and morbidity, especially in high volume centers[3]. Improvement in imaging modalities has led to better delineation of resectable disease and spares patients from unnecessary surgery[4]. Yet, of those patients who undergo potentially curative resections, the 5-year survival remains abysmal at 20%[1].

Despite the fact that the progress has been slow, there has been improvement in systemic therapies for the treatment of pancreatic cancer. Gemcitabine remained the standard of care option for unresectable pancreatic cancer for a long time. Recently, two randomized clinical trials have demonstrated superior efficacy over single agent gemcitabine in the setting of metastatic and locally advanced disease. Conroy *et al*[5] reported a phase Ⅲ trial comparing the combination of 5-fluorouracil, folinic acid, oxaliplatin and irinotecan (FOLFIRINOX) to gemcitabine. The median survival was significantly better with FOLFIRINOX at 11.1 mo compared to 6.8 mo with single agent gemcitabine. The response rates were higher in the combination group as well (31.6% *vs* 9.4%). However, increased grade 3 or 4 toxicities with FOLFIRINOX limits this therapy to highly selected patients. The addition of nab-paclitaxel to gemcitabine has demonstrated improvement in median survival (8.5 mo *vs* 6.7 mo), progression free-survival (5.5 mo *vs* 3.7 mo) and response rates (23% *vs* 7%)[6]. The higher response rates observed with this regimen makes them very appealing for downstaging tumors. Further, since the objective of systemic treatment for borderline resectable pancreatic cancer is the possibility of margin negative surgery and potentially cure, higher toxicities may be acceptable in this group of patients. This is in contrast to patients with metastatic disease where the primary aim is to improve survival by a few months while maintaining a good quality of life.

Involvement of blood vessels by tumor frequently renders the possibility of resection with negative margins problematic in patients with non-metastatic pancreatic cancer. Patients with negative margins have significantly improved survival compared to patients who have gross disease at the resection margin[7]. The term "borderline resectable pancreatic cancer" has no universal definition but, in general, denotes patients with pancreatic cancer that abuts regional blood vessels such that there is a high risk for margin-positive resection[8]. Tumor abutment refers to solid tumor contact of ⩽ 180 degrees of circumference of blood vessel and encasement refers to greater than 180 degree of contact. Unfortunately, the current pancreatic staging system by the American Joint Committee on Cancer (AJCC) does not differentiate this subgroup of patients with those tumors encasing blood vessels termed locally advanced disease. In this staging system, patients with portal vein, superior mesenteric vein or superior mesenteric artery involvement are considered unresectable. All patients with vascular involvement and no metastatic disease are grouped under stage Ⅲ disease.

Staging work up

Pre-operatively, diagnostic imaging is utilized for differentiating pancreatic cancer into resectable, borderline resectable or unresectable disease. The National Comprehensive Cancer Network (NCCN) recommends multidetector computerized tomography (CT) angiography, acquiring thin, preferably sub-millimeter sections using a pancreatic protocol. The images are to be obtained in the non-contrast, arterial, pancreatic parenchymal and portal venous phase contrast enhancement. The multiphasic protocol helps in assessment of vascular invasion of tumors by selective visualization of arterial (superior mesenteric artery, celiac axis, gastroduodenal artery) and venous (superior mesenteric vein, portal vein, splenic vein) structures. Pancreatic protocol CT has an excellent sensitivity (89%-97%) and negative predictive value[9]. However, CT is not very accurate for predicting resectability (45%-79%) as it is not very sensitive to detect small hepatic and peritoneal metastases[9]. Pancreatic magnetic resonance imaging (MRI) can also be used as an adjunct for staging, especially for patients with a contrast allergy. MRI is similar to CT in respect to providing details of tumor anatomy for resectability status but is less widely utilized. The role of positron emission tomography (PET) scan for patients with borderline resectable disease remains unclear. PET scans may help, however, in detecting metastatic disease in addition to CT scans and spare patients from unnecessary surgery[10,11]. Thus, PET scans may be used as adjuncts to CT scans especially in patients with a high risk of advanced disease.

Endoscopic ultrasound (EUS) is a complementary modality to CT scan and is utilized in many centers.

Table 1 Criteria for resectability

	NCCN	AHPBA/ SSAT/SSO	MD Anderson	Intergroup (Alliance)
Celiac artery	No abutment for pancreatic head cancer. For body/tail, ≤ 180° contact	No abutment or encasement	Abutment	Tumor-vessel interface < 180° of vessel wall circumference
CHA	Solid tumor contact ≤ 180° allowing for reconstruction	Abutment or short segment encasement	Abutment or short-segment encasement	Reconstructable short-segment interface of any degree
SMA	Solid tumor contact ≤ 180°	Abutment	Abutment	Tumor-vessel wall interface < 180° of vessel wall circumference
SMV/PV	Solid tumor contact > 180° or contact of ≤ 180° with contour irregularity or thrombosis allowing for safe reconstruction	Occlusion	Occlusion	Tumor-vessel interface ≥ 180° of vessel wall circumference and/or reconstructible occlusion

CHA: Common hepatic artery; SMA: Superior mesenteric artery; SMV: Superior mesenteric vein; PV: Portal vein; NCCN: National Comprehensive Cancer Network; AHPBA/SSAT/SSO: Americas Hepato-Pancreato-Biliary Association/Society for Surgery of the Alimentary Tract/Society of Surgical Oncology.

It is particularly useful for assessment of vascular invasion, especially of the portal vein. EUS is not a good modality for involvement of the superior mesenteric artery. EUS is routinely performed for patients with borderline pancreaticcancer for pathologic diagnosis. Tissue confirmation is not necessary for patients undergoing upfront surgery but should be obtained prior to initiation of neoadjuvant therapy. EUS-guided fine needle aspiration or biopsy is safe and is associated with a low complication rate[12-14]. Further, there is decreased potential for peritoneal seeding compared to percutaneous biopsy.

Staging laparoscopy is performed routinely at selected centers to detect occult metastatic disease, especially peritoneal involvement. It can thus be performed prior to surgery or prior to initiation of neoadjuvant therapy to avoid non-curative surgery and potentially prevent unnecessary complications associated with laparotomy[15]. At some institutions laparoscopy is reserved for patients with a higher chance of metastatic disease, including markedly elevated tumor markers or symptomatic patients. Despite the fact that staging laparoscopy can detect occult disease even in patients who had undergone good quality imaging studies, this procedure is not routinely utilized.

Classification

The definition of borderline resectable pancreatic cancer (BRPC) is not uniform. Some series have included patients based on anatomic imaging criteria for BRPC alone while others include patients with clinical factors. Recently, attempts have been made to clearly define borderline resectable disease and differentiate it from clearly resectable or unresectable disease. Table 1 lists the different classification systems utilized for defining borderline resectable pancreatic cancer including those proposed by the National Comprehensive Cancer Network (NCCN), MD Anderson, Americas Hepato-Pancreato-Biliary Association/Society of Surgical Oncology/Society for Surgery of the Alimentary Tract (AHPBA/SSO/SSAT) and the Intergroup[16-18]. Due to complexities involved in making these distinctions, it is very important that all cases of non-metastatic pancreatic cancer are discussed by a multidisciplinary team in high volume centers.

The NCCN panel has recently updated the guidelines and the definition of borderline resectable pancreatic cancer is included in the Table 1.

Vascular involvement

One of the key concepts for defining borderline resectable pancreatic cancer is the possibility of benefit of surgery in patients with vessel involvement. Vascular reconstruction is frequently the limiting factor during pancreatectomy in these patients. Siriwardana *et al*[19] in 2006 reported outcomes on 1646 patients from 52 studies with portal vein or superior mesenteric vein resections. Median postoperative morbidity was 42% with mortality of 5.9%. Median survival was only 13 mo with 5-year survival of only 7%. This study concluded that pancreatic surgery requiring resection of the portal vein did not improve outcomes. However, this study was limited by relatively older studies from 1996-2005 and heterogeneity of the studies included in the review. Since then, multiple single institution studies from high volume centers have demonstrated similar morbidity, mortality and survival for patients who underwent pancreatic surgery with or without venous involvement[20-24]. Zhou *et al*[25] in 2012 published a meta-analysis of 19 nonrandomized studies comprising 2247 patients. There was no difference in perioperative morbidity, mortality or 5-year survival among patients who underwent pancreatic surgery with or without venous resection. These studies suggest that venous resection with pancreatectomy is safe and feasible and can lead to improvement in long term outcomes. However, the results should be interpreted with caution as there may be publication bias as well as underreporting of morbidity data. Further, studies using National Surgery Quality Improvement Program database and National Inpatient Sample database demonstrated increases in morbidity and mortality with the addition of venous resection to pancreatic resection[26,27]. However, the limitations of these studies include the use of an administrative database, no distinction between venous or arterial resection and the

inability to differentiate between planned and unplanned vascular resections.

There is even limited data for arterial resection during pancreatectomy for pancreatic cancer. Some studies have demonstrated similar morbidity and mortality with the addition of arterial resection to pancreatic surgery[28,29]. However, a meta-analysis including 366 patients from 26 studies demonstrated significantly greater peri-operative morbidity and mortality with arterial resection[30]. This study also found that despite increased complications, patients undergoing pancreatic and arterial resection had improved survival compared to those patients who did not undergo resection. Similar results have been reported in other studies from high volume centers[31,32]. Thus, arterial resection should be limited to highly selected patients.

Treatment

Patients with borderline resectable pancreatic cancer are preferentially treated with neoadjuvant therapy to enhance the potential to facilitate margin negative, or R0, resection. Some patients with micrometastatic disease initially may have progressive disease on subsequent restaging scans after neoadjuvant therapy and thus are spared from unnecessary surgery. These patients would have been unlikely to benefit from pancreatic resection. It is generally acceptable that multimodality treatment is required for this patient population, although some centers have pursued a strategy of neoadjuvant chemotherapy alone[33]. In the adjuvant setting, up to 25% of patients are unable to receive treatment secondary to post-operative complications[34,35]. For these reasons, at some centers, neoadjuvant therapy is recommended even for resectable pancreatic cancer but is not the standard of care at this time[36].

There is no standard of care for the type of neoadjuvant therapy in this patient population. Treatment typically consists of a combination of radiation therapy and chemotherapy. The treatment regimens are usually reported from a single institution experience and are largely retrospective in nature. The chemotherapy regimen, dose and duration of radiation and type of radiation are different in these reports making cross-comparison very difficult. Moreover, the definitions of resectability have not been uniform in these studies. The most commonly cited resectability criteria are similar to the NCCN and MD Anderson anatomic imaging criteria while some studies have classified patients as borderline if they have a marginal performance status for surgery or have findings on imaging indeterminate for metastases.

After neoadjuvant therapy, depending on the case series, approximately 50% of the patients are able to undergo resection. After treatment, the change in tumor size by the Response Evaluation Criteria In Solid Tumors (RECIST) is low, around 10%-20%. RECIST response did not correlate with survival among patients who underwent pancreatic resection after neoadjuvant therapy, suggesting that RECIST criteria is a poor determinant of benefit in these patients[37]. There is the possibility that the tumor near the vessel can be replaced by fibrous tissue which may not be easily discernible on CT scan[38].

There have been four small prospective trials reported in the literature that have evaluated neoadjuvant therapy for patients with borderline resectable cancer (Table 2). Landry *et al*[39] reported the multi-institutional randomized phase II trial comparing two neoadjuvant regimens. Patients in arm A (n = 10), received concurrent gemcitabine and radiation while patients in arm B (n = 11) received induction chemotherapy with gemcitabine, cisplatin and 5-fluorouracil followed by 5-flourouracil based radiation. Three patients in arm A and two patients in arm B underwent resection. The median survival of resected patients was 26.3 mo. These outcomes were consistent with previous retrospective studies[40,41]. The trial was terminated early due to poor accrual. Another phase II trial evaluated the role of neoadjuvant therapy in patients with resectable or borderline resectable pancreatic cancer[42]. Thirty nine patients with borderline resectable disease were identified using NCCN criteria and were treated with gemcitabine and oxaliplatin for two cycles. Radiation was administered with the first cycle of chemotherapy to a total dose of 30 Gy in 15 fractions. Pancreatic resection was performed in 63% of patients and 84% of those patients had R0 resection. The median survival of resected patients was 25.4 mo. Similar results were observed with other small clinical trials[43,44].

The data on clinical outcomes after neoadjuvant therapy for borderline pancreatic cancer is primarily derived from retrospective single institution experience. One of the first restrospective studies from MD Anderson included 160 patients with pancreatic cancer who received pre-operative therapy, including 84 patients who met radiologic criteria for borderline resectable disease[40]. Patients were treated with a variety of neoadjuvant regimens including chemotherapy or chemoradiotherapy with a gemcitabine based regimen being most common. Resection was performed in 38% of the patients with negative margins in 97% of the subjects. The median survival for resected patients was 40 mo and for all patients was 21 mo. In the follow up report, 115 patients who met AHPBA/SSO/SSAT criteria for borderline resectable pancreatic cancer were included[37]. Despite the fact that partial response by RECIST criteria was observed in only 12% of the patients, 70% of the patients underwent resection and only 5% of the patients had positive margins.

Stokes *et al*[41] evaluated capecitabine based chemo-radiation in 40 patients with borderline resectable pancreatic cancer. Patients received external bean radiation in conventional fractionation (50.4 Gy in 28 fractions) or in an accelerated protocol (50 Gy in 20 fractions). Radiation was targeted at the gross tumor as

Table 2 Selected neoadjuvant studies for borderline resectable pancreatic cancer

Ref.	Study type	*n*	Regimen	Resection	R0 resection	Median OS (resected patients)	Median OS (all patients)	Definition
Katz *et al*[40]	Retrospective	84	5-FU, paclitaxel, gemcitabine or capecitabine + RT; Gemcitabine based chemotherapy	38%	97%	40 mo	21	MDA
Turrini *et al*[70]	Retrospective	49	5-FU/cis + RT 45 Gy for 5 wk	18%	100%	24 mo	14 mo	MDA
Chun *et al*[71]	Retrospective	74	5-FU or gem + RT	100%	59%	23	23	Other
Stokes *et al*[41]	Retrospective	40	Capecitabine + RT	46%	75%	23	12	MDA
Katz *et al*[37]	Retrospective	115	Gem followed by gem or 5-FU or capecitabine + RT; Gem or 5-FU or capecitabine + RT	70%	95%	33	22	NCCN
Mellon *et al*[45]	Retrospective	110	GTX X 3 cycles followed by SBRT	51%	96%	19	34	NCCN
Landry *et al*[39]	Randomized phase II	21	Gem + RT; Gem/cis/5-FU followed by 5-FU/RT	24%	100%	26	19.4 mo; 13.4 mo	Other
Lee *et al*[44]	Prospective trial	18	Gem/capecitabine X 3-6 cycles	61%	82%	23	16	NCCN
Kim *et al*[42]	Phase II study	39	Gem/Ox + RT	63%	84%	25	18	NCCN
Motoi *et al*[43]	Phase II study	16	Gem/S1 X 2 cycles	NA	87%	NA	18	MDA
Takahashi *et al*[46]	Prospective	80	Gem + RT followed by Gem	54%	98%	NA	NA	Other

NCCN: National Comprehensive Cancer Network; MDA: MD Anderson; 5-FU: 5-flurouracil; NA: Not available; RT: Radiation therapy.

well as draining lymphatics with a margin ranging from 0.5-2 cm (excluding the para-aortic and porta-hepatis location) utilizing intensity modulated radiation therapy (IMRT) and image guided radiation therapy. Pancreatic resection was performed in 46% of the patients with R0 resection in 87.5% of patients. Accelerated fraction radiation wasn't associated with increased severe toxicities. A report from Moffitt Cancer Center included 110 patients with BRPC treated with induction chemotherapy followed by stereotactic body radiation therapy (SBRT)[45]. The majority of the patients received combination of gemcitabine, docetaxel and capecitabine for 3 cycles. Surgical resection of the tumor was performed in 51% of the patients with R0 resection rate of 96%. Interestingly, 4 (7%) patients had complete pathologic response and a total of 28 (50%) patients had College of American Pathology Tumor Regression Grade 0-1. The median survival for all BRPC was 19 mo.

Radiation type

The neoadjuvant radiation strategies presented above for borderline pancreatic cancer vary greatly from center to center with respect to dose and technique. This ranges from a conventionally fractionated approach all the way to a SBRT approach and everywhere in between. Moreover, some series report the integration of radiosensitizing chemotherapy, consisting largely of continuous infusion 5-flurouracil (5-FU) or gemcitabine.

Standard fractionation has been used in upfront resectable patients with good outcomes and has been adopted at many centers as a strategy for borderline resectable patients[41,46-48]. With standard fractionation, > 90% pathologic response was achieved in 16%-37% and resection rates are around 50%[41,46]. In the report by Stokes *et al*[41], there was a trend for increased survival and a statistically significant increase in > 90% pathologic response in patients that received accelerated fractionation. Takeda *et al*[49] report their results of a phase Ⅰ and Ⅱ trial looking at accelerated hyperfractionation in borderline pancreatic cancer patients. A total of 35 patients were treated with concurrent gemcitabine and accelerated hyperfractionated radiation 1.5 Gy given twice daily to a total dose of 30 Gy (phase Ⅰ) or 36 Gy (phase Ⅱ) targeting the tumor and regional metastatic lymph nodes with a > 1 cm margin utilizing a 4-field technique. No acute grade ⩾ 3 non-hematologic toxicity was observed. Three fourth of the patients underwent surgical resection with all being R0 resections. Greater than 90% pathologic response to neoadjuvant treatment was observed in 23% of patients. Median survival was 41.2 mo in the patients that underwent surgical resection. This, along with the report by Stokes *et al*[41], suggests a benefit in response rates with accelerated fractionation concurrent with chemotherapy.

The radiation dose and volume treated depends on many factors including technique as well as chemotherapy used. Patients treated with the radiation sensitizing chemotherapy agent 5-FU can be treated to a higher dose and a larger volume, targeting the gross tumor as well as draining lymphatics[41]. When concurrent full dose gemcitabine is utilized, caution on the total dose of radiation as well as the volume being treated is indicated. In the prospective trial, only the gross tumor with a 1 cm margin and a total dose of 30 Gy in standard fractionation was used[42].

IMRT and/or SBRT can be used to increase the biologically effective dose and data suggests there may be potential for improved outcomes in the setting of pancreatic cancer not amenable to upfront resection.

The University of Michigan data reporting dose escalation with IMRT (recommended dose of 55 Gy in 25 fractions) in the locally advanced setting with full dose gemcitabine shows promising results as far as toxicity and R0 resection rates[50]. The most recent Radiation Therapy Oncology Group 1201 trial is a phase II trial looking at local *vs* systemic treatment escalation stratified by SMAD4 expression[51]. SMAD4 has been identified and shown to correlate with patterns of failure, either locally destructive failure *vs* metastatic disease in a rapid autopsy study done at John Hopkins[52]. These results will add to the knowledge of dose escalation with IMRT. SBRT along with chemotherapy prior to or after was initially established in locally advanced pancreatic cancer and was shown to be an effective treatment strategy with low rates of toxicity[53-57]. More recently, results from a phase Ⅱ trial reported by Herman *et al*[58], showed that in locally advanced pancreatic cancer patients treated with SBRT (33 Gy in 5 fractions) there were minimal acute and late toxicity (2% and 11%, respectively). The results published by group at Moffitt Cancer Center incorporating SBRT demonstrated that 51% of the BRPC patients underwent surgical resection with 96% being R0 resections[59]. The median dose was 30 Gy (range 28-30) to the gross disease and 40 Gy (25-50 Gy) to the area of vessel abutment. No prophylactic draining lymphatics were in the treatment volume. There were few acute and late grade $\geqslant$ 3 toxicity (7%). With 14 mo of follow up, there were no recurrences in this subset of patients and there was a rate of pathologic complete response of 7%. SBRT allows for escalating and personalizing the dose to each patient based on specific tumor location, vasculature abutment, and proximity to critical normal tissues with no increase in toxicity or peri-operative mortality and allows for the time course from systemic therapy to potential resection to be shorter since the duration of therapy is only one week. No prospective data is yet available in the BRPC setting incorporating SBRT but the available evidence merits further investigation of this novel approach.

Lastly, interest has been generated on the potential of proton therapy to improve outcomes for pancreatic cancer patients. Proton therapy over five days has been successfully integrated with capecitabine for upfront resectable patients on a phase Ⅰ/Ⅱ study with low rates of toxicity[60]. MD Anderson has compared 3-dimentional conformal radiation (3DCRT), IMRT, and passive-scattering proton therapy dose escalation (72 Gy) plans for pancreatic tumors[61]. Overall they found 3DCRT to be inadequate for coverage and IMRT to be more conformal in high gradient dose regions which would be beneficial for dose escalation in patients with organs at risk in close proximity, as seen in pancreatic cancer. Proton therapy had the advantage of a low integral dose but this would not affect dose escalation. Thompson *et al*[62] reported their dosimetric comparison of IMRT, double scattering and pencil beam scanning proton therapy. They found again that proton beam therapy would unlikely result in dose escalation over IMRT. Proton therapy resulted in decreased dose in the low-intermediate dose range but increased dose in the mid to high dose region, with unclear clinical significance.

The optimal technique and dose of radiation therapy is unclear; however, dose escalation with IMRT and/or SBRT show promising results in increasing R0 resection rates with low toxicity.

DISCUSSION

The margin status is very important to the clinical outcomes after pancreatic resection. The goal of the resection is to obtain R0 resection as patients with gross disease at the margins (R2 resection) do not benefit from surgical resection and have similar outcomes as patients without surgery[63-65]. Microscopic disease at the margin (R1 resection) is associated with a poor prognosis but is not consistent across all studies[63,66,67]. The definition of R1 resection has not been uniform in the past which makes interpretation of data from various studies problematic. AJCC criteria define positive resection margins when tumor cells are present at the edge of resected specimen whereas European criteria defines positive margins if tumor cells are present within $\leqslant$ 1 mm of resected margins[68]. The location of margins has prognostic impact as well. In one study, R1 status at the anterior or posterior margins was not relevant for outcomes[69].

Recently, there has been improvement in systemic therapies for metastatic pancreatic cancers that has improved response rates over single agent gemcitabine. The FOLFIRINOX regimen and gemcitabine/nab-paclitaxel combination is associated with response rates of 31% and 23% compared to less than 10% with single agent gemcitabine. These regimens may increase the probability of margin negative resection and the ability to obtain an R0 resection. There are additional toxicities associated with these combination regimens, especially FOLFIRINOX, including neutropenic fever. The Intergroup trial (ALLIANCE A021101) is evaluating neoadjuvant FOLFIRINOX followed by capecitabine based chemoradiotherapy. The dose of 5-FU has been modified to make it more tolerable. Patients who undergo resection will also receive adjuvant gemcitabine. The criteria for resection have been clearly defined through consensus and may become the new standard for resectability.

CONCLUSION

Management of borderline resectable pancreatic cancer continues to evolve. Prior studies have been complicated by low accruing trials, largely retrospective single institution experiences, and different classification criteria, chemotherapy regimens and radiotherapy type and schedule. There is an urgent need to apply uniform criteria for defining borderline pancreatic cancer. The patients should be classified and treated with a

multidisciplinary approach at high volume centers. Patients should undergo a pancreas protocol CT scan and EUS to determine the resectability status. Ideally, these patients should be treated on a clinical trial protocol. The ability to obtain negative margins is of the utmost importance for improving the outcomes of these patients. Newer aggressive chemotherapy regimens may help improve the resectability rate. These regimens followed by SBRT or IMRT may have a role in treatment. Induction chemotherapy followed by chemoradiation is the most commonly utilized approach but is not uniform. Newer trial designs incorporating uniform classification and treatment strategy will help standardize treatment for patients with borderline resectable pancreatic cancer.

REFERENCES

1 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin* 2015; **65**: 5-29 [PMID: 25559415 DOI: 10.3322/caac.21254]

2 **Stathis A**, Moore MJ. Advanced pancreatic carcinoma: current treatment and future challenges. *Nat Rev Clin Oncol* 2010; **7**: 163-172 [PMID: 20101258 DOI: 10.1038/nrclinonc.2009.236]

3 **Gupta PK**, Turaga KK, Miller WJ, Loggie BW, Foster JM. Determinants of outcomes in pancreatic surgery and use of hospital resources. *J Surg Oncol* 2011; **104**: 634-640 [PMID: 21520092 DOI: 10.1002/jso.21923]

4 **Lee ES**, Lee JM. Imaging diagnosis of pancreatic cancer: a state-of-the-art review. *World J Gastroenterol* 2014; **20**: 7864-7877 [PMID: 24976723 DOI: 10.3748/wjg.v20.i24.7864]

5 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]

6 **Von Hoff DD**, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J, Renschler MF. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**: 1691-1703 [PMID: 24131140 DOI: 10.1056/NEJMoa1304369]

7 **Liles JS**, Katz MH. Pancreaticoduodenectomy with vascular resection for pancreatic head adenocarcinoma. *Expert Rev Anticancer Ther* 2014; **14**: 919-929 [PMID: 24833085 DOI: 10.1586/14737140.2014.919860]

8 **Katz MH**, Crane CH, Varadhachary G. Management of borderline resectable pancreatic cancer. *Semin Radiat Oncol* 2014; **24**: 105-112 [PMID: 24635867 DOI: 10.1016/j.semradonc.2013.11.006]

9 **Wong JC**, Lu DS. Staging of pancreatic adenocarcinoma by imaging studies. *Clin Gastroenterol Hepatol* 2008; **6**: 1301-1308 [PMID: 18948228 DOI: 10.1016/j.cgh.2008.09.014]

10 **Farma JM**, Santillan AA, Melis M, Walters J, Belinc D, Chen DT, Eikman EA, Malafa M. PET/CT fusion scan enhances CT staging in patients with pancreatic neoplasms. *Ann Surg Oncol* 2008; **15**: 2465-2471 [PMID: 18551347 DOI: 10.1245/s10434-008-9992-0]

11 **Rijkers AP**, Valkema R, Duivenvoorden HJ, van Eijck CH. Usefulness of F-18-fluorodeoxyglucose positron emission tomography to confirm suspected pancreatic cancer: a meta-analysis. *Eur J Surg Oncol* 2014; **40**: 794-804 [PMID: 24755095 DOI: 10.1016/j.ejso.2014.03.016]

12 **Agarwal B**, Abu-Hamda E, Molke KL, Correa AM, Ho L. Endoscopic ultrasound-guided fine needle aspiration and multidetector spiral CT in the diagnosis of pancreatic cancer. *Am J Gastroenterol* 2004; **99**: 844-850 [PMID: 15128348 DOI: 10.1111/j.1572-0241.2004.04177.x]

13 **Chang KJ**, Nguyen P, Erickson RA, Durbin TE, Katz KD. The clinical utility of endoscopic ultrasound-guided fine-needle aspiration in the diagnosis and staging of pancreatic carcinoma. *Gastrointest Endosc* 1997; **45**: 387-393 [PMID: 9165320]

14 **Bang JY**, Magee SH, Ramesh J, Trevino JM, Varadarajulu S. Randomized trial comparing fanning with standard technique for endoscopic ultrasound-guided fine-needle aspiration of solid pancreatic mass lesions. *Endoscopy* 2013; **45**: 445-450 [PMID: 23504490 DOI: 10.1055/s-0032-1326268]

15 **Hariharan D**, Constantinides VA, Froeling FE, Tekkis PP, Kocher HM. The role of laparoscopy and laparoscopic ultrasound in the preoperative staging of pancreatico-biliary cancers--A meta-analysis. *Eur J Surg Oncol* 2010; **36**: 941-948 [PMID: 20547445 DOI: 10.1016/j.ejso.2010.05.015]

16 **Al-Hawary MM**, Francis IR, Chari ST, Fishman EK, Hough DM, Lu DS, Macari M, Megibow AJ, Miller FH, Mortele KJ, Merchant NB, Minter RM, Tamm EP, Sahani DV, Simeone DM. Pancreatic ductal adenocarcinoma radiology reporting template: consensus statement of the society of abdominal radiology and the american pancreatic association. *Gastroenterology* 2014; **146**: 291-304.e1 [PMID: 24355035 DOI: 10.1053/j.gastro.2013.11.004]

17 **Katz MH**, Marsh R, Herman JM, Shi Q, Collison E, Venook AP, Kindler HL, Alberts SR, Philip P, Lowy AM, Pisters PW, Posner MC, Berlin JD, Ahmad SA. Borderline resectable pancreatic cancer: need for standardization and methods for optimal clinical trial design. *Ann Surg Oncol* 2013; **20**: 2787-2795 [PMID: 23435609 DOI: 10.1245/s10434-013-2886-9]

18 **Varadhachary GR**, Tamm EP, Abbruzzese JL, Xiong HQ, Crane CH, Wang H, Lee JE, Pisters PW, Evans DB, Wolff RA. Borderline resectable pancreatic cancer: definitions, management, and role of preoperative therapy. *Ann Surg Oncol* 2006; **13**: 1035-1046 [PMID: 16865597 DOI: 10.1245/ASO.2006.08.011]

19 **Siriwardana HP**, Siriwardena AK. Systematic review of outcome of synchronous portal-superior mesenteric vein resection during pancreatectomy for cancer. *Br J Surg* 2006; **93**: 662-673 [PMID: 16703621 DOI: 10.1002/bjs.5368]

20 **Adham M**, Mirza DF, Chapuis F, Mayer AD, Bramhall SR, Coldham C, Baulieux J, Buckels J. Results of vascular resections during pancreatectomy from two European centres: an analysis of survival and disease-free survival explicative factors. *HPB* (Oxford) 2006; **8**: 465-473 [PMID: 18333103 DOI: 10.1080/13651820600839944]

21 **Abrams RA**, Lowy AM, O'Reilly EM, Wolff RA, Picozzi VJ, Pisters PW. Combined modality treatment of resectable and borderline resectable pancreas cancer: expert consensus statement. *Ann Surg Oncol* 2009; **16**: 1751-1756 [PMID: 19390900 DOI: 10.1245/s10434-009-0413-9]

22 **Callery MP**, Chang KJ, Fishman EK, Talamonti MS, William Traverso L, Linehan DC. Pretreatment assessment of resectable and borderline resectable pancreatic cancer: expert consensus statement. *Ann Surg Oncol* 2009; **16**: 1727-1733 [PMID: 19396496 DOI: 10.1245/s10434-009-0408-6]

23 **Illuminati G**, Carboni F, Lorusso R, D'Urso A, Ceccanei G, Papaspyropoulos V, Pacile MA, Santoro E. Results of a pancreatectomy with a limited venous resection for pancreatic cancer. *Surg Today* 2008; **38**: 517-523 [PMID: 18516531 DOI: 10.1007/s00595-007-3661-y]

24 **Fukuda S**, Oussoultzoglou E, Bachellier P, Rosso E, Nakano H, Audet M, Jaeck D. Significance of the depth of portal vein wall invasion after curative resection for pancreatic adenocarcinoma. *Arch Surg* 2007; **142**: 172-179; discussion 180 [PMID: 17309969 DOI: 10.1001/archsurg.142.2.172]

25 **Zhou Y**, Zhang Z, Liu Y, Li B, Xu D. Pancreatectomy combined with superior mesenteric vein-portal vein resection for pancreatic cancer: a meta-analysis. *World J Surg* 2012; **36**: 884-891 [PMID: 22350478 DOI: 10.1007/s00268-012-1461-z]

26 **Castleberry AW**, White RR, De La Fuente SG, Clary BM, Blazer DG, McCann RL, Pappas TN, Tyler DS, Scarborough JE. The impact of vascular resection on early postoperative outcomes after pancreaticoduodenectomy: an analysis of the American College

of Surgeons National Surgical Quality Improvement Program database. *Ann Surg Oncol* 2012; **19**: 4068-4077 [PMID: 22932857 DOI: 10.1245/s10434-012-2585-y]

27 **Worni M**, Castleberry AW, Clary BM, Gloor B, Carvalho E, Jacobs DO, Pietrobon R, Scarborough JE, White RR. Concomitant vascular reconstruction during pancreatectomy for malignant disease: a propensity score-adjusted, population-based trend analysis involving 10,206 patients. *JAMA Surg* 2013; **148**: 331-338 [PMID: 23715922 DOI: 10.1001/jamasurg.2013.1058]

28 **Martin RC**, Scoggins CR, Egnatashvili V, Staley CA, McMasters KM, Kooby DA. Arterial and venous resection for pancreatic adenocarcinoma: operative and long-term outcomes. *Arch Surg* 2009; **144**: 154-159 [PMID: 19221327 DOI: 10.1001/archsurg.2008.547]

29 **Bachellier P**, Rosso E, Lucescu I, Oussoultzoglou E, Tracey J, Pessaux P, Ferreira N, Jaeck D. Is the need for an arterial resection a contraindication to pancreatic resection for locally advanced pancreatic adenocarcinoma? A case-matched controlled study. *J Surg Oncol* 2011; **103**: 75-84 [PMID: 21105000 DOI: 10.1002/jso.21769]

30 **Mollberg N**, Rahbari NN, Koch M, Hartwig W, Hoeger Y, Büchler MW, Weitz J. Arterial resection during pancreatectomy for pancreatic cancer: a systematic review and meta-analysis. *Ann Surg* 2011; **254**: 882-893 [PMID: 22064622 DOI: 10.1097/SLA.0b013e31823ac299]

31 **Ouaissi M**, Hubert C, Verhelst R, Astarci P, Sempoux C, Jouret-Mourin A, Loundou A, Gigot JF. Vascular reconstruction during pancreatoduodenectomy for ductal adenocarcinoma of the pancreas improves resectability but does not achieve cure. *World J Surg* 2010; **34**: 2648-2661 [PMID: 20607257 DOI: 10.1007/s00268-010-0699-6]

32 **Bockhorn M**, Burdelski C, Bogoevski D, Sgourakis G, Yekebas EF, Izbicki JR. Arterial en bloc resection for pancreatic carcinoma. *Br J Surg* 2011; **98**: 86-92 [PMID: 21136564 DOI: 10.1002/bjs.7270]

33 **Rose JB**, Rocha FG, Alseidi A, Biehl T, Moonka R, Ryan JA, Lin B, Picozzi V, Helton S. Extended neoadjuvant chemotherapy for borderline resectable pancreatic cancer demonstrates promising postoperative outcomes and survival. *Ann Surg Oncol* 2014; **21**: 1530-1537 [PMID: 24473642 DOI: 10.1245/s10434-014-3486-z]

34 **Spitz FR**, Abbruzzese JL, Lee JE, Pisters PW, Lowy AM, Fenoglio CJ, Cleary KR, Janjan NA, Goswitz MS, Rich TA, Evans DB. Preoperative and postoperative chemoradiation strategies in patients treated with pancreaticoduodenectomy for adenocarcinoma of the pancreas. *J Clin Oncol* 1997; **15**: 928-937 [PMID: 9060530]

35 **Breslin TM**, Hess KR, Harbison DB, Jean ME, Cleary KR, Dackiw AP, Wolff RA, Abbruzzese JL, Janjan NA, Crane CH, Vauthey JN, Lee JE, Pisters PW, Evans DB. Neoadjuvant chemoradiotherapy for adenocarcinoma of the pancreas: treatment variables and survival duration. *Ann Surg Oncol* 2001; **8**: 123-132 [PMID: 11258776]

36 **Roland CL**, Yang AD, Katz MH, Chatterjee D, Wang H, Lin H, Vauthey JN, Pisters PW, Varadhachary GR, Wolff RA, Crane CH, Lee JE, Fleming JB. Neoadjuvant therapy is associated with a reduced lymph node ratio in patients with potentially resectable pancreatic cancer. *Ann Surg Oncol* 2015; **22**: 1168-1175 [PMID: 25352267 DOI: 10.1245/s10434-014-4192-6]

37 **Katz MH**, Fleming JB, Bhosale P, Varadhachary G, Lee JE, Wolff R, Wang H, Abbruzzese J, Pisters PW, Vauthey JN, Charnsangavej C, Tamm E, Crane CH, Balachandran A. Response of borderline resectable pancreatic cancer to neoadjuvant therapy is not reflected by radiographic indicators. *Cancer* 2012; **118**: 5749-5756 [PMID: 22605518 DOI: 10.1002/cncr.27636]

38 **Tzeng CW**, Fleming JB, Lee JE, Xiao L, Pisters PW, Vauthey JN, Abdalla EK, Wolff RA, Varadhachary GR, Fogelman DR, Crane CH, Balachandran A, Katz MH. Defined clinical classifications are associated with outcome of patients with anatomically resectable pancreatic adenocarcinoma treated with neoadjuvant therapy. *Ann Surg Oncol* 2012; **19**: 2045-2053 [PMID: 22258816 DOI: 10.1245/s10434-011-2211-4]

39 **Landry J**, Catalano PJ, Staley C, Harris W, Hoffman J, Talamonti M, Xu N, Cooper H, Benson AB. Randomized phase II study of gemcitabine plus radiotherapy versus gemcitabine, 5-fluorouracil, and cisplatin followed by radiotherapy and 5-fluorouracil for patients with locally advanced, potentially resectable pancreatic adenocarcinoma. *J Surg Oncol* 2010; **101**: 587-592 [PMID: 20461765 DOI: 10.1002/jso.21527]

40 **Katz MH**, Pisters PW, Evans DB, Sun CC, Lee JE, Fleming JB, Vauthey JN, Abdalla EK, Crane CH, Wolff RA, Varadhachary GR, Hwang RF. Borderline resectable pancreatic cancer: the importance of this emerging stage of disease. *J Am Coll Surg* 2008; **206**: 833-846; discussion 846-848 [PMID: 18471707 DOI: 10.1016/j.jamcollsurg.2007.12.020]

41 **Stokes JB**, Nolan NJ, Stelow EB, Walters DM, Weiss GR, de Lange EE, Rich TA, Adams RB, Bauer TW. Preoperative capecitabine and concurrent radiation for borderline resectable pancreatic cancer. *Ann Surg Oncol* 2011; **18**: 619-627 [PMID: 21213060 DOI: 10.1245/s10434-010-1456-7]

42 **Kim EJ**, Ben-Josef E, Herman JM, Bekaii-Saab T, Dawson LA, Griffith KA, Francis IR, Greenson JK, Simeone DM, Lawrence TS, Laheru D, Wolfgang CL, Williams T, Bloomston M, Moore MJ, Wei A, Zalupski MM. A multi-institutional phase 2 study of neoadjuvant gemcitabine and oxaliplatin with radiation therapy in patients with pancreatic cancer. *Cancer* 2013; **119**: 2692-2700 [PMID: 23720019 DOI: 10.1002/cncr.28117]

43 **Motoi F**, Ishida K, Fujishima F, Ottomo S, Oikawa M, Okada T, Shimamura H, Takemura S, Ono F, Akada M, Nakagawa K, Katayose Y, Egawa S, Unno M. Neoadjuvant chemotherapy with gemcitabine and S-1 for resectable and borderline pancreatic ductal adenocarcinoma: results from a prospective multi-institutional phase 2 trial. *Ann Surg Oncol* 2013; **20**: 3794-3801 [PMID: 23838925 DOI: 10.1245/s10434-013-3129-9]

44 **Lee JL**, Kim SC, Kim JH, Lee SS, Kim TW, Park do H, Seo DW, Lee SK, Kim MH, Kim JH, Park JH, Shin SH, Han DJ. Prospective efficacy and safety study of neoadjuvant gemcitabine with capecitabine combination chemotherapy for borderline-resectable or unresectable locally advanced pancreatic adenocarcinoma. *Surgery* 2012; **152**: 851-862 [PMID: 22682078 DOI: 10.1016/j.surg.2012.03.010]

45 **Mellon EA**, Hoffe SE, Springett GM, Frakes JM, Strom TJ, Hodul PJ, Malafa MP, Chuong MD, Shridhar R. Long-term outcomes of induction chemotherapy and neoadjuvant stereotactic body radiotherapy for borderline resectable and locally advanced pancreatic adenocarcinoma. *Acta Oncol* 2015; **54**: 979-985 [PMID: 25734581 DOI: 10.3109/0284186X.2015.1004367]

46 **Takahashi H**, Ohigashi H, Gotoh K, Marubashi S, Yamada T, Murata M, Ioka T, Uehara H, Yano M, Ishikawa O. Preoperative gemcitabine-based chemoradiation therapy for resectable and borderline resectable pancreatic cancer. *Ann Surg* 2013; **258**: 1040-1050 [PMID: 23799421 DOI: 10.1097/SLA.0b013e31829b3ce4]

47 **Evans DB**, Varadhachary GR, Crane CH, Sun CC, Lee JE, Pisters PW, Vauthey JN, Wang H, Cleary KR, Staerkel GA, Charnsangavej C, Lano EA, Ho L, Lenzi R, Abbruzzese JL, Wolff RA. Preoperative gemcitabine-based chemoradiation for patients with resectable adenocarcinoma of the pancreatic head. *J Clin Oncol* 2008; **26**: 3496-3502 [PMID: 18640930 DOI: 10.1200/JCO.2007.15.8634]

48 **Varadhachary GR**, Wolff RA, Crane CH, Sun CC, Lee JE, Pisters PW, Vauthey JN, Abdalla E, Wang H, Staerkel GA, Lee JH, Ross WA, Tamm EP, Bhosale PR, Krishnan S, Das P, Ho L, Xiong H, Abbruzzese JL, Evans DB. Preoperative gemcitabine and cisplatin followed by gemcitabine-based chemoradiation for resectable adenocarcinoma of the pancreatic head. *J Clin Oncol* 2008; **26**: 3487-3495 [PMID: 18640929 DOI: 10.1200/JCO.2007.15.8642]

49 **Takeda Y**, Nakamori S, Eguchi H, Kobayashi S, Marubashi S, Tanemura M, Konishi K, Yoshioka Y, Umeshita K, Mori M, Doki Y, Nagano H. Neoadjuvant gemcitabine-based accelerated hyperfractionation chemoradiotherapy for patients with borderline resectable pancreatic adenocarcinoma. *Jpn J Clin Oncol* 2014; **44**: 1172-1180 [PMID: 25425728 DOI: 10.1093/jjco/hyu143]

50 **Ben-Josef E**, Schipper M, Francis IR, Hadley S, Ten-Haken R, Lawrence T, Normolle D, Simeone DM, Sonnenday C, Abrams R, Leslie W, Khan G, Zalupski MM. A phase I/II trial of intensity modulated radiation (IMRT) dose escalation with concurrent fixed-dose rate gemcitabine (FDR-G) in patients with unresectable pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2012; **84**:

1166-1171 [PMID: 22543215 DOI: 10.1016/j.ijrobp.2012.02.051]
51 **Denson AC**, Mahipal A. Participation of the elderly population in clinical trials: barriers and solutions. *Cancer Control* 2014; **21**: 209-214 [PMID: 24955704]
52 **Iacobuzio-Donahue CA**, Fu B, Yachida S, Luo M, Abe H, Henderson CM, Vilardell F, Wang Z, Keller JW, Banerjee P, Herman JM, Cameron JL, Yeo CJ, Halushka MK, Eshleman JR, Raben M, Klein AP, Hruban RH, Hidalgo M, Laheru D. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol* 2009; **27**: 1806-1813 [PMID: 19273710 DOI: 10.1200/JCO.2008.17.7188]
53 **Koong AC**, Le QT, Ho A, Fong B, Fisher G, Cho C, Ford J, Poen J, Gibbs IC, Mehta VK, Kee S, Trueblood W, Yang G, Bastidas JA. Phase I study of stereotactic radiosurgery in patients with locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2004; **58**: 1017-1021 [PMID: 15001240 DOI: 10.1016/j.ijrobp.2003.11.004]
54 **Schellenberg D**, Kim J, Christman-Skieller C, Chun CL, Columbo LA, Ford JM, Fisher GA, Kunz PL, Van Dam J, Quon A, Desser TS, Norton J, Hsu A, Maxim PG, Xing L, Goodman KA, Chang DT, Koong AC. Single-fraction stereotactic body radiation therapy and sequential gemcitabine for the treatment of locally advanced pancreatic cancer. *Int J Radiat Oncol Biol Phys* 2011; **81**: 181-188 [PMID: 21549517 DOI: 10.1016/j.ijrobp.2010.05.006]
55 **Mahadevan A**, Miksad R, Goldstein M, Sullivan R, Bullock A, Buchbinder E, Pleskow D, Sawhney M, Kent T, Vollmer C, Callery M. Induction gemcitabine and stereotactic body radiotherapy for locally advanced nonmetastatic pancreas cancer. *Int J Radiat Oncol Biol Phys* 2011; **81**: e615-e622 [PMID: 21658854 DOI: 10.1016/j.ijrobp.2011.04.045]
56 **Rwigema JC**, Parikh SD, Heron DE, Howell M, Zeh H, Moser AJ, Bahary N, Quinn A, Burton SA. Stereotactic body radiotherapy in the treatment of advanced adenocarcinoma of the pancreas. *Am J Clin Oncol* 2011; **34**: 63-69 [PMID: 20308870 DOI: 10.1097/COC.0b013e3181d270b4]
57 **Didolkar MS**, Coleman CW, Brenner MJ, Chu KU, Olexa N, Stanwyck E, Yu A, Neerchal N, Rabinowitz S. Image-guided stereotactic radiosurgery for locally advanced pancreatic adenocarcinoma results of first 85 patients. *J Gastrointest Surg* 2010; **14**: 1547-1559 [PMID: 20839073 DOI: 10.1007/s11605-010-1323-7]
58 **Herman JM**, Chang DT, Goodman KA, Dholakia AS, Raman SP, Hacker-Prietz A, Iacobuzio-Donahue CA, Griffith ME, Pawlik TM, Pai JS, O'Reilly E, Fisher GA, Wild AT, Rosati LM, Zheng L, Wolfgang CL, Laheru DA, Columbo LA, Sugar EA, Koong AC. Phase 2 multi-institutional trial evaluating gemcitabine and stereotactic body radiotherapy for patients with locally advanced unresectable pancreatic adenocarcinoma. *Cancer* 2015; **121**: 1128-1137 [PMID: 25538019 DOI: 10.1002/cncr.29161]
59 **Chuong MD**, Springett GM, Freilich JM, Park CK, Weber JM, Mellon EA, Hodul PJ, Malafa MP, Meredith KL, Hoffe SE, Shridhar R. Stereotactic body radiation therapy for locally advanced and borderline resectable pancreatic cancer is effective and well tolerated. *Int J Radiat Oncol Biol Phys* 2013; **86**: 516-522 [PMID: 23562768 DOI: 10.1016/j.ijrobp.2013.02.022]
60 **Hong TS**, Ryan DP, Borger DR, Blaszkowsky LS, Yeap BY, Ancukiewicz M, Deshpande V, Shinagare S, Wo JY, Boucher Y, Wadlow RC, Kwak EL, Allen JN, Clark JW, Zhu AX, Ferrone CR, Mamon HJ, Adams J, Winrich B, Grillo T, Jain RK, DeLaney TF, Fernandez-del Castillo C, Duda DG. A phase 1/2 and biomarker study of preoperative short course chemoradiation with proton beam therapy and capecitabine followed by early surgery for resectable pancreatic ductal adenocarcinoma. *Int J Radiat Oncol Biol Phys* 2014; **89**: 830-838 [PMID: 24867540 DOI: 10.1016/j.ijrobp.2014.03.034]
61 **Bouchard M**, Amos RA, Briere TM, Beddar S, Crane CH. Dose escalation with proton or photon radiation treatment for pancreatic cancer. *Radiother Oncol* 2009; **92**: 238-243 [PMID: 19454367 DOI: 10.1016/j.radonc.2009.04.015]
62 **Thompson RF**, Mayekar SU, Zhai H, Both S, Apisarnthanarax S, Metz JM, Plastaras JP, Ben-Josef E. A dosimetric comparison of proton and photon therapy in unresectable cancers of the head of pancreas. *Med Phys* 2014; **41**: 081711 [PMID: 25086521 DOI: 10.1118/1.4887797]
63 **Sohn TA**, Yeo CJ, Cameron JL, Koniaris L, Kaushal S, Abrams RA, Sauter PK, Coleman J, Hruban RH, Lillemoe KD. Resected adenocarcinoma of the pancreas-616 patients: results, outcomes, and prognostic indicators. *J Gastrointest Surg* 2000; **4**: 567-579 [PMID: 11307091]
64 **Neoptolemos JP**, Stocken DD, Dunn JA, Almond J, Beger HG, Pederzoli P, Bassi C, Dervenis C, Fernandez-Cruz L, Lacaine F, Buckels J, Deakin M, Adab FA, Sutton R, Imrie C, Ihse I, Tihanyi T, Olah A, Pedrazzoli S, Spooner D, Kerr DJ, Friess H, Büchler MW. Influence of resection margins on survival for patients with pancreatic cancer treated by adjuvant chemoradiation and/or chemotherapy in the ESPAC-1 randomized controlled trial. *Ann Surg* 2001; **234**: 758-768 [PMID: 11729382]
65 **Winter JM**, Cameron JL, Campbell KA, Arnold MA, Chang DC, Coleman J, Hodgin MB, Sauter PK, Hruban RH, Riall TS, Schulick RD, Choti MA, Lillemoe KD, Yeo CJ. 1423 pancreaticoduodenectomies for pancreatic cancer: A single-institution experience. *J Gastrointest Surg* 2006; **10**: 1199-1210; discussion 1210-1211 [PMID: 17114007 DOI: 10.1016/j.gassur.2006.08.018]
66 **Yeo CJ**, Abrams RA, Grochow LB, Sohn TA, Ord SE, Hruban RH, Zahurak ML, Dooley WC, Coleman J, Sauter PK, Pitt HA, Lillemoe KD, Cameron JL. Pancreaticoduodenectomy for pancreatic adenocarcinoma: postoperative adjuvant chemoradiation improves survival. A prospective, single-institution experience. *Ann Surg* 1997; **225**: 621-633; discussion 633-636 [PMID: 9193189]
67 **Allison DC**, Piantadosi S, Hruban RH, Dooley WC, Fishman EK, Yeo CJ, Lillemoe KD, Pitt HA, Lin P, Cameron JL. DNA content and other factors associated with ten-year survival after resection of pancreatic carcinoma. *J Surg Oncol* 1998; **67**: 151-159 [PMID: 9530884]
68 **Bockhorn M**, Uzunoglu FG, Adham M, Imrie C, Milicevic M, Sandberg AA, Asbun HJ, Bassi C, Büchler M, Charnley RM, Conlon K, Cruz LF, Dervenis C, Fingerhutt A, Friess H, Gouma DJ, Hartwig W, Lillemoe KD, Montorsi M, Neoptolemos JP, Shrikhande SV, Takaori K, Traverso W, Vashist YK, Vollmer C, Yeo CJ, Izbicki JR. Borderline resectable pancreatic cancer: a consensus statement by the International Study Group of Pancreatic Surgery (ISGPS). *Surgery* 2014; **155**: 977-988 [PMID: 24856119 DOI: 10.1016/j.surg.2014.02.001]
69 **Jamieson NB**, Foulis AK, Oien KA, Going JJ, Glen P, Dickson EJ, Imrie CW, McKay CJ, Carter R. Positive mobilization margins alone do not influence survival following pancreatico-duodenectomy for pancreatic ductal adenocarcinoma. *Ann Surg* 2010; **251**: 1003-1010 [PMID: 20485150 DOI: 10.1097/SLA.0b013e3181d77369]
70 **Turrini O**, Viret F, Moureau-Zabotto L, Guiramand J, Moutardier V, Lelong B, Giovannini M, Delpero JR. Neoadjuvant chemoradiation and pancreaticoduodenectomy for initially locally advanced head pancreatic adenocarcinoma. *Eur J Surg Oncol* 2009; **35**: 1306-1311 [PMID: 19576722 DOI: 10.1016/j.ejso.2009.06.005]
71 **Chun YS**, Milestone BN, Watson JC, Cohen SJ, Burtness B, Engstrom PF, Haluszka O, Tokar JL, Hall MJ, Denlinger CS, Astsaturov I, Hoffman JP. Defining venous involvement in borderline resectable pancreatic cancer. *Ann Surg Oncol* 2010; **17**: 2832-2838 [PMID: 20725860 DOI: 10.1245/s10434-010-1284-9]

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TOPIC HIGHLIGHT

2015 Advances in Pancreatic Cancer

Genomic alterations in pancreatic cancer and their relevance to therapy

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Abstract

Pancreatic cancer is a highly lethal cancer type, for which there are few viable therapeutic options. But, with the advance of sequencing technologies for global genomic analysis, the landscape of genomic alterations in pancreatic cancer is becoming increasingly well understood. In this review, we summarize current knowledge of genomic alterations in 12 core signaling pathways or cellular processes in pancreatic ductal adenocarcinoma, which is the most common type of malignancy in the pancreas, including four commonly mutated genes and many other genes that are mutated at low frequencies. We also describe the potential implications of these genomic alterations for development of novel therapeutic approaches in the context of personalized medicine.

Key words: Pancreatic cancer; Genomic alterations; Signaling pathways; Therapeutic targets; Personalized medicine

Core tip: With the advance of sequencing technologies for global genomic analysis, the landscape of genomic alterations in pancreatic cancer is becoming increasingly well understood. In this review, we summarize the latest knowledge of genomic alterations in pancreatic ductal adenocarcinoma including commonly mutated genes and many other genes that are mutated at low frequencies. We also describe the potential implications of these genomic alterations for development of novel therapeutic approaches in the context of personalized medicine.

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INTRODUCTION

Pancreatic cancer was the seventh leading cause of death in the world in 2012, and is responsible for about 331000 deaths per year[1]. The 5-year survival of pancreatic cancer patients is approximately 5%, and this figure has remained constant in recent decades. Because of the absence of effective methods for early detection and the aggressive nature of this disease, the majority of patients present with locally advanced or metastatic cancer which is not eligible for surgical resection. Chemotherapeutic options for treatment of advanced pancreatic cancer are still limited, and gemcitabine has been the standard chemotherapeutic drug for patients with advanced disease for many years, even though this drug alone provides only a modest survival advantage[2-4]. Since the approval of gemcitabine in United States, many randomized clinical trials have been performed to evaluate combinations of gemcitabine with other drugs, such as 5-fluorouracil (5-FU), cisplatin, oxaliplatin and irinotecan[5], but few of them show a significant survival advantage compared with gemcitabine alone. The combination of gemcitabine with the epidermal growth factor receptor (EGFR) inhibitor, erlotinib, does confer a survival advantage over gemcitabine monotherapy, but the overall survival of patients with advanced disease was extended by only 10 d on average[6]. The combination of gemcitabine with nab-paclitaxel (albumin-bound paclitaxel) was recently shown to be superior to gemcitabine alone, probably because of depletion of tumor stroma, which leads to improved delivery of gemcitabine to tumor cells[7]. Other than gemcitabine-based chemotherapies, 5-FU-based chemotherapeutic regimens have also been evaluated. FOLFIRINOX (folinic acid, fluorouracil, irinotecan and oxaliplatin) improved the median overall survival from 6.8 to 11.1 mo compared with gemcitabine, although significant toxicities associated with this regimen limit its utility in a wide range of patients[8]. It seems that a deeper understanding of the molecular biology of pancreatic cancer is needed to develop novel therapeutic approaches.

In recent years, advances in sequencing technologies have enabled us to perform genome-wide analysis to establish the genetic alterations underlying pancreatic carcinogenesis and progression. In this review, we summarize current knowledge of genomic alterations in pancreatic ductal adenocarcinoma (PDAC), which is the most common type of malignancy in the pancreas, and we discuss their implications for development of novel therapeutic strategies.

GENOMIC ALTERATIONS OF PANCREATIC CANCER

Jones *et al*[9] have shown that PDAC harbors an average of 63 genome alterations, of which the majority are point mutations. Four key genes are frequently altered in PDAC: *KRAS*, *CDKN2A*, *TP53* and *SMAD4*. The most common gene alteration is in *KRAS* (v-ki-ras2 Kirsten rat sarcoma viral oncogene homolog), where mutations occur in codons 12, 13 and 61[9,10]. More than 90% of PDAC contains *KRAS* mutation, and such mutations are also present in about 45% of low-grade pancreatic intraepithelial neoplasia (PanIN) lesions[11,12]. *KRAS* encodes a GTPase that activates various downstream signaling pathways, including the mitogen-activated protein kinase (MAPK) cascades[13]. Mutations in *KRAS* result in constitutive activation. Ras proteins are involved in a variety of cellular functions, including proliferation, differentiation and survival[14,15]. *P16*, cyclin-dependent kinase inhibitor 2A gene (*CDKN2A*) is also inactivated in up to 90% of PDAC, due to intragenic mutation in association with allelic loss, homozygous deletion, or hypermethylation of the gene promoter[16-18]. *CDKN2A* encodes a cyclin-dependent kinase inhibitor that controls G1-S transition in the cell cycle. Mutations in *CDKN2A* are thought to be subsequent to those of *KRAS,* because of the higher prevalence of *KRAS* mutations in early-stage precursor lesions and the fact that most PanIN lesions containing *CDKN2A* inactivation also harbor *KRAS* mutation[19]. *TP53* is one of the most frequently mutated genes in many types of cancer[20-22], and is inactivated in about 75% of PDAC, mainly due to point mutations or small deletions[21,22]. p53 is a transcription factor that determines cell fate by inducing expression of a variety of genes related to cell cycle arrest and apoptosis, and plays an important role as a master regulator of cellular stress responses. *SMAD4* (*DPC4*, SMAD family member 4 gene) is inactivated in up to 55% of PDAC by homozygous deletion or intragenic mutation in association with allelic loss[23]. *SMAD4* encodes a transcription factor that mediates signaling of the transforming growth factor-β (TGF-β) superfamily. *TP53* and *SMAD4* genes are mutated in late-stage precursor lesions, typically in high-grade PanIN[24,25].

In addition to these four frequently altered genes, various other genes are mutated at relatively low frequencies in pancreatic cancer. Jones *et al*[9] reported alterations in genes related to chromatin remodeling (*ARID1A*, *MLL3*). Furthermore, they proposed that core signaling pathways exist in pancreatic cancer (Figure 1), and noted that the pathway components altered in individual tumors may vary widely[9]. Whole-exome sequencing analysis of 99 pancreatic cancers found many significantly mutated genes, including genes

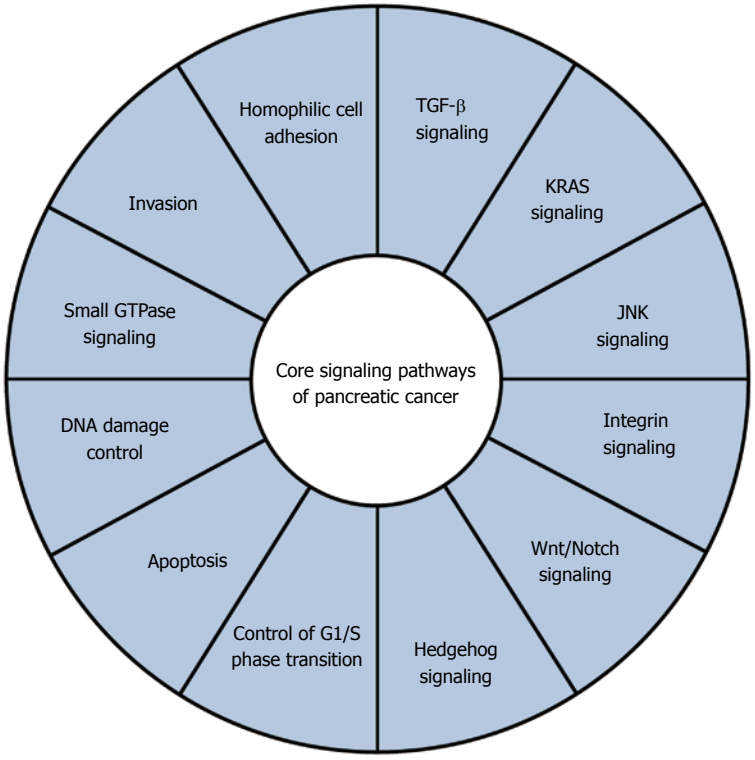

Figure 1 Core signaling pathways of pancreatic cancer. Twelve signaling pathways and cellular processes that are important in pancreatic cancer have been identified based on whole-exome sequencing analysis[9]. Various component genes associated with each pathway are mutated in most pancreatic cancers. Targeting one or more of these pathways, rather than specific gene alterations that occur within a pathway, would be a new strategy for treatment of pancreatic cancer. KRAS: V-ki-ras2 Kirsten rat sarcoma viral oncogene homolog; JNK: C-jun N-terminal kinase; TGF-β: Transforming growth factor-β.

related to chromatin remodeling (*EPC1*, *ARID2*) and DNA damage repair (*ATM*)[26]. In addition to the core signaling pathways mentioned above[9], they identified significant alterations in genes related to the axon guidance pathway, including *ROBO1/2* and *SLIT2*[26]. More recently, whole-genome analysis of 100 PDACs provided a comprehensive picture of the genomic alterations in this disease[27]. In addition to genes known to be important in PDAC (*TP53*, *SMAD4*, *CDKN2A*, *ARID1A* and *ROBO2*), chromosomal rearrangements affecting *KDM6A* and *PREX2* were identified. *KDM6A* is related to chromatin remodeling, and is mutated in renal cell carcinoma and medulloblastoma[28,29]. The RAC1 guanine nucleotide exchange factor, *PREX2*, is mutated in melanoma[30]. Copy number analysis also uncovered a number of amplifications in genomic regions including *KRAS* and *GATA6*[27], in accordance with a previous report[31]. Most importantly, they demonstrated that a small fraction of patients (1%-2%) harbor focal amplifications in druggable genes, including *ERBB2*, *MET*, *FGFR1*, *CDK6*, *PIK3CA* and *PIK3R3*[27].

Some germline mutations are known to be associated with familial clusters of pancreatic cancer. For example, inactivation of *BRCA2*, which encodes a protein involved in DNA damage repair, is related to familial pancreatic cancer. Indeed, *BRCA2* mutation is associated with a 3.5- to 10-fold increased risk of pancreatic cancer, as well as increased risk of breast cancer and ovarian cancer[32,33]. Germline mutations in the Fanconi anemia genes, such as *FANCC*, *FANCG* and *PALB2* (also known as *FANCN*), are also implicated in familial pancreatic cancer[34-37]. In addition, germline mutation of *ATM* has recently been identified in subsets of familial pancreatic cancer[38].

IMPLICATIONS OF GENOMIC ALTERATIONS FOR TREATMENT OF PANCREATIC CANCER

The development of powerful sequencing technologies has led to a detailed knowledge of the human cancer genome, and it has become evident that some types of cancer can be effectively treated by targeted therapies based on their specific gene alterations. Here we discuss potential approaches for gene alteration-based treatment of pancreatic cancer.

The most prevalent oncogenic alteration, in *KRAS*, seems an obvious target for cancer therapy, because mutant KRAS protein has been experimentally demonstrated to play a pivotal role in maintenance of PDAC[39,40]. Activating mutations at *KRAS* codons 12, 13 and occasionally 61 are currently the most common gene alterations in pancreatic cancer. A therapeutic effect of blocking G12D mutant *KRAS* has been demonstrated by using siRNA and a novel siRNA delivery system, both *in vitro* and *in vivo*[41]. Although great efforts have been made to develop small-molecular inhibitors of mutant *KRAS*, no clinically effective antagonist has yet been identified[42]. Instead, some indirect approaches, such as targeting post-transcriptional processes, have been tried. Farnesylation of KRAS allows the protein to associate with the membrane and interact with Ras activating proteins, including Ras-GEFs. Farnesyltransferase is the key enzyme involved in addition of a 15-carbon isoprenoid chain to

KRAS protein. However, despite *in vitro* and xenograft studies[43], farnesyltransferase inhibitors, such as tipifarnib, have proven unsuccessful in combination with gemcitabine[44,45]. This can be attributed to the existence of an alternative post-transcriptional mechanism, geranyl-geranylation, that compensates for inhibition of farnesyltransferase[46]. A dual inhibitor of farnesyltransferase and geranylgeranyltransferase (L-778,123) was tested in a Phase Ⅰ clinical trial in combination with radiotherapy for locally advanced PDAC, and showed acceptable toxicity[47]. Some groups have recently investigated strategies targeting localization of KRAS to the membrane. Deltarasin is a small molecule that binds to the farnesyl-binding pocket of the delta subunit of phosphodiesterase (PDEδ) and inhibits translocation of KRAS to the membrane by blocking the interaction between PDEδ and farnesylated KRAS[48,49]. On the other hand, Salirasib blocks KRAS activation by dislodging the farnesylated protein from the membrane[50]. The results of preclinical and clinical trials suggest that salirasib may be effective[51].

Targeting downstream effectors of KRAS may be an alternative approach to block the KRAS signaling pathway. The MEK/MAPK and PI3K/Akt/mTOR pathways are the principal downstream pathways of KRAS. But, although several MEK inhibitors, such as CI-1040 and PD0325901, have been investigated in clinical trials, they failed to deliver meaningful therapeutic benefit[52,53]. In addition, trametinib, another MEK1/2 inhibitor, was recently tested in combination with gemcitabine for patients with metastatic pancreatic cancer, but failed to improve the clinical outcome[54]. Activation of the PI3K/Akt/mTOR pathway also plays an important role in maintenance of pancreatic cancer[55-57]. An inhibitor of PI3K, LY294002, was reported to induce apoptosis *in vitro* and to inhibit tumor growth *in vivo*[58]. In addition, everolimus, a mammalian target of rapamycin (mTOR) inhibitor, has been reported to inhibit tumor growth *in vivo*[59]. However, everolimus had minimal activity in patients with gemcitabine-resistant PDAC in a phase Ⅱ study[60,61]. It was recently found that tumors with activated *KRAS* and mutant *TP53* did not respond to mTOR inhibition, whereas tumors with *KRAS* activation and *PTEN* loss are responsive to mTOR inhibition[62].

Since the MEK/MAPK and PI3K/Akt/mTOR pathways are both downstream of KRAS, it is possible that inhibition of one pathway induces compensatory activation of the other pathway. Therefore, inhibition of both pathways may have a synergistic effect in treatment of pancreatic cancer[63,64]; thus, simultaneous blockade of MEK/MAPK and PI3K/Akt/mTOR seems to warrant further investigation as a candidate therapy for pancreatic cancer.

In addition to *KRAS*, *CDKN2A*, *TP53* and *SMAD4* are also commonly altered in pancreatic cancer. However, therapeutic approaches targeting these proteins are considered to be difficult for various reasons, including cellular location and multifunctionality. Although a number of therapeutic strategies targeting these genes have been examined for various types of cancer, none has yet been implemented for treatment of pancreatic cancer.

Focusing on signaling pathways in pancreatic cancer may be a better strategy than targeting particular gene alterations for treatment of pancreatic cancer. The core signaling pathways of pancreatic cancer[9] include several druggable pathways. For example, the Wnt/Notch pathway is important, and inhibition of the Notch pathway by inhibiting γ-secretase has been suggested as a potential treatment strategy[65]. The combination of γ-secretase inhibitor MRK003 with gemcitabine has been shown to provide a survival benefit *in vivo*[66]. It has also been reported that pancreatic cancer cells that harbor inactivating mutations of *RNF43* are sensitive to LGK974, a Wnt pathway inhibitor currently in a phase 1 clinical trial[67]. Inhibition of the Hedgehog pathway with a natural hedgehog antagonist, cyclopamine, decreases growth of various types of tumor, including PDAC[68,69]. Clinical use of cyclopamine, however, is problematic because of its side effects and suboptimal pharmacokinetics. A novel, orally bioavailable, small-molecular Hedgehog inhibitor, IPI-269609, has been shown to inhibit tumor initiation and metastasis of pancreatic cancer[70]. Interestingly, blockade of the Hedgehog pathway has also been proposed as a means to target the tumor stroma and improve delivery of gemcitabine *in vivo*[71]. Small-molecular inhibitor Saridegib (IPI-926) was tested in combination with gemcitabine in patients with pancreatic cancer. However, the Phase Ⅰ/Ⅱb trial was stopped because patients receiving the combination had higher rates of progressive disease and lower overall survival in 2012[72].

Although the frequencies are low, mutations of several familial pancreatic cancer-related genes are associated with drug sensitivity. Inactivation of *BRCA2* is found in about 7% of western PDAC patients[32,73]. BRCA2 plays a crucial role in homologous recombination-based DNA damage repair processes[74]. Poly ADP-ribose polymerase (PARP) is an important enzyme in the DNA repair mechanism mediated by BRCA2, and PARP inhibitors induce extreme genome instability and death of *BRCA*-mutated cancer cells[75]. As well as PARP inhibitors, DNA-crosslinking agents such as mitomycin C, cisplatin and carboplatin are also effective for treatment of BRCA-inactivated pancreatic cancer[76]. As *PALB2* encodes a protein that interacts with BRCA2, *PALB2* mutations are expected to disrupt BRCA2-mediated repair of DNA double strand breaks. *PALB2* mutations in PDAC patients confer sensitivity to DNA-damaging agents[77]. Tumors with mutations in *ATM*, another familial pancreatic cancer-related gene, might also be sensitive to PARP inhibitors[78].

Overall, pancreatic cancer is characterized by substantial genomic heterogeneity with numerous infrequently mutated genes[9,26,27]. Although the common mutations in pancreatic cancer, *KRAS*, *TP53*, *CDKN2A* and *SMAD4*, are currently not druggable, stratified therapeutic strategies based on genomic alterations

that occur at low frequency might be beneficial for treatment of pancreatic cancer. Recently, Jones *et al*[79] identified somatic alteration in potentially druggable genes in approximately 20% of PDAC patients. In Australia, the Individualized Molecular Pancreatic Cancer Therapy (IMPaCT) trial screens patients for actionable molecular phenotypes, with the aim of developing personalized therapies for pancreatic cancer[80]. IMPaCT is a randomized phase Ⅱ clinical trial designed to assess standard therapy (gemcitabine) *vs* genotype-guided target therapies in patients with recurrent or metastatic pancreatic cancer. Initially, three subgroups with pre-defined actionable mutations, *i.e., HER2*-amplified (gemcitabine + trastuzumab), DNA damage response-defective (gemcitabine + PARP inhibitor) and anti-EGFR-responsive (gemcitabine + erlotinib), are being tested. This clinical trial was designed so that other arms could be added as novel subgroups or agents are identified. This approach could facilitate development of personalized therapies for pancreatic cancer.

CONCLUSION

Comprehensive genomic studies have provided extensive information on the pancreatic cancer genome, including its heterogeneity and core signaling pathways. These findings should be useful for the development of novel therapeutic strategies. For example, it might be helpful for early detection of pancreatic cancer to identify individuals with a genetic predisposition for the disease, including familial pancreatic cancer-related genes, so that periodic follow-up screening can be performed. Analysis of clonal evolution of pancreatic cancer indicates that it takes more than 10 years from occurrence of the initiating genomic alteration to formation of the parental clone[81]. Thus, there appears to be a substantial time window for early detection. Current sensitive sequencing technologies allow us to detect tumor DNA of various types of cancer in plasma (circulating tumor DNA, ctDNA)[82], and indeed, ctDNA has been detected in plasma from patients with early-stage breast and lung cancers[83,84]. Such an approach could also be applicable to patients with pancreatic cancer. More comprehensive genomic analysis may also be useful for identifying actionable mutations. Furthermore, ctDNA is thought to reflect the genetic heterogeneity of cancer, since it may contain tumor DNA derived from various regions, including metastases. Novel strategies based on genomic information seem likely to revolutionize pancreatic cancer therapy over the next few years, and may ultimately lead to fully personalized medicine.

REFERENCES

1 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

2 **Verslype C**, Van Cutsem E, Dicato M, Cascinu S, Cunningham D, Diaz-Rubio E, Glimelius B, Haller D, Haustermans K, Heinemann V, Hoff P, Johnston PG, Kerr D, Labianca R, Louvet C, Minsky B, Moore M, Nordlinger B, Pedrazzoli S, Roth A, Rothenberg M, Rougier P, Schmoll HJ, Tabernero J, Tempero M, van de Velde C, Van Laethem JL, Zalcberg J. The management of pancreatic cancer. Current expert opinion and recommendations derived from the 8th World Congress on Gastrointestinal Cancer, Barcelona, 2006. *Ann Oncol* 2007; **18** Suppl 7: vii1-vii10 [PMID: 17600091 DOI: 10.1093/annonc/mdm210]

3 **Hidalgo M**. Pancreatic cancer. *N Engl J Med* 2010; **362**: 1605-1617 [PMID: 20427809 DOI: 10.1056/NEJMra0901557]

4 **Burris HA**, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403-2413 [PMID: 9196156]

5 **Stathis A**, Moore MJ. Advanced pancreatic carcinoma: current treatment and future challenges. *Nat Rev Clin Oncol* 2010; **7**: 163-172 [PMID: 20101258 DOI: 10.1038/nrclinonc.2009.236]

6 **Moore MJ**, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960-1966 [PMID: 17452677 DOI: 10.1200/JCO.2006.07.9525]

7 **Von Hoff DD**, Ramanathan RK, Borad MJ, Laheru DA, Smith LS, Wood TE, Korn RL, Desai N, Trieu V, Iglesias JL, Zhang H, Soon-Shiong P, Shi T, Rajeshkumar NV, Maitra A, Hidalgo M. Gemcitabine plus nab-paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. *J Clin Oncol* 2011; **29**: 4548-4554 [PMID: 21969517 DOI: 10.1200/JCO.2011.36.5742]

8 **Vaccaro V**, Sperduti I, Milella M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **365**: 768-779; author reply 769 [PMID: 21864184 DOI: 10.1056/NEJMc1107627]

9 **Jones S**, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 2008; **321**: 1801-1806 [PMID: 18772397 DOI: 10.1126/science.1164368]

10 **Almoguera C**, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. *Cell* 1988; **53**: 549-554 [PMID: 2453289 DOI: 10.1016/0092-8674(88)90571-5]

11 **Rozenblum E**, Schutte M, Goggins M, Hahn SA, Panzer S, Zahurak M, Goodman SN, Sohn TA, Hruban RH, Yeo CJ, Kern SE. Tumor-suppressive pathways in pancreatic carcinoma. *Cancer Res* 1997; **57**: 1731-1734 [PMID: 9135016]

12 **Löhr M**, Klöppel G, Maisonneuve P, Lowenfels AB, Lüttges J. Frequency of K-ras mutations in pancreatic intraductal neoplasias associated with pancreatic ductal adenocarcinoma and chronic pancreatitis: a meta-analysis. *Neoplasia* 2005; **7**: 17-23 [PMID: 15720814 DOI: 10.1593/neo.04445]

13 **Jancík S**, Drábek J, Radzioch D, Hajdúch M. Clinical relevance of KRAS in human cancers. *J Biomed Biotechnol* 2010; **2010**: 150960 [PMID: 20617134 DOI: 10.1155/2010/150960]

14 **Campbell SL**, Khosravi-Far R, Rossman KL, Clark GJ, Der CJ. Increasing complexity of Ras signaling. *Oncogene* 1998; **17**: 1395-1413 [PMID: 9779987 DOI: 10.1038/sj.onc.1202174]

15 **Malumbres M**, Barbacid M. RAS oncogenes: the first 30 years. *Nat Rev Cancer* 2003; **3**: 459-465 [PMID: 12778136 DOI: 10.1038/

nrc1097]
16 **Caldas C**, Hahn SA, da Costa LT, Redston MS, Schutte M, Seymour AB, Weinstein CL, Hruban RH, Yeo CJ, Kern SE. Frequent somatic mutations and homozygous deletions of the p16 (MTS1) gene in pancreatic adenocarcinoma. *Nat Genet* 1994; **8**: 27-32 [PMID: 7726912 DOI: 10.1038/ng0994-27]
17 **Schutte M**, Hruban RH, Geradts J, Maynard R, Hilgers W, Rabindran SK, Moskaluk CA, Hahn SA, Schwarte-Waldhoff I, Schmiegel W, Baylin SB, Kern SE, Herman JG. Abrogation of the Rb/p16 tumor-suppressive pathway in virtually all pancreatic carcinomas. *Cancer Res* 1997; **57**: 3126-3130 [PMID: 9242437]
18 **Wilentz RE**, Geradts J, Maynard R, Offerhaus GJ, Kang M, Goggins M, Yeo CJ, Kern SE, Hruban RH. Inactivation of the p16 (INK4A) tumor-suppressor gene in pancreatic duct lesions: loss of intranuclear expression. *Cancer Res* 1998; **58**: 4740-4744 [PMID: 9788631]
19 **Moskaluk CA**, Hruban RH, Kern SE. p16 and K-ras gene mutations in the intraductal precursors of human pancreatic adenocarcinoma. *Cancer Res* 1997; **57**: 2140-2143 [PMID: 9187111]
20 **Nigro JM**, Baker SJ, Preisinger AC, Jessup JM, Hostetter R, Cleary K, Bigner SH, Davidson N, Baylin S, Devilee P. Mutations in the p53 gene occur in diverse human tumour types. *Nature* 1989; **342**: 705-708 [PMID: 2531845 DOI: 10.1038/342705a0]
21 **Barton CM**, Staddon SL, Hughes CM, Hall PA, O'Sullivan C, Klöppel G, Theis B, Russell RC, Neoptolemos J, Williamson RC. Abnormalities of the p53 tumour suppressor gene in human pancreatic cancer. *Br J Cancer* 1991; **64**: 1076-1082 [PMID: 1764370 DOI: 10.1038/bjc.1991.467]
22 **Redston MS**, Caldas C, Seymour AB, Hruban RH, da Costa L, Yeo CJ, Kern SE. p53 mutations in pancreatic carcinoma and evidence of common involvement of homocopolymer tracts in DNA microdeletions. *Cancer Res* 1994; **54**: 3025-3033 [PMID: 8187092]
23 **Hahn SA**, Schutte M, Hoque AT, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, Yeo CJ, Hruban RH, Kern SE. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science* 1996; **271**: 350-353 [PMID: 8553070 DOI: 10.1126/science.271.5247.350]
24 **Wilentz RE**, Iacobuzio-Donahue CA, Argani P, McCarthy DM, Parsons JL, Yeo CJ, Kern SE, Hruban RH. Loss of expression of Dpc4 in pancreatic intraepithelial neoplasia: evidence that DPC4 inactivation occurs late in neoplastic progression. *Cancer Res* 2000; **60**: 2002-2006 [PMID: 10766191]
25 **Lüttges J**, Galehdari H, Bröcker V, Schwarte-Waldhoff I, Henne-Bruns D, Klöppel G, Schmiegel W, Hahn SA. Allelic loss is often the first hit in the biallelic inactivation of the p53 and DPC4 genes during pancreatic carcinogenesis. *Am J Pathol* 2001; **158**: 1677-1683 [PMID: 11337365 DOI: 10.1016/S0002-9440(10)64123-5]
26 **Biankin AV**, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL, Miller DK, Wilson PJ, Patch AM, Wu J, Chang DK, Cowley MJ, Gardiner BB, Song S, Harliwong I, Idrisoglu S, Nourse C, Nourbakhsh E, Manning S, Wani S, Gongora M, Pajic M, Scarlett CJ, Gill AJ, Pinho AV, Rooman I, Anderson M, Holmes O, Leonard C, Taylor D, Wood S, Xu Q, Nones K, Fink JL, Christ A, Bruxner T, Cloonan N, Kolle G, Newell F, Pinese M, Mead RS, Humphris JL, Kaplan W, Jones MD, Colvin EK, Nagrial AM, Humphrey ES, Chou A, Chin VT, Chantrill LA, Mawson A, Samra JS, Kench JG, Lovell JA, Daly RJ, Merrett ND, Toon C, Epari K, Nguyen NQ, Barbour A, Zeps N, Australian Pancreatic Cancer Genome I, Kakkar N, Zhao F, Wu YQ, Wang M, Muzny DM, Fisher WE, Brunicardi FC, Hodges SE, Reid JG, Drummond J, Chang K, Han Y, Lewis LR, Dinh H, Buhay CJ, Beck T, Timms L, Sam M, Begley K, Brown A, Pai D, Panchal A, Buchner N, De Borja R, Denroche RE, Yung CK, Serra S, Onetto N, Mukhopadhyay D, Tsao MS, Shaw PA, Petersen GM, Gallinger S, Hruban RH, Maitra A, Iacobuzio-Donahue CA, Schulick RD, Wolfgang CL, Morgan RA, Lawlor RT, Capelli P, Corbo V, Scardoni M, Tortora G, Tempero MA, Mann KM, Jenkins NA, Perez-Mancera PA, Adams DJ, Largaespada DA, Wessels LF, Rust AG, Stein LD, Tuveson DA, Copeland NG, Musgrove EA, Scarpa A, Eshleman JR, Hudson TJ, Sutherland RL, Wheeler DA, Pearson JV, McPherson JD, Gibbs RA, Grimmond SM. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature* 2012; **491**: 399-405 [PMID: 23103869 DOI: 10.1038/nature11547]
27 **Waddell N**, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P, Johns AL, Miller D, Nones K, Quek K, Quinn MC, Robertson AJ, Fadlullah MZ, Bruxner TJ, Christ AN, Harliwong I, Idrisoglu S, Manning S, Nourse C, Nourbakhsh E, Wani S, Wilson PJ, Markham E, Cloonan N, Anderson MJ, Fink JL, Holmes O, Kazakoff SH, Leonard C, Newell F, Poudel B, Song S, Taylor D, Waddell N, Wood S, Xu Q, Wu J, Pinese M, Cowley MJ, Lee HC, Jones MD, Nagrial AM, Humphris J, Chantrill LA, Chin V, Steinmann AM, Mawson A, Humphrey ES, Colvin EK, Chou A, Scarlett CJ, Pinho AV, Giry-Laterriere M, Rooman I, Samra JS, Kench JG, Pettitt JA, Merrett ND, Toon C, Epari K, Nguyen NQ, Barbour A, Zeps N, Jamieson NB, Graham JS, Niclou SP, Bjerkvig R, Grützmann R, Aust D, Hruban RH, Maitra A, Iacobuzio-Donahue CA, Wolfgang CL, Morgan RA, Lawlor RT, Corbo V, Bassi C, Falconi M, Zamboni G, Tortora G, Tempero MA, Gill AJ, Eshleman JR, Pilarsky C, Scarpa A, Musgrove EA, Pearson JV, Biankin AV, Grimmond SM. Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* 2015; **518**: 495-501 [PMID: 25719666 DOI: 10.1038/nature14169]
28 **Varela I**, Tarpey P, Raine K, Huang D, Ong CK, Stephens P, Davies H, Jones D, Lin ML, Teague J, Bignell G, Butler A, Cho J, Dalgliesh GL, Galappaththige D, Greenman C, Hardy C, Jia M, Latimer C, Lau KW, Marshall J, McLaren S, Menzies A, Mudie L, Stebbings L, Largaespada DA, Wessels LF, Richard S, Kahnoski RJ, Anema J, Tuveson DA, Perez-Mancera PA, Mustonen V, Fischer A, Adams DJ, Rust A, Chan-on W, Subimerb C, Dykema K, Furge K, Campbell PJ, Teh BT, Stratton MR, Futreal PA. Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. *Nature* 2011; **469**: 539-542 [PMID: 21248752 DOI: 10.1038/nature09639]
29 **Robinson G**, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, Phoenix TN, Hedlund E, Wei L, Zhu X, Chalhoub N, Baker SJ, Huether R, Kriwacki R, Curley N, Thiruvenkatam R, Wang J, Wu G, Rusch M, Hong X, Becksfort J, Gupta P, Ma J, Easton J, Vadodaria B, Onar-Thomas A, Lin T, Li S, Pounds S, Paugh S, Zhao D, Kawauchi D, Roussel MF, Finkelstein D, Ellison DW, Lau CC, Bouffet E, Hassall T, Gururangan S, Cohn R, Fulton RS, Fulton LL, Dooling DJ, Ochoa K, Gajjar A, Mardis ER, Wilson RK, Downing JR, Zhang J, Gilbertson RJ. Novel mutations target distinct subgroups of medulloblastoma. *Nature* 2012; **488**: 43-48 [PMID: 22722829 DOI: 10.1038/nature11213]
30 **Berger MF**, Hodis E, Heffernan TP, Deribe YL, Lawrence MS, Protopopov A, Ivanova E, Watson IR, Nickerson E, Ghosh P, Zhang H, Zeid R, Ren X, Cibulskis K, Sivachenko AY, Wagle N, Sucker A, Sougnez C, Onofrio R, Ambrogio L, Auclair D, Fennell T, Carter SL, Drier Y, Stojanov P, Singer MA, Voet D, Jing R, Saksena G, Barretina J, Ramos AH, Pugh TJ, Stransky N, Parkin M, Winckler W, Mahan S, Ardlie K, Baldwin J, Wargo J, Schadendorf D, Meyerson M, Gabriel SB, Golub TR, Wagner SN, Lander ES, Getz G, Chin L, Garraway LA. Melanoma genome sequencing reveals frequent PREX2 mutations. *Nature* 2012; **485**: 502-506 [PMID: 22622578 DOI: 10.1038/nature11071]
31 **Zhong Y**, Wang Z, Fu B, Pan F, Yachida S, Dhara M, Albesiano E, Li L, Naito Y, Vilardell F, Cummings C, Martinelli P, Li A, Yonescu R, Ma Q, Griffin CA, Real FX, Iacobuzio-Donahue CA. GATA6 activates Wnt signaling in pancreatic cancer by negatively regulating the Wnt antagonist Dickkopf-1. *PLoS One* 2011; **6**: e22129 [PMID: 21811562 DOI: 10.1371/journal.pone.0022129]
32 **Goggins M**, Schutte M, Lu J, Moskaluk CA, Weinstein CL, Petersen GM, Yeo CJ, Jackson CE, Lynch HT, Hruban RH, Kern SE. Germline BRCA2 gene mutations in patients with apparently sporadic pancreatic carcinomas. *Cancer Res* 1996; **56**: 5360-5364 [PMID: 8968085]
33 **Klein AP**. Genetic susceptibility to pancreatic cancer. *Mol Carcinog* 2012; **51**: 14-24 [PMID: 22162228 DOI: 10.1002/mc.20855]

34 **van der Heijden MS**, Yeo CJ, Hruban RH, Kern SE. Fanconi anemia gene mutations in young-onset pancreatic cancer. *Cancer Res* 2003; **63**: 2585-2588 [PMID: 12750283]

35 **Rogers CD**, van der Heijden MS, Brune K, Yeo CJ, Hruban RH, Kern SE, Goggins M. The genetics of FANCC and FANCG in familial pancreatic cancer. *Cancer Biol Ther* 2004; **3**: 167-169 [PMID: 14726700]

36 **Couch FJ**, Johnson MR, Rabe K, Boardman L, McWilliams R, de Andrade M, Petersen G. Germ line Fanconi anemia complementation group C mutations and pancreatic cancer. *Cancer Res* 2005; **65**: 383-386 [PMID: 15695377]

37 **Jones S**, Hruban RH, Kamiyama M, Borges M, Zhang X, Parsons DW, Lin JC, Palmisano E, Brune K, Jaffee EM, Iacobuzio-Donahue CA, Maitra A, Parmigiani G, Kern SE, Velculescu VE, Kinzler KW, Vogelstein B, Eshleman JR, Goggins M, Klein AP. Exomic sequencing identifies PALB2 as a pancreatic cancer susceptibility gene. *Science* 2009; **324**: 217 [PMID: 19264984 DOI: 10.1126/science.1171202]

38 **Roberts NJ**, Jiao Y, Yu J, Kopelovich L, Petersen GM, Bondy ML, Gallinger S, Schwartz AG, Syngal S, Cote ML, Axilbund J, Schulick R, Ali SZ, Eshleman JR, Velculescu VE, Goggins M, Vogelstein B, Papadopoulos N, Hruban RH, Kinzler KW, Klein AP. ATM mutations in patients with hereditary pancreatic cancer. *Cancer Discov* 2012; **2**: 41-46 [PMID: 22585167 DOI: 10.1158/2159-8290.CD-11-0194]

39 **Brummelkamp TR**, Bernards R, Agami R. Stable suppression of tumorigenicity by virus-mediated RNA interference. *Cancer Cell* 2002; **2**: 243-247 [PMID: 12242156]

40 **Fleming JB**, Shen GL, Holloway SE, Davis M, Brekken RA. Molecular consequences of silencing mutant K-ras in pancreatic cancer cells: justification for K-ras-directed therapy. *Mol Cancer Res* 2005; **3**: 413-423 [PMID: 16046552 DOI: 10.1158/1541-7786.MCR-04-0206]

41 **Zorde Khvalevsky E**, Gabai R, Rachmut IH, Horwitz E, Brunschwig Z, Orbach A, Shemi A, Golan T, Domb AJ, Yavin E, Giladi H, Rivkin L, Simerzin A, Eliakim R, Khalaileh A, Hubert A, Lahav M, Kopelman Y, Goldin E, Dancour A, Hants Y, Arbel-Alon S, Abramovitch R, Shemi A, Galun E. Mutant KRAS is a druggable target for pancreatic cancer. *Proc Natl Acad Sci USA* 2013; **110**: 20723-20728 [PMID: 24297898 DOI: 10.1073/pnas.1314307110]

42 **Ledford H**. Cancer: The Ras renaissance. *Nature* 2015; **520**: 278-280 [PMID: 25877186 DOI: 10.1038/520278a]

43 **Omer CA**, Kohl NE. CA1A2X-competitive inhibitors of farnesyl-transferase as anti-cancer agents. *Trends Pharmacol Sci* 1997; **18**: 437-444 [PMID: 9426472 DOI: 10.1016/S0165-6147(97)01129-2]

44 **Van Cutsem E**, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, Schoffski P, Post S, Verslype C, Neumann H, Safran H, Humblet Y, Perez Ruixo J, Ma Y, Von Hoff D. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. *J Clin Oncol* 2004; **22**: 1430-1438 [PMID: 15084616 DOI: 10.1200/JCO.2004.10.112]

45 **Appels NM**, Beijnen JH, Schellens JH. Development of farnesyl transferase inhibitors: a review. *Oncologist* 2005; **10**: 565-578 [PMID: 16177281 DOI: 10.1634/theoncologist.10-8-565]

46 **Whyte DB**, Kirschmeier P, Hockenberry TN, Nunez-Oliva I, James L, Catino JJ, Bishop WR, Pai JK. K- and N-Ras are geranylgeranylated in cells treated with farnesyl protein transferase inhibitors. *J Biol Chem* 1997; **272**: 14459-14464 [PMID: 9162087 DOI: 10.1074/jbc.272.22.14459]

47 **Martin NE**, Brunner TB, Kiel KD, DeLaney TF, Regine WF, Mohiuddin M, Rosato EF, Haller DG, Stevenson JP, Smith D, Pramanik B, Tepper J, Tanaka WK, Morrison B, Deutsch P, Gupta AK, Muschel RJ, McKenna WG, Bernhard EJ, Hahn SM. A phase I trial of the dual farnesyltransferase and geranylgeranyltransferase inhibitor L-778,123 and radiotherapy for locally advanced pancreatic cancer. *Clin Cancer Res* 2004; **10**: 5447-5454 [PMID: 15328183 DOI: 10.1158/1078-0432.CCR-04-0248]

48 **Zimmermann G**, Papke B, Ismail S, Vartak N, Chandra A, Hoffmann M, Hahn SA, Triola G, Wittinghofer A, Bastiaens PI, Waldmann H. Small molecule inhibition of the KRAS-PDEδ interaction impairs oncogenic KRAS signalling. *Nature* 2013; **497**: 638-642 [PMID: 23698361 DOI: 10.1038/nature12205]

49 **Chandra A**, Grecco HE, Pisupati V, Perera D, Cassidy L, Skoulidis F, Ismail SA, Hedberg C, Hanzal-Bayer M, Venkitaraman AR, Wittinghofer A, Bastiaens PI. The GDI-like solubilizing factor PDEδ sustains the spatial organization and signalling of Ras family proteins. *Nat Cell Biol* 2012; **14**: 148-158 [PMID: 22179043 DOI: 10.1038/ncb2394]

50 **Weisz B**, Giehl K, Gana-Weisz M, Egozi Y, Ben-Baruch G, Marciano D, Gierschik P, Kloog Y. A new functional Ras antagonist inhibits human pancreatic tumor growth in nude mice. *Oncogene* 1999; **18**: 2579-2588 [PMID: 10353601 DOI: 10.1038/sj.onc.1202602]

51 **Laheru D**, Shah P, Rajeshkumar NV, McAllister F, Taylor G, Goldsweig H, Le DT, Donehower R, Jimeno A, Linden S, Zhao M, Song D, Rudek MA, Hidalgo M. Integrated preclinical and clinical development of S-trans, trans-Farnesylthiosalicylic Acid (FTS, Salirasib) in pancreatic cancer. *Invest New Drugs* 2012; **30**: 2391-2399 [PMID: 22547163 DOI: 10.1007/s10637-012-9818-6]

52 **Lorusso PM**, Adjei AA, Varterasian M, Gadgeel S, Reid J, Mitchell DY, Hanson L, DeLuca P, Bruzek L, Piens J, Asbury P, Van Becelaere K, Herrera R, Sebolt-Leopold J, Meyer MB. Phase I and pharmacodynamic study of the oral MEK inhibitor CI-1040 in patients with advanced malignancies. *J Clin Oncol* 2005; **23**: 5281-5293 [PMID: 16009947 DOI: 10.1200/JCO.2005.14.415]

53 **Haura EB**, Ricart AD, Larson TG, Stella PJ, Bazhenova L, Miller VA, Cohen RB, Eisenberg PD, Selaru P, Wilner KD, Gadgeel SM. A phase II study of PD-0325901, an oral MEK inhibitor, in previously treated patients with advanced non-small cell lung cancer. *Clin Cancer Res* 2010; **16**: 2450-2457 [PMID: 20332327 DOI: 10.1158/1078-0432.CCR-09-1920]

54 **Infante JR**, Somer BG, Park JO, Li CP, Scheulen ME, Kasubhai SM, Oh DY, Liu Y, Redhu S, Steplewski K, Le N. A randomised, double-blind, placebo-controlled trial of trametinib, an oral MEK inhibitor, in combination with gemcitabine for patients with untreated metastatic adenocarcinoma of the pancreas. *Eur J Cancer* 2014; **50**: 2072-2081 [PMID: 24915778 DOI: 10.1016/j.ejca.2014.04.024]

55 **Schlieman MG**, Fahy BN, Ramsamooj R, Beckett L, Bold RJ. Incidence, mechanism and prognostic value of activated AKT in pancreas cancer. *Br J Cancer* 2003; **89**: 2110-2115 [PMID: 14647146 DOI: 10.1038/sj.bjc.6601396]

56 **Agbunag C**, Bar-Sagi D. Oncogenic K-ras drives cell cycle progression and phenotypic conversion of primary pancreatic duct epithelial cells. *Cancer Res* 2004; **64**: 5659-5663 [PMID: 15313904 DOI: 10.1158/0008-5472.CAN-04-0807]

57 **Kong B**, Wu W, Cheng T, Schlitter AM, Qian C, Bruns P, Jian Z, Jäger C, Regel I, Raulefs S, Behler N, Irmler M, Beckers J, Friess H, Erkan M, Siveke JT, Tannapfel A, Hahn SA, Theis FJ, Esposito I, Kleeff J, Michalski CW. A subset of metastatic pancreatic ductal adenocarcinomas depends quantitatively on oncogenic Kras/Mek/Erk-induced hyperactive mTOR signalling. *Gut* 2015 Jan 19; Epub ahead of print [PMID: 25601637 DOI: 10.1136/gutjnl-2014-307616]

58 **Bondar VM**, Sweeney-Gotsch B, Andreeff M, Mills GB, McConkey DJ. Inhibition of the phosphatidylinositol 3'-kinase-AKT pathway induces apoptosis in pancreatic carcinoma cells in vitro and in vivo. *Mol Cancer Ther* 2002; **1**: 989-997 [PMID: 12481421]

59 **O'Reilly T**, McSheehy PM, Wartmann M, Lassota P, Brandt R, Lane HA. Evaluation of the mTOR inhibitor, everolimus, in combination with cytotoxic antitumor agents using human tumor models in vitro and in vivo. *Anticancer Drugs* 2011; **22**: 58-78 [PMID: 20890178 DOI: 10.1097/CAD.0b013e3283400a20]

60 **Javle MM**, Shroff RT, Xiong H, Varadhachary GA, Fogelman D, Reddy SA, Davis D, Zhang Y, Wolff RA, Abbruzzese JL. Inhibition of the mammalian target of rapamycin (mTOR) in advanced pancreatic cancer: results of two phase II studies. *BMC Cancer* 2010; **10**: 368 [PMID: 20630061 DOI: 10.1186/1471-2407-10-368]

61 **Wolpin BM**, Hezel AF, Abrams T, Blaszkowsky LS, Meyerhardt JA, Chan JA, Enzinger PC, Allen B, Clark JW, Ryan DP, Fuchs CS. Oral mTOR inhibitor everolimus in patients with gemcitabine-refractory metastatic pancreatic cancer. *J Clin Oncol* 2009; **27**: 193-198 [PMID: 19047305 DOI: 10.1200/JCO.2008.18.9514]

62 **Morran DC**, Wu J, Jamieson NB, Mrowinska A, Kalna G, Karim SA, Au AY, Scarlett CJ, Chang DK, Pajak MZ, Oien KA, McKay CJ, Carter CR, Gillen G, Champion S, Pimlott SL, Anderson KI, Evans TR, Grimmond SM, Biankin AV, Sansom OJ, Morton JP. Targeting mTOR dependency in pancreatic cancer. *Gut* 2014; **63**: 1481-1489 [PMID: 24717934 DOI: 10.1136/gutjnl-2013-306202]

63 **Williams TM**, Flecha AR, Keller P, Ram A, Karnak D, Galbán S, Galbán CJ, Ross BD, Lawrence TS, Rehemtulla A, Sebolt-Leopold J. Cotargeting MAPK and PI3K signaling with concurrent radiotherapy as a strategy for the treatment of pancreatic cancer. *Mol Cancer Ther* 2012; **11**: 1193-1202 [PMID: 22411900 DOI: 10.1158/1535-7163.MCT-12-0098]

64 **Alagesan B**, Contino G, Guimaraes AR, Corcoran RB, Deshpande V, Wojtkiewicz GR, Hezel AF, Wong KK, Loda M, Weissleder R, Benes C, Engelman JA, Bardeesy N. Combined MEK and PI3K inhibition in a mouse model of pancreatic cancer. *Clin Cancer Res* 2015; **21**: 396-404 [PMID: 25348516 DOI: 10.1158/1078-0432.CCR-14-1591]

65 **Hu H**, Zhou L, Awadallah A, Xin W. Significance of Notch1-signaling pathway in human pancreatic development and carcinogenesis. *Appl Immunohistochem Mol Morphol* 2013; **21**: 242-247 [PMID: 23235341 DOI: 10.1097/PAI.0b013e3182655ab7]

66 **Cook N**, Frese KK, Bapiro TE, Jacobetz MA, Gopinathan A, Miller JL, Rao SS, Demuth T, Howat WJ, Jodrell DI, Tuveson DA. Gamma secretase inhibition promotes hypoxic necrosis in mouse pancreatic ductal adenocarcinoma. *J Exp Med* 2012; **209**: 437-444 [PMID: 22351932 DOI: 10.1084/jem.20111923]

67 **Jiang X**, Hao HX, Growney JD, Woolfenden S, Bottiglio C, Ng N, Lu B, Hsieh MH, Bagdasarian L, Meyer R, Smith TR, Avello M, Charlat O, Xie Y, Porter JA, Pan S, Liu J, McLaughlin ME, Cong F. Inactivating mutations of RNF43 confer Wnt dependency in pancreatic ductal adenocarcinoma. *Proc Natl Acad Sci USA* 2013; **110**: 12649-12654 [PMID: 23847203 DOI: 10.1073/pnas.1307218110]

68 **Feldmann G**, Habbe N, Dhara S, Bisht S, Alvarez H, Fendrich V, Beaty R, Mullendore M, Karikari C, Bardeesy N, Ouellette MM, Yu W, Maitra A. Hedgehog inhibition prolongs survival in a genetically engineered mouse model of pancreatic cancer. *Gut* 2008; **57**: 1420-1430 [PMID: 18515410 DOI: 10.1136/gut.2007.148189]

69 **Kelleher FC**. Hedgehog signaling and therapeutics in pancreatic cancer. *Carcinogenesis* 2011; **32**: 445-451 [PMID: 21186299 DOI: 10.1093/carcin/bgq280]

70 **Feldmann G**, Fendrich V, McGovern K, Bedja D, Bisht S, Alvarez H, Koorstra JB, Habbe N, Karikari C, Mullendore M, Gabrielson KL, Sharma R, Matsui W, Maitra A. An orally bioavailable small-molecule inhibitor of Hedgehog signaling inhibits tumor initiation and metastasis in pancreatic cancer. *Mol Cancer Ther* 2008; **7**: 2725-2735 [PMID: 18790753 DOI: 10.1158/1535-7163.MCT-08-0573]

71 **Olive KP**, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Rückert F, Grützmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J, Tuveson DA. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 2009; **324**: 1457-1461 [PMID: 19460966 DOI: 10.1126/science.1171362]

72 **Lou KJ**. Stromal uncertainties in pancreatic cancer. *SciBX* 2014; **7**: 23

73 **Schutte M**, da Costa LT, Hahn SA, Moskaluk C, Hoque AT, Rozenblum E, Weinstein CL, Bittner M, Meltzer PS, Trent JM. Identification by representational difference analysis of a homozygous deletion in pancreatic carcinoma that lies within the BRCA2 region. *Proc Natl Acad Sci USA* 1995; **92**: 5950-5954 [PMID: 7597059 DOI: 10.1073/pnas.92.13.5950]

74 **Howlett NG**, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, Persky N, Grompe M, Joenje H, Pals G, Ikeda H, Fox EA, D'Andrea AD. Biallelic inactivation of BRCA2 in Fanconi anemia. *Science* 2002; **297**: 606-609 [PMID: 12065746 DOI: 10.1126/science.1073834]

75 **Fong PC**, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, Ashworth A, Carmichael J, Kaye SB, Schellens JH, de Bono JS. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *N Engl J Med* 2009; **361**: 123-134 [PMID: 19553641 DOI: 10.1056/NEJMoa0900212]

76 **van der Heijden MS**, Brody JR, Gallmeier E, Cunningham SC, Dezentje DA, Shen D, Hruban RH, Kern SE. Functional defects in the fanconi anemia pathway in pancreatic cancer cells. *Am J Pathol* 2004; **165**: 651-657 [PMID: 15277238 DOI: 10.1016/S0002-9440(10)63329-9]

77 **Villarroel MC**, Rajeshkumar NV, Garrido-Laguna I, De Jesus-Acosta A, Jones S, Maitra A, Hruban RH, Eshleman JR, Klein A, Laheru D, Donehower R, Hidalgo M. Personalizing cancer treatment in the age of global genomic analyses: PALB2 gene mutations and the response to DNA damaging agents in pancreatic cancer. *Mol Cancer Ther* 2011; **10**: 3-8 [PMID: 21135251 DOI: 10.1158/1535-7163.MCT-10-0893]

78 **McCabe N**, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, Giavara S, O'Connor MJ, Tutt AN, Zdzienicka MZ, Smith GC, Ashworth A. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. *Cancer Res* 2006; **66**: 8109-8115 [PMID: 16912188 DOI: 10.1158/0008-5472.CAN-06-0140]

79 **Jones S**, Anagnostou V, Lytle K, Parpart-Li S, Nesselbush M, Riley DR, Shukla M, Chesnick B, Kadan M, Papp E, Galens KG, Murphy D, Zhang T, Kann L, Sausen M, Angiuoli SV, Diaz LA, Velculescu VE. Personalized genomic analyses for cancer mutation discovery and interpretation. *Sci Transl Med* 2015; **7**: 283ra53 [PMID: 25877891 DOI: 10.1126/scitranslmed.aaa7161]

80 **Cowley MJ**, Chang DK, Pajic M, Johns AL, Waddell N, Grimmond SM, Biankin AV. Understanding pancreatic cancer genomes. *J Hepatobiliary Pancreat Sci* 2013; **20**: 549-556 [PMID: 23660961 DOI: 10.1007/s00534-013-0610-6]

81 **Yachida S**, Jones S, Bozic I, Antal T, Leary R, Fu B, Kamiyama M, Hruban RH, Eshleman JR, Nowak MA, Velculescu VE, Kinzler KW, Vogelstein B, Iacobuzio-Donahue CA. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 2010; **467**: 1114-1117 [PMID: 20981102 DOI: 10.1038/nature09515]

82 **Bettegowda C**, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, Antonarakis ES, Azad NS, Bardelli A, Brem H, Cameron JL, Lee CC, Fecher LA, Gallia GL, Gibbs P, Le D, Giuntoli RL, Goggins M, Hogarty MD, Holdhoff M, Hong SM, Jiao Y, Juhl HH, Kim JJ, Siravegna G, Laheru DA, Lauricella C, Lim M, Lipson EJ, Marie SK, Netto GJ, Oliner KS, Olivi A, Olsson L, Riggins GJ, Sartore-Bianchi A, Schmidt K, Shih lM, Oba-Shinjo SM, Siena S, Theodorescu D, Tie J, Harkins TT, Veronese S, Wang TL, Weingart JD, Wolfgang CL, Wood LD, Xing D, Hruban RH, Wu J, Allen PJ, Schmidt CM, Choti MA, Velculescu VE, Kinzler KW, Vogelstein B, Papadopoulos N, Diaz LA. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014; **6**: 224ra24 [PMID: 24553385 DOI: 10.1126/scitranslmed.3007094]

83 **Beaver JA**, Jelovac D, Balukrishna S, Cochran RL, Croessmann S, Zabransky DJ, Wong HY, Valda Toro P, Cidado J, Blair BG, Chu D, Burns T, Higgins MJ, Stearns V, Jacobs L, Habibi M, Lange J, Hurley PJ, Lauring J, VanDenBerg DA, Kessler J, Jeter S, Samuels ML, Maar D, Cope L, Cimino-Mathews A, Argani P, Wolff AC, Park BH. Detection of cancer DNA in plasma of patients with early-stage breast cancer. *Clin Cancer Res* 2014; **20**: 2643-2650 [PMID:

24504125 DOI: 10.1158/1078-0432.CCR-13-2933]

84 **Newman AM**, Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA, Liu CL, Neal JW, Wakelee HA, Merritt RE, Shrager JB, Loo BW, Alizadeh AA, Diehn M. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med* 2014; **20**: 548-554 [PMID: 24705333 DOI: 10.1038/nm.3519]

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CASE REPORT

Paraneoplastic leukemoid reaction in pancreatic cancer: A case report

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Abstract

Paraneoplastic leukemoid reaction is a rare syndrome defined by a leukocyte count exceeding 50 Giga/Liter (G/L), mostly described with progressive lung or renal carcinoma. We report a case of a 68-year-old man with recurrent pancreatic carcinoma presenting a leukemoid reaction with a white blood cell count of 63.87 G/L without identified infectious, iatrogenic or hematologic causes. His overall condition quickly degraded and he died three weeks after the discovery of the leukemoid reaction. This is the first case in French literature of leukemoid reaction in a patient with pancreatic carcinoma with poor prognostic value.

Key words: Leukemoid reaction; Pancreatic neoplasms; Paraneoplastic syndrome; Prognosis

Core tip: Paraneoplastic leukemoid reaction is a rare syndrome which seems to be associated with aggressive tumors, rapid clinical deterioration, and short survival. We report a rare presentation of pancreatic cancer with leukemoid reaction in a 68-year-old man who died three weeks after its discovery. This paper may contribute to clinical practice when encountering such a patient because of its poor prognostic value.

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INTRODUCTION

Carcinoma is the most common (90%) and gravest type of pancreatic tumor with 5-year global survival

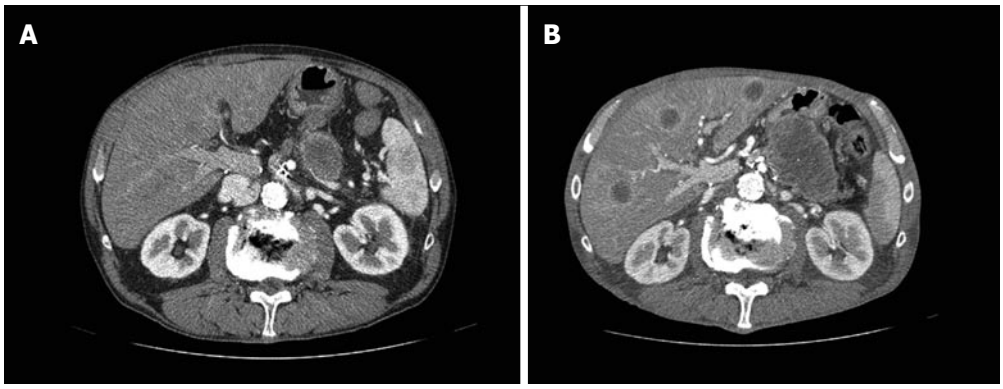

Figure 1 Computer tomography before (A) and after (B) leukemoid reaction. Pancreatic and hepatic evolution.

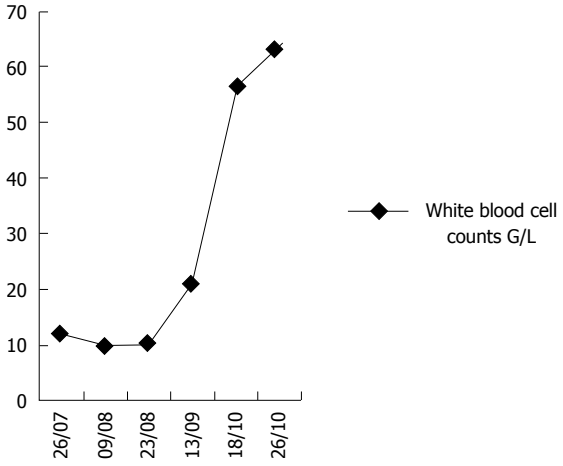

Figure 2 Evolution of white blood cell counts associated with leukemoid reaction. Leukocytosis rapid increase.

rates around 5%. In France, it is the fifth cause of cancer-related deaths and its incidence is increasing fast with approximately 8000 annual new cases. Paraneoplastic syndromes can occur in a minority of cancer cases (less than 10%) and are not directly related to the physical effects of the tumor. Those most frequently associated with pancreatic carcinoma are Trousseau's syndrome, Cushing's syndrome, and the unexplained prolonged fever. They can reveal the disease or arise during progression. They can decline under treatment, even disappear with the cure and reappear in case of relapse. Paraneoplastic leukemoid reaction is defined as leukocytosis exceeding 50 Giga/Liter (G/L). Its diagnosis rests essentially on the exclusion of infectious, hematologic or iatrogenic causes such as growth factor or corticosteroid therapy[1]. This syndrome is most frequently associated with carcinomas, in particular lung and renal[2,3], and is rarely described in cancers of the digestive tract, including pancreatic cancers.

CASE REPORT

We report the case of a 68-year-old man with pancreatic carcinoma, who was diagnosed with paraneoplastic leukemoid reaction in the absence of plausible differential diagnoses.

Our patient was diagnosed with pT2N0M0 carcinoma of the head of the pancreas, discovered by jaundice, and operated by cephalic duodenopancreatectomy. He then received adjuvant chemotherapy with 6 cycles of gemzar. One year later, tumor markers (carbohydrate antigen 19-9 and carcinoembryonic antigen) increased and a positron emission tomography scan detected a local recurrence. Radiological stabilization and a decrease of markers were obtained after 4 cycles of folfox. Therefore, 6 additional cycles were administered.

Follow-up imaging revealed local evolution and hepatic metastases. Tumor marker levels were increased. A new line of chemotherapy was begun with folfiri. After 4 cycles, hepatic (Figure 1) and pulmonary evolution were observed associated with a progressive generalized weakness. Nevertheless, due to the patient's strong insistence on treatment and a relatively stable overall condition, a third line of 5-fluorouracil (5-FU)/cisplatin was considered. During the first cycle, a white blood cell count showed extreme leukocytosis of 63.87 G/L (Figure 2), with neutrophil predominance of 92.7%, associated with a myelaemia of 1%, without abnormal eosinophilia, basophilia or anomaly of the other cell lines (hemoglobin 10.5 g/dL and platelets 207 G/L).

The patient had not received granulocyte colony-stimulating factors (G-CSF) or corticosteroids. Standard infectious investigations found no obvious sign of infection: C-reactive protein was slightly elevated at 138 mg/L, central and peripheral blood cultures as well as urine culture were negative, and a chest radiograph was normal. Moreover, a skeletal scintigraphy was performed and found no evidence of bone metastases. A cytological bone marrow examination showed a massively increased granulopoiesis with predominant neutrophils, complete maturation, without excess of blast cells or other anomalies that might suggest the existence of an acute leukaemia (Figure 3).

Molecular genetic analysis did not find a BCR-ABL fusion gene or a V617F mutation in the *JAK2* gene. The serum level of G-CSF was within normal range (< 40 pg/mL) and interleukin-6 (IL-6) was at 10 pg/mL (reference range: 0-10 pg/mL).

Only one cure of chemotherapy by 5-FU/cisplatin was

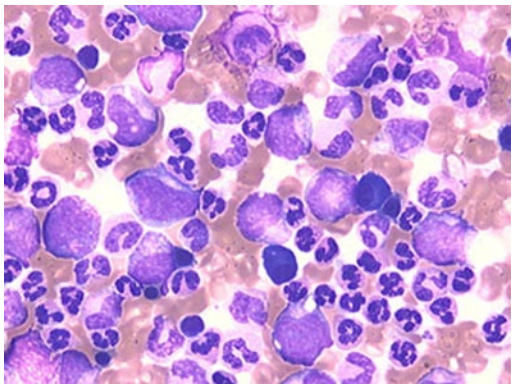

Figure 3 Bone marrow cytology (original magnification × 500). Increased granulopoiesis up to the neutrophils with a complete maturation and without blast cells.

administered, because of the patient's rapid deterioration. He died three weeks after the development of the leukemoid reaction. During this period, leukocyte count remained above 50 G/L.

DISCUSSION

Paraneoplastic leukemoid reaction has rarely been described in cancers of the digestive tract, in particular pancreatic carcinoma, with only four cases found in the literature[4-7]. This seems to be the first case of leukemoid reaction in a patient with pancreatic cancer reported in the French literature.

Making this diagnosis requires eliminating an infection, a treatment with corticoids or G-CSF, and the existence of hematologic neoplasia. This paraneoplastic syndrome has a poor prognostic value without a fast effective anti-tumor treatment, as illustrated by other reviews of the literature. Indeed, it is associated with aggressive tumors, rapid clinical deterioration, and short survival. The mechanism of this reaction is still not formally identified. Some data, concerning essentially lung cancers, suggest a secretion by tumor cells of hematopoietic growth factors such as G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF) inducing extreme leucocytosis[8,9]. Other mechanisms could also be involved in this reaction, in particular the production of pro-inflammatory cytokines in response to tumor progression or necrosis[10,11].

In our case, there was no elevation of G-CSF or IL-6, although serum levels were tested only once because of the fast change in the patient's overall condition. No elevations of these levels were found in other reports, implying the existence of other factors.

Paraneoplastic leukemoid reaction is rarely associated with pancreatic cancer.

The mechanisms, prognosis, and management of this syndrome are poorly understood. More data are needed to conclude.

Leukemoid reaction appears at an advanced stage and may be a prognostic indicator in patients with pancreatic cancer. It is advisable to quickly diagnose the condition, after elimination of other plausible causes, because of its poor prognostic value.

COMMENTS

Case characteristics

A 68-year-old man with pancreatic carcinoma presented a paraneoplastic leukemoid reaction.

Clinical diagnosis

Rapid clinical deterioration with generalized weakness.

Differential diagnosis

Infection, treatment with corticoids or granulocyte colony-stimulating factors and hematologic neoplasia.

Laboratory diagnosis

White blood cell count showed extreme leukocytosis of 63.87 G/L.

Imaging diagnosis

Computer tomography scans revealed progression of local, liver and lung disease.

Pathological diagnosis

Carcinoma of the pancreas.

Treatment

The tumor was treated by cephalic duodenopancreatectomy associated with adjuvant chemotherapy, and three additional lines of chemotherapy for metastatic disease.

Related reports

Poor prognostic value is also illustrated by other reviews of the literature with short survival. The mechanism of this reaction is still not formally identified, but some data suggest a secretion by tumor cells of hematopoietic growth factors or pro-inflammatory cytokines.

Term explanation

Paraneoplastic leukemoid reaction is defined as leukocytosis exceeding 50 G/L.

Experiences and lessons

Paraneoplastic leukemoid reaction is a rare syndrome, infrequently described with pancreatic cancer, which seems to be associated with poor prognostic value.

Peer-review

A very rare complication of pancreatic cancer with very rare occurence in gastrointestinal cancers and pancreatic cancer in peculiar, worth publishing to inform physicians. It is a step forward on the way of clarifying the pathogeny of this syndrome.

REFERENCES

1 **Halkes CJ**, Dijstelbloem HM, Eelkman Rooda SJ, Kramer MH. Extreme leucocytosis: not always leukaemia. *Neth J Med* 2007; **65**: 248-251 [PMID: 17656811]

2 **Riesenberg H**, Müller F, Görner M. Leukemoid reaction in a patient with adenocarcinoma of the lung: a case report. *J Med Case Rep* 2012; **6**: 211 [PMID: 22812671 DOI: 10.1186/1752-1947-6-211]

3 **Huang W**, Wang F, Li Y, Duan F, Yu Z. Leukemoid reaction in sarcomatoid renal cell carcinoma: a two-case report. *World J Surg Oncol* 2014; **12**: 100 [PMID: 24745762 DOI: 10.1186/1477-7819-12-100]

4 **Qureshi KM**, Raman AK, Tan D, Fakih MG. Leukemoid reaction in pancreatic cancer: a case report and review of the literature. *JOP*

2006; **7**: 631-634 [PMID: 17095843]
5 **Godquin B**. [Cancer of the pancreas and paraneoplastic leukemoid syndrome]. *Chirurgie* 1973; **98**: 785-787 [PMID: 4711525]
6 **Akoun G**, Duhamel G, Duvaldestin P, Koubi G, Brocard H. Myeloid leukemoid reaction in cancer of the pancreas: paraneoplastic syndrome or myeloid leukosis? *Sem Hop* 1972; **48**: 861-863 [PMID: 4339445]
7 **Akoun G**, Duhamel G, Duvaldestin P, Koubi G, Brocard H. Paraneoplastic leukemoid reaction of a myeloid type or myeloid leukemia in cancer of the pancreas? *Presse Med* 1971; **79**: 2327 [PMID: 5288840]
8 **Hocking W**, Goodman J, Golde D. Granulocytosis associated with tumor cell production of colony-stimulating activity. *Blood* 1983; **61**: 600-603 [PMID: 6600635]
9 **Jardin F**, Vasse M, Debled M, Dominique S, Courville P, Callonnec F, Buchonnet G, Thiberville L, Tilly H. Intense paraneoplastic neutrophilic leukemoid reaction related to a G-CSF-secreting lung sarcoma. *Am J Hematol* 2005; **80**: 243-245 [PMID: 16247754 DOI: 10.1002/ajh.20454]
10 **Inoue M**, Minami M, Fujii Y, Matsuda H, Shirakura R, Kido T. Granulocyte colony-stimulating factor and interleukin-6-producing lung cancer cell line, LCAM. *J Surg Oncol* 1997; **64**: 347-350 [PMID: 9142195 DOI: 10.1002/(SICI)1096-9098(199704)64]
11 **Chen YM**, Whang-Peng J, Liu JM, Chao Y, Lai CR, Wang SY, Perng RP. Leukemoid reaction resulting from multiple cytokine production in metastatic mucoepidermoid carcinoma with central necrosis. *Jpn J Clin Oncol* 1995; **25**: 168-172 [PMID: 7545252]

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