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MECHANISMS OF DISEASE

Molecular pathways in colorectal cancer

Sam Al-Sohaily,*,†,§ Andrew Biankin,*,†,¶ Rupert Leong,†,** Maija Kohonen-Corish*,‡ and Janindra Warusavitarne*,[1]

*Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, †South Western Sydney Clinical School and ‡St Vincent's Clinical School, University of New South Wales, Sydney, §Departments of Gastroenterology, Campbelltown Hospital, Campbelltown, and ¶Departments of Surgery and **Gastroenterology, Bankstown Lidcombe Hospital, Bankstown, New South Wales, Australia

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Correspondence
Dr Sam Al-Sohaily, Cancer Research Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia. Email: s.al-sohaily@garvan.org.au

[1]Present address: Department of Surgery, St Mark's Hospital and Imperial College, London, UK.

Abstract

Colorectal cancer (CRC) is the second most common newly diagnosed cancer and accounts for the second highest number of cancer related deaths in Australia, the third worldwide and of increasing importance in Asia. It arises through cumulative effects of inherited genetic predispositions and environmental factors. Genomic instability is an integral part in the transformation of normal colonic or rectal mucosa into carcinoma. Three molecular pathways have been identified: these are the chromosomal instability (CIN), the microsatellite instability (MSI), and the CpG Island Methylator Phenotype (CIMP) pathways. These pathways are not mutually exclusive, with some tumors exhibiting features of multiple pathways. Germline mutations are responsible for hereditary CRC syndromes (accounting for less than 5% of all CRC) while a stepwise accumulation of genetic and epigenetic alterations results in sporadic CRC. This review aims to discuss the genetic basis of hereditary CRC and the different pathways involved in the process of colorectal carcinogenesis.

Introduction

Colorectal cancer (CRC) is a major cause for morbidity and mortality globally. Worldwide, CRC is the fourth most common cancer in men and the third most common cancer in women.[1] In the United States, approximately 142 000 new diagnoses and 50 000 deaths are reported annually from the disease.[2] The disease burden is similar in Australia, where CRC is the second most common newly diagnosed cancer, with over 14 000 new cases reported each year and it accounts for the second highest number of cancer related deaths behind lung cancer.[3] Lifetime risk for developing CRC is 1 in 17 for men and 1 in 26 for women. CRC costs the Australian government $235 million a year in direct costs, accounting for 8.1% of total cancer cost.[4]

Relative CRC risk is defined by genetic predisposition and environmental factors, with age being the most important risk factor for sporadic CRC. The risk of developing CRC increases with age, and over 90% of sporadic CRCs occur in individuals over the age of 50.[5] Other risk factors include family history of CRC, a diet low in fibers and folate and high in fat and red meat, alcohol, cigarette smoking, sedentary occupation, obesity, and diabetes.[6] Approximately 5% of all CRC are due to inherited genetic mutations. Of the remaining 95% of cases, approximately 20% have a positive family history but cannot be categorized to any hereditary CRC syndrome.[7] These are probably caused by genetic alterations secondary to an inherited predisposition, or common dietary and environmental factors. Advances in microarray technology allow genotyping of hundreds of thousands of single nucleotide polymorphisms (SNP) with high accuracy. Using this technology, genome wide association studies (GWAS) aim to find susceptibility loci for CRC. In principle, GWAS compare the frequencies of genetic variants between affected individuals (cases) and unaffected individuals (controls) in a family based or case-control design.[8,9] Multiple susceptibility loci have been identified; however, their value in CRC risk prediction remains low.[10] The predictive value will likely improve with more variants being discovered.

Colorectal cancer evolves through a stepwise accumulation of genetic and epigenetic alterations, leading to the transformation of normal colonic mucosa into invasive cancer. Most CRC arise within pre-existing adenomas which harbor some of the genetic fingerprints of malignant lesions. This transformation is believed to take 10–15 years, giving clinicians a window of opportunity to screen and subsequently remove these premalignant or early malignant lesions. The time to progression varies based on the polyp characteristics; high risk features for rapid malignant transformation include large size ($\geq$ 1 cm in diameter), multiple adenomas ($\geq$ 3), adenomas with villous change, and adenomas with high grade dysplasia.[11] The recently described sessile serrated adenomas (SSA) demonstrate distinct molecular and pathological changes not commonly seen in traditional adenomas. These lesions are thought to progress to cancer via a different pathway—the serrated neoplasia pathway.[12] The optimum surveillance strategy for patients with SSA is yet to be determined and will require further investigation.

Identification of different molecular pathways of colorectal carcinogenesis has demonstrated the heterogeneous nature of CRC. The first model was proposed by Fearon and Vogelstein,[13] in this model, there are three important features: first, colorectal neoplasia arises as a result of mutational activation of oncogenes coupled with mutational inactivation of tumor suppressor genes; second, mutations of at least 4 to 5 different genes are required for cancer to develop; and third, the accumulation of genetic alterations rather than their order is responsible for determining the biologic behavior of the tumor. The discovery of other CRC pathways beyond the Fearon and Vogelstein model[13] highlights the importance of understanding the molecular nature of CRC. In the past two decades, two important molecular discoveries have been made: first, the discovery of Microsatellite Instability (MSI) caused by defective Mismatch Repair (MMR) genes, an important feature in a subset of hereditary and in about 15% of sporadic CRC; and second, discovering the role of epigenetics, in particular hypermethylation, in silencing of gene function. Concordant methylation of the CG di-nucleotides in the promoter region of multiple genes is called CpG Island Methylator Phenotype (CIMP). Patients with CIMP tumors have distinct clinical and pathological characteristics. Classifying CRC based on the presence of MSI and CIMP was suggested by Jeremy Jass.[14] This classification describes five molecular subtypes, each with a different molecular profile and clinico-pathological features. These are:

1. CIMP high/MSI high (12% of CRC); originates in serrated adenomas and is characterized by BRAF mutation and MLH1 methylation.
2. CIMP high/MSI low or microsatellite stable (8%); originates in serrated adenomas and is characterized by BRAF mutation and methylation of multiple genes.
3. CIMP low/MSI low or microsatellite stable (20%); originates in tubular, tubulovillus, or serrated adenomas and is characterized by chromosomal instability (CIN), *K-ras* mutation, and *MGMT* methylation.
4. CIMP negative/microsatellite stable (57%); originates in traditional adenoma and is characterized by CIN.
5. Hereditary Non Polyposis Colorectal Cancer (HNPCC); CIMP negative/MSI high; negative for *BRAF* mutations

This review aims to provide a general overview of the different molecular pathways involved in colorectal carcinogenesis. Characterizing the genetic basis of the hereditary syndromes has led to a better understanding of the molecular biology of the more common sporadic CRC and will therefore be presented first.

Hereditary colorectal cancer syndromes

Hereditary CRC syndromes result from germline mutations in genes involved in colorectal carcinogenesis. They account for less than 5% of all CRC cases.[15] Many syndromes are identified; the most common are Familial Adenomatous Polyposis and Lynch syndrome (also called Hereditary Non Polyposis Colorectal Cancer [HNPCC]). Mutational analysis for at-risk patients and their families is available to identify the specific mutations, allowing appropriate surveillance and treatment. A brief review of hereditary CRC and their genetic basis will be discussed in this article. Management and screening strategies are beyond the purpose of this review and therefore will not be presented.

Familial Adenomatous Polyposis. Familial Adenomatous Polyposis (FAP) was the first recognized and best characterized colonic polyposis syndrome. It is a highly penetrant autosomal dominant disorder caused by germline mutations of the Adenomatous Polyposis Coli (*APC*) gene.[16–18] FAP accounts for less than 1% of all CRC.[19] FAP serves as a model for the adenoma-carcinoma sequence described by Fearon and Vogelstein.[13] Clinically, patients with FAP present with hundreds to thousands of colorectal adenomatous polyps, usually in the second decade of life. The life time risk of CRC approaches 100% and patients with FAP are also at risk of extra-colonic manifestations such as cutaneous lesions, osteomas, dental anomalies, congenital hypertrophy of the retinal pigment epithelium, desmoid tumors, and extra-colonic cancers (liver, pancreas, gastric and small bowel, periampullary, thyroid, and central nervous system).[20]

Attenuated FAP (AFAP) is a less aggressive form of the disease; it is characterized by delayed age of onset and fewer colorectal adenomatous polyps. Extra-colonic manifestations are less common in attenuated FAP.[21]

APC *gene.* Adenomatous Polyposis Coli gene is a tumor suppressor gene located on chromosome 5q21; it was first localized in 1987[16] and cloned in 1991.[17] The *APC* gene has 15 exons and encodes a 310 kDa protein with multiple functional domains. The location of the mutation within the *APC* gene seems to correlate with disease severity and the presence of extra-colonic manifestation in FAP patients.[22] The majority of the mutations are frameshift or nonsense mutations that lead to premature truncation of protein synthesis.[23,24] APC protein is an important regulator of epithelial homeostasis. In particular, it regulates degradation of cytoplasmic β-catenin.[25] APC and β-catenin are components of the Wnt signaling pathway, a signal transduction pathway important for colorectal tumorigenesis. When *APC* is mutated, cytoplasmic β-catenin accumulates and binds to the Tcf family of transcription factors (Fig. 1), altering the expression of various genes affecting proliferation, differentiation, migration, and apoptosis. APC also plays a role in controlling cell cycle progression and stabilizing microtubules, thus promoting chromosomal stability.[21]

MYH-Associated Polyposis. MYH-Associated Polyposis (MAP) is characterized by the presence of colorectal adenomatous polyps and an increased risk of CRC. It is an autosomal recessive disorder caused by bi-allelic mutations in the *MYH* gene.[26] The *MYH* gene is located on chromosome 1p35 and is a base excision repair (BER) gene primarily targeting oxidative DNA damage.[7] The MAP carcinogenesis pathway appears to be distinct from CIN or MSI. It involves a high frequency of somatic APC mutations, a low frequency of loss of heterozygosity (LOH), and the tumors are usually microsatellite stable.[27] Clinically, patients with MAP have multiple adenomatous polyps, with varying numbers (ranging from 10 to more than 100). CRC develop in about 65% of patients, and usually presents at an older age than classic FAP.[28] One third of patients with MAP could have upper gastrointestinal lesions, but other extra-colonic manifestations are less common than classic FAP.[28] Phenotypically, MAP can be indistinguishable from FAP or attenuated FAP, and therefore genetic testing for *MYH* mutations should be performed in patients with suspected FAP or attenuated FAP and negative APC germline mutations.

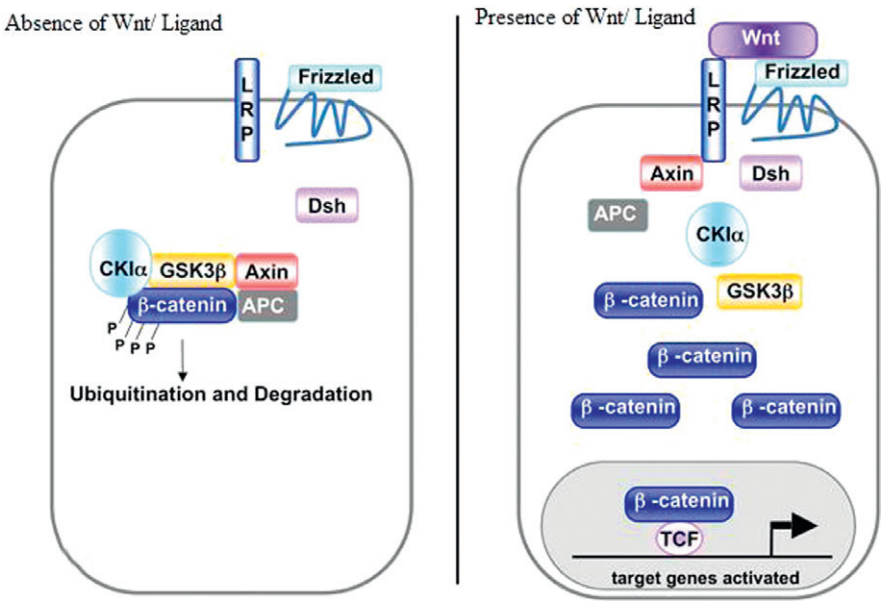

Figure 1 Canonical Wnt/ beta-catenin pathway. Binding of Wnts to Frizzled receptors activates Dishevelled (Dsh), which blocks the function of a destruction complex based on the scaffold proteins axin/ conductin. In the absence of Wnts, the axin/conductin complexes promote phosphorylation of β-catenin by GSK3β. Phosphorylated β-catenin becomes multi-ubiquitinated and subsequently degraded in proteasomes. In the presence of Wnts or after mutations of *APC*, phosphorylation and degradation of β-catenin is blocked, which allows the nuclear transfer of β-catenin. The TCF/β-catenin complexes bind to DNA and activate Wnt target genes.

Lynch syndrome, Hereditary Non Polyposis Colorectal Cancer. Hereditary Non Polyposis Colorectal Cancer is an autosomal dominant condition caused by germline mutations in DNA mismatch repair (MMR) genes. Loss of MMR activity leads to replication errors with an increased rate of mutations and a higher potential for malignancy. It is the most common hereditary CRC syndrome, accounting for 2–3% of all CRC cases.[29] The hallmark of HNPCC is the presence of microsatellite instability (MSI); this will be discussed in more detail later in the article. Patients with HNPCC develop CRC at a younger age than the general population, have a predilection for proximal colon cancers (70–85% of colon cancers are right sided), and are at a higher risk for synchronous CRCs.[30,31] Patients are at a higher risk of developing extra-colonic tumors including endometrial, ovarian, gastric, small bowel, pancreatic, hepatobiliary, skin, brain, and urethral tumors. The cumulative lifetime risk of an extra-colonic malignancy in females and males is 47% and 27%, respectively.[32]

Several guidelines are set out to help clinicians identify patients at risk of HNPCC. It is recommended that MSI testing be carried out on patients fulfilling these criteria and then be referred for further assessment by a cancer geneticist if MSI testing is positive. The revised Amsterdam II criteria[33] and the Bethesda criteria[34] are summarized in Table 1.

Hereditary Non Polyposis Colorectal Cancer is caused by a germline mutation in one of the MMR genes. These include *MLH1* (MutL homolog 1)[35] located on chromosome 3p21, *MSH2* (MutS homolog 2)[35] located on chromosome 2p21-22, *PMS2* (post-meiotic segregation 2)[36] located on chromosome 7p22, and *MSH6* (MutS homolog 6)[37] located on chromosome 2p16. Bi-allelic inactivation of any of these MMR genes results in defective DNA repair and hence the accumulation of repetitive short nucleotide sequences called microsatellites. Mutations in *MLH1* or *MSH2* account for the majority of all mutations causing HNPCC.[38] Recently, germline deletions in the *TACSTD1* gene (a gene directly upstream of *MSH2*), which encodes the epithelial cell adhesion molecule Ep-CAM, has been identified as the causative mutation in some families with HNPCC.[39] The risk of developing cancer in HNPCC patients and families differs depending on the gene mutation present. Families with *MSH2* mutations have more extra-colonic cancers than *MLH1* mutation carriers. Families that harbor *MSH6* mutations develop CRC at a more advanced age, and have a higher risk of developing endometrial cancers.[31]

Table 1 Guidelines for identifying Hereditary Non Polyposis Colorectal Cancer (HNPCC)

Revised Bethesda guidelines:[34]
1. CRC diagnosed in a patient who is younger than 50 years of age.
2. Presence of synchronous, metachronous colorectal, or other HNPCC associated tumor regardless of age.
3. CRC with MSI-H histology diagnosed in a patient younger than 60 years of age.
4. CRC diagnosed in ≥ 1 first degree relatives with an HNPCC related tumor, with one of the cancers being diagnosed under age 50 years.
5. CRC diagnosed in ≥ 2 first or second degree relatives with HNPCC related tumor, regardless of age.

Amsterdam II criteria:[33]
1. Three or more family members with HNPCC related cancers, one of whom is a first degree relative of the other two.
2. Two successive affected generations.
3. One or more of the HNPCC related cancers diagnosed under the age of 50 years.
4. FAP has been excluded

CRC, colorectal cancer; FAP, Familial Adenomatous Polyposis; MSI-H, microsatellite instability high.

Hamartomatous polyposis syndromes. These include Peutz-Jeghers syndrome (PJS), Juvenile Polyposis syndrome (JPS), and Cowden syndrome.

Peutz-Jeghers syndrome is an autosomal dominant syndrome caused by germline mutation in *STK11/LKB1*. It is characterized by the presence of hamartomatous polyps throughout the gastrointestinal (GI) tract, predominantly in the small bowel, and mucocutaneous pigmentation, typically on the lips, buccal mucosa, and periorbital area. As well, there is an increased risk of GI and extra-GI malignancies.[7]

Juvenile polyposis syndrome is a rare autosomal dominant disorder with multiple juvenile polyps throughout the GI tract. It is associated with increased risk of GI and pancreatic cancers. Germline mutations in *BMPR1A* and *SMAD4* have been reported in JPS.[26]

Cowden syndrome is caused by a germline mutation in *PTEN* and is characterized by hamartomatous polyps throughout the GI tract. Patients with Cowden syndrome are at increased risk of extra-GI malignancies including breast, thyroid, and endometrial cancer.[7]

Molecular pathways in sporadic colorectal cancer: An overview

Colorectal cancer is a heterogeneous disease with different molecular pathways leading to different phenotypes. Genetic and epigenetic alterations act to dysregulate conserved signaling pathways involved in cellular metabolism, proliferation, differentiation, survival, and apoptosis. Understanding the molecular basis of colorectal carcinogenesis has important ramifications in both prognosis and treatment of CRC. Optimizing the screening and surveillance protocols, better assessment of the disease stage, and individualizing therapy based on pathologic and molecular characteristics of the tumors may improve outcomes.

Different gene mutations have been linked with colorectal carcinogenesis,[40] but the exact role of many of these genes in the initiation and progression of the disease is yet to be confirmed. Only a limited number of these genes, most notably *APC, K-ras, and p53*, have been found to be altered in a sizable proportion of CRC, but the combination of these mutations in the same cancer is un-common.[41]

Colorectal cancer develops through a series of events that lead to the transformation of normal mucosa to adenoma and then to carcinoma. Genomic instability is an integral part in this transformation process. To date, three distinct molecular pathways have been recognized. These are the Chromosomal Instability (CIN) pathway, Microsatellite Instability (MSI) pathway, and the CpG Island Methylator Phenotype (CIMP) pathway. These pathways are not mutually exclusive, with some tumors exhibiting features of more than one pathway.[42]

Chromosomal instability pathway. Chromosomal instability is the most common cause of genomic instability in CRC. It accounts for 65–70% of sporadic CRC. It is characterized by gain or loss of whole chromosomes or chromosomal regions harboring genes integral for the process of colorectal carcinogenesis. CIN results from defects in chromosome segregation with subsequent aneuploidy, telomere dysfunction, or defects in the DNA damage response mechanisms.[43] The consequence is an imbalance in chromosome number (aneuploidy), chromosomal genomic amplifications, and a high frequency of LOH.[43]

Broad (greater than half a chromosomal arm) amplifications have been identified on chromosomes 7, 8q, 13q, 20, and X, and broad deletions on chromosomes 1, 4, 5, 8p, 14q, 15q, 17p, 18, 20p, and 22q. In addition, focal gains or losses are found in regions containing important cancer genes, e.g. *VEGF*, *MYC*, *MET*, *LYN*, *PTEN*, and others.[44] Chromosomes 1, 5, 8, 17, and 18 have the highest frequency of allele loss (46–78%).[45] Whole chromosome loss is more frequent for chromosome 18, while other chromosomes are predominantly affected by partial loss.[45] Coupled with these karyotypic abnormalities is the accumulation of mutations in oncogenes and tumor suppressor genes. The most common single genetic alterations are mutations in the *APC* and *K-ras* genes.

K-ras *oncogene.* The *K-ras* proto-oncogene is mutated in 30–60% of CRC and large adenomas.[46–48] It is proposed that activated K-ras may play an important role in the transition from adenoma to carcinoma through activation of downstream targets including *BCL-2*, *H2AFZ*, *RAP1B*, *TBX19*, *E2F4*, and *MMP1*.[46] The *K-ras* gene product, a 21 kDa membrane bound protein involved in signal transduction, is activated in response to extracellular signals. The mutated protein is locked in the active form due to impaired GTPase activity, which hydrolyses GTP to GDP. Most activating mutations are found in codons 12 and 13 of exon 1.[48] Ras activation affects multiple cellular pathways that control cellular growth, differentiation, survival, apoptosis, cytoskeleton organization, cell motility, proliferation, and inflammation.[43]

Loss of 5q allele. Allelic loss of chromosome 5q has been reported in 20–50% of sporadic CRC.[13] Two important genes are located on the long arm of chromosome 5; these are the *APC* and the Mutated in Colorectal Cancer (*MCC*) genes. Somatic *APC* mutations are seen in 60–80% of CRC as well as in a large percentage of colorectal precursor lesions (adenomas), indicating that *APC* mutation is an early event in the process of colorectal tumourigenesis.[49] APC was described as the "gatekeeper" of cellular proliferation in the colon. It belongs to the canonical Wnt/ wingless pathway. APC protein forms a complex with β-catenin, axin, and glycogen synthase kinase 3 (GSK3).[50] Loss of both alleles is required for loss of APC function, complying with the Knudson's two-hit hypothesis.[51]

The Wnt pathway plays a central role in supporting intestinal epithelial renewal.[52] APC binds to β-catenin and induces its degradation, thereby acting as a negative regulator of β-catenin.[53] Loss of APC function (through mutation, LOH, or promoter methylation) results in accumulation of cytoplasmic β-catenin, leading to nuclear translocation and binding of β-catenin to T-cell factor (TCF)/ lymphoid enhancer factor (LEF). A simplified scheme of the Canonical Wnt/ beta-catenin pathway is illustrated in Figure 1.

The Wnt target genes affect multiple cellular functions including regulators of cell cycle progression (c-myc and cyclin D1), cell proliferation, angiogenesis, and apoptosis.[53] Therefore, it appears that the Wnt signaling pathway is important for both initiation and progression of CRC. It represents a "final common pathway", as other signaling pathways converge and interact with this pathway. Loss of APC function is not the only trigger for Wnt activation; alternatives include activating *β-catenin* mutations, which render β-catenin resistant to degradation (found in less than 5% of all CRC), mutations in *AXIN1* and *AXIN2* (which are important for

β-catenin degradation), or activating mutations in the transcription factor TCF-4.[54]

The *MCC* gene is located on 5q21. It is commonly silenced in colorectal cancers through promoter hypermethylation.[55,56] *MCC* has been identified as one of the "driver genes" of colorectal carcinogenesis in a mouse model.[40] It is a cell cycle regulatory protein that induces cell cycle arrest in response to DNA damage.[57] In addition, a recent study suggested that MCC can also inhibit Wnt/β-catenin signal transduction independent of APC.[55]

Loss of 8p allele. Allelic loss of 8p is seen in over 50% of CRC.[58] A common region of deletion has been identified in 8p21, suggesting the presence of tumor suppressor genes in this locus. Candidate genes have been identified but no specific gene mutation has been found.[59] Loss of chromosome 8p has been associated with advanced stage disease and increased metastatic potential, with the region 8p21-22 representing a hot-spot for tumor progression and a metastatic susceptibility locus.[60] Loss of this locus increases the potential for metastasis.

Loss of 17p allele. Loss of 17p is reported in 75% of CRC but not in adenomas, suggesting that loss of this segment, which contains the tumor suppressor gene *p53* is a late event in the process of colorectal tumourigenesis.[61] In CRC, allelic loss of 17p is commonly associated with mutations in *p53* in the second allele, and this may mediate the transition of adenoma to carcinoma.[62]

p53 is a transcription factor with tumor suppressor activity that binds to a specific DNA sequence and activates a number of genes involved in cell cycle arrest, apoptosis, senescence, autophagy, and cellular metabolism. In addition, it has a number of transcription independent cellular activities important for the maintenance of genomic stability.[63] p53 facilitates the cellular adaptation in response to different cellular stresses including DNA damage by mutagens, oncogenic stimulation, hypoxia, and telomere erosion.[63]

Loss of 18q allele. The long arm of chromosome 18 contains many candidate tumor suppressor genes, including *Cables*, *Deleted in Colorectal Cancer* (*DCC*), *Smad2*, and *Smad4*. 18q LOH is detected in 50–70% of CRC and is a marker of poor prognosis in stage II and III CRC.[64–66] Cables is a cell cycle regulatory protein that interacts with cdk2, cdk3, and cdk5.[67] Reduced expression through mutation or promoter methylation of Cables has been reported in 65% of CRC.[68] *DCC* encodes a 170–190 kDa protein of the immunoglobulin superfamily; it plays a role in the regulation of cell adhesion and migration.[69] In addition, DCC induces apoptosis in the absence of its ligand (netrin-1).[70] Smad proteins are transcription factors involved in the transforming growth factor β (TGF-β) signaling pathway. Loss of Smad4 protein expression correlates with poor prognosis and advanced stage CRC. Smad proteins regulate the transcription of key target genes, including *c-myc*, *CBFA1*, *FLRF*, and *furin*.[71] Smad4 also downregulates claudin-1, a potential metastatic modulator, in a TGF-β independent manner.[72]

Microsatellite instability pathway. Microsatellites are short repeat nucleotide sequences that are spread out over the whole genome and are prone to errors during replication due to their repetitive manner. The DNA mismatch repair (MMR) system recognizes and repairs base-pair mismatches that occur during DNA replication. Instability of microsatellites is a reflection of the inability of the MMR system to correct these errors and is recognized by frameshift mutations in the microsatellite repeats. The discovery of MSI in 1993, its linkage to HNPCC, and the subsequent cloning of MMR genes have led to the recognition of MSI as an alternative pathway in colorectal carcinogenesis. Germline mutation in MMR genes results in HNPCC, while somatic mutation or hypermethylation silencing of MMR genes accounts for about 15% of sporadic CRC. Members of the MMR system identified include MSH2, MLH1, MSH6, PMS2, MLH3, MSH3, PMS1, and Exo1.[73] Sporadic MSI-High CRC is usually caused by hypermethylation silencing of MLH1.

MSI-high, MSI-low, and microsatellite stable. In 1997, the National Cancer Institute sponsored "The International Workshop on Microsatellite Instability" at which approximately 120 investigators convened to discuss MSI.[74] In this workshop, a panel of five microsatellite loci were recommended for identification of MSI. The panel consists of two mononucleotide repeats (BAT25 and BAT26) and three dinucleotide repeats (D5S346, D2S123, and D17S250). MSI-high is defined by instability of at least two markers, MSI-low is defined by instability in one marker, and tumors are called MSS when there is no apparent instability. Subsequently, other researchers proposed higher sensitivity and specificity by testing five mononucleotide repeat markers (BAT25, BAT26, NR21, NR24, and NR27).[75] Elevated microsatellite alterations at selected tetranucleotide repeats (EMAST) is another MSI form seen in about 60% of all CRC. MSI-low and EMAST are thought to be related to downregulation of MSH3 resulting in dinucleotide and tetranucleotide instability.[73] MSI-low tumors are associated with worse patient survival when compared with MSS tumours.[76]

Clinicopathological features of MSI-high tumors. Sporadic CRC with MSI-High molecular features have a distinct phenotype. They are more common in older women, and predominantly located in the right colon, proximal to the splenic flexure.[77] Pathological characteristics include increased lymphocytic infiltration (Crohn's disease-like reaction), mucinous histology, and poor differentiation.[78]

In vitro studies indicate resistance of MSI-high tumors to various chemotherapeutic agents, such as 5-Fluorouracil (5-FU)[79,80] and cisplatin.[81] Clinical data on use of MSI as a chemotherapy predictive marker are conflicting, although most studies suggest poor response of MSI-H tumors to 5-FU.[82–84] A recent meta-analysis showed no difference in recurrence-free survival, irrespective of the use of 5-FU based chemotherapy.[85] Another meta-analysis confirmed better response to 5-FU based chemotherapy in patients with MSS tumours.[86] Despite the adverse pathological features of MSI-high tumors, they are associated with improved overall survival.[86]

Molecular features and mechanisms of carcinogenesis in MSI-high tumors. Microsatellite instability-high tumors tend to be diploid with less LOH. They have fewer mutations in *K-ras* and *p53*.[87] *BRAF* V600E mutations are frequently seen in sporadic MSI-high CRC but not in HNPCC.[88] Mutation in the polyadenine tract of *Transforming growth factor β type II receptor*

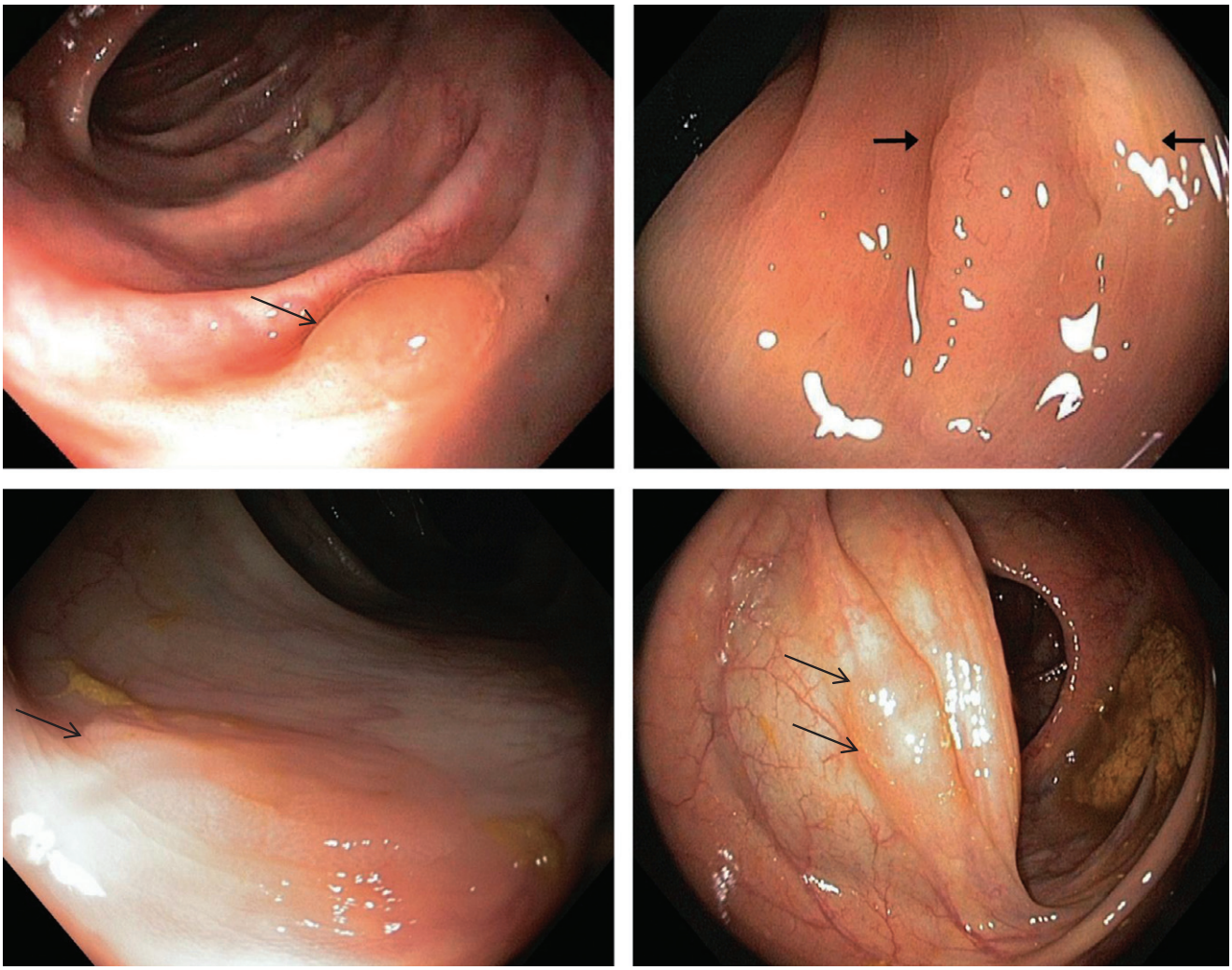

Figure 2 Colonoscopic examples of sessile serrated adenomas (arrow points to the lesion).

(*TGFβRII*) inactivates gene function[89] and has been observed in 90% of CRC with MSI.[90] TGFβ-II signaling inhibits cellular proliferation, and therefore alterations in the gene function represent a possible mechanism in MSI carcinogenesis. There is a large list of genes containing coding repeats that are susceptible to mutations in the presence of defective MMR function. It includes genes involved in DNA repair (e.g. *RAD50, MSH3, MSH6, BLM, MBD4, and MLH3*), apoptosis (e.g. *APAF1, BAX, BCL-10*, and *Caspase 5*), signal transduction (e.g. *TGFβRII, ACTRII, IGFIIR, and WISP-3*), cell cycle (*PTEN* and *RIZ*), and the transcription factor TCF-4.[77]

CpG Island Methylator Phenotype pathway. Epigenetic alterations refer to changes in gene expression or function without changing the DNA sequence of that particular gene. In humans, epigenetic changes are usually caused by DNA methylation or histone modifications.[91] DNA methylation occurs commonly at the 5′-CG-3′ (CpG) dinucleotide. Methylation of gene promoter region results in gene silencing, hence providing an alternative mechanism for loss of function of tumor suppressor genes.[91] Genes involved in colorectal carcinogenesis are found to be silenced by DNA hypermethylation include *APC*, *MCC*, *MLH1*, *MGMT*, and several others. A classic example is the hypermethylation silencing of *MLH1* in sporadic MSI-high CRC.[92] Environmental factors including smoking and advanced age have been shown to correlate with increased methylation.[92–94]

CpG Island Methylator Phenotype (CIMP) refers to the presence of concomitant hypermethylation of multiple genes. Five markers have been chosen to serve as markers for CIMP: these are CACNA1G, IGF2, NEUROG1, RUNX3, and SOCS1. CIMP positivity is defined by methylation of at least three markers.[95] CIMP-high CRC accounts for 15–20% of sporadic CRC and has distinct characteristics. It is more common in females, older patients, and proximal location (right colon). Pathologically, CIMP-high tumors are often poorly differentiated, of mucinous or signet ring histology, microsatellite unstable, and harbor *BRAF* mutation.[96] Patients with CIMP-high tumors may not benefit from 5-FU-based adjuvant chemotherapy.[97] The precursor lesions are usually the SSAs. These account for 9% of colorectal polyps and have distinct features; usually flat or minimally elevated, have a strong predilection for the

cecum and ascending colon, exhibit *BRAF* mutations and extensive DNA methylation (CIMP pattern).[98,99] SSA can be subtle, therefore extra vigilance and enhanced endoscopy techniques are often required to minimize the risk of missing these lesions (Fig. 2).

Hyperplastic Polyposis Syndrome. Hyperplastic Polyposis Syndrome (HPP) is a newly described rare syndrome in which up to 50% develop CRC.[100] The World Health Organization lists three independent criteria for diagnosis of HPP: (i) At least five hyperplastic polyps proximal to the sigmoid colon, two of which are larger than 1 cm; or (ii) The presence of hyperplastic polyps proximal to the sigmoid colon in a subject with a first degree relative of HPP regardless of the number of polyps; or (iii) More than 30 hyperplastic polyps throughout the colon regardless of the size.[99] HPP can be familial, but the genetic basis of its inheritance is yet to be determined. It is important that all clinicians become aware of the malignant potential of some hyperplastic polyps (or hyperplastic-like lesions). In general, large, atypical, or dysplastic lesions are at higher risk.[101] Failure to identify and remove SSA could explain the higher rate of interval CRC in the right colon.

Invasion and metastasis

Malignant tumors are characterized by their invasive and metastatic capabilities. This process involves detachment of tumor cells from its primary site, migration, invasion of blood or lymphatic vessels, dissemination, and finally settlement in the distant site. Tumor cells at the invasive front de-differentiate to attain a mesenchymal-like phenotype to enable invasion and metastasis of tumor cells; this process is often called "epithelial-mesenchymal transition (EMT)".[102] The finding that metastatic deposits usually exhibit morphologic features of the primary tumor (and not that of the invasive mesenchymal phenotype) indicates that migrating cells re-differentiate after settling in the distant site or undergo "mesenchymal-epithelial transition (MET)".[103] Epithelial cells must undergo functional and morphologic changes for EMT to happen. This process involves multiple signaling pathways, including stimulation of TGF-β signaling, which in turn stimulates other EMT related pathways such as Wnt signaling, and altered expression of transcription factors such as the snail family, which leads to repression of the intercellular adhesion protein E-cadherin.[104,105]

Conclusion

Since the description of the adenoma-carcinoma model of carcinogenesis by Fearon and Vogelstein in 1990, understanding of the genetics of CRC has revealed the heterogeneity of the disease. The importance of molecular pathways in determining the CRC phenotype and prognosis has been highlighted in this article. However, the role of molecular markers in determining tailored therapy in CRC is yet to be fully determined, and remains an important avenue for future research.

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