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1 Molecular Biology of Colon Cancer

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Summary

Colorectal cancer affects approx 140,000 people in the United States each year, resulting in more than 55,000 deaths. Colorectal cancer develops as the result of the progressive accumulation of genetic and epigenetic alterations that lead to the transformation of normal colonic epithelium to colon adenocarcinoma. The loss of genomic stability is a key molecular and pathophysiological step in this process and serves to create a permissive environment for the occurrence of alterations in tumor suppressor genes and oncogenes. Alterations in these genes, which include *APC, CTNNB1, KRAS2, BRAF, MADH4*/*SMAD4*, *TP53*, *PI3KCA*, and *TGFBR2*, appear to promote colon tumorigenesis by perturbing the function of signaling pathways, such as the transforming growth factor-β and PI3K signaling pathways, or by affecting genes that regulate genomic stability, such as the mutation mismatch repair genes.

Key Words: Colon cancer; mutation; oncogene; tumor suppressor gene; DNA methylation.

1. INTRODUCTION

Colorectal cancer (CRC) arises as the consequence of the progressive accumulation of genetic and epigenetic alterations that drive the evolution of normal colonic epithelial cells to colon adenocarcinoma cells. This process of colon carcinogenesis, which has been termed the polyp-carcinoma sequence, is believed to typically take place over 10–15 yr and involves concurrent histological and molecular changes. The subsequent effect of these genetic and epigenetic alterations on the cell and molecular biology of the cancer cells in which they occur is the acquisition of key biological characteristics that are central to the malignant phenotype. From the analysis of the molecular genetics of colon cancer, it has become clear that the formation of colon cancer involves a multistage process, which is currently characterized at the molecular level by the underlying form of genomic instability (i.e., the loss of the ability to maintain

AU: CRC = colorectal cancer? Abbreviation must be defined on first use.

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the wild-type DNA coding sequence and repair DNA mutations) present in the cancers. In this background of genomic instability, genetic and epigenetic alterations accumulate and cooperate with each other to drive the initiation and progression of colon cancer *(1–3)*.

Colon cancer appears to be most commonly initiated by alterations that affect the Wingless/Wnt signaling pathway. The initiated colon cancer then progresses as the result of the accumulation of sequential genetic or epigenetic events that either activate oncogenes or deactivate tumor suppressor genes that are involved in other signaling pathways, such as the *RAF-RAS-MAPK* pathway, transforming growth factor (TGF)-β pathway, and the phosphatidylinositol 3 kinase (PI3K)-AKT pathway *(4,5)*. Some of the alterations that have been convincingly shown to promote colon carcinogenesis affect *KRAS2*, *TP53,* the gene for p53, and elements of the TGF-β signaling pathway, such as *TGFBR2* and *MADH4*/*SMAD4*. The identification of these alterations has provided potential targets for the development of new therapies for the prevention and/or treatment of colon tumors (Fig. 1).

Fig. 1

2. POLYP-CARCINOMA SEQUENCE

The evolution of normal epithelial cells to adenocarcinoma usually follows a predictable progression of histological changes and concurrent genetic and epigenetic changes. These gene mutations and epigenetic alterations provide a growth advantage and lead to the clonal expansion of the altered cells. This process leads to the progression of adenomas to adenocarcinomas by the serial acquisition of genetic and epigenetic alterations that produce clonal heterogeneity followed by Darwinian evolution at the cellular level. Until recently, it was believed that only adenomatous polyps had the potential to undergo malignant transformation; however, it now also appears that a subset of hyperplastic polyps may have the potential to transform through a hyperplastic polyp-serrated adenoma-adenocarcinoma progression sequence *(6)*. Colon cancers arising through a hyperplastic polyp-serrated adenoma-colon cancer pathway appear to have a unique molecular as well as histological pathway through which they arise.

3. GENOMIC INSTABILITY

Genomic instability, which is the loss of the ability of the cell to maintain the fidelity of the DNA, is a fundamental aspect of the tumorigenesis process. At least three forms of genomic instability have been identified in colon cancer: (1) microsatellite instability (MSI), (2) chromosome instability (CIN; i.e., aneusomy, gains and losses of chromosomal regions), and (3) chromosomal translocations *(7)*. The etiology of CIN has only been identified in a small subset of colon cancers; however, MSI is known to result from inactivating mutations or the aberrant methylation of genes in the DNA mutation mismatch

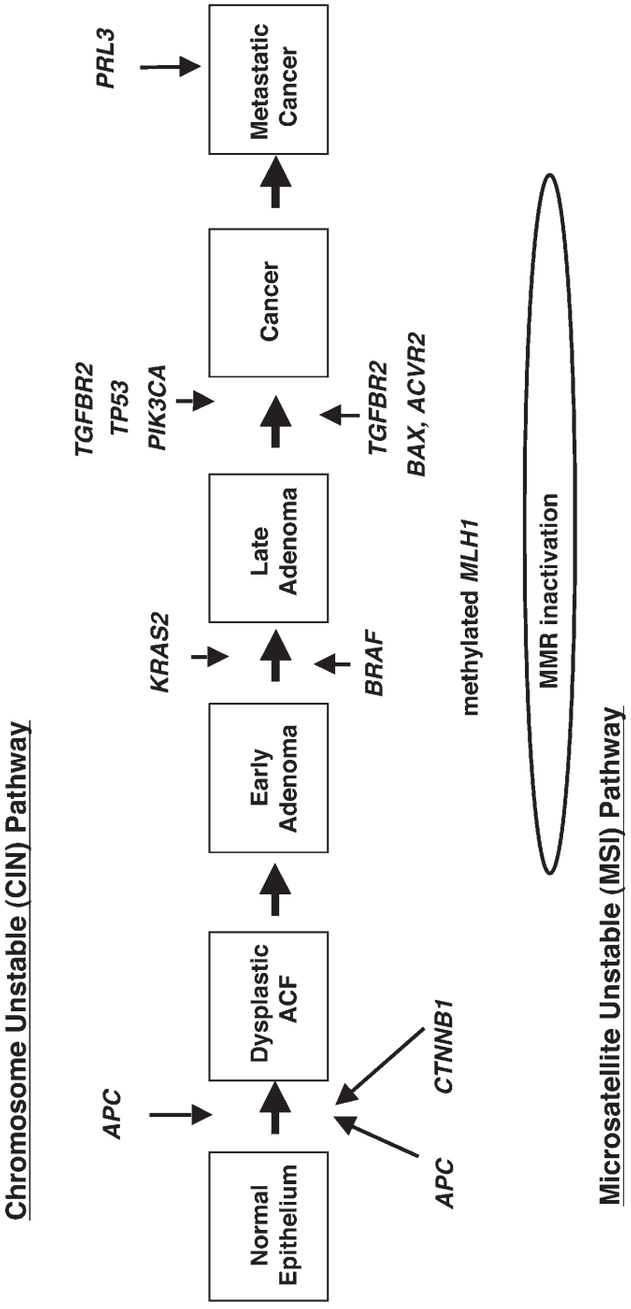

Fig. 1. Schematic representation of polyp-carcinoma progression sequence.

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repair (MMR) family, which repairs DNA base-pair mismatches that arise during DNA replication. Genomic instability contributes to the accumulation of mutations in tumor suppressor genes and oncogenes that drive the polyp-cancer progression sequence. The timing of the loss of genomic stability, either CIN or MSI, appears to be after adenoma formation but before progression to frank malignancy. In fact, both CIN and MSI can be detected in colon adenomas *(8–14)*. Shih et al. demonstrated that more than 90% of early adenomas (1–3 mm in size) exhibited allelic imbalance (also known as loss of heterozygosity [LOH]) of at least one of four chromosomes tested *(8)*. Ried et al. detected a stepwise increase in the average number of copy alterations using comparative genomic hybridization as adenomas progressed from low- to high-grade and then finally to carcinoma *(13)*. Despite the accumulation of data demonstrating the presence of genomic instability in early colon tumors, the causative role of genomic instability in cancer remains a source of considerable controversy *(2,7)*. Nonetheless, genomic instability is an attractive target for anticancer therapies because it is nearly ubiquitous in colon cancer and is a unique characteristic of cancer cells that is not present in normal epithelial cells. The feasibility of targeting genomic instability for anticancer treatments has been shown in in vitro systems *(15)*.

3.1. DNA Mismatch Repair Pathway/Inactivation of MMR Genes

Genomic instability arises because of inactivation of the normal mechanisms used by the cell to maintain its DNA fidelity. Defects in two of the systems that regulate DNA fidelity, the MMR system and Base Excision Repair (BER), have been identified in independent subsets of colon cancer. The DNA mismatch repair system (also known as the MMR system) consists of a complex of proteins that recognize and repair base-pair mismatches that occur during DNA replication. Inactivation of the MMR system occurs in 1–2% of CRCs owing to germline mutations in members of the MMR system, *MLH1, MSH2, PMS2*, and *MSH6*, and is the cause of the colon cancer family syndrome, hereditary nonpolyposis colon cancer syndrome (HNPCC) *(16,17)*. In addition to HNPCC-related colon cancers, approx 15% of sporadic colon cancers have inactivated MMR systems owing to the aberrant methylation of MLH1 (*see* below) *(18)*. MSI occurs as the consequence of inactivation of the MMR system and is recognized by frameshift mutations in microsatellite repeats located throughout the genome. Because many colon cancers demonstrate frameshift mutations at a small percentage of microsatellite repeats, the designation of a colon adenocarcinoma as showing MSI depends on the detection of at least two unstable loci out of five from a panel of loci that were selected at a National Cancer Institute consensus conference *(19)*.

AU: "(*see* below)" Please be more specific about where below the reader should look.

Study of the biochemistry of the MMR proteins has revealed that recognition of the base–base mismatches and insertion/deletion loops is performed by a

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heterodimer of either MSH2 and MSH6 or MSH2 and MSH3. Of interest, the MSH2–MSH3 heterodimer preferentially recognizes insertion/deletion loops and thus cannot compensate for loss of hMSH6. Consequently, cancers arising with a loss of MSH6 function display MSI only in mononucleotide repeats and may display an attenuated form of MSI called MSI-low *(20)*. The MLH1, PMS2, and PMS1 proteins appear to operate primarily in performing the repair of the base–base mismatches and insertion/deletion loops. A heterodimer of MLH1–PMS2 operates as a "molecular matchmaker" and is involved in executing the repair of the mismatches in conjunction with DNA-polymerase ∂ and the replication factors proliferating cell nuclear antigen (PCNA), riobonuclease protection assay, and replication factor C, as well as the 5′,3′ exo/endonucleases EXO1 and FEN1 and other unidentified 3′,5′ exonucleases and helicases *(20,21)*.

AU: was the symbol "⟶" intended between 5′,3′ exo/endonucleases and 3′,5′ exonucleases?

AU: Correct definitions for RPA and RFC? If not, please provide definitions

The MSI that results from loss of MMR activity affects mono-, di-, and trinucleotide tracts predominantly. However, cell lines from these tumors also show up to a 1000-fold increased mutation rate at expressed gene sequences, and in particular show instability of short sequence repeats with expressed sequences *(22)*. Genes that possess such "microsatellite-like" repeats in their coding regions appear to be the targets relevant to carcinogenesis. This pathway to tumor formation appears to be distinct from that seen in colon cancers that are microsatellite stable (MSS) *(23)*. The most frequently targeted gene for mutation in this pathway is the TGF-β receptor type II tumor suppressor (*TGFBR2*) gene, which is discussed in greater detail below. Other, less frequently targeted genes include the *IGF2* receptor; *BAX* and *CASPASE 5,* proteins which regulate apoptosis; *ACVR2,* a receptor for activin; *MSH3* and *MSH6*, DNA mismatch repair proteins; *RIZ*, the retinoblastoma protein-interacting zinc finger gene; and *CDX2*, an intestinal homeobox factor *(23–28)*. Importantly, MSI and the subsequent target gene mutations appear to occur throughout the adenoma-to-carcinoma progression. The timing of many of these events during tumor formation remains to be mapped, but preliminary studies have shown they occur at distinct phases of tumor progression *(10)*. Thus, MSI creates a favorable state for accumulating mutations in vulnerable genes that promote tumorigenesis, and these alterations ultimately lead to the generation of colon cancers.

AU: "(*TGFBR2*) gene, which is discussed in greater detail below." Please be more specific about where below the gene will be discussed.

The relationship between the MSI pathway and other genetic alterations frequently found in colon cancer is only partially understood. Alteration of the Wnt/Wingless pathway can be observed in tumors irrespective of MSI status *(29)*. Mutations in *APC* and *CTNNB1* can be found in 21 and 43% of MSI tumors, respectively *(30,31)*. In addition, the incidence of *KRAS2* mutations appears to be as high as 22–31%, which is similar to the incidence observed in MSS colon cancers *(32,33)*. Mutations in *TP53* are less frequent in MSI cancers than in MSS cancers. The mutation incidence in MSI colon cancers ranges

between 0 and 40%, whereas the incidence in MSS tumors is between 31 and 67% *(30,32,34,35)*. Of interest, monoallelic and biallelic *BAX* mutations are found frequently in MSI colon cancers and may serve to replace the role of mutant *TP53* in colon carcinogenesis. Thus, the microsatellite mutator pathway appears to be initiated through changes in the Wnt/Wingless pathway and to share some alterations with the MSS colon cancer pathway. However, other events, such as *TP53* and *TGFBR2* mutations, occur at different frequencies in the MSI vs the MSS pathway.

The impact of MSI on the clinical behavior of CRCs has been intensely investigated, but remains only partly understood to date. Several retrospective studies have shown mixed results regarding the effect of MSI on prognosis. Watanabe et al. found that 18qLOH correlated with a reduction in 5-yr survival from 74 to 50% in stage III CRC patients and that *TGFBR2 BAT-RII* mutations correlated with improved 5-yr survival in tumors with MSI, 74 vs 46% *(36)*. In addition, a systematic review of MSI revealed that there was a combined hazard ratio estimate for overall survival associated with MSI of 0.65 (95% confidence interval [CI], 0.59 to 0.71) *(37)*. Finally, at present, no definite conclusions regarding the effect of MSI on CRC treated with adjuvant therapy can be made.

AU: HR = hazard ratio?

4. BER DEFECTS AND COLON CANCER

Inactivation of a second "DNA caretaker" mechanism, the BER system, is found in a subset of colon cancer cell lines and is a cause of an autosomal recessive form of adenomatous polyposis, called the MYH adenomatous polyposis (MAP) syndrome *(38)*. Germline mutations in *MYH*, which encodes for a protein involved in BER, is the cause of adenomatous polyposis in up to 5–10% of individuals who have an adenomatous polyposis syndrome. *MYH* germline mutations were discovered as a cause of adenomatous polyposis when investigators identified an excessive number of somatic G:C → A:T mutations in neoplasms of people with adenomatous polyposis but no detectable germline mutations in *APC (39–41)*. This type of mutation is commonly a consequence of oxidative damage to DNA that results in 8-oxo-7,8-dihydro2′deoxyguanosine (8-oxodG), which is one of the most stable deleterious products of oxidative DNA damage *(38,42)*. The BER system is responsible for repairing this form of DNA damage, which led these investigators to assess candidate genes involved in this process, *OGG1, MTHF1*, and *MYH* (Fig. 2). This assessment revealed biallelic germline mutations in a subset of people with adenomatous polyposis, but who did not have germline mutations in *APC*. The most common mutations are Tyr165Cys and Gly382Asp, which account for 82% of the mutant alleles detected to date *(41)*. Somatic *MYH* mutations do not appear to be common in sporadic colon cancer. A study of 1042 unselected patients with CRC in Finland revealed no somatic *MYH* mutations *(38,43)*. Of interest, the

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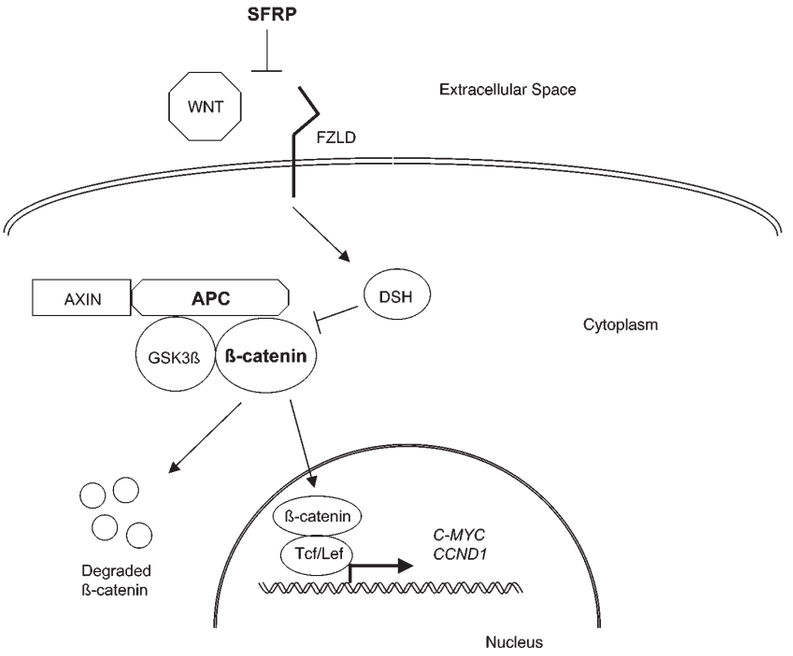

Fig. 2. Schematic representation of base-excision repair system.

tumors arising in the setting of biallelic *MYH* germline mutations do not show differences in the frequency of *TP53, SMAD4,* or *TGFBR2* mutations but do show an absence of MSI or CIN, suggesting that they have a unique molecular pathogenesis *(44)*. The discovery of *MYH* germline mutations in people with a hereditary colon cancer syndrome provides more evidence for the importance of genomic instability in cancer formation.

5. EPIGENTIC ALTERATIONS

Heritable phenomenon that regulate gene expression without involving changes of the DNA base-pair code are defined as epigenetic. Recently, epigenetic alterations have been increasingly recognized as being common and likely pathogenic in a variety of cancers. DNA methylation, the most commonly studied epigenetic phenomenon that appears to be altered in cancer, is normally present throughout the majority of the genome and is maintained in relatively stable patterns, which are established during development *(45)*. In humans, approx 70% of CpG dinucleotides are methylated. However, there are regions that contain higher proportions of CpG dinucleotides, called CpG islands, which are present in the 5′ region of approx 50–60% of genes and are normally

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AU: Please define CpG if appropriate

maintained in an unmethylated state. In cancers, many of these CpG islands become aberrantly methylated, and this aberrant methylation can be accompanied by transcriptional repression *(46,47)*. An ever-increasing number of genes have been shown to be aberrantly methylated in CRCs, including *CDKN2A, HLTF, MGMT, p14, TIMP3, TSP1*, and others.

The significance of these epigenetic alterations has been a point of significant controversy. For instance, whether aberrant methylation is generally a cause or an effect of cancer formation remains unresolved because the mechanism responsible for aberrant DNA methylation has yet to be identified *(48,49)*. Nonetheless, there is substantial data that the aberrant methylation of at least some genes, such as *MLH1*, is pathogenetic in cancer *(18,50,51)*. Inactivation of *MLH1*, a member of the MMR system, presumably plays an initiating role in the pathogenesis of colon cancers. Thus, the demonstration of aberrant methylation of *MLH1* in sporadic MSI colon cancers, and the restoration of *MLH1* expression by demethylating the *MLH1* promoter in MSI colon cancer cell lines, strongly suggests that such aberrant methylation could be a cause rather than a consequence of colon carcinogenesis *(18,50,51)*. Moreover, it is likely that the aberrant hypermethylation of 5′ CpG dinucleotides that has been demonstrated to silence a variety of known tumor suppressor genes in colon cancer, including *CDKN2A/p16*, *MGMT,* and $p14^{ARF}$, may be similarly pathogenetic in colon cancer *(46,50–54)*. Of specific note, methylation of *CDKN2A/p16,* a canonical tumor suppressor gene, is detected in 40% of colon cancers *(53)* and has been found not only in colon cancer but also in colon adenomas, as have other aberrantly methylated genes *(55,56)*. This observation demonstrates that aberrant promoter methylation is occurring early in the adenoma sequence, although it does not confirm that the aberrant *CDKN2A/p16* methylation is a primary rather than a secondary event in the tumorigenesis process. More broadly, early work has suggested that colon cancers that hypermethylate *MLH1* and/or *CDKN2A/p16* may belong to a distinct subclass of colon cancers, termed the CpG island methylator phenotype (CIMP), that demonstrate genome-wide aberrant methylation of gene promoters and that may arise by a distinct and unique mechanism *(53,54,57)*.

Also of note is recent progress in our understanding of mechanisms through which DNA methylation may affect transcription. DNA methylation may impair transcription by direct inhibition between methylated promoters and transcription factors, such as AP-2, CREB, E2F, and NF-κB *(45)*. CpG island methylation also can mediate transcriptional silencing by recruiting methyl-binding proteins, MeCP2, MBD2, and MBD3, that recognize methylated sequence and recruit histone deacetylases (HDACs). The HDACs then induce changes in chromatin structure that impede the access of transcription factors to the promoter *(46)*. The relationship between DNA methylation and posttranslational modification of histones appears to be complex, as other studies have

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shown that changes in the methylation state of H3-lysine 9 and H3-lysine 4 precede changes in DNA methylation, suggesting that the histone modification state and chromatin structure may cause the DNA methylation changes *(45)*. There is considerable interest in targeting these histone changes for anticancer therapies, using drugs such as histone deacetylases inhibitors.

6. GENETIC ALTERATIONS

6.1. The Wingless/Wnt Signaling Pathway

6.1.1. Adenomatous Polyposis Coli

The role of genetic alterations in colon cancer formation was initially suggested by the colon cancer family syndrome, familial adenomatous polyposis (FAP). FAP is a hereditary colon cancer predisposition syndrome that is characterized by the development of hundreds of intestinal adenomatous polyps. The gene responsible for this syndrome, adenomatous polyposis coli (*APC*), was identified as the result of the discovery of an interstitial deletion on chromosome 5q in a patient affected with FAP and from classical linkage analysis of families affected by FAP *(58–60)*. The *APC* gene has 15 exons and encodes a large protein (310 kDa, 2843 amino acids) that possesses multiple functional domains that mediate oligomerization as well as binding to a variety of intracellular proteins, including β-catenin, γ-catenin, glycogen synthase kinase (GSK)-3β, axin, tubulin, EB1, and hDLG *(3)*. Germline mutations in *APC* result in FAP or one of its variants: Gardner's syndrome, attenuated FAP, Turcott's syndrome, or the flat adenoma syndrome *(61–64)*.

AU: Appropriate to expand EB1 or hDLG?

APC is mutated in up to 70% of all sporadic colon adenocarcinomas, and these mutations are present beginning in the earliest stages of colon cancer formation and precede the other alterations observed during colon cancer formation *(31,65–68)*. In fact, dysplastic aberrant crypt foci, presumptive precursor lesions to colon cancer, have been found by some investigators to harbor *APC* mutations *(69,70)*. The mutations observed in sporadic colon cancer are observed most frequently in the 5′ end of exon 15, between amino acid residues 1280 and 1500 *(71)*. Mutations in this region can affect the domains between amino acid residues 1020–1169 and 1324–2075, which have been implicated in β-catenin interactions. These mutations can also affect the SAMP (Ser-Ala-Met-Pro) domains located between amino acids 1324–2075 and thus disrupt *APC*'s interaction with axin *(72–74)*. The vast majority of *APC* mutations (>90%) result in premature stop codons and truncated gene products *(75)*. As mentioned previously, these mutations are often accompanied by chromosomal deletion of the residual wild-type allele, but biallelic inactivation of *APC* can also occur by second somatic mutations *(76)*.

One of the central tumor promoting effects of these mutations is to lead to over-activation of the Wingless/Wnt signaling pathway with the subsequent

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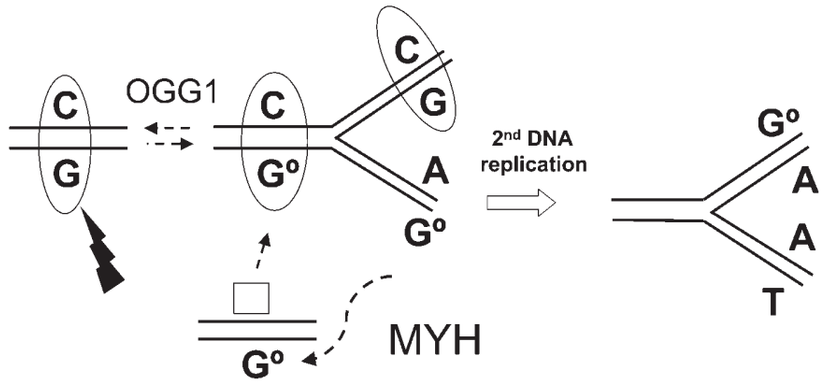

Fig. 3. Wnt signaling pathway diagram.

Fig. 3

expression of genes that favor cell growth (Fig. 3). The disruption of the association of APC with β-catenin leads to over-activation of the Wnt signaling pathway, which leads to the transcription of genes that favor tumor formation, such as *c-MYC* or *MATRILYSIN (65,77)*. Normally, GSK-3β forms a complex with APC, β-catenin, and axin, and phosphorylates these proteins. The phosphorylation of β-catenin targets it for ubiquitin-mediated proteasomal degradation. Truncating *APC* mutations prevent this process from happening and cause an increase in the amount of cytoplasmic β-catenin, which can then translocate to the nucleus and interact with other transcription factors like T-cell factor/lymphoid-enhancing factor (TCF/LEF). TCF-4 is the predominant TCF family member expressed in colonic epithelium. Consistent with the concept that increased Wnt-β-catenin pathway activity is a central tumor-promoting effect of *APC* mutations, oncogenic mutations in the β-catenin gene (*CTNNB1*) have been observed in some CRCs, as has methylation of *SFRP2* and *SFRP4*, members of a family of secreted Wnt antagonists called secretory frizzled related proteins *(78–80)*.

The clinical effects of *APC* mutations are best understood in the context of FAP, in which the location of the mutations associates with the severity of the phenotype and the occurrence of extraintestinal tumors, such as desmoid tumors *(33,81–83)*. Polymorphisms in the *APC* gene that associate with a slight increased risk of CRC have also been identified and include *I1307K* and *E1317Q* polymorphisms. *APC* I1307K occurs exclusively in people of Ashkenazi Jewish descent and results in a twofold increased risk of colonic adenomas and adenocarcinomas compared to the general population *(84,85)*. The *I1307K* polymorphism results from a transition from T to A at nucleotide 3920 in the *APC* gene and appears to create a region of hypermutability.

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6.2. β-CATENIN (CTNNB1)

β-Catenin is a member of the APC/β-catenin/TCF-LEF pathway that plays a role in the formation of a subset of colon cancers. β-Catenin is a homolog of armadillo, and its expression is increased by activation of the Wnt signaling pathway *(86–88)*. APC interacts with β-catenin and forms a macromolecular complex with it and GSK-3β. β-Catenin is consequently directed toward degradation as a result of phosphorylation by GSK-3β *(89–91)*. Mutations of *CTNNB1* or *APC* often render β-catenin insensitive to APC/β-catenin/GSK-3β-mediated degradation *(92,93)*. One of the functions of β-catenin is to bind members of the TCF family of transcription factors and activate gene transcription. Accordingly, cancers with *APC* or *CTNNB1* mutations have increased β-catenin/TCF-mediated transcription, which leads to the over-expression of genes such as *CYCLIN D1* and *c-MYC (94,95)*. The majority of these mutations are in a portion of exon 3 encoding for the GSK-3β phosphorylation consensus region of β-catenin. These mutations are often missense mutations in the highly conserved aspartic acid 32 and presumably impair the ability of GSK-3β to phosphorylate β-catenin *(96)*. Caca et al. found *CTNNB1* mutations in the NH2-terminal phosphorylation sites of β-catenin and found increased TCF/LEF transcriptional activity in association with this mutation *(97)*. Mutations that abolish β-catenin binding with E-cadherin have also been identified and have been shown to impair cell adhesion *(98,99)*. Like *APC* mutations, *CTNNB1* mutations have an essential role in early colon tumor formation. Mouse models with conditional alleles that lead to the stabilization of *Ctnnb1* in the intestinal tract, resulting in an FAP phenotype, have provided functional evidence that *CTNNB1* mutations lead to the formation of adenomas *(100)*. Interestingly, the incidence of *CTNNB1* mutations decreases from 12.5% in benign adenomas to 1.4% in invasive cancers, suggesting that *CTNNB1* mutations do not favor the progression of adenomas to adenocarcinomas *(101)*. Frameshift mutations in a polyadenine tract in *TCF-4* have also been identified in microsatellite unstable tumors, although their functional significance is unknown *(102)*.

AU: Correctio ns okay in sentence: "Mouse models with conditional alleles…l ead to the formation of adenomas *(100)*"?

6.3. KRAS2*, and* RAS-RAF-MAPK *Signaling Pathway*

One of the most prominent proto-oncogenes in colon carcinogenesis is a member of the *RAS* family of genes, *KRAS2*. The *RAS* oncogenes, which include *HRAS, NRAS*, and *KRAS2*, were initially discovered as the transforming genes of the Harvey and Kirsten murine sarcoma viruses (Ha-MSV, Ki-MSV) *(103,104)*. *KRAS2* is the most commonly mutated *RAS* family member in colon cancer, although *N-RAS* mutations are also observed in a small percentage of colon cancers *(105)*.

The *RAS* family genes encode a highly conserved family of 21-kDa proteins, which are involved in signal transduction. One major function of the ras protein family is to couple growth factors to the Raf-mitogen-activated protein (MAP)

AU: "MAP" was already defined as "MYH adenomatous polyposis (MAP)" earlier in this chapter. Each abbreviation can only stand for one term, so please decide which one to keep.

kinase kinase-MAP kinase signal transduction pathway, which leads to the nuclear expression of early response genes *(106)*. *KRAS2* consists of four exons that produce either a 188- or 189-amino acid peptide, depending on whether the fourth exon is alternatively spliced *(107)*. The protein encoded by *KRAS2* has three domains that either: (1) bind guanosine triphosphate or diphosphate (GTP/GDP); (2) attach the protein to the inner side of the plasma membrane after post-translational modification (isoprenylation) of the carboxy terminus; or (3) interact with cellular targets. Inactive KRAS2 binds GDP, and upon its activation GDP is exchanged for GTP. The activated KRAS2 then interacts with downstream signaling molecules to propagate cell proliferation. The activated KRAS2 is normally immediately deactivated by intrinsic GTP hydrolysis. Oncogenic mutations of *KRAS2* disrupt the GTPase activity of KRAS2 and allow it to remain in an activated state *(107)*. In fact, the most common mutations observed in human cancers involve codons 12, 13, and 61, which correspond to areas in the GTP-/GDP-binding domains in the KRAS2 protein. The consequence of these mutations is that approx 30% of the KRAS2 protein is in the GTP-bound state as compared to less than 0.3% in cells with wild-type *KRAS2 (108)*. The increased fraction of activated KRAS2 leads to activation of the RAF-RAS-MAPK signaling pathway, which promotes cell proliferation and increased survival, as well as other protumorigenic effects (Fig. 4).

AU: "Raf-mitogen-activated protein (MAP) kinase kinase-MAP kinase signal transduction pathway", were all those kinases intended?

Fig. 4

Mutation of *KRAS2* and *KRAS2* amplification has been observed in a large percentage of gastrointestinal tract tumors. As in other tumors, the *KRAS2* mutations observed in colon cancer almost always affect codons 12, 13, and 61. *KRAS2* mutations can be detected in 37–41% of colon cancers, and codon 12 is the most commonly mutated in CRC and usually undergoes a missense mutation *(68,109–111)*. The *KRAS2* mutations appear to follow *APC* mutations and are associated with advanced adenomatous lesions *(68)*. Evidence for this model comes from the observation that small adenomas with *APC* mutations carry *KRAS2* mutations in approx 20% of the tumors; whereas approx 50% of more advanced adenomas have been found to have *KRAS2* mutations *(66,112)*. Thus, alterations of *KRAS2* appear to promote colon cancer formation early in the adenoma-carcinoma sequence by mediating adenoma growth. Of interest, owever, they do not appear necessary for the malignant conversion of adenomas to adenocarcinomas.

More recently, mutations in *BRAF*, which is a kinase in the RAS-RAF signaling pathway, have also been recognized. *BRAF* mutations can be found in 27–31% of MSI colon cancers and 5% of MSS colon cancers and can be detected in ACFs, adenomas, and adenocarcinomas *(113–115)*. Of all the mutations, 80% are V600E mutations, which are predominantly found in MSI cancers and which lead to activation of the ERK and NF-κB pathways *(116)*. *BRAF* mutations appear to be mutually exclusive from *KRAS2* mutations, suggesting that mutations in either gene affect tumor formation by activating the

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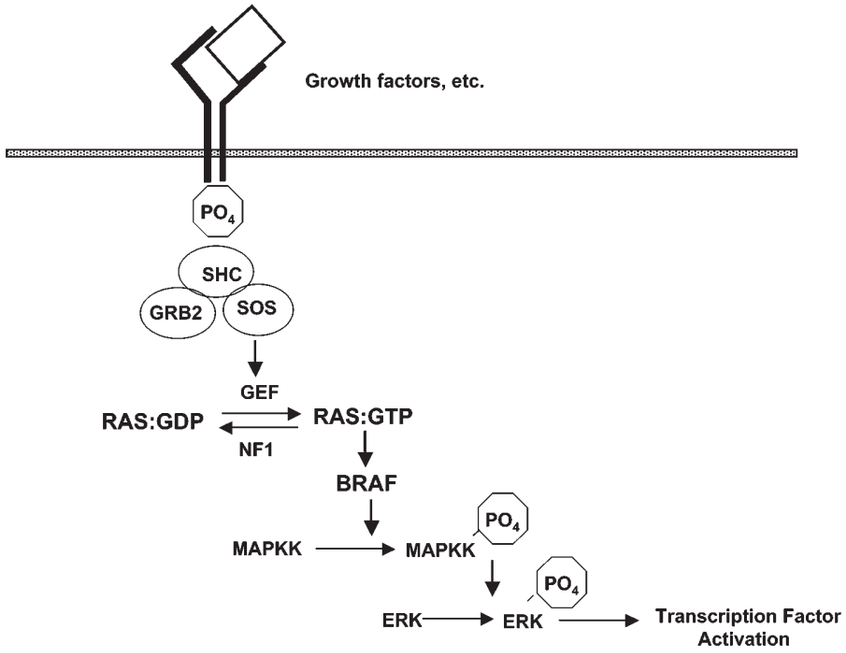

Fig. 4. Raf-Ras-MAPK signaling pathway diagram.

RAS-RAF-MAPK pathway. *BRAF* mutations also appear to occur rarely in MSI colon cancers that occur in the setting of HNPCC and instead are tightly associated with CIMP colon cancers, suggesting that there may be two distinct molecular pathways for the formation of sporadic MSI colon cancers *(57,117–119)*.

6.4. p53 (TP53)

The p53 protein was initially identified as a protein forming a stable complex with the SV40 large T-antigen, and was originally suspected to be an oncogene *(120)*. Subsequent studies demonstrated that *TP53* is located at 17p13.1 and is mutated in 50% of primary human tumors, including tumors of the gastrointestinal tract *(121)*. p53 is currently appreciated to be a transcription factor that is involved in maintaining genomic stability through the control of cell cycle progression and apoptosis in response to genotoxic stress *(121)*. The protein encoded by p53 has been structurally divided into four domains: (1) an acidic amino-terminal domain (codons 1–43) required for transcriptional activation; (2) a central core sequence-specific DNA-binding domain (codons 100–300); (3) a tetramerization domain (codons 324–355); and (4) a C-terminal regulatory

domain (codons 363–393), rich in basic amino acids and believed to regulate the core DNA-binding domain *(121)*. The spectrum of mutations in *TP53* seen in colon cancer appears similar to that seen in other tumors with mutations of *TP53* clustering at four hot spots in highly conserved regions (domains II–V). *TP53* is mutated in more than 50% of colon adenocarcinomas and the mutations localize primarily to exons 5–8 *(68,122)*. The mutations found to occur commonly in colon carcinoma are G:C to A:T transitions at CpG dinucleotide repeats, and in general interfere with the DNA-binding activity of the protein *(123,124)*. The mutation of *TP53* in colon cancer is commonly accompanied by allelic loss at 17p consistent with its role as a tumor suppressor gene *(125)*. In colon cancers, *TP53* mutations have not been observed in colon adenomas but rather appear to be late events in the colon adenoma-carcinoma sequence that may mediate the transition from adenoma to carcinoma *(68)*. Furthermore, mutation of *TP53* coupled with LOH of the wild-type allele was found to coincide with the appearance of carcinoma in an adenoma, providing further evidence of its role in the transition to malignancy *(125–128)*.

p53 normally serves to regulate cell growth and division in the context of genotoxic stress. It is expressed at very low levels in cells until it is activated, by poorly understood mechanisms, by DNA damage resulting from γ-irradiation, ultraviolet irradiation, or chemotherapeutic agents *(129)*. Its activation results in the transcription of genes that directly regulate cell cycle progression and apoptosis. These genes include $p21^{WAF1/CIP1}$, *GADD45*, *MDM2, 14-3-3-σ, BAX, B99, TSP1, KILLER/DR5, FAS/APO1, CYCLIN G,* and others *(121)*. Expression of many of these genes effectively halts DNA replication and induces DNA repair *(130–133)*. This function of p53 to recognize DNA damage and induce cell cycle arrest and DNA repair or apoptosis has led to p53 being called the "guardian of the genome" *(129)*. Thus, *TP53* normally acts as a tumor suppressor gene by inducing genes that can cause cell cycle arrest or apoptosis and also by inhibiting angiogenesis through the induction of *TSP1 (134,135)*. Mutant p53 protein can block these functions through forming oligomers with wild-type p53, causing diminished DNA-binding specificity *(136)*. Furthermore, the majority of p53 mutations occur in the sequence-specific DNA-binding region and serve to interfere with binding to the consensus sequence, 5′-PuPuPuC(A/T)-3′ *(137)*.

With regards to *TP53* mutation status as a prognostic or predictive marker for CRC response to treatment, there are conflicting results in the literature. *TP53* mutations are common in CRC and are believed to play a fundamental role in deregulating the cell cycle and inducing resistance to apoptosis in CRC. The over-expression of p53 by idiopathic hemochromatosis has been interpreted to indicate the presence of mutant p53 protein because the mutant forms of p53 have prolonged protein half-lives. Using this method or DNA mutation analysis for assessing *TP53* mutations, p53 has not consistently shown any prognostic or

AU: IHC = idiopathic hemochromatosis? If not, please define correctly.

predictive value in colorectal cancer *(138,139)*. It is possible that the prognostic value of *TP53* mutations will only be appreciated when specific *TP53* mutations are correlated with clinical outcomes.

6.5. The Phosphatidylinositol 3-Kinase (PI3K) Pathway

The PI3Ks are a family of lipid kinases that regulate the activity of kinases such as AKT and p70S6K, which ultimately regulate cell proliferation, apoptosis, and cell motility, hallmark biological functions that are commonly deregulated in cancer *(140)*. Multiple isoforms of PI3K can be identified in mammalian cells and can be divided into three classes, including notably the class I PI3Ks, which are composed of a p110 catalytic subunit and a regulatory adapter subunit. The class I PI3K members share homologous domains that include the lipid kinase domain, the helical domain, the C2 domain, a Ras-binding domain (RBD), and a NH2-terminal domain that interacts with the regulatory subunit *(141)*. Recently, large-scale mutational analysis studies of members of the PI3K signaling pathway have identified mutations that activate this pathway in a large proportion of colon cancers *(4,142)*. Gain-of-function mutations in *PI3KCA*, the p110α catalytic subunit of PI3K, have been found in 32% of colon cancers *(142)*. Of the *PI3KCA* mutations, 75% occur in two small clusters in the regions encoding the helical and kinase domains of the protein, which are highly evolutionarily conserved. One of the most common mutations, H1074R, has been shown to increase lipid kinase activity in in vitro studies, and a broader screen of other mutation hot spots identified in colon cancers, including E542K, E454K, and five other PI3KCA mutations, revealed that all of these mutations increased lipid kinase activity of PI3KCA *(142,143)*. Analysis of 76 colon adenomas and 199 colon cancers detected *PI3KCA* mutations only in advanced adenomas or CRCs, suggesting that these mutations influence the transition of the adenomas to adenocarcinomas *(142)*. In addition to mutations in *PI3KCA*, mutations in other members of the PI3K pathway have been detected in a series of 180 colorectal cancers, including mitogen activated protein-kinase kinase-4 (*MKK4*/*JNKK1*), myosin light-chain kinase-2 (*MYLK2*), phosphoinositide-dependent protein kinase-1 (*PDK1*), p21-activated kinase 4 (*PAK4*), v-akt murine thymoma viral oncogene homolog-2 kinase (*AKT2*), MAP/microtubule affinity-regulating kinase 3 (*MARK3*), cell division cycle-7 kinase (*CDC7*), a hypothetical casein kinase (*PDIK1L*), insulin related receptor (*INSRR*), and v-Erb-B erythroblastic leukemia viral oncogene homolg *(ERBB4) (4)*. Amplification of insulin-receptor substrate *IRS2* was also detected in a subset of colon cancers. In addition, inactivating mutations in *PTEN*, a lipid dual-specificity phosphatase, and in *PIK3R1*, the p85α regulatory subunit of PI3K, have been demonstrated in 5 and 2% of colon cancers, respectively *(140,144)*. Remarkably, mutations that affect the PI3K pathway can be detected in nearly 40% of CRCs and these mutations are nearly mutually exclusive, suggesting

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that they have equivalent tumorigenic effects through the activation of the PI3K pathway. These results suggest the PI3K pathway is an attractive pathway for targeted therapies *(4)*.

6.6. TGF-β Superfamily and Signaling Pathways

TGF-β is a multifunctional cytokine that can induce growth inhibition, apoptosis, and differentiation in intestinal epithelial cells *(145,146)*. Evidence of TGF-β's role in colon cancer formation first came from studies that demonstrated colon cancer cell lines were resistant to the normal growth inhibitory effects of TGF-β *(147)*. Furthermore, this pathway is deregulated in approx 75% of colon cancer cell lines, suggesting it is an important tumor suppressor pathway in colon cancer *(148)*. TGF-β mediates its effects on cells through a heteromeric receptor complex that consists of type I (TGFBR1) and type II (TGFBR2) components. TGFBR1 and TGFBR2 are serine-threonine kinases that phosphorylate downstream, signaling proteins upon activation *(149)*. The receptor complex is activated by TGF-β binding to the TGFBR2 component of the receptor complex, causing formation of the heteromeric R1–R2 receptor complex. The activated TGFBR2 component then phosphorylates the TGFBR1 component in the glutamine synthetase (GS) box of TGFBR1, a glycine-serine-rich region of the receptor. TGFBR1 then propagates the signal from the receptor through the phosphorylation of downstream proteins, including the Smad proteins, Smad2 and Smad3, and non-Smad proteins, such as PI3K, p38MAPK, and RhoA *(145,150)*. The Smad pathway is the most extensively characterized post-TGF-β receptor pathway. Upon activation, Smad2 and Smad3 form a hetero-oligomeric complex, which can also include Smad4, and translocate to the nucleus *(149,151)*. In the nucleus, they modulate transcription of specific genes through *cis*-regulatory Smad-binding sequences and through binding with other transcription factors such as p300/CBP, TFE3, Ski, and c-jun *(65,152,153)* (Fig. 5).

AU: First "smad" capitalized for consistency. Is this okay?

Fig. 5

The downstream transcriptional targets of the TGF-β signaling pathway are involved in the regulation of cell proliferation, extracellular matrix production, and immune surveillance. These functions not only are an integral part of tissue homeostasis but also are logical targets for dysregulation in colon carcinogenesis. Elements involved in growth regulation that have been clearly shown to be controlled in part by TGF-β include the cyclin-associated proteins cyclin D1, cdk4, p21, p27, p15, and Rb *(154–159)*. C-myc is also a downstream target of TGF-β and has been shown to be transcriptionally repressed in MvLu1 cells after treatment with TGF-β1 *(158,160)*. In addition to the cyclin-associated proteins, the extracellular matrix proteins and regulators of extracellular matrix proteins, fibronectin, tenascin, and plasminogen activator inhibitor 1, also appear to be regulated by TGF-β *(161,162)*.

The disruption of the normal extracellular matrix production may play a role in tumor invasion. In support of this concept, *TGFBR2* mutations in MSI colon

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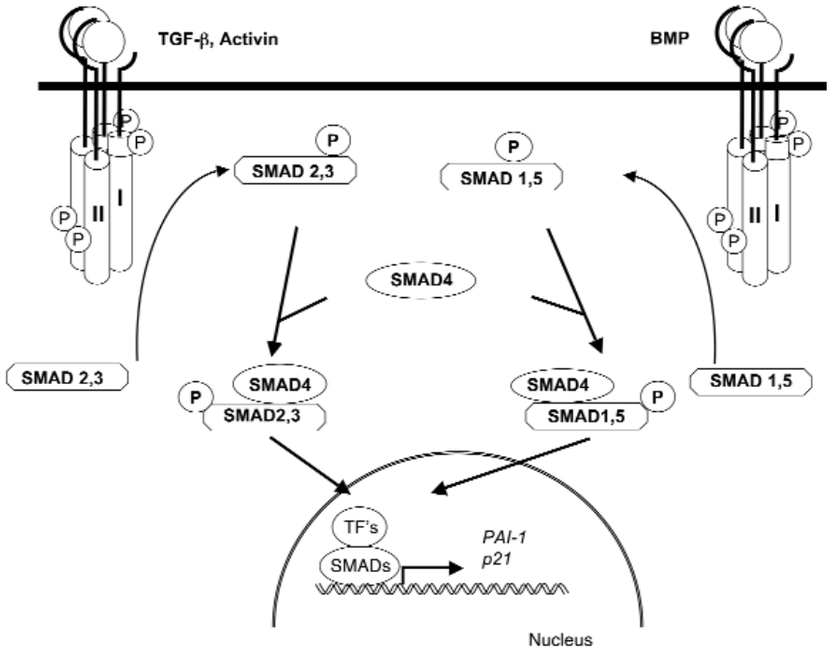

Fig. 5. TGF-β-SMAD signaling pathway diagram.

adenomas are only detected in areas of high-grade dysplasia or in adenomas with concurrent adenocarcinoma, suggesting that TGFBR2 inactivation promotes the malignant transition of colon adenomas to adenocarcinomas *(10)*. Furthermore, analysis of neoplasms that form in an in vivo mouse model that is knocked out for *Tgfbr2* in the colon (*Fabp*$^{4xat\text{-}132}$ Cre;*Tgfbr2*$^{flx/flx}$) suggest TGFBR2 inactivation promotes the progression of adenomas to adenocarcinomas *(7)*.

6.7. TGFBR2

A common mechanism through which colon cancers acquire TGF-β resistance is through genetic alterations of the *TGFBR2* gene. Functionally significant alterations of *TGFBR2* have been identified in up to 30% of colon cancers and are the most common mechanism identified to date for inactivating the TGF-β signaling pathway *(24,148)*. No alterations in *TGFBR1* or the type III TGF-β receptor (*TGFBR3)* have been observed in studies of TGF-β-resistant colon cancer cell lines, suggesting mutational inactivation of *TGFBR2* is a particularly favorable event that leads to tumor formation. Markowitz et al. have demonstrated that mutational inactivation of *TGFBR2* is an extremely common event in MSI colon cancers because *TGFBR2* has a microsatellite-like region in

exon 3 that consists of a 10-base-pair polyadenine tract, making it particularly susceptible to mutation in the setting of MSI *(24,163,164)*. The mutations in this region, which has been named *BAT-RII* (Big Adenine Tract in TGF-β Receptor type II), are frameshift mutations that result in the insertion or deletion of one or two adenines between nucleotides 709 and 718, introducing nonsense mutations that encode a truncated TGFBR2 protein lacking the intracellular serine-threonine kinase domain *(24)*. In a series of 110 MSI colon cancers, 100 were found to carry *BAT-RII* mutations, and in almost all of these cases the mutations were biallelic consistent with the tumor suppressor function of TGFBR2 *(163)*. *TGFBR2*'s role as a tumor suppressor gene in colon cancer has been further elucidated by studies showing that reconstitution of wild-type *TGFBR2* in colon cancer cell lines with mutant *TGFBR2* suppresses the tumor phenotype of the cell line *(148,165)*. Further support for *TGFBR2*'s role as a tumor suppressor gene in colon cancer in general was provided by the demonstration of *TGFBR2* mutations in colon cancer cell lines that are MSS. *TGFBR2* mutations have been found in 15% (n = 3/14) of TGF-β-resistant MSS colon cancer cell lines. These mutations are not frameshift mutations in *BAT-RII* but are inactivating missense in the kinase domain or putative binding domain of *TGFBR2 (148)*. In aggregate, the overall incidence of *TGFBR2* mutation in both MSS and MSI colon cancers appears to be 30% *(148)*. Interestingly, in a study of colon cancer cell lines, the incidence of TGF-β resistance was found to be 55% despite frequently having wild-type *TGFBR1* and *TGFBR2 (148)*. These cancers have presumably inactivated the TGF-β signaling pathway through genetic or epigenetic alterations in post-receptor defects, further underscoring the significance of the TGF-β signaling pathway in colon cancer formation.

6.8. SMAD2 and SMAD4

LOH occurs commonly at 5q, 18q, and 17p in colon cancer and suggests that there are tumor suppressor genes at these loci. LOH of chromosome 18q occurs in approximately 70% of colon adenocarcinomas. The incidence of 18q LOH is approx 10% in early-stage colon adenomas and 30% in later-stage, larger adenomas, demonstrating that the incidence of LOH involving 18q increases through the adenoma-carcinoma sequence *(68,122)*. A region of deletion on 18q that is shared among colon cancers that demonstrate allelic loss involving a contiguous segment of 18q has been observed and is the locus of a number of tumor suppressor genes implicated in colon cancer formation, including *DCC*, *SMAD2*, and *SMAD4*. All of these genes have been shown to be mutated in CRCs *(166–168)*. Other genes that are candidate tumor suppressor genes and map at 18q21-qter include *BCL-2*, gastrin-releasing peptide, and the cellular homolog of *YES-1*; however, none of these have been shown to be altered in CRCs *(169)*.

The most likely tumor suppressor genes that are the targets of 18q LOH are *SMAD2*, *SMAD4*, and *DCC*. The Smad proteins are a family of proteins that serve as intracellular mediators to regulate TGF-β superfamily signaling. The Smad proteins compose an evolutionarily conserved signaling pathway that has been demonstrated *in Caenorhabditis elegans*, *Drosophila melanogaster*, *Xenopus*, and humans. These proteins are characterized by two regions that are homologous to the *Drosophila* ortholog, Mad, and that are located at the N- and C-termini of the protein. These regions are termed the Mad-homology domains MH1 and MH2, respectively, and are connected by a less well-conserved, proline-rich linker domain. Numerous studies have identified three major classes of Smad proteins: (1) the receptor-regulated Smads (R-Smads), which are direct targets of the TGF-β receptor family type I kinases and include Smads1, 2, 3, and 5; (2) the common Smads (Co-Smads: Smad4), which form heteromeric complexes with the R-Smads and propagate the TGF-β-mediated signal; and (3) the inhibitory Smads (I-Smads: Smad6 and Smad7), which antagonize TGF-β signaling through the Smad pathway. Ligand binding to the TGF-β receptor complex results in TGF-β receptor type I mediated phosphorylation of Smad2 and Smad3 on two serine residues in a conserved –SS(M/V)S motif located at the C-terminus of the R-Smads *(170,171)*. Phosphorylation of these serine residues is required for downstream signaling pathway activation *(172,173)*.

In light of the known tumor suppressor effects of the TGF-β signaling pathway and the role the Smad proteins play in propagating this signal, it is not surprising that alterations of some of the *SMAD* genes have been found in colon cancer. Mutational inactivation of *SMAD2* and *SMAD4* has been observed in a high percentage of pancreatic cancers and in 5–10% of colon cancers *(167,168,174,175)*. *SMAD4* alterations have been found in up to 16% of colon cancers *(167)*. The effect of these mutations on colon carcinogenesis is being investigated in a number of different animal models. One murine model, a compound heterozygote $Smad4^{-/+}/Apc^{\Delta 716}$, develops colon cancer unlike the $Apc^{\Delta 716}$ mouse, which only develops small-intestinal adenomas *(176)*. This model suggests that *SMAD4* inactivation may play a role in the progression of colon cancers as opposed to their initiation. However, in some contexts *SMAD4* mutations also appear to initiate tumor formation and contribute to tumor initiation while in a state of haploid insufficiency. The $Smad4^{-/+}$ mouse develops gastric and intestinal juvenile polyps and invasive gastric cancer after several months; however, it does not appear to develop colon cancer *(177,178)*. Furthermore, germline mutations in *SMAD4*/*MADH4* have been found in approximately one-third of individuals with Juvenile Polyposis (JPS), an autosomal dominant syndrome characterized by gastrointestinal hamartomatous polyps and an increased risk of gastrointestinal cancer, consistent with the concept that haploid insufficiency of *SMAD4* may contribute to tumor initiation *(179–181)*. Importantly, though, the polyps observed in JPS and the invasive

cancers in the *Smad4*$^{-/+}$ mouse have been shown to have allelic loss of *SMAD4*, supporting the idea that biallelic inactivation of *SMAD4* is needed for cancer formation *(178,182)*. Taken together, these studies suggest that *SMAD4* is a tumor suppressor gene in colon cancer and is one of the targets of 18q LOH. However, given the frequency of 18q LOH vs detected *SMAD4* mutations or deletions, there are likely other tumor suppressor loci on 18q21.

Although also located at 18q21 and presumably a target for inactivation in colon carcinogenesis, mutations in *SMAD2* occur infrequently in colon cancer and have been found in only 0–5% of cancers *(168,175,183)*. The other *SMAD* genes do not appear to be frequently altered in colon cancer, despite the fact that *SMAD3* and *SMAD6* are located on chromosome 15q21–22, which is a frequent site of allelic loss in colon cancer *(175,184,185)*. Interestingly, and in contrast to the studies of human colon cancer, *Smad3*$^{-/-}$ mice have a high frequency of invasive colon carcinoma, but Smad2 inactivation does not appear to affect intestinal tumor formation in mouse models *(186,187)*. In conclusion, *SMAD* mutations appear to play a role in tumor formation in a subset of colon cancers, but are not as common as *TGFBR2* mutations. This observation raises the possibility that there are non-Smad TGF-β signaling pathways that play an important role in the tumor suppressor activity of *TGFBR2*.

The effect of 18q LOH, and thus presumably inactivation of the tumor suppressor genes at this locus, on the clinical behavior of colon carcinomas has been subjected to intense scrutiny with inconclusive results to date. Several different groups have assayed for LOH of 18q using microsatellite markers in stage II colon cancer and have found either no association with the clinical behavior of the cancer or an association with more aggressive cancer behavior *(169,188–191)*. The reason for the discrepancy is unclear but may be related to different microsatellite loci assessed in each study and thus the specific region of 18q that was assessed by each investigator. Adding to this confusion, SMAD4 diploidy and *TGFBR2 BAT-RII* mutations have been shown to associate with improved survival after adjuvant chemotherapy *(36,192)*.

6.9. TGF-β Superfamily Receptors: ACVR2 and BMPR1A

The TGF-β superfamily includes not only TGF-β1, TGF-β2, and TGF-β3, but also the BMPs (bone morphogenetic proteins), activin, nodal, growth and differentiation factors, and inhibin. The identification of germline mutations in signaling elements of the BMP signaling pathway in individuals with JPS, a hereditary colon cancer syndrome, and somatic mutations in the activin receptor in colon cancers has globally implicated deregulation of the TGF-β superfamily in the pathogenesis of colon cancer. Germline mutations in *MADH4*/*SMAD4* and *BMPR1A*, a type I receptor for a class of TGF-β superfamily ligands called BMPs, in families with JPS has implicated inactivation of BMP signaling in this subset of hereditary colon cancers. Howe et al. found nonsense

and missense germline mutations in *BMPR1A* in four families with JPS, 44-47delTGTT, 715C>T, 812G>A, and 961delC affecting exons 1, 7, 7, and 8, respectively *(193)*. MADH4/SMAD4 germline mutations have been found in 5–56% of families with JPS *(179,194)*.

The BMPs are disulfide-linked dimeric proteins that number at least 15 in total and include BMP-2, BMP-4, and BMP-7 (OP-1). They have a wide range of biological activities, including the regulation of morphogenesis of various tissues and organs during development, as well as the regulation of growth, differentiation, chemotaxis, and apoptosis in monocytes, epithelial cells, mesenchymal cells, and neuronal cells *(195)*. The BMPs transduce their signals through a heteromeric receptor that consists of a type I and type II receptor. BMPR1A is one of two different type I BMP receptors (BMPR1A and BMPR1B). It serves to predominantly bind BMP-4 and BMP-2 as well as other BMPs and transduces their signals when partnered with a BMP type II receptor. As with the TGF-β receptor, the best understood post-BMP receptor pathway is the Smad pathway. The R-Smads, Smads 1 and 5, partner with Smad4 (Co-Smad) to transduce BMP-mediated signals from the BMP receptors *(195)* (Fig. 5). Thus, the identification of both *BMPR1A* and *MADH4/SMAD4* germline mutations in families with JPS strongly implicates BMP signaling disruption in the pathogenesis of this syndrome. Furthermore, mice that overexpress Noggin, a soluble antagonist for the BMPs, or a dominant negative *Bmpr1a* in the intestinal epithelium, display ectopic crypt formation and a phenotype reminiscent of JPS *(196,197)*.

With regards to activin, activin is a secreted dimeric ligand, composed of either Activin βA and/or Activin βB, that activates intracellular signaling pathways, including the SMAD2/3-SMAD4 pathway, via a heteromeric receptor that is composed of a type I receptor (ACVRL1, ActRIA, or ActRIB) and a type II receptor (ACVR2 or ACVR2B) *(198)*. Mutations in *ACVR2* have been found to occur in 58–90% of MSI colon cancers as the result of a polyadenine tract in the coding region of the gene *(199,200)*. The identification of mutations that affect activin, TGF-β, and BMP signaling broadly implicate the TGF-β family as a tumor suppressor pathway in colon cancer.

6.10. Genes Associated With Colorectal Metastases

One of the clear challenges in cancer biology is the identification of genes that contribute to the metastatic and lethal cancer phenotype. Intense investigation in this area has led to the identification of promising candidate genes that may influence the metastatic potential of the primary colon cancer. PRL3, a phosphatase, was found overexpressed in 12 of 12 colon cancer liver metastases, but not in matched colon cancer primaries from the same patients *(201)*. Moreover, in 3 of 12 cases, PRL3 overexpression was accompanied by marked *PRL3* gene amplification, suggesting that *PRL3* overexpression is a

primary genetic event selected during metastasis. Osteopontin is a protein that also appears to have potential to predict the metastatic potential of CRC. Osteopontin was identified through a global screen using expression arrays and is 15-fold overexpressed in primary colon cancers and 27-fold overexpressed in liver metastases *(202)*. Osteopontin is a phosphoglycoprotein that can bind to several integrins, as well as CD44, and has been shown to contribute to the malignant phenotype in breast cancer *(202,203)*. To date, neither PRL3 or osteopontin has been shown to have the ability to predict the metastatic potential of CRC in a prospective clinical trial.

7. CONCLUSION

Investigation of the molecular pathogenesis of CRC has yielded many insights into the mechanisms driving the tumorigenesis process and to the identification of many potential therapeutic targets. Key insights from the assessment of the molecular genetics and epigenetics of colon cancer include the multistep nature of carcinogenesis, the central role of tumor suppressor pathways, the role of DNA repair genes and genomic stability in cancer formation, and the role of TGFβ signaling in tumor suppression. Nonetheless, many challenges remain. The molecular genesis of the metastatic phenotype that directly accounts for cancer lethality remains unknown. A mechanistic understanding of the basis of chromosomal instability, aneuploidy, and aberrant methylation of the cancer genome has yet to be achieved. In addition, the translation of molecular genetics to new diagnostic, prognostic, and therapeutic modalities appears promising but has yet to have a major impact on the clinical management of CRC. The promise for the future is that this field of inquiry will yield the important answers to these and other key questions.

ED: Some of these references have bracketed expressions in the titles, such as "[see comment]". These are not part of the actual titles and I'm not sure what their significance is.

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