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Pathways of Colorectal Carcinogenesis

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Abstract

Colorectal cancer is a heterogeneous disease that develops via stepwise accumulation of well-characterized genetic and epigenetic alterations. We review the genetic changes associated with the development of pre-cancerous colorectal adenomas and their progression to tumors, as well as the effects of defective DNA repair, chromosome instability, microsatellite instability, and alterations in the serrated pathway and DNA methylation. We provide insights into the different molecular subgroups of colorectal tumors that develop via each of these different mechanisms and their associations with patient outcomes.

Keywords

CRC; genomic instability; DNA mismatch repair; serrated polyp

Since Fearon and Vogelstein proposed their multi-hit genetic model of colorectal carcinogenesis,¹ accumulated insights have refined our understanding of the diverse genetic and epigenetic changes that underlie the initiation and progression of the adenoma-carcinoma sequence. A comprehensive analysis of mutations in 276 colorectal tumors by the Cancer Genome Atlas Network revealed that among all malignancies, colorectal tumors have one of the highest mutational burdens; dozens of somatic mutations have been

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identified in multiple colorectal tumors. Colorectal tumors can be broadly categorized as hypermutated (more than 12 mutations per 10^6 bases) or non-hypermutated (fewer than 8.24 mutations per 10^6 bases).² Parallel efforts to subtype colorectal cancers based upon gene expression profiles resulted in a new classification system, comprising 4 consensus molecular subtypes (CMSs 1–4). Each CMS has unique histopathologic features and correlations with progression and clinical outcomes.^{3–6}

Although colorectal tumors are heterogenous at a genetic level, they appear to develop via several distinct pathways. The pathways of chromosome instability (CIN), microsatellite instability (MSI), and serrated neoplasia provide some information about their mechanisms of pathogenesis, although and these pathways have some overlap. In addition, entirely new pathways continue to be recognized. Integrating data on gene expression signatures with tumor genotype has updated and refined these classifications.

We review the molecular changes associated with the transformation of normal colonic epithelium to histologically distinct precursor adenomatous lesions, and ultimately, malignant colorectal cancer (CRC). We discuss alterations in tumor suppressor genes, oncogenes, and DNA repair genes that contribute to colorectal carcinogenesis and review the correlations between these alterations and tumor progression.

Adenoma–Carcinoma Sequence

Most colorectal tumors arise from pre-cancerous polyps that are broadly categorized as either traditional tubular adenomas or serrated polyps. Adenomas develop when normal mechanisms that regulate DNA repair and cell proliferation are altered. Constant epithelial renewal is required due to the continued loss of surface cells from the intestinal mucosa; proliferation occurs only at the crypt base. As mutant cells advance towards the colonic lumen, the typically predictable process of terminal differentiation and eventual apoptosis is disrupted and discrete adenomas form. Over time, adenomatous polyps increase in size, develop increasingly dysplastic features, and can eventually acquire invasive potential.

Sequential alterations in key growth regulatory genes mark the transition from normal to hyperproliferative epithelium. This stepwise progression that couples specific genetic alterations with advancing histologic features has served as a paradigm for solid tumorigenesis. Mutations in the adenomatous polyposis coli gene, (*APC*) which encodes a tumor suppressor,⁷ or the *BRAF* oncogene,^{8,9} are initiating events that give rise to traditional adenomas or a serrated polyps, respectively. The subsequent events vary depending upon the specific pathway engaged (Fig. 1). Additional genetic alterations mark the progression from early to intermediate neoplasias, then to advanced polyps with high-grade dysplasia, and later, invasive tumors. However, not all adenomas advance to cancer—the accumulation of specific mutations in a particular order is essential for progression to malignancy. The timeline depends upon the specific pathway of tumorigenesis. For example, tumorigenesis via the CIN pathway can take 10 years or more, whereas tumor development via the comparatively accelerated MSI pathway can occur in a few years.

CIN pathway

The CIN pathway, observed in 65%–70% of sporadic colorectal tumors,^{10, 11} is characterized by chromosome changes that include somatic copy number alterations (SCNA) caused by aneuploidy, deletions, insertions, amplifications, or loss of heterozygosity. Tumors that develop through this pathway are considered non-hypermethylated because of the relative paucity of base-pair mutations in coding sequences (Fig. 1). Mechanisms that lead to CIN are usually characterized by defects in chromosomal segregation, such as those that control sister chromatid separation; disordered cell senescence, induced by telomere shortening and culminating in genomic reorganization; dysfunctional DNA damage-response machinery; and loss of heterozygosity (LOH) at a tumor suppressor gene.

These karyotypic abnormalities are coupled with mutations in the tumor suppressor genes *APC* and *TP53* and activating mutations in *KRAS* and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*). Mutation of *APC* appears to be the earliest genetic event in colorectal tumorigenesis^{1, 12}. Loss of APC activity results in nuclear translocation of beta-catenin and activation of the Wnt signaling pathway.

In the absence of Wnt signaling, APC, axin, and glycogen synthase kinase 3 beta (GSK3B) complex with beta-catenin in the cytosol. This complex phosphorylates beta-catenin, marking it for degradation by the ubiquitin-mediated proteasomal pathway (in the presence of Wnt signaling, beta-catenin is stable). Mutant *APC* has decreased binding affinity for this multi-protein destruction complex, resulting in translocation of beta-catenin to the nucleus, where it heterodimerizes with the TCF and LEF family of transcription factors. This results in constitutive activation of genes that are regulated by Wnt signaling and are associated with tumorigenesis, including *MYC*, the cyclin D1 gene (*CCND1*), vascular endothelial growth factor (VEGF) genes, and the peroxisome proliferator activated receptor delta gene.¹³ Mutations in other genes regulated by the Wnt pathway, such as *AXIN1*, *AXIN2*, or *CTNNB1*, can amplify Wnt signaling in the absence of an *APC* mutation.¹⁴ The Wnt signaling pathway is therefore an important regulator of intestinal epithelial cell proliferation and provides an example for how the disruption of any component in specific pathway can affect transcription of multiple genes to promote tumorigenesis.^{15–17}

The Wnt pathway is activated in nearly all CIN tumors, and *APC* mutations have identified in approximately 80% of these tumors.^{2, 3} In an effort to harmonize prior colorectal tumor functional profiles, which had only modest inter-study agreement,^{4, 6, 18, 19} the CRC Subtyping Consortium aggregated findings from more than 30 gene expression studies, performed with different platforms and sample preparation methods, into a single unified framework. Their efforts resulted in 4 classes of colorectal tumors based on robust, uniformly processed tumor gene expression profiles. Consensus molecular subtype 2 (CMS2) tumors are characterized by activation the Wnt and MYC signaling pathways and frequent SCNAs—features consistent with the CIN phenotype.³

MYC is a component of a heterodimeric basic helix-loop-helix transcription factor complex that regulates cell cycle progression, metabolism, and apoptosis. Mutations in *MYC* are not

found in most colorectal tumors, but amplifications of *MYC* have been found in colorectal and other tumor types.²⁰ Expression of *MYC* can be upregulated via activation of the Wnt signaling pathway.²¹ However, a meta-analysis found no clear association between tumor level of c-MYC protein and overall or disease-specific survival times.²²

Autosomal dominant mutations in *APC* cause familial adenomatous polyposis (FAP), characterized by hundreds to thousands of adenomatous polyps.²³ Biallelic germline variants in the mutY DNA glycosylase gene (*MUTYH*), which encodes a base excision repair protein, cause *MUTYH*-associated polyposis (MAP). Colon adenomas that develop in patients with MAP or FAP each have mutations in *APC*.^{24–26}

Activating mutations in *KRAS* often arise after mutations in *APC* and are found in nearly 40% of colorectal tumors.^{27, 28} *KRAS* is a component of several growth factor signaling pathways, including the epidermal growth factor receptor (EGFR) pathway. In this pathway, activation of *KRAS* results in constitutive activation of the Raf-MEK-ERK pathway, phosphoinositide 3 kinase (PI3K) signaling via MTOR, and the transcription factor NF-κB. Proteins in the Raf family are serine/threonine kinases that activate MEK1 and MEK2, resulting in phosphorylation of ERK1 and ERK2 and then phosphorylation of enzymes that promote cell cycle progression.²⁹ This pathway is activated in many tumors, including colorectal tumors, and might be targeted by therapeutic agents—especially metastatic colorectal tumors. Inhibitors of EGFR are used in treatment of metastatic tumors, but they are not effective in colorectal tumors with mutations in *KRAS* or *BRAF*, because these tumors maintain activity of this pathway.^{30–32}

Analyses of gene expression patterns in colorectal tumors have identified a relationship between *KRAS* mutations and CMS3 (also called the metabolic phenotype). CMS3 tumors are characterized by CIN, but with fewer SCNAs than the CMS2 subtype. Gene set mRNA enrichment analysis of CMS3 tumors found evidence for dysregulation of metabolic pathways, including those that involve sugars (such as glucose and fructose), amino acids (such as glutamine), lysophospholipids, and fatty acids. These metabolic aberrations might support tumor growth³ and are consistent with reports that activation of *KRAS* affects glucose metabolism and hypoxia.^{33, 34}

Many colorectal tumors with mutations in *KRAS* also contain mutations in genes encoding catalytic subunits of PI3K. PI3K is a heterodimeric lipid kinase that phosphorylates phosphatidylinositol, an cell signaling molecule. Increased activity of PI3K can increase prostaglandin synthesis to inhibit apoptosis in colorectal tumor cells.³⁵ Activating mutations in *PIK3CA* arise late in the adenoma-carcinoma sequence and are found in 10%–20% of colorectal tumors, as well as in breast, brain, ovarian, liver, and lung tumors.^{36, 37} *PIK3CA* regulates cell proliferation and survival, inactivating proteins that promote apoptosis such as forkhead box protein O1 (FOXO1) and the Rac family of GTPases. Gain of function mutations in *PIK3CA* activate AKT signaling via MTOR to promote cell proliferation. Mutations in *PIK3CA* have been associated with tumors in female patients, with proximal location, and with MSI and mutations in *KRAS*.^{38–42} Interestingly, the ratio of mutational burden at exons 9 and 20 in tumors associate with their tissue of origin (colorectal, breast, gastric, or endometrial) and with survival times of patients with CRC.^{36, 43}

Prostaglandin-endoperoxide synthase 2 (PTGS2, also called COX-2) is also involved in CRC development. COX-2 is an immediate to early response enzyme located on the luminal side of the endoplasmic reticulum and nuclear membrane.⁴⁴ It can be activated by cytokines and other stimuli and is overexpressed in adenomas and colorectal tumors,⁴⁵ although no somatic mutations in *COX-2* have been found in colorectal tumors. During colorectal carcinogenesis, COX-2 might convert free arachidonic acid into prostanoids, including prostaglandins such as PGE₂, which regulate proliferation of colorectal tumor cells.⁴⁶ Mice with disruption of *Ptgs2* have a decreased incidence of small- and large-bowel neoplasia.^{47, 48} COX-2 also regulates angiogenesis and promotes tumor vascularization.^{49, 50}

COX-2 might contribute to invasive and migratory activities of colorectal cancer cells. During early stages of colorectal tumorigenesis, expression of COX-2 is increased in the stroma and connective tissue, rather than the colonic epithelium.^{48, 51, 52} These observations might account for the association between use of COX inhibitors such as non-steroidal anti-inflammatory drugs (including aspirin and selective COX-2 inhibitors) and reduced risk of lesions in adenoma-carcinoma sequence, including metastatic CRC.^{53–58} Supported by large-scale epidemiology studies, as well as data from primary prevention trials for cardiovascular diseases (CVD), the United States Preventative Services Task Force recommends low-dose aspirin therapy for primary prevention of CVD and CRC in adults 50–59 years old with an increased risk of CVD, no increased risk for bleeding complications, a life expectancy of at least 10 years, and a willingness to adhere to regular, long-term use.⁵⁹

The *TP53* gene, on human chromosome 17p, is the most commonly mutated gene in cancer. Its product, P53, regulates transcription of genes that regulate DNA repair and cell responses to oxidative stress. Loss of function alterations in P53 are detected more frequently in colorectal tumors than adenomas with microscopic foci of invasive cancer, and more frequently in these invasive adenomas than benign adenomas⁶⁰. This association between the frequency of *TP53* mutations and lesion stage indicates that mutant forms of P53 promote tumor development, at a late stage of tumorigenesis.^{61, 62} Approximately 60% of CIN tumors contain inactivating mutations in *TP53*.² Patients with the Li-Fraumeni syndrome, characterized by germline mutations in *TP53*, have a modest increase in risk of CRC compared to the overall population. Analysis of data from patient registries found an association between germline alterations of *TP53* and young-onset CRC (diagnosed in patients younger than 50 years).⁶³ This observation is important because cases of young-onset CRC have increased greatly in the United States and parts of Europe and Asia, in contrast to reported decreases in CRC incidence in other age groups. Inherited genetic factors might contribute to a subset of these cases.^{64–69}

LOH at chromosome 18q is found in more than 70% of colorectal tumors (only of advanced stages).¹ This observation indicates the presence of colorectal tumor suppressor genes at this region. Candidates include the DCC netrin 1 receptor gene (*DCC*) and genes that encode proteins in the transforming growth factor beta (TGFB) pathway SMAD2 and SMAD4.^{70–72} However, mutations in these genes are rare in human colorectal tumors and deletions of any of these genes in mice have not been associated with colorectal tumorigenesis. LOH at 18q has been associated with shorter survival times, although this result might be confounded by

the association of 18q LOH with other negative prognostic factors, including high body mass index, higher tumor grade, and hypomethylation at long interspersed nucleotide element 1 elements of genomic DNA.^{73–75}

MSI pathway

In contrast to the CIN pathway, characterized by a high frequency of genomic copy number alterations, colorectal tumors can also develop through hypermutable pathways, characterized by frequent somatic DNA base pair mutations. The MSI pathway is the primary mechanism for this hypermutable phenotype. Broadly, mutations in DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*) or *EPCAM* (encodes a protein that regulates *MSH2*) cause instability within microsatellite regions. DNA microsatellites are repeated tandem sequences consisting of mono-, di-nucleotide, or even higher-order nucleotide repeats. These areas accumulate errors, as DNA polymerase struggles to efficiently bind these repeating genome sequences.

Failure to correctly assess the number of bases to insert into these invariable areas or even slippage during new strand synthesis (which generates a temporary insertion deletion loop) can produce a frameshift mutation that results in a truncated or non-functional protein. Ordinarily, in the absence of deleterious MMR gene mutations, the mismatch repair system recognizes these mistakes and performs DNA excision repair prior to daughter strand replication (see Fig. 2). Cells in tumors with the MSI phenotype do not properly detect and repair mismatched DNA, allowing them to maintain and replicate their mutations and acquire additional mutations (a hypermutation phenotype).

MSI is observed in nearly 15% of sporadic colorectal tumors and nearly all colorectal tumors that develop in patients with Lynch syndrome, the most common hereditary colon cancer syndrome, caused by germline mutations in DNA MMR genes.⁷⁶ However, most colorectal tumors with MSI are sporadic. The most common cause of the MSI phenotype is epigenetic silencing of the *MLH1* gene, through promoter hypermethylation. Colorectal tumors of the MSI phenotype usually have high levels of methylation at regulatory regions throughout the genome, including the CpG island methylator phenotype (CIMP). MSI tumors also have an increased frequency of a mutation that encodes a V to E substitution in amino acid 600 of BRAF (in BRAF^{V600E}), but a low frequency of *APC* and *TP53* mutations, compared colorectal tumors that develop via the CIN pathway.^{8, 77, 78} In patients with Lynch syndrome, colorectal tumors that arise via a germline mutation in a DNA MMR gene also have MSI but do not usually have mutations in *BRAF*.⁷⁹

MSI mutations include those in the TGFβ receptor-2 gene (*TGFBR2*), which encodes a protein that prevents colon epithelial cell proliferation. *TGFBR2* is mutated in more than 90% of MSI colorectal tumors.⁸⁰ Mutations in *TGFBR2* accumulate within a specific polyadenine tract to inactivate the receptor, so it can no longer signal to prevent proliferation. Other genes disrupted by MSI include those that encode proteins that regulate proliferation (*GRB1*, *TCF4*, *WISP3*, *ACVR2*, *IGF2R*, *AXIN2*, and *CDX*), cell cycle arrest or apoptosis (*CASP5*, *PRDM2*, *BCL10*, *PTEN*, *PA2G4*, and *FAS*), and DNA repair (*MBD4*, *BLM*, *CHK1*, *MLH3*, *RAD50*, *MSH3*, and *MSH6*).^{81, 82}

The events that initiate tumorigenesis via the MSI pathway vary. *APC* mutations are found in 35%–50% of MSI tumors,^{2,3} so the initiating event of adenoma formation might be shared by MSI and CIN tumors. However, it is important to note that a distinct set of MSI tumors can develop via an initiating *BRAF* mutation (Fig. 1).^{8,9} These tumors share traits of the MSI and serrated pathways. Once MSI occurs, colorectal tumors develop more rapidly than via the CIN pathway. Tumors with high levels of MSI (MSI-high) are associated with shorter times of progression, attributed to the hypermutable environment; CRC develops within 1–3 years as opposed to the decades required by CIN tumors.⁸³

MSI status is determined using a standard panel of microsatellite markers defined by the NCI/Bethesda consensus guidelines. Tumors with frameshifts in 30% or more of marker genes are classified as MSI-high tumor. Tumors with frameshift mutations in fewer than 30% of marker genes are classified as MSI-low. Finally, those without instability are deemed microsatellite stable (MSS).⁸⁴ PCR can reliably detect the presence of MSI, although immunohistochemical analysis of MMR proteins is the most common method. Tumor cells that lack MMR proteins, based on immunohistochemical analysis, are categorized as MSI-high. Genotypes of patients' colorectal tumors are routinely analyzed, to identify factors that might be targeted therapeutically. MSI status can also be determined by DNA sequencing.⁸⁵

Within the CMS framework, CMS1 tumors are characterized by hypermethylation of DNA, MSI-high, and infiltration by immune cells.³ Hypermethylation of promoter regions of the *MLH1* gene, in particular, results in its silencing, accumulation of DNA mutations, and expression of many mutant forms of proteins. These are immunogenic, resulting in tumor infiltration by cytotoxic T cells and natural killer cells^{87–89}, followed by expression of checkpoint molecules that inhibit T-cell activation and cytokine production, such as programmed cell death-ligand 1 (PD-L1). MSI-high tumors are therefore susceptible to treatment with PD-1 inhibitors,^{90,91} resulting in a paradigm shift in treatment of colorectal cancer.

A high proportion of CMS1 tumors have the *BRAF* V600E mutation and CIMP-high status. However, patients with these tumors have a better prognosis than patients with other CRC subtypes.⁸⁶ These tumors have distinctive clinical and histopathologic features, in that they tend to arise in the proximal colon, contain mucin, and be poorly differentiated. There is growing consensus that adjuvant, single-agent, fluoropyrimidine-based chemotherapy is not effective, and might even be harmful, for patients with MSI-high colorectal tumors.^{92–96} Although these tumors have characteristics such as poor differentiation, the presence of MSI indicates a good prognosis, so determining MSI status is now routinely recommended for the staging of incident CRC.⁹⁷ Among patients with localized tumors, those with MSI-high tumors had longer survival times than patients with MSS or MSI-low tumors of a similar stage.^{88,96,98–100}

Serrated Neoplasia Pathway

Adenomas with intestinal type dysplasia are not the only precursor lesions to CRC. Advances in endoscopic technology have improved detection of serrated polyps—saw-toothed lesions with a varied spectrum of histopathologic correlates. Serrated polyps are

believed to give rise to nearly 15% of CRCs, via the serrated neoplasia pathway.¹⁰¹ Serrated polyps are a heterogeneous group of lesions characterized by a stellate pattern of crypt infolding and include benign hyperplastic polyps, pre-cancerous sessile serrated adenomas or polyps, or traditional serrated adenomas (TSAs).¹⁰² A large proportion of interval CRCs (those that develop within recommended surveillance periods, typically 3–5 years) are believed to arise via the serrated pathway.^{103–105}

Hyperplastic polyps are the most frequently occurring subtype of serrated polyps, accounting for nearly two-thirds of all serrated lesions, and rarely undergo malignant transformation.^{106–108} Hyperplastic polyps have narrow crypt bases with serration confined to the upper portion and may be further subdivided by predominant mucin type: microvesicular, goblet cell rich, and mucin-poor hyperplastic polyps.

The second most common form of serrated lesions are sessile serrated adenomas and polyps, which are believed to account for one-third of all serrated lesions, although precise overall prevalence estimates have proven elusive.^{106, 107} Features that may make their identification challenging include a flat or sessile morphology that is easily missed by white-light imaging without chromoendoscopy and subtle distinguishing histopathologic traits that confound inter-observer reliability, even among expert gastrointestinal pathologists.^{109–111} Nuanced attributes such as abnormal proliferation, branching and dilated T- or L-shaped crypts, or subtle cellular dysplasia contribute to the heterogeneity of risk estimates.

TSAs account for a smaller proportion of serrated lesions. TSAs are histologically defined by protuberant growth patterns with villiform projections. These defining factors make distinctions between TSAs and tubulovillous adenomas more challenging. Cytologic dysplasia manifests as nuclear atypia with reduced differentiation and eosinophilic cytoplasm.¹¹²

The serrated pathway is a distinct mechanism of colorectal carcinogenesis. The pace of tumor progression is not fully characterized, but the subset of serrated tumors that acquire MSI are associated with a comparatively accelerated progression from precancerous lesion to carcinoma, like all MSI-high tumors. A distinguishing trait of the serrated pathway is the activating V600E mutation in *BRAF*, a component of the MAPK pathway.⁸ A large proportion of microvesicular hyperplastic polyps contain *BRAF* mutations, so this mutation is believed to occur early in the serrated pathway, causing constitutive activation of the MAPK—ERK pathway and uncontrolled cell division.^{8, 78, 113–117} *BRAF* is mutated in most sessile serrated adenomas, but rarely in conventional adenomas, supporting the concept that the serrated pathway is an alternative route to CRC.¹¹⁷

After mutation of *BRAF*, serrated tumors develop via 2 different routes (Fig. 1). One route converges with the MSI pathway—mutations in an MMR gene result in the MSI-high phenotype. These tumors typically develop from sessile serrated adenomas and share features with CMS1 tumors. Alternatively, tumors with mutations in *BRAF* can acquire *TP53* mutations and activate several oncogenic pathways, including Wnt signaling, TGF β signaling, and the epithelial to mesenchymal transition; these do not result in MSI-high but rather MSS tumors (Fig. 1).³ These tumors typically develop through the traditional serrated

adenoma as an intermediate lesion and have features of CMS4 (mesenchymal subtype) tumors. Unlike CMS1 tumors, CMS4 tumors have MSS with CIN, low levels of hypermutation, and high SCNA. CMS4 tumors have activation of pathways that facilitate an immunosuppressive microenvironment and permit stromal inflammation and tumor invasion, such as the angiogenic pathway. These factors may contribute to the abilities of CMS4 tumors to evade the immune response, resulting in the lowest survival rates of the CMSs.^{118, 119} Compared with classic CIN tumors (MSS and no mutations in *BRAF*), patients with MSS and mutant *BRAF* tumors had shorter times of overall survival (hazard ratio, 2.16) and disease-specific survival (hazard ratio, 2.59). In contrast, patients with MSI and mutant *BRAF* tumors have longer times of overall survival (hazard ratio, 0.74) and disease-specific survival (hazard ratio, 0.74), respectively.¹¹⁹

Although *BRAF* mutations are the first detected event in the serrated pathway, it is not uncommon for the Wnt pathway to also become activated.¹²⁰ In this scenario, the Wnt pathway is activated not by truncating mutations in *APC*, but rather by alternate routes including missense *APC* mutations or *RNF43* mutations (Fig. 1).^{121, 122} RNF43 is an E3 ubiquitin ligase that can inhibit Wnt signaling through R-spondin. Up to 85% of MSI-high tumors with methylation at *MLH1* have a somatic mutation in RNF43.¹²²

Despite successes in other tumor types that carry *BRAF* mutations, such as melanoma, use of single agent BRAF inhibitors in *BRAF*-mutant colorectal cancers has been disappointing.^{123, 124} This has been attributed to the frequent development of resistance mutations in compensatory molecular pathways, such as upregulation of EGFR.¹²⁵ Recently, a strategy that combines inhibition of BRAF with inhibitors of MEK and EGFR has demonstrated promising results in *BRAF*-mutant colon tumors, and this multi-drug approach is currently recommended by National Comprehensive Cancer Network (NCCN).¹²⁶

Tumors that develop through either arm of the serrated neoplasia pathway also typically exhibit high levels of CpG island methylation. CpG islands are dense clusters of cytosine/guanine (CpG) dinucleotides linked by a phosphodiester bond that are particularly enriched in gene promoter regions.¹²⁷ Hypermethylation of these promoter islands, particularly those upstream from tumor suppressor genes, abrogates their transcription, resulting in gene silencing and eventual tumor formation. The full spectrum of molecular mechanisms underlying the hypermethylation phenotype are not fully elucidated. However, one important mechanism depends upon the transcriptional repressor MAFG, which recruits BACH1, CHD8 and DNMT3B to bind and hypermethylate specific gene promoters including *MLH1*.¹²⁸ Importantly, mutant BRAF upregulates levels of MAFG to enhance its binding to promoters.

The CpG island methylation phenotype (CIMP) has multiple definitions. Tumors were initially classified as CIMP-high (≥ 3) or CIMP-low (< 2) based on the number of positive methylation markers found at locations of 5 genes (*MINT1*, *MINT2*, *MINT31*, *CDKN2A*, and *hMLH1*).¹²⁹ Since then, multiple other panels have been proposed that include different genes (*CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3*, and *SOC3*).¹³⁰ In addition, assessment of CIMP can now be made through genome-wide methylation analyses.¹³¹ This lack of consistency has made it challenging to compare independent studies. Nevertheless, CIMP-

high status is identified in approximately 20% of colorectal tumors, and this most often occurs in combination with a *BRAF* mutation and hypermethylation of *MLH1*, features that describe a large fraction of MSI-H tumors.^{130, 131} Although CIMP can be found in most sporadic MSI-H tumors, it is not restricted to MSI-H tumors. In fact, half of all tumors with CIMP do not have methylation of *MLH1* or MSI.^{132, 133}

CIMP can be first observed in early stages of tumorigenesis. For example, microvesicular hyperplastic polyps can be CIMP-high, and the frequency of CIMP increases compared with sessile serrated adenomas, TSAs, and other more advanced lesions.^{130, 134, 135} There is also an association between the CIMP in normal tissue and the occurrence of serrated polyps, indicating a field effect.¹³⁶ Meta-analyses found that CIMP status correlated with mucinous or poorly differentiated tumors that contain *BRAF* mutations and MSI, often in the right colon, predominantly in older and female patients.^{137, 138} However, there does not appear to be a clear and reproducible prognostic role for the CIMP.

Other Pathways

Efforts to genotype large panels of colorectal tumors in an unbiased manner have revealed recurrent mutations in genes not previously associated with colorectal cancer pathogenesis. For example, The Cancer Genome Atlas project completed a comprehensive molecular analysis of 276 colorectal tumors, and in addition to expected mutations in *APC*, *TP53*, and *KRAS*, frequent mutations were identified in *POLE*, *ARID1A*, *SOX9*, and *FAM123B*.² The identification of DNA polymerase protein mutations has specifically led to the characterization of a new molecular pathway. *POLE*, a catalytic subunit of DNA polymerase epsilon, and *POLD1*, its lagging-strand equivalent for DNA polymerase delta, each provide proofreading capabilities to their respective enzyme complexes. Mutations in *POLE* generate a hypermutated phenotype with high frequency of single nucleotide variants in the absence of aneuploidy or MSI (Fig. 1).¹³⁹ *APC* mutations appear to be the initiating event in this pathway, but the full spectrum of downstream mutations that develop in the context of this hypermutated environment is not yet described. Although these tumors do not have MSI, the hypermutated milieu nevertheless makes these tumors promising candidates for immunotherapy.^{140, 141}

A new subgroup of colorectal tumors has been proposed; these tumors appear to have stable genomes, without significant levels of aneuploidy or hypermutation. These tumors also are initiated through *APC*, have modest levels of DNA hypermethylation (CIMP-low), and acquire mutations in *KRAS*, *PIK3CA*, *SOX9*, and *PCBP1*.¹³⁹ The gene expression pattern of this subset of tumors most closely resembles the pattern of the CMS3 metabolic subtype, consistent with the frequent activation of *KRAS*. *TP53* mutations were less frequently observed, which might account for the low rates of aneuploidy in these tumors. The outcomes of patients with this group of tumors have not been defined. More detailed characterizations of these subtypes are underway.

Implications for Diagnostics

Identification of molecular features of colorectal tumors could lead to new diagnostic tools. Multi-target stool-based tests have been developed to non-invasively screen for CRC and its precursors. These tests detect methylation patterns and genetic mutations in stool samples that are associated with the adenoma-carcinoma sequence.¹⁴² Heralded as the first commercialized CRC-based screening assay the Cologuard test detects mutant *KRAS*, aberrantly methylated promoter regions of *BMP3* and *NDRG4*, and fecal hemoglobin through an immunological assay. Combined results from Cologuard and the fecal immunohistochemical test (FIT) identified patients with CRC with 92% sensitivity, compared to 74% sensitivity for the FIT alone, but with lower specificity (87% for the combined tests vs 95% for the FIT alone).

In 2016, the Food and Drug Administration approved a second-generation blood assay for a tumor-associated DNA marker for CRC screening. The septin 9 gene (*SEPT9*) encodes an eponymous GTP-binding protein involved in apoptosis, pseudopod protrusion, tumor cell migration, and invasion.¹⁴³ *SEPT9* is hypermethylated in CRC tissue compared with normal colon.^{144–146} Tests to detect methylated *SEPT9* in plasma samples (Epi proColon 2.0) detect CRC with only 48% sensitivity and 92% specificity.¹⁴⁷ Although the performance characteristics are inferior to those of the FIT, the *SEPT-9* remains an option for patients unwilling to pursue other CRC screening strategies.

Quantification of circulating tumor cells or circulating cell-free DNA derived from tumor cells (circulating tumor DNA, or ctDNA) can be used to monitor for CRC recurrence and response to treatment, and is a promising area of investigation. Apart from the *SEPT9* assay, the utility of so-called liquid biopsies has not been firmly established for CRC screening. Rather, the amount of CTCs or ctDNA detected in blood might be measured as a marker of disease recurrence after curative treatment.¹⁴⁸ Serial, non-invasive surveillance of patients undergoing treatment for CRC might allow for more timely identification of resistant clonal evolution. There is evidence for the rapid emergence of cancer cells with mutations that mediate resistance to EGFR inhibitors that can be detected in circulating DNA, even before recurrence is detected by radiology.^{149–152}

Future Directions

A wide variety of genetic and molecular changes mediate colorectal carcinogenesis. CRC is no longer considered to be a single disease entity. Advancement of care for patients with CRC will depend upon continued characterization of the different subtypes of colorectal tumors and development of therapeutic strategies based on these differences. In an effort to evaluate and summarize this progress, an expert panel represented by the American Society for Clinical Oncology, American Society for Clinical Pathology, Association for Molecular Pathology, and College of American Pathologists in 2017 offered 21 guideline statements for the use in analysis of colorectal tumors and care of patients.¹⁵³

Identification of the colorectal tumor subtypes leads to important areas for future studies. For example, although the CIMP is well-described, we do not know how aberrations in DNA

methylation occur or how reverse or overcome them. As we elucidate the effects of modifiable lifestyle and environmental factors on CRC risk, we will have to learn how features of the diet, obesity, and sedentary behavior contribute to colorectal tumor development. The association between *Fusobacterium* and other intestinal microbes with CRC risk will lead to many studies of the effects of the composition of the microbiome on intestinal epithelial cell transformation and tumorigenesis. There have been accomplishments, such as the discovery that immune checkpoint inhibitors are effective against MSI tumors, that non-steroidal anti-inflammatory and COX-2 inhibitors reduce risk of CRC, and that EGFR inhibitors are effective against tumors without mutations in *KRAS*. As we learn more about mechanisms of CRC pathogenesis and the different types of colorectal tumors, new therapeutic and diagnostic approaches will arise.

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REFERENCES

1. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61:759–67. [PubMed: 2188735]
2. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012;487:330–7. [PubMed: 22810696]
3. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015;21:1350–6. [PubMed: 26457759]
4. Marisa L, de Reynies A, Duval A, et al. Gene expression classification of colon cancer into molecular subtypes: characterization, validation, and prognostic value. *PLoS Med* 2013;10:e1001453. [PubMed: 23700391]
5. De Sousa EMF, Wang X, Jansen M, et al. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. *Nat Med* 2013;19:614–8. [PubMed: 23584090]
6. Sadanandam A, Lyssiotis CA, Homicsko K, et al. A colorectal cancer classification system that associates cellular phenotype and responses to therapy. *Nat Med* 2013;19:619–25. [PubMed: 23584089]
7. Sparks AB, Morin PJ, Vogelstein B, et al. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. *Cancer Res* 1998;58:1130–4. [PubMed: 9515795]
8. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature* 2002;417:949–54. [PubMed: 12068308]
9. Rad R, Cadinanos J, Rad L, et al. A genetic progression model of Braf(V600E)-induced intestinal tumorigenesis reveals targets for therapeutic intervention. *Cancer Cell* 2013;24:15–29. [PubMed: 23845441]
10. Vogelstein B, Papadopoulos N, Velculescu VE, et al. Cancer genome landscapes. *Science* 2013;339:1546–58. [PubMed: 23539594]
11. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. *Gastroenterology* 2008;135:1079–99. [PubMed: 18773902]
12. Powell SM, Zilz N, Beazer-Barclay Y, et al. APC mutations occur early during colorectal tumorigenesis. *Nature* 1992;359:235–7. [PubMed: 1528264]
13. Mann B, Gelos M, Siedow A, et al. Target genes of beta-catenin-T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. *Proc Natl Acad Sci U S A* 1999;96:1603–8. [PubMed: 9990071]

14. Salahshor S, Woodgett JR. The links between axin and carcinogenesis. *J Clin Pathol* 2005;58:225–36. [PubMed: 15735151]
15. Pinto D, Clevers H. Wnt control of stem cells and differentiation in the intestinal epithelium. *Exp Cell Res* 2005;306:357–63. [PubMed: 15925592]
16. Pinto D, Gregorieff A, Begthel H, et al. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev* 2003;17:1709–13. [PubMed: 12865297]
17. Schepers A, Clevers H. Wnt signaling, stem cells, and cancer of the gastrointestinal tract. *Cold Spring Harb Perspect Biol* 2012;4:a007989. [PubMed: 22474007]
18. Budinska E, Popovici V, Tejpar S, et al. Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer. *J Pathol* 2013;231:63–76. [PubMed: 23836465]
19. Schlicker A, Beran G, Chresta CM, et al. Subtypes of primary colorectal tumors correlate with response to targeted treatment in colorectal cell lines. *BMC Med Genomics* 2012;5:66. [PubMed: 23272949]
20. Dang CV. MYC on the path to cancer. *Cell* 2012;149:22–35. [PubMed: 22464321]
21. He TC, Sparks AB, Rago C, et al. Identification of c-MYC as a target of the APC pathway. *Science* 1998;281:1509–12. [PubMed: 9727977]
22. He WL, Weng XT, Wang JL, et al. Association Between c-Myc and Colorectal Cancer Prognosis: A Meta-Analysis. *Front Physiol* 2018;9:1549. [PubMed: 30483143]
23. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996;87:159–70. [PubMed: 8861899]
24. Sieber OM, Lipton L, Crabtree M, et al. Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. *N Engl J Med* 2003;348:791–9. [PubMed: 12606733]
25. Jones S, Emmerson P, Maynard J, et al. Biallelic germline mutations in MYH predispose to multiple colorectal adenoma and somatic G:C→T:A mutations. *Hum Mol Genet* 2002;11:2961–7. [PubMed: 12393807]
26. Venesio T, Molatore S, Cattaneo F, et al. High frequency of MYH gene mutations in a subset of patients with familial adenomatous polyposis. *Gastroenterology* 2004;126:1681–5. [PubMed: 15188161]
27. Santini D, Loupakakis F, Vincenzi B, et al. High concordance of KRAS status between primary colorectal tumors and related metastatic sites: implications for clinical practice. *Oncologist* 2008;13:1270–5. [PubMed: 19056857]
28. Tsuchida N, Ryder T, Ohtsubo E. Nucleotide sequence of the oncogene encoding the p21 transforming protein of Kirsten murine sarcoma virus. *Science* 1982;217:937–9. [PubMed: 6287573]
29. Pruitt K, Der CJ. Ras and Rho regulation of the cell cycle and oncogenesis. *Cancer Lett* 2001;171:1–10. [PubMed: 11485822]
30. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006;66:3992–5. [PubMed: 16618717]
31. Laurent-Puig P, Cayre A, Manceau G, et al. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. *J Clin Oncol* 2009;27:5924–30. [PubMed: 19884556]
32. Lievre A, Bachet JB, Boige V, et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol* 2008;26:374–9. [PubMed: 18202412]
33. Ying H, Kimmelman AC, Lyssiotis CA, et al. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. *Cell* 2012;149:656–70. [PubMed: 22541435]
34. McDonald PC, Chafe SC, Brown WS, et al. Regulation of pH by Carbonic Anhydrase 9 Mediates Survival of Pancreatic Cancer Cells With Activated KRAS in Response to Hypoxia. *Gastroenterology* 2019.
35. Wang D, Fu L, Sun H, et al. Prostaglandin E2 Promotes Colorectal Cancer Stem Cell Expansion and Metastasis in Mice. *Gastroenterology* 2015;149:1884–1895 e4. [PubMed: 26261008]

36. Liao X, Morikawa T, Lochhead P, et al. Prognostic role of PIK3CA mutation in colorectal cancer: cohort study and literature review. *Clin Cancer Res* 2012;18:2257–68. [PubMed: 22357840]
37. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science* 2004;304:554. [PubMed: 15016963]
38. Benvenuti S, Frattini M, Arena S, et al. PIK3CA cancer mutations display gender and tissue specificity patterns. *Hum Mutat* 2008;29:284–8. [PubMed: 18022911]
39. Taylor JG, DiSario JA, Buchi KN. Argon laser therapy for hemorrhagic radiation proctitis: long-term results. *Gastrointest Endosc* 1993;39:641–4. [PubMed: 8224685]
40. Nosho K, Kawasaki T, Ohnishi M, et al. PIK3CA mutation in colorectal cancer: relationship with genetic and epigenetic alterations. *Neoplasia* 2008;10:534–41. [PubMed: 18516290]
41. Kato S, Iida S, Higuchi T, et al. PIK3CA mutation is predictive of poor survival in patients with colorectal cancer. *Int J Cancer* 2007;121:1771–8. [PubMed: 17590872]
42. Baldus SE, Schaefer KL, Engers R, et al. Prevalence and heterogeneity of KRAS, BRAF, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases. *Clin Cancer Res* 2010;16:790–9. [PubMed: 20103678]
43. Barbi S, Cataldo I, De Manzoni G, et al. The analysis of PIK3CA mutations in gastric carcinoma and metanalysis of literature suggest that exon-selectivity is a signature of cancer type. *J Exp Clin Cancer Res* 2010;29:32. [PubMed: 20398348]
44. Chandrasekharan NV, Simmons DL. The cyclooxygenases. *Genome Biol* 2004;5:241. [PubMed: 15345041]
45. Eberhart CE, Coffey RJ, Radhika A, et al. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994;107:1183–8. [PubMed: 7926468]
46. Greenhough A, Smartt HJ, Moore AE, et al. The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. *Carcinogenesis* 2009;30:377–86. [PubMed: 19136477]
47. Oshima M, Murai N, Kargman S, et al. Chemoprevention of intestinal polyposis in the Apcdelta716 mouse by rofecoxib, a specific cyclooxygenase-2 inhibitor. *Cancer Res* 2001;61:1733–40. [PubMed: 11245490]
48. Oshima M, Dinchuk JE, Kargman SL, et al. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 1996;87:803–9. [PubMed: 8945508]
49. Fukuda R, Kelly B, Semenza GL. Vascular endothelial growth factor gene expression in colon cancer cells exposed to prostaglandin E2 is mediated by hypoxia-inducible factor 1. *Cancer Res* 2003;63:2330–4. [PubMed: 12727858]
50. Williams CS, Tsujii M, Reese J, et al. Host cyclooxygenase-2 modulates carcinoma growth. *J Clin Invest* 2000;105:1589–94. [PubMed: 10841517]
51. Nakagawa H, Liyanarachchi S, Davuluri RV, et al. Role of cancer-associated stromal fibroblasts in metastatic colon cancer to the liver and their expression profiles. *Oncogene* 2004;23:7366–77. [PubMed: 15326482]
52. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature* 2004;432:332–7. [PubMed: 15549095]
53. Dube C, Rostom A, Lewin G, et al. The use of aspirin for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. *Ann Intern Med* 2007;146:365–75. [PubMed: 17339622]
54. Cole BF, Logan RF, Halabi S, et al. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. *J Natl Cancer Inst* 2009;101:256–66. [PubMed: 19211452]
55. Labayle D, Fischer D, Vielh P, et al. Sulindac causes regression of rectal polyps in familial adenomatous polyposis. *Gastroenterology* 1991;101:635–9. [PubMed: 1650315]
56. Giardiello FM, Hamilton SR, Krush AJ, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. *N Engl J Med* 1993;328:1313–6. [PubMed: 8385741]
57. Higuchi T, Iwama T, Yoshinaga K, et al. A randomized, double-blind, placebo-controlled trial of the effects of rofecoxib, a selective cyclooxygenase-2 inhibitor, on rectal polyps in familial adenomatous polyposis patients. *Clin Cancer Res* 2003;9:4756–60. [PubMed: 14581346]

58. Steinbach G, Lynch PM, Phillips RK, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 2000;342:1946–52. [PubMed: 10874062]
59. Bibbins-Domingo K, Force USPST. Aspirin Use for the Primary Prevention of Cardiovascular Disease and Colorectal Cancer: U.S. Preventive Services Task Force Recommendation Statement. *Ann Intern Med* 2016;164:836–45. [PubMed: 27064677]
60. Russo A, Bazan V, Iacopetta B, et al. The TP53 colorectal cancer international collaborative study on the prognostic and predictive significance of p53 mutation: influence of tumor site, type of mutation, and adjuvant treatment. *J Clin Oncol* 2005;23:7518–28. [PubMed: 16172461]
61. Leslie A, Carey FA, Pratt NR, et al. The colorectal adenoma-carcinoma sequence. *Br J Surg* 2002;89:845–60. [PubMed: 12081733]
62. Baker SJ, Preisinger AC, Jessup JM, et al. p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer Res* 1990;50:7717–22. [PubMed: 2253215]
63. Yurgelun MB, Masciari S, Joshi VA, et al. Germline TP53 Mutations in Patients With Early-Onset Colorectal Cancer in the Colon Cancer Family Registry. *JAMA Oncol* 2015;1:214–21. [PubMed: 26086041]
64. Ahnen DJ, Wade SW, Jones WF, et al. The increasing incidence of young-onset colorectal cancer: a call to action. *Mayo Clin Proc* 2014;89:216–24. [PubMed: 24393412]
65. Siegel RL, Fedewa SA, Anderson WF, et al. Colorectal Cancer Incidence Patterns in the United States, 1974–2013. *J Natl Cancer Inst* 2017;109.
66. Siegel RL, Miller KD, Jemal A. Colorectal Cancer Mortality Rates in Adults Aged 20 to 54 Years in the United States, 1970–2014. *JAMA* 2017;318:572–574. [PubMed: 28787497]
67. Bailey CE, Hu CY, You YN, et al. Increasing disparities in the age-related incidences of colon and rectal cancers in the United States, 1975–2010. *JAMA Surg* 2015;150:17–22. [PubMed: 25372703]
68. Larsen IK, Bray F. Trends in colorectal cancer incidence in Norway 1962–2006: an interpretation of the temporal patterns by anatomic subsite. *Int J Cancer* 2010;126:721–32. [PubMed: 19688825]
69. Vuik FER, Nieuwenburg SAV, Bardou M, et al. Increasing incidence of colorectal cancer in young adults in Europe over the last 25 years. *Gut* 2019;gutjnl-2018–317592.
70. Mehlen P, Fearon ER. Role of the dependence receptor DCC in colorectal cancer pathogenesis. *J Clin Oncol* 2004;22:3420–8. [PubMed: 15310786]
71. Takagi Y, Kohmura H, Futamura M, et al. Somatic alterations of the DPC4 gene in human colorectal cancers in vivo. *Gastroenterology* 1996;111:1369–72. [PubMed: 8898652]
72. Takagi Y, Koumura H, Futamura M, et al. Somatic alterations of the SMAD-2 gene in human colorectal cancers. *Br J Cancer* 1998;78:1152–5. [PubMed: 9820171]
73. Sheffer M, Bacolod MD, Zuk O, et al. Association of survival and disease progression with chromosomal instability: a genomic exploration of colorectal cancer. *Proc Natl Acad Sci U S A* 2009;106:7131–6. [PubMed: 19359472]
74. Jen J, Kim H, Piantadosi S, et al. Allelic loss of chromosome 18q and prognosis in colorectal cancer. *N Engl J Med* 1994;331:213–21. [PubMed: 8015568]
75. Ogino S, Nosho K, Irahara N, et al. Prognostic significance and molecular associations of 18q loss of heterozygosity: a cohort study of microsatellite stable colorectal cancers. *J Clin Oncol* 2009;27:4591–8. [PubMed: 19704056]
76. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998;58:5248–57. [PubMed: 9823339]
77. Giannakis M, Hodi E, Jasmine Mu X, et al. RNF43 is frequently mutated in colorectal and endometrial cancers. *Nat Genet* 2014;46:1264–6. [PubMed: 25344691]
78. Rajagopalan H, Bardelli A, Lengauer C, et al. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. *Nature* 2002;418:934. [PubMed: 12198537]
79. Bessa X, Balleste B, Andreu M, et al. A prospective, multicenter, population-based study of BRAF mutational analysis for Lynch syndrome screening. *Clin Gastroenterol Hepatol* 2008;6:206–14. [PubMed: 18096441]

80. Parsons R, Myeroff LL, Liu B, et al. Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. *Cancer Res* 1995;55:5548–50. [PubMed: 7585632]
81. Duval A, Hamelin R. Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability. *Cancer Res* 2002;62:2447–54. [PubMed: 11980631]
82. Mori Y, Yin J, Rashid A, et al. Instabilitytyping: comprehensive identification of frameshift mutations caused by coding region microsatellite instability. *Cancer Res* 2001;61:6046–9. [PubMed: 11507051]
83. Umar A, Risinger JI, Hawk ET, et al. Testing guidelines for hereditary non-polyposis colorectal cancer. *Nat Rev Cancer* 2004;4:153–8. [PubMed: 14964310]
84. Dietmaier W, Wallinger S, Bocker T, et al. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. *Cancer Res* 1997;57:4749–56. [PubMed: 9354436]
85. Latham A, Srinivasan P, Kernel Y, et al. Microsatellite Instability Is Associated With the Presence of Lynch Syndrome Pan-Cancer. *J Clin Oncol* 2019;37:286–295. [PubMed: 30376427]
86. Fessler E, Drost J, van Hooff SR, et al. TGFbeta signaling directs serrated adenomas to the mesenchymal colorectal cancer subtype. *EMBO Mol Med* 2016;8:745–60. [PubMed: 27221051]
87. Boland CR. The molecular biology of gastrointestinal cancer: implications for diagnosis and therapy. *Gastrointest Endosc Clin N Am* 2008;18:401–13, vii. [PubMed: 18674693]
88. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993;260:816–9. [PubMed: 8484122]
89. Schwitalle Y, Kloor M, Eiermann S, et al. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. *Gastroenterology* 2008;134:988–97. [PubMed: 18395080]
90. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017;357:409–413. [PubMed: 28596308]
91. Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015;372:2509–20. [PubMed: 26028255]
92. Ribic CM, Sargent DJ, Moore MJ, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003;349:247–57. [PubMed: 12867608]
93. Twelves C, Wong A, Nowacki MP, et al. Capecitabine as adjuvant treatment for stage III colon cancer. *N Engl J Med* 2005;352:2696–704. [PubMed: 15987918]
94. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005;23:609–18. [PubMed: 15659508]
95. Carethers JM, Smith EJ, Behling CA, et al. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* 2004;126:394–401. [PubMed: 14762775]
96. Lanza G, Gafa R, Santini A, et al. Immunohistochemical test for MLH1 and MSH2 expression predicts clinical outcome in stage II and III colorectal cancer patients. *J Clin Oncol* 2006;24:2359–67. [PubMed: 16710035]
97. Amin MB, Greene FL, Edge SB, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more “personalized” approach to cancer staging. *CA Cancer J Clin* 2017;67:93–99. [PubMed: 28094848]
98. Lothe RA, Peltomaki P, Meling GI, et al. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. *Cancer Res* 1993;53:5849–52. [PubMed: 8261392]
99. Gryfe R, Kim H, Hsieh ET, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *N Engl J Med* 2000;342:69–77. [PubMed: 10631274]
100. Sinicrope FA, Mahoney MR, Smyrk TC, et al. Prognostic impact of deficient DNA mismatch repair in patients with stage III colon cancer from a randomized trial of FOLFOX-based adjuvant chemotherapy. *J Clin Oncol* 2013;31:3664–72. [PubMed: 24019539]

101. Toyota M, Ahuja N, Ohe-Toyota M, et al. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A* 1999;96:8681–6. [PubMed: 10411935]
102. Bosman F, F C, Hruban RH, et al. WHO Classification of Tumours of the Digestive System, 2010.
103. Nishihara R, Wu K, Lochhead P, et al. Long-term colorectal-cancer incidence and mortality after lower endoscopy. *N Engl J Med* 2013;369:1095–105. [PubMed: 24047059]
104. Burgess NG, Tuticci NJ, Pellise M, et al. Sessile serrated adenomas/polyps with cytologic dysplasia: a triple threat for interval cancer. *Gastrointest Endosc* 2014;80:307–10. [PubMed: 24890425]
105. Arain MA, Sawhney M, Sheikh S, et al. CIMP status of interval colon cancers: another piece to the puzzle. *Am J Gastroenterol* 2010;105:1189–95. [PubMed: 20010923]
106. Hazewinkel Y, de Wijkerslooth TR, Stoop EM, et al. Prevalence of serrated polyps and association with synchronous advanced neoplasia in screening colonoscopy. *Endoscopy* 2014;46:219–24. [PubMed: 24254386]
107. Carr NJ, Mahajan H, Tan KL, et al. Serrated and non-serrated polyps of the colorectum: their prevalence in an unselected case series and correlation of BRAF mutation analysis with the diagnosis of sessile serrated adenoma. *J Clin Pathol* 2009;62:516–8. [PubMed: 19126563]
108. Weston AP, Campbell DR. Diminutive colonic polyps: histopathology, spatial distribution, concomitant significant lesions, and treatment complications. *Am J Gastroenterol* 1995;90:24–8. [PubMed: 7801943]
109. Higuchi T, Sugihara K, Jass JR. Demographic and pathological characteristics of serrated polyps of colorectum. *Histopathology* 2005;47:32–40. [PubMed: 15982321]
110. Aust DE, Baretton GB, Members of the Working Group GIPotGSoP. Serrated polyps of the colon and rectum (hyperplastic polyps, sessile serrated adenomas, traditional serrated adenomas, and mixed polyps)-proposal for diagnostic criteria. *Virchows Arch* 2010;457:291–7. [PubMed: 20617338]
111. Farris AB, Misdraji J, Srivastava A, et al. Sessile serrated adenoma: challenging discrimination from other serrated colonic polyps. *Am J Surg Pathol* 2008;32:30–5. [PubMed: 18162767]
112. Snover DC. Update on the serrated pathway to colorectal carcinoma. *Hum Pathol* 2011;42:1–10. [PubMed: 20869746]
113. O'Brien MJ, Yang S, Mack C, et al. Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. *Am J Surg Pathol* 2006;30:1491–501. [PubMed: 17122504]
114. Spring KJ, Zhao ZZ, Karamatic R, et al. High prevalence of sessile serrated adenomas with BRAF mutations: a prospective study of patients undergoing colonoscopy. *Gastroenterology* 2006;131:1400–7. [PubMed: 17101316]
115. Rosenberg DW, Yang S, Pleau DC, et al. Mutations in BRAF and KRAS differentially distinguish serrated versus non-serrated hyperplastic aberrant crypt foci in humans. *Cancer Res* 2007;67:3551–4. [PubMed: 17440063]
116. Chan AO, Broaddus RR, Houlihan PS, et al. CpG island methylation in aberrant crypt foci of the colorectum. *Am J Pathol* 2002;160:1823–30. [PubMed: 12000733]
117. Kambara T, Simms LA, Whitehall VL, et al. BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. *Gut* 2004;53:1137–44. [PubMed: 15247181]
118. Sinicrope FA, Shi Q, Allegra CJ, et al. Association of DNA Mismatch Repair and Mutations in BRAF and KRAS With Survival After Recurrence in Stage III Colon Cancers : A Secondary Analysis of 2 Randomized Clinical Trials. *JAMA Oncol* 2017;3:472–480. [PubMed: 28006055]
119. Blaker H, Alwers E, Arnold A, et al. The Association Between Mutations in BRAF and Colorectal Cancer-Specific Survival Depends on Microsatellite Status and Tumor Stage. *Clin Gastroenterol Hepatol* 2019;17:455–462 e6. [PubMed: 29660527]
120. Borowsky J, Dumenil T, Bettington M, et al. The role of APC in WNT pathway activation in serrated neoplasia. *Mod Pathol* 2018;31:495–504. [PubMed: 29148535]

121. Bond CE, McKeone DM, Kalimutho M, et al. RNF43 and ZNRF3 are commonly altered in serrated pathway colorectal tumorigenesis. *Oncotarget* 2016;7:70589–70600. [PubMed: 27661107]
122. Yan HHN, Lai JCW, Ho SL, et al. RNF43 germline and somatic mutation in serrated neoplasia pathway and its association with BRAF mutation. *Gut* 2017;66:1645–1656. [PubMed: 27329244]
123. Kopetz S, Desai J, Chan E, et al. Phase II Pilot Study of Vemurafenib in Patients With Metastatic BRAF-Mutated Colorectal Cancer. *J Clin Oncol* 2015;33:4032–8. [PubMed: 26460303]
124. Hyman DM, Puzanov I, Subbiah V, et al. Vemurafenib in Multiple Nonmelanoma Cancers with BRAF V600 Mutations. *N Engl J Med* 2015;373:726–36. [PubMed: 26287849]
125. Prahallad A, Sun C, Huang S, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 2012;483:100–3. [PubMed: 22281684]
126. NCCN Clinical Practice Guidelines in Oncology: Colon Cancer. 2.2019 ed, 2019.
127. Bird AP. CpG-rich islands and the function of DNA methylation. *Nature* 1986;321:209–13. [PubMed: 2423876]
128. Fang M, Ou J, Hutchinson L, et al. The BRAF oncoprotein functions through the transcriptional repressor MAFK to mediate the CpG Island Methylator phenotype. *Mol Cell* 2014;55:904–915. [PubMed: 25219500]
129. Chan AO, Issa JP, Morris JS, et al. Concordant CpG island methylation in hyperplastic polyposis. *Am J Pathol* 2002;160:529–36. [PubMed: 11839573]
130. Weisenberger DJ, Siegmund KD, Campan M, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet* 2006;38:787–93. [PubMed: 16804544]
131. Hinoue T, Weisenberger DJ, Lange CP, et al. Genome-scale analysis of aberrant DNA methylation in colorectal cancer. *Genome Res* 2012;22:271–82. [PubMed: 21659424]
132. Samowitz WS, Albertsen H, Herrick J, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. *Gastroenterology* 2005;129:837–45. [PubMed: 16143123]
133. Hawkins N, Norrie M, Cheong K, et al. CpG island methylation in sporadic colorectal cancers and its relationship to microsatellite instability. *Gastroenterology* 2002;122:1376–87. [PubMed: 11984524]
134. Fernando WC, Miranda MS, Worthley DL, et al. The CIMP Phenotype in BRAF Mutant Serrated Polyps from a Prospective Colonoscopy Patient Cohort. *Gastroenterol Res Pract* 2014;2014:374926. [PubMed: 24812557]
135. Yang S, Farraye FA, Mack C, et al. BRAF and KRAS Mutations in hyperplastic polyps and serrated adenomas of the colorectum: relationship to histology and CpG island methylation status. *Am J Surg Pathol* 2004;28:1452–9. [PubMed: 15489648]
136. Worthley DL, Whitehall VL, Buttenshaw RL, et al. DNA methylation within the normal colorectal mucosa is associated with pathway-specific predisposition to cancer. *Oncogene* 2010;29:1653–62. [PubMed: 19966864]
137. Advani SM, Advani P, DeSantis SM, et al. Clinical, Pathological, and Molecular Characteristics of CpG Island Methylator Phenotype in Colorectal Cancer: A Systematic Review and Meta-analysis. *Transl Oncol* 2018;11:1188–1201. [PubMed: 30071442]
138. Zong L, Abe M, Ji J, et al. Tracking the Correlation Between CpG Island Methylator Phenotype and Other Molecular Features and Clinicopathological Features in Human Colorectal Cancers: A Systematic Review and Meta-Analysis. *Clin Transl Gastroenterol* 2016;7:e151. [PubMed: 26963001]
139. Liu Y, Sethi NS, Hinoue T, et al. Comparative Molecular Analysis of Gastrointestinal Adenocarcinomas. *Cancer Cell* 2018;33:721–735 e8. [PubMed: 29622466]
140. Boland PM, Ma WW. Immunotherapy for Colorectal Cancer. *Cancers (Basel)* 2017;9.
141. Bourdais R, Rousseau B, Pujals A, et al. Polymerase proofreading domain mutations: New opportunities for immunotherapy in hypermutated colorectal cancer beyond MMR deficiency. *Crit Rev Oncol Hematol* 2017;113:242–248. [PubMed: 28427513]
142. Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Multitarget stool DNA testing for colorectal-cancer screening. *N Engl J Med* 2014;370:1287–97. [PubMed: 24645800]

143. Shankar J, Messenberg A, Chan J, et al. Pseudopodial actin dynamics control epithelial-mesenchymal transition in metastatic cancer cells. *Cancer Res* 2010;70:3780–90. [PubMed: 20388789]
144. Ahmed D, Danielsen SA, Aagesen TH, et al. A tissue-based comparative effectiveness analysis of biomarkers for early detection of colorectal tumors. *Clin Transl Gastroenterol* 2012;3:e27. [PubMed: 23324654]
145. Wasserkort R, Kalmar A, Valcz G, et al. Aberrant septin 9 DNA methylation in colorectal cancer is restricted to a single CpG island. *BMC Cancer* 2013;13:398. [PubMed: 23988185]
146. Lofton-Day C, Model F, Devos T, et al. DNA methylation biomarkers for blood-based colorectal cancer screening. *Clin Chem* 2008;54:414–23. [PubMed: 18089654]
147. Church TR, Wandell M, Lofton-Day C, et al. Prospective evaluation of methylated SEPT9 in plasma for detection of asymptomatic colorectal cancer. *Gut* 2014;63:317–25. [PubMed: 23408352]
148. Yamada T, Matsuda A, Koizumi M, et al. Liquid Biopsy for the Management of Patients with Colorectal Cancer. *Digestion* 2019;99:39–45. [PubMed: 30554222]
149. Misale S, Yaeger R, Hobor S, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* 2012;486:532–6. [PubMed: 22722830]
150. Siravegna G, Mussolin B, Buscarino M, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. *Nat Med* 2015;21:795–801. [PubMed: 26030179]
151. Diaz LA Jr., Williams RT, Wu J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature* 2012;486:537–40. [PubMed: 22722843]
152. Misale S, Arena S, Lamba S, et al. Blockade of EGFR and MEK intercepts heterogeneous mechanisms of acquired resistance to anti-EGFR therapies in colorectal cancer. *Sci Transl Med* 2014;6:224ra26.
153. Sepulveda AR, Hamilton SR, Allegra CJ, et al. Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology. *J Clin Oncol* 2017;35:1453–1486. [PubMed: 28165299]

Figure 1. Pathways of Colorectal Carcinogenesis

Activation of the Wnt pathway (primarily via APC mutation) or a mutation in BRAF can initiate colorectal tumorigenesis. BRAF mutations promote tumorigenesis via the serrated neoplasia pathway, leading to MSI with or without hypermutation (indicated in figure). Colorectal tumor classifications include CIN, MSI, and the serrated pathway (see CMS). L, low; H, high; EMT, epithelial to mesenchymal transition.

Figure 2. MMR of Single-pair DNA Mismatches and Insertion or Deletion Loops

Upon detection of a DNA mismatch, MSH2 heterodimerizes with MSH6 or MSH3 to form MutS α (to correct single base-base or short insertion deletion loop mismatches) or MutS β (principally to correct larger insertion deletion loop), respectively. These MutS complexes recognize the replication errors and form larger complexes, with MutL α , β , or γ (for example, MLH1 binds to PMS2, PMS1, or MLH3) to direct the action of exonuclease-1 and proliferating cell nuclear antigen (PCNA) and remove the misincorporated nucleotide(s). DNA polymerase and DNA ligase then reconstruct and reanneal the daughter strand to accurately reflect the parent strand.