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REVIEW ARTICLE

MOLECULAR ORIGINS OF CANCER

Molecular Basis of Colorectal Cancer

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EVERY YEAR IN THE UNITED STATES, 160,000 CASES OF COLORECTAL CANCER are diagnosed, and 57,000 patients die of the disease, making it the second leading cause of death from cancer among adults.[1] The disease begins as a benign adenomatous polyp, which develops into an advanced adenoma with high-grade dysplasia and then progresses to an invasive cancer.[2] Invasive cancers that are confined within the wall of the colon (tumor–node–metastasis stages I and II) are curable, but if untreated, they spread to regional lymph nodes (stage III) and then metastasize to distant sites (stage IV).[3-5] Stage I and II tumors are curable by surgical excision, and up to 73% of cases of stage III disease are curable by surgery combined with adjuvant chemotherapy.[3,4,6] Recent advances in chemotherapy have improved survival, but stage IV disease is usually incurable.[3,4]

The clinical behavior of a colorectal cancer results from interactions at many levels (Fig. 1). The challenges are to understand the molecular basis of individual susceptibility to colorectal cancer and to determine factors that initiate the development of the tumor, drive its progression, and determine its responsiveness or resistance to antitumor agents. This review summarizes areas of current knowledge, recognizing that the topics presented are only fragments of the total picture.

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GENOMIC INSTABILITY

The loss of genomic stability can drive the development of colorectal cancer by facilitating the acquisition of multiple tumor-associated mutations. In this disease, genomic instability takes several forms, each with a different cause (Table 1).[7-26]

CHROMOSOMAL INSTABILITY

The most common type of genomic instability in colorectal cancer is chromosomal instability, which causes numerous changes in chromosomal copy number and structure.[7] Chromosomal instability is an efficient mechanism for causing the physical loss of a wild-type copy of a tumor-suppressor gene, such as *APC*, *P53*, and SMAD family member 4 (*SMAD4*), whose normal activities oppose the malignant phenotype.[2,27,28] In colorectal cancer, there are numerous rare inactivating mutations of genes whose normal function is to maintain chromosomal stability during replication, and in the aggregate, these mutations account for most of the chromosomal instability in such tumors.[8] In contrast to some other cancers, colorectal cancer does not commonly involve amplification of gene copy number[29] or gene rearrangement.

DNA-REPAIR DEFECTS

In a subgroup of patients with colorectal cancer, there is inactivation of genes required for repair of base–base mismatches in DNA, collectively referred to as mis-

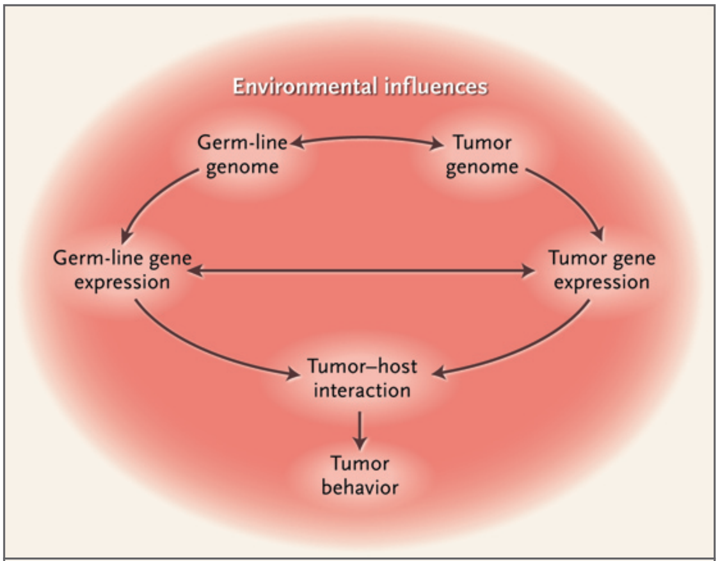


Figure 1. Multifactorial Colorectal Carcinogenesis.

The molecular events that drive the initiation, promotion, and progression of colorectal cancer occur on many interrelated levels. This dynamic process involves interactions among environmental influences, germ-line factors dictating individual cancer susceptibility, and accumulated somatic changes in the colorectal epithelium.

match-repair genes (Fig. 2 and 3). The inactivation can be inherited, as in hereditary nonpolyposis colon cancer (HNPCC), also known as the Lynch syndrome, or acquired, as in tumors with methylation-associated silencing of a gene that encodes a DNA mismatch-repair protein.

In patients with HNPCC, germ-line defects in mismatch-repair genes (primarily *MLH1* and *MSH2*) confer a lifetime risk of colorectal cancer of about 80%, with cancers evident by the age of 45 years, on average.[10-13,30,31] The loss of mismatch-repair function in patients with HNPCC is due not only to the mutant germ-line mismatch-repair gene but also to somatic inactivation of the wild-type parental allele.[31] Genomic instability arising from mismatch-repair deficiency dramatically accelerates the development of cancer in patients with HNPCC — some cancers arise within 36 months after normal results on colonoscopy.[32] For this reason, yearly colonoscopy is recommended for carriers of an HNPCC mutation,[30,32] and prophylactic colectomy should be considered for patients with high-grade lesions. Germ-line mutations of another mismatch-repair gene, *MSH6*, attenuates the predisposition to familial cancer.[9,33,34] Somatic inactivation of mismatch-repair genes occurs in approximately 15% of patients with nonfamilial colorectal cancer. In these patients, biallelic silencing of the promoter region of the *MLH1* gene by promoter methylation inactivates mismatch repair[15-17] (Fig. 2 and 3).

The loss of mismatch-repair function is easy to recognize by the associated epiphenomenon of microsatellite instability, in which the inability to repair strand slippage within repetitive DNA sequence elements changes the size of the mononucleotide or dinucleotide repeats (microsatellites) that are scattered throughout the genome. Mismatch-repair deficiency can also be detected by immunohistochemical analysis, which can identify the loss of one of the mismatch-repair proteins.[14,35-37] Cancers characterized by mismatch-repair deficiency arise primarily in the proximal colon, and in sporadic cases, they are associated with older age and female sex.[30] In mismatch-repair deficiency, tumor-suppressor genes, such as those encoding transforming growth factor β (TGF-β) receptor type II (*TGFBR2*) and BCL2-associated X protein (*BAX*), which have functional regions that contain mononucleotide or dinucleotide repeat sequences, can be inactivated.[2,27,28]

An alternative route to colorectal cancer involves germ-line inactivation of a base excision repair gene, mutY homologue (*MUTYH*, also called *MYH*).[25,33] The MYH protein excises from DNA the 8-oxoguanine product of oxidative damage to guanine.[24,25,33] In persons who carry two inactive germ-line *MYH* alleles, a polyposis phenotype develops, with a risk of colorectal cancer of nearly 100% by the age of 60 years.[33] *MYH*-associated polyposis is increasingly recognized: one third of all persons in whom 15 or more colorectal adenomas develop have *MYH*-associated polyposis.[33] The diagnosis requires genetic testing, which is facilitated by two mutations, Y165C and G382D, that together account for 85% of cases.[33] Thus far, somatic inactivation of *MYH* has not been detected in colorectal cancer.

ABERRANT DNA METHYLATION

Epigenetic silencing of genes, mostly mediated by aberrant DNA methylation, is another mecha-

Table 1. Patterns of Genomic Instability in Colorectal Cancer.*

Type of Instability and Syndrome	Type of Defect	Genes Involved	Phenotype
Chromosomal instability — loss of heterozygosity at multiple loci	Somatic	Loss of heterozygosity at *APC, TP53, SMAD4*[7,8]	Characteristic of 80 to 85% of sporadic colorectal cancers, depending on stage
DNA mismatch-repair defects			
Hereditary nonpolyposis colon cancer	Germ-line	*MLH1, MSH2, MSH6* germ-line gene mutations[9-14]	Multiple primary colorectal cancers, accelerated tumor progression, and increased risk of endometrial, gastric, and urothelial tumors
Sporadic colorectal cancer with mismatch-repair deficiency	Somatic	*MLH1* somatic methylation[15-17]	Colorectal cancer with increased risk of poor differentiation, more commonly located in right colon, less aggressive clinical behavior than tumors without mismatch-repair deficiency
CpG island methylator phenotype — methylation target loci	Somatic	Target loci *MLH1, MINT1, MINT2, MINT3*[18-23]	Characteristic of 15% of colorectal cancers, with most showing mismatch-repair deficiency from loss of tumor *MLH1* expression
Base excision repair defect — MYH-associated polyposis	Germ-line	*MYH*[24-26]	Development of 15 or more colorectal adenomas with increased risk of colorectal cancer

* MYH denotes mutY homologue.

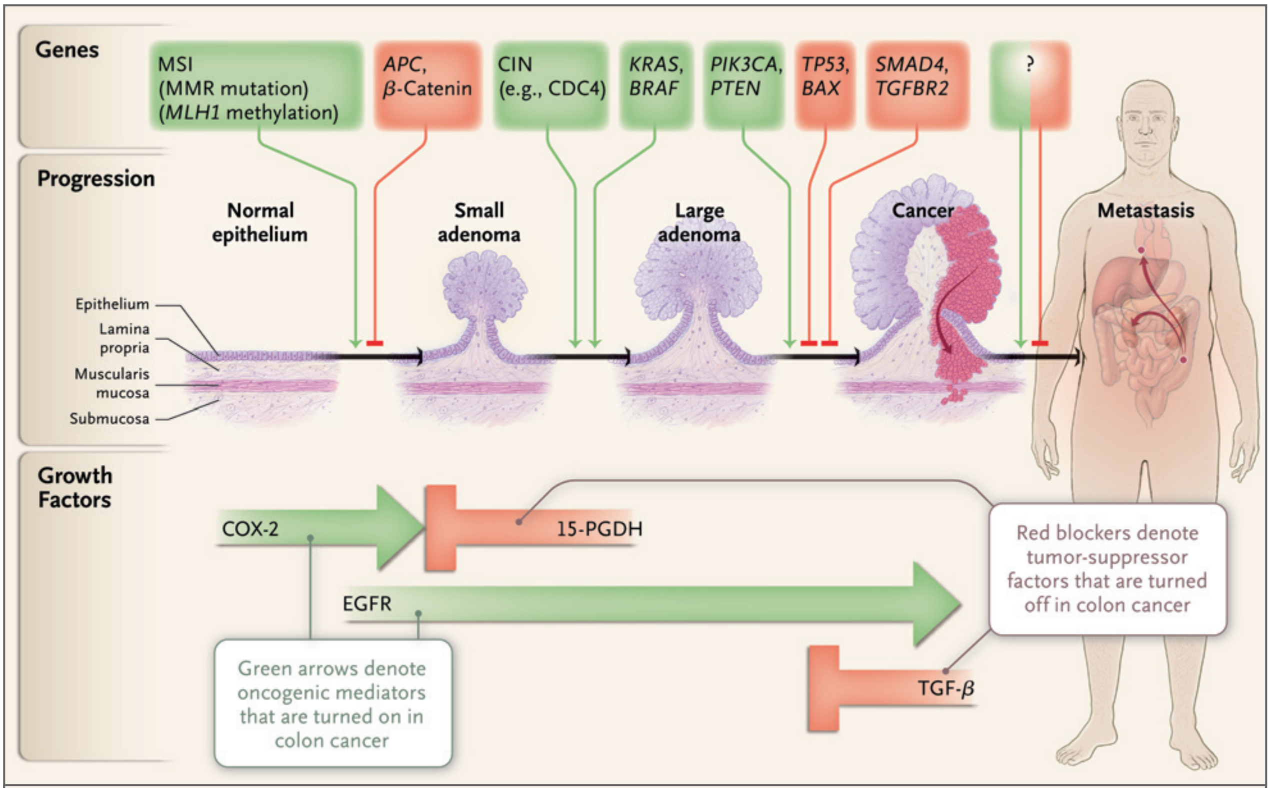
nism of gene inactivation in patients with colorectal cancer.[18,20] A methylated form of cytosine in which a methyl group is attached to carbon 5 (5-methylcytosine) defines a fifth DNA base, introduced by DNA methylases that modify cytosines within CpG dinucleotides.[18] In the normal genome, cytosine methylation occurs in areas of repetitive DNA sequences outside of exons; it is largely excluded from the CpG-rich "CpG islands" in the promoter regions of approximately half of all genes.[18] By comparison, in the colorectal-cancer genome, there is a modest global depletion of cytosine methylation but considerable acquisition of aberrant methylation within certain promoter-associated CpG islands.[18] This aberrant promoter-associated methylation can induce epigenetic silencing of gene expression.[18] In sporadic colorectal cancer with microsatellite instability, somatic epigenetic silencing blocks the expression of *MLH1*.[18]

Among the loci that can undergo aberrant methylation in colorectal cancer, a subgroup seems to become aberrantly methylated as a group, a phenomenon called the CpG island methylator phenotype (CIMP, or CIMP-high).[18,19] The molecular mechanism for CIMP remains unknown, but the phenomenon is reproducibly observed in about 15% of colorectal cancers and is present in nearly all such tumors with aberrant methylation of *MLH1*[18,19,21,38] (Fig. 2 and 3). The pathogenetic consequence of MLH1 silencing is well established, but the contribution of other epigenetic silencing events to colorectal carcinogenesis remains an area of ongoing study. An intermediate level of aberrant methylation in CIMP may define a subtype (i.e., CIMP2 and CIMP-low) that is thought to account for 30% of CIMP cases.[22,23] A third pattern of aberrant methylation is exemplified by exon 1 of the gene encoding vimentin. Although this locus is not expressed by normal colon mucosa or colorectal cancer, it is aberrantly methylated in 53 to 83% of patients with colorectal cancer in a pattern that is independent of CIMP.[39,40]

MUTATIONAL INACTIVATION OF TUMOR-SUPPRESSOR GENES

APC

Colorectal cancers acquire many genetic changes, but certain signaling pathways are clearly singled



Green arrows denote oncogenic mediators that are turned on in colon cancer

Red blockers denote tumor-suppressor factors that are turned off in colon cancer

Figure 2. Genes and Growth Factor Pathways That Drive the Progression of Colorectal Cancer.

In the progression of colon cancer, genetic alterations target the genes that are identified at the top of the diagram. The microsatellite instability (MSI) pathway is initiated by mismatch-repair (MMR) gene mutation or by aberrant *MLH1* methylation and is further associated with downstream mutations in *TGFBR2* and *BAX*. Aberrant *MLH1* methylation and *BRAF* mutation are each associated with the serrated-adenoma pathway. The question mark indicates that genetic or epigenetic changes specific to metastatic progression have not been identified. Key growth factor pathways that are altered during colon neoplasia are shown at the bottom of the diagram. CIN denotes chromosomal instability, EGFR epidermal growth factor receptor, 15-PGDH 15-prostaglandin dehydrogenase, and TGF-β transforming growth factor β.

out as key factors in tumor formation (Fig. 2 and Table 2).[41-62] One of these changes, the activation of the Wnt signaling pathway, is regarded as the initiating event in colorectal cancer.[2,28,43] Wnt signaling occurs when the oncoprotein β-catenin binds to nuclear partners (members of the T-cell factor–lymphocyte enhancer factor family) to create a transcription factor that regulates genes involved in cellular activation.[2,28,43] The β-catenin degradation complex controls levels of the β-catenin protein by proteolysis. A component of this complex, APC, not only degrades β-catenin but also inhibits its nuclear localization.

The most common mutation in colorectal cancer inactivates the gene that encodes the APC protein. In the absence of functional APC — the brake on β-catenin — Wnt signaling is inappropriately and constitutively activated. Germ-line *APC* mutations give rise to familial adenomatous polyposis, an inherited cancer-predisposition syndrome in which more than 100 adenomatous polyps can develop; in carriers of the mutant gene, the risk of colorectal cancer by the age of 40 years is almost 100%.[2,30,43] Somatic mutations and deletions that inactivate both copies of *APC* are present in most sporadic colorectal adenomas and cancers.[2,43] In a small subgroup of tumors with wild-type *APC*, mutations of β-catenin that render the protein resistant to the β-catenin degradation complex activate Wnt signaling.[2,41-43]

TP53

The inactivation of the p53 pathway by mutation of *TP53* is the second key genetic step in colorectal cancer. In most tumors, the two *TP53* alleles are inactivated, usually by a combination of a missense mutation that inactivates the transcriptional activity of p53 and a 17p chromosomal deletion that eliminates the second *TP53* allele.[2,27,28,44,45] Wild-type p53 mediates cell-cycle arrest and a cell-death checkpoint, which can be activated by multiple cellular stresses.[63] The inactivation of *TP53* often coincides with the transition of large adenomas into invasive carcinomas.[64] In many colorectal cancers with mismatch-repair defects, *TP53* remains wild-type, though in these cancers the activity of the p53 pathway is probably attenuated by mutations in the BAX inducer of apoptosis.[2,28]

TGF-*β* TUMOR-SUPPRESSOR PATHWAY

The mutational inactivation of TGF-*β* signaling is a third step in the progression to colorectal cancer.[50] In about one third of colorectal cancers, somatic mutations inactivate *TGFBR2*.[47,49,50,65,66] In tumors with mismatch-repair defects, *TGFBR2* is inactivated by distinctive frameshift mutations in a polyadenine repeat within the *TGFBR2* coding sequence.[47] In at least half of all colorectal cancers with wild-type mismatch repair, TGF-*β* signaling is abolished by inactivating missense mutations that affect the TGFBR2 kinase domain or, more commonly, mutations and deletions that inactivate the downstream TGF-*β* pathway component SMAD4 or its partner transcription factors, SMAD2 and SMAD3.[29,47,49-51,65-68] Mutations that inactivate the TGF-*β* pathway coincide with the transition from adenoma to high-grade dysplasia or carcinoma.[69]

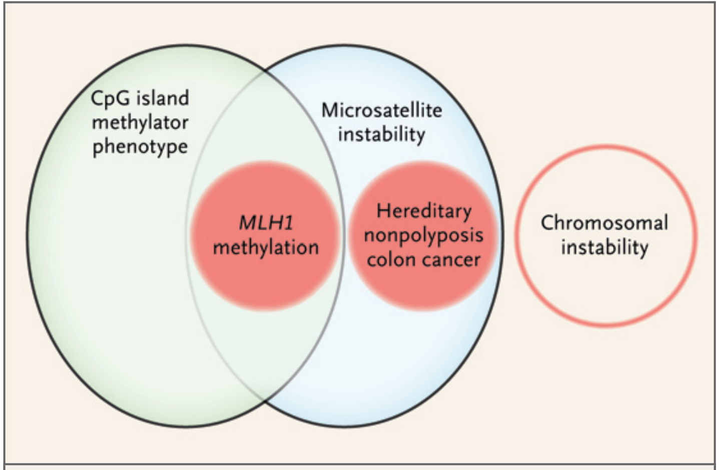

CpG island methylator phenotype
Microsatellite instability
MLH1 methylation
Hereditary nonpolyposis colon cancer
Chromosomal instability

Figure 3. **Genetic Instability Pathways That Drive Colon Neoplasias.**

Shown are the overlapping relationships that define the major pathways of genomic instability in colon cancers: chromosomal instability, microsatellite instability caused by defects in DNA mismatch-repair genes that are either inherited as germ-line defects (e.g., in hereditary nonpolyposis colon cancer) or somatically acquired (e.g., by aberrant methylation and epigenetic silencing of *MLH1*), and the CpG island methylator phenotype.

ACTIVATION OF ONCOGENE PATHWAYS

RAS AND *BRAF*

Several oncogenes play key roles in promoting colorectal cancer (Fig. 2 and Table 2). Oncogenic mutations of *RAS* and *BRAF*, which activate the mitogen-activated protein kinase (MAPK) signaling pathway, occur in 37% and 13% of colorectal cancers, respectively.[21,55,57,70,71] *RAS* mutations, principally in *KRAS*, activate the GTPase activity that signals directly to RAF. *BRAF* mutations signal BRAF serine–threonine kinase activity, which further drives the MAPK signaling cascade.[70,71] *BRAF* mutations are detectable even in small polyps,[21] and as compared with *RAS* mutations, they are more common in hyperplastic polyps, serrated adenomas, and proximal colon cancers, particularly in those with the CIMP phenotype (Fig. 3). Patients with numerous and large hyperplastic lesions, a condition termed the hyperplastic polyposis syndrome, have an increased risk of colorectal cancer, with disease progression occurring through an intermediate lesion with a serrated luminal border on histologic analysis.[18,22,38,58,59]

PHOSPHATIDYLINOSITOL 3-KINASE

One third of colorectal cancers bear activating somatic mutations in *PI3KCA*, which encodes the catalytic subunit of phosphatidylinositol 3-kinase (PI3K).[72] Less common genetic alterations that may substitute for *PI3KCA* mutations include loss of PTEN, an inhibitor of PI3K signaling, as well as amplification of insulin receptor substrate 2 (IRS2), an upstream activator of PI3K signaling, and coamplification of AKT and PAK4, which are downstream mediators of PI3K signaling.[73]

SEQUENCING THE COLORECTAL-CANCER GENOME

Advances in DNA sequencing technology have made it possible to sequence the entire coding genome of a human cancer. Colorectal cancer provided the first example of the power of this technology, with high-throughput sequencing of 18,000

Table 2. Tumor-Suppressor Genes and Oncogenes Commonly Associated with Colorectal Cancer.*

Affected Gene	Frequency %	Nature of Defect	Comments
APC	85	Activation of Wnt signaling due to inability to degrade the β-catenin oncoprotein[41,42]	Germ-line mutation in familial adenomatous polyposis; somatic inactivation found in 85% of sporadic colorectal cancers[43]
MLH1, MSH2, MSH6	15–25	DNA single-nucleotide mismatch-repair defect permitting the accumulation of oncogenic mutations and tumor-suppressor loss[10-14,31,35]	Germ-line mutation in hereditary nonpolyposis colorectal cancer[30]; epigenetic silencing causes loss of tumor MLH1 protein expression
TP53	35–55	Encoding a protein responsible for cell-cycle regulation[44,45]; inactivating missense mutations paired with loss of heterozygosity at 17p	Germ-line mutation in Li–Fraumeni syndrome[46]
TGFBR2	25–30	Receptor responsible for signaling pathways mediating growth arrest and apoptosis; inactivated by frameshift mutation in polyA repeat within TGFBR2 coding sequence in patients with mismatch-repair defects[47] or by inactivating mutation of kinase domain[48,49]	Mutation present in >90% of tumors with microsatellite instability and 15% of microsatellite-stable colon cancers[50]
SMAD4	10–35	Critical components of transforming growth factor β pathway signaling, along with related proteins SMAD2 and SMAD3; SMAD4 and SMAD2 are located on chromosome 18q, a frequent site of loss of heterozygosity in colorectal cancers; inactivated by homozygous deletion or mutation[51,52]	Germ-line mutations in familial juvenile polyposis, with a risk of colorectal cancer as high as 60% over three to four decades[53]
KRAS	35–45	Encoding the KRAS G-protein, with constitutive activation resulting in activation of both the PI3K–PDK1–PKB and RAF–MEK–ERK1/2 signaling pathways, thereby promoting cell survival and apoptosis suppression[54,55]	Germ-line mutation in the cardiofaciocutaneous syndrome[56]
BRAF V600E	8–12	Activating mutation in the BRAF serine–threonine kinase, a downstream mediator of signaling through the RAF–MEK–ERK1/2 pathway, which mimics the biologic consequences of KRAS mutation[38,57]	Associated with hyperplastic polyposis, with increased incidence in serrated adenomas[58,59]; like KRAS, germ-line mutation in the cardiofaciocutaneous syndrome[56]
PTEN	10–15	Promotion of the activation of PI3K pathway signaling through loss of function by inactivating mutation, resulting in cell-survival signaling and apoptosis suppression	Germ-line mutation in Cowden's syndrome, which carries a high risk of breast cancer, with 10% increased risk of colorectal cancer; possible role in maintenance of chromosomal stability[60-62]

* ERK denotes extracellular signal–regulated kinase, MAPK mitogen-activated protein kinase, MEK MAPK kinase, PDK1 pyruvate dehydrogenase kinase isozyme 1, PI3K phosphatidylinositol 3-kinase, and PKB protein kinase B.

members of the Reference Sequence (RefSeq) database of the National Center for Biotechnology Information.[65,66] Cancer-associated somatic mutations were identified in 848 genes. Of these, 140 were identified as candidate cancer genes that probably contributed to the cancer phenotype because they were mutated in at least two colorectal cancers and when corrected for gene size showed more mutations than expected by chance.

The average stage IV colorectal-cancer genome bears 15 mutated candidate cancer genes and 61 mutated passenger genes (very-low-frequency mutational events). The predominance of low-frequency mutations in candidate cancer genes implies enormous genetic heterogeneity among colorectal cancers, which mirrors the heterogeneity of the clinical behavior of colorectal cancers. The high degree of genetic heterogeneity makes it difficult to determine the clinical effect of individual mutational events. Moreover, these initial results are probably conservative, because some mutations, which were initially labeled as rare "passengers" in colorectal cancer, have subsequently emerged as common and are probably pathogenetic in other cancer types (e.g., an *IDH1* mutation noted initially in one colorectal cancer but subsequently in many gliomas).[65,66,74]

High-throughput sequencing of the colorectal-cancer genome has identified new common mutational targets. These include the ephrin receptors *EPHA3* and *EPHB6* (receptor tyrosine kinases), which together are mutated in 20% of colorectal cancers, and *FBXW7*, which functions in a protein degradation pathway that regulates levels of cyclin E and is mutated in 14% of colorectal cancers.[65,66,75] An important challenge is to reduce the complexity of the 140 candidate cancer genes by identifying the biologic pathways and processes that are common downstream targets of multiple mutational events.

GENOMIC CHANGES AND TUMOR PROGRESSION

The sequence of transformation from adenoma to carcinoma, as initially formulated,[2,28,43] was a model of the development of colorectal cancer in which specific tumor-promoting mutations are progressively acquired. This model predicts the presence of mutations that dictate specific tumor characteristics, such as the presence of regional or distant metastases (Fig. 2). Unexpectedly, the examination of results of full-genome sequencing from primary colorectal cancers and distant metastases in the same patient showed no new mutations in the metastases,[76] implying that new mutations are not required to enable a tumor cell to leave the primary tumor and seed a distant site. Because the ongoing generation of mutations serves as a molecular clock, the finding that all the mutations in a metastasis are also present in the primary tumor implies that metastatic seeding is rapid, requiring less than 24 months from the appearance of the final mutation in the primary tumor.[76]

GROWTH FACTOR PATHWAYS

ABERRANT REGULATION OF PROSTAGLANDIN SIGNALING

The activation of growth factor pathways is common in colorectal cancer (Fig. 2). An early and critical step in the development of an adenoma is the activation of prostaglandin signaling.[77,78] This abnormal response can be induced by inflammation or mitogen-associated up-regulation of COX-2, an inducible enzyme that mediates the synthesis of prostaglandin E_2, an agent strongly associated with colorectal cancer.[78] Prostaglandin E_2 activity can also be increased by the loss of 15-prostaglandin dehydrogenase (15-PGDH), the rate-limiting enzyme in catalyzing degradation of prostaglandin.[79-81] Increased levels of COX-2 are found in approximately two thirds of colorectal cancers,[78,82] and there is loss of 15-PGDH in 80% of colorectal adenomas and cancers.[79] Clinical trials have shown that the inhibition of COX-2 by nonsteroidal antiinflammatory drugs prevents the development of new adenomas[83-86] and mediates regression of established adenomas.[87]

EPIDERMAL GROWTH FACTOR RECEPTOR

Epidermal growth factor (EGF) is a soluble protein that has trophic effects on intestinal cells. Clinical studies have supported an important role of signaling through the EGF receptor (EGFR) in a subgroup of colorectal cancers.[88-91] EGFR mediates signaling by activating the MAPK and PI3K signaling cascades. Recent clinical data have shown that advanced colorectal cancer with tumor-promoting mutations of these pathways — including activating mutations in *KRAS*,[92-94] *BRAF*,[95,96] and the p110 subunit of PI3K[97] — do not respond to anti-EGFR therapy.

VASCULAR ENDOTHELIAL GROWTH FACTOR

Vascular endothelial growth factor (VEGF) that is produced in states of injury or during the growth of normal tissue drives the production of new stromal blood vessels (angiogenesis). Clinical studies have suggested a role for angiogenic pathways in the growth and lethal potential of colorectal cancer. Treatment with the anti-VEGF antibody bevacizumab added an average of 4.7 months to the overall survival of patients with advanced colorectal cancer (15.6 months with standard therapy).[98] The identification of molecular distinctions between cancers that benefit from this treatment and those that do not remains a challenge.

STEM-CELL PATHWAYS

Stem cells in colorectal cancers are believed to be uniquely endowed with the capacity to renew themselves.[99-102] Single colorectal-cancer stem cells, by definition, can lodge in a permissive site, such as the liver, and produce a metastasis. Currently, it is not possible to isolate individual colorectal-cancer stem cells, although certain cell-surface proteins (e.g., CD133, CD44, CD166, and aldehyde dehydrogenase 1) are promising markers. Normal stem cells that reside in the colonic crypt rely on adhesive and soluble stromal–epithelial interactions to maintain division and differentiation. The extent of alterations in these regulatory mechanisms in colorectal-cancer stem cells is a promising area of investigation, since agents that control the growth of colorectal-cancer stem cells could theoretically be used for cancer prevention and treatment.

PREDICTIVE AND PROGNOSTIC MARKERS

One ongoing challenge is to translate the wealth of knowledge regarding colorectal-cancer genomics into clinically applicable predictive or prognostic tests (Table 3). The relation between mutations in EGFR signaling components RAS and BRAF and anti-EGFR therapy is currently the only application of colorectal-cancer genomics to treatment.[92-96] A few genomic markers are useful for prognosis. For example, germ-line mutations in tumor-suppressor genes, such as *APC*, *MLH1*, and *MSH2*, indicate a very high risk of colorectal cancer and guide the frequency of colorectal-cancer surveillance and recommendations for prophylactic surgery. Other somatic markers have modest or unconfirmed prognostic value and are not currently used to direct care. Sporadic colorectal cancers with a mismatch-repair deficiency generally have a favorable prognosis[35,103,105,108]; poor survival in stage II and III colon cancers is associated with the loss of p27 (a proapoptotic regulator of the cell cycle[109]) or the loss of heterozygosity at chromosomal location 18q.[105]

NONINVASIVE MOLECULAR DETECTION

The development of molecular diagnostics for the early detection of colorectal cancer is an important translation of colon-cancer genetics into clinical practice. One example is the development of assays to detect mutations that are specific to colorectal cancer and cancer-associated aberrant DNA methylation in fecal DNA from patients with colorectal cancer or advanced adenomas. These assays have a sensitivity of 46 to 77% for detecting early-stage colorectal cancer, which is superior to the sensitivity of testing for fecal occult blood although their superiority in preventing death from cancer has not been shown.[39,110-113] Stool DNA testing for colorectal cancer has been added to the cancer-screening guidelines of the American Cancer Society[114] and appears to be equally sensitive for detecting advanced adenomas.[115] Although still in the developmental stage, assays for detecting plasma cell-free DNA may also be clinically useful,[115] and assays for tumor-specific plasma protein or RNA profiles also remain targets of research. Questions that remain to be resolved are

Table 3. Prognostic and Predictive DNA Markers in Colorectal Cancer.*

DNA Marker	Comments
Prognostic	
APC	A germ-line mutation defines the colorectal-cancer predisposition syndrome, familial adenomatous polyposis, with an 80 to 100% lifetime risk of colorectal cancer. Patients with germ-line *APC* mutations undergo prophylactic colectomy or proctocolectomy.
MLH1, MSH2, MSH6	A germ-line mutation in these and, less commonly, in other mismatch-repair genes defines hereditary nonpolyposis colon cancer, with a 40 to 80% lifetime risk of colorectal cancer, as well as an increased risk of endometrial cancer. Patients with germ-line mismatch-repair gene mutations undergo frequent colonoscopic surveillance and may be considered for prophylactic colectomy and hysterectomy.
MLH1 methylation–associated silencing	The somatic inactivation of *MLH1* in primary colorectal cancers is evidenced by either detection of DNA microsatellite instability or loss of tumor MLH1 protein expression on immunohistochemical analysis, and is more frequent in early-stage colorectal cancers than in advanced disease. Such inactivation may be a marker of more indolent disease or a better prognosis in the absence of adjuvant chemotherapy.[103,104]
18q Loss of heterozygosity	The somatic loss of heterozygosity at chromosomal location 18q, a site containing genes associated with colorectal cancer (e.g., *SMAD4* and *SMAD2*), is associated with a poorer outcome in patients with stage II or stage III colon cancer than that in patients with tumors retaining both parental alleles at 18q.[105]
Predictive	
KRAS	The somatic mutation produces unrestricted activity of signaling through the MAPK and PI3K cascades. Patients with stage IV colorectal cancer and activating mutations in *KRAS* do not have a response to EGFR-inhibitor therapy.[92-94]
BRAF V600E	The somatic mutation activating this kinase causes unrestricted MAPK pathway signaling. Patients with stage IV colorectal cancer and the activating *BRAF* V600E mutation do not have a response to EGFR-inhibitor therapy.[95]
MLH1 methylation-associated silencing	The loss of the mismatch-repair function contributes to the loss of other tumor suppressors (e.g., TGFBR2 and BAX). Patients with mismatch-repair–deficient tumors may not have a response to fluorouracil and may have an improved response to irinotecan-containing regimens.[106,107]

* BAX denotes BCL2-associated X protein, EGFR epidermal growth factor receptor, MAPK mitogen-activated protein kinase, PI3K phosphatidylinositol 3-kinase, and TGFBR2 transforming growth factor receptor β type II.

the optimal interval between serial tests and the performance and cost-effectiveness of stool DNA testing as compared with those of newer immunochemical fecal occult-blood tests.[116]

GENETIC INFLUENCES IN POPULATION SUSCEPTIBILITY

Genetic epidemiology and twin studies indicate that 35 to 100% of colorectal cancers and adenomas develop in persons with an inherited susceptibility to the disease.[117-119] In addition, an HNPCC-like syndrome occurs in some families without any evidence of defects in mismatch repair.[120] Several genomic loci that could harbor highly penetrant susceptibility genes have been identified with the use of linkage approaches,[121-123] but the underlying mutations are unknown. Genomewide association studies have also identified germ-line DNA variants that are strongly associated with susceptibility, but the clinical use of these results is probably limited, since the relative risk associated with these variants is low.[124-129]

CONCLUSIONS

Studies that aid in the understanding of colorectal cancer on a molecular level have provided important tools for genetic testing for high-risk familial forms of the disease, predictive markers for selecting patients for certain classes of drug therapies, and molecular diagnostics for the noninvasive detection of early cancers. In addition, biologic pathways that could form the basis of new therapeutic agents have been identified. Although some high-frequency mutations are attractive targets for drug development, common signaling pathways downstream from these mutations may also be tractable as therapeutic targets. Recent progress in molecular assays for the early detection of colorectal cancer indicates that understanding the genes and pathways that control the earliest steps of the disease and individual susceptibility can contribute to clinical management in the near term.

An understanding of the signals that dictate the metastatic phenotype will provide the information necessary to develop drugs to control or prevent advanced disease. The considerable recent advances encourage us to believe that improvements in our knowledge of the molecular basis of colorectal cancer will continue to reduce the burden of this disease.

Dr. Markowitz reports being listed on patents licensed to Exact Sciences and LabCorp and is entitled to receive royalties on sales of products related to methylated vimentin DNA, in accordance with the policies of Case Western Reserve University. No other potential conflict of interest relevant to this article was reported.

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