

The Genetic Basis of Colorectal Cancer: Insights Into Critical Pathways of Tumorigenesis

DANIEL C. CHUNG

Gastrointestinal Unit, Massachusetts General Hospital, Boston, Massachusetts

The impact that modern molecular biology has had on elucidating the genetic basis of neoplasia is best illustrated by the paradigm of colon cancer. Several unique features of colon cancer biology have served to accelerate the discovery process. The development of a readily evident precursor lesion, the adenomatous polyp, has made it possible to construct a model of the sequential genetic events in cancer initiation and progression. Insights into the molecular basis of the relatively rare inherited syndromes familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) have had profound implications for tumorigenesis in the more widespread sporadic forms of colon cancer.

Information on the genetics of neoplasia has increased at an exponential rate; the goal of this review is to focus on the pathways that seem to be uniquely relevant to colorectal cancer pathogenesis. It is clear that multiple events involving tumor-suppressor genes, oncogenes, and DNA mismatch repair genes contribute to the development of colorectal cancer, and the order in which these mutations occur is critical. Table 1 outlines some of the key genetic alterations that have been identified.

In 1990, a stepwise model of colorectal tumorigenesis was developed in which (1) mutations in the adenomatous polyposis coli tumor-suppressor gene (*APC*) were proposed to occur early during the development of polyps, (2) oncogenic *KRAS* mutations arose during the adenomatous stage, and (3) mutations of *TP53* and deletions on chromosome 18q coincided with the transition to malignancy.¹ This model has been generally validated. Subsequent work has identified additional genetic events as well as the specific molecular pathways perturbed by each of these mutations. These pathways regulate important cellular processes in neoplastic cells including proliferation and apoptosis. Ras and p53 pathways underlie tumorigenesis in a broad array of tumor types, and have been extensively reviewed.^{2–5} This article will focus on three critical mechanistic pathways that characteristically affect colonic epithelia: the APC/ β -catenin, DNA mismatch repair, and transforming growth factor (TGF)- β /SMAD pathways.

The final section will address the role of epigenetics in tumor development. While clonal gene mutations that provide a selective growth advantage are considered the sine qua non of molecular tumor pathogenesis, the importance of epigenetic mechanisms that regulate gene expression is becoming increasingly clear. Defects in DNA methylation may play an especially critical role in colon cancer pathogenesis.

The APC/ β -Catenin Pathway

The clinical and hereditary features of the FAP syndrome implied the existence of a single gene that regulates the formation of adenomatous polyps, the precursor for most colorectal cancers. The identification of an interstitial deletion on chromosome 5q in a patient with the Gardner's variant of FAP combined with classical linkage analysis facilitated the positional cloning of the *APC* gene in 1991.^{6,7} The 15-exon gene encodes a multifunctional 2843–amino acid protein of 310 kilodaltons that, when mutated in the germline, results in either FAP or Gardner's syndrome.

Subsequent studies have suggested that the *APC* tumor-suppressor gene also plays a critical early role in sporadic colon cancer, and *APC* is mutated in more than 70% of all colorectal cancers. This high mutational rate is unique to colorectal cancer. Although germline inactivating mutations of *APC* are distributed throughout the entire gene, somatic mutations are somewhat clustered at the 5' end of exon 15 between amino acid residues 1280–1500.⁸ This overlaps with the 15–amino acid repeat domains between residues 1020 and 1169 and 20–amino acid repeat domains between residues 1324 and 2075 that have been implicated in β -catenin

Abbreviations used in this paper: APC, adenomatous polyposis coli; COX, cyclooxygenase; DNMT, DNA methyltransferase; FAP, familial adenomatous polyposis; GSK, glycogen synthase kinase; HNPCC, hereditary nonpolyposis colorectal cancer; LOH, loss of heterozygosity; MSI, microsatellite instability; PPAR, peroxisome proliferator-activated receptor; TCF, T-cell factor; TGF, transforming growth factor.

© 2000 by the American Gastroenterological Association

0016-5085/00/\$10.00

doi:10.1053/gast.2000.16507

Table 1. Overall Prevalence of Genetic Mutations in Colorectal Cancer

Gene	Chromosomal location	Prevalence of mutations	Comment
Oncogenes			
<i>KRAS2</i>	12p	~50%	Consensus activating point mutations at codons 12 and 13 Identified in 50% of tumors without APC mutations; most mutations in exon 3 at GSK-3β phosphorylation sites Identified only in metastatic tumors
<i>CTNNB1</i>	3p22	~4%–15%	
<i>SRC</i>	20q11	2%	
Tumor-suppressor genes			
<i>APC</i>	5q21	~70%	Most mutations are truncating and occur early at adenoma stage Rarely identified in benign adenomas; mutant p53 protein detectable immunohistochemically >70% of colorectal tumors show allelic loss on 18q; many potential tumor-suppressor gene targets including <i>SMAD4</i> , <i>SMAD2</i> , and <i>DCC</i>
<i>TP53</i>	17p13	~50%–70%	
<i>SMAD4</i>	18q21	16%	
<i>SMAD2</i>	18q21	6%	
<i>DCC</i>	18q21	3%	
DNA mismatch repair genes			
<i>hMSH2</i>	2p21	~15% ^a	>90% of identified germline mutations are in <i>hMSH2</i> or <i>hMLH1</i> ; limited mutational data in sporadic tumors, but maximum of 26% with MSI have somatic <i>hMSH2</i> mutations
<i>hMLH1</i>	3p21		
<i>hPMS1</i>	2q31–33		
<i>hPMS2</i>	7p22		
<i>hMSH6</i>	2p21		

^aOverall, 15% of tumors display MSI.

interactions,^{9,10} as well as the SAMP (Ser-Ala-Met-Pro) domains located between the 20–amino acid repeats that interact with axin.¹¹ Consistent with the model of tumor-suppressor gene inactivation first proposed by Knudsen, mutations in 1 copy of *APC* are typically coupled with chromosomal deletions of the second wild-type allele. However, biallelic inactivation may occur through alternative routes. In some tumors from FAP patients with a germline *APC* mutation, deletions of the wild-type *APC* allele are not observed. Rather, there are deletions of the inherited mutant allele, and these are accompanied by new somatic mutations in the remaining allele.^{8,12}

Regardless of the mechanism through which they are acquired, more than 90% of *APC* mutations result in a premature stop codon and thus a truncated gene product.^{8,13} This observation serves as the basis for the most commonly used clinical assay for *APC* gene testing, the *in vitro* synthesized protein truncation test (Figure 1).¹⁴ As predicted by the stepwise model of colonic tumorigenesis cited above, alterations in *APC* are characteristically present in benign polyps and the prevalence does not increase with advancing tumor stage.^{13,15}

Several fascinating correlations between genotype and phenotype have been found in families with germline *APC* mutations. Mutations between codons 463 and 1387 are associated with congenital hypertrophy of the retinal pigmented epithelium.¹⁶ An increased risk of desmoid tumor formation is observed in families with germline mutations between codons 1445 and 1578.¹⁷ In addition, mutations at the 5' end of the gene proximal to

codon 157¹⁸ or at the 3' end distal to codon 1900¹⁹ are associated with a more attenuated form of the disease (attenuated APC), in which fewer polyps develop than found in classic FAP and the onset of cancer is delayed until the sixth decade of life. However, these correlations are not uniform, and families with identical mutations may display variable phenotypes.⁷

Specific polymorphisms of *APC* are also associated with an increased risk of colorectal cancer. Individuals with the I1307K polymorphism (a transition from T to A at nucleotide 3920 of *APC*) have a 2-fold increase in the risk of colon cancer compared with the general population.²⁰ Tumors develop when new inactivating mutations in *APC* arise on the same allele carrying the I1307K polymorphism; curiously, most such mutations are situated within 36 nucleotides of the 8–base pair polyA tract that results from this polymorphism. The mechanisms underlying this unusual susceptibility to somatic mutations associated with certain *APC* alleles remain to be defined.

The *APC* gene product is a large protein with multiple functional domains that mediate both oligomerization and binding to many intracellular proteins including β -catenin, γ -catenin, glycogen synthase kinase (GSK)-3 β , axin, tubulin, EB1, and hDLG.²¹ The greatest progress in understanding the function of *APC* has been made in studying its interaction with GSK-3 β and β -catenin, each an essential component of the Wnt/Wingless signaling pathway, which was first characterized in *Drosophila* and mice. As outlined in Figure 2, GSK-3 β complexes with and phosphorylates APC, β -catenin, and

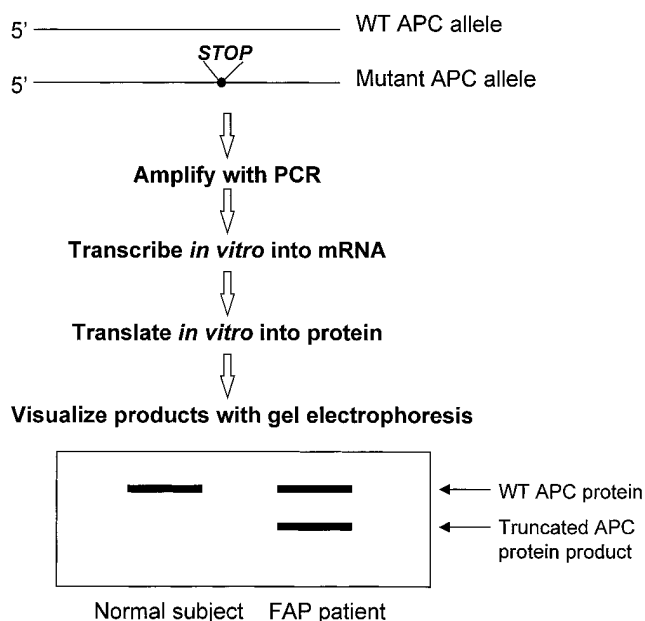


Figure 1. The APC protein truncation assay. Genomic DNA or complementary DNA is harvested from peripheral blood leukocytes, and fragments of the *APC* gene are amplified with polymerase chain reaction. Each fragment is then transcribed and translated *in vitro*, and the synthesized protein products are visualized with gel electrophoresis. A truncated protein product is detected in addition to the normal full-length product in patients who carry an *APC* mutation in 1 allele. This assay will detect germline mutations in 80% of patients with classic FAP. WT, wild-type.

axin. Phosphorylation targets β -catenin for degradation via a ubiquitin-mediated proteasomal pathway.²² Truncation of APC results in the disruption of complex formation and ultimately increased cytoplasmic levels of β -catenin. Free β -catenin is translocated to the nucleus, where it interacts with T-cell factor (TCF)/lymphoid enhancer factor high-mobility group transcription factors. TCF-4 is the predominant member of this family of transcription factors in colonic epithelial cells, and activation of this pathway up-regulates the oncogenes c-Myc and cyclin D1, as well as matrix metalloproteinase-9 (MMP-9) and urokinase-type plasminogen activator receptor through binding to TCF-response elements.^{23–26} Another recently identified target is the peroxisome proliferator-activated receptor (PPAR) δ gene, which is also regulated by nonsteroidal anti-inflammatory drugs (NSAIDs).²⁷ It is likely that additional important gene targets of TCF still remain to be identified.

Collectively, these findings suggest that β -catenin is a “driver” that up-regulates TCF-responsive genes critical for proliferation and transformation of colonic epithelial cells. In this context, it is noteworthy that gain-of-function mutations in the β -catenin gene (*CTNNB1*) have been identified in as many as 50% of colon tumors with intact *APC*.²⁸ Overall, 4%–15% of all sporadic

colorectal adenomas and carcinomas harbor *CTNNB1* mutations,^{28,29} and these localize predominantly to the amino-terminal regulatory region (amino acids 29–45) of exon 3 where critical serine and threonine phosphorylation sites are altered. Because mutant forms of β -catenin constitutively activate TCF-promoter elements and transform cells in culture,^{30,31} it is now clear that β -catenin can function as an oncogene.

Consistent with the essential role of this pathway in early tumorigenesis, β -catenin mutations are also identified in benign adenomatous polyps. Because it is “downstream” of APC, mutations in β -catenin can promote polyp formation even when *APC* is intact. In contrast to *APC*, the frequency of mutations in benign adenomas (12.5%) decreases significantly in invasive cancers (1.4%), suggesting that adenomas with alterations in β -catenin are less likely to progress to malignancy.²⁹ Interestingly, no germline mutations in *CTNNB1* have yet been described in FAP kindreds with wild-type *APC*.³² Similarly, no mutations of GSK-3 β or axin have

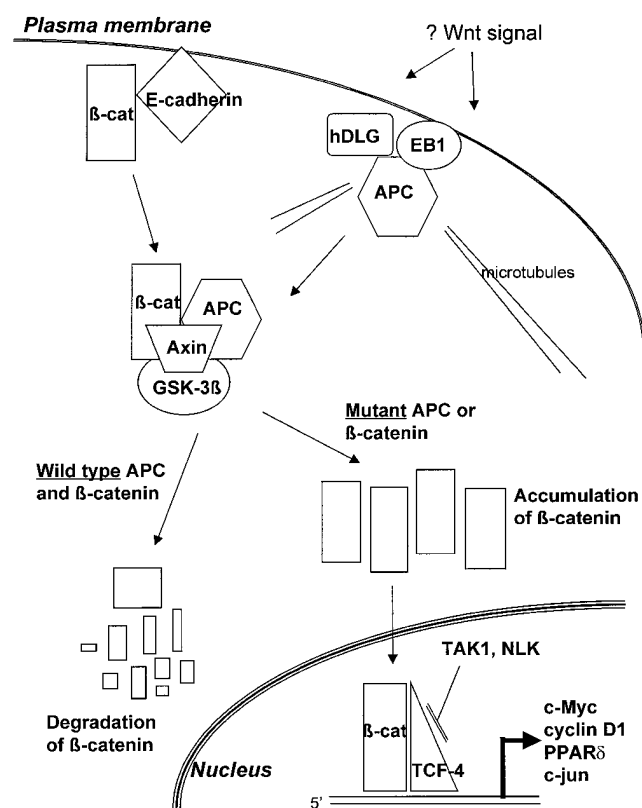


Figure 2. The APC/ β -catenin pathway. APC together with GSK-3 β provides a signal that targets the degradation of cytoplasmic β -catenin (β -cat). Axin functions as a scaffold for this multimeric complex. In the presence of either mutant APC or β -catenin, this process is disrupted. Excess β -catenin is translocated to the nucleus where it binds with TCF-4 to up-regulate target genes including c-myc, cyclin D1, PPAR δ , and c-jun. β -Catenin binds to APC in a mutually exclusive manner with E-cadherin at the plasma membrane. The nature of the extracellular Wnt signal that may regulate colonic epithelial cells is unknown.

yet been found in colorectal tumors with intact *APC* and *CTNNB1*. *TCF4* mutations have been identified, but only in a unique subset of colon tumors; 39% (19/49) of tumors with microsatellite instability (MSI) harbored a 1-base pair deletion within a polyA tract,³³ although the functional significance of this frameshift has not yet been defined.

The mechanisms that counterbalance signaling through this pathway are only beginning to be characterized. The mitogen-activated protein kinase-related enzymes TAK1 and NLK are mammalian homologues of the MOM-4 and LIT-1 kinases that regulate Wnt signaling in *Caenorhabditis elegans*. Both TAK1 and NLK inhibit β -catenin-mediated up-regulation of a TCF-response element, and this inhibition results from direct phosphorylation of TCF-4 by NLK.³⁴ The B56 subunit of protein phosphatase 2A also regulates β -catenin signaling, and its effects may be mediated through dephosphorylation of GSK-3 β .³⁵ Mutations of a phosphatase 2A subunit were identified in 2 of 13 primary colon tumors, suggesting that alterations in these counterregulatory pathways may play a role in the pathogenesis of colorectal cancer.³⁶

Specific localization of the APC protein to discrete puncta at the ends of microtubules implies an important role in regulation of the cytoskeleton and cellular migration.³⁷ In colonic epithelial cells, APC also interacts with hDLG through its COOH terminus, and hDLG and APC colocalize along the lateral plasma membrane.³⁸ However, animal studies suggest that this interaction may not be essential for adenoma formation. Mice with a truncating *Apc* mutation at codon 1638 (*Apc1638T*) that eliminates the COOH-terminus DLG-binding domain do not develop intestinal adenomas.³⁹ In contrast, ani-

mals carrying a nonsense *Apc* mutation at either codon 850 or 716 eliminating β -catenin interaction sites develop widespread intestinal polyposis.^{40,41} A similar polyposis phenotype is observed in mice with a mutant β -catenin gene (Table 2).⁴²

The DNA Mismatch Repair Pathway

Identification of the genes responsible for the HNPCC syndrome has led to insights into a novel mechanism of tumorigenesis in colon cancer. Mutations in five different genes (*bMSH2*, *bMSH6*, *bMLH1*, *bPMS1*, and *bPMS2*) have now been associated with the syndrome.⁴³ As many as 45%–70% of HNPCC families who meet the Amsterdam criteria have mutations in one of these genes, most frequently either *bMSH2* or *bMLH1*.^{44,45} Each of the five genes encodes a protein involved in DNA mismatch repair, a complex enzymatic proofreading system that corrects base pair mismatches that arise during DNA replication.

In yeast, MSH2 complexes with either MSH6 to recognize single base pair mismatches, insertions, or deletions or MSH3 to recognize larger 2- to 4-base pair insertions or deletions.⁴⁶ The MLH1 and PMS2 gene products then excise the mismatched nucleotides from the newly synthesized strand. Cells with a defective mismatch repair system display a mutator phenotype in which many genes exhibit mutations because of uncorrected errors in DNA replication. Genes with simple repetitive sequences such as polyA tracts or CA-repeat microsatellites are particularly susceptible targets. Tumors with such alterations are said to display MSI or the replication error phenotype. A recent National Cancer Institute workshop proposed a consensus panel of five

Table 2. Summary of Selected Genetic Mouse Models of Colorectal Cancer

Model	Genetic alteration	Intestinal tumors				Comment
		+++	++	+	o	
<i>Apc</i> -Min +/-	Truncation at <i>Apc</i> codon 850	✓				
× <i>Dnmt1</i> +/-	Heterozygous inactivation of DNMT in Min mice				✓	
× <i>p53</i> -/-	Homozygous inactivation of <i>p53</i> in Min mice	✓				No change in intestinal tumors
<i>Apc716</i> +/-	Truncation at <i>Apc</i> codon 716	✓				
× <i>Ptgs2</i> -/-	Homozygous inactivation of <i>cox2</i> in <i>Apc716</i> mice				✓	
× <i>Smad4</i> +/-	Heterozygous inactivation of <i>Smad4</i> in <i>Apc716</i> mice	✓				Tumors are more malignant
<i>Apc1638T</i> +/- or -/-	Truncation at <i>Apc</i> codon 1638				✓	
<i>Apc1638N</i> +/-	Insertion of neomycin gene at <i>Apc</i> codon 1638		✓			Results in an unstable <i>Apc</i> protein
× <i>Mlh1</i> -/-	Homozygous deletion of <i>Mlh1</i> in <i>Apc1638N</i> mice	✓				Tumors are also more malignant
<i>Ctnnb ex3</i> +/-	Heterozygous deletion of exon 3 of β -catenin gene	✓				
<i>Msh2</i> -/-	Homozygous deletion of <i>Msh2</i>				✓	Frequent lymphomas
<i>Mlh1</i> -/-	Homozygous deletion of <i>Mlh1</i>		✓			Frequent lymphomas
<i>Msh3</i> -/-	Homozygous deletion of <i>Msh3</i>				✓	
<i>Msh6</i> -/-	Homozygous deletion of <i>Msh6</i>				✓	Frequent lymphomas
× <i>Msh3</i> -/-	Homozygous deletion of <i>Msh3</i> in <i>Msh6</i> null mice		✓			

mononucleotide and dinucleotide microsatellite markers for MSI testing. On this basis, tumors could be classified as demonstrating high frequency MSI (MSI-H, ≥ 2 markers unstable), low frequency MSI (MSI-L, 1 marker unstable), or microsatellite stability (MSS, 0 markers unstable).⁴⁷

HNPCC accounts for as much as 3%–6% of colorectal cancer; virtually all of these tumors display MSI. Up to 15% of sporadic colorectal tumors exhibit MSI. However, only a small fraction (4%–26%) of these have identifiable somatic mutations in *bMSH2*,^{48,49} and only 6%–10% of unselected sporadic cases with MSI harbor germline mutations in *bMSH2* or *bMLH1*.^{50,51} Tumors with MSI show distinctive clinical features including a proximal colonic location, mucinous or undifferentiated histology, prominent infiltration of lymphocytes, and improved prognosis compared with stage-matched microsatellite stable controls.^{47,52} The molecular basis for these unique clinical phenotypes remains to be defined.

Germline mutations in *bMSH2* and *bMLH1* are widely distributed throughout the 16 exons spanning 73 kilobases of *bMSH2* or the 19 exons spanning 58 kilobases of *bMLH1*.⁵³ The majority are point mutations arising from single base pair substitutions, deletions, or insertions. In *bMSH2*, this results primarily in frameshifts (60%) or premature truncations (23%), whereas frameshifts (40%) or missense mutations of unknown significance (31%) are typically observed in families with a mutant *bMLH1* gene. Simple assays to determine the functional consequences of these frequent missense mutations are not yet available. The absence of either consensus mutational hotspots or a high frequency of truncating mutations has hampered efforts to develop an inexpensive and simple mutational assay. Correlations between genotype and phenotype have not yet been defined, with the exception of an increased risk of extraintestinal tumors of the urinary tract, stomach, and ovary in carriers of germline *bMSH2* mutations.⁵⁴

Inactivation of both alleles of *bMSH2* or *bMLH1* is necessary to generate the MSI-H phenotype, and this occurs through several routes. Loss of heterozygosity (LOH) is often coupled with point mutations of *bMSH2* or *bMLH1*, consistent with the two-hit model of tumor-suppressor gene inactivation.⁵⁰ Epigenetic mechanisms may also play a role because *bMLH1* can be silenced through hypermethylation of CpG islands in its promoter. More than 80% of MSI-positive sporadic colorectal tumors show hypermethylation of the *bMLH1* promoter, suggesting that this may be a primary mechanism underlying the MSI phenotype in sporadic tumors.⁵⁵ Furthermore, both alleles of *bMLH1* were hypermethy-

lated in five of six MSI-positive colon cancer cell lines without identifiable mismatch repair gene mutations, and the demethylating agent 5-azacytidine restored the expression of both *bMLH1* alleles as well as functional mismatch repair activity.^{55,56} A third mechanism of gene inactivation is a dominant negative effect caused by a germline truncating *bPMS2* mutation at codon 134.⁵⁷ In this instance, MSI occurs in cells that still contain a wild-type *bPMS2* allele. In mice, homozygous inactivation of DNA mismatch repair genes can lead to intestinal tumorigenesis^{58–60}; some of these models are outlined in Table 2.

DNA mismatch repair gene defects in HNPCC syndrome are presumed to lead to tumor development as a result of the accumulation of widespread mutations within short repetitive sequences. Because most stretches of microsatellite DNA lie in noncoding sequences, many such alterations are unlikely to be functionally significant. However, mutations also occur in genes with repetitive sequences in coding exons. The type II TGF- β receptor gene was the first such target to be identified, and frameshift mutations occurred in a short GT-repeat sequence or a stretch of 10 adenines as a consequence of either an insertion or deletion of the repeated nucleotides.⁶¹ This finding provided independent validation of the importance of the TGF- β signaling pathway in colorectal tumorigenesis because TGF- β is a potent inhibitor of colonic epithelial cell growth.^{62,63} Additional mutational targets include the apoptosis regulator BAX,⁶⁴ the insulin-like growth factor II receptor,⁶⁵ the cell cycle-regulated transcription factor E2F-4,⁶⁶ TCF-4,³³ caspase 5,⁶⁷ the intestinal homeobox factor CDX2,⁶⁸ and even the mismatch repair genes hMSH3 and hMSH6 themselves.⁶⁹ The latter provides a unique positive feedback loop in which tumors with MSI can enhance their own phenotype. Table 3 summarizes the unique association between this specific class of frameshift mutations and MSI-positive tumors. Although the relative importance of these alterations in tumorigenesis is unknown, they are likely to play a pathogenic role because no mutations of short repeat sequences are identified in genes (e.g., histone) that are not intimately linked to the tumorigenic process.⁷⁰

The relationship between the MSI phenotype and mutations of *APC*, *KRAS*, or *TP53* remains unsettled. Initial reports suggested that *APC* mutations were present in only 21% of tumors with MSI,⁷¹ but more recent studies have noted that 43% of tumors with wild-type *APC* harbor *CTNNB1* gene mutations.⁷² Thus, alterations in the APC/ β -catenin pathway are common in tumors irrespective of MSI. Although some

Table 3. Prevalence of Somatic Frameshift Mutations in Colorectal Tumors With and Without MSI

Gene	Mutated target sequence	MSI ⁺ tumors (HNPCC)	MSI ⁺ tumors (sporadic)	MSS tumors
TGFβRII	(A) ₁₀	78%–83%	82%–90%	0% ^a
IGF2R	(G) ₈	13%	9%	0%
BAX	(G) ₈	52%–55%	13%–50%	0%
hMSH3	(A) ₈	50%–58%	39%–46%	0%
hMSH6	(C) ₈	33%	28%–36%	0%
E2F4	(CAG) ₁₃	71%	42%–57%	0%
Caspase-5	(A) ₁₀	—	62%	0%
TCF4	(A) ₉	—	39%	2%
CDX2	(G) ₇	5%	—	0%

MSS, microsatellite stable; —, data not available.

^aFifteen percent of MSS colorectal cancer cell lines exhibit TGFβRII mutations that are not frameshifts in the polyA tract.

investigators have identified *KRAS* mutations in only 7% of MSI-positive tumors,⁷¹ others have noted frequencies as high as 22%–31%, similar to the rates seen in control tumors with MSS.^{16,73} Finally, fewer p53 mutations seem to be present in tumors with MSI; mutation rates range from 0%–5%, 13%–40%, and 31%, compared with rates of 33%–50%, 55%–67%, and 55%, respectively, for tumors without MSI.^{71,73,74}

Collectively, these observations establish the overriding importance of the APC/β-catenin pathway in the initiation of tumors regardless of MSI status. The mechanisms that then govern the accumulation of additional mutations after initial polyp formation diverge depending on the underlying basis for genetic instability. In contrast to MSI-positive tumors that are typically diploid and possess a narrowly defined mutator phenotype affecting simple sequence repeats, the larger population of MSS tumors shows more global chromosomal instability manifested by aneuploidy and frequent LOH.⁷⁵ The molecular basis for this type of chromosomal instability is beginning to be understood, and it seems that alterations in mitotic checkpoint genes such as *hBUB1* may be important.⁷⁶ Both types of genetic instability can be observed in benign polyps, and the specific rates at which subsequent mutations develop during the transition to malignancy are unknown. However, the clinical observation that HNPCC-associated polyps become malignant more rapidly than sporadic MSS polyps suggests that the MSI mutator phenotype may accelerate tumor progression. This contrasts with FAP-associated polyps, in which the process of tumor initiation is accelerated. Figure 3 summarizes the timing of the key genetic events in colon cancer pathogenesis, based on whether there is underlying chromosomal instability or MSI.

The TGF-β/SMAD Pathway

TGF-β and related family members mediate a wide variety of biological effects, the most characteristic being its potent inhibition of proliferation.⁷⁷ TGF-β initiates its broad spectrum of cellular responses through binding to the type II TGF-β receptor (TGFβRII). Binding to TGFβRII recruits and activates the type I receptor through phosphorylation of specific serine and threonine residues; signaling to the nucleus is then mediated through the so-called SMAD proteins, which are phosphorylated by the type I receptor. To date, nine SMAD proteins have already been identified. In mammalian cells, the type I receptor kinase first phosphorylates SMAD2 and SMAD3; SMAD4 then joins the complex. After translocation to the nucleus, this heterooligomeric complex modulates transcription of specific genes through cis-regulatory SMAD-binding sequences. The SMAD pathway has been shown to regulate genes encoding plasminogen activator inhibitor1,⁷⁸ the CDK inhibitors p15 and p21,^{79,80} cyclin D1,⁸¹ and TGF-β itself,⁸² among others.

The identification of TGFβRII mutations in most colorectal tumors with MSI first suggested that genetic alterations in this pathway may contribute to colorectal tumorigenesis. Functional studies confirmed that these mutations render cells resistant to the effects of TGF-β.⁶¹ Type II receptor mutations are also present in 15% of MSS colorectal cancer lines. Not surprisingly, these are not frameshifts of the (A)₁₀ tract typically mutated in tumors with MSI.⁸³ However, the number of mutations identified in the multiple SMAD genes has been relatively small; 16% (5/31) of primary colorectal tumors harbor alterations of *SMAD4*,⁸⁴ and missense mutations of *SMAD2* are identified in 6% (4/66) of cases.⁸⁵ *SMAD4* and *SMAD2* (as well as *DCC*, the “deleted in colon cancer” locus) lie on chromosome 18q21 within 10 cM of each other, and all three are thus potential tumor-suppressor gene targets of the LOH on 18q present in as many as 70% of colorectal cancers.⁸⁶ So far, no mutations of the remaining SMADs (*SMAD1*, *SMAD3*, *SMAD5*, or *SMAD6*) have been documented.⁸⁷ Of note, deletion of *Smad4* in *Apc* null mice results in more malignant intestinal tumors than those in *Apc* null mice alone (Table 2), suggesting that SMAD4 plays a functional role in tumor progression.⁸⁸

The importance of the TGF-β pathway in colorectal tumorigenesis is further underscored by the description of a colon cancer family exhibiting a germline mutation in the TGFβRII gene.⁸⁹ Colorectal tumors developed in members of this kindred with the loss of the remaining wild-type allele. In contrast to classic HNPCC syn-

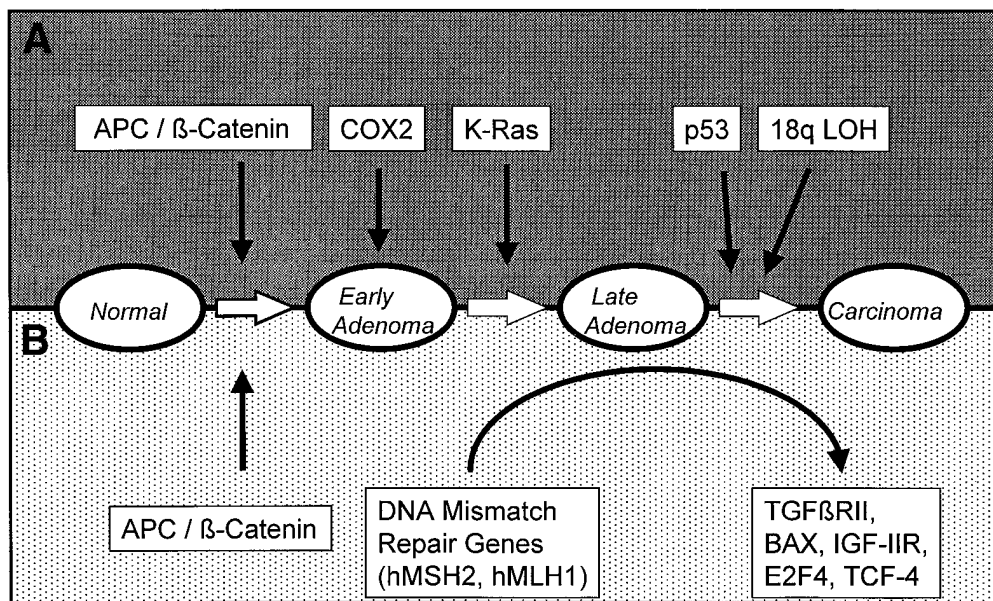


Figure 3. Overall scheme of key genetic events in colorectal tumorigenesis. The critical genes involved in the development of colorectal polyps and cancer are illustrated. The specific timing of each genetic event is crucial in tumor pathogenesis. These genetic events can be grouped into two broad categories. Eighty-five percent of tumors exhibit chromosomal instability but stability of microsatellite DNA; the typical genetic events associated with these tumors are illustrated in A. The remaining 15% of tumors are characterized by MSI; the key genetic events in these tumors are illustrated in B.

drome, tumors in this family were diagnosed after age 60 and no metachronous lesions were found. Furthermore, no extracolonic malignancies were reported, illustrating the unique dependence of intestinal epithelia on the actions of TGF- β .

Additional Genetic Events in Colorectal Tumorigenesis

KRAS is mutated in approximately 50% of colorectal tumors, and alterations in this small GTP-binding protein almost uniformly occur as activating point mutations in codons 12, 13, and to a lesser extent, 61. Among their many functions, K-ras pathways transduce signals from extracellular growth factors to regulate progression through the cell cycle and proliferation.^{2,3} *KRAS* mutations are associated with up-regulation of DNA methyltransferase, cyclin D1, and gastrin, all of which may be important in colon cancer pathogenesis.^{90–92} Mutations are frequently present in aberrant crypt foci, which may represent the earliest precursor of an adenoma. However, these mutations are present in 80%–92% of nondysplastic or hyperplastic aberrant crypt foci but only 57% of dysplastic or adenomatous aberrant crypt foci,⁹³ casting doubt on their importance in tumorigenesis given that hyperplastic changes are generally not thought to be precursors of adenomas.

TP53 mutations are also identified in 50% or more of colorectal tumors; mutations localize primarily to exons 5–8. The *TP53* tumor-suppressor gene product (p53) is a sequence-specific transcriptional activator that plays diverse and complex roles in the regulation of cell cycle progression and apoptosis.^{4,5} Most p53 mutations result

in a protein with an abnormally long half-life, and the accumulation of mutant p53 can be detected immunohistochemically. Inactivating mutations in p53 occur as a late event during the transition from adenoma to carcinoma and are typically accompanied by deletions of the remaining wild-type allele on chromosome 17p.

The *DCC* tumor-suppressor gene was cloned in 1990 and encodes a cell surface receptor for the ligand netrin. Point mutations have been detected in 2 of 30 human colorectal cancer samples.⁹⁴ The large size of the gene (>1300 kilobases) has hampered efforts to analyze a greater number of tumors for mutations in this gene. Loss of DCC protein is associated with a poor prognosis, suggesting an important functional role for the protein.⁹⁵ Data on the specific biological activities of DCC are limited, but regulation of apoptosis has been implicated.⁹⁶

SRC encodes a nonreceptor tyrosine kinase that is frequently up-regulated in advanced colonic malignancies. A truncating mutation at codon 531 was found in 12% of metastatic colorectal tumors in one study.⁹⁷ Truncation results in increased src kinase activity and tumorigenicity in both colony-formation assays and nude mice.

PPAR γ , a member of the nuclear hormone receptor superfamily, is a key regulator of adipocyte differentiation. Interestingly, PPAR γ is also expressed in colorectal cancers, and in vitro studies indicate that ligand activation can induce differentiation of colon cancer cells, partially reversing the malignant phenotype.^{98,99} However, there are conflicting data because ligand activation in *Apc^{min}* mice results in enhanced tumorigenesis.^{100,101}

Monoallelic inactivating mutations of PPAR γ have been found in 4 of 55 sporadic colon cancers, suggesting that ligand signaling through PPAR γ may prevent colon tumor development in humans.¹⁰²

Several genes are also consistently up-regulated but not mutated in colon cancer. Cyclooxygenase (COX)-2 is overexpressed in as many as 43% of adenomas and 86% of carcinomas, and the increased levels of COX-2 play a role in regulating angiogenesis and apoptosis.^{103–105} Furthermore, inhibition of COX-2 activity blocks the growth of cultured colon cancer cells¹⁰⁶ and dramatically reduces the number of intestinal polyps in *Apc*-mutant mice (Table 2).¹⁰⁷ Interestingly, several mouse models of colonic tumorigenesis suggest that it is the stromal cells within these tumors that overexpress COX-2.¹⁰⁸ Overexpression of insulin-like growth factor II and incompletely processed forms of gastrin also seem to play a role in colorectal tumorigenesis.^{109–111} Up-regulation of these tumor-promoting genes cannot be easily classified as a clonal genetic event underlying tumor formation. Although these events seem to be secondary, they likely play an important role in tumor pathogenesis.

The number of genes already implicated in colorectal cancer development is impressive, but the list is undoubtedly incomplete. Initial allelotyping studies performed over a decade ago suggested critical tumor-suppressor gene loci on 5q, 8p, 17p, 18q, and 22q.¹¹² Although the *APC*, *TP53*, and *DCC/SMAD4/SMAD2* genes have been mapped to 5q, 17p, and 18q, respectively, no candidates have yet been identified on 8p or 22q. LOH on 22q has been confirmed in independent analyses, inferring the presence of a novel tumor-suppressor gene.¹¹³

Epigenetic Mechanisms in Colorectal Tumorigenesis

The transformation of a normal colonic epithelial cell to a malignant clone is the consequence of a complicated program in which the inappropriate activation of some genes is accompanied by the specific inactivation of others. Tumorigenesis requires that this altered program is passed on to subsequent generations of cells. Thus far, this review has focused primarily on the specific alterations in gene sequence (i.e., mutations) that fulfill these criteria. However, epigenetic mechanisms, especially DNA methylation, also seem to play an important role.

Widespread genomic hypomethylation in both benign adenomas and malignant colon cancers was first reported in 1985.¹¹⁴ Interestingly, this global hypomethylation occurs in the setting of localized hypermethylation.¹¹⁵ As

noted above, hypermethylation of CpG islands within promoter sequences of specific tumor-suppressor genes can have a potent silencing effect. In addition to *hMLH1*, hypermethylation of the *INK4A^{p16}* gene is observed in 28%–55% of colon tumors.^{90,116}

DNA methyltransferase (DNMT) enzymes catalyze the transfer of methyl groups from S-adenosyl methionine to cytosine residues. DNMT-1 is the best studied form in eukaryotic cells. The specific mechanisms that regulate DNMT activity and the methylation status of the genome are poorly understood. Mutant K-ras signaling is associated with up-regulation of DNA methyltransferase activity.⁹⁰ Furthermore, elevated DNA methyltransferase activity is detected in colon cancer cells,¹¹⁷ and overexpression of *DNMT1* can lead to cellular transformation.¹¹⁸ The most compelling in vivo data directly implicating altered methylation in colorectal tumorigenesis come from *Apc^{min}* mice crossed with *Dnmt1* null mice. A dramatic reduction in the number of intestinal polyps is observed, suggesting that DNA methylation plays a critical role in tumor pathogenesis.¹¹⁹

Implications for Clinical Management

Dramatic insights into the genetic basis of colon cancer have been achieved. These advances will undoubtedly have many direct applications to clinical management of patients with colorectal cancer. The feasibility of K-ras and p53 molecular analysis of stool for the diagnosis of colon cancer has already been shown and awaits further refinement.^{120,121} Germline testing for the FAP and HNPCC syndromes is now commercially available and plays a valuable role in the counseling of high-risk families. The search for molecular prognostic markers that may predict outcomes is ongoing and will hopefully build on initial observations that the status of chromosome 18q LOH and loss of DCC protein staining can be used to stratify patients.^{95,122} It is clear that optimal responses to chemotherapy are influenced by the underlying tumor genotype, e.g., the efficacy of DNA alkylating agents is dependent on an intact DNA mismatch repair system.¹²³ Similarly, p53-deficient colorectal cancer cells display an enhanced sensitivity to doxorubicin but are resistant to the commonly used antimetabolite, 5-fluorouracil.¹²⁴ Finally, advances in our understanding of COX-2 up-regulation in colon cancer are already enabling strategies for the prevention of colon cancer with NSAIDs.¹²⁵

References

1. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61:759–767.

2. Rommel C, Hafen E. Ras: a versatile cellular switch. *Curr Opin Genet Dev* 1998;8:412–418.
3. Pronk GJ, Bos JL. The role of p21ras in receptor tyrosine kinase signalling. *Biochim Biophys Acta* 1994;1198:131–147.
4. Ko LJ, Prives C. p53: puzzle and paradigm. *Genes Dev* 1996;10:1054–1072.
5. Prives C. Signaling to p53: breaking the MDM2-p53 circuit. *Cell* 1998;95:5–8.
6. Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, Joslyn G, Stevens J, Spirio L, Robertson M, Sargeant L, Krapcho K, Wolff E, Burt R, Hughes JP, Warrington J, McPherson J, Wasmuth J, Le Paslier D, Abderrahim H, Cohen D, Leppert M, White R. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 1991;66:589–600.
7. Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, Koyama K, Utsunomiya J, Baba S, Hedge P, Markham A, Krush AJ, Petersen G, Hamilton SR, Nilbert MC, Levy DB, Bryan TM, Preisinger AC, Smith KJ, Su L-K, Kinzler KW, Vogelstein B. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 1991;253:665–669.
8. Miyake M, Konishi M, Kikuchi-Yanoshita R, Enomoto M, Igari T, Tanaka K, Muraoka M, Takahashi H, Amada Y, Fukayama M, Maeda Y, Iwama T, Mishima Y, Mori T, Koike M. Characteristics of somatic mutation of the adenomatous polyposis coli gene in colorectal tumors. *Cancer Res* 1994;54:3011–3020.
9. Su L-K, Vogelstein B, Kinzler KW. Association of the APC tumor suppressor protein with catenins. *Science* 1993;262:1734–1737.
10. Rubinfeld B, Souza B, Albert I, Muller O, Chamberlain SH, Marszalek FR, Munemitsu S, Polakis P. Association of the APC gene product with β -catenin. *Science* 1993;262:1731–1734.
11. Behrens J, Jerchow B-A, Wurtele M, Grimm J, Asbrand C, Wirtz R, Kuhl M, Wedlich D, Birchmeier W. Functional interaction of an axin homolog, conductin, with beta-catenin, APC, and GSK3beta. *Science* 1998;280:596–599.
12. Spirio L, Samowitz W, Robertson J, Robertson M, Burt RW, Leppert M, White R. Alleles of APC modulate the frequency and classes of mutations that lead to colon polyps. *Nat Genet* 1998;20:385–388.
13. Miyoshi Y, Nagase H, Ando H, Horii A, Ichii S, Nakatsuru S, Aoki T, Miki Y, Mori T, Nakamura Y. Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. *Hum Mol Genet* 1992;1:229–233.
14. Powell SM, Petersen GM, Krush AJ, Booker S, Jen J, Giardiello FM, Hamilton SR, Vogelstein B, Kinzler K. Molecular diagnosis of familial adenomatous polyposis. *N Engl J Med* 1993;329:1982–1987.
15. Powell SM, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, Vogelstein B, Kinzler KW. APC mutations occur early during colorectal tumorigenesis. *Nature* 1992;359:235–237.
16. Olschwang S, Tiet A, Laurent-Puig P, Muleris M, Parc R, Thomas G. Restriction of ocular fundus lesions to a specific subgroup of APC mutations in adenomatous polyposis coli patients. *Cell* 1993;75:959–968.
17. Caspari R, Olschwang S, Friedl W, Mandl M, Boisson C, Boker T, Augustin A, Kadmon M, Moslein G, Thomas G, Al E. Familial adenomatous polyposis: desmoid tumors and lack of ophthalmic lesions (CHRPE) associated with APC mutations beyond codon 1444. *Hum Mol Genet* 1995;4:337–340.
18. Spirio L, Olschwang S, Groden J, Robertson M, Samowitz W, Joslyn G, Gelbert L, Thliveris A, Carlson M, Otterud B, Lynch H, Watson P, Lynch P, Laurent-Puig P, Burt R, Hughes JP, Thomas G, Leppert M, White R. Alleles of the APC gene: an attenuated form of familial polyposis. *Cell* 1993;75:951–957.
19. Gardner RJM, Kool D, Edkins E, Walpole IR, Macrae FA, Nasio-ulas S, Scott WJH. The clinical correlates of a 3' truncating mutation (codons 1982–1983) in the adenomatous polyposis coli gene. *Gastroenterology* 1997;113:326–331.
20. Laken SJ, Petersen GM, Gruber SB, Oddoux C, Ostrer H, Giardiello FM, Hamilton SR, Hampel H, Markowitz A, Klimstra D, Jhanwar S, Winawer S, Offit K, Luce MC, Kinzler KW, Vogelstein B. Familial colorectal cancer in Ashkenazim due to a hypermutable tract in APC. *Nat Genet* 1997;17:79–83.
21. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996;87:159–170.
22. Rubinfeld B, Albert I, Porfiri E, Fiol C, Munemitsu S, Polakis P. Binding of GSK3 β to the APC- β -catenin complex and regulation of complex assembly. *Science* 1996;272:1023–1026.
23. He T-C, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW. Identification of c-MYC as a target of the APC pathway. *Science* 1998;281:1509–1512.
24. Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 1999;398:422–426.
25. Mann B, Gelos M, Siedow A, Hanski ML, Gratchev A, Ilyas M, Bodmer WF, Moyer MP, Riecken EO, Buhr HJ, Hanski C. Target genes of beta-catenin-T-cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. *Proc Natl Acad Sci U S A* 1999;96:1603–1608.
26. Crawford HC, Fingleton BM, Rudolph-Owen LA, Heppner Goss KJ, Rubinfeld B, Polakis P, Matrisian LM. The metalloproteinase matrilysin is a target of β -catenin transactivation in intestinal tumors. *Oncogene* 1999;18:2883–2891.
27. He T-C, Chan TA, Vogelstein B, Kinzler KW. PPAR δ is an APC-regulated target of nonsteroidal anti-inflammatory drugs. *Cell* 1999;99:335–345.
28. Sparks AB, Morin PJ, Vogelstein B, Kinzler KW. Mutational analysis of the APC/ β -catenin/Tcf pathway in colorectal cancer. *Cancer Res* 1998;58:1130–1134.
29. Samowitz WS, Powers MD, Spirio LN, Nollet F, Van Roy F, Slattey ML. Beta-catenin mutations are more frequent in small colorectal adenomas than in larger adenomas and invasive carcinomas. *Cancer Res* 1999;59:1442–1444.
30. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW. Activation of β -catenin-Tcf signaling in colon cancer by mutations in β -catenin of APC. *Science* 1997;275:1787–1790.
31. Kolligs FT, Hu G, Dang CV, Fearon ER. Neoplastic transformation of RK3E by mutant β -catenin requires deregulation of Tcf/Lef transcription but not activation of c-myc expression. *Mol Cell Biol* 1999;19:5696–5706.
32. Muller O, Nimrich I, Finke U, Friedl W, Hoffmann I. A beta-catenin mutation in a sporadic colorectal tumor of the RER phenotype and absence of beta-catenin germline mutations in FAP patients. *Genes Chromosomes Cancer* 1999;22:37–41.
33. Duval A, Gayet J, Zhou X-P, Iacopetta B, Thomas G, Hamelin R. Frequent frameshift mutations of the TCF-4 gene in colorectal cancers with microsatellite instability. *Cancer Res* 1999;59:4213–4215.
34. Ishitani T, Ninomiya-Tsuji J, Nagai S, Nishita M, Meneghini M, Barker N, Waterman M, Bowerman B, Clevers H, Shibuya H, Matsumoto K. The TAK1-NLK-MAPK-related pathway antagonizes signalling between β -catenin and transcription factor TCF. *Nature* 1999;399:798–802.
35. Seeling JM, Miller JR, Gil R, Moon RT, White R, Virshup DM. Regulation of β -catenin signaling by the B56 subunit of protein phosphatase 2A. *Science* 1999;283:2089–2091.
36. Wang SS, Esplin ED, Li JL, Huang L, Gazdar A, Minna J, Evans GA. Alterations of the PPP2R1B gene in human lung and colon cancer. *Science* 1998;282:284–287.
37. Nathke IS, Adams CL, Polakis P, Sellin JH, Nelson WJ. The adenomatous polyposis coli tumor suppressor protein localizes to plasma membrane sites involved in active cell migration. *J Cell Biol* 1996;134:165–179.

38. Matsumine A, Ogai A, Senda T, Okumura N, Satoh K, Baeg G-H, Kawahara T, Kobayashi S, Okada M, Toyoshima K, Akiyama T. Binding of APC to the human homolog of the *Drosophila* discs large tumor suppressor protein. *Science* 1996;272:1020–1023.
39. Smits R, Kielman MF, Breukel C, Zurcher C, Neufeld K, Jagmohan-Changur S, Hofland N, van Dijk J, White R, Edelmann W, Kucherlapati R, Khan PM, Fodde R. Apc1638T: a mouse model delineating critical domains of the adenomatous polyposis coli protein involved in tumorigenesis and development. *Genes Dev* 1999;13:1309–1321.
40. Su L-K, Kinzler KW, Vogelstein B, Preisinger AC, Moser AR, Luongo C, Gould KA, Dove WF. Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. *Science* 1992;256:668–670.
41. Oshima M, Oshima H, Kitagawa K, Kobayashi M, Itakura C, Taketo M. Loss of APC heterozygosity and abnormal tissue building in nascent intestinal polyps in mice carrying a truncated Apc gene. *Proc Natl Acad Sci U S A* 1995;92:4482–4486.
42. Harada N, Tamai Y, Ishikawa T, Sauer B, Takaku K, Oshima M, Taketo MM. Intestinal polyposis in mice with a dominant stable mutation of the beta-catenin gene. *EMBO J* 1999;18:5931–5942.
43. Chung DC, Rustgi AK. DNA mismatch repair and cancer. *Gastroenterology* 1995;109:1685–1699.
44. Wijnen JT, Vasen HFA, Khan PM, Zwiderman AH, van der Klift H, Mulder A, Tops C, Moller P, Fodde R. Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. *N Engl J Med* 1998;339:511–518.
45. Liu B, Parsons R, Papadopoulos N, Nicolaides NC, Lynch HT, Watson P, Jass JR, Dunlop M, Wyllie A, Peltomaki P, De La Chapelle A, Hamilton SR, Vogelstein B, Kinzler KW. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. *Nat Med* 1996;2:169–174.
46. Kolodner R. Biochemistry and genetics of eukaryotic mismatch repair. *Genes Dev* 1996;10:1433–1442.
47. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute workshop in microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998;58:5248–5257.
48. Borresen AL, Lothe RA, Meling GI, Lystad S, Morrison P, Lipford J, Kane MF, Rognum TO, Kolodner RD. Somatic mutations in the hMSH2 gene in microsatellite unstable colorectal carcinomas. *Hum Mol Genet* 1995;4:2065–2072.
49. Bubb VJ, Curtis LJ, Cunningham C, Dunlop MG, Carothers AD, Morris RG, White S, Bird CC, Wyllie A. Microsatellite instability and the role of hMSH2 in sporadic colorectal cancer. *Oncogene* 1996;12:2641–2649.
50. Liu B, Nicolaides NC, Markowitz S, Willson JKV, Parsons RE, Jen J, Papadopoulos N, Peltomaki P, de la Chapelle A, Hamilton SR, Kinzler KW, Vogelstein B. Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. *Nat Genet* 1995;9:48–55.
51. Wu Y, Nystrom-Lahti M, Osinga J, Looman MW, Peltomaki P, Aaltonen LA, de la Chapelle A, Hofstra RM, Buys CH. MSH2 and MLH1 mutations in sporadic replication error-positive colorectal carcinoma as assessed by two-dimensional DNA electrophoresis. *Genes Chromosomes Cancer* 1997;18:269–278.
52. Gryfe R, Kim H, Hsieh ETK, Aronson MD, Holowaty EJ, Bull SB, Redston M, Gallinger S. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *N Engl J Med* 2000;342:69–77.
53. Peltomaki P, Vasen HFA. Mutations predisposing to hereditary nonpolyposis colorectal cancer: database and results of a collaborative study. *Gastroenterology* 1997;113:1146–1158.
54. Vasen HFA, Wijnen JT, Menko FH, Kleibeuker JH, Taal BG, Griffioen G, Nagengast FM, Meijers-Heijboer EH, Bertario L, Varesco L, Bisgaard M, Mohr J, Fodde R, Khan PM. Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. *Gastroenterology* 1996;110:1020–1027.
55. Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, Issa JJ, Markowitz S, Willson JKV, Hamilton SR, Kinzler KW, Kane MF, Kolodner RD, Vogelstein B, Kunkel TA, Baylin SB. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci U S A* 1998;95:6870–6875.
56. Veigl ML, Kasturi L, Olechnowicz J, Ma A, Lutterbaugh JD, Periyasamy S, Li G-M, Drummond J, Modrich PL, Sedwick WD, Markowitz SD. Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing MSI cancers. *Proc Natl Acad Sci U S A* 1998;96:8698–8702.
57. Nicolaides NC, Littman SJ, Modrich P, Kinzler KW, Vogelstein B. A naturally occurring hPMS2 mutation can confer a dominant negative mutator phenotype. *Mol Cell Biol* 1998;18:1635–1641.
58. de Wind N, Dekker M, Berns A, Radman M, te Riele H. Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell* 1995;82:321–330.
59. Edelman W, Yang K, Kuraguchi M, Heyer J, Lia M, Kneitz B, Fan K, Brown AMC, Lipkin M, Kucherlapati R. Tumorigenesis in Mlh1 and Mlh1/Apc1638N mutant mice. *Cancer Res* 1999;59:1301–1307.
60. de Wind N, Dekker M, Claij N, Jansen L, van Klink Y, Radman M, Riggins G, van der Valk M, van't Wout K, te Riele H. HNPCC-like cancer predisposition in mice through simultaneous loss of Msh3 and Msh6 mismatch-repair protein functions. *Nat Genet* 1999;23:359–362.
61. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan R, Zborowska E, Kinzler K, Vogelstein B, Brattain M, Willson J. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 1995;268:1336–1338.
62. Kurokawa M, Lynch K, Podolsky DK. Effects of growth factors on an intestinal epithelial cell line: transforming growth factor beta inhibits proliferation and stimulates differentiation. *Biochem Biophys Res Commun* 1987;142:775–782.
63. Markowitz S, Roberts A. Tumor suppressor activity of the TGF-beta pathway in human cancers. *Cytokine Growth Factor Rev* 1996;7:93–102.
64. Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, Perucho M. Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science* 1997;275:967–969.
65. Souza R, Appel R, Yin J, Wang S, Smolinski KN, Abraham JM, Zou T, Shi Y, Lei J, Cottrell J, Cymes K, Biden K, Simms L, Leggett B, Lynch PM, Frazier M, Powell SM, Harpaz N, Sugimura H, Young J, Meltzer SJ. Microsatellite instability in the insulin-like growth factor II receptor gene in gastrointestinal tumours. *Nat Genet* 1996;14:255–257.
66. Yoshitaka T, Matsubara N, Ikeda M, Tanino M, Hanafusa H, Tanaka N, Shimizu K. Mutations of E2F-4 trinucleotide repeats in colorectal cancer with microsatellite instability. *Biochem Biophys Res Commun* 1996;227:553–557.
67. Schwartz S, Yamamoto H, Navarro M, Maestro M, Reventos J, Perucho M. Frameshift mutations at mononucleotide repeats in caspase-5 and other target genes in endometrial and gastrointestinal cancer of the microsatellite mutator phenotype. *Cancer Res* 1999;59:2995–2302.
68. Wicking C, Simms LA, Evans T, Walsh M, Chawengsaksophak K, Beck F, Chenevix-Trench G, Young J, Jass J, Leggett B, Wainwright B. CDX2, a human homologue of *Drosophila* caudal, is mutated in both alleles in a replication error positive colorectal cancer. *Oncogene* 1998;17:657–659.

69. Malkhosyan S, Rampino N, Yamamoto H, Perucho M. Frame-shift mutator mutations. *Nature* 1996;382:499–500.
70. Ouyang H, Shiwaku H, Hagiwara H, Miura K, Abe T, Kato Y, Ohtani H, Shiiba K, Souza R, Meltzer S, Horii A. The insulin-like growth factor II receptor gene is mutated in genetically unstable cancers of the endometrium, stomach, and colorectum. *Cancer Res* 1997;57:1851–1854.
71. Konishi M, Kikuchi-Yanoshita R, Kiyoko T, Masatoshi M, Onda A, Okumura Y, Kishi N, Iwama T, Mori T, Koike M, Ushio K, Chiba M, Nomizu S, Konishi F, Utsunomiya J, Miyaki M. Molecular nature of colon tumors in hereditary nonpolyposis colon cancer, familial polyposis, and sporadic colon cancer. *Gastroenterology* 1996;111:307–317.
72. Miyaki M, Iijima T, Kimura J, Yasuno M, Mori T, Hayashi Y, Koike M, Shitara N, Iwama T, Kuroki T. Frequent mutation of beta-catenin and APC genes in primary colorectal tumors from patients with hereditary nonpolyposis colorectal cancer. *Cancer Res* 1999;59:4506–4509.
73. Fujiwara T, Stolker J, Watanabe T, Rashid A, Longo P, Eshleman J, Booker S, Lynch H, Jass J, Green JS, Kim H, Jen J, Vogelstein B, Hamilton SR. Accumulated clonal genetic alterations in familial and sporadic colorectal carcinomas with widespread instability in microsatellite sequences. *Am J Pathol* 1998;153:1063–1078.
74. Olschwang S, Hamelin R, Laurent-Puig P, Thuille B, De Rycke Y, Li Y, Muzeau F, Girodet J, Salmon R, Thomas G. Alternative genetic pathways in colorectal carcinogenesis. *Proc Natl Acad Sci U S A* 1997;94:12122–12127.
75. Lengauer C, Kinzler KW, Vogelstein B. Genetic instability in colorectal cancers. *Nature* 1997;386:623–627.
76. Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JKV, Markowitz SD, Kinzler KW, Vogelstein B. Mutations of mitotic checkpoint genes in human cancers. *Nature* 1998;392:300–303.
77. Heldin C-H, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature* 1997;390:465–471.
78. Keeton MR, Curriden SA, van Zonneveld AJ, Loskutoff DJ. Identification of regulatory sequences in the type I plasminogen activator inhibitor gene responsive to transforming growth factor β . *J Biol Chem* 1991;266:23048–23052.
79. Li J-M, Nichols MA, Chandrasekharan S, Xiong Y, Wang X-F. Transforming growth factor β activates the promoter of cyclin-dependent kinase inhibitor p15INK4B through an Sp1 consensus site. *J Biol Chem* 1995;270:26750–26753.
80. Datto MB, Yu Y, Wang X-F. Functional analysis of the transforming growth factor β responsive elements in the WAF1/Cip1/p21 promoter. *J Biol Chem* 1995;270:28623–28628.
81. Ko TC, Sheng HM, Reisman D, Thompson EA, Beauchamp RD. Transforming growth factor- β 1 inhibits cyclin D1 expression in intestinal epithelial cells. *Oncogene* 1995;10:177–184.
82. Kim SJ, Angel P, Lafyatis R, Hattori K, Kim KY, Sporn MB, Karin M, Roberts AB. Autoinduction of transforming growth factor β 1 is mediated by the AP-1 complex. *Mol Cell Biol* 1990;10:1492–1497.
83. Grady W, Myeroff L, Swinler S, Rajput A, Thiagalingam S, Lutterbaugh J, Neumann A, Brattain M, Chang J, Kim S, Kinzler K, Vogelstein B, Willson J, Markowitz S. Mutational inactivation of transforming growth factor beta receptor type II in microsatellite stable colon cancers. *Cancer Res* 1999;59:320–324.
84. Takagi Y, Kohmura H, Futamura M, Kida H, Tanemura H, Shimokawa K, Saji S. Somatic alterations of the DPC4 gene in human colorectal cancers in vivo. *Gastroenterology* 1996;111:1369–1372.
85. Eppert K, Scherer SW, Ozcelik H, Prone R, Hoodless P, Kim H, Tsui LC, Bapat B, Gallinger S, Andrulis IL, Thomsen GH, Wrana JL, Attisano L. MADR2 maps to 18q21 and encodes a TGF-beta-regulated MAD-related protein that is functionally mutated in colorectal carcinoma. *Cell* 1996;86:543–552.
86. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988;319:525–532.
87. Riggins GJ, Kinzler KW, Vogelstein B, Thiagalingam S. Frequency of Smad gene mutations in human cancers. *Cancer Res* 1997;57:2578–2580.
88. Takaku K, Oshima M, Miyoshi H, Matsui M, Seldin MF, Taketo MM. Intestinal tumorigenesis in compound mutant mice of both Dpc4 (Smad4) and Apc genes. *Cell* 1998;92:645–656.
89. Lu S-L, Kawabata M, Imamura T, Akiyama Y, Nomizu T, Miyazono K, Yuasa Y. HNPCC associated with germline mutation in the TGF- β type II receptor gene. *Nat Genet* 1998;19:17–18.
90. Guan RJ, Fu Y, Holt PR, Pardee AB. Association of K-ras mutations with p16 methylation in human colon cancer. *Gastroenterology* 1999;116:1063–1071.
91. Aktas H, Cai H, Cooper GM. Ras links growth factor signaling to the cell cycle machinery via regulation of cyclin D1 and the Cdk inhibitor p27KIP1. *Mol Cell Biol* 1997;17:3850–3857.
92. Nakata H, Wang S-L, Chung DC, Westwick JK, Tillotson LG. Oncogenic ras induces gastrin gene expression in colon cancer. *Gastroenterology* 1998;115:1144–1153.
93. Takayama T, Katsuki S, Takahashi Y, Ohi M, Nojiri S, Sakamaki S, Kato J, Kogawa K, Miyake H, Niitsu Y. Aberrant crypt foci of the colon as precursors of adenoma and cancer. *N Engl J Med* 1998;339:1277–1284.
94. Cho KR, Oliner JD, Simons JW, Hedrick L, Fearon ER, Preisinger AC, Hedge P, Silverman GA, Vogelstein B. The DCC gene: structural analysis and mutations in colorectal carcinomas. *Genomics* 1994;19:525–531.
95. Shibata D, Reale MA, Lavin P, Silverman M, Fearon ER, Steele G, Jessup JM, Loda M, Summerhayes IC. The DCC protein and prognosis in colorectal cancer. *N Engl J Med* 1996;335:1727–1732.
96. Chen YQ, Hsieh JT, Yao F, Fang B, Pong RC, Cipriano SC, Krepulat F. Induction of apoptosis and G2/M cell cycle arrest by DCC. *Oncogene* 1999;18:2747–2754.
97. Irby RB, Mao W, Coppola D, Kang J, Loubeau JM, Trudeau W, Karl R, Fujita DJ, Jove R, Yeatman TJ. Activating SRC mutation in a subset of advanced human colon cancers. *Nat Genet* 1999;21:187–190.
98. Sarraf P, Mueller E, Jones D, King FJ, DeAngelo DJ, Partridge JB, Holden SA, Chen LB, Singer S, Fletcher C, Spiegelman BM. Differentiation and reversal of malignant changes in colon cancer through PPAR γ . *Nat Med* 1998;4:1046–1052.
99. Brockman JA, Gupta RA, DuBois RN. Activation of PPAR γ leads to inhibition of anchorage-independent growth of human colorectal cancer cells. *Gastroenterology* 1998;115:1049–1055.
100. Levebvre A-M, Chen I, Desreumaux P, Najib N, Fruchart J-C, Geboes K, Briggs M, Heyman R, Auwerx J. Activation of the peroxisome proliferator-activated receptor γ promotes the development of colon tumors in C57BL/6J-Apmin⁺ mice. *Nat Med* 1998;4:1053–1056.
101. Saez E, Tontonoz P, Nelson MC, Alvarez JGA, Tze MU, Baird SM, Thomazy VA, Evans RM. Activators of the nuclear receptor PPAR γ enhance colon polyp formation. *Nat Med* 1998;4:1058–1061.
102. Sarraf P, Mueller E, Smith WM, Wright HM, Kum JB, Aaltonen LA, de la Chapelle A, Spiegelman BM, Eng C. Loss-of-function mutations in PPAR γ associated with human colon cancer. *Mol Cell* 1999;3:799–804.
103. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, Dubois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1997;107:1183–1188.

104. DuBois RN, Shao J, Tsujii M, Sheng H, Beauchamp RD. G1 delay in cells overexpressing prostaglandin endoperoxide synthase-2. *Cancer Res* 1996;56:733–737.
105. Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M, DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. *Cell* 1998;93:705–716.
106. Sheng H, Shao J, Kirkland SC, Isakson P, Coffey RJ, Morrow J, Beauchamp RD, DuBois RN. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. *J Clin Invest* 1997;99:2254–2259.
107. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM. Suppression of intestinal polyposis in Apc delta 716 knockout mice by inhibition in cyclooxygenase 2 (COX-2). *Cell* 1996;87:803–809.
108. Shattuck-Brandt RL, Varilek GW, Radhika A, Yang F, Washington MK, Dubois RN. Cyclooxygenase 2 expression is increased in the stroma of colon carcinomas from IL10^{-/-} mice. *Gastroenterology* 2000;118:337–345.
109. Singh P, Rubin N. Inuslinlike growth factors and binding proteins in colon cancer. *Gastroenterology* 1993;105:1218–1237.
110. Kochman ML, Delvalle J, Dickinson CJ, Boland CR. Post-translational processing of gastrin in neoplastic human colonic tissues. *Biochem Biophys Res Commun* 1992;189:1165–1169.
111. van Solinge WW, Neilsen FC, Friis-Hansen L, Falkmer U, Rehfeld JF. Expression but incomplete processing of progastrin in colorectal carcinomas. *Gastroenterology* 1993;104:1099–1107.
112. Vogelstein B, Fearon ER, Kern SE, Hamilton SR, Preisinger AC, Nakamura Y, White R. Allelotype of colorectal carcinomas. *Science* 1989;253:49–53.
113. Castells A, Ino Y, Louis DN, Ramesh V, Gusella JF, Rustgi AK. Mapping of a target region of allelic loss to a 0.5-cM interval on chromosome 22q13 in human colorectal cancer. *Gastroenterology* 1999;117:831–837.
114. Goelz SE, Vogelstein B, Hamilton SR, Feinberg AP. Hypomethylation of DNA from benign and malignant human colon neoplasms. *Science* 1985;228:187–190.
115. Jones PA, Laird PW. Cancer epigenetics comes of age. *Nat Genet* 1999;21:163–167.
116. Liang J-T, Chang K-J, Chen J-C, Lee C-C, Cheng Y-M, Hsu H-C, Wu M-S, Wang S-M, Lin J-T, Cheng A-L. Hypermethylation of the p16 gene in sporadic T3N0M0 stage colorectal cancers: association with DNA replication error and shorter survival. *Oncology* 1999;57:149–156.
117. el Deiry WS, Nelkin BD, Celano P, Yen RW, Falco JP, Hamilton SR, Baylin SB. High expression of the DNA methyltransferase gene characterizes human neoplastic cells and progression stages of colon cancer. *Proc Natl Acad Sci U S A* 1991;88:3470–3474.
118. Wu J, Issa JP, Herman J, Bassett DE, Nelkin BD, Baylin SB. Expression of an exogenous eukaryotic DNA methyltransferase gene induces transformation of NIH 3T3 cells. *Proc Natl Acad Sci U S A* 1993;90:8891–8895.
119. Laird PW, Jackson-Grusby L, Fazeli A, Dickinson SL, Jung WE, Li E, Weinberg RA, Jaenisch R. Suppression of intestinal neoplasia by DNA hypomethylation. *Cell* 1996;81:197–205.
120. Sidransky D, Tokino T, Hamilton SR, Kinzler KW, Levin B, Frost P, Vogelstein B. Identification of ras oncogene mutations in the stool of patients with curable colorectal tumors. *Science* 1992;256:102–105.
121. Eguchi S, Kohara N, Komuta K, Kanematsu T. Mutations of the p53 gene in the stool of patients with resectable colorectal cancer. *Cancer* 1996;77:1707–1710.
122. Jen J, Kim H, Piantadosi S, Liu Z-F, Levitt RC, Sistonen P, Kinzler KW, Vogelstein B, Hamilton SR. Allelic loss of chromosome 18q and prognosis in colorectal cancer. *N Engl J Med* 1994;331:213–221.
123. Carethers JM, Hawn MT, Chauhan DP, Luce MC, Marra G, Koi M, Boland CR. Competency in mismatch repair prohibits clonal expansion of cancer cells treated with N-methyl-N'-nitro-N-nitrosoguanidine. *J Clin Invest* 1996;98:199–206.
124. Bunz F, Hwang PM, Torrance C, Waldman T, Zhang Y, Dillehay L, Williams J, Lengauer C, Kinzler KW, Vogelstein B. Disruption of p53 in human cancer cells alters the responses to therapeutic agents. *J Clin Invest* 1999;104:263–269.
125. Williams CS, Smalley W, DuBois RN. Aspirin use and potential mechanisms for colorectal cancer prevention. *J Clin Invest* 1997;100:1325–1329.

Received January 31, 2000. Accepted May 3, 2000.

Address requests for reprints to: Daniel C. Chung, M.D., Gastrointestinal Unit, GRJ 825, Massachusetts General Hospital, Boston, Massachusetts 02114. e-mail: d_chung@helix.mgh.harvard.edu.