J Gastroenterol 2006; 41:185–192
DOI 10.1007/s00535-006-1801-6

Journal of Gastroenterology

Review

Colorectal cancer: genetics of development and metastasis

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It has been well documented that there are two major pathways in colorectal carcinogenesis. One is the chromosomal instability pathway (adenoma–carcinoma sequence), which is characterized by allelic losses on chromosome 5q (*APC*), 17p (*p53*), and 18q (*DCC/SMAD4*), and the other is a pathway that involves microsatellite instability. Recent progress in molecular biology, however, has shown that colorectal carcinogenesis is not necessarily clearly divided into these two pathways, but is in fact more complicated. Other routes, including the transforming growth factor-β/SMAD pathway, the serrated pathway, and the epigenetic pathway, have been reported. Cross talk among these pathways has also been reported. In the invasion and metastasis steps of colorectal cancers, many more genes have now been identified as being involved in proteolysis, adhesion, angiogenesis, and cell growth. Recently accumulated evidence indicates that colorectal cancer is a genetically heterogeneous and complicated disease.

Key words: colorectal cancer, chromosomal instability, microsatellite instability, metastasis

Introduction

It is now generally accepted that colorectal cancer develops by genetic alterations. Analysis of the molecular mechanism makes it possible to develop a more targeted approach to prevention and treatment of this cancer. There are two major pathways in colorectal carcinogenesis. One is the chromosomal instability pathway (adenoma–carcinoma sequence), which is characterized by allelic losses, and the other is a pathway involving microsatellite instability (MSI). However, recent studies have suggested that colorectal carcinogenesis is not necessarily clearly divided into these two pathways. Other routes, including the serrated and epigenetic pathways, have been reported. There is also some cross talk among these pathways. Moreover, in the progression and metastasis steps of colorectal cancers, many more gene alterations are involved.

In this review, we describe the latest molecular biology of carcinogenesis and metastasis in colorectal cancers.

Received: March 1, 2006 / Accepted: March 6, 2006
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Chromosomal instability pathway

Fearon and Vogelstein[1] proposed a multistep model of colorectal carcinogenesis, in which mutations in the adenomatous polyposis coli (*APC*) gene occur early during the development of polyps, *K-ras* mutations arise during the adenomatous stage, and mutations of *p53* and deletions on chromosome 18q occur concurrently with the transition to malignancy. This pathway is characterized by allelic losses on chromosome 5q (*APC*), 7p (*p53*), and 18q (*DCC/SMAD4*), and is therefore called the chromosomal instability (CIN) pathway. One of the cornerstones of the CIN pathway is the model represented by familial adenomatous polyposis (FAP), in which multiple small adenomas form as a result of initiation of tumorigenesis, namely, two hits in the *APC* gene, followed by mutations of *K-ras*, and subsequently mutations of *p53* and deletion on chromosome 18q. It has been surmised that this same mechanism is also applicable to sporadic colorectal carcinogenesis. The genes that have been reported to be involved in this pathway are listed in Table 1.

APC/β-catenin

APC is a tumor suppressor gene on chromosome 5q whose germline mutation is responsible for FAP.[2,3] APC

Table 1. Oncogene genes and tumor suppressor genes in colorectal cancers

Gene	Chromosomal location	Prevalence of mutation (%)	Function of Gene product	References
Tumor suppressor genes				
APC	5q21	34–72	Inhibition of cell growth via β-catenin degradation	8–11
P53	17p12	40–50	G1 cell-cycle arrest, apoptosis induction	18–21
SMAD	18q21	16–25	Growth arrest via p15 and p21 induction	53–55
DCC	18q21	6	Binding to netrin	34
Oncogenes				
K-ras	12p12	40–65	Growth promotion via RAF/MAPK, JNK, and PI3-K	15, 23–24
β-catenin	31q21	5	Transcription of growth promoting genes (*cyclin D1*, *c-myc*, etc.)	12

APC, adenomatous polyposis coli; DCC, deleted in colorectal cancer

protein, a member of the Wnt signal pathway, normally binds to β-catenin to form a complex with axin and GSK-3β, which is degraded through ubiquitylation.[4–6] When it is inactivated, accumulated β-catenin translocates from the lateral cell membrane to the nucleus, where it drives the transcription of multiple genes implicated in tumor growth and invasion. The large majority of *APC* mutations result in a premature stop codon and thus a truncated protein.[7] *APC* mutations are identified in approximately 30%–70% of sporadic adenomas and in 34%–72% of sporadic cancers.[8–11] About 50% of sporadic tumors with intact *APC* are reported to show mutations of β-catenin itself, resulting in the accumulation of β-catenin.[12] Thus, the APC/β-catenin pathway is considered to play a major role in colorectal carcinogenesis.

However, recent studies have claimed that the APC/β-catenin pathway is not necessarily invaluable first genetic alterations in colorectal cancer. For example, Umetani et al.[13] reported that the frequency of the *APC* mutation is 7% in flat tubular adenomas and 36% in polypoid tubular adenomas. We analyzed APC mutations in aberrant crypt foci (ACF), putative precursors of adenoma and cancer, by an in vitro synthesized protein (IVSP) assay and found no APC mutations in dysplastic or nondysplastic ACF.[14,15] Moreover, no β-catenin accumulation was observed in these lesions. Although one study reports APC mutations in dysplastic ACF, the positive rate was low.[16]

p53

p53 is the most commonly mutated tumor suppressor gene in various kinds of malignant tumors. p53 protein normally induces G1 cell-cycle arrest to facilitate DNA repair during replication, or it induces apoptosis. *p53* mutations are generally considered to occur at the time of the transition from adenoma to cancer.[1] Most mutations occur in highly conserved areas of exons 5 to 8.[17] Moreover, the majority (approximately 80%) are missense mutations (GC to AT), which occur principally in five hotspot codons (175, 245, 248, 273, and 282).[18] *p53* mutations have been identified in 40%–50% of sporadic colorectal cancers.[19] The frequency of *p53* mutations is higher in distal colon and rectal cancers than in proximal colon cancers.[20] Patients with cancers involving a *p53* mutation have a worse outcome and shorter survival time than patients whose cancers do not have a mutation in this gene.[21]

K-ras

K-ras mutations have been detected in various kinds of cancers, particularly in gastroenterological cancers, including colorectal, pancreatic, and bile duct cancers. They have been found in 15%–68% of sporadic colorectal adenomas and in 40%–65% of cancers.[15,22–24] The majority of *K-ras* mutations occur as an activating point mutation in codons 12, 13, and 64.[25–27] Mutated K-ras protein activates a variety of effector pathways, including RAF/MAPK, JNK, and phosphatidylinositol 3-kinase (PI3-K), leading to constitutive growth promotion. Some of the downstream gene targets of K-ras include the cyclin D1, DNA methyltransferase, and vascular endothelial growth factor (*VEGF*) genes.[28–30]

DCC/SMAD

As noted above, allelic losses on chromosome 18q have been identified in approximately 70% of primary colorectal cancers, particularly in advanced colorectal cancers with hepatic metastasis, implying that an 18q deletion may contribute to the progression of colorectal cancers.[1] The *DCC* (deleted in colorectal cancer) gene was long ago proposed as a candidate tumor suppressor gene on 18q.[31] Point mutations of the *DCC* gene have been identified in approximately 6% of sporadic colorectal cancers.[32] However, the candidacy of this gene has recently been called into question. Mice het-

erozygous for *DCC* have been reported to lack the tumor predisposition phenotype.[33] Moreover, other tumor suppressor genes, including *SMAD4/2,* have been reported on 18q.[34,35] In particular, *SMAD4* is currently a candidate gene because the inactivation of SMAD4 has been causally associated with progression of cancers. The detailed role of *DCC* in colorectal cancers needs further study.

Microsatellite instability pathway

Microsatellite instability (MSI) is characterized by expansions or contractions in the number of tandem repeats of simple DNA sequences (microsatellites). MSI has been identified in colorectal cancer associated with hereditary nonpolyposis colorectal cancer (HNPCC) syndrome,[36–38] and DNA mismatch repair (MMR) enzymes, including hMSH2, hMLH1, hPMS1, hPMS2, and hMSH6, have since been shown to be responsible for MSI.[39–43] Moreover, mutations in microsatellites of target genes such as the transforming growth factor-β (*TGF-β*) gene (Table 2) have also been identified in MSI positive tumors.[44–50] Interestingly, MSI is also present in sporadic colorectal cancers: MSI-H (high-frequency MSI) is present in 10%–20% and MSI-L (low-frequency MSI) in 5%–50% of such cancers. In approximately 80% of MSI-H sporadic colorectal cancers, hypermethylation of the *hMLH1* promoter is observed.[51] Sporadic cancers with the MSI-H phenotype demonstrate distinct clinicopathological features compared with MSS (microsatellite stable) or MSI-L tumors, occurring predominantly in the proximal colon and more frequently in women than in men.[52] These cancers are also characterized by distinct histopathological features, including mucinous or signet-ring cell differentiation, medullary features, and excess lymphocyte infiltrations. However, neither MSI-L nor MSS tumors demonstrate such characteristic features. Moreover, MSI-L and MSS tumors more frequently have *K-ras* and *p53* mutations and loss of heterozygosity (LOH) at 5q, 19p, and 18q. Therefore, it is still controversial whether MSI-L and MSS tumors are really different from each other. There is a possibility that MSI-L tumors develop and progress in association with both the MSI and CIN pathways. In addition, approximately 30%–40% of sporadic MSI-H cancers have *APC* mutations. Similarly, approximately 36% of sporadic MSI-H cancers have *p53* mutations. Thus, a subset of colorectal cancers develop in association with both MSI and *APC* or *p53* mutations.

TGF-β/SMAD signaling pathway

The TGF-β/SMAD signaling pathway is composed of TGF-β receptor type I (TGFβRI) and type II (TGFβRII) and SMAD proteins. When TGF-β binds to TGFβRII, which then complexes with TGFβRI, TGFβRI phosphorylates SMAD2, which binds to SMAD4. This complex translocates into the nucleus and induces the Cdk inhibitors, p15 and p21, leading to growth arrest. Mutations leading to the inactivation of the *SMAD4* gene have been found in 16%–25% of colorectal cancer cases.[53–55] Alterations of SMAD2 have been identified in 6% of cases.[34] In contrast, a *TGFβRII* mutation is frequently identified in the 10-bp polyA tract in MSI-positive tumors.[44] In MSI-positive tumors without the *TGFβRII* mutation, mutations of the IGF-II receptor have been frequently identified.[45] Both *TGFβRII* mutation and *SMAD* mutation are reported to occur with the same timing during carcinogenesis, at the transition from adenoma to carcinoma.

Recently, studies suggesting cross talk between the TGF-β and Wnt signal pathways have attracted much attention. A direct physical interaction between TGF-β and Wnt pathway components has been reported. That is, the TGF-β and Wnt pathways synergistically promote carcinogenesis of colorectal cancers through direct interaction of SMAD proteins and LEF-1.[56] Moreover, in heterozygote mice of both *APC* and

Table 2. Target genes of MSI in colorectal cancer

Gene	DNA repetitive sequence (nucleotides)	Incidence of mutations (%)		Function of the gene products	References
		HNPCC	Sporadic MSI positive tumor		
TGFβRII	$(A)_{10}$ (709–718)	75–83	80–90	Inhibition of cell growth	44
BAX	$(G)_8$ (114–121)	50–55	11–50	Induction of apoptosis	45
IGFIIR	$(G)_8$ (4089–4096)	13	9	Growth promotion	46
hMSH6	$(C)_8$ (3049–3056)	33	25–36	Mismatch repair enzyme	47
hMSH3	$(A)_8$ (1141–1148)	50–58	35–46	Mismatch repair enzyme	48
PTEN	$(A)_6$ (795–800)	—	18.8	Inhibition of cell growth	49
E2F-4	$(CAG)_{13}$ (918–956)	71	42–57	Progression of cell-cycle	50

TGFβRII, TGF-β receptor II; IGFIIR, IGF-II receptor; MSI, microsatellite instability; HNPCC, hereditary nonpolyposis colorectal cancer

Table 3. Gene alterations in serrated and classical adenoma

Gene	Serrated adenomas (%)	Classical adenomas (%)	References
K-ras	15–20	15–68	58, 60
APC	0–20	30–70	58
p53	5–8	0–25	58, 60
MSI			
MSI-H	17–21	5	58, 59
MSI-L	5–50	11	58, 59, 62

MSI-H, high-frequency microsatellite instability; MSI-L, low-frequency microsatellite instability

SMAD4 genes, intestinal polyps develop into more malignant tumors compared with those of *APC* alone.[57] Thus, one signaling pathway is coordinately associated with another signaling pathway in the process of carcinogenesis.

Serrated pathway

Recently, the potential role of serrated adenomas and hyperplastic polyps in the genesis of colorectal cancer has gained considerable attention. Serrated adenomas are histologically defined as adenomas that have the morphological features of hyperplastic polyps but which also contain cytological features of conventional adenomas. It has been reported that 30%–50% of serrated adenomas display MSI, mostly at a low level (MSI-L), whereas they show a low rate of *K-ras* and *APC* mutations (Table 3).[58–60] In serrated adenomas with the MSI-H phenotype, aberrant methylation [CpG island methylator phenotype (CIMP)] of the *hMLH-1* gene, and loss of its expression have been frequently noted.[61] Moreover, mutations of the same target genes as those in MSI-H cancers, for example, *TGFβRII*, *BAX*, and *IGFIIR*, have also been reported.[45–47] The genetic alterations of *p53* are still controversial. However, several recent studies have shown low incidences of *p53* mutations, similar to those in classical adenoma.[58,60] Thus, evidence of the MSI-H serrated pathway in colorectal cancers has accumulated. Regarding serrated adenoma with the MSI-L phenotype, expression of the DNA repair gene *O*-6-methylguanine DNA methyltransferase (*MGMT*) has been reported to be lost by methylation.[62,63] However, details remain unclear.

Epigenetic mechanism of colon carcinogenesis

Recent molecular biology studies have revealed that an epigenetic mechanism plays an important role in colorectal carcinogenesis. CpG-rich regions located at the 5′ end of coding sequences can undergo hypermethylation, leading to the silencing of the gene. Cancers demonstrating methylation and silencing of multiple genes are described as CIMP positive. The *hMLH1* gene is a frequent target of hypermethylation, which leads to the MSI-H phenotype, as described above. Many genes other than *hMLH1*, including $p16^{INK4A}$, *MGMT*, estrogen receptor (*ER*), *APC*, and *COX-2*, have been reported to undergo hypermethylation and silencing in human colorectal cancers.[64] Recently colorectal adenomas, in particular, villous and tubulovillous adenomas as well as cancers, have been reported to show CIMP.

Genes related to invasion and metastasis

The conventional paradigm of invasion and metastasis of colorectal cancer consists of a complex series of steps, including proteolysis of the local extracellular matrix (ECM), adhesion, angiogenesis, dissemination, and cell growth. Many gene alterations are complexly involved in these processes (Table 4).

Genes for proteolysis

In the proteolysis step, proteinases, which are produced by cancer cells or fibroblasts, degrade ECM components and enable cancer cells to detach from the primary site. Of the many kinds of proteinases, matrix metalloproteinases (MMPs), which currently are known to comprise more than 25 enzymes, appear to exert a dominant effect. MMPs are collectively able to degrade virtually all ECM components, that is, collagens, laminin, fibronectin, vitronectin, enactin, and proteoglycans. In particular, MMP-7 (matrylysin) is reported to play an important role in the degradation of ECM. Matrylysin is overexpressed in the majority of colorectal cancers, and its expression is positively correlated with metastatic potential.[65] MMPs other than matrylysin, including collagenases, gelatinases, and stromelysin, are also reported to be involved in proteolysis of ECM.[66–71] On the other hand, tissue inhibitors of metalloproteinase (TIMP) in colorectal cancer tissues protect against ECM degradation.[72] Urokinase

Table 4. Genes related to invasion and metastasis in colorectal cancers

Genes	Characters of gene products	References
Genes for proteolysis		
MMP-7 (matrylisin)	Digestion of fibronectin, laminin, collagen IV, and protoglycans	65, 71
MMP-2, -9 (gelatinases)	Digestion of gelatins and collagen IV	66, 68
MMP-1, -8, -13 (collagenases)	Digestion of collagens I, II, III, IV, VI, IX, X, and XI	66, 67
MMP-3 (stromelysin-1)	Digestion of fibronectin and laminin	70
TIMP-1	Tissue inhibitors of MMP	72
uPAR	Activation of plasmin-plasminogen system	73
Genes for adhesion		
Integrins	Binding to laminin, collagen, fibronectin, and vitronectin	74, 75
Cadherins	Cell–cell adhesion	76, 77
CD44	Binding to hyaluronan	78
CEA	Binding to a receptor on Kupffer cells	79–81
Genes for angiogenesis		
VEGF	Angiogenesis, MMP-9 induction	82, 84–88
PD-ECGF	Angiogenesis	83
Genes for cell survival and others		
TRAIL-R	Binding to TRAIL to induce apoptosis	89–93
CXCR4	Binding to SDF-1 to enhance migration and invasiveness	94, 95
Drg-1	Cell differentiation	96
c-Met	Binding to HGF to enhance motility and invasiveness	97, 98

MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; uPAR, urokinase plasminogen activator receptor; CEA, carcinoembryonic antigen; VEGF, vascular endothelial growth factor; PD-ECGF, platelet-derived endothelial cell growth factor; TRAIL, tumor necrosis factor related apoptosis-inducing ligand; TRAIL-R, TRAIL receptor; SDF, stromal cell-derived factor; HGF, hepatocyte growth factor

plasminogen activator receptor (uPAR) is another factor that has been implicated in this process.[73]

Genes for adhesion

Many adhesion molecules, including integrins, cadherins, selectins, CD44, ICAM-1, VCAM-1, and carcinoembryonic antigen (CEA), have been identified in colorectal cancers.[74–82] For example, integrins can bind many ECM molecules, such as laminin, collagen, fibronectin, and vitronectin. Cancer cells expressing these adhesion molecules are more likely to adhere to the ECM, leading to subsequent invasion and metastasis.

E-cadherin is a cell–cell adhesion molecule that participates in a homotypic, calcium-dependent interaction to form an epithelial adherens junction. The invasiveness and metastatic potential of a broad range of cancers are often associated with downregulation of E-cadherin expression.[76] Previously, we also reported that expression of E-cadherin is inversely correlated with tumor aggressiveness.[77] CEA, a well-known human tumor marker, has also been reported to function as an intercellular adhesion molecule.[79–81] It is well documented that CEA expression is positively correlated with liver metastasis. However, the CEA receptor molecule and the mechanism of their binding are not yet clarified.

Genes for angiogenesis

Angiogenesis is an important step in the outgrowth of a primary tumor and also provides a source for hematogenous dissemination, progression, and metastasis. Many potential angiogenic factors have been characterized, including VEGF and platelet-derived endothelial cell growth factor (PD-ECGF).[82,83] Of these factors, VEGF is the most important, and it has been well examined for its role in the invasion and metastasis of cancer cells. Currently, six VEGF molecules (VEGF A–F), are known, and they induce angiogenesis by acting as highly specific mitogens for endothelial cells. Signal transduction involves binding to tyrosine kinase receptors (VEGF receptors; VEGFR) and results in endothelial cell proliferation, migration, and new vessel formation, as well as increased vascular permeability.[84,85] This process is also closely associated with other signal transductions such as mitogen-activated protein kinase.[86] Colorectal cancers with increased *VEGF* expression are well known to be associated with a poor prognosis.[87,88]

Genes related to cell growth and evasion of the immune system

Only a small population of tumor cells lodged in distant organs have the ability to survive, grow, and evade the immune system. Many molecular factors are involved in this process (Table 4). For instance, tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a mem-

ber of the TNF family, is known to be expressed in human hepatic NK cells.[89,90] Recent studies have revealed that a tumor cell that expresses TRAIL receptor 1 (TRAIL-R1) or 2 (TRAIL-R2) is destroyed by NK cells through apoptosis in the liver, whereas a tumor cell expressing TRAIL receptor 3 (TRAIL-R3) or 4 (TRAIL-R4) can survive by resisting apoptosis.[91,92] Patients with colorectal cancers with high TRAIL-R1 expression have been reported show a significantly poorer prognosis than those with low TRAIL-R1 expression.[93]

The gene for chemokine receptor CXCR4, whose ligand is the chemokine stromal cell-derived factor (SDF-1) has also been reported to be involved in metastasis of colorectal cancers.[94] There is growing evidence that CXCR4 and SDF-1 regulate migration and metastasis of cancer cells. Zeelenberg et al.[95] reported that CXCR4-deficient colon cancer cells did not proliferate but stayed as single cells in the liver, although the control cells grew there, indicating that CXCR4 plays an important role after the cancer cells colonize the liver. It has also been reported that other genes such as *Drg-1* and *c-Met* play a role in this process of metastasis.[96–98]

The gene alterations involved in invasion and metastasis occur by various mechanisms, including chromosomal instability, MSI, and promoter methylation.

Epilogue

Rapid advances are being achieved in the understanding of the molecular genetics and epigenetics of colorectal cancers. Accumulating evidence has shown that colorectal cancer is heterogeneous and complex. However, we believe that detailed genetic and molecular biological analyses of colorectal cancer will contribute to its prevention and diagnosis and to effective therapeutics in the future.

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