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Our Current Understanding of Genetic Alterations in Colorectal Cancer, and Implications for Clinical Use

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ABSTRACT

In recent years, we have unfolded some of the intimate mechanisms that drive the genesis and progression of colorectal cancer (CRC). Sporadic cases of CRC present a wide variety of genetic alterations, such as genomic deletions at several chromosomal loci and mutations in APC, p53 and K-ras, which affect tumor suppressor genes and oncogenes. Epigenetic alterations and dysregulation of the patterns of gene expression are also a common hallmark, exemplified by the aberrant methylation and inactivation of the tumor suppressor and DNA repair genes $p16^{INK4a}$, $p14^{ARF}$, hMLH1, and O^6 methylguanine DNA methyltransferase (MGMT). The genetic background of the major inherited CRC syndromes—familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, and Peutz-Jeghers syndrome—has also been characterized. In addition, analysis of the corresponding germline mutations in APC, hMLH1/hMSH2, and LKB1 can be obtained. All of these molecular alterations are starting to be used as biomarkers for early detection, tumor extension, and prediction of tumor behavior. Future management of patients with CRC will benefit from our evolving understanding.

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INTRODUCTION

Colorectal cancer (CRC) ranks as the third most common cancer in the Western countries, and is a major socioeconomic problem. In 1990, an estimated 738,000 new CRC cases were diagnosed worldwide, and 437,000 deaths occurred (8.4% of the world total).[1] Unlike most other tumor types, incidence and mortality of CRC are similar between males and females.[2] The incidence of CRC is higher in developed countries than in developing countries—the lifetime probability of developing CRC in developed countries is 4.6% and 3.2% in men and women, respectively. The highest incidences are in Australia/New Zealand, North America, and Northern and Western Europe.

The 5-year relative survival rate for CRC patients is 61%. When CRC is detected early and when it is localized, the 5-year survival rate is 90%; however, only 37% of CRCs are diagnosed at an early stage. When there are metastases, the 5-year survival rate is only 8%.[2] A personal or family history of CRC, polyps, or inflammatory bowel disease is associated with an increased CRC risk. Other factors, such as high-fat or low fiber-diet, as well as diets low in fruits and vegetables, have been reported to be risk factors for CRC.[3]

CHARACTERISTIC GENETIC AND EPIGENETIC ALTERATIONS OF SPORADIC CRC

Genes implicated in cancer development can be roughly divided into oncogenes and tumor suppressor genes (TSGs). Oncogenes are those genes whose alterations in primary tumors lead to a gain of function of protein. Alteration at one of the two alleles in an oncogene is sufficient to increase the protein's function. The common genetic mechanisms for oncogene activation are point mutations, chromosomal translocations, and gene amplification. The most important oncogene in CRC is K-*ras*. The *ras* genes encode a 21-kilodalton protein with homology to G proteins. These proteins participate in the transduction of mitogenic signals from the cell membrane to the nucleus.[4] Specific point mutations are found in approximately 50% of CRCs. Most (85%) of the mutations identified are localized to codons 12 and 13, while the remaining 15% target codon 61.[5]

Conversely, TSGs are those genes whose alterations lead to a loss of protein function. The common mechanisms for TSG inactivation are point mutations, loss of heterozygosity, homozygous deletions, and promoter hypermethylation. The paradigm of TSG in CRC and in almost all other tumor types is p53. The p53 gene is located in

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Newly discovered biomarkers based in genetic alterations could be used in colorectal cancer.

Defects in DNA repair may have an impact in predicting chemotherapy response.

A good genetic screening of familial colorectal cancer can reduce further procedures.

Optimum care of colorectal cancer patients can be achieved in the inherited forms of the disease.

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chromosome 17p13 and is commonly altered by point mutations and loss of heterozygosity (50–70% of CRCs harbor p53 alterations).[6] P53 protein expression is very low in normal cells; however, p53 levels increase when a cell is subjected to different types of stress such as radiation, drug-induced DNA damage, and hypoxia. At present, p53 is believed to maintain genomic stability, and it has been called "the guardian of the genome." At low or repairable levels of DNA damage, p53 mediates the delay or arrest of cell replication; this allows the cell to repair the damage and avoid the fixation and propagation of gene alterations that may lead to carcinogenesis. Upon high or irreparable DNA damage, p53 promotes the cells toward apoptosis.[7] Mutations in the p53 gene are present in the germline of patients with the Li-Fraumeni syndrome. People with this syndrome have a high incidence of many cancers, such as lymphoma, leukemia, sarcomas, and breast cancer at early ages. Although the frequency of p53 mutations in CRC is very high, the occurrence of CRC is not common in the Li-Fraumeni syndrome.[8]

Other frequently altered genes in CRC are APC and B-catenin. As we discuss below, germline mutations in the APC gene are responsible for the familial adenomatous polyposis (FAP) syndrome;[9] however, this gene also plays an important role in sporadic colorectal tumors. The APC and B-catenin proteins belong to the same biochemical pathway in which APC is an inhibitor of the B-catenin-mediated gene transcription.[10] The APC gene acts as a tumor suppressor gene and the mechanisms of inactivation include point mutation, loss of heterozygosity, and promoter hypermethylation.[11,12] It has been estimated that 80% of sporadic colorectal tumors show APC gene alterations. Alternatively, the B-catenin gene acts as an oncogene and is inactivated through point mutations. B-catenin gene mutations are present in about 10–20% of sporadic colorectal tumors, and alterations at the APC and B-catenin genes are mutually exclusive.

Other genetic and epigenetic changes in sporadic CRC are chromosomal losses and gains, and promoter hypermethylation in specific genes. The frequent detection of loss of heterozygosity or genetic amplification in specific chromosomal regions are hallmarks of TSGs or oncogenes, respectively. Perhaps the most common region for loss of heterozygosity in CRC is 18q. Losses at 18q have been reported in 75% of sporadic colorectal tumors.[13] Several candidate TSGs have been identified in this region, but efforts to pinpoint positively the target gene or genes have been hampered by the inability to identify frequent intragenic mutations. Losses at chromosomes 5q and 17p are also very common; however, in most of the cases the targeted genes are APC and p53, respectively. Table 1 lists the most common gene mutations in sporadic CRC.

As described above, the CRC cell differs from a normal colonic mucosal cell in its genotype. The recent completion of the human genome sequence gives us the tool to define each change in DNA specific to a tumor cell. However, the malignant cell has also acquired a different epigenotype.[14] The inheritance of information based on gene expression levels is known as epigenetics, as opposed to genetics, which refers to information transmitted on the basis of gene sequence. The main epigenetic modification in humans is methylation of the cytosine nucleotide. In a healthy cell, DNA methylation patterns are conserved through cell divisions, allowing the expression of the particular set of genes necessary for that cell type and blocking the expression of exogenous sequences. In a cancer cell, there is a clear distortion in the expression profiles, and these distortions can now be studied using microarray technology. Changes in DNA methylation patterns are one of the "guilty parties" in CRC.

One of the main aberrant methylation changes in cancer occurs at the beginning of the gene. In this region, which is where RNA originates, approximately half of genes have a very rich density of CpG dinucleotides. The "CpG island," as it is called, stays unmethylated in the normal state, allowing the correct pattern of gene expression. However, in CRC and most other human neoplasms, some CpG islands become hypermethylated and shut down gene expression, contributing to tumorigenesis.[14] In colorectal tumors, the genes affected by promoter CpG island hypermethylation cover all the cellular pathways: the tumor suppressor genes $p16^{INK4a}$ and $p14^{ARF}$ alter the cell cycle and p53 regulation, and the DNA repair genes hMLH1 and MGMT cause the appearance of genetic instability (microsatellite alterations) and mutations (transitions in K-*ras*), respectively.[15-19] Even the gate-keeper gene for colorectal carcinogenesis, APC, can be occasionally methylated.[12] Table 1 lists the most common targets of gene promoter hypermethylation in sporadic CRC.

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In addition to the generation of genetic and epigenetic alterations in CRC, the timing in which these alterations accumulate during tumor development may be critical. Most of them, such as K-*ras* mutations and hypermethylation of $p16^{INK4a}$, $p14^{ARF}$, and MGMT, are detectable in colorectal adenomas.[16,18] Using genetic screening of different preneoplastic colorectal lesions and colorectal tumors at different stages, Vogelstein and colleagues have postulated a genetic model for colorectal carcinogenesis.[20] We have slightly updated this (Figure 1).

FAMILIAL TYPES OF CRC

Familial CRC can be divided into two general groups: those with multiple benign colorectal polyps (polyposis) and those without polyposis. Types of polyposis include FAP coli, Peutz-Jeghers syndrome, juvenile polyposis syndrome (JP), and Cowden syndrome. Table 2 shows the genes associated with these syndromes and their respective chromosomal locations. The FAP syndrome has been estimated to be present in about 1 in 7,000 individuals in the United States. A person affected with FAP develops hundreds to thousands of adenomatous polyps during his lifetime. Genetically, the FAP syndrome is inherited in an autosomal-dominant fashion, and the gene responsible is APC (adenomatous polyposis coli).[9,21]

Peutz-Jeghers syndrome (PJS) has two clinical hallmarks: mucocutaneous melanin pigmentation and intestinal harmatomatous polyposis. PJS patients are at increased risk for cancer, especially malignant tumors of the gastrointestinal tract, breast, uterine, cervix, and ovary. The incidence of the PJS has been estimated as 1/8,000 to 1/29,000 live births. It is inherited as an autosomal dominant disease and it is caused by germline mutations in the LKB1 gene (also named STK11).[22]

Cowden syndrome is an autosomal-dominant disorder characterized by multiple hamartomas (benign disorganized growths) and higher risk of breast and thyroid cancers. It usually presents by the late 20s. From informal population studies, it has been estimated that the frequency of Cowden syndrome in the general population is 1 in 1 million; however, the true rate is likely to be much higher. Its most common clinical manifestations are gastrointestinal hamartomas, mucocutaneous lesions, thyroid abnormalities (including benign tumors), fibrocystic disease, breast cancer, macroencephaly, and mental retardation. Occasionally the benign tumors will develop a malignancy (10% of benign thyroid

TABLE 1. GENES COMMONLY ALTERED IN SPORADIC COLORECTAL CARCINOMAS AND THE MECHANISMS OF GENE ALTERATIONS

Gene altered	Frequency (%)	Mechanism
APC	80	MUT, LOH, MET
p53	50	LOH, MUT
K-*ras*	50	MUT
B-catenin	15	MUT
p16INK4a	35	MET, LOH
p14ARF	30	MET, LOH
MGMT	30	MET
hMLH1	15	MET

LOH=loss of heterozygosity (genomic deletion); MUT=point mutation; MET=promoter hypermethylation.

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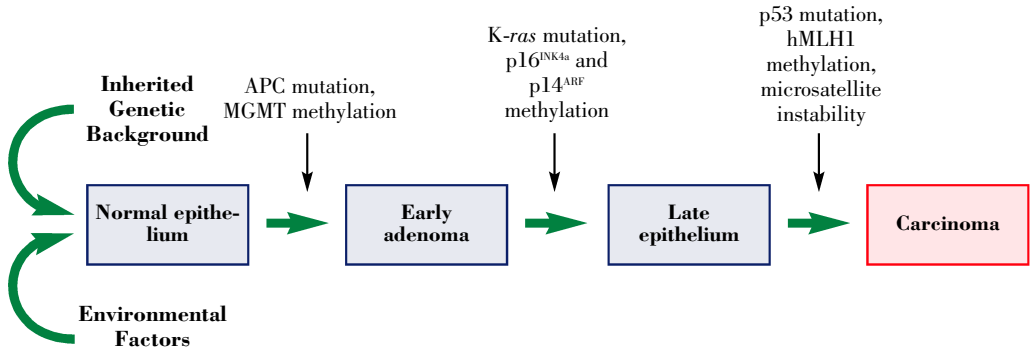
FIGURE 1. A MODEL FOR GENETIC AND EPIGENETIC ALTERATIONS IN COLORECTAL TUMORIGENESIS



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tumors and 50% of benign breast lesions).[23] The PTEN gene is responsible for this syndrome. This gene encodes for a protein with phosphatase activity whose biological function is not yet completely elucidated.[24]

Juvenile polyposis is an autosomal-dominant syndrome characterized by multiple hamartomatous polyps of the gastrointestinal tract. Patients may have polyps in the stomach, small intestine, and/or colon. Patients have an approximately 50% risk for developing gastrointestinal cancer, most of which are colorectal tumors.[25] It is not yet clear which gene is responsible for this syndrome, although mutations at the PTEN gene have been described in some patients.

The most common CRC syndrome without polyposis is hereditary nonpolyposis CRC (HNPCC). Affected individuals are principally at increased risk for CRC, however, tumors in the endometrium, ovary, stomach, urinary tract, and brain have also been associated with HNPCC. HNPCC syndrome accounts for approximately 3–4% of all CRCs and its frequency in the general population is estimated at about 1 per 500 persons. Most individuals with HNPCC develop tumors at a very early age. The mean age for cancer diagnosis is about 45 years; however, many tumors occur in individuals in their 20s or even teens.[26] As defined by the Amsterdam criteria, the HNPCC families include: (1) at least three affected relatives with verified CRC; (2) at least one who is a first-degree relative of the other two; (3) exclusion of FAP; (4) at least 2 successive generations that have been affected; and (5) diagnosis of CRC in at least one family member younger than 50.[27] However, not all HNPCC families, diagnosed genetically, meet the Amsterdam criteria. The HNPCC syndrome is inherited in an autosomal-dominant manner. The genes responsible are the DNA-mismatch repair genes,[28,29] hMSH2, hMLH1, hPMS1, or hPMS2 (Table 2). Mutations in any of these genes lead to a hypermutable phenotype or general genetic instability, which was first observed as a deletion or insertion of DNA in simple repetitive elements named microsatellites. Thus, germline mutations at any of these genes leave the individual susceptible to the development of hypermutability, which induces mutations at critical genes that eventually lead to cancer.[30] We do not yet have a suitable explanation for why specific organs are at selective risk to develop cancer.

IMPACT OF GENETIC AND EPIGENETIC STUDIES IN CRC PATIENTS

The first obvious benefit derived from study of CRC genetics has been the identification of inherited forms of colorectal tumors. We can analyze the mutational status of APC (FAP), mismatch repair genes, hMLH1 and hMSH2 (HNPCC,) and LKB1/STK11 (Peutz-Jeghers), which allows presymptomatic diagnosis in affected families. If a person has a positive test result, rigorous screening can then be implemented. Changes in lifestyle or prophylactic surgery can be considered. We should also point out that the demonstration of no germline mutation in a high-risk family can have a significant positive impact, in that it can reduce the discomfort and anxiety associated with disease expectation, and lessen the economic expenses of repeated medical examinations.

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The study of molecular alterations in sporadic cases of colorectal tumors, which comprise 90% of cases, can also be of diagnostic and prognostic value. The finding of a mutation in such oncogenes as K-*ras* has been demonstrated in stool samples, which allows presymptomatic detection.[31] More molecular markers are likely to be added to this approach to identify CRCs in a cost-effective manner. Other tumor-specific alterations can help predict the biologic virulence of that particular colorectal tumor. For example, chromosome 17p and 18q losses and simultaneous presence of K-*ras* mutation and p16INK4a methylation are associated with poorer prognosis.[13,32] Analysis of genetic and epigenetic alterations can also be extremely useful to pathologists in determining tumor extent. The finding of p53 or K-*ras* mutations or

TABLE 2. GENETIC DISEASES AND GENES ASSOCIATED WITH CRC PREDISPOSITION

Disease	Gene	Chromosomal location
FAP	APC	5q21
Peutz-Jeghers	LKB1/STK11	19p13.3
Cowden syndrome	PTEN	10q23
Juvenile polyposis	PTEN	10q23
HNPCC	hMLH1	3p21
HNPCC	hMSH2	2p15–16
HNPCC	hPMS1	2q31
HNPCC	hPMS2	7p22

FAP=familial adenomatous polyposis; HNPCC=hereditary nonpolyposis colorectal cancer.

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p16INK4a methylation in a hepatic lymph node of a CRC patient, for example, strongly suggests that cancer cells have reached (and probably surpassed) this localization.[33]

Recently, we described one of the first molecular markers to predict response to alkylating chemotherapy in human cancer: aberrant methylation of the DNA repair gene MGMT renders brain tumors sensitive to carmustine.[34] Similar biopredictors could be developed in colorectal neoplasms to design a personalized chemotherapy. For example, some patients respond to 5-fluorouracil, a front-line component in many chemotherapies, while others are resistant from the beginning. The presence or absence of a genetic variant in the dihydropyrimidine dehydrogenase gene (DPD), responsible for 5-fluorouracil metabolism, could be used to predict a patient's likelihood of achieving a response to fluorouracil.[35] While we wait for "magic bullets" against colorectal tumors (efficient gene therapy, highly tumor-specific monoclonal antibodies, chemical inhibitors of oncogenes, targeted demethylation drugs, and useful antiangiogenic compounds), we can take advantage of our current knowledge of the molecular genetics of colorectal tumors.

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