Abstract—Each year over 1.83 million people are diagnosed with colorectal cancer (CRC) worldwide, with an annual mortality of more than 694,000 making it the third most common cause of cancer mortality among men and women. The disease results from the progressive accumulation of genetic and epigenetic alterations that lead to the transformation of normal colonic epithelium to colon adenocarcinoma. The challenges are to understand the molecular basis of individual receptivity to colorectal cancer and to determine factors that initiate the development of the tumor, drive its progression, and determine its responsiveness or resistance to antitumor agents. Colorectal cancer is a heterogeneous disease, with four known major molecular aspects. The first is that the genetic and epigenetic alterations that cause colorectal cancer formation promote the cancer formation process because they provide a clonal growth advantage to the cells that acquire them. The second concept is that cancer arises via a multi-step progression at both the molecular and the morphologic level. The third is the loss of genomic stability can drive the development of colorectal cancer by facilitating the acquisition of multiple tumor-associated mutations. The fourth is that hereditary cancer syndromes frequently correspond to germ line forms of key genetic disorders whose somatic occurrences drive the emergence of sporadic colorectal cancers. In this paper we investigate the roles and relationships of these concepts and their interactions in colorectal cancer.

Keywords- Colorectal cancer, metastasis, tumour suppressor genes, oncogenes, KRAS, EGFR, Genetic instability.

INTRODUCTION

The colon is one of the fundamental parts of the digestive tract, as the largest and first of the segments of the large intestine, located between the small intestine and the rectum. Its principal functions are the absorption of water, minerals, and nutrients, and to serve as a storage area for the waste material that forms the feces. It consists of four sections: the ascending colon located on the right side; the transverse colon; the descending colon located on the left side, and the sigmoid colon or sigmoid. Together they constitute an irregular and thick organ because of the longitudinal disposition of muscular fibres, with a less developed submucosa, but a very evident mucosa as it is full of lymph nodes which confer its characteristic appearance. The mucosa, which is thicker than that of the small intestine, has multiple tubular invaginations called ‘crypts of Lieberkühn’, which are wide, deep, and numerous, along the surface of its epithelium, and in which the regeneration of the epithelium takes place [1]. Because of its biological nature, the colon has a high level of cellular regeneration and a physiological role in of physical, chemical, and biological nature, which increases the possibility of developing diverse pathologies, including cancer. Colorectal cancers are classified as well-differentiated, moderately well differentiated, or poorly differentiated on the degree of preservation of normal glandular architecture and cytologic features. Progressively more poor differentiation is presumably a histologic marker of further underlying genetic mutations, but the mutations associated with
poor differentiation are currently unknown. About 20% of cancers are poorly differentiated. They have a poor prognosis[2]. About 15% of colon cancers are classified as mucinous or colloid because of prominent intracellular accumulation of mucin. These cancers are more aggressive[3].

Colorectal cancer is recently staged according to the tumor–node–metastases (TNM) classification by mural depth of the primary tumor (T), by presence of local lymph node metastases (N), and by presence of distant metastases (M)[4]. This classification is particularly helpful in endosonographic staging of colorectal cancer[5]. In the TNM classification, invasive colon cancer is classified from stage I to IV. Stage I in the TNM classification corresponds to Dukes A or B1 lesions, stage II corresponds to a Dukes B2 lesion, stage III corresponds to a Dukes C lesion, and stage IV corresponds to a Dukes D lesion. Pathologic stage is highly correlated with cancer prognosis[6].

**GENETICS AND EPIGENETICS ALTERATIONS IN CRC**

Colorectal cancer results from the progressive accumulation of genetic and epigenetic alterations that lead to the transformation of normal colonic epithelium to colon adenocarcinoma. From the analysis of the molecular genesis of colorectal cancer, four central tenets concerning the pathogenesis of cancer have been established. The first is that the genetic and epigenetic alterations that underlie colon cancer formation promote the cancer formation process because they provide a clonal growth advantage to the cells that acquire them. The second tenet is that cancer emerges via a multi-step progression at both the molecular and the morphologic levels[7]. The third is that loss of genomic stability is a key molecular step in cancer formation[8]. The fourth is that hereditary cancer syndromes frequently correspond to germ line forms of key genetic defects whose somatic occurrences drive the emergence of sporadic colorectal cancers[9].

**A. GENETIC ALTERATIONS**

Colorectal cancer is believed caused by a cascade of genetic mutations leading to progressively disordered local DNA replication and accelerated colonocyte replication. The progressive accumulation of multiple genetic mutations results in the transition from normal mucosa to benign adenoma to severe dysplasia to frank carcinoma (Table 1). A progression from normal mucosa to adenoma to carcinoma was supported by the demonstration of accumulating mutations in genes of APC, K-RAS, P53 and DCC, all of which are thought to be of significance, but are not able successfully to account for all CRCs. Mutations of the mismatch repair genes are believed to account for about 15% of sporadic colorectal cancers[10]. APC mutation is believed to account for about 80% of sporadic colorectal cancers[10]. Alterations in APC, which result in overactivation of the Wingless/Wnt signalling pathway, appear to initiate tumour formation in the colon. Subsequent alterations in other genes then play a role in tumour growth and the eventual acquisition of other malignant characteristics such as tissue invasiveness and the ability to metastasize.

**APC**

The Adenomatous polyposis coli (APC) gene encodes a protein that possesses multiple functional domains that mediate oligomerization as well as binding to a variety of intracellular proteins including β-catenin, γ-catenin, glycogen synthase kinase (GSK)-3β, axin, tubulin, EB1, and Hdlg [9]. The activation of the Wnt signaling pathway, is regarded
as the initiating event in colorectal cancer. Wnt signaling occurs when the oncoprotein β-catenin binds to nuclear partners (members of the T-cell factor–lymphocyte enhancer factor family) to create a transcription factor that regulates genes involved in cellular activation[11][12][13]. The β-catenin degradation complex controls levels of the β-catenin protein by proteolysis. A component of this complex, APC, not only degrades β-catenin but also inhibits its nuclear localization.

One of the central tumour promoting effects of these mutations results in overactivation of the Wingless/Wnt signaling pathway, with the subsequent expression of genes that favor cell growth. APC mutations disrupt the association of APC with β-catenin, resulting in excessive amounts of β-catenin and overactivation of the Wnt signaling pathway. Consequently, genes that promote tumour formation are transcribed. Truncating APC mutations prevent this process from happening and cause an increase in the amount of cytoplasmic β-catenin, which can then translocate to the nucleus and interact with other transcription factors.

P53

The normal tumour suppressor gene p53 product arrests the cell cycle following DNA injury to permit either DNA repair if the damage is correctable, or apoptosis if the damage is too severe. The wild-type p53 protein product is up-regulated after cell stress from radiation exposure, DNA injury, or other noxious events to prevent new DNA synthesis and halt cell division. Loss of function can promote genomic instability as genetic errors are replicated without check, resulting in loss of heterozygosity. Mutation of the p53 gene is believed to be important in the transition from late adenoma to frank carcinoma. About 50% of lesions with high-grade dysplasia and about 75% of frank cancers exhibit loss of normal p53 function, usually from a missense point mutation of one allele and deletion of the other, wild-type, allele[14][15].

In colorectal cancers, P53 mutations have not been observed in colon adenomas, but rather appear to be late events in the colon adenoma-carcinoma sequence that may mediate the transition from adenoma to carcinoma[16]. Furthermore, mutation of P53 coupled with loss of heterozygosity (LOH) of the wild-type allele was found to coincide with the appearance of carcinoma in an adenoma, thus providing further evidence of its role in the transition to malignancy [17][18]. The function of P53 to recognize DNA damage and induce cell cycle arrest and DNA repair or apoptosis has led to P53 being called the “guardian of the genome”[19].

DCC

The DCC (deleted in colon cancer) gene encodes for a neural cell adhesion molecule receptor and normally promotes apoptosis and suppresses tumors. Loss of the normal DCC gene is believed to be important in the transition from an intermediate to a late adenoma. Its role in this transition is supported by its frequent allelic deletion during this transformation[16] One of the most frequent genetic abnormalities that occur in advanced colorectal cancer is loss of heterozygosity (LOH) of DCC in region 18q21. DCC elimination is not believed to be a key genetic change in tumour formation, but one of many alterations that can promote existing tumour growth.

K-RAS
Kirstein rat sarcoma (K-RAS) is a member of the RAS family of genes and present one of the most prominent proto-oncogenes in colon carcinogenesis. The RAS family genes encode highly conserved proteins that are involved in signal transduction. One major function of the RAS protein family is to couple growth factors to the Raf-mitogen-activated protein (MAP) kinase-MAP signal transduction pathway, which leads to the nuclear expression of early response genes[22]. The K-ras gene encodes for a protein involved in signal transduction from the cell membrane to the nucleus[20]. Specific mutations of this gene result in constitutive activation of this signal pathway and increased colonocyte replication. These mutations are associated with exophytic growth of adenomas in the transition to carcinoma[21].

K-RAS mutations have been found in 37% - 41% of colon carcinomas and appear to occur relatively early in colorectal cancer formation[23][24]. The K-RAS mutations appear to follow APC mutations and are associated with advanced adenomatous lesions. Evidence for this model comes from the observation that small adenomas with APC mutations carry K-RAS mutations in approximately 20% of the tumours, whereas approximately 50% of more advanced adenomas have K-RAS mutations. Thus, alterations of K-RAS appear to promote colorectal cancer formation early in the adenoma-carcinoma sequence by mediating adenoma growth[25][26].

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome location</th>
<th>Normal physiologic function of encoded protein</th>
<th>Clinical manifestations of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>5q</td>
<td>Regulates cell growth and apoptosis</td>
<td>Homozygous somatic mutation associated with colonic adenomas.</td>
</tr>
<tr>
<td>K-RAS family</td>
<td>12q</td>
<td>Encodes a small GTP binding protein on cell membrane involved in transduction of mitogenic signals across cell membrane</td>
<td>Mutated in about one half of colon cancers; may act in an intermediate stage of carcinogenesis; mutation common in hyperplastic polyps.</td>
</tr>
<tr>
<td>p53</td>
<td>17q</td>
<td>Regulates G1 cell cycle and apoptosis</td>
<td>Critical in transition from late adenoma to early cancer.</td>
</tr>
<tr>
<td>DCC</td>
<td>18q</td>
<td>Encodes a neural cell adhesion molecule, facilitates apoptosis, tumor suppressor</td>
<td>Believed to promote progression to frank carcinoma.</td>
</tr>
<tr>
<td>Mismatch repair genes</td>
<td>Located on several chromosomes</td>
<td>Recognize errors in nucleotide matching of complementary chromosome strand and initiate excision of erroneous strand</td>
<td>Progressive accumulation of mutations throughout the genome in affected cells leading to hyper mutability and genetic chaos; mutations of oncogenes or tumor suppressor genes can lead to colon cancer</td>
</tr>
</tbody>
</table>
Figure 1: Genes and Growth Factor Pathways That Drive the Progression of Colorectal Cancer.

B. Epigenetic Alterations

Epigenetic silencing of genes, mostly mediated by aberrant DNA methylation, is another mechanism of gene inactivation in patients with colorectal cancer[27][28]. The term DNA methylation refers to the methylation of cytosine residues (5-methyl cytosine) at CpG sites found throughout the genome[29]. These epigenetic alterations are characteristically clustered in called CpG islands in gene promoter regions, and hypo and hyper methylation of these regions are related to activation and inhibition of transcription, respectively. This type of gene regulation is essential to cell differentiation as well as embryological development [30]. This aberrant promoter associated methylation can induce epigenetic silencing of gene expression[27]. In sporadic colorectal cancer with microsatellite instability, somatic epigenetic silencing blocks the expression of MLH1[27].

Genetic classification of CRC

Colorectal cancer is classified into three forms: Sporadic (60%) comprises patients with no notable family history and, by definition, with no identifiable inherited gene mutation that accelerates cancer development, Familial (30%) refers to patients who have at least one blood relative with CRC or an adenoma, but with no specific germline mutation or clear pattern of inheritance, and hereditary syndromes (10%) which result from germline inheritance of mutations in highly penetrant cancer susceptibility genes[31].
Figure 2: Types of colorectal cancer cases that arise in various family risk setting

Genetic instability of CRC

The loss of genomic stability can drive the development of colorectal cancer by facilitating the acquisition of multiple tumor-associated mutations. In this disease, genomic instability takes several forms, each with a different cause table 2[32]. The most common is termed the chromosomal instability pathway and accounts for 70% to 85% of colorectal cancers. These tumours are characterized by mutations in APC, P53, and KRAS and by frequent allelic loss at 18q[33] The microsatellite instability (MSI) pathway, comprising the remaining 15% of colorectal cancers, is characterized by loss of proficiency of the DNA mismatch repair (MMR) system and MSI.

<table>
<thead>
<tr>
<th>Type of Instability and Syndrome</th>
<th>Type of Defect</th>
<th>Genes Involved</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomal instability - loss of heterozygosity at multiple loci</td>
<td>Somatic</td>
<td>Loss of heterozygosity at APC, TP53, SMAD4</td>
<td>Characteristic of 70 to 85% of sporadic CRCs, depending on Stage.</td>
</tr>
<tr>
<td>DNA mismatch-repair defects</td>
<td>Germ-line</td>
<td>MLH1, MSH2, MSH6 germ-line gene mutations</td>
<td>Multiple primary CRCs, accelerated tumor progression, and increased risk of endometrial, gastric, and urothelial tumors.</td>
</tr>
<tr>
<td>Hereditary nonpolyposis colon cancer</td>
<td>germ-line</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sporadic colorectal cancer with mismatch-repair deficiency</td>
<td>Somatic</td>
<td>MLH1somatic methylation</td>
<td>CRC with increased risk of poor differentiation, more commonly located in right colon, less aggressive clinical behavior than tumors without mismatch-repair deficiency.</td>
</tr>
<tr>
<td>MSI-CpG island methylator phenotype — methylation target loci</td>
<td>Somatic</td>
<td>Target, loci MLH1, MINT1, MINT2, MINT3</td>
<td>Characteristic of 15% of CRC, with most showing mismatchrepair deficiency from loss of tumor MLH1 expression</td>
</tr>
<tr>
<td>Base excision repair defect—MYH-associated polyposis</td>
<td>Germ-line</td>
<td>MYH (denotesmutY homologue.)</td>
<td>Development of 15 or more colorectal adenomas with increased risk of colorectal cancer.</td>
</tr>
</tbody>
</table>
SUMMARY

Colorectal cancer is probably caused by a complex interaction between many genetic and environmental factors over time. More and large studies with informations on life style factors are required to assess these very possible gene-environment interactions. Studies that aid in the understanding of colorectal cancer on a molecular level have provided important tools for genetic testing for high-risk familial forms of the disease, predictive markers for selecting patients for certain classes of drug therapies, and molecular diagnostics for the noninvasive detection of early cancers.

REFERENCES


