Neoplasia in the GI tract remains one of the most frequent diseases gastroenterologists encounter. Advances in our understanding of the cellular and molecular basis of GI neoplasia have provided a foundation for the development of novel preventive, diagnostic, and therapeutic approaches. Although some features of carcinogenesis are tissue-site specific, many mechanisms are universal to all sites throughout the GI tract. This chapter reviews mechanisms of normal cell growth and the fundamental cellular and molecular alterations that facilitate malignant transformation. The basic concepts discussed in this chapter provide the framework for discussion of specific GI neoplasms in later chapters.

### MECHANISMS OF NORMAL CELL HOMEOSTASIS

#### Cellular Proliferation

Neoplasia results from the disruption of an intricate network of homeostatic mechanisms regulating cell cycle progression, differentiation, senescence, and programmed cell death. Proliferation occurs as cells traverse the cell cycle (Fig. 1-1). In preparation for cell division, there is a period of biosynthetic activity called the G1 phase that is typically associated with an increase of cell size. This phase is followed by precise duplication of the genome, designated the S phase. After an intervening gap period designated the G2 phase, mitosis occurs in the M phase.

The commitment to proceed to DNA replication occurs during the G1 phase at the G1/S checkpoint or restriction (R) point. Cells may exit this cycle of active proliferation before reaching the S point and enter a quiescent phase, G0. Cells can subsequently re-enter the cell cycle from the G0 state (see Fig. 1-1). Another checkpoint exists at the boundary between the G2 and M phases. The G2/M checkpoint ensures that mitosis does not proceed prior to the repair of any damaged DNA after genome replication. Impaired function of these checkpoints is frequently observed in cancers.

Regulation of cell cycle progression appears to be achieved principally by cyclins and cyclin-dependent kinase activity at the G1/S and G2/M checkpoints. Cyclins A and B are predominantly expressed during the S and G2 phases, respectively (see Fig. 1-1). In contrast, cyclins D and E are most active during the G1 phase. Overexpression of cyclin D1 in fibroblasts results in more rapid entry of cells into the S phase. Cyclin D1 is frequently overexpressed in a number of GI and non-GI malignancies.

Each cyclin forms a complex with a cyclin-dependent kinase (CDK) in a cell cycle–dependent fashion. Cyclins function as catalysts for CDK activity (see Fig. 1-1). The cyclin-CDK complexes regulate cell cycle progression through phosphorylation of key target proteins, including the retinoblastoma gene product (pRb) as well as the Rb family members p107 and p107. The final result is progression out of G1 into the S phase of the cell cycle. The cell cycle is also regulated by multiple CDK inhibitors; p21CIP1/WAF1 and p27KIP1 are inhibitors of cyclin E/CDK2. Originally discovered to be part of the complex containing cyclin D1 and CDK4/6, p16INK4A is transcriptionally activated by several tumor suppressor genes, most notably TP53.

Another CDK inhibitor, p15INK4B, specifically inhibits CDK4 and CDK6 and is part of a larger family of related inhibitors that includes p14, p15, and p18; p16INK4A is frequently

<table>
<thead>
<tr>
<th>Mechanisms of Normal Cell Homeostasis</th>
<th>Tumor Metabolism</th>
<th>Environmental and Microenvironmental Influences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular Proliferation</td>
<td>Chemical Carcinogenesis</td>
<td>Dietary Factors</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>Microenvironment</td>
<td>Inflammation and Cancer</td>
</tr>
<tr>
<td>Senescence</td>
<td>Tumor Metabolism</td>
<td>Tumor Metabolism</td>
</tr>
<tr>
<td>Signaling Pathways That Regulate Cellular Growth</td>
<td>Environmental and Microenvironmental Influences</td>
<td>Biological Features of Tumor Metastasis</td>
</tr>
<tr>
<td>Intestinal Tumor Development</td>
<td>Genetic Influences</td>
<td>Epigenetics</td>
</tr>
<tr>
<td>Multistep Formation</td>
<td>Noncoding RNAs</td>
<td>Noncoding RNAs</td>
</tr>
<tr>
<td>Clonal Expansion</td>
<td>Oncogenic Signaling Pathways</td>
<td>Oncogenic Signaling Pathways</td>
</tr>
<tr>
<td>Cancer Stem Cells</td>
<td>Epigenetics</td>
<td>Epigenetics</td>
</tr>
<tr>
<td>Neoplasia-Associated Genes</td>
<td>Cell Cycle</td>
<td>Oncogenic Signaling Pathways</td>
</tr>
<tr>
<td>Oncogenes</td>
<td>DNA Repair Genes</td>
<td>DNA Repair Genes</td>
</tr>
<tr>
<td>Tumor Suppressor Genes</td>
<td>Oncogenic Signaling Pathways</td>
<td>Oncogenic Signaling Pathways</td>
</tr>
<tr>
<td>DNA Repair Genes</td>
<td>Noncoding RNAs</td>
<td>Noncoding RNAs</td>
</tr>
<tr>
<td>Oncogenic Signaling Pathways</td>
<td>Oncogenic Signaling Pathways</td>
<td>Oncogenic Signaling Pathways</td>
</tr>
<tr>
<td>Noncoding RNAs</td>
<td>Oncogenic Signaling Pathways</td>
<td>Oncogenic Signaling Pathways</td>
</tr>
<tr>
<td>Epigenetics</td>
<td>Oncogenic Signaling Pathways</td>
<td>Oncogenic Signaling Pathways</td>
</tr>
</tbody>
</table>
in neoplasia, these tumor-suppressive mechanisms are bypassed or lost. When grown in vitro, most primary cells have a limited replicative potential and eventually undergo replicative senescence.11 During carcinogenesis, these tumor-suppressive mechanisms are bypassed or lost. When grown in vitro, most primary cells have a limited replicative potential and eventually undergo replicative senescence.11 During carcinogenesis, these tumor-suppressive mechanisms are bypassed or lost. When grown in vitro, most primary cells have a limited replicative potential and eventually undergo replicative senescence.11 During carcinogenesis, these tumor-suppressive mechanisms are bypassed or lost. When grown in vitro, most primary cells have a limited replicative potential and eventually undergo replicative senescence.11 During carcinogenesis, these tumor-suppressive mechanisms are bypassed or lost. When grown in vitro, most primary cells have a limited replicative potential and eventually undergo replicative senescence.11 During carcinogenesis, these tumor-suppressive mechanisms are bypassed or lost. When grown in vitro, most primary cells have a limited replicative potential and eventually undergo replicative senescence.11 During carcinogenesis, these tumor-suppressive mechanisms are bypassed or lost. When grown in vitro, most primary cells have a limited replicative potential and eventually undergo replicative senescence.11

**FIGURE 1-1. Regulation of the cell cycle by cyclins (cycs), cyclin-dependent kinases (cdks), and cdk inhibitors. In the normal cell cycle, DNA synthesis (in which chromosomal DNA is duplicated) occurs in the S phase, whereas mitosis (in which nuclei first divide to form a pair of new nuclei, followed by actual cellular division to form a pair of daughter cells) takes place in the M phase. The S and M phases are separated by 2 gap phases, the G1 phase after mitosis and before DNA synthesis, and the G2 phase following the S phase. During these gap phases, the cell is synthesizing proteins and metabolites, increasing its mass, and preparing for the S phase and M phase. Cell cycle progression is regulated primarily at 2 points, the G1/M and G2/S checkpoints, through the coordinated activities of cyclins and CDKs, which in turn are negatively regulated by CDK inhibitors (INK4 and CIP/KIP families). The mid-G1 phase is characterized by the interaction between cyclin D and cdk4/6. This complex hyperphosphorylates the retinoblastoma protein (pRb) and its family members (e.g., p130). Another important complex at the G1/S boundary is that of cdk2 and cyclin E (cyc E). The result is to release transcription factors such as E2F that are complexed with pRb. In turn, E2F binds to and activates the promoters of genes important in DNA synthesis.

**Apoptosis**

Apoptosis (programmed cell death) is an important mechanism that counterbalances cell proliferation, and escape from normal apoptotic mechanisms plays a critical role in oncogenesis. Apoptosis is characterized by distinctive features that include chromatin compaction, condensation of cytoplasm, and mild convolution of the nucleus and cytoplasm. These changes are followed by nuclear fragmentation and marked convolution of the cell surface. Eventually, membrane-bound apoptotic bodies that represent the cellular residue are produced and phagocytosed.

Apoptosis may be triggered by internal or external stimuli. Apoptosis routinely occurs during normal development to facilitate tissue patterning. Internal stimuli of apoptosis may include nutrient deprivation, hypoxia, DNA damage, or other stressors. Ultimately, these internal apoptotic signals converge to increase permeability of the mitochondrial membrane and collapse the electrical gradient required for aerobic respiration (Fig. 1-2). Small mitochondria-derived activators of caspases (SMACs) and cytochrome c are released into the cytoplasm. SMACs and the so-called apoptosome complex (cytochrome c, caspase 9, and Apaf1) then activate downstream caspases, such as caspase 3, precipitating cell death. Caspases are intracellular cysteine proteases and are key mediators of programmed cell death in mammalian cells.

The Bcl-2 family of proteins has been shown to modulate the activity of mitochondrial permeability pores. Bax and Bak help form the pore, while Bcl-2, Bcl-xL, and Mcl-1 inhibit pore formation. The stoichiometric ratio between pro-apoptotic and anti-apoptotic members of the Bcl-2 family can determine the balance between cell survival and cell death.15 In neoplasia, this balance is skewed toward anti-apoptotic factors.

Apoptosis may also be stimulated by external signals. Activation of the TNF receptors, TNFR1 and TNFR2, by TNF cytokines results in activation of caspases. Activation of Fas receptor by the Fas ligand also results in the death-induced signaling complex that activates caspases. In addition to these well-characterized pathways, toxins, chemical signals, and pathogens may trigger apoptosis (see Fig. 1-2).

**Senescence**

Senescence is the process by which cells permanently lose their ability to divide. Senescence may occur in response to the stress induced by activation of oncogenes, DNA damage, or after a fixed number of cellular divisions (replicative senescence). These processes limit dysregulated or excessive proliferation. However, these mechanisms also contribute to aging and depletion of stem cells.15 During carcinogenesis, these tumor-suppressive mechanisms are bypassed or lost. When grown in vitro, most primary cells have a limited replicative potential and eventually undergo replicative senescence.11 Telomeres are repetitive DNA sequences at the ends of all chromosomes that regulate chromosomal stability. Telomeres shorten with each cell division, and when they have been reduced to a certain critical length, senescence typically occurs through activation of DNA damage signaling. Cancer cells are able to maintain their telomere length despite multiple cell divisions through reactivation of telomerase enzyme activity, which adds additional telomeres to the end of chromosomes.16 Aberrant DNA damage signaling in cancers may result in chromosomal fusions and aneuploidy when telomeres are exhausted.

**Signaling Pathways That Regulate Cellular Growth**

Cellular proliferation is achieved through transition of cells from G0 arrest into the active cell cycle (see Fig. 1-1). Although progression through the cell cycle is controlled by the regulatory mechanisms just described, overall proliferation is also modulated by external stimuli. Growth factors that bind to specific transmembrane receptors on the cell surface may be especially important. The cytoplasmic tails of these transmembrane receptor proteins activate intracellular signaling pathways.
attenuation of their own activity to effect an intramolecular feedback regulatory mechanism. The receptors for many peptide growth factors, including EGF, belong to this receptor class.

Other receptors on the cell surface possess kinase activity directed toward serine or threonine residues rather than tyrosine. These receptors also phosphorylate a variety of cellular proteins, leading to a cascade of biological responses. Multiple sites of serine and threonine phosphorylation are present on many growth factor receptors, including the tyrosine kinase receptors, suggesting the existence of significant interactions among various receptors present on a single cell.

The receptors for many peptide growth factors contain intrinsic tyrosine kinase activity within their intracellular tail. After ligand binding, tyrosine kinase activity is stimulated, leading to phosphorylation of tyrosine residues in target proteins within the cell. Most receptors also autophosphorylate tyrosine residues present in the receptors themselves to magnify signaling and, in some cases, this also causes attenuation of their own activity to effect an intramolecular feedback regulatory mechanism. The receptors for many peptide growth factors, including EGF, belong to this receptor class.

Other receptors on the cell surface possess kinase activity directed toward serine or threonine residues rather than tyrosine. These receptors also phosphorylate a variety of cellular proteins, leading to a cascade of biological responses. Multiple sites of serine and threonine phosphorylation are present on many growth factor receptors, including the tyrosine kinase receptors, suggesting the existence of significant interactions among various receptors present on a single cell.

The transforming growth factor (TGF)-β receptor complex is one important example of a serine-threonine kinase-containing transmembrane receptor.

Many receptors are members of the so-called 7-membrane-spanning receptor family. These receptors are coupled to guanine nucleotide binding proteins and designated G proteins. G proteins undergo a conformational change that is
dependent on the presence of guanosine phosphates.\textsuperscript{15} Activation of G proteins can trigger a variety of intracellular signals, including stimulation of phospholipase C and the generation of phosphoinositides (most importantly, inositol 1,4,5-triphosphate) and diacylglycerol through hydrolysis of membrane phospholipids, as well as modulation of the second messengers cyclic adenosine monophosphate (cAMP) and guanosine monophosphate (GMP).\textsuperscript{16} Somatostatin receptors exemplify a G protein-coupled receptor prevalent in the GI tract.

Binding of growth factors and cytokines to cell surface receptors typically produces alterations in a variety of cellular functions that influence growth. These functions include ion transport, nutrient uptake, and protein synthesis. However, the ligand-receptor interaction must ultimately modify one or more of the homeostatic mechanisms discussed to affect cellular proliferation.

The Wnt pathway is one important example of a signaling pathway that regulates a diverse number of homeostatic mechanisms to control proliferation of intestinal epithelial cells (Fig. 1-3). Evolutionarily conserved among several species, Wnt signaling, as a rule, ultimately results in accumulation of $\beta$-catenin in the nucleus, where it binds with the transcription factor Tcf-4 to activate a set of target genes.\textsuperscript{17} In normal cells, this signal is initiated by secreted Wnt ligands that bind to cell surface receptors of the Frizzled family. Inhibition of the Wnt signal in mice can be achieved by deletion of Tcf-4 or overexpression of the Wnt inhibitor Dickkopf1, which results in dramatic hypoproliferation of the intestinal epithelium.\textsuperscript{18,19} Tissue homeostasis is also maintained by growth-inhibiting signals that counterbalance proliferative signals. TGF-$\beta$ is a potent growth-inhibiting factor that mediates arrest of the cell cycle at the G\textsubscript{1} phase. TGF-$\beta$ not only induces transcription of the cell cycle inhibitors p15\textsuperscript{INK4B} and p21\textsuperscript{CIP1/WAF1}, it also enhances the inhibitory activity of p27\textsuperscript{KIP1} on the cyclin E/CDK2 complex (see Fig. 1-1).\textsuperscript{20} These effects of TGF-$\beta$ are mediated intracellularly through the Smad family of proteins.

### INTESTINAL TUMOR DEVELOPMENT

#### Multistep Formation

Multiple sequential genetic alterations are required for the transformation of normal intestinal epithelium to neoplasia. This multistep nature of tumorigenesis is most directly illustrated by the changes that accrue in the development of colonic neoplasia (see Chapter 127). The accumulation of genetic and epigenetic alterations parallels the progression from normal

---

**FIGURE 1-3.** The Wnt signaling pathway is an important regulator of intestinal epithelial cell proliferation and tumorigenesis. In the absence of a Wnt signal (left top), cytosolic $\beta$-catenin forms a cytoplasmic complex with APC, Axin, and glycogen synthase kinase-3$\beta$ (GSK-3$\beta$). This $\beta$-catenin destruction complex phosphorylates $\beta$-catenin and targets it for degradation via the ubiquitin-mediated proteasomal pathway. In the presence of an active Wnt signal (right top), $\beta$-catenin is stabilized, and excess cytoplasmic $\beta$-catenin is translocated to the nucleus, where it interacts with the Tcf-4 transcription factor to regulate the expression of many key target genes. APC, adenomatous polyposis coli; P, phosphate group; VEGF, vascular endothelial growth factor.
Chapter 1  Cellular Growth and Neoplasia

epithelium through adenomatous polyps to malignant neoplasia. Studies on the molecular pathogenesis of colon cancer have served as a paradigm for the elucidation of genetic alterations in other GI cancers, including gastric and pancreatic cancer.

A genetically unstable environment is necessary for the development of the multiple alterations that ultimately result in cancer. Genomic instability is observed in almost all cancers, regardless of organ site. Instability of the genome may result from several mechanisms. In colon cancer, there are now 3 well-recognized forms of genetic/epigenetic instability that promote carcinogenesis, and they have been termed chromosomal instability, microsatellite instability, and CpG island methylator phenotype (CIMP). Chromosomal instability results in tumor cells that display frequent aneuploidy, large chromosomal deletions, and chromosomal duplications. In contrast, tumors that display microsatellite instability are often diploid or near-diploid on a chromosomal level but harbor frequent alterations in smaller tracts of microsatellite DNA (see later discussion on DNA repair). CIMP-high tumors have excessive gene promoter CpG-island methylation, which results in gene silencing. Thus, there are at least 3 distinct routes to the formation of a colorectal cancer, depending on the nature of the underlying genetic or epigenetic instability (Fig. 1-4). It is important to note that involvement by these pathways is not mutually exclusive.

FIGURE 1-4. Multistep models of colorectal cancer based on underlying genetic instability. As shown on the left, there are 3 major pathways: chromosomal instability (top pathway), microsatellite instability (middle pathway), and serrated (lower pathway). The progression from normal colonic epithelium to carcinoma is associated with the acquisition of several genetic and epigenetic alterations. In the chromosomal instability pathway (top pathway), these alterations include the concomitant activation of oncogenes (e.g., K-ras) through a point mutation and inactivation of tumor suppressor genes (e.g., APC, TP53) through a point mutation or deletion. An increasing aggregate number of mutations can be correlated with progression from early benign adenoma to cancer, as reflected by analysis of polyps by size. In the microsatellite instability model (middle pathway), mutations in DNA mismatch repair genes create a mutator phenotype in which mutations accumulate in specific target genes (see section on DNA mismatch repair). Tumors develop much more rapidly through this pathway than through the chromosomal instability pathway (horizontal arrows). In the serrated pathway (lower pathway), the initiating event is hypothesized to be a BRAF or KRAS activating mutation that results in a serrated adenoma. Serrated adenomas may undergo extensive promoter hypermethylation (CpG island methylator phenotype [CIMP]) to become sporadic microsatellite unstable cancers (MSI-H) through silencing of genes encoding for MLH1 and p16. Alternatively, serrated adenomas can undergo a pathway similar to that of chromosomal instability to become microsatellite stable tumors.
Clonal Expansion

Clonal expansion is essential to tumor development.23 Whereas germline mutations may lead to altered expression of a gene in all cells in a tissue, subsequent additional somatic mutations generally occur only in a small subpopulation of cells. Clonal expansion of these mutated cells occurs if a specific gene mutation results in a survival advantage for the cells. A second round of clonal expansion occurs when a cell within this population sustains still another genetic alteration that further enhances its growth properties. This iterative process of selection, with accumulating genetic alterations, results in cellular transformation and malignancy. Once frank malignancy has developed, the catalog of mutations harbored may vary between cancer cells. Referred to as tumor heterogeneity, this ongoing process may give certain cells selection advantages.24 Metastasis may be facilitated by the evolution of a subset of tumor cells that acquire the capability of traversing the circulatory system and thriving in a new environment.

Cancer Stem Cells

These observations of tumor heterogeneity have led to the cancer stem cell hypothesis, which asserts that there exists a subset of tumor cells that have stem cell-like properties. Cancer stem cells (CSCs) are believed to be the tumor-initiating cells from which clonal expansion occurs. Moreover, it is hypothesized that eradication of these cells is a key therapeutic goal because failure to do so may result in relapse of disease. Within this CSC hypothesis, there are 2 models.25 The first is a hierarchical model in which CSCs may serve as progenitors of cancer cells with limited reproductive potential. The second stochastic model posits that each cancer cell has the same potential to be a CSC, but this determination is stochastically based on internal factors in addition to external environmental cues. Analysis of putative CSCs demonstrate transcriptional programs and markers shared with normal intestinal stem cells. Markers such as Lgr5 and EphB2 have been used to identify and purify colon CSCs.26

NEOPLASIA-ASSOCIATED GENES

The genes that collectively play an important role in oncogenesis generally lead to disruption of the orderly mechanisms of normal cell proliferation. Since normal cell proliferation appears to depend on a wide variety of genes, it is not surprising that alterations in the expression of a diverse set of genes confer part or all of the phenotypic features of transformation. Despite this diversity, all these genes that become altered appear to belong to 1 of 2 distinct groups: (1) oncogenes, which actively confer a growth-promoting property, or (2) tumor suppressor genes, the products of which normally restrain growth or proliferation. An important category within tumor suppressor genes includes DNA repair genes, which prevent accumulation of new mutations. Activation of oncogenes or inactivation of tumor suppressor genes contributes to malignant transformation. Transcriptionally active sites of the genome that do not encode for proteins also play a significant role in regulation of gene expression and carcinogenesis. These noncoding RNAs may harbor oncogenic and tumor suppressive functions as well.

Oncogenes

Typically, oncogenes are genes that encode a normal cellular protein expressed at inappropriately high levels or mutated genes that produce a structurally altered protein that exhibits inappropriately high activity. For example, several genes that encode tyrosine kinase–containing growth factor receptors become oncogenes after a mutation results in unregulated tyrosine kinase activity that is no longer dependent on the presence of the appropriate ligand. The normal cellular genes from which the oncogenes derive are designated proto-oncogenes. Most of these genes are widely expressed in many different types of tumor cells.

Several mechanisms can lead to oncogene activation. These include gene transduction or insertion, point mutation, gene rearrangement, and gene amplification. Gene transduction and insertion generally result from retroviral infection. Point mutations result in constitutively active oncogene products. Gene rearrangements can result in oncogenic fusion proteins, and gene amplifications lead to uncontrolled overexpression of a normal gene product.

The proteins encoded by oncogenes comprise at least 4 distinct groups—peptide growth factors that may be secreted into the extracellular milieu, protein kinases, signal-transducing proteins associated with the inner cell membrane surface (membrane-associated G proteins), and transcriptional regulatory proteins located in the nucleus.

Peptide Growth Factor Oncogenes

The transforming effects of enhanced expression of a variety of growth factors have been demonstrated both in vitro and in vivo. Several growth factor–related proteins encoded by oncogenes have now been recognized, including the family of Wnt proteins and Sis, which encodes the β chain of platelet-derived growth factor. Cancer cells may engage in autocrine signaling to promote their growth, or coax the adjacent stroma to hypersecrete such growth-stimulating factors.

Protein Kinase–Related Oncogenes

The largest family of oncogenes encodes proteins with protein kinase activity. These oncogenes encompass the full variety of protein kinases, including receptor/nonreceptor tyrosine kinases and cytoplasmic serine/threonine kinases. Many members of this large oncogene group are expressed by neoplasms of the GI tract, and these include the receptor tyrosine kinases of the EGF receptor family (ERBB1-4) and the Src nonreceptor tyrosine kinase that associates with the inner surface of the plasma membrane.

Signal Transduction–Related Oncogenes (Membrane-Associated G Proteins)

Intermediate steps that effectively translate ligand-receptor binding to an intracellular signal are essential in mediating functional responses of the cell. Mutations in genes that encode key proteins that participate in signal transduction can also lead to cellular transformation. G proteins regulate signaling of the large family of G protein–coupled receptors (GPCRs) through the exchange of guanosine triphosphate (GTP) with guanosine diphosphate (GDP). Altered ras genes, a family of proteins related to G proteins, are among the most commonly detected oncogenes in GI tract cancers. The ras family contains 3 genes: H-ras, K-ras, and N-ras. Point mutations that result in amino acid substitutions at critical hot spot positions convert the normal gene into an oncogene.

To date, almost all ras mutations in GI malignancies occur in the K-ras oncogene. The highest mutation frequency is found in tumors of the exocrine pancreas (>90%).27 Ras genes activated through point mutation have been identified in
approximately 50% of colonic cancers as well as a subset of serrated tumors (see Fig. 1-4).26

Most oncogenic mutations in ras cause biochemical changes that maintain it in the active, GTP-bound state by reducing guanosine triphosphatase (GTPase) activity or by destabilizing the inactive GDP-bound form. However, several ras mutants retain significant GTPase activity; therefore, other mechanisms that convert ras to a transforming protein may be involved.27

A functional consequence of ras activation is phosphorylation of key serine/threonine kinases. One important downstream signaling target of ras is B-raf. In colon cancers without an identifiable K-ras mutation, 20% possess an activating B-raf mutation,28 consistent with the concept that activation of an oncogenic pathway can be achieved through an alteration in any of several sequential components of a particular pathway.

**Nuclear Oncogenes**

Many cellular oncogenes encode proteins that localize to the nucleus. In essence, these nuclear oncogene products are the final mediators of signal transduction pathways that are also affected by cytoplasmic and plasma membrane-bound oncoproteins, because they act as transcription factors that regulate expression of certain genes that enhance cellular proliferation and suppress normal differentiation.

The role of nuclear oncogenes is illustrated by the myc family. The c-Myc protein product is involved in critical cellular functions like proliferation, differentiation, apoptosis, transformation, and transcriptional activation of key genes.30 Frequently, c-Myc is overexpressed or amplified in many GI cancers. c-Myc has been found to be a transcriptional target of the β-catenin/TCF-4 complex in colorectal cancers (see Fig. 1-3), which may explain the overexpression of c-Myc observed in this cancer type.31

**Tumor Suppressor Genes**

The products of tumor suppressor genes prevent acquisition of the transformed phenotype in vitro and have similar functional properties in vivo. Mutations that disrupt the biological function of these genes are associated with all GI cancers. Germline mutations of this class of gene underlie most of the known inherited cancer syndromes in which a specific gene has been implicated. A number of these genes and their products have been identified and characterized (Table 1-1).

Initial recognition of the existence of tumor suppressor genes was derived from linkage analyses of cancer-prone families. In the GI tract, hereditary colon cancer, gastric cancer, and pancreatic cancer syndromes are the best described and are discussed elsewhere in this text. A number of features are common to GI cancer syndromes with Mendelian patterns of inheritance. Most importantly, the marked increase in risk for tumors often develops within the target tissue, and tumors in these affected members typically arise at a younger age than they do in the general population. Finally, affected individuals are sometimes at risk for tumors outside the GI tract.

These observations led Knudson to hypothesize that tumors in familial cancer syndromes might derive from independent mutations in the 2 alleles of a specific tumor suppressor gene (Fig. 1-5). Specifically, he proposed that the first mutation was present in 1 copy of the gene inherited in the germline and therefore present in all cells in affected family members.32 A somatic mutation of the remaining normal allele of the tumor suppressor gene that might occur in any cell would then lead to tumor development. The same gene might play a role in the development of the same tumor type in the general population (sporadic cancer), but 2 independent somatic mutations of each of the 2 alleles would be required. However, this combination of events should be uncommon and would explain the lower frequency and later age of diagnosis of similar tumors in the general population. Comings was the first to suggest that the relevant gene in a familial cancer syndrome might encode a tumor-suppressing gene product.33 Although this 2-hit model has been generally

---

**TABLE 1-1 Mutations Associated with Hereditary Gastrointestinal Cancer Syndromes**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Gene(s) Mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAP, AFAP</td>
<td>APC</td>
</tr>
<tr>
<td>Lynch syndrome (HNPCC)</td>
<td>MSH2, MLH1, MSH6, MSH2, PMS2, EpCAM</td>
</tr>
<tr>
<td>MUTYH polyposis</td>
<td>MUTYH</td>
</tr>
<tr>
<td>Peutz-Jeghers syndrome</td>
<td>LKB1/STK11</td>
</tr>
<tr>
<td>Cowden’s disease</td>
<td>PTEN</td>
</tr>
<tr>
<td>Juvenile polyposis</td>
<td>SMAD4, BMPR1A</td>
</tr>
<tr>
<td>Hereditary diffuse gastric cancer</td>
<td>CDH1</td>
</tr>
<tr>
<td>Hereditary pancreatic cancer</td>
<td>ATM, BRCA1, BRCA2, PALB2, PALLD, CDKN2A, PRSS1, SPINK1, PRSS2, CTRC, CTRF</td>
</tr>
<tr>
<td>MEN1</td>
<td>Menin</td>
</tr>
</tbody>
</table>

AFAP, attenuated FAP; APC, adenomatous polyposis coli; FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer; MEN1, multiple endocrine neoplasia, type 1; MUTYH, mutY homolog.

---

**FIGURE 1-5.** Knudson’s 2-hit hypothesis. In an inherited cancer syndrome, 1 chromosome has an inactive tumor suppressor gene locus because of a germline mutation. The counterpart tumor suppressor gene on the remaining paired chromosome is subsequently inactivated by a somatic mutation, leading to tumor formation. In contrast, in a sporadic cancer, the 2 alleles of the tumor suppressor gene become inactivated through 2 independent somatic mutations, an unlikely event within a single cell.
observed for mendelian cancer syndromes, there are exceptions. Some tumor suppressors may function to increase cancer risk when only 1 allele is mutated. These genes may be so critical that the reduction in gene expression by 1 mutant allele is sufficient to drive tumorigenesis. Also, 1 mutant allele may function in a dominant-negative fashion, blocking the effect of the intact protein encoded by the normal allele.

**Tumor Suppressor Gene Inactivation**

Some tumor suppressor genes were first cloned through detection of regions of gene deletion in tumor samples from cancer-prone kindreds by DNA screening for markers scattered throughout the genome. These deletions targeted the second wild-type allele and served to pinpoint the chromosomal location of the disease-causing gene present on the other allele. More recently, our knowledge of the genetic variation observed in tumors has greatly increased by next-generation sequencing technologies. By analyzing the genetic changes in tumors in comparison to normal mucosa, we are now aware of the types of genetic changes that occur in cancer cells. **Single nucleotide variants (SNVs)** refer to changes in a single base pair of the genetic code. While many of these mutations are silent, others can result in significant changes in gene expression or function. Missense mutations result in a change in the amino acid encoded by the codon. **Nonsense mutations** refer to the introduction of a premature stop codon. SNVs at splice-acceptor or donor sites may result in exon loss or misexpression of intrinsic sequences. SNVs in the promoter or untranslated regulatory regions of a gene may dramatically change gene expression. Another type of genetic variation includes insertions or deletions. Small insertion or deletion mutations may result in frameshift mutations within a gene. Larger-scale insertion and deletions are also seen. Each type of variant may result in inactivation of a given gene, and they represent important mechanisms of inactivation of 1 copy of tumor suppressor genes. Another mechanism of tumor suppressor gene inactivation includes promoter hypermethylation. Transcriptional silencing can result from methylation of CpG islands in gene promoters; this has been demonstrated to occur in the genes encoding p16\(^{INK4A}\) and E-cadherin. Excess CpG island methylation has been implicated as a cardinal feature in the seriated pathway to colon cancer (see Fig. 1-4).

Tumor suppressor genes do not function identically in every tissue type. Consequently, inactivation of a particular tumor suppressor gene is tumorigenic only in certain tissues. For example, the tumor suppressor genes RFI and VHL play crucial roles in retinoblastomas and renal cell cancer, respectively, but are rarely mutated in GI malignancies. Three tumor suppressor genes shown to have a critical role in the pathogenesis of GI malignancies, APC, TP53, and SMAD4, are described below.

**Adenomatous Polyposis Coli Gene**

Genetic linkage analysis revealed markers on chromosome 5q21 that were tightly linked to polypos development in affected members of kindreds with familial adenomatous polyposis (FAP) and Gardner’s syndrome. Further work led to identification of the gene responsible for FAP, the adenomatous polyposis coli (APC) gene. The full spectrum of adenomatous polyposis syndromes attributable to APC is discussed in detail in Chapter 126. Somatic mutations in APC have also been found in most sporadic colon polyps and cancers. Mutations in APC are characteristically identified in the earliest adenomas, indicating that APC plays a critical role as the gatekeeper in the multistep progression from normal epithelial cell to colon cancer (see Fig. 1-4).

The APC gene comprises 15 exons and encodes a predicted protein of 2843 amino acids, or approximately 310 kd. Most germline and somatic APC gene mutations result in a premature stop codon and therefore a truncated APC protein product. Mutations occurring in the APC amino terminal are associated with a rare variant of FAP, attenuated familial adenomatous polyposis (AFAP). APC mutations result in functional changes in key protein-protein interactions. As discussed earlier, APC is a negative regulator of the Wnt signaling pathway (see Fig. 1-3). Mutant APC proteins are unable to interact with β-catenin, resulting in uncontrolled activation of the Wnt signaling pathway and the subsequent oncogenic phenotype.

**TP53 Gene**

Named for a 53-kd-sized gene product, p53 is a nuclear phosphoprotein that plays a key role in cell cycle regulation and apoptosis. The p53 protein was first detected in tumors as the product of a mutated gene that was mapped to chromosome 17p, a region found to exhibit loss of heterozygosity in many tumors. Point mutations in TP53 have been identified in as many as 50% to 70% of sporadic colon cancers (see Fig. 1-4) but only a small subset of colonic adenomas. Point mutations in TP53 have also been found in all cancers of the GI tract. Interestingly, aflatoxin appears to induce a mutation in a single hot spot codon (codon 249) of TP53 in many hepatocellular carcinomas. In addition to the TP53 point mutations in sporadic cancers, germline TP53 mutations have been observed in the Li-Fraumeni syndrome, an autosomal dominant familial disorder in which breast carcinoma, soft tissue sarcoma, osteosarcoma, leukemia, brain tumor, and adrenocortical carcinoma can develop in affected persons.

The sequence-specific transcription factor p53 is induced in conditions of cellular stress, such as ionizing radiation, growth factor withdrawal, or cytotoxic therapy (see Fig. 1-2). As a consequence of genotoxic damage, p53 arrests cells at the G\(_1\) phase to facilitate DNA repair, senescence, or trigger apoptosis. Factor p53 mediates some of these responses through induction of the p21\(^{kip1/waf1}\) inhibitor of the cell cycle or pro-apoptotic genes, including PUMA, and c-Myc appears to play a role in this cell fate decision.

**SMAD4 Gene**

SMAD4 is a tumor suppressor gene located on chromosome 18q and is deleted or mutated in most pancreatic adenocarcinomas and a subset of colon cancers. This gene encodes Smad4, an essential intracellular mediator of the growth inhibitory effects of TGF-β. The Smad4 protein has 2 important domains, the mad homology domains 1 and 2 (MH1 and MH2), which are essential for DNA binding and for oligomerization with other Smad proteins, respectively. Mutant Smad4 blocks TGF-β-induced inhibition of proliferation. Germline mutations in SMAD4 result in the juvenile polyposis syndrome (see Chapter 126).

**DNA Repair Genes**

Cellular mechanisms have evolved to preserve the fidelity of DNA. Errors can be introduced into the genome through multiple physiologic and pathologic mechanisms. These errors include spontaneous mismatching of nucleotides during normal DNA replication, oxidative damage of nucleotides, and complete double-strand breaks. Numerous discrete systems exist to repair these types of DNA damage that can arise from a variety of insults, including carcinogens, irradiation, and reactive oxygen species. One type of error that
develops during replication may occur in stretches of microsatellite DNA, which involves regions of mononucleotide (e.g., poly-A) or dinucleotide (e.g., poly-CA) repeats.36 The DNA mismatch repair system corrects these errors. The enzymes bind mismatched DNA, cut the DNA strand with the mismatched nucleotide, unwind the DNA fragment, fill in the gap with the correct nucleotide, and finally reseal the remaining nick. The family of DNA mismatch repair genes includes MSH2, MSH3, MSH4, MSH5, MSH6, MLH1, MLH3, PMS1, and PMS2.

MLH1 and MSH2 are the 2 DNA mismatch repair genes that are most frequently mutated at the germline level in Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC).37,38 Mutations can lead to functional alterations that allow strand slippage during replication. Affected cells are called replication error (RER) positive, in contrast to the RER-negative phenotype.32,35 Because microsatellite DNA sequences are primarily affected by this type of genetic instability, the tumor cells are said to display microsatellite instability (MSI). Mechanistically, the absence of DNA repair does not directly cause cancer. Rather, the DNA repair defect creates a milieu that permits accumulation of mutations in a variety of other genes that contain microsatellite DNA sequences, such as the TGF-β type II receptor, IGF II receptor, BAX, and E2F-4. This MSI pathway represents a novel mechanism for the accumulation of mutations within a tumor (see Fig. 1-4). It is characteristic of all Lynch-related tumors and is observed in approximately 15% of all sporadic colon cancers. Increasing evidence has emerged that these sporadic MSI tumors result from the serrated pathway and MLH1 promoter hypermethylation (see Fig. 1-4).

Errors can also be introduced when individual nucleotides are damaged by chemical factors; the base excision repair system corrects these types of errors. 8-Oxoguanine residues can result from oxidative DNA damage, and these altered bases will inappropriately pair with adenosines, ultimately leading to somatic G:C→T:A mutations if uncorrected. MUTYH is a DNA glycosylase that participates in the repair of these oxidized guanine nucleotides. An autosomal recessive adenomatous polyposis syndrome caused by germ line mutations in the MUTYH repair gene has been identified.32,35 Interestingly, G:C→T:A mutations in the APC gene were almost universally found in the polyps of patients with the autosomal recessive polyposis MUTYH mutations, indicating that there are important similarities in the molecular pathogenesis of polypos in the MUTYH and FAP syndromes.

**Oncogenic Signaling Pathways**

Individual oncogenes or tumor suppressor genes do not necessarily induce cellular transformation directly but typically function as components of larger oncogenic signaling pathways. Some of the pathways that are particularly relevant for GI tumorigenesis include the Wnt and Ras signaling pathways. These are pathways that regulate normal tissue homeostasis but become oncogenic when the signals are transduced in an aberrant or amplified manner. The key features of Wnt signaling are illustrated in Figure 1-3. β-catenin is translocated from the inner plasma membrane to the cytoplasm. There, it forms a macromolecular complex with the APC protein Axin and glycogen synthase kinase-3β (GSK-3β). Phosphorylation of β-catenin by GSK-3β triggers its degradation. In the presence of an active Wnt signal, β-catenin is stabilized, and it enters the nucleus where it interacts with the transcription factor Tcf-4 to up-regulate a number of key target genes, including c-Myc, cyclin D1, and VEGF. As discussed earlier, Wnt signaling is essential for regulating proliferation of normal intestinal epithelium, and dysregulated Wnt signaling is an almost universal feature of all colorectal cancers. The latter can result from a mutation in the APC, Axin, or β-catenin genes, although alterations in the APC tumor suppressor gene are the most common. An alteration in just 1 of these components is sufficient to activate the entire pathway. Thus, it is essential to consider individual genetic alterations in the context of the overall signaling pathway in which they function.

Because pathways are typically not linear, additional levels of complexity arise. There is frequent overlap among pathways, and the distinction between pathways can be somewhat arbitrary. For example, mutations in the K-ras oncogene result in activation of multiple distinct signaling pathways, including Raf/ERK/MAPK, PI3K/Akt, and NF-κB, all of which play an important role in tumorigenesis (Fig. 1-6). Crosstalk between these effector pathways serves to modulate the cellular responses further. For example, Akt, a target of PI3K, can phosphorylate Raf and thereby regulate signaling through the MAPK pathway.56 Finally, each of these signaling pathways regulates multiple biological processes related to tumorigenesis, including cell cycle progression, apoptosis, senescence, angiogenesis, and invasion.

Another pathway that plays a particularly important role in GI tumors is the cyclooxygenase-2 (COX-2) pathway. The enzyme COX-2 is a key regulator of prostaglandin synthesis that is induced in inflammation and neoplasia. Although no mutations of COX-2 have been described, overexpression of COX-2 in colonic adenomas and cancers is associated with tumor progression and angiogenesis, primarily through induction of prostaglandin E2 synthesis. Inhibition of COX-2 with a variety of agents (aspirin, nonsteroidal anti-inflammatory drugs, or COX-2 selective inhibitors) is associated with a reduced risk of colorectal adenomas and cancer.58

**Noncoding RNAs**

Although previously referred to as “junk DNA,” a significant portion of the non–protein coding genome remains transcriptionally active. The RNA products, termed non-coding RNAs (ncRNAs), consist of a broad category of active RNA molecules including long noncoding RNAs (IncRNAs) and micro
RNAs (miRNAs) that are frequently dysregulated in cancers. Initially processed into small interfering RNAs (siRNAs) by the protein Dicer into 20- to 25-nucleotide sequences, microRNAs play a critical role in transcript silencing. These siRNAs bind to complementary mRNA sequences, and this binding then facilitates the activity of the RNA-induced silencing complex to target the mRNA for cleavage and degradation. LncRNAs may perform diverse functions like gene silencing, splicing, and extension of telomeres.

Epigenetics

Epigenetics refers to changes in the genome that result in change in expression or phenotype without a change in the sequence of the DNA. Often these changes can result from structural alterations of the genome. One major mechanism is promoter CpG-island hypermethylation. The promoters of many genes are enriched with these CG sites (“CpG islands”). Methylation of the cytosine residues in these islands can result in silencing of the downstream gene.

Many cancers exhibit promoter hypermethylation and silencing of important tumor suppressor genes. In approximately 15% to 20% of colorectal cancers, this process becomes a dominant feature of carcinogenesis. Characterized as CpG island methylator phenotype (CIMP) positive, these tumors have excessive levels of promoter hypermethylation of tumor suppressor genes. Notably, MLH1 is frequently hypermethylated, resulting in sporadic microsatellite unstable cancers. The mechanisms underlying this promoter hypermethylation remain undefined, but recent studies demonstrate a link between tumor metabolism and global methylation status. Mutations in IDH1 can induce a CIMP-high phenotype in glioblastomas.

TUMOR METABOLISM

Metabolic cues and nutrient availability play a critical role in cell growth and homeostasis. As previously described, a lack of available nutrients or mitochondrial dysfunction may signal growth arrest or apoptosis. However, tumor cells exhibit abnormal metabolic profiles to facilitate their growth and anabolic needs. Observations in 1924 from Nobel Laureate Otto Heinrich Warburg revealed that tumor cells displayed dramatic increases in aerobic glycolysis and diminished mitochondrial respiration. This hypothesis, known as the Warburg hypothesis, has been validated and is a hallmark feature of most malignancies. Many of the genes implicated in GI cancers (p53, K-Ras, PI3K, mTOR, HIF, Myc) can in fact regulate metabolic pathways. Moreover, germline mutations in metabolic regulators (e.g., subunits of succinate dehydrogenase [SDH]) that are not classical oncogenes or tumor suppressor genes have been associated with a high risk of tumorigenesis (pheochromocytoma and paraganglioma). The selection advantage of increased glycolysis in cancer cells may include greater tolerance to hypoxic environments and shunting of metabolic byproducts to other biosynthetic pathways. These altered metabolic pathways are promising new targets for therapy.

ENVIRONMENTAL AND MICROENVIRONMENTAL INFLUENCES

Fundamentally, cancer is a genetic disorder. Environmental factors play an important role in tumorigenesis, but they ultimately lead to expression of abnormal genes or inappropriate expression of normal genes, the products of which confer the malignant phenotype. Genetic mutation is the common denominator of agents or mechanisms that contribute to the development of neoplasia.

Chemical Carcinogenesis

Metabolic activation by the host is a key determinant of the carcinogenic potential of many compounds. The initial compound, the procarcinogen, is converted by host enzymes to an electrophilic derivative, which then chemically modifies DNA. Mutations result from errors that occur during DNA replication as a result of distorted base pairs. Factors that influence the potency of any chemical carcinogen include the equilibrium between activation of the procarcinogen and deactivation or degradation of the carcinogen. Deactivation typically occurs through a conjugation reaction, usually in the liver.

These principles are exemplified by experimental colonic carcinomas that arise in rodents fed cycasin, a glucosylated compound present in the cycad nut. The glucose residue of cycasin is cleaved in the rat liver by β-glucosidase to form methylazoxymethanol, which is subsequently deformylated by enzymes in the liver and colon to give rise to methylidazo- nium, a carcinogen. These same metabolites are formed through hepatic enzymatic modification of the compound dimethylhydrazine and result in colon cancer in the rat.

In humans, regular tobacco use is strongly associated with a higher risk of multiple GI cancers, including pancreatic and colon cancer. Among active smokers with long-term tobacco use, the risk for pancreatic cancer can be elevated 2-fold. Multiple carcinogenic agents including arsenic, benzene, and ethylene oxide have been identified in cigarettes, but the chemicals linked specifically to the development of pancreatic or colon cancer have not yet been defined.

Dietary Factors

Chemical mutagenesis may be especially important in the development of cancers within the GI tract and related organs. The mucosal surfaces from which most primary cancers in the GI tract develop are in constant contact with a complex mixture of dietary constituents that are potential carcinogens or procarcinogens. The ability of dietary factors to act as mutagens in humans was demonstrated directly in 1995. The frequency of contamination of foodstuffs with aflatoxins, a fungal metabolite, parallels the incidence of hepatocellular carcinoma in various areas of the world. Studies demonstrating that aflatoxins cause mutations in the TP53 gene in hepatocellular carcinoma have provided a compelling link between genes and the environment.

Nitrates present in many foods appear to be additional dietary constituents that may act as procarcinogens in the GI tract. Diet-derived nitrates can be converted by bacterial action in a hypochlorhydric stomach to nitrates and subsequently to mutagenic nitrosamines. These events may underlie the documented correlation between dietary intake of foods high in nitrates and the incidence of gastric cancer in different populations.

Other dietary factors may modulate the biological potency of dietary procarcinogens. Variations in the relative and absolute amounts of dietary fats may lead to alterations in the composition of the colonic microflora and their metabolic characteristics, resulting in modulation of the production of enzymes that convert dietary constituents into potentially mutagenic compounds. Changes in dietary fiber content
can alter the transit time of luminal contents in the bowel, thereby changing the duration of exposure of the mucosa to potential mutagens. Bile salt content may be an additional luminal factor that can modulate the biological effect of procarcinogens. Deconjugated bile salts may promote carcinogenesis through mucosal injury and enhanced epithelial proliferation.

These mechanisms could explain well-documented correlations between the intake of various dietary constituents and the incidence of colorectal cancer in certain populations. Populations that have a high fiber intake and resulting fast colonic transit times generally exhibit a lower incidence of colorectal cancer than populations with low fiber intake and delayed transit. The incidence of colorectal cancer in Japanese immigrants to the United States who consume a Western diet is much higher than that of native Japanese who consume a traditional Japanese diet.

Microbiome

The human body possesses over 10 trillion microbes. The interaction between these organisms and the host is an area of great interest, particularly for a broad range of autoimmune, metabolic, and neoplastic disorders. The Human Microbiome Project seeks to develop a map for these organisms throughout the body, with the goal of correlating specific bacterial species with disease states. Although the results of this track of investigation are preliminary, evidence is accumulating that the composition of the gut microbiome may affect cancer risk. Altered bacterial populations have the potential to influence metabolic pathways and inflammatory indices in the GI tract.

Viruses also can lead to disruption of normal genes by integration into the host genome in a position that disrupts normal gene sequences (insertional mutagenesis) or through the introduction of aberrant genes present in the virus’s own genetic material. Viruses that appear to play a role in oncogenesis in the GI tract through insertionally mutagenesis include human papillomavirus in squamous cell cancers of the esophagus and anus, Epstein-Barr virus in gastric lymphoepithelial malignancies, and hepatitis B virus in hepatocellular carcinoma.

Inflammation and Cancer

A number of chronic inflammatory conditions increase the site-specific risk of cancer, such as ulcerative colitis (Chapter 116), chronic gastritis (Chapter 52), chronic pancreatitis (Chapter 59), Barrett’s esophagus (Chapter 45), and chronic viral hepatitis (Chapters 79 and 80). In addition to the direct proliferative stimuli, the influences of inflammation on the development of neoplasia are multifaceted and complex. Immune cells may promote remodeling of the vascular network and promote angiogenesis (discussed later). Inflammation may also induce epigenetic changes in cells to favor gene silencing of tumor suppressor genes through DNA damage from cytokine-stimulated production of reactive oxygen species. In addition, cytokines produced by inflammatory cells can lead to activation of nuclear factor (NF)-κB in tumor cells that can serve to inhibit apoptosis and stimulate proliferation.

Although chronic inflammation creates a pro-tumorigenic environment, it should be noted that the immune system also plays an important role in tumor suppression through tumor surveillance. Immunosuppressive therapies are associated with an increased risk of malignancy. Maintenance of this tight balance of immunoregulation is critical to prevent the development of a pro-tumorigenic environment.

BIOLOGICAL FEATURES OF TUMOR METASTASIS

The establishment of distant metastases requires multiple processes, many of which involve alterations in interactions between tumor cells and normal host cells. To metastasize, a cell or group of cells must detach from the primary tumor, gain access to the lymphatic or vascular space, adhere to the endothelial surface at a distant site, penetrate the vessel wall to invade the second tissue site, and finally proliferate as a second tumor focus. Angiogenesis is necessary for proliferation of the primary tumor and tumor metastases. Tumor cells must also overcome host immune cell killing. As a result, few circulating tumor cells (<0.01%) successfully initiate metastatic foci. A “survival of the fittest” view of metastasis has been proposed, in which selective competition favors metastasis of a subpopulation of cells from the primary site. Clonal expansion occurs again after formation of a metastatic focus.

Epithelial-Mesenchymal Transition

Modulation of tumor cell interactions with adjacent cells and with the extracellular matrix is an essential step as epithelial tumor cells invade through the basement membrane and ultimately metastasize to distant sites. A similar process occurs during normal embryogenesis, when polarized epithelial cells no longer recognize the boundaries imposed by adjacent epithelial cells or their basement membrane and adopt features of migratory mesenchymal cells. This phenomenon, designated epithelial-mesenchymal transition (EMT), has provided insight into understanding tumor progression (Fig. 1-7). E-cadherin is a critical component of adherens junctions that maintain epithelial cell-cell interactions, and loss of E-cadherin is one of the key features of the EMT phenotype. Mutations
in E-cadherin are common in many GI cancers, particularly gastric cancer. Germline mutations in E-cadherin are linked to hereditary diffuse gastric cancer.

The epithelial basement membrane consists of a dense matrix of collagen, glycoproteins, and proteoglycans and normally does not permit passive penetration of cells. The transmigration of tumor cells through the basement membrane likely involves production of key proteolytic activities. Alternatively, the tumor cell may produce factors capable of activating proenzymes present in the extracellular matrix. For example, the tumor may produce urokinase, itself a protease, or plasminogen activator. Having gained access to the interstitial stromal compartment, tumor cells can then enter lymphatic and blood vessels and metastasize.

Angiogenesis and Lymphangiogenesis

Angiogenesis is essential to sustain continued growth of the primary tumor. If new vessels are not developed as the primary tumor expands, cells most distant from available vessels are deprived of an adequate source of nutrition, and central necrosis occurs. Neovascularization is also an important permissive factor in facilitating metastatic dissemination of tumors. A number of protein growth factors produced by malignant tumor cells and stromal cells have been found to be potent stimuli of angiogenesis, including vascular endothelial growth factor (VEGF)-A, basic fibroblast growth factor (bFGF), and TGF-β. VEGF-A is perhaps the most critical factor that is up-regulated in most tumor types, including colorectal cancer. Multiple genetic pathways implicated in GI carcinogenesis modulate VEGF-A expression, including Wnt and mutant ras. Therapeutic strategies that inhibit VEGF-A are now standard-of-care therapies in metastatic colorectal cancer (see Chapter 127).

Angiogenesis occurs in an ordered series of events. Endothelial cells in the parent vessel are stimulated to degrade the endothelial basement membrane, migrate into the perivascular stroma, and initiate a capillary sprout. The sprout develops into a tubular structure that in turn develops into a capillary network. In vitro models that recapitulate the early events of angiogenesis indicate that this process involves a balance between proteases and protease inhibitors in a manner similar to that during tumor invasion. Indeed, functional parallels between tumor invasion and angiogenesis are evident in their mutual requirement for cellular motility, basement membrane proteolysis, and cell growth.

In addition to angiogenesis, lymphangiogenesis plays an important role in tumor metastasis. Some important clues into the molecular basis of tumor lymphangiogenesis have been obtained. VEGF-C or VEGF-D bind to the VEGF receptor-3 on lymphatic endothelial cells to stimulate formation of new lymphatic vessels. This results in the development of new lymphatic channels within the tumor mass and, consequently, enhanced dissemination of tumor cells to regional lymph nodes. Strategies to inhibit tumor lymphangiogenesis are being actively pursued.

Molecular Diagnostics

Progress in the identification of cancer-associated genes coupled with the inherent power of molecular biological techniques to analyze exquisitely small amounts of DNA and protein are leading to more effective diagnostic markers. The most immediate application is assessment of cancer risk in members of cancer-prone kindreds. Strategies have been developed to identify germline mutations in patients with a variety of inherited GI cancer syndromes, including FAP, Lynch syndrome, and hereditary diffuse gastric cancer (HGDG) (see Table 1-1). Genetic testing is a powerful tool to identify high-risk families and define the cancer risk for individual family members. Application of genetic testing must take into consideration the sensitivity and specificity of the assay as well as issues of patient confidentiality and potential impact on medical insurability. For these reasons, genetic counseling is an essential component of the genetic testing process.

Improved detection of sporadic GI cancers and their precursor lesions has also been the focus of research studies. Small numbers of shed cells obtained from stool or fluid aspiration from cysts can be assessed for the presence of mutations or epigenetic alterations in specific tumor-associated genes (B-rif, K-ras, APC, TP53, etc.). MSI testing can be performed on archived colon tumor samples and serves as a useful screening test to identify individuals whose colorectal cancers may have developed as a manifestation of the Lynch syndrome or the serrated pathway to colorectal cancer. Loss of MSH2, MLH1, PMS2, or MSH6 immunohistochemical staining may provide similar information. Studies have demonstrated that the MSI status of a colon tumor is predictive of the response to 5-fluorouracil-based chemotherapy. Therapies that target specific signaling pathways are likely to increase as our molecular understanding of GI cancers increases. Antibodies that target EGF receptors and block the EGF receptor signaling pathway have proved therapeutic benefit in colorectal cancer. However, their benefit has been shown only in cancers lacking activating mutations in K-ras. Testing for K-ras mutations in colorectal cancers is now standard of care before administration of such targeted therapy.

In addition, small molecule tyrosine kinase inhibitors of the c-KIT oncogene now constitute routine treatment of GI stromal tumors (see Chapter 32). Molecular techniques may also find a role in the staging of disease. For example, capture of small numbers of circulating tumor cells prior to the discovery of metastasis may yield prognostic and therapeutic benefits. Finally, as more tests for genetic markers become available, monitoring for disease recurrence after surgery may become another important application.

Genome-wide Association Studies

Although 11% of individuals with colorectal cancer have 2 close family members with the disease, only a small fraction of those occur within an already defined mendelian cancer syndrome. Moreover, identical twin studies of colorectal cancer only demonstrate a 35% risk in the sibling. Identification of other genetic variants that confer an increased risk of colorectal cancer remains a high priority. Given the development of genotyping and deep-sequencing technologies, many such variants have been discovered. Two underlying hypotheses, which are not mutually exclusive, have driven the search for these variants.

The common disease–common variant hypothesis is based on the idea that the heritable risk for illnesses like colorectal cancer is based on the summation of the small effects from genetic variants that are common (minor allelic frequency >5%) in the general population. Thus far, many loci have been identified. However, the small relative risk of each associated common variant has not yielded any more predictive information than family history for diseases like colorectal cancer. Despite this limited clinical applicability, identification of
novel genes not previously associated with the disease raises the possibility of new therapeutic and diagnostic approaches. Another caveat of such studies is that such variants are not necessary causal but merely associated, since other variants may be in linkage disequilibrium with the variant of interest.

The common disease–rare variant hypothesis is based on the premise that the genetic risk of diseases such as colorectal cancer are primarily driven by a heterogeneous set of rare or de novo mutations. In most studies, rare variants are defined as those with a minor allelic frequency of less than 1% in the general population. Compared to common variants, rare variants are more likely to have larger effect sizes owing to the effect of purifying selection. Recent studies, however, demonstrate a bulk of the rare variants likely occurred over the past 5000 years and were due to population expansion and relatively weaker purifying selection of these variants. Advantages of rare variant studies are that the identified variant is more likely to be directly implicated in disease, given the lack of linkage disequilibrium with other variants. Given the larger effect sizes, these variants may also play a key role in clinical decision making.

Whole Genome Sequencing and Exome Sequencing

Given the decline in DNA sequencing costs, considerable interest exists in incorporating the full genomic profile of tumors and cancers into clinical care, with the goal of identifying tailored therapeutics suitable for each individual. At present, 2 strategies are being actively pursued. The first is whole genome sequencing, where the entire genome of the tumor is detailed. As our understanding of the non–protein coding genome evolves, the expectation is that we may discover novel prognostic and therapeutic strategies based on non–protein coding regions of the genome. Another method is to exclusively focus on the exome, the protein-coding portion of the genome. Although only comprising 1% of the genome, the exome is believed to contain approximately 85% of the mutations associated with disease, and the cost of exome sequencing is a fraction of whole genome sequencing. Multiple efforts, including the National Cancer Institute–sponsored Cancer Genome Atlas Project and International Cancer Genome Consortium, are underway to catalog the variation in a large number of cancers.

KEY REFERENCES

Full references for this chapter can be found on www.expertconsult.com.

REFERENCES


