Dysplastic Lesions in Inflammatory Bowel Disease
Molecular Pathogenesis to Morphology

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Context.—Inflammatory bowel disease (IBD) is a long-standing chronic active inflammatory process in the bowel with increased risk for the development of colorectal carcinoma. Several molecular events involved in chronic active inflammatory processes contribute to multistage progression of human cancer development, including reactive oxygen and nitrogen species, aberrant arachidonic acid metabolites and cytokines/growth factors, and immune dysfunction. These molecular events in IBD lead to genetic abnormality and promote aberrant cell proliferation, which further lead to epithelial changes encompassing a broad spectrum from inflammation-induced hyperplasia to dysplasia.

Objective.—To review the (1) epidemiologic and molecular pathogenesis of the risk for colorectal cancer in IBD, (2) morphologic characterization, biomarker(s), and classification of dysplastic lesions, and (3) clinical management of dysplastic lesions arising in IBD.

Data Sources.—The different IBD-related dysplastic lesions are illustrated by using morphology in conjunction with molecular pathways, and the “field cancerization” theory and its potential significance are discussed with a review of the literature.

Conclusions.—Patients with IBD are at increased risk of developing colorectal cancer. The risk of developing carcinoma is related to the extent/duration/activity of the patient’s disease. There is no consensus regarding the extent of carcinoma risk associated with IBD; however, all would agree that patients with IBD represent a group at significant risk for developing carcinoma and as such, warrant adequate surveillance and prevention. With better screening modalities and detection/characterization of dysplastic lesions, IBD-associated serrated lesions, and “field cancerization,” we will improve our understanding of and approach to risk stratification.


Chronic inflammation is a well-recognized risk factor for human cancer development, and at least one-third of all human cancers have been associated with inflammation, for example, long-standing chronic inflammatory bowel disease. Two recognized entities, ulcerative colitis (UC) and Crohn disease (CD), constitute chronic inflammatory bowel diseases (IBDs). Both mainly affect the gastrointestinal tract; however, they can also exhibit extraintestinal inflammatory manifestations. Ulcerative colitis is a continuous and diffuse inflammatory process extending proximally from the rectum, with inflammation limited to the mucosa. In contrast, Crohn disease is characterized by discontinuous, transmural inflammation involving both the small and large intestine. The transmural nature of Crohn disease frequently results in fibrosis, obstructive symptoms, microperforations, and fistula formation. The chronic inflammatory state in patients with IBD places them at a greater risk for developing colorectal carcinoma. Identifying and eliminating precursor dysplastic lesions in IBD is a practical approach to preventing the development of invasive adenocarcinoma; the classification of these precancerous lesions and their pathogenic nature has significant patient management implications. In this review, in combination with our experience, we outline the epidemiologic and molecular pathogenesis of the risk for colorectal cancer in IBD, and further focus on illustrating the different IBD-related dysplastic lesions, by using morphology in conjunction with molecular pathways, and discuss the “field cancerization” theory and its potential significance.

Epidemiology and Possible Susceptibility Genes of Inflammatory Bowel Diseases

Numerous studies have evaluated the epidemiology of IBD. The incidence varies as a function of geography; IBD is most common in Western countries, including the United States and United Kingdom, and less frequent in Asia, Japan, and South America. Incidence varies from approximately 2 to 14 cases per 100,000 persons/y for UC and approximately 3 to 14 cases per 100,000 persons/y for CD in the United States. Owing to the long-standing nature of the disease, prevalence rates are very high, ranging from approximately 37 to 246 cases per 100,000 persons for UC and approximately 26 to 201 cases per 100,000 persons for CD. Interestingly, incidence/prevalence of disease decreases from north to south latitudes, with lower rates in the southern United States when compared to the Northeast,
Table 1. Susceptibility Risk Loci Shared Between Crohn Disease and Ulcerative Colitis and Their Functional Role

<table>
<thead>
<tr>
<th>Locus</th>
<th>Associated Gene</th>
<th>Function/Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>REL</td>
<td>Proto-oncoprotein c-Rel</td>
<td>Restitution and innate mucosal defense</td>
</tr>
<tr>
<td>PTGER4</td>
<td>Prostaglandin E receptor 4</td>
<td>Restitution</td>
</tr>
<tr>
<td>NKX2-3</td>
<td>Homeobox protein</td>
<td>Restitution</td>
</tr>
<tr>
<td>XBP1</td>
<td>X-box-binding protein 1</td>
<td>Paneth cells and stress</td>
</tr>
<tr>
<td>CARD9</td>
<td>Caspase recruitment domain-containing protein 9</td>
<td>Innate mucosal defense and oxidative stress</td>
</tr>
<tr>
<td>MST1</td>
<td>Hepatocyte growth factor-like protein</td>
<td>Innate cell recruitment and apoptosis</td>
</tr>
<tr>
<td>IL23R</td>
<td>Interleukin 23 receptor</td>
<td>IL23/Th17</td>
</tr>
<tr>
<td>JAK2</td>
<td>Janus kinase 2</td>
<td>IL23/Th17</td>
</tr>
<tr>
<td>TYK2</td>
<td>Nonreceptor tyrosine-protein kinase 2</td>
<td>IL23/Th17</td>
</tr>
<tr>
<td>ICOSLG</td>
<td>ICOS ligand</td>
<td>IL23/Th17</td>
</tr>
<tr>
<td>TNFSF15</td>
<td>Vascular endothelial growth inhibitor</td>
<td>T-cell regulation</td>
</tr>
<tr>
<td>TNFSF8</td>
<td>CD153</td>
<td>T-cell regulation</td>
</tr>
<tr>
<td>IL12B</td>
<td>Subunit β of interleukin 12</td>
<td>T-cell regulation</td>
</tr>
<tr>
<td>IL23R</td>
<td>Interleukin 23 receptor</td>
<td>T-cell regulation</td>
</tr>
<tr>
<td>PRDM11</td>
<td>PR domain zinc finger protein 1</td>
<td>T-cell regulation</td>
</tr>
<tr>
<td>ICOSLG</td>
<td>ICOS ligand</td>
<td>T-cell regulation</td>
</tr>
<tr>
<td>IL10</td>
<td>Interleukin 10</td>
<td>Immune tolerance</td>
</tr>
<tr>
<td>CREM</td>
<td>cAMP-responsive element modulator</td>
<td>Autophagy</td>
</tr>
<tr>
<td>CUL2</td>
<td>Cullin-2</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>PUS10</td>
<td>Pseudouridylate synthase 10</td>
<td>Stress</td>
</tr>
<tr>
<td>ORMDL3</td>
<td>Unknown</td>
<td>Carbohydrate metabolism</td>
</tr>
<tr>
<td>SLC2A4R</td>
<td>SLC2A4 regulator</td>
<td>Intracellular logistics</td>
</tr>
<tr>
<td>KIF21B</td>
<td>Kinesin-like protein</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>UTS2</td>
<td>Urotensin-2</td>
<td>Oxidative stress</td>
</tr>
<tr>
<td>PEX13</td>
<td>Peroxosomal membrane protein PEX13</td>
<td>Oxidative stress</td>
</tr>
</tbody>
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Abbreviations: cAMP, cyclic adenosine monophosphate; ICOS, Inducible T-cell costimulator; IL, interleukin; Th, T-helper.

Midwest, and West Coast.7 Recent findings suggest that the incidence of IBD may have changed over time. In the early 20th century, UC was more common than CD in northern Europe and the United States. From the mid to late 1900s, a rise in incidence of CD was observed, while that of UC remained unchanged. At present, the incidence of UC and CD appears similar in the United States and Europe.

The exact etiology of IBD remains unknown; however, a multifactorial concept involving genetic susceptibility, immune dysfunction, as well as environmental and microbial factors is generally believed to play a role. At present, 2 dichotomous theories regarding the role of autoimmunity in the pathogenesis are proposed. One hypothesizes that IBD is a normal immune response to abnormal environmental stimuli, while the other hypothesizes an abnormal immune response to normal environmental stimuli, resulting in IBD.8,9 Most recently, major advances in understanding the pathogenesis of IBD have been derived from animal models and analyses of lesions from patients.10,11

Inflammatory bowel disease is predominately a disease of late adolescence and early adulthood, with peak incidence in the third decade.12 Inflammatory bowel disease affects whites more commonly; however, the gender “trend” varies among studies and appears to reflect the study population.13 Many studies have demonstrated a genetic contribution from the susceptibility of certain ethnic groups to UC; however, it should be noted that other studies have shown that environmental factors, such as smoking and infection, play an important role.14,15 Inflammatory bowel disease is common in Northern Europeans, and there is a higher incidence in persons of Ashkenazi Jewish background.16,17 High concordance (30%–50%) among monozygotic twins with Crohn disease has been documented, with less concordance for UC.18,19 Additionally, first-degree relatives of patients with IBD are affected at a rate of 15%, while 9% of parents or siblings are affected with the disease.

With important technical advances in polymerase chain reaction technique and the completion of the human genome project, there has been a remarkable acceleration in our understanding of molecular genetics during the last 20 years. Genome-wide linkage analysis and single-nucleotide polymorphism array studies are the most common approaches to identifying susceptibility genes in disorders with a significant environmental interaction. After the identification of a susceptibility locus for Crohn disease on chromosome 16 in 1996,20 and the subsequent identification of CARD15 gene in 2001,21 significant research efforts have been underway to identify novel genes of susceptibility for IBD. Recently, it has been reported that there are almost 100 nonoverlapping risk loci identified in IBD, with approximately 25 loci shared between Crohn disease and ulcerative colitis22,23 (see Table 1). The loci are heterogeneous and are associated with a wide range of genes, including those involved in innate immune regulation, barrier function, autophagy, endoplasmic reticulum stress, and other areas of cellular and intestinal homeostasis.

RISK FOR COLORECTAL CANCER IN IBD

Patients with long-standing IBD have an increased risk of developing colorectal carcinoma. The risk of developing precursor lesions such as dysplasia or invasive carcinoma increases exponentially with the duration of the disease.24 The exact extent of risk is uncertain as much variation has been reported in the literature. Some studies use data from tertiary referral centers or population-based studies, while others, from small case series or individual case reports. The patients at greatest risk for the development of colorectal cancer are those with disease in the colon extending to the hepatic flexure or even more proximally (pancolitis). Approximately 8 to 10 years after the onset of symptoms, the risk for cancer begins to increase when compared to age-matched controls.25,26 The approximate cumulative incidence of cancer is 5% to 10% after 20 years and 12%
to 20% after 30 years of disease. In contrast, when colitis is limited to the left colon, most studies have found that the risk of developing colorectal cancer increases after 15 to 20 years. It should be noted that the rates of colorectal cancer and dysplasia were similar to those seen in pancolitis. Ulcerative proctitis likely does not place patients at greater risk for colorectal cancer. In a meta-analysis of 116 studies by Eaden et al, the risk for cancer in patients with ulcerative colitis was approximately 2% after 10 years, 8% after 20 years, and 18% after 30 years of disease. For comparison, the lifetime probability of developing colon cancer for the general US population is approximately 5%. More recently, data from population-based studies suggest that the increased use of aminosalicylates, more frequent screening, and/or early colectomy may have decreased the risk of developing colon cancer over time. The risk for colorectal cancer in long-standing Crohn disease involving the colon is probably comparable to that for ulcerative colitis. However, owing to inconsistencies with prior studies examining Crohn disease, wide variation in the risk for the development of colorectal cancer has been reported. These previous studies limited their focus to patients with small-bowel disease or colonic resections, while others did not consider the duration of disease. In the meta-analyses that adjusted for the duration of disease, the standardized incidence ratio was 2.5 (95% confidence interval [CI], 1.3–4.7) and the relative risk was 4.5 (95% CI, 1.3–14.9). The risk for the development of colorectal cancer was similar in studies that included patients with ulcerative colitis and Crohn disease. Therefore, it appears that extensive Crohn disease should raise the same concerns for the development of colorectal cancer as does ulcerative colitis. As a result, surveillance for dysplasia is recommended for patients with involvement by ulcerative colitis or Crohn disease of greater than 10 years’ duration.

### PATHOGENESIS OF RISK FOR COLORECTAL DYSPLASIA AND CANCER

Like other cancers, IBD-associated colorectal carcinogenesis is believed to follow a multistep process from inflamed, regenerative epithelium, to hyperplastic epithelium, to flat dysplasia, and finally invasive adenocarcinoma (see Figure 1, A through C). The interaction of macrophages and neutrophils with the colonic epithelial cell plays a pivotal role in IBD-induced carcinogenesis. The interplay between reactive oxygen/nitrogen species overproduction, key arachidonic acid metabolites and cytokines/growth factors, activated inflammation-associated signal transduction pathways, along with immune system dysfunction, may contribute to the multistep progression of IBD-associated carcinogenesis. Molecular alterations in IBD-associated colon cancer, similar to sporadic colorectal cancer, include accumulation of gene mutations in tumor suppressor genes, oncogenes, and DNA repair genes, as well as genomic

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**Figure 1.** Carcinoma sequence pathways. The inflammatory bowel disease (IBD)–associated carcinoma pathway with earliest identified molecular changes in p53, followed by chromosomal instability, and finally β-catenin/WNT signaling (A). The adenoma–carcinoma sequence established in 1988 by Vogelstein as a stepwise progression of mutational activation of oncogenes and inactivation of tumor suppressor genes, resulting in cancer (B). The serrated polyp/adenoma–carcinoma sequence established by Jass in 1992 reveals activation of MAPK signaling through BRAF mutation and abnormal DNA methylation with ensuing microsatellite instability (C). Abbreviations: CIMP, CpG island methylator phenotype; MSI, microsatellite instability.
instabilities such as aneuploidy, chromosome instability, and microsatellite instability. Although similarities exist in the molecular pathogenesis of IBD-associated and sporadic colorectal cancer, there are also many differences. The timing and frequency of the molecular genetic alterations are unique and are believed to result from different etiologic factors and cellular microenvironments.

Oxidative stress secondary to chronic inflammation plays a pivotal role in IBD-associated colorectal carcinogenesis. Iron deficiency, mainly due to bleeding from ulcerations and nutritional deficiency, and subsequent supplementation, may further alter or enhance oxidative stress and inflammation-associated carcinogenesis. Oxygen and nitrogen free radicals bind target DNA, RNA, proteins, or lipid, resulting in gene alterations, genetic instability, and aberrant methylation. Chromosomal instability resulting from oxygen or nitrogen free radicals occurs secondary to DNA translocations/deletions/amplifications, DNA strand breakage, or telomere damage. Telomere shortening in ulcerative colitis has been linked with the development of dysplasia. Lipid peroxidation occurs when oxygen and nitrogen free radicals interact with cell membranes, causing DNA adducts leading to transition mutations and frequently involving the p53 tumor suppressor gene. In addition, these free radicals inhibit DNA repair proteins and are believed to be initiators of microsatellite instability.

Aberrant activation of arachidonic acid metabolic pathways has also been shown to be important in IBD-associated colorectal carcinogenesis. COX2 is expressed in sporic carcinogenesis, although not in normal mucosa. Previous data have demonstrated elevated COX2 protein and messenger RNA expression in inflamed mucosa, dysplastic lesions, as well as carcinomas arising in ulcerative colitis. COX2 activates procarcinogens, promotes angiogenesis, and indirectly increases free radical production. Inhibitors of COX2 and other nonsteroidal anti-inflammatory drugs have been shown to be protective against carcinogenesis in humans.

Epithelial proliferation and regeneration as a result of inflammation lead to enhanced cell turnover with resultant increase in the number of mutations. Errors in replication and transcription occur at a rate at which they are no longer manageable.

**MOLECULAR PATHWAYS IN COLON CANCER:**

**SPORADIC VERSUS IBD-ASSOCIATED COLORECTAL CARCINOGENESIS**

The molecular events in both the “classic” adenoma-to-carcinoma and serrated adenoma-carcinoma sequences have been implicated in IBD-associated malignant transformation, and chromosomal instability remains an important event with a unique p53 mutation pattern in IBD-associated carcinogenesis. The molecular characteristics of common colorectal cancers and IBD-associated carcinogenesis are discussed in this section.

**Sporadic Colorectal Carcinogenesis**

Sporadic colorectal cancer results from genomic instability; 85% from chromosomal instability and 15% from microsatellite instability (MSI). Chromosomal instability results in abnormal segregation of chromosomes and aneuploidy. As a result, loss of heterozygosity often occurs with loss of function of key tumor suppressor genes such as adenomatous polyposis coli (APC), p53, retinoblastoma (Rb), and deleted in colon cancer (DCC). Mutations can also render these same genes nonfunctional. Accumulation of molecular changes in tumor suppressor genes drives adenoma to carcinoma progression, and is also known as the “suppressor pathway” (as seen in Figure 1). Loss of APC function is an early event in sporadic colon cancer and is therefore often referred to as the “gatekeeper” of the colon. As the adenoma increases in size and degree of dysplasia, additional genetic alterations occur including induction of the v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) oncogene and loss of function of tumor suppressor genes on chromosome arm 18q in the region of DCC and deleted in pancreatic cancer (DPC4) genes. Loss of p53 function occurs late and is believed to be the defining event that drives the adenoma to carcinoma. These tumors are microsatellite stable. The remaining 15% of sporadic colon cancers occur via the MSI or “mutator” pathway. The MSI pathway involves the primary loss of function of genes that repair DNA base-pair mismatches, which occur as a result of normal DNA replication in dividing cells. The 2 most commonly affected genes are human Mu1L homolog-1 (hMLH1) and human mutS homolog-2 (hMSH2), but other genes are also affected, such as those prone to incorrect copying because of short nucleotide repeats, including transforming growth factor, β receptor II (TGFB2), insulin-like growth factor 2 receptor (IGF2R), and Bcl-2-associated X protein (BAX). Colon cancers with MSI are more commonly seen in the proximal colon, have mucinous and poorly differentiated histologic appearance, with a lymphocytic infiltration, exhibit diploidy, and most importantly, have a more favorable prognosis.

**IBD-Associated Colon Carcinogenesis**

Many of the molecular changes responsible for sporadic colon cancer development also play a role in IBD-associated colon carcinogenesis. Distinguishing features of IBD-associated colorectal carcinogenesis are differences in the timing and frequency of these alterations, as seen in Figure 1. In contrast to sporadic colon cancer, loss of APC function is much less frequent and usually occurs late in the Collins-associated dysplasia-carcinoma sequence. There is more evidence implicating p53 as the key factor in IBD-associated colorectal carcinogenesis. Methylation of CpG islands in several genes seems to precede dysplasia, and is more widespread throughout the mucosa of patients with UC, and is assuming increasing importance as a mechanism contributing to the genetic alterations in IBD-associated colon cancer. A more detailed discussion of the molecular alterations identified in IBD-associated colorectal carcinogenesis is provided below.

**Chromosomal Instability and MSI.**—Chromosomal instability is the most frequently occurring form of genomic instability in UC-associated cancer. Aneuploidy, a marker for genomic instability, is more frequent in high-risk patients with duration of disease longer than 10 years, and is frequently associated with dysplasia. Aneuploidy commonly results from chromosomal instability, caused by various chromosomal abnormalities such as translocations, deletions, and amplifications. Chromosomal abnormalities are found in nondysplastic, dysplastic, and cancerous epithelia in the IBDs, but are more frequently observed in higher-grade lesions. Another cause for genomic instability may be telomere shortening. Many studies have been performed in an effort to determine if abnormal DNA content, or aneuploidy, is a more reliable predictor of UC-associated
cancer development than dysplasia. Regions of aneuploidy in the colons of patients with UC are frequently associated with dysplasia, and possibly precede overt histologic changes.\(^{67,86}\) Aneuploid tissue samples are more frequent in “high-risk” patients with disease duration of longer than 10 years, but aneuploidy has also been detected in colon samples of low-risk patients.\(^{67}\) Aneuploidy in noncancerous mucosa adjacent to UC-associated cancers is greatly increased as compared with the mucosa of noncancer patients, suggesting that carcinoma arises from a field of genetically abnormal epithelia.\(^{59}\) Chromosomal instability is the most frequently occurring form of genomic instability in UC-associated cancers, as revealed by studies using fluorescence in situ hybridization and comparative genomic hybridization.\(^{54,65}\) Fluorescence in situ hybridization analysis of tissue from colectomy samples has shown that UC-associated carcinoma and dysplasia exhibit monosomies and polysomies. These abnormalities are frequently conserved between nondysplastic and dysplastic epithelium, and between dysplasia and cancer. Comparative genomic hybridization analysis of tissue from the same site revealed an increasing frequency of chromosome losses or gains from nondysplastic epithelium to dysplasia and carcinoma. Conversely, comparative genomic hybridization of samples from patients with UC who are at low risk for carcinoma development (defined as disease of <8 years’ duration) revealed no chromosomal anomalies.\(^{64}\) The number of sites with chromosomal alterations increased with the histologic progression to carcinoma, as did the number of alterations per site.\(^{64}\) These results suggest that chromosomal instability is an early event in the progression to UC-associated carcinoma and may contribute to widespread aneuploidy and eventually dysplasia. Microsatellite instability is characteristic of a genome-wide deficiency in the faithful replication of repetitive DNA sequences. The frequency of MSI has ranged from 8% to 21% in UC-associated carcinomas and 13% to 19% in dysplasias.\(^{71-73}\) Microsatellite instability has also been detected in inflamed and regenerative epithelia.\(^{73-75}\) Brentnall et al\(^{64}\) detected MSI in 50% of nondysplastic but chronically inflamed colonic mucosa samples,\(^{76}\) whereas the frequency was much lower in the study by Noffsinger et al.\(^{73}\) Thus, MSI-positive, diploid cancers comprise approximately 10% to 15% of UC-associated carcinomas. The mechanistic role of microsatellite alterations in UC-associated carcinogenesis requires further study. The relatively high frequency of MSI in nondysplastic, inflamed epithelia, as compared with dysplasia, suggests that MSI may be associated with chronic inflammation, perhaps oxidative stress. However, most UC-associated lesions appear to emerge from a pathway involving chromosomal instability and aneuploidy. Ulcerative colitis and the associated carcinogenic progression are characterized by accelerated telomere shortening.\(^{50}\) Conceivably, loss of telomere integrity could contribute to a degeneration of chromosomal stability as well as changes in chromosome number.

**Tumor Suppressor p53 Gene.**—p53 tumor suppressor gene is one of the most important suppressor genes involved in IBD-associated carcinogenesis and its alteration occurs as an early genomic event in the multistep pathway.\(^{76}\) p53 mutations occur as a result of cellular DNA damage from intrinsic and extrinsic factors.\(^{77}\) Several studies have investigated the status of the p53 tumor suppressor gene and p53 protein expression during the progression to UC-associated carcinoma. p53 protein accumulation, which is associated with p53 mutation as well as wild-type p53 overexpression, is frequently detected in UC dysplasias and carcinomas by immunohistochemistry.\(^{78-80}\) Up to 6% of normal cases, 9% in the category “indefinite for dysplasia,” 33% with low-grade dysplasia, 63% with high-grade dysplasia, and 50% to 85% of cases with cancer have been found to have a deletion of TP53.\(^{81,82}\) The extent of p53 mutations has been found to correlate with the degree of IBD-associated dysplasia in several studies.\(^{76,78,80,83,84}\) Interestingly, p53 mutations have also been identified in adjacent, nondysplastic epithelium, suggesting that ongoing chronic inflammation can predispose to genomic changes.\(^{72,83}\) Approximately 80% of identified p53 mutations are transition mutations\(^{78,79,82-84}\) and appear to be strongly associated with inflammation-induced and oxidative stress–induced DNA damage. In patients with UC, frequent transition mutations in p53 codons 247 and 248 have been identified in inflamed mucosa.\(^{85}\) Codon 248 appears to be a hotspot for mutation in the p53 gene, but hotspots unique to UC-associated cancers have not been reported.\(^{78,79,82,84,85}\) Thus, the early appearance of p53 alteration makes it a clinically useful marker in the screening for UC-associated dysplasia and in the assessment of cancer risk.

**Adenomatous Polyposis Coli Gene.**—The loss of APC is considered the initiating event of sporadic colorectal carcinogenesis.\(^{86}\) In contrast, APC alterations are relatively less frequent and occur as a late event in the sequence of genetic alterations in IBD-associated colorectal carcinomas.\(^{61,62,83}\) In patients with IBD and low-grade dysplasia or cancer, APC mutations have been found in fewer than 14% of samples.\(^{60,62,87}\) Moreover, allelic deletions of APC occur in fewer than approximately 30% of cases.\(^{87}\) In contrast to TP53, APC alterations have not been detected in adjacent inflamed, nondysplastic mucosa.\(^{88,89}\)

**KRAS Oncogene.**—The presence of activating mutations of the KRAS oncogene is not a frequent event in IBD-associated carcinogenesis as compared with sporadic colorectal carcinomas. KRAS mutations were identified in approximately 15% of cases with inflamed mucosa, in 23% of dysplasias, and 24% of carcinomas.\(^{61,78,81,83,90-92}\) KRAS mutation does seem to play a significant role in the later stages of UC-associated carcinogenesis, although studies on earlier histologic changes are limited.

**DNA Repair Genes.**—Alterations in mismatch repair (MMR) genes, or microsatellite instability, has been identified in 8% to 50% of dysplasias and carcinomas arising in patients with ulcerative colitis.\(^{71,72,74,75}\) In contrast, the mucosa of normal controls, those with benign inflammatory conditions, as well as those with Crohn disease did not exhibit alterations in MMR genes.\(^{74,93}\) Germline splice site substitutions of MSH2 were identified in 26% of patients with UC-associated carcinoma or dysplasia.\(^{94}\) In contrast, no relationship between the germline MSH2 alteration and UC-associated dysplasia or carcinoma incidence, or the occurrence of the microsatellite unstable phenotype, was observed in a separate study.\(^{73}\) Genetic or epigenetic alterations of mismatch repair proteins, including certain MLH1 gene haplotypes,\(^{95}\) MLH1 promoter hypermethylation,\(^{96}\) and loss of MSH2 protein expression,\(^{72}\) may lead to high-level microsatellite instability in UC-associated lesions. On the other hand, low-level microsatellite instability has not been associated with such mismatch repair gene alterations, although other mismatch repair genes such as PMS1 and MSH6 have not been studied. Microsatellite instability appears to inactivate some tumor suppressor genes. In microsatellite-unstable, UC-associated carcino-
mas, TGFBR2 has been shown to be mutated, although at much lower rates, than in microsatellite-unstable sporadic carcinomas. For microsatellite-stable, UC-associated lesions, mutations in TGFBR2 were quite low (2%). In addition, microsatellite mutations in the IGF2R gene and transcription factor E2F-4 gene have also been detected.\(^{97,98}\)

**Aberrant Gene Methylation.**—CpG island methylator phenotype was recently recognized as a possible molecular alteration in IBD-associated colorectal carcinogenesis\(^ {99}\) The methylated genes are frequently involved in cell cycle control, cell adhesion, and DNA repair.\(^ {100}\) The methylation of these CpG islands can contribute to genomic instability, occurs before dysplasia, and appears to be predominant in the mucosa of patients with UC.\(^ {63}\) Hypermethylation of cyclin-dependent kinase inhibitor 2A (CDKN2A or p16\(^ {INK4A}\)), a cell cycle inhibitor involved in sporadic colorectal cancer, is common in UC-associated neoplasia.\(^ {101}\) Promoter hypermethylation leads to the loss of alternate reading frame product of the CDKN2A locus (p14ARF), a product of the p16 tumor suppressor gene and modulator of p53 protein levels.\(^ {102}\) Hypermethylation of p14ARF was detected in 50% of UC-associated adenocarcinomas, 33% of dysplasia lesions, and 60% of noncancerous but inflamed samples.\(^ {101}\) It has been suggested that methylation of p16 exon 1 is characteristic of age-related methylation, whereas methylation of the p16 promoter occurs in cancers. Increased methylation of other genes, including estrogen receptor (ER) and myoblast determination protein 1 (MYOD), was also observed in the normal mucosa of patients with dysplasia, suggesting the intriguing possibility that this may be due to the elevated rate of cell turnover and oxidative stress characteristic of long-standing UC.\(^ {63}\)

**“FIELD CANCERIZATION”: A COMMON PHENOMENON IN IBD**

Essentially all colorectal carcinomas develop from a dysplastic precursor lesion. In sporadic colon cancer, the dysplastic precursor is the tubular adenoma, a discrete focus of premalignant tissue often completely excised by endoscopic polypectomy. In contrast, the dysplasia in IBD can be polypoid or flat, localized or diffuse.\(^ {103-105}\) In the study by Rubin et al,\(^ {104}\) approximately 89% of dysplasias associated with IBD were elevated. In IBD, multifocal dysplastic lesions and cancer are another common feature.\(^ {106,107}\) In addition to the stepwise progression model of IBD-associated carcinogenesis, “field cancerization” is proposed. Slaughter et al in 1953 first postulated the concept of field cancerization as follows: “the entire epithelial surface of the upper aerodigestive tract has an increased risk for the development of premalignant lesions because of multiple genetic abnormalities in the whole tissue region.” This concept has been widely accepted and applied to other organs including colon, particularly important in screening for IBD-associated dysplasia. Thus, this concept in IBD is further defined as “the formation of a histologically indistinguishable area of clonally derived, mutant cells within the inflamed segment of intestinal tract in IBD.”\(^ {108}\)

Molecular studies have provided strong evidence for this theory, particularly in identification of the mutation status of individual crypts in patients with IBD who have neoplasia. The same mutation spectrum in either Tp53 or KRAS is detected across entire neoplastic lesions as well as in nondysplastic crypts, suggesting that the mutant clone(s) are involved in the field of IBD mucosa.\(^ {106,108}\) Most field changes appear to be induced by chronic active inflammation, which implies key inflammatory events/“carcinogen”-induced field cancerization. Thus, frequent field changes create a different view on tumor excision and pathology examination. Once dysplasia is identified in a patient with IBD, the entire colon is at a greater risk for developing carcinoma or “field cancerization.” This finding often warrants surgical removal of the entire colon and rectum and an extensive histopathologic evaluation for the identification of multiple dysplastic lesions and cancer.

**MORPHOLOGIC CHARACTERIZATION AND CLASSIFICATION OF DYSPLASTIC LESIONS**

Surveillance colonoscopy is currently the most widely used method to detect dysplasia and cancer in patients with IBD.\(^ {109-112}\) Multiple biopsies are necessary to adequately screen the entire length of the colon. In one study, the authors suggest that in order to achieve 90% confidence in detecting dysplasia, approximately 33 biopsies are required.\(^ {67}\) Even when jumbo biopsies are performed, less than 1% of colonic mucosa is sampled with random biopsies. With the advent of chromoendoscopy and other newer endoscopic technology, better and more efficient ways for screening dysplasia are on the horizon.

**IBD-Associated Flat Dysplasia Versus Inflammatory Injur-
Induced Epithelial Regeneration/Hyperplasia**

Owing to extensive inflammation, epithelial regeneration and dysplasia may be difficult to distinguish histologically.\(^ {113}\) Regenerative changes are frequently identified at the base of the crypts and exhibit surface maturation\(^ {42}\) (Figure 2, A and B), while true dysplasia involves the entire crypt and surface epithelium. Some of the characteristic morphologic features present in dysplastic epithelium include crowding of glands, nuclear hyperchromasia and pleomorphism, nuclear stratification, lack of surface maturation, and increased mitoses, as shown in Figure 3, A.\(^ {42,114-116}\) Colonoscopic biopsies obtained from patients with a history of IBD should be characterized pathologically as negative, indefinite, or positive for dysplasia. Dysplasia should be further subclassified as either low-grade dysplasia (LGD) or high-grade dysplasia (HGD), by the extent of cytologic atypia.\(^ {42,113,117-119}\) Among pathologists, there appears to be a high level of agreement on both negative for dysplasia and HGD, while the diagnoses of LGD or indefinite for dysplasia often have little agreement.\(^ {42,113,117-119}\) In flat LGD, the incidence of an associated carcinoma is less than 20%. As a result, patients usually undergo increased surveillance.\(^ {31,109,120-124}\) In contrast, flat HGD carries a higher probability (~60%) of a coexisting colon cancer and of progression to carcinoma,\(^ {120,125}\) and as such is treated with colectomy.

**Dysplasia-Associated Lesion or Mass Versus Sporadic Tubular Adenoma**

In 1981, Blackstone et al\(^ {126}\) first described the term dysplasia-associated lesion or mass (DALM) in patients with ulcerative colitis and found that 7 of 12 DALMs were associated with an underlying invasive carcinoma. More recently, raised dysplastic lesions or DALMs with the appearance of sporadic adenomas have been termed adenoma-like dysplasia-associated lesions or masses, as seen in Figure 2, C and D, and Figure 3, D.\(^ {127}\) To differentiate these lesions from sporadic adenomas can be quite challenging. Theoretically, sporadic adenomas have a
“top-down” growth pattern; specifically, the dysplastic cells are present at the luminal surface of the crypts, while the cells at the base of the crypts are morphologically unremarkable \(^{128}\) (Figure 3, G through I). The dysplastic cells exhibit alterations in \(APC\) and neoplasia-associated patterns of gene expression, whereas the cells at the base did not contain mutations. In contrast, DALM lesions usually have a “bottom-up” growth pattern (Figure 3, D through F). Another important histologic feature that helps to identify a DALM lesion is that it usually arises in a background mucosa with active chronic inflammation. A set of immunohistochemical stains may also aid in the distinction and will be discussed in the section below.

**IBD-Associated Serrated Lesion Versus Hyperplastic Polyps**

The serrated neoplasia pathway was recently proposed in sporadic colorectal carcinomas, as seen in Figure 1. These tumors have defective DNA mismatch repair resulting in microsatellite instability \(^{129}\). In addition to errors in DNA repair, these cancers are associated with activating mutations in serine/threonine protein kinase B-Raf (\(BRAF\)) and are rarely associated with mutations in \(KRAS\) \(^{129-131}\). The precursor lesions are serrated polyps and include hyperplastic polyps, traditional serrated adenomas, and sessile serrated polyps/adenoma \(^{132}\). The presence of the V600E mutation of the \(BRAF\) gene in hyperplastic polyps strongly supports this entity as a specific marker for the serrated neoplasia pathway \(^{132}\). In a recent study, Bossard et al \(^{133}\) found that serrated lesions, such as hyperplastic polyps and sessile serrated polyps/adenomas, accounted for approximately 7% of premalignant lesions in the inflamed mucosa in patients with IBD, as shown in Figure 2, E and F, and Figure 3, J through L. In that study, all serrated lesions contained \(BRAF\) mutations, while adjacent inflamed mucosa and conventional tubular adenomas had activating mutations in \(KRAS\) with wild-type \(BRAF\). Traditional serrated adenomas and sessile serrated polyps/adenomas were microsatellite stable, while the serrated malignant lesion (mucinous adenocarcinoma) demonstrated microsatellite instability via immunohistochemistry. A rare \(p53\) mutation was identified in this lesion. These findings are interesting and further studies are warranted to fully assess their potential risk for carcinoma development in patients with IBD.

**PRACTICAL BIOMARKERS FOR IDENTIFYING AND DISTINGUISHING DYSPLASTIC LESIONS IN IBD**

As indicated previously, there are distinct differences in the timing and frequency of genomic alterations observed in IBD-associated carcinogenesis and common sporadic colorectal carcinoma. These differences have been used to identify and characterize dysplastic lesions in IBD \(^{134-136}\). Some altered molecular processes have been studied for their potential as surrogate biomarkers of IBD dysplasia involving cell proliferation, cell cycle, oxidative stress/DNA damage and repair, oncogenes, tumor suppressors, Wnt pathway, and others \(^{137}\). For example, these biomarkers, along with histologic evaluation, are helpful in identifying dysplastic lesions from inflammatory reactive changes and in distinguishing low- and high-grade dysplasia as well as DALM lesions and sporadic tubular adenoma.

Mutation of \(p53\) is probably an early and important event in IBD-associated carcinogenesis. A strong correlation between \(p53\) mutations and the histologic progression from low-grade dysplasia to invasive carcinoma in patients with...
IBD has been shown.\textsuperscript{338} Monoclonality pattern of p53 mutation has been further identified in dysplastic crypts as well as tumor.\textsuperscript{336} The nuclear accumulation of p53, as detected by immunohistochemical analysis, has been well studied as a biomarker to analyze the mutant form of p53, by the tendency of mutated p53 to have an increased half-life when compared to wild-type p53.\textsuperscript{339} For IBD, immunohistochemical analysis of the nuclear p53 accumulation has been helpful in distinguishing dysplastic epithelium from inflammatory regenerative epithelium.\textsuperscript{340,341} However, there is concern that the nuclear accumulation of wild-type p53 protein may be a part of the cellular response to various types of stress including inflammatory nitro-oxidative stress that directly damages DNA.\textsuperscript{3} Noffsinger et al\textsuperscript{141} reported a unique basal pattern of p53 expression in ulcerative colitis that is associated with mutation in the p53 gene, as seen in Figure 3, B and C. From data in the literature, as well as our clinical experience, mutant p53 protein accumulation in IBD

Figure 3. Morphologic and immunohistochemical alterations in patients with inflammatory bowel disease. Flat dysplasia (A) with increased Ki-67 proliferative index (B) and nuclear p53 expression (C). An “adenoma-like dysplasia-associated lesion or mass” (D) also demonstrates an elevated proliferative index (E) and increased nuclear p53 expression levels (F). In contrast, the sporadic tubular adenoma (G) has an increased proliferative index (H) with low levels of p53 expression (I). The serrated lesion (J) reveals a very low proliferative index (K) and an absence of p53 staining (L) (hematoxylin-eosin, original magnifications $\times$400 [A and J], and $\times$200 [D and G]; Ki-67, original magnifications $\times$400 [B], $\times$200 [E and H], and $\times$600 [K]; p53, original magnifications $\times$400 [C], $\times$200 [F and I], and $\times$400 [L]).
Dysplastic lesions display a clonal pattern in which the immunostaining intensity and positive cell numbers of nuclear mutant p53 protein (Figure 3, C and F) are equal to/greater than those for Ki-67–labeled cell proliferation (Figure 3, B and E). The unique immunostaining patterns of p53 and Ki-67 are significant for differentiating the dysplastic lesions in IBD (Figure 3, B, C, E, and F) from a sporadic tubular adenoma (Figure 3, H and I). Thus, the combination of p53 and Ki-67 is a useful biomarker to identify dysplastic lesions in IBD biopsy specimens.

β-Catenin is a cell membrane protein that accumulates within the nucleus, most frequently in sporadic adenomas and colon cancer rather than DALMs. In contrast, mutations of the tumor suppressor gene p53 occur more frequently in sporadic tubular adenomas. In a study by Walsh et al, almost 30% of adenoma-like DALMs expressed p53, whereas nuclear β-catenin was more frequently expressed in sporadic adenomas (40%).

Cellular senescence is the phenomenon by which normal diploid cells lose the ability to divide; this often occurs after about 50 cell divisions in vitro. Some cells become senescent after fewer replication cycles, commonly due to DNA double-strand breaks, toxins, and other causes. Senescent cells are abundant in low-grade premalignant lesions, whereas they are rarely found in malignant tumors. This finding is important since senescence markers, such as senescence-associated heterochromatin foci, senescence-associated β-galactosidase, or the transcription factor known as deleted in esophageal cancer 1 (DECI), may potentially be used to differentiate low-grade dysplasia from high-grade dysplasia or carcinoma. Remarkably, a recent study shows that low-grade dysplasia displays the shortest telomeres and the highest levels of senescence with overexpression of DECI, whereas high-grade dysplasia exhibits the opposite pattern. This finding is crucial for IBD-associated carcinogenesis. It is, however, important to confirm these results in studies with several different senescence markers and a large cohort of patients, and to further confirm that senescence is a reliable biomarker of premalignancy in IBD.

α-Methylacyl coenzyme A racemase (AMACR) is an enzyme that catalyzes the racemization of α-methyl-branched carboxylic coenzyme A thiocarboxylates. AMACR has been shown to be the most promising biomarker for diagnosing prostate cancer and other cancers. It has been suggested that immunohistochemical analysis of AMACR could serve a similar purpose in IBD as that in prostate cancer. An early study shows that in patients with IBD, AMACR is not expressed in any foci considered negative for dysplasia, but is significantly increased in low-grade dysplasia (96%), high-grade dysplasia (80%), and invasive carcinoma (71%), while only 1 of 7 cases (14%) that are indefinite for dysplasia are focally positive. A recent study also shows that p53 and AMACR show positivity in most cancers (77.3% and 80.3%, respectively) and dysplasias (94.4% and 94.4%, respectively), but only rarely in nonneoplastic epithelium (1.6% and 9.4%, respectively). The combination of p53 and AMACR is a stronger distinction for neoplastic tissues and shows 75.8% coexpression of AMACR and p53.

| Table 2. Dysplasia and Colorectal Cancer Surveillance Recommendations of the Major Gastroenterologic Societies |
|---------------------------------|--------------------------------------------------|
| **Ulcrovac colitis**            | * Surveillance should begin after 8 years in patients with pancolitis. |
| **AGA recommendations:**        | * Surveillance should begin after 8 years in patients with pancolitis. |
| **ACG recommendations:**        | * Surveillance should begin after 15 years in patients with colitis involving only the left colon. |
| **ASGE recommendations:**       | * Colonoscopy should be repeated every 1 to 2 years. |
| **AGA recommendations:**        | * Surveillance should begin after 8 years in patients with pancolitis. |
| **ACG recommendations:**        | * Surveillance should begin after 15 years in patients with left-sided colitis. |
| **ASGE recommendations:**       | * Surveillance is not indicated in ulcerative proctitis. |
| **AGA recommendations:**        | * Any suspicious lesion/mass should be biopsied. |
| **ASGE recommendations:**       | * Colonoscopy should be repeated every 1–3 years. |
| **AGA recommendations:**        | * The finding of carcinoma or high-grade dysplasia is an indication for colectomy. |
| **ASGE recommendations:**       | * Colectomy is also indicated for any degree of dysplasia associated with a lesion or mass. |

**CLINICAL MANAGEMENT OF DYSPLASIA ARISING IN IBD**

The current American Gastroenterologic Association, the American College of Gastroenterology (ACG), and the American Society for Gastrointestinal Endoscopy (ASGE) acknowledge that patients with IBD are at increased risk of developing colon cancer. Colorectal cancer in most instances develops from dysplasia and as such, at present it serves as the best marker of cancer risk in patients with IBD. Recommendation for dysplasia and carcinoma surveillance in IBD is based on disease duration and extent of disease, with colonoscopic screening to begin 8 years after diagnosis in patients with pancolitis and after 15 years in patients with left-sided disease only (see Table 2). Adequate biopsies should be taken and if there is no evidence of dysplasia, patients should return for a repeat colonoscopy every 1 to 2 years for the remainder of their lifetime or until the detection of dysplasia or carcinoma (see Figure 4; adapted from Itzkowitz and Harpaz). Similar guidelines have been established by other societies such as the ACG and the ASGE.
Areas of dysplasia that appear endoscopically as raised lesions, irrespective of their location to active colitis, have a favorable prognosis following complete excision with polypectomy and close follow-up. Kisiel and colleagues recently determined the natural history of polypectomy in sporadic adenomas and adenoma-like DALMs. In their study, 49% of patients had an adenoma-like DALM and 51% had sporadic adenomas. Upon subsequent screening, the authors identified a higher risk for nonadenoma-like DALM or cancer (6%), higher than that previously reported. The cumulative risk for carcinoma or nonadenoma-like DALM after polypectomy ranged from 5% to 28% after 5 years. The 5-year cumulative risk of requiring colectomy in patients with adenoma-like DALM originally managed with polypectomy was approximately 30% and was not statistically different between the groups. The 5-year overall survival was 91% irrespective of the group. Although polypectomy appears to be a safe strategy for the management of adenoma-like DALMs and sporadic tubular adenomas in ulcerative colitis, this approach is fraught with a higher-than-previously-expected risk of developing nonadenoma-like DALM or carcinoma after polypectomy. In contrast, patients with nonadenoma-like DALM or those with flat high-grade dysplasia should be treated with colectomy, since the incidence of an undiagnosed synchronous cancer will be identified.

CONCLUSIONS

Patients with IBD, including ulcerative colitis and Crohn disease, are at increased risk of developing colorectal cancer. The risk of developing carcinoma is related to the extent of the patient’s disease (pancolitis versus left-sided disease), duration of disease, and level of activity. Despite numerous studies, there is no consensus regarding the extent of carcinoma risk associated with IBD; however, all would agree that patients with IBD represent a group at significant risk for developing carcinoma and as such, warrant adequate surveillance and prevention. With better screening modalities such as chromoendoscopy, detection and characterization of dysplastic lesions, including flat and DALM dysplasia, IBD-associated serrated lesions, and “field cancerization,” we will improve our understanding of and approach to risk stratification.

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References


