Inflammation and Cancer

IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation

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Inflammation and Cancer, IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physiol 287: G7–G17, 2004; 10.1152/ajpgi.00079.2004.—Patients with ulcerative colitis and Crohn’s disease are at increased risk for developing colorectal cancer. To date, no known genetic basis has been identified to explain colorectal cancer predisposition in these inflammatory bowel diseases. Instead, it is assumed that chronic inflammation is what causes cancer. This is supported by the fact that colon cancer risk increases with longer duration of colitis, greater anatomic extent of colitis, the concomitant presence of other inflammatory manifestations such as primary sclerosing cholangitis, and the fact that certain drugs used to treat inflammation, such as 5-aminosalicylates and steroids, may prevent the development of colorectal cancer. The major carcinogenic pathways that lead to sporadic colorectal cancer, namely chromosomal instability, microsatellite instability, and hypermethylation, also occur in colitis-associated colorectal cancers. Unlike normal colonic mucosa, however, inflamed colonic mucosa demonstrates abnormalities in these molecular pathways even before any histological evidence of dysplasia or cancer. Whereas the reasons for this are unknown, oxidative stress likely plays a role. Reactive oxygen and nitrogen species produced by inflammatory cells can interact with key genes involved in carcinogenic pathways such as p53, DNA mismatch repair genes, and even DNA base excision-repair genes. Other factors such as NF-κB and cyclooxygenases may also contribute. Administering agents that cause colitis in healthy rodents or genetically engineered cancer-prone mice accelerates the development of colorectal cancer. Mice genetically prone to inflammatory bowel disease also develop colorectal cancer especially in the presence of bacterial colonization. These observations offer compelling support for the role of inflammation in colon carcinogenesis.

Inflammatory bowel disease; colorectal cancer; colitis; oxidative stress; animal models

Patients with inflammatory bowel disease (IBD), both ulcerative colitis (UC) and Crohn’s colitis, are at increased risk of developing colorectal cancer (CRC). Indeed, IBD ranks among the top three high-risk conditions for CRC, together with the hereditary syndromes of familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC). Unlike the latter two conditions that have a well-defined genetic etiology, CRC risk in IBD appears to be related more to chronic inflammation of the gastrointestinal mucosa than to any clear-cut genetic predisposition.

Regardless of the underlying condition, essentially all CRCs develop from a dysplastic precursor lesion. In sporadic CRC, the dysplastic precursor is the adenomatous polypl (adenoma), a discrete focus of neoplasia that is typically removed by simple endoscopic polypectomy. In contrast, dysplasia in patients with IBD can be polypoid or flat, localized, diffuse, or multifocal and, once found, marks the entire colon as being at heightened risk of neoplasia, thereby warranting surgical removal of the entire colon and rectum. These differences in morphology and biological behavior not only make clinical cancer surveillance in IBD patients more challenging than in the general population, but they raise the important question of how chronic inflammation contributes to the development of CRC. The “adenoma-carcinoma” sequence found in the sporadic setting becomes the “inflammation-dysplasia-carcinoma” sequence in IBD. The object of this theme article is to first review the clinical and molecular features of CRC in IBD and then discuss how inflammation may contribute to CRC pathogenesis.

Clinical features of colitis-associated CRC

Compared with sporadic colorectal carcinoma (SCC), CRC arising in patients with IBD has several distinguishing clinical features. Colitis-associated colorectal cancer (CAC) affects individuals at a younger age than the general population. They more often have a mucinous or signet ring cell histology, there is a higher rate of two or more synchronous primary CRCs, and in some studies, they demonstrate a more proximal distribution in the colon. Curiously, these same features are found in CRCs arising in individuals with HNPCC, although there has been no clear genetic etiology to CAC. A germline hMSH2 mutation (a gene responsible for HNPCC) was reported to be more frequent in UC patients who developed high-grade dysplasia (HGD) and cancer than in those who did not (11), but this has not been substantiated by other investigators (60). The fact that a positive family history of CRC confers a twofold greater risk of developing CRC to the patient with IBD can be viewed as evidence for a genetic etiology, but this same degree of risk attributed to a positive family history of CRC also applies to the general population in whom no clear genetic etiology has yet been elucidated. There must be other factors besides genetic predisposition that contribute to cancer development in IBD.

Several lines of evidence implicate chronic inflammation as a key predisposing factor to CRC in IBD (46). First, the risk for developing CRC increases with longer duration of colitis. Although, for some reason, CRC is rarely encountered before seven years of colitis; thereafter, the risk increases at a rate of ~0.5–1.0% per year. Second, the extent of colitis is another important risk factor; the more colonic surface that is involved with colitis, the greater the colon cancer risk. Paradoxically, however, patients who have inflammation limited only to the rectum do not have an appreciably increased risk of cancer. Third, the risk of CRC is much greater in the small subset of
IBD patients who also have primary sclerosing cholangitis, an idiopathic condition characterized by chronic inflammation of the bile ducts, which predisposes not only to CRC but also to biliary tract cancer. Fourth, evidence is mounting to suggest that anti-inflammatory medications, especially 5-aminosalicylates but possibly corticosteroids, can reduce the development of colorectal dysplasia and cancer in IBD. This situation is similar to healthy individuals and even those with FAP, in whom the use of aspirin or other nonsteroidal anti-inflammatory agents has been shown to diminish the growth and subsequent development of colorectal neoplasia.

Despite all of the evidence strongly implicating chronic inflammation as the culprit, surprisingly little research has directly addressed the question of whether inflammation per se correlates with CRC risk in IBD. In fact, historically, the degree of colitis activity has been considered not to be an independent risk factor for CRC, probably because of the way that disease activity was defined. For example, when activity of disease was measured according to the frequency of clinical (symptomatic) exacerbations, there did not appear to be a correlation with CRC risk (25, 64). However, when colitis activity has been defined histologically, a recent case-control study found that greater degrees of histologically active inflammation were indeed associated with increased risk of CRC (74). New evidence also indicates that CRC can arise in areas of microscopic colitis proximal to areas of gross colitis, suggesting that histological change, even without colonscopc alteration, is a better determinant of inflammation for the purposes of cancer risk (34). It is important to realize though, that patients with the most severe inflammation often undergo colectomy early in their disease because they are not responding to medical therapy. As such, they are no longer at risk for developing CRC. Ironically then, many patients who develop CRC in IBD have clinically, as well as histologically, quiescent inflammation. Active inflammation in colorectal mucosa is characterized by a predominant neutrophilic infiltration with crypt abscesses and ulceration of the epithelium. Inactive, or quiescent, inflammation is marked by a predominance of lymphocytes. Thus better insight into the pathogenesis of CAC will likely come from studying the role of the immune cells and their products. This will be reviewed below, following a brief overview of the molecular pathogenesis of CAC.

**MOLECULAR PATHOGENESIS OF SPORADIC CRC**

To place the molecular pathogenesis of CAC in proper perspective, it is first helpful to appreciate the molecular events involved in the development of SCC. SCC arises as a result of genomic instability. The two main types of genomic instability that contribute to colon carcinogenesis are chromosomal instability (CIN) and microsatellite instability (MSI), accounting for 85 and 15% of SCC, respectively. Chromosomal instability results in abnormal segregation of chromosomes and abnormal DNA content (aneuploidy). As a result, loss of chromosomal material [loss of heterozygosity (LOH)] often occurs, contributing to the loss of function of key tumor suppressor genes such as adenomatous polyposis coli (APC) and p53. These genes can also be rendered nonfunctional by mutation. In either event, it is the accumulation of molecular disturbances mainly in tumor suppressor genes that drives the sporadic adenoma-carcinoma progression, and therefore this pathway has sometimes been referred to as the “suppressor pathway.” Colon cancers arising in patients with FAP tend to proceed via this pathway.

Loss of APC function is typically an early event in SCC pathogenesis, giving the APC gene the moniker of “gatekeeper” of the colon (Fig. 1). Although some have argued that APC mutation may not be the universal initiating event but may instead occur at somewhat later stages of adenoma progression (48), APC still contributes to the process of CIN (32). During the progression of the adenoma, whereby increases in adenoma size, degree of dysplasia, and degree of villous histology take place, other changes in genetic regulation occur, such as induction of the k-ras oncogene and loss of function of tumor suppressor genes on chromosome 18q in the region of the deleted in colon cancer (DCC) and deleted in pancreatic cancer (DPC4) genes. Loss of p53 gene function occurs late and is believed to be the defining event that drives the adenoma to carcinoma.

**SPORADIC COLON CANCER**

![Figure 1](https://example.com/fig1.png)

**COLITIS-ASSOCIATED COLON CANCER**

![Figure 2](https://example.com/fig2.png)
Tumors that arise via the CIN/tumor suppressor gene pathway are typically microsatellite stable (MSS). The remaining 15% of sporadic CRCs arise through the MSI pathway. This pathway is preferred by the vast majority of CRCs that occur in patients with HNPCC. The MSI pathway involves the primary loss of function of genes that usually repair DNA base-pair mismatches that occur during the normal process of DNA replication in dividing cells. Although two of these genes, human MutL homolog-1 (hMLH1) and human MutS homolog-2 (hMSH2), are most commonly affected by loss of function in CRC, several other DNA mismatch repair (MMR) genes cooperate to repair DNA base mismatches, and the mutation or loss of any one of them gives rise to DNA replication errors throughout the genome. These errors preferentially affect target genes such as transforming growth factor (TGF)βII, IGF2R, and BAX, which contain in their coding regions short nucleotide repeats that are intrinsically unstable and therefore prone to be copied incorrectly during DNA replication. The resulting microsatellite instability renders these genes incapable of normal colonocyte homeostasis resulting in malignant growth. This pathway of colon carcinogenesis has been referred to as the “mutator” pathway because of the many mutations in key genes involved. Compared with MSS sporadic colon cancers, MSI sporadic colon cancers are more likely to be diploid (normal DNA content), located in the proximal colon, mucinous, poorly differentiated, show lymphocytic infiltration, and associated with a more favorable prognosis (48). MSI-positive CRCs can be further classified into those with high (MSI-H) or low (MSI-L) degrees of MSI depending on how many markers of a consensus panel are found to be unstable (8).

Epigenetic alterations also contribute to altered gene expression in colon carcinogenesis. A recently recognized molecular alteration is the CpG island methylator phenotype (CIMP) (85). CpG islands are dense aggregates of cytosine-guanine dinucleotide sequences that may occur in the promoter region of genes. Extensive methylation of the cytosine bases is associated with promoter silencing and loss of gene expression. Many genes involved in cell cycle control, cell adhesion, and DNA repair can be methylated in colon cancer (75). So-called type A methylation, i.e., the estrogen receptor, occurs as a function of age and is found in both normal colon and colon cancer. Type C methylation, however, is cancer associated, leading to pathogenic silencing of genes such as hMLH1, MGMT, p16, and p14.

In general, tumors manifest either the CIN or the MSI phenotype. However, there can be overlap between the CIMP and MSI phenotype. For example, hypermethylation of hMLH1 can produce the MSI-H cancer phenotype. By the same token, methylation of MGMT rather than hMLH1 underlies MSI-L cancers (48). The process of methylation is an area of intense investigation, and it is anticipated that this line of research will help to better define the molecular pathways involved in CRC in a variety of clinical settings including IBD.

MOLECULAR PATHOGENESIS OF COLITIS-ASSOCIATED CRC

Cancers in the setting of IBD are believed to occur by a progression from no dysplasia to indefinite dysplasia to low-grade dysplasia (LGD) to HGD to carcinoma (Fig. 1). It is important to realize that in clinical practice, cancers can arise without proceeding through each of these steps (47). Similar to sporadic CRC, colon carcinogenesis in IBD is a consequence of sequential episodes of somatic genetic mutation and clonal expansion. However, unlike SCC, where dysplastic lesions arise in one or two focal areas of the colon, in colitic mucosa, it is not unusual for dysplasia or cancer to be multifocal, reflecting a broader “field change.” Careful mapping studies using DNA aneuploidy as a marker of genomic instability indicate that individual cell populations are observed in the same locations of the colon on repeated colonoscopic examinations and became more widely distributed over time, occupying larger areas of the mucosa (72). Moreover, within an aneuploid area, additional subclones of aneuploidy seem to emerge from their predecessors. Thus genomic instability of increasingly dysregulated subclones of cells with clonal expansion of mutant cell populations occurs at the expense of the normal surrounding epithelium. For the most part, in IBD, neoplastic lesions arise within areas of the mucosa that have been involved with colonic inflammation. It is not known whether the reepithelialization of large patches of colonic mucosa by abnormal clones is simply a consequence of the healing response to ulceration caused by chronic inflammation or whether the epithelial cells of IBD patients have an innate ability to replace surrounding epithelium. Regardless, aneuploidy is often more widespread than dysplasia, and this indicates that substantial genomic alterations can occur in colitic mucosa without disturbing morphology.

Perhaps not surprisingly, many of the molecular alterations responsible for SCC development also play a role in colitis-associated colon carcinogenesis. In fact, emerging evidence suggests that in CAC, the frequency of CIN (85%) and MSI (15%) is roughly the same as in SCC (87). Distinguishing features of CAC, however, are differences in the timing and frequency of these alterations (Fig. 1). For example, APC loss of function, considered to be a very common early event in SCC, is much less frequent and usually occurs late in the colitis-associated dysplasia-carcinoma sequence (4, 68, 84). Mutations in APC are rarely, if ever, encountered in colitic mucosa that is negative or indefinite for dysplasia, and fewer than 14% of tissues manifesting LGD or cancer harbor APC mutations (4, 68, 86). Likewise, allelic deletion of APC occurs in fewer than 33% of colitis-associated neoplasms (86).

Greater evidence implicates p53 as playing an instrumental role in CAC. Allelic deletion of p53 occurs in ~50–85% of cancers (13, 89). Indeed, p53 LOH correlates with malignant progression, occurring in 6% of biopsies without dysplasia, 9% with indefinite dysplasia, 33% with LGD, 63% with HGD, and 85% with cancer (13). In the setting of colitis, p53 mutations occur early and are often detected in mucosa that is nondysplastic or only indefinite for dysplasia (10, 13). In carefully mapped colectomy specimens, p53 mutation was an early molecular change that occurred before aneuploidy, which, in turn, preceded p53 LOH (Fig. 1) (10). In fact, a high frequency of p53 mutations was found in inflamed mucosa from UC patients who did not have cancer, indicating that chronic inflammation predisposes to these early mutations (see below) (43).

Methylation is assuming increasing importance as a mechanism contributing to the genetic alterations in CAC (Fig. 1). Indeed, methylation of CpG islands in several genes seems to precede dysplasia and is more widespread throughout the
mucosa of UC patients (45). In colitis-associated neoplasms, hMLH1 hypermethylation was observed in 6 of 13 (46%) MSI-H, 1 of 6 (16%) MSI-L, and 4 of 27 (15%) MSS specimens, implicating this epigenetic change as a cause of microsatellite instability (31). The cell cycle inhibitor p16 INK4a, the loss of which has been implicated in sporadic CRC, is commonly hypermethylated in UC neoplasms (42). Approximately 10% of biopsies without dysplasia already demonstrate p16 promoter hypermethylation, the rate increasing with higher grades of dysplasia and reaching 100% in cancer specimens. p14ARF is an indirect regulator of p53, and it resides at the same locus as p16INK4a. Loss of p14ARF function by promoter hypermethylation has been reported in 50% of adenocarcinomas, 33% of dysplastic lesions, and even in 60% of mucosal samples without dysplasia from patients with UC (76).

THE ROLE OF CHRONIC INFLAMMATION IN COLON CARCINOMENESIS

Thus the three main molecular pathways of colon carcinogenesis (the APC/tumor suppressor gene/CIN pathway, the MSI pathway, and the CIMP pathway) can all be induced early in the process of CAC, in colitic mucosa before dysplasia develops (Fig. 1). This strongly suggests that inflammation plays a causative role. In addition to the above-mentioned tumor-associated genes that are progressively expressed in the process of CAC carcinogenesis, in UC patients, several inflammation-associated genes such as cyclooxygenase-2 (COX-2) (1), nitric oxide (NO) synthase-2 (NOS)-2 (43), and the interferon-inducible gene 1–8U (38) are also increased in inflamed mucosa and remain elevated in colonic neoplasms. The central question is how inflammation results in neoplastic transformation and progression.

For one thing, the colonic mucosa of patients with IBD demonstrates enhanced epithelial cell turnover. Compared with normal colonic biopsies taken from patients with sporadic adenomas, mucosal biopsies from patients with UC demonstrate higher rates of mitosis as well as apoptosis, especially in areas of active, as opposed to quiescent, inflammation (3). However, whereas increased epithelial cell turnover likely contributes to carcinogenesis, it is insufficient to cause cancer. Rather, in the setting of heightened epithelial cell turnover, mutagenic assault and sustained DNA damage caused by factors within an inflammatory cell-rich microenvironment appear to drive the carcinogenic process. As such, tumors behave similar to wounds that fail to heal (20).

A leading theory is that the oxidative stress that accompanies chronic inflammation contributes to neoplastic transformation. Indeed, IBD is considered one of the main “oxygen radical overload” diseases whereby chronic inflammation, be it inherited or acquired, results in a cancer-prone phenotype (44). Oxidative stress, with its associated cellular damage, is thought to play a key role in the pathogenesis of the colitis itself as well as in colon carcinogenesis (Fig. 2). Colitis is triggered in a genetically susceptible individual by an environmental insult such as gastrointestinal infection, NSAID use, or other environmental toxins. The inflammatory cells that contribute to the colitis generate reactive oxygen and nitrogen species (RONS). Neutrophils and macrophages, which are important in the acute inflammatory process, generate free radicals and other prooxidant molecules. Inflamed tissues from patients with active UC or Crohn’s colitis demonstrate increased expression of NOS and other RONS (40, 43, 50, 66). With the use of GAPDH enzyme as a molecular marker, McKenzie et al. (55) showed that oxidation of thiols in the active site of GAPDH, with subsequent inhibition of enzyme activity, occurred in colonic epithelial cells from inflamed mucosa of Crohn’s disease and UC but not from paired samples of unaffected mucosa. Measurements of 8-hydroxydeoxyguanosine (8-OHdG), a mutagen formed by the action of hydroxyl radical at the C8 position of deoxyguanosine DNA base, in mucosal biopsies of patients with UC were reported to be increased in patients with UC compared with normal controls, with levels even higher in UC patients who had dysplasia (23). Interestingly, 8-OHdG levels were increased with longer duration of UC and were lowest in the rectum, suggesting that the mesalamine enemas that most of these patients were using might have had an antioxidant effect.

Direct support that RONS participate in colonic inflammation comes from interventional studies in animals. Intrarectal administration of peroxyinitrite causes colonic inflammation in rats (67). The use of oxygen radical scavengers such as superoxide dismutase, catalase, and NOS inhibitors attenuate colonic inflammation in animal models of chemical-induced colonic injury (78). Likewise, inducible NOS knockout mice (iNOS−/−) manifest an attenuated colitis in response to injury (41), and in the APCMin/+ mouse, which is genetically susceptible to multiple intestinal adenomas, crossing them with iNOS−/− mice or treating them with an iNOS inhibitor significantly reduced the prevalence of adenomas (2). Curiously, however, treating APCMin/+ mice with 5-ASA did not appreciably reduce adenoma size or number, although treatment with other NSAIDs (piroxicam, sulindac) was effective (70). Although the APCMin/+ mouse is not a model of colon cancers that arises in the setting of inflammation, these data still offer insights into mechanisms of colon carcinogenesis. The role...
played by the NO/iNOS system in both colonic inflammation and tumor development is quite complex and does not permit simple conclusions or extrapolation to the issue of carcinogenesis in UC at the present time (6, 22). The fact that mice deficient in glutathione peroxidase enzymes develop inflammation and cancer of the small and large intestine further supports the notion that antioxidant pathways are important for preventing the inflammation-neoplasia sequence (17).

How then might oxidative stress contribute to colorectal carcinogenesis? Free radicals have the potential to affect a large array of metabolic processes, because their targets include DNA, RNA, proteins, and lipids (44, 53). If key genes or proteins responsible for colonic yeast fermentation are targeted, dysplasia and subsequent carcinoma arise. The p53 tumor suppressor gene is one important target. Hussain et al. (43) examined the mutation spectrum of p53 at codons 247 and 248 in biopsies taken from inflammatory colonic mucosa of UC patients compared with normal, non-UC controls. They noted that more than half of the UC cases exhibited a higher frequency of G-to-A transitions at the CpG site of codon 248 and C-to-T transitions at the third base of codon 247 compared with controls. Moreover, in paired biopsies from UC patients, this pattern of mutation was only seen in inflamed rather than noninflamed mucosa. Increased NOS-2 activity was associated with these p53 mutations, suggesting that oxidative stress was playing a role. These investigators also observed that increases in posttranslational modifications of p53 were associated with increased iNOS activity in inflamed tissues from UC patients, further implicating activated p53 in response to oxidant injury (40).

More direct evidence for the role of RONS in colon carcinogenesis comes from the work of Gasche et al. (33), who used model colon cancer cell lines to observe that hydrogen peroxide (H$_2$O$_2$) produced frameshift mutations in a reporter gene. In their system, cells that were genetically deficient in DNA MMR activity were particularly susceptible to frameshift mutations, but even at higher concentrations of H$_2$O$_2$, cells with normal MMR activity displayed frameshift mutations. In subsequent studies, these investigators found that H$_2$O$_2$ inactivated the DNA MMR system, apparently by damaging the protein complexes responsible for DNA base mismatch repair (15). Conceivably therefore, even in the absence of mutations of the relevant genes, oxidative stress may put enough “pressure” on the DNA MMR system to create microsatellite instability, and this presumably contributes to the MSI phenotype seen both in nonneoplastic and in neoplastic mucosa of UC patients. A recent discovery suggests that increased activity of enzymes responsible for the process of base excision-repair might also contribute to MSI in UC tissues (39). MSI can be detected in chronically inflamed mucosa from UC patients, even in those with rather short disease duration before the risk of neoplasia ostensibly rises (9). This lends credence to the concept that inflammation alone can cause MSI. Curiously, however, MSI is not found in normal colonic mucosa from healthy controls or from patients with other types of benign inflammatory colitis including Crohn’s colitis (9, 59). It is tempting to speculate that certain RONS may cause MSI and that the spectrum of RONS differs in the local tissue microenvironment depending on the inflammatory background of the underlying disease.

As mentioned above, chromosomal instability represents the major pathway by which cancers seem to arise in IBD patients. It is not yet clear whether chronic inflammation contributes to this molecular pathway or whether some other factor(s) predispose. For example, using fluorescent in situ hybridization with probes specific for chromosomes 8, 11, 17, and 18, Rabinovitch et al. (65) reported that patients with UC who had a neoplasm (HGD or cancer) in the colon (so-called progressors) demonstrated CIN both in the neoplastic lesions themselves as well as in nondysplastic rectal mucosa remote from the neoplastic areas. Although normal mucosa from control subjects without UC did not display CIN, inflamed but nondysplastic mucosa of UC patients who did not harbor a neoplasm in their colon (nonprogressors) also did not exhibit CIN. This observation was reinforced by studies in which DNA fingerprinting demonstrated substantial genomic instability in both the dysplastic as well as nondysplastic mucosa of UC patients harboring a neoplasm (16). These findings suggest that widespread genomic instability occurs in patients with UC who develop colonic neoplasia but not in patients with UC who have not yet developed neoplasia despite comparable disease duration and the presence of inflammation. Thus this type of genomic instability may be a marker of cancer risk in UC perhaps apart from the inflammatory process. It has been observed that a possible mechanism to explain the chromosomal instability associated with UC is telomere shortening (63). Given the importance of the CIN pathway in CAC, it will be important to more directly study the role of inflammation in this process.

Oxidants can also alter DNA methylation patterns (78). It is not yet known whether the hypermethylation of genes involved in CAC (45) are affected by oxidant injury.

In addition to damaging DNA and other macromolecules, oxyradicals can also induce key genes involved in the inflammatory and carcinogenic process. For example, the transcription factor NF-κB can stimulate iNOS to generate NO and COX-2 to generate prostanoids that have proinflammatory and carcinogenic effects (88). Activated NF-κB is found in inflamed mucosal biopsies of patients with IBD (71). Among the factors that can regulate NF-κB activity, TNF-α induces NF-κB, whereas peroxisome proliferator-activated receptor-γ (PPARγ) attenuates NF-κB. At the present time, it is not clear whether TNF-α itself plays a role in carcinogenesis nor is it known whether inhibiting TNF-α, which is so effective in reducing the inflammation of Crohn’s disease, can abrogate the carcinogenic process. PPARγ ligands have been shown to inhibit intestinal inflammation (83), and impaired expression of PPARγ has been described in colonic epithelial cells of patients with UC (24). Treatment of APC deficient mice with PPARγ ligands has yielded mixed results, with both increases and decreases in adenoma growth reported (12, 58). The role of PPARγ in colitis-associated cancer has not been investigated.

COX-2 activity plays an important role in sporadic carcinogenesis where it has been shown that normal colonic mucosa does not express COX-2, but with the adenoma-carcinoma sequence, this enzyme is induced. Among the many effects of cyclooxygenases, they can activate procarcinogens, indirectly produce free radicals, and promote angiogenesis (81). In animal models, COX-2 inhibition can dramatically reduce the development of colon cancers, just as in humans, use of COX-2 inhibitors and other NSAIDs is protective against colon cancer (34). In UC, COX-2 expression is somewhat enhanced in inflamed mucosa, but it is further induced in dysplastic and...
cancerous lesions (1). Whether COX-2 inhibition would be chemopreventive against CRC in the setting of UC is not known. Concern has been raised by a study in the IL-10-deficient mouse model of chronic colitis, where COX-2 inhibitors paradoxically enhanced the frequency of colonic dysplasia (see below) (35).

LESSONS FROM ANIMAL MODELS

To help better understand the role of inflammation in vivo, investigators have turned to animal models, and indeed, the existing data suggest that inflammation predisposes to colorectal cancer. Three main approaches have been used: 1) inducing inflammation with injurious agents such as dextran sulfate sodium (DSS) in otherwise healthy rodents and monitoring for the development of neoplasia; 2) inducing inflammation in mice with a genetic predisposition to colon cancer; and 3) studying mice with a genetic predisposition to inflammatory bowel disease for the development of neoplasia. Of the three approaches, the latter is the most physiologically relevant to human disease.

DSS COLITIS IN HEALTHY RODENTS

Dysplasia and cancer can be induced in healthy mice or rats when colitis is induced by repeated cycles (19, 52, 61) or rarely after a single cycle (19) of DSS. In this model, longer disease duration (3–6 mo) was associated with an increased rate of neoplasia, even in mice given the identical initial colitis insult and in the setting of clinical remission (19). The localization of dysplasia and/or cancer is ~20, 44, and 36% for the proximal, middle, and distal colon, respectively (19). Animals that developed neoplasia demonstrated more severe degrees of inflammation, especially in the distal colon (19, 61). The histology of dysplastic and cancerous lesions resembles that of IBD neoplasms, but in contrast to the human situation, none of the cancers and only 7% of dysplasias induced by DSS manifested nuclear p53 immunostaining (19). Parenthetically, in the DSS model, treatment with the antioxidant N-acetylcysteine reduced both the inflammation and tumor incidence (77). In general, these observations support the concept that prolonged and repetitive inflammation promotes colon cancer.

DSS COLITIS IN CANCER-PRONE MICE

The induction of chronic inflammation by DSS results in more frequent development of cancer and dysplasia in mice prone to MSI (51). For example, 28/30 MSH2−/− mice developed dysplasia or cancer in response to DSS, and the frequency of HGD was 47, 23, and 8% for MSH2−/−, MSH2+/−, and MSH2+/+, respectively. Perhaps not surprisingly, 89% of colonic dysplasias or cancers from MSH2−/− mice were MSI positive, whereas the majority of neoplasms arising in DSS-treated heterozygotes or wild-type mice developed independently of the mismatch repair system, because very little MSI was found in these groups. The inflamed and uninflamed colonic mucosa remote from dysplastic lesions in the MSH2−/− mice also demonstrated MSI, implying that, similar to humans, MSI is induced by colonic inflammation and can precede dysplasia.

The APCMin/+ mouse has also been studied in the context of DSS-induced colitis (18). No cancers arose in APCMin/+ mice that did not receive DSS. In contrast, the frequency of cancers among mice receiving only one or two cycles of DSS was 22 and 40%, respectively. Likewise, DSS induced a very high frequency of dysplasia that arose in areas of healed mucosa as well as those of acute and chronic inflammation. Animals with higher inflammation scores had significantly more dysplastic lesions. Thus colitis markedly accelerates the development of neoplasia in APC mutant mice.

CANCER AND DYSPLASIA IN IBD-PRONE MICE

Of greater relevance to human CAC, the genetically engineered immunodeficient mice have provided new insights into aspects of the inflammatory response that may be responsible for instigating neoplastic progression (Table 1).

IL-2-Deficient, IL-2−, and β2-Microglobulin Double Knockout Mice

IL-2 is an essential regulatory cytokine of the immune system. Among IL-2−/− mice, 50% die within 9 wk with splenomegaly, lymphadenopathy, and severe autoimmune hemolytic anemia (37). The rest develop chronic colitis resembling UC and a systemic wasting disease resulting in death within 6 mo. Within the limited lifespan, dysplasia (37) but not cancers (80) has been observed. The IL-2null and β2-Mnull double knockout mouse develops pancolitis similar to IL-2 knockout mice, but these mice have milder overall disease and are able to survive >6 mo. This offers an opportunity to study colon carcinogenesis. With prolonged observation (6–12 mo), 32% of these mice develop adenocarcinoma in the proximal colon. In addition, LGD and HGD are also noted in these animals (80). Molecular characterization of these cancers revealed that all of the cancers harbored APC gene mutations, more than one-half had p53 gene mutations, and most exhibited MSI (80).

IL-10 KNOCKOUT MICE

When the IL-10 gene is disrupted, mice develop spontaneous generalized enterocolitis under conventional conditions. Specific pathogen-free (SPF) housing conditions reduce the severity and limit the disease to the proximal colon (7). The development of colitis in these mice is dependent on IL-12 and requires the presence of enteric bacteria (57). Under SPF conditions, 100% of the mice develop colitis after 3 mo of age. After 3 and 6 mo, 25 and 60% of the mice, respectively, develop adenocarcinoma. Cancers arise mainly in the proximal colon, and they often express COX-2 in regions containing inflammatory cells and myofibroblasts (79). Ironically, these cancers do not seem to manifest alterations of APC, p53, K-ras, or Msh2 genes, and although circulating TGF-β levels are elevated, there does not appear to be any downregulation or truncation of the TGF-β receptor type II (82). Thus the molecular mechanism of these tumors remains elusive. IL-10 treatment was shown to ameliorate the colitis in all animals and decreased the cancer occurrence by half even after the colitis had been established (7). Likewise, in a small study (62), probiotics also reduced the prevalence of colon cancer and mucosal inflammatory activity. On the other hand, pure cultures of Enterococcus faecalis induced both IBD and, after 20 wk, dysplasia and cancer of the rectum in IL-10−/− mice (5). Paradoxically, COX-2 inhibitors have been shown to exacerbate, rather than ameliorate, the severity of colitis and the
IL-10 in IBD has also been studied in other colitis models (57). In this animal, significant colitis results in hyperplasia of the cecum and colon, with a primarily granulocytic inflammation of the submucosa. This model shares similarities with Rag2-deficient mice, including the location of colitis, the universal development of both dysplasia and adenocarcinoma, and the requirement of *H. hepaticus* for the induction of colitis and colon tumors (26–29). The most significant difference, perhaps, is the genetic background of these two models. In this model, colitis is required but not sufficient for the development of adenocarcinoma.

Table 1. Summary of colorectal neoplasia in genetically engineered IBD animal models

<table>
<thead>
<tr>
<th>Knockout Mouse (Ref)</th>
<th>Background Strain</th>
<th>Inflammation</th>
<th>Tumor Histology</th>
<th>Tumor Location</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2/β2M DKO (80)</td>
<td>C57BL/6 × 129</td>
<td>UC-like</td>
<td>Carcinoma (32%)</td>
<td>Rectum</td>
<td>Spontaneous development of colitis and carcinoma.</td>
</tr>
<tr>
<td>IL-10 KO (7)</td>
<td>C57BL/6 × 129</td>
<td>Colitis with some features of Crohn’s, duodenitis</td>
<td>Carcinoma (60%)</td>
<td>Colon</td>
<td>Colitis induced by normal flora; colitis and cancer caused by Enterococcus faecalis.</td>
</tr>
<tr>
<td>Rag2 KO (28,29)</td>
<td>129/SvEv</td>
<td>Induced by infecting with <em>Helicobacter hepaticus</em></td>
<td>Dysplasia, tubular adenoma, carcinoma in situ, adenocarcinoma (100%)</td>
<td>Cecum</td>
<td>1. Both colitis and neoplasia are induced by <em>H. hepaticus</em>.</td>
</tr>
<tr>
<td>Rag2/Tgfb1 DKO (27)</td>
<td>129S6 × CF1</td>
<td>Colitis</td>
<td>Dysplasia, adenocarcinoma (100%)</td>
<td>Cecum</td>
<td>Require <em>H. hepaticus</em> to induce inflammation and neoplasms.</td>
</tr>
<tr>
<td>TCRβ/δp53 DKO (49)</td>
<td>C57BL/6jcl</td>
<td>UC-like</td>
<td>Dysplasia, adenocarcinoma (70%)</td>
<td>Ileocecum, Cecum</td>
<td>Normal flora is required to produce inflammation and tumors.</td>
</tr>
<tr>
<td>Gpx1/Gpx2 DKO (17)</td>
<td>Mixed C57BL/6J and 129Sv/J or 129S3</td>
<td>Ileocolitis</td>
<td>Dysplasia, adenocarcinoma (28%); signet ring cell carcinoma</td>
<td>Ileum</td>
<td>Require bacterial flora, including <em>Helicobacter</em> species.</td>
</tr>
<tr>
<td>N-cadherin dominant negative (36)</td>
<td>129/Sv→B6</td>
<td>Crohn’s-like</td>
<td>Adenomas, not cancer</td>
<td>Small bowel</td>
<td>Chimeric mouse; adenomas form in inflamed and non-inflamed areas.</td>
</tr>
<tr>
<td>Gα12 KO (73)</td>
<td>129/Sv</td>
<td>UC-like</td>
<td>Carcinoma (31%)</td>
<td>Colon</td>
<td>Colitis and cancer occur even in SPF barrier facility.</td>
</tr>
</tbody>
</table>

KO, knockout; DKO, double knockout; IBD, inflammatory bowel disease; UC, ulcerative colitis; GPx, glutathione peroxidase; TCR, T cell receptor; SPF, specific-pathogen free.

The frequency of dysplasia in IL-10−/− mice (35). The effect of IL-10 in IBD has also been studied in other colitis models (57). In SCID mice, transfer of CD4+ CD45RBhi T cells from normal donors into CB-17 SCID mice resulted in severe colitis. IL-10 has been shown to prevent colitis in this setting. Oral administration of *Lactococcus lactis*-secreting IL-10 reduced inflammation. In this model, colitis is required but is not sufficient for the development of adenocarcinoma. The chronic inflammation further contributed to the formation of adenocarcinoma.

**RAG2-DEFICIENT MICE**

Rag2-deficient mice lack functional T and B lymphocytes because of an inability to properly rearrange antigen receptors. This mouse has a valuable model to study the function of various subsets of lymphocytes by transferring specific lymphocytes from immunocompetent mice. Colitis can be induced in this mouse by adoptive transfer of CD4+ CD45RBhi T cells. This colitis is preventable by cotransfer of CD4+ CD25+ or CD4+ CD45RBhi regulatory T cells. The latter subset of T cells is believed to harbor regulatory cells that inhibit intestinal inflammation. In this animal, significant colitis may also be induced by infection with a widespread enteric mouse bacterial pathogen, *Helicobacter hepaticus*, in the absence of T cells (28). Interestingly, after the infection, these mice not only contract colitis, but also inevitably develop colonic tumors, including sessile tubular adenoma, dysplasia, carcinoma in situ, and adenocarcinoma (100%) (28). However, they do not produce pedunculated or villous adenomas. In contrast, if they are maintained in a *H. hepaticus*-free environment, these animals do not have colon tumors or colitis. Adoptive transfer of CD4+ CD45RBhi regulatory T cells to these mice significantly suppressed colitis. After the regulatory T cell transfer, the majority of animals had minimal or no colitis and did not develop neoplasia; neoplasia was found in those animals that developed moderate to severe local colitis. It is believed that IL-10 produced by these regulatory T cell is pivotal in inhibiting inflammation and interrupting carcinogenesis, because transferring IL-10-deficient CD4+ CD45RBhi regulatory cells did not protect these animals from colitis and carcinoma (29).

Tgfb1−/− mice develop an autoimmune disease with inflammatory lesions in multiple organs. Crossing these mice with Rag2 knockout mice rescues them from the autoimmune phenotype and permits them to live as long as 8 mo. They develop hyperplasia of the cecum and colon, with a primarily granulocytic inflammation of the submucosa. This model shares similarity with Rag2-deficient mice, including the location of colitis, the universal development of both dysplasia and adenocarcinoma, and the requirement of *H. hepaticus* for the induction of colitis and colon tumors (26–29). The most significant difference, perhaps, is the genetic background of these two models. In this model, colitis is required but is not...
sufficient for cancer formation; a genetic predisposition to cancer appears to contribute. Whether the enteric bacteria directly affect carcinogenesis or induce inflammation that results in neoplastic events is unclear. The cancers in this model do not demonstrate MSI.

**T CELL RECEPTOR-β KNOCKOUT MICE**

T cell receptor (TCR-β)-deficient mice have defective intestinal mucosal immune systems and develop UC-like colitis. When raised under conventional conditions, TCR-β and p53 double knockout mice are not only susceptible to chronic inflammation (90%, most limited to ileocecum and cecum) but are also prone to dysplasia (50–70%) and adenocarcinoma (70%) (49). Germ-free conditions prevent both chronic inflammation and neoplasms.

**GLUTATHIONE PEROXIDASE-1/-2 DOUBLE KNOCKOUT MICE**

Glutathione peroxidases (GPX) are a family of intracellular antioxidant enzymes that reduce H₂O₂ and organic hydroperoxides by oxidizing glutathione. Mice with targeted disruption of both Gpx1 and Gpx2 develop ileocolitis between 2 and 7 wk of age. After 4 mo of age, ~40% of animals develop tumors (28% adenocarcinomas) that are often nonpolyoid and primarily located in the distal ileum (17). Higher tumor rates were correlated with higher inflammation scores in the ileum but not the colon, suggesting that inflammation is necessary but not sufficient to cause tumors. Tumor incidence was highest in colonies that were raised in non-SPF conditions. Essentially no tumors developed in germ-free or SPF colonies nor were there any tumors noted in animals that had at least one wild-type Gpx1 or Gpx2 allele. This model is instructive, because these mice have otherwise intact immune systems and mucosal barrier function, and it highlights the importance of the antioxidant system in protecting against inflammation and neoplasia in the setting of bacterial infection.

**N-CADHERIN DOMINANT-NEGATIVE MICE**

These mice are immunologically intact but have a defect in their intestinal mucosal barrier. Cadherins are essential for mediating cell adhesion and normal development. Expression of a dominant-negative form of N-cadherin lacking extracellular domain (NCADΔ) resulted in loss of endogenous E-cadherin from the cell surface. Because complete disruption of cadherin production results in embryonic lethality in mice, a chimeric mouse model was created that expressed dominant-negative N-cadherin in the small intestine (36). This was generated by introducing genetically manipulated embryonic stem cells from 129/Sv strain (NCADΔ) into normal B6 blastocysts. The resultant 129/Sv→B6 chimeric mouse intestine contained patches of 129/Sv (NCADΔ)-derived crypt-villus units and patches of crypt-villus units of B6 background that were easily distinguishable from 129/Sv by their lectin-binding properties. All chimeric mice developed IBD similar to Crohn’s disease, presumably because disruption of the epithelial barrier results in inflammation. Inflammation is present only in 129/Sv (NCADΔ) patches but not in B6 epithelium, and consequently, foci of dysplasia and adenoma were only found in 129/Sv (NCADΔ) areas. No adenocarcinomas were observed during the 19 mo of the experiment. This model supports the association between inflammation and tumorogenesis. However, the fact that adenomas occurred in both inflamed and noninflamed 129/Sv (NCADΔ) patches indicates that a noninflammatory-driven process, such as aberrant cell migration, may also play a role.

**Gα₂/⁰ MICE**

Mice deficient in the G protein subunit αi2 develop profound alterations in thymocyte maturation and function (73). From 13 wk of age onward, these mice develop severely active colitis, especially in the distal colon, and the intensity and extent of colitis progress with age. By 15–36 wk of age, 31% of mice developed cancer that was not polypoid or metastatic. Large pools of mucin were found in some cancers.

**FUTURE DIRECTIONS**

Chemoprevention of CRC is an area of great interest and intense investigation. Just as aspirin and NSAIDs appear to prevent CRC in the general population, evidence is accumulating that 5-aminosalicylic acid (5-ASA) compounds may prevent colorectal neoplasia in patients with UC (21). Whereas the mechanism of this is not known, it has been shown experimentally that 5-ASA is able to prevent damage to GAPDH caused by exposure to hypochlorite, the most potent oxidant produced by neutrophils (56). This ability of 5-ASA to scavenge RONS was not shared by other medications used to treat IB and methylprednisolone, 6-mercaptopurine, or metronidazole (56). Rectal administration of 5-ASA in patients without IB has been shown to induce apoptosis selectively in tumor cells but not in normal epithelial cells (14). The chemopreventive role of 5-ASA in IB deservers further study in humans and in animal models. In addition, given the importance of prooxidants in causing molecular damage to colonic epithelium, further investigating the potential role of antioxidants as chemopreventive agents is also worthwhile. Moreover, more work is needed to identify the molecular underpinnings of how inflammation might contribute to chromosomal instability and abnormal methylation of genes. Another area of interest is the role of bacteria in causing cancer. Although an infectious origin to human CRC is not usually considered, recent data suggest that polyoma virus infection can cause CIN in human colonic epithelial cells (69). Whether bacteria can also cause CRC in humans, analogous to H. pylori causing gastric cancer, is not known. Because several of the animal models of CRC in IB implicate bacteria in the pathogenesis of both the inflammation and cancer, insights from these studies, in addition to asking why some colitis models do not get CRC, may provide further food for thought. Finally, to date, researchers in the field of CAC have typically drawn insights from the work performed on SCC. Curiously, a recent study (30) showing elevated C-reactive protein levels in individuals who were prone to sporadic CRC suggests that inflammation may play more of a role in SCC than previously appreciated. So, just as understanding the uncommon conditions FAP and HNPCC have taught us about the pathogenesis of SCC, lessons learned from IB as a model of colonic inflammation might also help us further our knowledge about how inflammation might cause CRC in general.

**REFERENCES**

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ROLE OF INFLAMMATION IN COLITIS-ASSOCIATED COLORECTAL CANCER


