Inflammation and colorectal cancer
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Patients with long-standing inflammatory bowel disease (IBD) have an increased risk of developing colorectal cancer (CRC). However, the underlying mechanisms are not entirely clear. A genetic basis for the increased risk of CRC in IBD patients is only a partial explanation. It is possible that high levels of inflammatory mediators that are produced in this setting may contribute to the development and progression of CRC. Growing evidence supports a role for various cytokines, released by epithelial and immune cells, in the pathogenesis of IBD-associated neoplasia. Two key genes in the inflammatory process, cyclooxygenase-2 (COX-2) and nuclear factor kappaB (NF-κB), provide a mechanistic link between inflammation and cancer while other factors such as, TNF-α and IL-6-induced signaling have been recently shown to promote tumor growth in experimental models of colitis-associated cancer. This article reviews the pathogenesis of IBD-related CRC and summarizes the molecular mechanisms underlying the development of intestinal neoplasia in the setting of chronic inflammation.

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Introduc 30

The functional relationship between inflammation and cancer has long been recognized and was already established two centuries ago. This link was made on the basis of several observations (reviewed in [1**]):

1. Tumors arise at sites of chronic inflammation.
2. Inflammatory cells, chemokines and cytokines are present in tumors.
3. Overexpression of cytokines and chemokines can induce cancer.
4. The same molecular targets and similar pathways are activated or shut down in inflammation, as well as in the carcinogenesis process.

5. Epidemiological studies have shown that chronic inflammatory states increase the risk of numerous cancers.
6. Non-steroidal anti-inflammatory drugs (NSAIDs) reduce the incidence and the mortality of several cancers.

The role of the immune system during cancer development is complex, involving extensive reciprocal interactions between genetically altered cells, adaptive and innate immune cells, their soluble mediators and structural components present in the tumor microenvironment [2].

In this review we discuss the model of colorectal cancer (CRC) and inflammation as the proof of concept linking between these two processes.

CRC remains a major health concern in the Western world. There is a body of literature suggesting a link between chronic inflammation and CRC, in which gastrointestinal (GI) inflammation may contribute to the promotion of CRC development. Anti-inflammatory treatment is known to reduce GI neoplasia, while CRC incidence is increased in inflammatory bowel disease (IBD) [3*].

Human CRC can be classified by etiology as: (1) inherited, including hereditary non-polyposis colorectal cancer (Lynch Syndrome/HNPCC) due to genetic instability, and familial adenomatous polyposis coli (FAP) due to a mutation in the adenomatous polyposis coli gene, APC; (2) inflammatory, including Crohn’s disease (CD) and ulcerative colitis (UC); or (3) sporadic, accounting for ~80% of CRCs but with poorly defined etiology.

Patients with long-standing IBD have an increased risk of developing CRC [4]. In fact, although patients with IBD represent only a small fraction of CRC cases (1–2%), these patients are among those at greatest risk of CRC in the general population. Moreover, in patients with prolonged (>30 years) and extensive colitis, the risk of CRC is much higher (18%). Risk factors for CRC include extent and duration of UC, primary sclerosing cholangitis, a family history of sporadic CRC, severity of bowel inflammation and early onset of the disease [3*].

Many of the molecular alterations responsible for sporadic CRC, namely chromosomal and microsatellite instability, and hypermethylation also play a role in colitis-associated CRC carcinogenesis. This article provides an overview on the pathogenesis of IBD-related colorectal neoplasia and...
Chronic inflammation promotes carcinogenesis by inducing gene mutations, inhibiting apoptosis, or stimulating angiogenesis and cell proliferation [6]. Inflammation also induces epigenetic alterations that are associated with cancer development. Two key genes in the inflammatory process, cyclooxygenase-2 (COX-2) and nuclear factor kappaB (NF-κB), provide a mechanistic link between inflammation and cancer and are targets for chemoprevention and particularly, in CRC [7].

NF-κB transcription factors have a key role in many physiological processes and disease conditions. Given its important role in mediating inflammatory signals, a lot of attention has been focused on the role of NF-κB and its upstream activator, IkB kinase (IKK), and their involvement in inflammation and cancer [8].

NF-κB is not a single gene but represents a family of closely related transcription factors that includes five genes NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), c-Rel and RelB. NF-κB regulates the expression of genes, many of which play important roles in the regulation of inflammation and apoptosis. Although RelA, RelB and NF-κB1 genetic alterations are rare in human cancer, these proteins are constitutively activated in a wide variety of human tumors and have been associated with tumor progression [9]. Constitutive NF-κB activation has been recently evaluated in CRC [10]. Thus, constitutively active NF-κB was found in 40% of CRC tissues and 67% of cell lines, and shown to promote tumor growth.

The upstream IkB kinases are also constitutively active in some types of cancer. Defective IKKα was found in several solid tumors such as breast, colon, ovarian, pancreatic, bladder, prostate carcinomas and melanoma [11]. A recent study has shown that IKKα is activated in colorectal tumors and associates to the chromatin of specific Notch targets, leading to the release of the co-repressor SMRT (silencing mediator of retinoic acid and thyroid hormone receptor) [12]. The Notch pathway plays an extensive role in the immune system and operates at various levels by acting in conjunction with defined immunological signals such as cytokines, T cell antigen receptor and co-stimulatory receptor-mediated signaling. A role for Notch signaling in cancer has extensively been reported (reviewed in [13]).

Many human cancers exhibit elevated prostaglandin (PG) levels owing to upregulation of COX-2, a key enzyme in eicosanoid biosynthesis. PGE(2) is a major downstream mediator of COX-2 that promotes cellular proliferation and angiogenesis, inhibits apoptosis, enhances invasiveness, and modulates immunosuppression. COX-2 is significantly overexpressed in a variety of tumors, including colorectal neoplasms, making it an attractive therapeutic target [14]. Although COX-2 is regularly expressed at low levels in colonic mucosa, its activity increases dramatically following mutation of the APC gene, suggesting that the APC-β-catenin/TCF signaling activity may regulate COX-2 gene expression.

Selective inhibitors of COX-2 (coxibs) have established efficacy in the treatment of pain and inflammation comparable to that of non-selective NSAIDs but with superior GI safety. Subsequently, additional pharmacologic activities have emerged outside of coxibs’ analgesic activity. Celecoxib (Celebrex®, Pfizer, USA) is unique among the coxibs and traditional NSAIDs, because this particular drug displays the greatest potency to induce apoptotic cell death and has clinically relevant anti-neoplastic applications, arising from additional activities that may exert anti-tumor functions independent of the COX-2 inhibitory activity [15].

Colitis-associated colorectal cancer
The association of IBD with CRC was first described by Burrill Crohn in 1925 [16]. More than two decades elapsed before the first description of a cancer of the colon complicating ‘regional enteritis’ [17]. Later on, additional case reports were published describing CRC in patients with CD [18]. However, no statistically significant increase in cancer risk among these patients was found, mainly because the absolute numbers of CD-associated cancer cases were usually small. Therefore, CRC in CD has been less well studied and most of our knowledge on the pathobiology of CRC is based on studies of patients with UC.

In the early 1980s, a first attempt to calculate direct comparisons between cancer incidences in CD and UC was described. Studies conducted at The Mount Sinai Hospital noted a parity of similar extent between the relative risks of colon cancer in CD and in left sided UC. Thus, it was suggested that when cases of ulcerative and Crohn’s colitis of similar anatomic extent are followed for similar durations of time, the two diseases may ultimately prove to have similar increases in risk for CRC [19]. Generally, both diseases are characterized by chronic, relapsing inflammation of the intestines. It is assumed that chronic inflammation is what causes cancer.
The neoplastic transformation in IBD is thought to be similar to the adenoma–carcinoma sequence in sporadic CRC (reviewed in [20]). However, unlike sporadic CRC where dysplastic lesions arise in one or two focal areas of the colon, in colitic mucosa, the dysplasia is usually multifocal, reflecting a broader ‘field effect’.

The transition from normal epithelium to adenoma to carcinoma is associated with acquired molecular events involving mutations in oncogenes and tumor suppressor genes, abnormal gene expression and genetic defects in a variety of genes. This tumor progression model was deduced by Fearon and Vogelstein from comparison of genetic alterations seen in normal colon epithelium, adenomas of progressively larger size, and malignancies [21]. Among the earliest events in CRC carcinogenesis is loss of the APC gene, which appears to be consistent with its important role in predisposing carriers of germline APC mutations to colorectal tumors. These studies showed that the molecular steps that occur after the activation of the APC/β-catenin/TCF pathway involve a non-linear accumulation of specific genetic changes, which include detectable losses at the molecular level of portions of chromosome 5q, chromosome 18q, and chromosome 17p, changes in methylation patterns and mutation of the K-ras oncogene. The important genes involved in these chromosome losses are APC(5q), DCC/MADH2/MADH4(18q), and p53(17p).

Many of the molecular alterations responsible for sporadic CRC development also play a role in colitis-associated colon carcinogenesis. However, there are several differences in the sequence of molecular events leading from dysplasia to invasive adenocarcinoma in IBD as compared with sporadic CRC. For example, APC loss of function, considered to be a common early event in sporadic CRC, is much less frequent and usually occurs late in the colitis-associated dysplasia–carcinoma sequence. On the contrary, p53 mutations in sporadic neoplasia usually occur late in the adenoma–carcinoma sequence, whereas in patients with colitis, p53 mutations occur early and are often detected in non-dysplastic mucosa [20].

Microsatellite instability and hypermethylation
Chromosomal instability, microsatellite instability (MSI), and hypermethylation are assuming increasing importance as mechanisms contributing to the genetic alterations in IBD. MSI due to mismatch repair deficiency has been reported to occur at variable frequencies in IBDb-associated intestinal neoplasias [22]. Moreover, the mutational events in target genes for instability in these cancers are similar to those in sporadic and hereditary colorectal cancers, indicating a colon-related repertoire of target gene alterations.

About 12–15% of sporadic CRCs display defective DNA mismatch repair, manifested as high levels of MSI. In this group of cancers, promoter hypermethylation of the mismatch repair gene hMLH1 is strongly associated with MSI. Silencing the hMLH1 gene expression by its promoter methylation stops the formation of MLH1 protein, and prevents the normal activation of the DNA repair gene. This is an important cause for microsatellite instability and cell proliferation to the point of colorectal cancer formation [23]. In IBD neoplasia, hMLH1 promoter hypermethylation is a frequent event. Furthermore, hMLH1 hypermethylation and MSI are strongly associated with reduced hMLH1 protein expression in IBD-related neoplasia, suggesting that its hypermethylation causes defective DNA mismatch repair in at least a subset of IBD cancers [24].

CpG island hypermethylation is a mechanism of gene silencing that can be used by neoplastic cells to inactivate undesirable genes. Methylation of CpG islands in several genes seems to precede dysplasia and is more widespread in UC patients [25]. Schulmann et al. have determined the frequency of high-level MSI and the mutational and methylation profile of IBD-related neoplasia, and found that the profiles of coding microsatellite mutations (instabilotypes) differ significantly between IBD cancers and sporadic CRCs [26]. Specifically, TGFBR2 and ACVR2 mutations are significantly rarer in IBD. Furthermore, HPP1 methylation occurs early, in non-dysplastic and dysplastic mucosa, whereas RAB32 methylation occurs at the transition to invasive growth, being rarer in dysplasias. Therefore, it is reasonable to assume that these mutations evolve through different pathways, leading to differentially expressed instabilotypes.

Tumor necrosis factor-alpha
Growing evidence supports a role for various cytokines, released by epithelial and immune cells, in the pathogenesis of colitis-associated cancer. Cytokines are essential mediators of the interactions between activated immune cells and non-immune cells, including epithelial and mesenchymal cells. Tumor necrosis factor-alpha (TNF-α) is a potent pro-inflammatory cytokine thought to be involved in the pathogenesis of IBD. Recent data indicate that TNF-α promotes tumor development in models of experimental colitis. Popivanova et al. have recently shown that blockade of TNF-α reduces the formation of colorectal tumors in mice lacking the TNF receptor, p55 (TNF-Rp55 knockout) [27]. Combined treatment with azoxymethane and dextran sodium sulfate (AOM/DSS), which causes severe colonic inflammation and the subsequent development of multiple tumors, induced the intracolonic expression of TNF-α, which in turn regulated the trafficking of inflammatory cells, a major source of COX-2. These findings are of particular interest because they suggest that blocking of TNF signaling can reverse tumorigenesis even when CRC is already present, probably by reducing the infiltration of inflammatory cells and, consequently,
the circulating levels of COX-2. Therefore, strategies targeting TNF-α could potentially provide effective anti-tumor therapies.

It should be noted that the regulation of TNF-α is partly genetic. Single nucleotide polymorphisms (SNPs) in the promoter of TNF-α were found associated with an increased risk for IBD and may genetically predispose towards developing colitis-associated CRC [28].

**Nuclear factor kappaB**

Although the molecular mechanisms linking IBD with CRC are not well understood, recent studies in pre-clinical models point to NF-κB as a central player. NF-κB regulates the expression of various cytokines and modulates the inflammatory processes in IBD. On the contrary, NF-κB stimulates the proliferation of tumor cells and enhances their survival through the regulation of anti-apoptotic genes.

Pro-inflammatory cytokines have been suggested to regulate pre-neoplastic growth during colitis-associated tumorigenesis. Interleukin 6 (IL-6) is a multifunctional NF-κB-regulated cytokine that acts on epithelial and immune cells. The importance of the IL-6 family of pro-inflammatory cytokines and their downstream effector STAT3 in colitis-associated CRC has been recently described (reviewed in [29]). Human patients with CRC have been reported to have elevated levels of IL-6 and increased levels of IL-6 have also been shown in murine experimental models of colitis-associated cancer induced by AOM/DSS [30].

STAT3, a nuclear transcription factor downstream of gp130, is necessary for the growth of colitis-associated CRC in mice [31,32]. It appears to function through increased epithelial proliferation and protection against AOM/DSS-induced epithelial cell apoptosis. Interestingly, DSS-induced mucosal inflammation or colitis was markedly increased in both IL-6-deficient mice and in mice lacking STAT3 in intestinal epithelial cells, which correlated with an increase in the expression of pro-inflammatory cytokines within the colonic mucosa.

**Oxidative stress**

The exact mechanism by which chronic inflammation results in carcinogenesis is still unclear. Persistent inflammation is believed to result in increased cell proliferation as well as oxidative stress, leading to the development of dysplasia [33]. Oxidative stress develops particularly in inflammatory reactions because the inflammatory cells, activated neutrophils, and macrophages produce large amounts of reactive oxygen and nitrogen species (RONs). DNA damage caused by oxidative stress in the characteristic damage-regeneration cycle is a major contributor to CRC development in UC patients. Thus, oxidative stress-induced cellular damage may provide a mechanistic basis for many of the events thought to drive UC-associated colon carcinogenesis in humans and animal models, including specific gene alterations, genetic instability and aberrant methylation. The available evidence suggests that DNA damage caused by oxidative stress in the characteristic damage-regeneration cycle is a major contributor to CRC development in UC patients. RONs produced by inflammatory cells can interact with key genes involved in carcinogenic pathways such as p53, DNA mismatch repair genes, and others. Factors such as NF-κB and COX-2 may also contribute.

Studies in animal models of UC have provided support as to the involvement of oxidative stress in inflammation-driven CRC. Seril et al. have recently examined the role of nitric oxide (NO) in UC-associated colorectal carcinogenesis using the DSS-induced and iron-enhanced model of chronic UC in inducible nitric oxide synthase (iNOS) knockout mice. These results showed that there is no difference in UC-associated cancer development in iNOS+/− and iNOS−/− mice, suggesting that in the absence of iNOS, other factors, such as eNOS, may play a role in nitrosative stress and UC-associated carcinogenesis in this model system [34].

Notably, UC patients frequently experience iron deficiency anemia owing to chronic disease and colonic blood loss, and anemia is corrected in these individuals by iron supplementation. It has been suggested that iron over nutrition may contribute to the carcinogenesis process by augmenting oxidative damage and inflammation-caused epithelial proliferation. However, further clinical studies are needed to clarify this issue [35].

**Transforming growth factor-beta and other factors**

Despite a primary tumor suppressor role, there is increasing evidence suggesting that transforming growth factor-beta (TGF-β) can promote tumor growth, invasion and metastasis in advanced stages of CRC. TGF-β has been shown to attenuate an anti-tumor immune response through the induction of regulatory T cells in spontaneous and inflammation-associated cancer. However, IL-6 signaling promotes tumor growth in experimental colitis-associated cancer, and this signaling has been shown to be inhibited by TGF-β [36]. Therefore, the role of TGF-β in this setting is not entirely clear.

Members of the IL-12 family, p35/p40 play an important role in T helper (Th) cell polarization and Th1 T cell differentiation. Recent findings have shown that both p35 and p40 can form other cytokines by heterodimerizing with different proteins (IL-23: p19/p40; IL-15: p35/EBI3). Furthermore, the cytokine IL-27 (EBI3/p28) was recently identified as a member of the IL-12 family. These cytokines have been implicated in the pathogenesis of colitis and IL-23, which plays a key role in the induction and maintenance of gut inflammation during
IBD, seems to be involved in inflammation-associated carcinogenesis (reviewed in [37]).

While some cytokines promote tumor growth, others such as IL-10, provide an anti-tumor effect. IL-10-deficient mice develop colitis and colitis-associated cancer within two to three weeks after birth. The IL-10 knockout mouse model results in a disease similar to human IBD and therefore, has been proven useful as an experimental model for developing new and effective therapies for active IBD and CD, in particular. However, a previous report aiming to identify possible mutations of oncogenes and tumor suppressor genes involved in colorectal tumorigenesis in these mice, showed no alterations in K-ras, p53, APC and MSH genes, suggesting that other genes are involved in the development of these tumors [38]. Therefore, despite the high similarities between these histopathological pattern of CRCs in IL-10-deficient mice and IBD-associated carcinomas, this model is apparently not appropriate for investigating IBD-associated carcinogenesis.

Since most of the above mentioned cytokines are involved in both, inflammation and carcinogenesis, it is difficult to assess their contribution to the individual steps during pathogenesis of colitis-associated cancer.

**Conclusion**

Patients with IBD have an increased risk of developing CRC. This risk appears to be closely associated to the cumulative effect of chronic inflammation and is in direct correlation with the severity of the inflammation and the extent and duration of the disease. The exact mechanism as to how chronic inflammation results in carcinogenesis is not resolved, although it is becoming clear that chronic inflammation and IBD in particular, is an important driving mechanism that promotes the development of cancer in the colon. Further studies are needed in order to elucidate the pathogenesis of colitis-associated CRC.

**References and recommended reading**

Papers of particular interest published within the period of review have been highlighted as:

- of special interest
- of outstanding interest

   This article reviews the current knowledge of the molecular and cellular pathways that link inflammation and cancer and describes how these pathways suppress anti-tumor immunity during cancer progression.


   This review discusses recent epidemiological trends and risk factors for CRC in patients with ulcerative colitis. The authors also describe the contribution of genetic factors to the pathogenesis of CRC in IBD.


   This is a perspective review that discusses the role of cytokines in causing symptoms in patients with cancer and their influence in the development and progression of cancer.


   This study evaluates the role of constitutive NF-κB activation in CRC cells and shows that this activation plays a role in angiogenesis and anti-apoptosis. These results suggest that NF-κB blockade may be a reasonable therapeutic approach for IBD and colitis-associated cancer.


   This study shows that IKK plays an NF-κB independent role in CRC and suggests a role for IKK in the regulation of NF-κB-independent genes such as, Notch targets.


   This review addresses the clinical and molecular features of colitis-associated CRC and also of other cancers in patients with IBD.


Cancer

IBD-associated intestinal neoplasias were screened for microsatellite instability. The study shows that the mutational events in target genes for instability were similar in IBD tumors and in CRCs.


This study demonstrates that TNF-α, through its effects on the immune system, plays a crucial role in promoting neoplastic transformation in the setting of chronic inflammation.


Using DNA extracted from formalin-fixed, paraffin-embedded tissues from ulcerative colitis-associated CRC cases and matched controls, the authors have demonstrated an association between TNF-α polymorphisms and UC-CRC. This is a novel finding supporting a genetic predisposition for the development of colorectal neoplasia in IBD patients.


This study demonstrates the importance of IL-6 and its downstream effector STAT3 in colitis-associated colon cancer. It also establishes a role for IL-6 and STAT3 signaling in IECs during inflammation-associated colon carcinogenesis. IL-6 was identified as a crucial NF-κB-dependent pro-tumorigenic cytokine. Both IL-6 and STAT3 are suggested as potential targets for the prevention and treatment of IBD neoplasia.


This study demonstrates that in addition to IKK-dependent NF-κB activation, STAT3 comprises a central checkpoint during inflammation-associated cancer. STAT3 expression and activation was genetically manipulated in the intestinal epithelium and was found to modulate colitis-associated tumorigenesis.


