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Review

From Inflammation to Cancer in Inflammatory Bowel Disease: Molecular Perspectives

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Abstract. *Inflammatory bowel diseases (IBD) are associated with an increased risk of colitis-associated colorectal carcinoma (CAC). CAC is one of the most important causes of morbidity and mortality in patients with Crohn's disease and ulcerative colitis. The aim of the present review was to discuss the most important signaling pathways and genetic alterations involved in carcinogenesis related to IBD, focusing on the molecular aspects of cancer stem cell physiology and the impact of the inflammatory microenvironment. Molecular mechanisms involved in CAC development differ from those in sporadic colorectal cancer, reflecting the prominent role of inflammation-induced carcinogenesis in the development of CAC. The alteration of the physiological microenvironment is thought to be responsible for the initiation of carcinogenesis in IBD. Furthermore, cancer stem cells seem to have a fundamental role in the generation and growth of CAC. We also address prevention and treatment modalities of CAC and its involvement in IBD.*

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Colonic carcinogenesis exemplifies the connection between chronic inflammation and the origin of cancer, and has long been discussed and investigated. Colitis-associated colorectal cancer (CAC) is a tumor that develops in the context of chronic inflammation, differs from sporadic colorectal cancer, and is considered the most serious complication of IBD. Patients with ulcerative colitis (UC) are more likely to develop colorectal cancer (CRC) (20-fold to 30-fold risk) compared to the general population (1). In addition, the risk of developing CAC increases notably 8-10 years after diagnosis of IBD (2). Moreover, CAC has a greater malignant potential than sporadic CRC and the typically advanced stage of CAC at diagnosis reduces life expectancy (3) (Figure 1).

The pathophysiological mechanisms that lie behind the elevated risk of colon cancer in IBD, particularly in UC, are not clear.

According to the cancer stem cell (CSC) concept, only a minority of CSCs or cancer-initiating cells are capable of generating a new tumor. Moreover, CSCs are known to have pluripotent and self-renewal potential (4). These cells were first discovered in leukemia and more recently in solid tumors. New research implies that the CSC concept also applies to CRC (5, 6).

The increased risk of cancer in patients with IBD may be associated with the chronic proliferation required to repair damage to the epithelial monolayer caused by constant inflammation. Tumor necrosis factor α (TNFα) plays a fundamental role in inflammation in IBD and has been the target of biological treatments. This cytokine provokes inflammation by stimulating the production of interleukine (IL) 1b and IL6, inducing expression of adhesion molecules, proliferation of fibroblasts, activation of pro-coagulant factors, and cytotoxicity of the acute-phase response (7).

CSCs, the microenvironment, and the immune system interact through many cytokines. In chronic inflammation, cytokines secreted by immune cells stimulate essential CSC pathways, such as the wingless-related integration site (WNT)–β-catenin, the TNFα–NFκB and the IL6–signal transducer and activator of transcription 3 (STAT3) pathways (8-10).

The Inflammatory Process: Focus on the Microenvironment (Figure 2)

Canonical IL6 signaling and trans signaling. IL6 is a pro-inflammatory cytokine related to carcinogenesis in various tissues. In murine models of colitis-associated cancer, the canonical IL6 receptor pathway triggers the production of Janus kinase 2 (JAK2), activating STAT3, which can allow evasion of immune surveillance, promote cell growth and increase survival signaling (11, 12). However, the pro-oncogenic effects of STAT3 are mostly evident following inactivation of the negative regulators of IL6 signaling in patients with UC, such as the suppressor of cytokine signaling 3 (*SOCS3*), a direct target gene of STAT3 which suppresses IL6 signaling *via* degeneration of the signaling intermediates (13). Interleukin-6 up-regulates STAT3-mediated transcription of *miR214* in human colonic tissues, which reduces levels of PDZ and LIM domain 2 (PDLIM2) and phosphatase and tensin homolog (PTEN), increases phosphorylation of protein kinase B (AKT), and activates NF-κB. Activation of this mechanism initiates the disease process in patients with UC, that progresses towards CRC. The transcription factor STAT3 is an important pro-tumorigenic IL6 effector. The depletion of this effector in intestinal epithelial cells prevents tumor multiplicity and progression (14).

During early CAC induction in mice, IL6 works as a tumor promoter and is mostly generated by myeloid cells. Nevertheless, other immune cell types, such as T-cells, and intestinal epithelial cells, also produce IL6. Bone marrow-derived cell IL6 is commonly associated with underlying chronic inflammation, but IL6 generated in epithelial and cancer cells may also produce new tumors (11). In IBD, IL6/STAT3 expression is higher in epithelial cells of biopsy specimens from patients with active UC than in patients with inactive UC and controls. Moreover, IL6/STAT3-positive staining on both epithelial and non-epithelial cells highlights an association with the severity of colitis (15). In addition, SOCS3 levels in biopsy specimens from patients with active UC appear to be higher than levels in healthy controls, which may explain the downstream effect of increased IL6/STAT3 signaling, despite insufficient activity of SOCS3 in this phase to fully impede IL6/STAT3 activation. This overactive IL6 signaling phase may be in perfect timing with therapeutic restriction of the IL6 signaling pathway.

Conversely, research on mouse models has shown that the absence of glycoprotein 130 (GP130)–IL6–STAT3 signaling in intestinal epithelial cells increases susceptibility to dextran sodium sulphate (DSS)–induced colitis. This may be due to a diminished epithelial cell survival and proliferation (16). Thus, the high constitutive expression of SOCS3 in epithelial cells in inactive UC may inhibit normal IL6–STAT3-mediated epithelial cell homoeostasis, resulting in high susceptibility of these epithelial cells to inflammatory damage. Li *et al*. (17) denoted significant silencing of *SOCS3* expression in the inflammation–dysplasia–carcinoma sequence in patients with UC, but lacking in patients with Crohn's disease (CD)-associated dysplasia and patients with CD-associated CRC. *SOCS3* down-regulation appears to delimit the extent of the dysplastic and cancerous area in patients with UC-associated dysplasia and UC-associated CRC, respectively. IL6 signaling can provoke an increase in methylation of SOCS3 by stimulating increased expression of DNA (cytosine-5-)-methyltransferase (*DNMT1*), which leads to increased signaling through STAT3. *DNMT1* is up-regulated in UC and its expression involves areas with high IL6 signaling and those affected by cancer. The *SOCS3* promoter STAT3-binding element is crucial for SOCS3 induction in response to IL6, and its methylation seems to establish a rate-limiting step in UC-associated CRC generation. IL6–STAT3 signaling has been shown in vitro to stimulate both colonic epithelial cell proliferation and to induce resistance to apoptosis, with recent investigations indicating that STAT3 expression can also inhibit inflammation in the colon *via* multiple but mainly tolerogenic processes (18, 13). IL6 also regulates self renewal of CSCs: this molecule may promote malignant phenotypes in neurogenic locus notch homolog protein 3 (NOTCH3)-expressing stem cells from human breast cancer and normal mammary glands (19), moreover it is noteworthy that a study by Lin *et al*. (6) suggests that IL6–STAT3–IL8 activation in colon cancer-initiating cells may contribute to the development of colonic cancer (9).

Signaling through membrane-bound IL6R is known as the classical or the *cis*-signaling pathway. The presence of soluble IL6R also allows IL6 to act through a so-called *trans*-signaling pathway (20). Soluble IL6R, generated by either mRNA alternative splicing (10%) or shedding of membrane-bound IL6R by metalloproteases (90%), is mainly produced

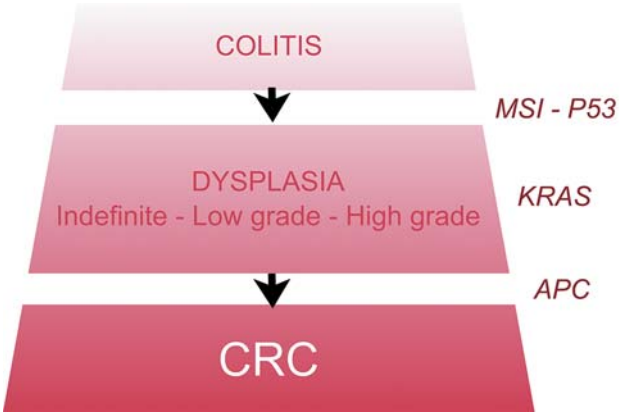

Figure 1. *Colitis-associated cancer (CAC) is a tumor that develops in the context of chronic inflammation and differs from sporadic colorectal cancer (CRC) and is considered the most serious complication of inflammatory bowel diseases. CAC has a higher malignant potential than sporadic CRC and the typically advanced stage of CAC at diagnosis reduces life expectancy. MSI: Microsatellite instability; KRAS: V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; APC: adenomatous polyposis coli.*

by neutrophils, macrophages, and some $CD4^+$ T-cells. Soluble IL6R forms a complex with IL6 and interacts with cell surface GP130. The fully assembled, activated IL6R complex is hexameric in structure and contains two of each IL6, soluble IL6R (or membrane-bound IL6R), and GP130 molecules, and this complex promotes the signaling pathway (JAK/STAT) in a larger number of cell types. Both classical and *trans*-signaling are mediated by GP130, and both activate the same intracellular pathway. The *trans*-signaling mechanism allows IL6 to act on non-leukocytes, including fibroblasts, epithelial cells, synoviocytes and cancer cells, that do not commonly react to IL6 (19). The activation of the IL6–STAT3 *via* IL6 *trans*-signaling is fundamental in the pathogenesis of IBD, which depends on mucosal macrophage-derived IL6/soluble IL6Ra, moreover IL6 *trans*-signaling is known to be involved in large-bowel cancer. The protein and mRNA expression levels of the membrane-bound form of IL6Ra seems to be higher in EGF-like module-containing mucin-like hormone receptor-like 1 $(F4/80)^+$ *lamina propria* macrophages during the development of CAC.

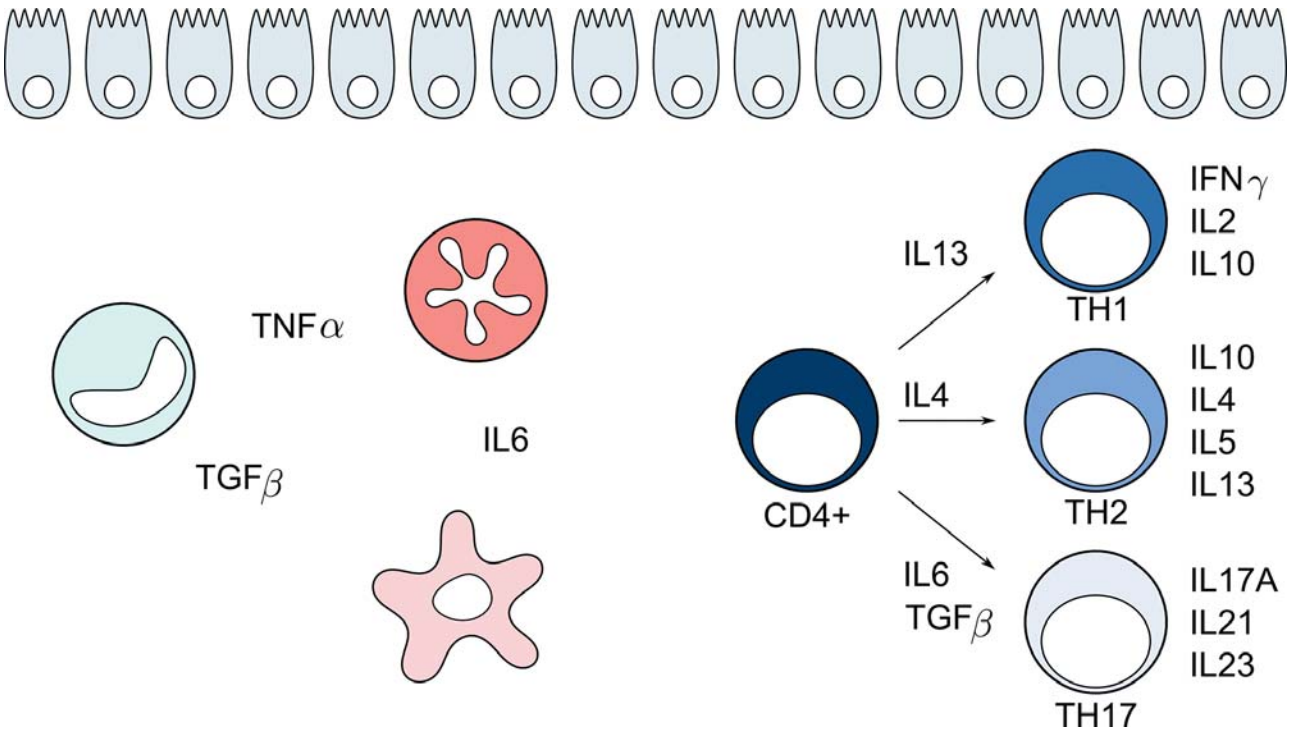

Figure 2. *The role of the microenvironment in the genesis of the inflammatory process. The figure shows the main cytokines underlying the inflammatory process underlying the genesis of tumor. TNFα: Tumor necrosis factor α; TGFβ : transforming drowth factor β; IL: Interleukin; TH: T Helper; CD: cluster differentiation; IFN γ: interferon γ.*

Furthermore, the induction of TNFα converting enzyme (*TACE*) mRNA in *lamina propria* mononuclear cells is related to a rise in soluble IL6Ra expression occurring in colonic epithelial cells during inflammation-based colonic carcinogenesis, together with a reduction of membrane-bound IL6Ra biosynthesis (21).

Matsumoto *et al*. (22) demonstrated that the treatment with soluble GP130Fc, a competitive inhibitor of IL6 *trans*-signaling, suppressed the development of CAC in BALB/c mice, which seems to be influenced by the inhibition of NF-κB.

TACE is a key enzyme involved in the shedding of soluble IL6Ra, TNFα, and the ligands of epithelial growth factor receptor from the cell membrane and according to Matsumoto *et al*., TACE induction in inflammatory macrophages is associated with the presence of enteric bacteria, while Kado *et al*. noted that the microflora in the intestine has an impact on the development of colonic adenocarcinoma in *Tcrb*$^{-/-}$ and *Tp53*$^{-/-}$ mice (23).

The infiltration of activated CD4^{+} T-cells into the colonic mucosa in colorectal cancer- and CAC-affected mice is also a key factor which may be owing to IL6 *trans*-signaling.

Other cytokines. TNFα: The pro-inflammatory cytokine TNFα is significant in IBD pathogenesis (11). Binding of TNFα to its receptor causes, in a process facilitated by NADPH oxidase, the activation of mitogen-activated protein kinases (MAPKs) and of the NF-κB pathway, which may influence barrier permeability. NF-κB activation may lead to increased transcription of pro-inflammatory cytokines, resulting in a continuous feeding of the inflammatory cycle, and increased expression of myosin light chain kinase (MLCK) (13,16), which stimulates permeabilization of the intestinal barrier. In this regard Greten and colleagues investigated the association of IkB kinase B–NF-κB in colonic carcinogenesis induced in mice by combined treatment with azoxymethane and dextran sulfate sodium. They revealed that ablation of inhibitor of nuclear factor kappa-B kinase beta (IKKB) in intestinal epithelial cells led to an increase in epithelial apoptosis and simultaneously reduced tumor incidence with no change in tumor size or inflammation. On the other hand, depletion of IKKB in myeloid cells reduced tumor size and inflammation without affecting apoptosis (24).

In addition Popivanova *et al*. observed that colonic inflammation, as tumor incidence and size, were reduced in a TNF–RP55-deficient (*Tnf-Rp55*$^{-/-}$) mouse UC model (25). In this study epithelial cell apoptosis increased in wild-type mice, and not in *Tnf-Rp55*$^{-/-}$ mice. Furthermore, both TNFα and TNF-RP55 were evident in infiltrating cells but not in epithelial cells, therefore it is likely that endogenously produced TNFα induces NF-κB in inflammatory cells *via* interaction with TNF-RP55. Moreover, *Tnf-Rp55* gene ablation and administration of TNF antagonist appear to reduce both cyclooxygenase 2 (COX2) expression and tumor angiogenesis. According to ongoing research, prostaglandin E_2 (PGE_2) seems to directly promote epithelial cell survival and intestinal adenoma formation (26). Moreover, PGE_2 presence seems to increase colonic cancer cell invasion. Of note is the promotion of nuclear translocation of β-catenin following PGE_2-induced hepatocyte growth factor receptor transactivation (27). Thus, COX2–derived PGE_2 may have a direct impact on carcinogenesis through regulation of the WNT signaling pathway. Therefore, interruption of TNF signaling is likely to restrain colonic carcinomas from progressing by reducing COX2 expression and subsequently arresting the WNT signaling pathway (25).

IL17-IL23: Inflammatory bowel events are frequent in patients with CD and UC in relation to excessive cell responses of Th1 and Th2, respectively. Nevertheless, recent studies have reported an increased production of a subset of Th17 cells in both conditions. Some studies have also demonstrated that this T-cell subset promotes immune-mediated variations in IBD (28, 29).

IL17 and IL23 seem to be responsible for the maintenance of the Th17 phenotype (30). Interleukin 23 receptors (IL23R) have been implicated in chronic inflammatory diseases through their role in initiating the differentiation of Th17 (31, 32). Recent evidence in the mouse indicates that TGFβ and IL6 drive the differentiation of Th17 cells from naïve T cells and induce IL23R expression on Th17 cells (33). Moreover it seems that IL23 stabilises the Th17 response and that in the intestine IL23 drives IL17-independent inflammatory pathways (34).

There are two forms of IL17: IL17A and IL17F. Peripheral blood and tissues levels of IL17A are increased in a variety of patients with cance, while IL17F appears to be down-regulated in human colonic cancer tissues. IL17A, generated from naive CD4^{+} cells, mediates inflammatory reactions in Th17 cells in IBD. Recent investigations have reported an association between IL17A and tumor formation, among them Wu *et al*. (35) and Chae *et al*. (36) reported potential inhibition of tumor formation after IL17A receptor blockade in mice models of colorectal tumorigenesis, while Hyun *et al*. showed that blocking IL17A considerably reduced the expression of IL6, STAT3, TNFα and IFNc in mice with *Il17a* knockout, thereby demonstrating reduced epithelial cell proliferation (37). The blockage of IL17A thus leads to an important modification in the microenvironment in IL17A-related inflammation and tumors. STAT3 determines IL6-induced Th17 cell differentiation, which subsequently leads to production of IL17A (38), showing that IL17A contributes to the tumor-initiation stage in the advancement of CAC. The dysregulation of cell-cycle seems to be also involved in IL17A related atypical cell proliferation, which will progress towards tumor development (39).

Cold-inducible RNA-binding protein (CIRP): This protein was initially found in the testis as the first mammalian cold-shock protein and is necessary for preserving neural stem cells. CIRP is generated through cellular stresses such as UV irradiation and hypoxia, and responds to such stress by migrating from the nucleus to the cytoplasm. As a result, it modifies the expression of its target mRNAs and, when present extracellularly, acts as a damage-associated molecular pattern molecule that eventually enhances the inflammatory processes. CIRP also has an impact on cell growth and cell death triggered by TNFα or genotoxic stress. In addition, activation of TNFα and IL23/IL17 signaling are immune responses driven by CIRP which have an influence on the proliferation of stem cells and enhance the expression of stem cell markers, such as doublecortin like kinase 1 (DCLK1) and sex determining region Y-box 2 (SOX2). Significantly, a decrease in the number of $SOX2^+$ and $DCLK1^+$ cells in tumor was observed upon CIRP deletion in the hematopoietic compartment (7).

IL21: A cytokine synthesized by $CD4^+$ Th cells, including Th1 and Th17 cells, activated natural killer (NK) cells, and T follicular helper cells, IL21 is a key regulator of the proliferation and effector function of B-cells, T-cells, and NK cells, and it also affects regulatory T-cells. IL21 is also able to modify the activity on non-immune cells, and plays an important role in allergic disorders and autoimmune diseases (40). IL21 is overexpressed in the mucosa of the colon in patients with UC (41), and positively regulates Th17 cell responses (42). Absence of IL21 resulted in reduced colonic inflammation and a decrease in both tumor incidence and size in *Il21* knockout mice according to Stolfi *et al*. (43); IL21 may engage an inflammatory circuit that enhances the progression of colonic cancer. Moreover, according to these authors, mice lacking in IL21 showed a reduction in STAT3 expression, both in the *lamina propria* and gut epithelium after azoxymethane and dextran sulfate sodium administration. Reduced expression of B-cell lymphoma-XL, a STAT3-induced anti-apoptotic protein, was also observed in these animals. The reduced expression of STAT3 in cancer cells may be due to the decrease of IL6 and IL17A associated with the lack of IL21 expression, as it has been demonstrated that human colonic epithelial cells express IL21R and respond to IL21 by up-regulating chemokine synthesis (44). Evidence has also shown that enhanced antitumor immunity elicits a reduction in tumor cell growth when IL21 is overexpressed (45). Like other cytokines, IL21 may have contrasting effects on tumor growth, depending on tissue type and local immune activation (43).

IL13: IL13 is secreted by activated T-cells, NK cells, human airway smooth muscle cells, renal cell carcinomas, and Hodgkin's Reed-Sternberg tumor cells, and plays an active role in allergic inflammation, fibrosis, goblet cell hyperplasia, and tumor cell growth. The biological effects of IL13 may be the result of mediation of a shared receptor composed of the IL4Rα and IL13Rα1 chains, and a decoy receptor containing high affinity IL13Rα2 protein. On hematopoietic cells, the IL13 receptor is composed of an IL13Rα1 chain with low binding affinity to IL13, the shared γC cytokine chain, and the IL4Rα chain. In non-hematopoietic cells lacking in γC, the receptor consists of IL13Rα1 and IL4Rα (46, 47). IL13Rα1 is also an accessory component of the IL4 signaling complex. The inhibitory IL13Rα2 chain has high affinity for IL13. Accordingly, the equilibrium between IL13Rα1 and IL13Rα2 expression enhances the potential of the IL13 signal (48). In patients with IBD, IL13Rα1 and IL13Rα2 expression is significantly increased in UC and CRC intestinal epithelial cells, when compared to control and cells in patients with CD. It is noteworthy that IL13 stimulation has no effect on STAT6 or MAPK pathways in UC but activates the STAT6 pathway in tumors of the colon. Moreover, IL13Rα2 acts both as an initiator in MAPK signaling at low concentrations and as an inhibitor or decoy receptor at high IL13Rα2 to IL13Rα1 ratios. Thus IL13Rα2 is both a decoy receptor and induces MAPK signal transduction, in relation to expression and the local concentration of IL13, which conjointly regulate the equilibrium and the strength of the signaling pathways initiated in UC and CRC (49). Inflammation of the colonic mucosa may be modified by overexpression of the Th1 T-cell response related to an increase in IFNγ and IL12 secretion or overexpression of Th2 T-cell response in relation to an increase in IL4, IL5 and IL13 secretion (50). IL4 and IL13 modify expression of activation-induced cytidine deaminase (AID) in a STAT6-dependent manner and promote atypical AID expression in cultured colonic epithelial cells (51). Aberrant AID expression is likely to target the *TP53* gene in human colonic epithelial cells, whereas longer AID activation may instigate further mutations. In contrast to the *TP53* gene, no nucleotide alterations were observed in the *APC* and *KRAS* genes, following 8 weeks of AID activation (52). Moreover a lack of endogenous AID reduces the incidence of somatic mutations in the *Trp53* gene, as well as reducing the risk of developing cancer of the colon in inflamed colonic mucosa, and colitis-associated colonic cancer (53).

Transforming growth factor β (TGFβ): In patients with IBD, elevated TGFβ1 expression was detected in the serum and mucosal tissue, evident in epithelial cells, adjacent fibroblasts and inflammatory cells (54-56). An increase in TGFβ1 expression under inflammatory conditions may be due to the down-regulation of the body's immune responses in an attempt to control inflammation. TGFβ1 plays an important role in the epithelial–mesenchymal transition (EMT) (57, 58). Schäfer *et al*. supported this hypothesis by demonstrating that TGFβ1 can elicit the expression of the adhesion molecule L1CAM in human NCM460 colonocytes regulated by the

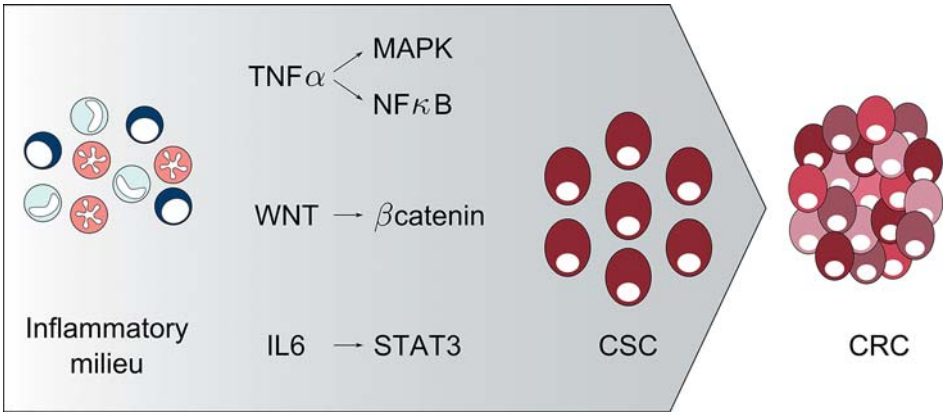

Figure 3. *The main molecular pathways involved in cancer initiation. TNFα: Tumor necrosis factor α; WNT: wingless-related integration site; NFkB: nuclear factor kappa-light-chain-enhancer of activated B-cells; IL: interleukin; CSC: cancer stem cell; CRC: colorectal cancer.*

activation of JNK (59). Therefore, macrophage-derived TGFβ1 induces a migratory and apoptosis-resistant phenotype of intestinal epithelial cells through SLUG induction and subsequent L1CAM gene expression. This cellular phenotype response leads to full epithelial–mesenchimal transition (EMT) induction or may develop independently of EMT in intestinal epithelial cells and subsequently in CRC cells. Moreover, in the work by Schäfer *et al*., L1CAM expression was elevated in intestinal epithelial cells in UC and CD tissues and associated with the presence of macrophages, but was not detectable in normal colonic tissues. In addition, a rise in epithelial L1CAM expression with duration of inflammation was observed in the colon of patients with IBD. Investigations on a murine UC colonic tumor model further confirmed these findings, suggesting involvement of anti-inflammatory macrophages in the development of cancer associated with inflammation (60).

Molecular Pathways Involved in Cancer Initiation (Figure 3)

Genetic instability. Reports have revealed several molecular alterations in long-standing UC which involve inactivation of tumor-suppressor genes, oncogene mutations, loss of heterozygosity, and chromosomal and microsatellite instability (MSI) (61). Distinct genetic modifications may be observed in the gradual progression of inflammation, so-called step-associated genetic alteration, considered fundamental to comprehending the molecular basis of neoplasms in IBD, Chen *et al*. demonstrated that instability is present in non-dysplastic mucosa of patients with UC-associated dysplasia or with cancer, and maintains the same progression during histological development to cancer. Genomic instability occurs in the colon only and is not present in the small bowel or in the organs outside the gastrointestinal tract. A higher level of genomic instability was observed in the negative biopsies of patients with UC with dysplasia compared to patients with UC who were dysplasia/cancer-free. Genomic instability and subsequent tumor development in a subset of patients with UC suggests that the colonic epithelium is damaged by reactive oxygen species (ROS) produced in the context of the inflammatory milieu and accounting for oxidative stress and cellular damage in terms of oxidation of proteins and DNA. Failure to remove or repair ROS-initiated damage can be either mutagenic or lethal to cells (62). Moreover, genomic instability does not increase during the neoplastic processbut occurs early and gradually in cancer development (63).

Epigenetic changes indicate that DNA methylation may be a molecular mediator of neoplastic modifications. For example, inactivation of tumor-suppressor genes [*e.g. TP53*, kruppel-like factor 6 (*KLF6*), APC, *KRAS*, and deleted in colorectal cancer (*DCC*)] has been detected in patients with IBD-associated cancer (64). Silencing of tumor-suppressor genes occurs in association with aberrant promoter methylation and in the absence of coding-region mutations. In the context of the progression of inflammation in IBD, DNA methylation is a key factor in a subset of tumors affected by the CpG island methylator phenotype, a pathway that emerges as a form of epigenetic instability (65). Gene-specific hypermethylation is most likely to be involved in the development of colonic cancer and is fundamental as a molecular marker in colonic neoplasia. Furthermore, aberrant DNA methylation of tumor-suppressor genes may arise secondary to a genetic predisposition or to a field-cancerization effect in the colon and may be considered as a molecular marker predicting development of colonic cancer.

The *TP53* mutation in sporadic CRC usually develops at a later stage in the adenoma–carcinoma sequence but in patients with IBD, *TP53* mutation develops early and is observable in nondysplastic mucosa (58). *TP53* expression is more frequently mutated in CAC than in sporadic CRC and the pattern is regularly altered in colonic mucosa in noncancer-inflamed IBD, common of histological features of inflammation. These results confirm the importance of inflammation in colorectal carcinogenesis in patients with IBD with colonic disease, with some studies showing both mutations and loss of heterozygosity in the p53 tumor suppressor gene in UC-associated CRC. Mutations are frequently observed in early UC-associated dysplastic lesions as in non-dysplastic, non-cancerous mucosa in UC, and precede loss of heterozygosity of *TP53* (66-69). In addition, Nathanson *et al*. found an association between *TP53* mutations and dysplasia and progression of dysplasia in patients with CD (70). Therefore, the *TP53* tumor-suppressor gene appears to be a key factor in the early stages of IBD-associated colorectal carcinogenesis. Research has further acknowledged that *TP53* mutation is the most common founder mutation in CAC, while the *KRAS* mutation is observable only in a small subset of patients with lesions. It appears that *TP53* abnormalities are driven by inflammation, this hypothesis being supported by the presence of increased TP53 expression in inflamed, nondysplastic, noncancerous colonic mucosa in IBD (71).

Fleisher *et al*. found that 15% of sporadic CRC exhibit defective DNA mismatch repair, manifested as MSI. Promoter hypermethylation of the mismatch repair gene mutL homolog 1 (*MLH1*) was significantly related to MSI in this group of carcinomas. In addition, investigations involving the same group hypothesized the correlation between *MLH1* hypermethylation and decreased MLH1 protein expression and MSI in neoplasms from patients with IBD. It was indeed reported that in IBD-associated neoplasia, *MLH1* promoter hypermethylation commonly occurred in the setting of MSI, in particular high MSI (72). Furthermore, *MLH1* hypermethylation and MSI are strongly related to decreased MLH1 protein expression in IBD-associated neoplasms. The role of 1219V polymorphism of *MLH1* has been investigated and is reported to influence the clinical characteristics of UC and the likehood of treatment resistance (28).

Bossard *et al*. showed the high sensitivity of the activating V600E *BRAF* mutation as a biomarker for the serrated neoplasia pathway in inflammatory mucosa in patients with IBD. Furthermore, the occurrence of a mucinous adenocarcinoma of the right colon displaying a *BRAF* mutation and MSI-H status close to a traditional serrated adenoma with a *BRAF* mutation and microsatellite stable status indicates that *BRAF* mutation may be an early molecular event preceding MSI, as in sporadic oncogenesis. (73). These activating mutations occur very early in IBD-related oncogenesis, since *KRAS* mutations are observable in inflammatory mucosa lacking dysplasia. Additionally, *KRAS* mutations are more frequent than *BRAF* mutations, as reported by Aust *et al*. and others, with 18% *versus* 9% in UC-related adenocarcinomas (74).

WNT–β-catenin. WNT proteins are secreted glycoproteins that interact with seven-pass transmembrane receptors of the Frizzled family and single-pass transmembrane co-receptors, such as lipoprotein receptor-related protein 5/6, neurotrophic tyrosine kinase, receptor-related 2 and related to receptor tyrosine kinase (75). The interactions between *WNT* ligands and their receptors lead to the activation of various intracellular signaling cascades that can be cross-connected or may act independently. *WNT* signaling may determine a number of different processes based on the activated pathway, including cell proliferation, differentiation, migration, and polarity and asymmetric cell division (76). *WNT* pathways fall into two general categories: canonical and noncanonical *WNT* signaling. Canonical *WNT* signaling is often referred to as the *WNT*–β-catenin pathway, due to the activation of β-catenin-dependent transcription following *WNT*-stimulated signals. In contrast, noncanonical *WNT* pathways, including the *WNT*/Ca^{2+} and *WNT*/JNK pathways, are β-catenin-independent and are likely to activate different intracellular signaling cascades (77-79). In KAD rats (homozygous for the *Apc* D2523 mutation), *WNT* signaling remains unchanged and the β-catenin binding sites are intact. The characteristic differences were ascribed to the physiological function of the *C*-terminus of APC. Research has confirmed that the onset and continuation of IBD is related to abnormal angiogenesis and that poor mucosal healing in IBD is due to microvessel dysfunction (80, 81). As with human IBD, decreased angiogenesis in the inflamed mucosa was also observed in KAD rats, and the KAD vascular endothelial cells revealed a reduced adhesive activity. The pathogenesis of IBD involves disks large homolog 5 (DLG5) in humans. In rats, expression of DLG5 can be induced by DSS treatment, and DLG5 binds to the C-terminus of APC, which is absent in KAD rats. Therefore, both APC and DLG5 may be involved in the pathogenesis of IBD (82).

Intestinal stem cells have several signaling pathways, such as WNT, bone morphogenetic proteins, PI3K/AKT, HEDGEHOG, and Notch. In particular, WNT and NOTCH signaling pathways are major pathways in stem cell proliferation and differentiation capacity (83). The WNT signaling pathway is able to stimulate progenitor cell proliferation, sustain a cycling cell type, and suppress differentiation, while the NOTCH pathway sustains the progenitor cells in an undifferentiated, proliferating state

(84). It seems that in IBD, canonical WNT and NOTCH signaling pathways are activated to stimulate the proliferation of intestinal stem cells in IBD, in order to reverse inflammation. The mutation of the *APC* tumor-suppressor gene in patients with familial adenomatous polyposis is characteristic of the presence of overactive WNT signaling in CRC. Non-canonical WNT signaling may be prevented by canonical signaling during inflammation (85). Two studies have suggested that WNT5A, involved in noncanonical WNT signaling, may counteract the oncogenic effects of the canonical WNT–β-catenin pathway (86). The WNT signaling pathway consists of proteins that transmit signals from the outside of the cell to the inside of the cell *via* cell-surface receptors. The pathway is activated by the binding of WNT protein ligands to a frizzled family receptor, which passes the biological signal to the dishevelled protein inside the cell. Early seminal studies by Shih *et al*. (87) and Reya and Clevers (88) revealed WNT signaling abnormalities in CSC and CRC cells and suggested the early involvement of CSC WNT signaling during CRC carcinogenesis. Recently, the role of epigenetic silencing of WNT-signaling pathway genes in the pathogenesis of IBD-associated neoplasia has been examined (64). The study by Dhir *et al*. (89) showed that methylation of the WNT-signaling genes occurs in early-stage IBD with a gradual rise in methylation of these genes during progression of IBD-associated CRC. Moreover, methylation of *APC1A, APC2,* secreted frizzled-related protein 1 (*SFRP1*), and *SFRP2* seems to indicate the development from IBD-colitis to IBD-associated CRC, and suggests that these genes may be useful as biomarkers for IBD-associated CRC (90).

TNFα-NFκB. The NF-κB family of proteins includes five members: NF-κB1 and NF-κB2, v-rel avian reticuloendotheliosis viral oncogene homolog A (RELA) (also known as p65), RELB and c-Rel. These proteins bind DNA as homo- and hetero-dimers that influence gene expression upon interaction with the basal transcriptional machinery. Activation of TNF receptor-1 stimulates the classical NF-κB pathway which progresses to the translocation of NF-κB1 p50/RELA or p50/c-Rel heterodimers into the nucleus thus inducing the expression of genes that encode pro-inflammatory cytokines and anti-apoptotic proteins. The latter results in the processing of the NF-κB2 p100 precursor and nuclear translocation of NF-κB2 p52/RELB heterodimers, which determine the expression of chemokines and cell adhesion molecules involved in lymphoid tissue development and B-cell maturation (91). Polymorphisms in *REL*, the human homolog of c-Rel, have been identified as an IBD susceptibility locus, with the minor allele conferring an increased risk of both CD (92) and UC (93). Signaling mediated by c-Rel is required to select appropriate damaged cycling cells within the colonic mucosa to allow senescence or apoptosis. Loss of this function may favor the survival of cells with mutations which could be central to the onset of colonic carcinogenesis. Investigations involving mice revealed greater colonic crypt survival in *c-Rel*$^{-/-}$ compared to wild-type mice after γ-irradiation. Conversely, NF-κB2-mediated signaling seems to be fundamental in the initiation of DSS-induced colitis. Hence *Nfkb2*$^{-/-}$ animals subjected to DSS and AOM may protect against the development of colitis-associated colonic neoplasia, primarily by an attenuated inflammatory response (94).

The basic helix-loop-helix transcription factor Atonal homolog 1 (*ATOH1*) is an important gene regarding cell formation, and is required to differentiate towards secretory lineages in the small and large intestines (95). The ATOH1 protein can be stably expressed in human colonic cancer cells induced by TNFα treatment, and its stabilization induces both a CSC phenotype and a mucinous phenotype, which leads to the attainment of chemoresistance. Reports have confirmed that stabilization of ATOH1 proteins is influenced by enzyme activity of glycogen synthase kinase 3 beta (GSK-3b) (96). It appears that TNFα suppresses the enzymatic activity of GSK-3b *via* AKT phosphorylation of GSK-3b. Other NFκB signal stimulating factors, such as lipopolysaccharide and flagellin, also stabilize ATOH1 protein in DLD1 cells, suggesting that NFκB signaling *via* Toll-like receptors may affect the activity of GSK-3b. This suggests that NFκB may have a role in regulating both the malignant transformation of CAC and the pathological type by stabilizing ATOH1 protein during the carcinogenesis in IBD patients. ATOH1 was detected in cells treated with fluorouracil and oxaliplatin, which induced the suppression of GSK-3a kinase activity (97). According to Fukushima *et al*., the apoptosis signal produced by fluorouracil and oxaliplatin incubation for 48 h is repressed in Atoh1 gene-transfected DLD1 cells regardless of TNFα treatment. It seems that the WNT signal is repressed when ATOH1 stimulates differentiation of intestinal epithelial cells in normal mucosa, which leads to the degradation of β-catenin protein. However, the stabilization of ATOH1 protein in cancer cells results in the colocalization of β-catenin protein, stabilized by aberrant WNT signaling. In addition, the expression of LGR5 which may be promoted by β-catenin (98,99), is further promoted by ATOH1 (100).

IL6-STAT3. Blocking signaling to STAT3 plays an important role in the survival and growth of tumor cells (101), as described above. This molecule is indeed useful as a therapeutic target for cancer. Additionally, Lin *et al*. (6) demonstrated that IL6–STAT3 is activated in colon cancer-initiating cells. Studies have revealed that IL6 stimulates STAT3 (102) and is important in the development of colonic cancer (11, 12, 103). Interestingly, Lin *et al*. confirmed that ALDH^{+}/CD133^{+} cells express higher levels of IL6, GP130,

and IL8 and secrete higher levels of IL6 than $ALDH^-/CD133^-$ cells. They also observed that STAT3 activation in $ALDH^+/CD133^+$ cells is IL6-dependent and that IL6 is down-regulated after STAT3 inhibition, indicating that IL6 may be regulated by STAT3. Furthermore, reports indicate that activated STAT3 selectively binds to *IL8* promoter and induces *IL8* transcription and that IL6 would generate the expression of IL8, suggesting that IL8 may be a downstream target of IL6/STAT3 in colon cancer cells. IL6–STAT3–IL8 activation in colon cancer–initiating cells may be crucial in the development of colonic cancer. In addition, it seems that the expression of ALDH1 and CD133 decreases after treatment with LLL12, an inhibitor of STAT3 phosphorylation. In fact STAT3 inhibitors eliminated the aldehyde dehydrogenase $(ALDH)^+$/cluster differentiation 133 $(CD133)^+$ subpopulation of cancer-initiating cells in human colonic cancer cell lines. Moreover they showed that IL6/STAT3 blockade by STAT3, IL6 short hairpin RNA (shRNA) and 5-hydroxy-9,10-dioxo-9,10-dihydroanthracene-1-sulfon- amide (LLL12), suppressed tumor-initiating cell growth *in vivo* in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice.

The IL6/STAT3 pathway may be generated in glioblastoma stem cells (104) and in breast cancer–initiating cells, thus showing that activated STAT3 is fundamental in colon cancer-initiating cells (6). Constitutively active STAT3 in CSCs promotes proliferation and survival in mice, as well as tumor growth, while STAT3 blockage by GO-Y030 seems to inhibit tumor cell growth in vitro and *in vivo*. GO-Y030 is a potent inhibitor of the STAT3 pathway, suppressing tumor growth of colon CSCs in mouse models *in vivo* (105).

Mao *et al*. demonstrated that transgenic15-LOX1 expression in colonic epithelial cells significantly reduced colorectal tumorigenesis in two mouse strains of different genetic backgrounds, FVB/N and C57BL/6, confirming that the observed effects were not mouse strain-specific. This suppression of CAC seems to be associated with the suppression of crypt proliferative zone expansion, acting as a mechanism to promote CAC tumorigenesis (106) and IL6/STAT3 signaling in CAC is inhibited in a manner dependent on 15-LOX1 transgenic expression levels. Moreover, MUC1 and NOTCH3, which are downstream pro-tumorigenic targets of IL6–STAT3, were suppressed by transgenic expression of 15-LOX1. The down-regulation of *IL6* seems to occur at the transcriptional level, *via* PPARd down-regulation, in fact PPARd overexpression seems to increase IL6/p-STAT3 signaling during colitis induction *in vivo* (107). PPARd promotes colonic inflammation and tumorigenesis. In addition, PGE_2 mediates the crosstalk between colonic tumor epithelial cells and macrophages *via* a self-amplifying loop between PPARd and COX2-derived PGE_2 signaling pathways (108).

Therapeutic Implications

Anti-inflammatory drugs are used in patients with IBD not only to treat their chronic disease, but also to prevent the development of CAC: Effective chemoprevention may reduce the incidence and risk of developing colorectal cancer. Therapeutics that may be appropriate in sporadic CRC prevention are mostly drugs that suppress inflammation. Sporadic CRC and CAC are, in fact, enhanced *via* similar pathways with the aforementioned differences. Extensive resection is generally recommended following identification of carcinoma or dysplasia. Total proctocolectomy is the only surgical procedure that eliminates all lesions present and prevents the development of other lesions in UC. Due to the segmental nature of CD, less extensive resections are to be considered. Nevertheless, decision-making regarding surgery should entail assessment of patient's physical conditions, which may not include total proctocolectomy. Nevertheless, these patients will require frequent surveillance of their remaining colon or rectum (109).

Classical therapies. Aminosalicylates are an integral part of maintenance therapy in patients with IBD considering they are generally safe and inexpensive. It was shown that CAC incidence can be reduced by more than 75% when mesalazine is used, while sulfasalazine is less effective (110). Many investigations confirm the importance of mesalazine and even sulfasalazine, to a degree, as chemopreventive substances in the progression of colitis-associated cancer, notwithstanding reservations regarding their protective role (111). Ursodesoxycholic acid may prevent progression of CAC and dysplasia in patients with UC when compared to untreated patients; it may also reduce oxidative stress (110). Glucocorticoids are employed in the treatment of acute burst of IBD, however their role in chemoprevention of CAC is limited. (112). Traditional treatments of IBD regarding chemoprevention from CAC exploit the anti-inflammatory properties of the agents to interrupt some of the molecular pathways described previously, thus inhibiting the carcinogenic mechanisms which occur in the inflammatory microenvironment.

Biologicals and immunomodulators. Data are currently lacking to support the preventative properties of biological agents for dysplasia or CAC. However, animal studies suggest a protective role of infliximab against CAC when compared to standard non-biological therapy (113). In a case–control study in North America, the use of immunosuppressive therapy or anti-TNFα was protective in terms of risk of IBD-related CRC (114). Other biological treatment, such as anti-IL6 receptor antibody tocilizumab or VEGF antibody bevacizumab, have recently been proposed for treatment of IBD and CAC (112). There is also insufficient evidence regarding the role of immunomodulators

in chemoprevention. Most retrospective studies (115, 116) on the protective role of azathioprine against CAC have not shown a significant difference in cancer development in patients with IBD treated with this drug, while the use of thiopurines seems to be associated with a decreased risk of advanced neoplasia in patients with IBD (117). Experimental evidence identifying TNFα as a mediator involved in initiation and progression of CAC has not yet been produced in clinical trials, however the risks associated with the use of these drugs constitute an obstacle to their employment for the sole aim of chemoprevention (118). Hence, IBD treatment and CAC prevention will benefit from the recently acquired knowledge on IBD molecular immunology. However, the elucidation of the molecular pathways involved in the development and growth of CAC is fundamental to developing new pharmacological mechanisms to treat this tumor type. New therapies with mesenchymal and hematopoietic stem cells, based on their reparative and immunomodulating properties, are favorable to treat patients with IBD.

Conclusion

In CAC, as in sporadic CRC, stem cells seem to have a peculiar role in the initiation of the malignancy and the most influential molecular pathways seem to be the WNT–β-catenin, TNFα–NFκB and the IL64STAT3. The significant association between the inflammatory process and the production of a favorable microenvironment for selection and growth of a sub-population of stem cells which are able to initiate and maintain CAC is noteworthy. Targeting these stem cells is a promising strategy for developing a curative cancer therapy. A better understanding of the underlying mechanisms leading to the development and growth of CSCs may lay the foundations for new preventative measures to prevent the onset of CAC in patients with IBD.

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Disclosure of Interest

The Authors declare no potential conflicts of interest.

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