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Inflammatory pathways in the early steps of colorectal cancer development

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Abstract

Colorectal cancer is a major cause of cancer-related death in many countries. Colorectal carcinogenesis is a stepwise process which, from normal mucosa leads to malignancy. Many factors have been shown to influence this process, however, at present, several points remain obscure. In recent years some hypotheses have been considered on the mechanisms involved in cancer development, expecially in its early stages. Tissue injury resulting from infectious, mechanical, or chemical agents may elicit a chronic immune response resulting in cellular proliferation and regeneration. Chronic inflammation of the large bowel (as in inflammatory bowel diseases), has been associated with the subsequent development of colorectal cancer. In this review we examine the inflammatory pathways involved in the early steps of carcinogenesis, with particular emphasis on colorectal. Firstly, we describe cells and proteins recently suggested as central in the mechanism leading to tumor development. Macrophages and neutrophils are among the cells mostly involved in these processes and proteins, as cyclooxygenases and resolvins, are crucial in these inflammatory pathways. Indeed, the activation of these pathways establishes an oxidative and anaerobic microenvironment with DNA damage to epithelial cells, and shifting from an aerobic to an anaerobic metabolism. Many cellular mechanisms, such as proliferation, apoptosis, and autophagy are altered causing failure to control normal mucosa repair and renewal.

Core tip: This paper examines the most important inflammatory pathways involved in the very early steps of colorectal carcinogenesis. In particular, it emphasizes the role played by cells of the immune system and key proteins, like cyclooxygenases, resistins, hypoxia-inducible factor 1, nuclear factor E2-related factor 2, and sirtuins, in fostering changes in mechanisms, like cell proliferation, DNA damage, apoptosis and autophagy, anaerobial metabolism and tissue remodeling, considered central for colorectal cancer development.

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INTRODUCTION

Colorectal cancer is still a major health concern. In recent

years new hypotheses have been considered on the mechanisms involved in the early stages of colorectal carcinogenesis. Among them, it has been postulated that inflammation, and in general colorectal mucosa injury caused by several environmental agents, can play an important role. Indeed, tissue injury resulting from infectious, mechanical, or chemical agents may elicit a chronic immune response resulting in cellular proliferation and regeneration. If the immune response fails to resolve injury, a microenvironment rich in cytokines, growth factors, and products of cellular respiration substaines a prolonged proliferation in attempt to repair, resulting in the accumulation of genetic errors and continued inappropriate proliferation. Evidence supports a role for inflammatory responses in the development of colorectal cancer. Chronic inflammation of the large bowel [as in inflammatory bowel diseases (IBD)], has been associated with the subsequent development of colorectal cancer.

Here we examine the inflammatory pathways involved in the early steps of carcinogenesis, with particular emphasis on colorectal. Firstly, we describe cells and proteins recently suggested as central in the mechanism leading to tumor development. A second chapter is targeted to the description of the tumor microenvironment and its oxidative and anaerobic metabolism. Finally, the role of inflammation in colorectal tissue remodelling is discussed.

INFLAMMATORY CELLS AND PROTEINS

Macrophages

Macrophages (Mfs) represent 10%-20% of all mononuclear cells found in the intestinal lamina propria making the intestine the largest reservoir of Mfs in humans.

Type I macrophages (M1) (classical activated) as cells able to produce large amounts of proinflammatory cytokines, are implicated in the mechanism of killing pathogens and tumor cells by secreting agents such as tumor necrosis factor α (TNF-α), interleukin (IL)-12, reactive nitrogen (iNOS), and oxygen intermediates (ROS). In contrast, Type II macrophages (M2) (alternative activated), generated by various signals which include IL-4, IL-13, IL-10, and glucocorticoid hormones, moderate the inflammatory response, eliminate cell wastes, promote angiogenesis and tissue remodeling, and release cytokines, including IL-10[1-4].

Macrophages in tumors, usually termed tumor-associated macrophages (TAMs), play important roles in determining the clinical outcome, and often express the M2 phenotype. M1 macrophages are often abundant in chronic inflammatory sites, and where tumors are initiated and start to develop. Moreover, it is possible that the macrophages switch to an M2-like phenotype as the tumor begins to invade, vascularize, and develop[5,6].

IL-23 is produced by macrophages within a few hours after the activation. This, in turn, triggers rapid IL-17 responses from tissue-resident macrophages. IL-17 promotes the production of IL-1, IL-6, IL-8, CXC lig-

and 1 and TNF-α in stromal, epithelial and endothelial cells, and also in a subset of monocytes. Together, these proinflammatory cytokines rapidly recruit neutrophils to the site of infection. Neutrophils normally traffic to peripheral tissues, where they are phagocytosed by Mfs after transmigration and apoptosis. Apoptotic cell phagocytosis might downregulate IL-23 secretion and then curb IL-17 and granulocyte colony stimulating factor (G-CSF) production and eventually granulopoiesis. If this processes were interrupted, tissue Mfs would continue to express IL-23. This could drive IL-17 expression and increase neutrophils retrieval in peripheral tissues[7-9].

The production of arginase has been associated with M2 type macrophages. The switch from (nitric oxide) NO production to induction of arginase in these "alternatively activated" cells up-regulates polyamine and proline biosynthesis, that can stimulate cell replication, collagen deposition, and tissue repair[10,11]. Some *in vivo* evidences indicate that an exacerbated local M1 macrophage-like inflammation favors oxidative microenvironment, while M2 macrophage-like inflammation substains progressive tumor growth[12-14] (Figure 1).

Immune cells are known to express specific recognition molecules for cell surface glycans, such as galectins, sialic acid binding Ig-like lectins (siglecs), and selectins. Some carbohydrate determinants are preferentially expressed in nonmalignant epithelial cells, whereas other determinants are expressed in association with cancers. The carbohydrate determinants associated with cancers, such as sialyl Lewis-A or sialyl Lewis-X, are clinically used as tumor markers. Most siglecs are known to inhibit excess activation of immune cells. It is noteworthy that only the glycans that are expressed in normal epithelial cells serve as ligands for siglec-7 and -9, whereas cancer-associated glycans do not. Their expression is lost at the early stage of colon carcinogenesis as a result of epigenetic silencing of glycol-genes involved in their synthesis. The majority of immune cells expressing siglec-7 and -9 in normal colonic mucosa are macrophages/monocytes. The ligation of siglec-7 and -9 suppresses lipopolysaccharide (LPS) induced cyclooxygenases (COX)-2 and PGE2 production. These results suggest that normal glycans of colonic epithelial cells exert a suppressive effect on tissue macrophage COX2 expression in colonic mucosa, thus maintaining immunological homeostasis in normal mucosal membranes. These results also imply that the cancer-associated impaired glycosylation of siglec-7 and -9 ligands serves to enhance COX2 production by mucosal macrophages[15-18].

Neutrophils

Neutrophils (polymorphonuclear cells, PMN) have a well-established role in the first line of defence against microbial pathogens but, because of their short life and fully differentiated phenotype, their role in cancer-related inflammation has long been considered negligible.

Upon encountering inflammatory signals, neutrophils change their responsiveness to allow directed migration
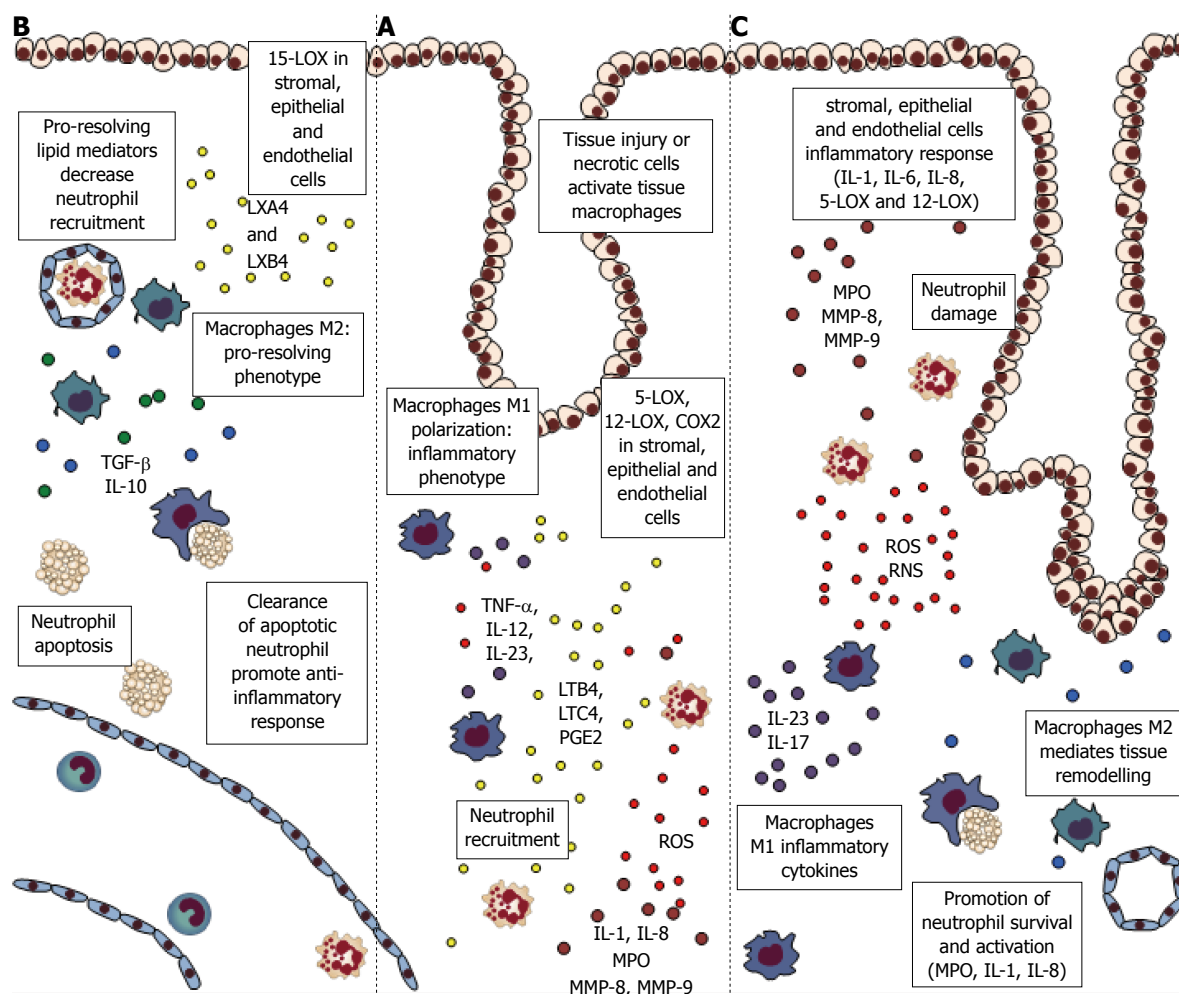
Figure 1 Inflammatory cells and proteins in the early phases of colorectal carcinogenesis. A: Inflammation and necrosis lead to monocytes recruitment and macrophages M1 polarization, with establishment of an inflammatory microenvironment and cytokines release [tumor necrosis factor α (TNFα), interleukin (IL)-12, IL-23]. Stromal, epithelial, and endothelial cells express lipooxygenases (5-LOX, 12-LOX), and cyclooxygenases 2 (COX2) proteins, with formation of inflammatory mediators leukotrienes and prostaglandins (*i.e.*, LTB4, PGE2) that drive neutrophils recruitment. Neutrophils, at the site of injury, amplify inflammation through myeloperoxydase (MPO), reactive oxygen species (ROS) and matrix metalloproteinases (MMP); B: If the inflammatory stimulus is switched-off the stromal and epithelial cells expressing 15-LOX drive the formation of pro-resolving mediators lipoxins (LXA4 and LXB4). These lipids block the neutrophils migration and stimulate the phagocytosis of apoptotic neutrophils by macrophages M1. The clearance of neutrophils sustain the switch to M2-phenotype, with secretion of anti-inflammatory cytokines such as IL-10 and transforming growth factor beta (TGFβ); C: If the stimulus is not resolved, the stromal and epithelial cells amplify the inflammatory signals (through IL-1, IL-8, 5-LOX and 12-LOX). In this way neutrophils apoptosis is inhibited, with continuous tissue and DNA damage by MPO, ROS and MMPs. The macrophages M1 support the inflammatory environment and the phagocyte afflux (IL-23 and IL-17), while M2 macrophages cause tissue remodeling.

and enhancement of microbicidal capacity. Neutrophil life-span is influenced during inflammation to enhance their anti-microbial action. Activated PMN are able to produce and release pro-inflammatory mediators, such as IL-1, IL-8, and macrophage inflammatory protein (MIP)-1s. PMN synthesize and store within cytoplasmic granules large quantities of serine proteinases (*e.g.*, neutrophil elastase), enzymes, including myeloperoxidase (MPO) and lysozyme, and ROS. The most abundant granule enzyme is MPO, which forms cytotoxic hypochlorous acid (HOCl) from the reaction of chloride anion with hydrogen peroxide produced after the respiratory burst[19,20].

In addition, cytokines IL-23 and IL-17 activate the inflammatory program of PMN by inducing the synthesis and the release of MPO and metalloproteinases {neutrophil collagenase [matrix metalloproteinases (MMP)-8], gelatinase B (MMP-9)} contributing, like ser-

ine proteinases, to tissue destruction through their proteolytic activity.

Once their physiological function has been performed in the tissues, neutrophils change their phenotype from a pro-inflammatory state, where they produce and release pro-inflammatory mediators such as LTB4 and PAF, to a more anti-inflammatory pro-resolution state whereby they release products (*e.g.*, lipoxins) that can influence the resolution phase of inflammation[8,9,21,22].

The resolution of inflammation therefore relies on the effective "switching off' of the neutrophil, the promotion of apoptosis and the successful recognition and uptake of cells by phagocytes such as macrophages. The apoptotic neutrophils stimulate macrophages into a pro-resolution phenotype, reducing the inappropriate inflammatory response further. In the intestine, the process of PMN apoptosis can be delayed or accelerated by a num-

ber of factors. Several host-derived cytokines, including IL-1, IL-8, and granulocyte-macrophage colony stimulating factor (GM-CSF), inhibit PMN apoptosis. There is now evidence that suggests MPO can act as a paracrine signalling molecule, promoting neutrophil survival. By contrast, the cytokines IL-10 and TNF-α and the products of respiratory burst, can induce apoptosis[23-26].

Under a persistent inflammation, this regulatory mechanism can be compromised. Indeed, it has been demonstrated that a low level of persistent inflammation in normal colorectal mucosa does exist in patients with colorectal cancer or adenomas[27]. Neutrophils continually accumulate within the intestinal mucosa and apoptotic neutrophils that are not eliminated by macrophages undergo secondary necrosis and release the contents of intracellular granules, which can induce pathological tissue damage[28,29] (Figure 1).

COX and resolvins
Lipid mediators such as eicosanoids, which are derived from the arachidonic acid, are among the earliest signals released in response to injury or an inflammatory stimulus. Two families of enzymes, namely, the cyclooxygenases (COX-1 and COX-2) and the lipoxygenases [5-lipoxygenase (5-LOX), 12-LOX, and 15-LOX], metabolize arachidonic acid to form lipid autacoids[30].

The 5-LOX pathway is closely related to chronic inflammation and carcinogenesis. Evidence suggests a potential role of 5-LOX products in early stages of colorectal carcinogenesis. 5-LOX is highly expressed in neutrophils and monocytes and is upregulated upon stimulation with IL-4 and IL-13. During cell activation, arachidonic acid released from membrane phospholipids is converted by 5-LOX in leukotriene B4 (LTB4) or LTC4. Two types of receptors, LTB4 receptor 1 (BLT1) and receptor 2 (BLT2) are known, and BLT1 is mainly involved in inflammatory responses. Overproduction of LTB4 in human colon cancer tissue and LTB4-mediated proliferation of colon cancer cells were reported. It has also been demonstrated a strong expression of BLT1 in the carcinomatous regions of human colon tissues, but not in the normal regions. Leukotriene B4 has been implicated in the pathogenesis of IBD[31-33].

The COX pathway contributes to neutrophil accumulation, and PGE2, a prominent product of the COX-2 pathway, plays a central role in checking leukocyte function by activating a specific PGE2 receptor. During the tissue progression of inflammatory events, PGE2 inhibits the production of proinflammatory cytokines, acts upreguling M2-type responses in Mfs but may also perpetuate chronic inflammatory responses by causing more prooxidant conditions, leading to DNA damage or reduced DNA repair. Thus, chronic inflammation leads to a chronic infiltration of neutrophils and macrophages with consequent damage to tissue. The increase in PGE2 production mediated by overexpression of COX-2, promotes colorectal tumorigenesis and activates the Wnt signaling pathway in colorectal cancer[34-37].

12-LOX metabolites promote cancer cell proliferation, metastasis, and angiogenesis, whereas 15-LOX metabolites seem to be protective against inflammation and carcinogenesis. 15-LOX is important for the resolution of inflammation and for the terminal differentiation of normal cells. 15-LOX enzymes are usually preferentially expressed in normal tissues and benign lesions, but not in carcinoma of the colon. In contrast, 5-LOX and 12-LOX are generally absent in normal epithelia, but they can be induced by pro-inflammatory stimuli, and are often constitutively expressed in various epithelial cancers including colonic ones. A strong correlation between 5-LOX expression and increased polyp size, higher tumor grade and histological epithelial localisation has been reported, and a 5-LOX overexpression has been seen in adenomatous colonic polyps and cancer compared to normal mucosa. This data support a role for 5-LOX in the early stages of colon cancer[38-41]. Signaling pathways leading to PGE2 and PGD2 in turn actively induce the formation of lipoxin (LX) A4 and lipoxin B4, which stop further recruitment of neutrophils and stimulate non-phlogistic monocyte infiltration. Both PGE2 and/or PGD2 switch eicosanoid biosynthesis from predominantly "proinflammatory" LTB4 to "antiinflammatory" LXA4 production. Specific lipoxins and the related members of the resolvin and protectin families provide potent signals that selectively stop neutrophil and eosinophil infiltration; stimulate non-phlogistic recruitment of monocytes (that is, without elaborating pro-inflammatory mediators); promote the uptake and clearance of apoptotic cells and microorganisms by macrophages; increase the exit of phagocytes from the inflamed site through the lymphatics; and stimulate the expression of molecules involved in antimicrobial defence. LXA4 treatment exerts anti-inflammatory responses in immune cells, reducing bowel inflammation *via* NF-κB and decreasing the damage caused to the intestinal epithelium, and some studies have shown that LXA4 analogs attenuated chemically induced colitis in rodents. Resolvin E1 (RvE1) reduces PMN transendothelial migration, superoxide generation and release, and attenuate colonic mucosal inflammation *in vivo*, probably by inhibiting phosphorylation of NF-κB and decreasing the levels of pro-inflammatory mediators. The resolvins have also recently been found to influence neutrophil apoptosis by suppressing MPO-induced survival mechanisms with improved resolution of inflammation[42-44].

Therefore, it has been hypothesized that the balance struck by linoleic and arachidonic acid metabolisms in the LOX pathway activity shifts from the antitumorigenic 15-LOX-1 and 15-LOX-2 pathways to the protumorigenic 5-LOX and 12-LOX pathways during tumorigenesis[45] (Figure 1).

Recently a role for the acyl-CoA synthetase 4 (ACSL4) in this shift has been reported. ACSL4 is an enzyme that esterifies arachidonic acid (AA) into arachidonoyl-CoA. It is poorly expressed in the gastrointestinal tract, but its expression is increased in colon cancer. It has been re-

ported that ACSL4 leads to increased COX-2 and LOX-5 levels and controls both lipooxygenase and cyclooxygenase metabolism of AA, resulting in inhibition of apoptosis and increase in cell proliferation. Thus, a new association therapy has been proposed, according to which a concomitant ACSL4, LOX and COX-2 inhibition may reduce side effects and improve cancer treatment[46,47].

OXIDATIVE MICROENVIRONMENT

DNA damage
The chronic inflammatory response represents a fine balance between active inflammation, repair, and destruction occurring in response to a persistent stimulus over a prolonged period of time. The activation of immune cells in response to a stimulus results in the elaboration of cytokines, chemokines, ROS, and reactive nitrogen species (RNS). Consequently, oxidative stress comes from the imbalance between endogenous generation of ROS and anti-oxidant defence systems that involve scavenging of low reactive ROS such as superoxide radical ($HO_2 \cdot$) and hydrogen peroxide (H_2O_2), the precursors of highly damaging hydroxyl radical ($OH \cdot$). The release of large amounts of ROS and RNS leads to oxidation of nucleic acids, proteins and lipids, and induction of several promutagenic DNA lesions. Indeed, DNA damage accumulation is associated with decrease of antioxidant defences[48-51].

It is estimated that ROS derived from chronic inflammatory cells may be a primary factor in the development of up to one-third of all cancers. Neutrophils and macrophages are a major source of oxidants that causes genetic alterations and may promote cancer development. Moreover, it has been reported a key role of MPO-mediated metabolic activation of inhaled chemical carcinogens in early stages of pulmonary carcinogenesis. Furthermore, it has been established that the type of DNA base modifications, as detected in target cells exposed to reagent H_2O_2, is highly comparable with the damage induced by activated neutrophils. In addition, HOCl also has been demonstrated to be an inhibitor of DNA strand break repair. Consequently the defects in DNA repair proteins genes may carry early to a mutated neoplastic clone, presumably as a result of markedly increased epithelial cell proliferation associated with inflammation, also in non-neoplastic colonic tissue. Unlike normal colonic mucosa, inflamed colonic mucosa shows abnormalities in these molecular pathways even before any histological evidence of dysplasia or cancer, and it has been reported by several works that the number of gene mutations in individually growing tumors was associated with the number of infiltrating neutrophils[52-54].

In addition, in colitis-associated colon carcinogenesis, ROS/RNS may contribute to the p53 mutations and can functionally impair the protein components of the DNA mismatch repair system[55]. iNOS expression is induced during inflammation and catalyzes the production of nitric oxide (NO). Moreover, depending on the concentration, genetic background, and NO enzyme involved, NO may induce protective effects. Clinical data show that iNOS levels are elevated in actively inflamed mucosa from inflammatory bowel diseases; however, there is controversy about its role in intestinal carcinogenesis[56,57].

Also carcinoma associated fibroblasts (CAFs), originated either by resident fibroblasts or by recruitment of circulating mesenchymal stem cells, are profoundly affected by oxidative stress. CAFs activation leads to lactate production and to lactate upload by neighbouring cancer cells, thus supporting their respiration and anabolic functions[58,59].

Thus, a persistent oxidative stress may, first, induce DNA damage such as modified base products and strand breaks that may lead to further mutation and chromosomal aberration of cancer (genomic instability) and, secondly, constantly activate transcription factors and induce expression of proto-oncogenes, such as NF-κB, c-fos, c-jun, and c-myc. In addition, ROS are involved in tumor angiogenesis, through the release of vascular endothelial growth factor, angiopoietin, and apoptosis evasion. The accumulation of tissue damage and the subsequent angiogenesis, remodeling, and connective tissue replacement, with a loss of cell cycle control, may contribute to tumor initiation.

Anaerobial metabolism
The inflammation sites are associated with changes in the tissue metabolism. More than 80 years ago, Otto Warburg suggested that cancer could be caused by a decrease in the energy metabolism of mitochondria in parallel with an increased glycolytic flux. In the following years it has been shown that cancer cells exhibit multiple alterations in structure, function and activity of mitochondria and glycolytic enzymes. The imbalance in glucose uptake and lactic acid production in the colonic neoplastic cells when compared with non-neoplastic cells has been well documented, and insulin signalling has been linked to an increased colon cancer risk. According to some studies, insulin pro-tumorigenic action may be due to an overproduction of ROS with subsequent DNA damage[60]. In this view, the mitochondria contribute to ROS generation, thus leading to DNA alteration. Both nuclear and mitochondrial DNA damage has been related to cancer development. Mitochondrial transcription factor A (TFAM) is a protein involved in transcription, replication and repair of mtDNA. It is essential also for mitochondrial biogenesis and function. Recent studies reported that its expression is related to clinical and pathological gradient of colorectal cancer, and that its loss can induce mtDNA instability with enhanced carcinogenic potential[61,62].

Hypoxia inducible factor 1
Inflammation can cause a significant hypoxia, resulting in the induction of hypoxia-response genes. Hypoxia leads to a coordinated transcriptional response mainly through
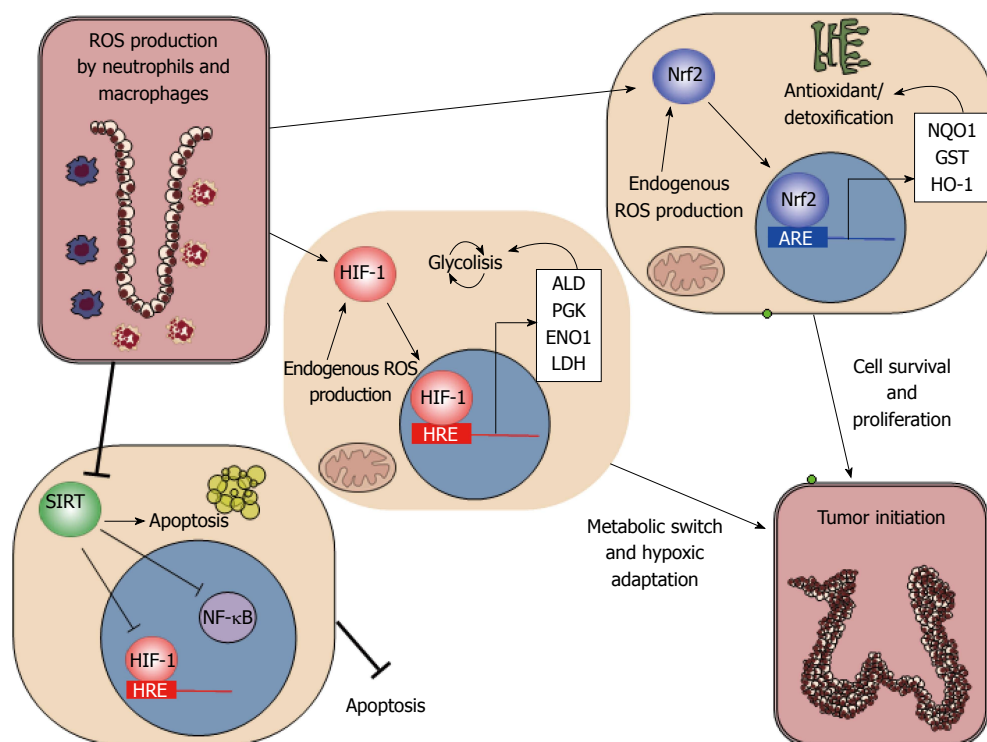
Figure 2 Oxidative microenvironment in the inflammatory milieu of colorectal mucosa. Inflammation leads to an oxidative microenvironment with consequent modification of cell metabolism. The major players of these changes is hypoxia inducible factor 1 (HIF1), nuclear factor erythroid 2-related factor 2 (Nrf2), and sirtuins (SIRT). HIF1 activation supports the metabolic switch to anaerobial metabolism [fructose-1,6 bisphosphate aldolase (ALD); phosphoglycerate kinase (PGK); enolase 1 (ENO1); lactate dehydrogenase (LDH)]. Nrf2 is involved in the antioxidant defences of epithelial cells [NAD(P)H dehydrogenase (NQO1); glutathione *S*-transferase (GST); heme oxygenase-1 (HO-1)], while sirtuins affect apoptosis and anti-inflammatory genes.

the activation of the transcription factor hypoxia inducible factor 1 (HIF1), which is composed by two subunits: HIF-1α that is oxygen-sensitive and HIF-1β [known also as aryl hydrocarbon receptor nuclear translocator] that is constitutively expressed.

HIF undergoes a negative regulation in normoxia. The stabilization of HIF-1α protein, however, is not limited to hypoxic conditions, and the so-called "hypoxic response" can also start with a suitable support of oxygen. This oxygen-independent hypoxic response can result from a wide variety of genetic abnormalities and dysfunctions in signaling pathways, such as tumor suppressor genes deletion (VHL, p53, PTEN), or by the activation of oncogenic pathways related to PI3K/Akt, Src, or activation of growth factors (such as EGF or IGF). Further, ROS, NO, and the heat shock promote HIF-1α expression in normoxic conditions. Pyruvate and oxaloacetate, the major products of the tricarboxylic acid cycle, contribute to the stabilization of HIF. Promoter analysis revealed that HIF-1α directly regulates more than 60 target genes, and genes induced by HIF-1α in hypoxic conditions are similar to those induced in normoxic conditions[63-67].

HIF-1 mediates adaptation to hypoxia through the activation of genes that increase the glycolysis, as the glucose transporter Glut1. This mechanism increases glucose entry into the cell and accelerates glycolysis. Enzymes such as aldolase, phosphoglycerate kinase [whose levels are increased already at the stage of aberrant crypt foci (ACF), early lesions in colorectal cancer development], Enolase, Lactate Dehydrogenase, and the carrier of lactate MCT4 contain consensus sequences for HIF. The increased induction of HIF target enzymes increases the environment acidity[68-71] (Figure 2).

It has been shown that the generation of mitochondrial reactive oxygen species during hypoxia promotes HIF stabilization. In turn, HIF-1α is also implicated in the control of mitochondrial activity. HIF-1α controls the expression of cytochrome c oxidase subunit IV (COX IV, isoform2) through HRE elements present on the gene. A continuous ROS production contributes to create mutations in the mitochondrial DNA. The presence of these mutations has been indicated as a factor promoting colorectal carcinogenesis[72-74].

A hypoxic microenvironment is established very early during the development of the tumor when the tumor has a volume of about 2-3 mm in diameter (possibly at the stage of aberrant crypt foci, ACF)[75]. HIF directly activates the genes coding for transferrin, vascular endothelial growth factor (VEGF), endothelin1 and nitric oxide synthase, which are involved in vasodilation and neovascularization. So, the tumor responds by increasing the glycolytic metabolism and angiogenic potential; thus, HIF is an important player in all the phases of neoplastic

growth by regulating survival, inhibition of apoptosis, neoangiogenesis and tumor metastasis[76,77].

Nuclear factor E2-related factor 2

Nrf2, or nuclear factor erythroid 2-related factor 2, is a positive regulator of the human antioxidant response element (ARE) that drives the expression of antioxidant enzymes such as NAD(P)H: quinone oxidoreductase 1 (NQO1), those involved in glutathione synthesis, and genes involved in limiting the inflammatory process[78,79].

Nrf2 signaling in physiological conditions acts as a switch that is turned on by the presence of stressors in the cellular microenvironment and that is rapidly deactivated when the insult is withdrawn and homeostasis is restored. However, under pathological conditions, the tight regulation of Nrf2 by rapid protein turnover is highly susceptible to being altered. This could result in the loss of responsiveness to cell stressors and subsequent vulnerability of the cell to various insults or in the acquisition of a constitutively active phenotype[80,81].

Constitutive signalling toward the expression of cytoprotective enzymes would confer cells a survival advantage under adverse conditions. Therefore, constitutive activation or augmented signalling of the Nrf2 pathway might be decisive for cell fate during tumorigenesis and affect the response to chemotherapy. Under these conditions, Nrf2 can be defined as a proto-oncogene[82] (Figure 2).

The involvement of Nrf2 in cancer pathogenesis is a controversial topic, provided a number of reports that still assign Nrf2 a role in cancer chemoprevention from genotoxic agents or inflammation[83].

Nrf2 knockout leads to an enhanced oxidative and inflammatory environment which would contribute to an increased level of free radicals, PGE2, LKTB4 and NO accumulation in the cells, leading to hyperproliferation of colonic crypts. However, some reports have shown that drugs that activate Nrf2 can promote cell growth, and an increasing number of works points to a potential role for Nrf2 and its transcriptional target genes in tumorigenesis. In conclusion Nrf2 can function as a proto-oncogene in plenty of solid tumors and leukemias. Nrf2 can be activated by numerous compounds and is also frequently deregulated in a wide variety of cancers by mutations, aberrant epigenetic or posttranslational regulation, or hyperactivation of oncogenic signalling pathways involving other transcription factors such as NF-κB, various protein kinases, structural proteins such as E-cadherin, or other regulators such as p62. Overexpressed or hyperactivated Nrf2 can participate in tumorigenesis by helping cells escape from diverse forms of stress through the induction of anti-oxidant target genes or by directly promoting cell survival, proliferation, and even metastasis[84,85].

SIRTUINS, INFLAMMATORY BOWEL DISEASE, AND COLORECTAL CANCER

Mammals express seven sirtuins (SIRT1-7) that have been demonstrated to play important roles in many physiological and pathophysiological conditions, including metabolism, cell survival, cancer, aging and caloric restriction-mediated longevity[86,87].

Sirtuins are a group of highly phylogenetically conserved proteins that catalyze the deacetylation of target proteins. The deacetylation reaction spends NAD$^+$, a key molecule in energy metabolism, thus linking protein regulatory control to metabolic conditions[88].

Mitochondrial SIRT3 is involved in tumor metabolism. SIRT3 induces fatty acid oxidation and regulates ROS homeostasis by targeting the mitochondrial enzymes Mn-SOD and SOD2. SIRT3 seems to maintain genomic stability by controlling ROS levels, that have been associated with mutagenesis promotion and genomic instability. ROS can modulate both cell survival and apoptotic pathways; thus SIRT3 may also promote tumorigenesis and prevent apoptosis, maintaining ROS at the appropriate level for a proliferative and aggressive phenotype. In contrast, some reports support a role for SIRT3 in inducing growth arrest and apoptosis in colorectal carcinoma[89,90].

SIRT1 regulates both apoptosis and autophagy by deacetylating p53 and other proteins involved in these pathways[91]. As a consequence, SIRT1 might be considered a facilitator for cancer development. Nevertheless, although pro-oncogenic effects of SIRT1 have been reported in some studies, there are also reports showing a tumor-suppressor role for this protein as well. Although information about the role of sirtuins in IBD is limited, there are several reports that show an antiinflammatory effect for these molecules. In fact, the best-known SIRT1 activator is resveratrol, that reverses colitis-associated decrease in SIRT1 gene expression, provokes the down-regulation of NF-κB and the increase of COX-2 expression, and other changes, in a dextran sulfate sodium-induced colitis, and resveratrol suppresses colon cancer associated with colitis[92,93]. In addition, SIRT1 is a negative regulator of NF-κB activity. With respect to colorectal cancer, several studies support the notion that SIRT1 could be involved in carcinogenesis, and SIRT1 has been found to be upregulated in various human cancers, including colon cancer[94,95]. SIRT1 expression is associated with microsatellite instability and CpG island methylator phenotype in human colorectal cancer. Conversely, there are also studies that indicate that SIRT1 can act as tumor suppressor. SIRT1 suppresses intestinal tumorigenesis and colon cancer growth in a β-catenin-driven mouse model of colon cancer[96,97]. SIRT1 has been shown to regulate Wnt signalling, to promote constitutive Wnt signalling and Wnt-induced cell migration, showing more a protumor action than an antitumor effect. In another study, SIRT1 has properties of a growth suppressor. Knockdown of SIRT1 increases the rate of tumor growth, whereas overexpression of SIRT1 reduces tumor formation in nude mice. Furthermore, pharmacological inhibition of SIRT1 increases the rate of cell proliferation in culture. These results together suggest that SIRT1 has properties of a
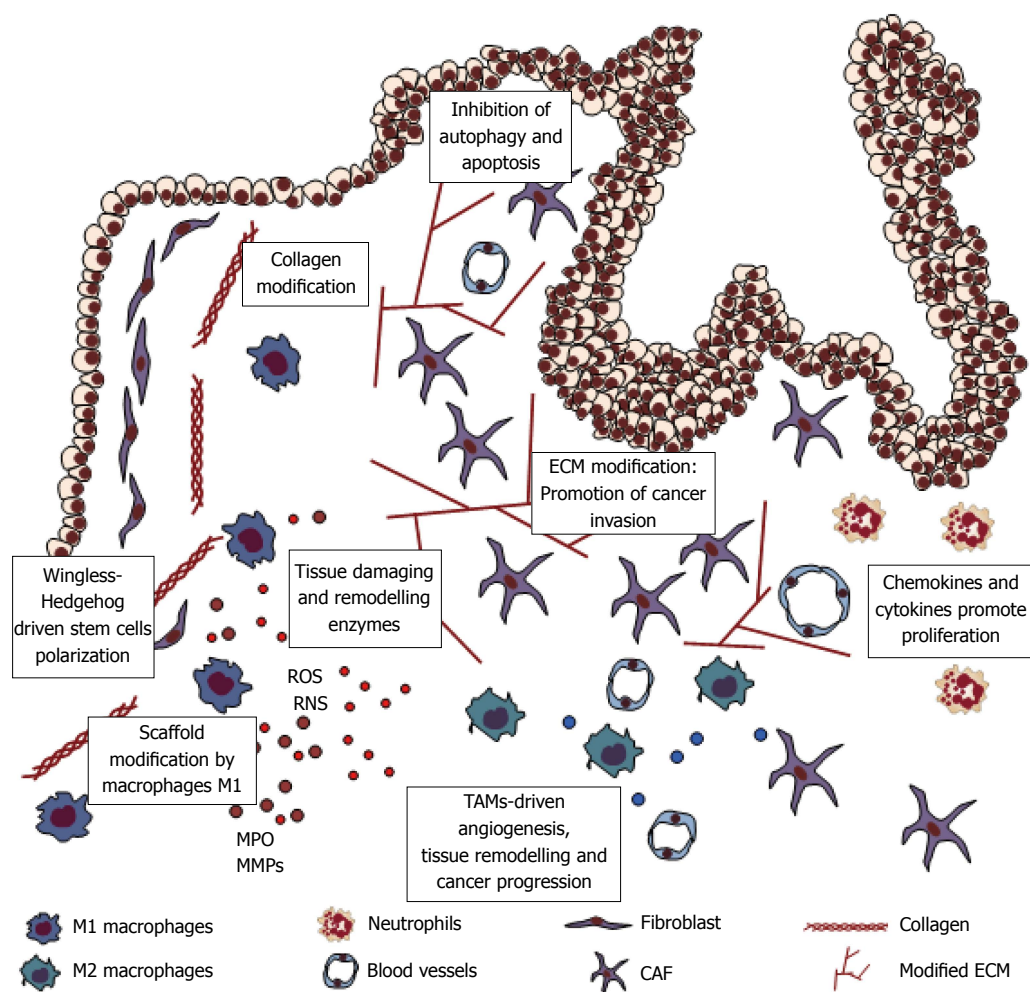
Figure 3 Inflammation and remodelling of colorectal mucosa. Macrophages and neutrophils cause tissue damage and DNA damage by reactive oxygen species (ROS) formation. Inflammatory cytokines stimulate crypt stem cells proliferation driven by Wingless and Hedgehog. Defects in apoptosis and autophagy systems cause accumulation and proliferation of transformed cells. Indeed, inflammatory cells cause extracellular matrix (ECM) modifications, sustaining disassembly of normal tissue architecture, angiogenesis and tumor invasion. CAF: Carcinoma associated fibroblasts; TAMs: Tumor-associated macrophages; MMP: Matrix metalloproteinases.

context-dependent tumor suppressor[98,99]. These results show that sirtuins have pleiotropic effects on cancer development (Figure 2).

INFLAMMATION AND REMODELLING

Generally, the repair of the damaged epithelium can rapidly be completed following the decrease of intestinal inflammation. Very few tissues in adult mammals have the ability for true regeneration; among them are the bone marrow, liver, intestinal epithelium, and epidermis of the skin.

There is accumulating evidence that loss of control over normal tissue repair or renewal mechanisms may lead to malignant transformation. Cancer has been described as a "wound that does not heal" or "the wound is a tumor that heals itself". But is there a link between tissue repair and cancer? The association between cancer and persistent inflammatory or regenerative states strongly suggests this connection[100,101] (Figure 3).

Further, PMNs recruited to the inflammation site act also by producing and releasing IL-22. IL-22 has a beneficial action on intestinal epithelial barrier by promoting cell proliferation, migration, and mucus production. This action is mediated probably by the IL-22 receptor (IL-22R), that is expressed by the epithelial cells of the gastrointestinal tract. There is also a soluble receptor for IL-22, IL-22BP, that acts by preventing the binding to the membrane-bound IL-22R and thus terminating the IL-22-induced regenerative program. So, as decreased levels of IL-22 are detrimental to the regeneration of epithelial monolayer, a defective control by IL-22BP can speed colon cancer development by sustaining a prolonged epithelial proliferation[102,103].

STROMAL INVOLVEMENT

Macrophages remove apoptotic neutrophils, the phagocytosis of which may lead to a change toward a more reparative (M2) macrophage phenotype and the resolution of the inflammatory phase of wound healing[104-106].

There is evidence for extracellular matrix (ECM)

proteins and activated ECs increasing the lifespan of neutrophils. The protection against neutrophil apoptosis is a result of adhesion to matrix proteins fibronectin and laminin, and activated EC-coated substrates, leading to an appropriate function[107,108].

The proliferative phase of wound healing involves new ECM deposition, including the deposition of dense fibrous connective tissue, within the site of injury. The architecture of the collagen scaffolds in tumors is severely altered. Tumor-associated collagens are often linearized and crosslinked, reflecting elevated deposition and significant posttranslational modification[109,110].

The ECM provides a physical scaffold for cell adhesion and migration, it influences tissue tension, and it signals to cells through ECM receptors. Proteolysis of the ECM regulates cellular migration by modifying the structure of the ECM scaffold and by releasing ECM fragments with biological functions. ECM proteolysis is therefore tightly controlled in normal tissues but typically deregulated in tumors[111-113].

Following the deposition of significant amounts of ECM (predominantly collagens type I and III) during the proliferative phase, the remodeling phase of wound healing begins. This phase is characterized by MMP and tissue inhibitor of metalloproteinase (TIMP)-mediated degradation and remodeling of the newly deposited collagen. An altered expression of some MMPs has also been reported in colorectal carcinogenesis[114,115].

TIMPs, which are secreted proteins, bind and inhibit enzymatically active MMPs at a 1:1 molar stechiometric proportion, thus inhibiting the proteolytic activity of MMPs. The impact of TIMPs is essential for the homeostasis of the ECM. The sensitive balance between MMPs and TIMPs is essential for many physiological processes in the gut[116].

Moreover, it has been demonstrated that serum antigen concentrations of MMP-9, TIMP-1 and TIMP-2, were significantly increased in patients with ulcerative colitis and crohn disease compared to controls. These results suggest that MMPs and TIMPs may contribute to the inflammatory and remodeling processes in IBD[117].

M2-like TAMs release a number of potent proangiogenic cytokines, such as VEGF-A, VEGF-C, TNF-α, IL-8, and bFGF. Additionally, these TAMs also express a broad array of proteases known to play roles in the angiogenic process. These proteases include urokinase-type plasminogen activator (uPA), the matrix metalloproteinases MMP-2, MMP-7, MMP-9, and MMP-12, and elastase uPA and MMP support angiogenesis by remodeling and breaking down the ECM. Degradation of ECM leads to the mobilization of growth factors and facilitates the migration of vascular cells into new environments[118,119].

Among the proteolytic enzymes expressed by TAMs there are several members of the cysteine cathepsin family, which have been implicated in cancer progression. Cysteine cathepsins are specifically involved in cancer, cysteine cathepsins B and L have been investigated most

intensively, and invariably their increased expression and/or activity correlates with malignant progression[120]. Several investigations have confirmed significantly higher levels of cathepsins D, L, H, and, in particular, cathepsin B in colorectal carcinoma[121].

Fibroblasts are among the most active cell types of the stroma. They are present in the stroma of normal tissues, including colorectal, where they perform tissue repair functions under certain physiological conditions, and in the stroma of tumors, in which they might represent the main component. They have been given various names: tumor-associated fibroblasts, CAF or myofibroblasts.

The differentiation of fibroblasts into myofibroblasts is an important step in tissue repair. Migration of colonic fibroblasts into and through the extracellular matrix during the initial phase of mucosal healing appears to be a fundamental component of wound contraction[122,123].

After differentiation, subepithelial myofibroblasts form a pericryptal fibroblast sheet adjacent to the basal lamina of colonic crypts. Intestinal subepithelial myofibroblasts contribute to the coordination of tissue regeneration by producing TGF-β, epidermal growth factor, basic fibroblast growth factor, proinflammatory cytokines, and the formation of new basement membrane.

In a state of permanent activation, fibroblasts can promote tumor growth and tumor progression, favoring a variety of tumor-specific mechanisms. These activated fibroblasts can be characterized molecularly by several markers that should be expressed by the fibroblasts in their activated state. Some of the most common CAF markers are α-smooth muscle actin, fibroblast-specific protein 1 (FSP1 or S100A4) and fibroblast activation protein. Together with M2 macrophages, and as previously stated above, CAF are a large component of the stroma and generally tumor promoting[124-126].

THE STEM CELL ROLE AND THE EPITHELIUM RESPONSE

Thus, rapid resealing of the epithelial surface barrier following injuries or physiological damage is essential to preserve the normal homeostasis. In a state of chronic injury or inflammation, stem cells are under a continuous stimulus of proliferation; pathway activation and presumed expansion of stem cell pools would persist so long as repeated injury prevents full regeneration[127].

Epithelial cell proliferation is stimulated in crypts near the damaged mucosal area to replenish the decreased cell pool. This appears as an elongation of the crypt, which may subsequently divide into two crypts. Maturation and differentiation of undifferentiated epithelial cells is needed to maintain the numerous functional activities of the mucosal epithelium[128,129].

Recent studies have revealed that the key signal regulating the proliferation of immature epithelial cells in the crypt may be Wnt signaling. Wnt signaling is an important part of normal epithelial renewal within the small and

large intestine. Wnt signaling has long been studied in the development of colon cancer, a disease characterized by the unregulated proliferation of intestinal epithelial cells. A series of studies in mice has revealed that Wnt signaling also regulates the proliferation of immature epithelial cells within the normal crypt. Two morphogenic signaling pathways, specifically Hedgehog (Hh) and Wingless (Wnt), serve to illustrate how pathways involved in stem cell proliferation during development, and regeneration have also been implicated in several different epithelial cancers. These observations suggest that cancer growth may represent the continuous operation of an unregulated state of tissue repair and that continuous Hh/Wnt pathway activities in carcinogenesis may represent a deviation from the return to quiescence that normally follows regeneration[130-133].

AUTOPHAGY

Autophagy is usually considered as a tumor-suppressing mechanism, though it can also enable tumor cell survival upon stress, and may promote metastasis formation. Autophagy is a key response mechanism to numerous extracellular and intracellular stresses. These include, for example, nutrient and growth factor deprivation and hypoxia. Autophagy is the only cellular catabolic process that can eliminate damaged or ROS-overproducing mitochondria, and thereby limit general oxidative damage. Nutrient or growth factor limitation, hypoxia and other cellular stressors are known to deactivate the signaling system that leads to autophagy induction and suppression of cell growth and proliferation[134,135].

Several pathways (including RAS/PKA, RAS/ERK, IRE1/JNK, TGF-β, WNT/GSK3, HIF) and transcription factors (TFs), such as NRF2, FoxO and p53 have been described to affect autophagy. Interestingly, these signaling pathways are also important in cell growth, proliferation, angiogenesis, immunity, cell survival and cell death, functions whose alteration are listed among the hallmarks of cancer. Thus, these data show that the control of autophagy is affected during tumorigenesis[136-138]. Numerous studies examined the role of autophagy in cancer, but the results are rather ambiguous. On the one hand, autophagy has tumor suppressing functions by suppressing chromosomal instability, restricting oxidative stress, promoting oncogene-induced senescence, and reducing intratumoral necrosis and local inflammation. On the other hand, enhanced autophagy represents a prominent mechanism used by tumor cells to escape from hypoxic, metabolic, detachment-induced and therapeutic stress as well as to develop metastasis and dormant tumor cells. During tumorigenesis, autophagy is frequently switched on and off, resulting in highly regulated anti- and pro-tumorigenic effects. Therefore, autophagy can be considered as a double-edged sword during tumorigenesis.

During tumorigenesis, cells not only increase their proliferative potential but also need to develop mecha-

nisms that allow them to escape their own tumor suppressor systems. Impairment or deregulation of the main apoptotic pathways is a major characteristic of cancer cells. In this regard, a cross link between Nrf2 and some effectors of the main apoptotic pathways has been proposed on several occasions. Tumor suppressor p53, which induces apoptosis upon DNA damage, partially in a ROS-dependent fashion, has been shown to inhibit the transcriptional activation of Nrf2 target genes in various cancer cell lines. This finding is supported by another report in which mice with decreased p53 levels showed enhanced expression of Nrf2 target genes after treatment with a genotoxic agent. These data suggest that Nrf2 inhibition is needed for p53-dependent apoptosis[139].

Many derangements in cell signaling occur within chronically inflamed tissues, which may lead to inappropriate suppression of apoptosis and subsequent tumorigenesis. Through careful microdissection of chronically inflamed and neoplastic tissues, several consistently upregulated survival signaling pathways have been identified, with subsequent attempts made to develop inhibitors to key pathway intermediates[140].

TARGETING THE NF-κB PATHWAY

NF-κB is a ubiquitously expressed transcription factor that plays a pivotal role in regulating cellular responses to environmental challenges, such as stress, infection, and inflammation. NF-κB is activated in response to cytokines and inflammatory mediators such as TNF-α, IL-1, LPS and ROS, and its regulatory products include growth factors, cytokines, immunoreceptors, and cell survival proteins, making it a complex modulator of the immune response.

Moreover, there is growing evidence of a connection between inflammation, NF-κB and tumor development. Viral oncogenes and some chemical and physical carcinogens, especially nicotine and carcinogens in tobacco, promote cell proliferation, survival, and inflammation *via* NF-κB activation. The role of NF-κB in promoting carcinogenesis is evidenced by numerous studies which indicate that this factor blocks apoptosis by regulating anti-apoptotic proteins, or by inhibiting the accumulation of ROS[141,142]. In chronic inflammation, the cytokines and chemokines produced by inflammatory cells activate NF-κB, which translocates into the nucleus, inducing the expression of certain tumorigenic, adhesion proteins, chemokines, and inhibitors of apoptosis that promote cell survival. Therefore, NF-κB may contribute to the development of colitis-associated colorectal cancer by sustaining the ongoing inflammatory process in the gut mucosa. NF-κB is also connected to the regulation of many genes differently expressed in invasion and metastasis: cyclin D1 and cMyc oncogenes, and VEGF and IL-8 are directly or indirectly enhanced by NF-κB activation. Several products have been suggested to inhibit NF-κB activation, including curcumin, ginseng extract, resveratrol, green tea extract, among others, and are known for their

Table 1 Main players in inflammatory pathways related to early phases of colorectal cancer development

Player	Role in early steps of colorectal cancer
IL-23	Induction of IL-17, activation of PMN respiratory burst
IL-17	Production of inflammatory cytokines, neutrophils recruitment and activation
Arginase	Stimulation of cell growth, collagen deposition, and tissue repair
sialyl Lewis-A and sialyl Lewis-X	Their accumulation on neoplastic cell escape the binding with siglec-7 and -9 in macrophages, thus enhancing COX-2 production
MPO	Hypochlorous acid (HOCl) formation, promotion of neutrophil survival
MMP-8, MMP-9	Tissue destruction and remodeling
5-LOX	Induced by IL-4 and IL-13, role in first step of leukotriene synthesis. Generally absent in normal epithelia
LTB4, LTC4, LTD4, LTE4	Chemoattraction of PMN, eosinophils, and macrophages. Activation of PMN. Increased microvascular permeability. Proliferation of colon cancer cells
COX-2	PGE production, neutrophil accumulation, perpetuate of inflammatory responses, Wnt signalling activation
12-LOX	Cancer cell proliferation, metastasis, and angiogenesis. Generally absent in normal epithelia
15-LOX	Resolution of inflammation. Generally absent in cancer cells
Lipoxins, resolvins and protectins	Resolution of inflammation (stop of PMN recruitment and superoxide generation and release, stimulation of non-phlogistic monocyte infiltration, promotion of the uptake and clearance of apoptotic cells, increase the exit of phagocytes from the inflamed site)
ACSL4	Increased COX-2 and LOX-5 levels, controls of COX-2 and LOX-5 metabolism of AA, inhibition of apoptosis and increased cell proliferation
TFAM	Regulation of mtDNA transcription, replication and repair. Essential for mitochondrial biogenesis and function
HIF-1	Activate also by ROS, mediates adaptation to hypoxia. Regulation of cell survival, inhibition of apoptosis, neoangiogenesis and tumor metastasis
Nrf2	Antioxidant enzymes expression, , promotion of cells survival by escaping to stress, cells proliferation, and metastasis
SIRT1	Regulation of both apoptosis and autophagy
SIRT3	Regulation of ROS homeostasis
IL-22	Promotion of epithelial repair
NF-κB	Activated by TNF-α, IL-1 and ROS, regulation of infection and inflammation. Apoptosis inhibition. Regulation of cyclin D1, cMyc, VEGF and IL-8

IL: Interleukin; MPO: Myeloperoxidase; MMPs: Matrix metalloproteinases; LOX: Lipoxygenase; ACSL4: Acyl-CoA synthetase 4; TFAM: Mitochondrial transcription factor A; HIF1: Hypoxia inducible factor 1; Nrf2: Nuclear factor erythroid 2-related factor 2; SIRT1–7: Seven sirtuins; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; PMN: Polymorphonuclear cells; TNF-α: Tumor necrosis factor α; VEGF: Vascular endothelial growth factor.

antiproliferative properties[143,144].

to draw a more definite one.

CONCLUSION

Several cells and proteins are involved in the early steps of colorectal carcinogenesis, and the most important are summarized in Table 1. They are components of a complex environment with continuous cross-talking between the epithelium and the stroma of the mucosal layer. Recent evidence has suggested that the stroma plays an important role in influencing important mechanisms both promoting and inhibiting the multistep process of carcinogenesis. Early injury to the colorectal mucosa caused by carcinogens coming from the environment, or any other agent damaging the mucosa may elicit an inflammatory process. Macrophages and neutrophils are among the cells mostly involved in these processes and proteins, as cyclooxygenases and resolvins, are crucial in these inflammatory pathways. Moreover, the activation of these pathways establishes an oxidative environment with further DNA damage to epithelial cells, and shifting from an aerobic to an anaerobic metabolism, thus awaking other proteins and altering other mechanisms, such as autophagy, proliferation and apoptosis, with final failure to control normal mucosal repair and renewal. However, the picture of the early events in colorectal carcinogenesis is still incomplete: future studies are needed in order

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