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REVIEW

Mechanisms of Immune Signaling in Colitis-Associated Cancer

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SUMMARY

This review discusses recent data on immune signaling pathways involved in the pathogenesis of colitis-associated cancer. These include molecular mechanisms activating the innate and adaptive immune system and thereby contributing to cancer initiation and promotion in inflammatory bowel diseases.

The inflammatory bowel diseases ulcerative colitis and Crohn's disease are associated with an increased risk for the development of colorectal cancer. During recent years, several immune signaling pathways have been linked to colitis-associated cancer (CAC), largely owing to the availability of suitable preclinical models. Among these, chronic intestinal inflammation has been shown to support tumor initiation through oxidative stress–induced mutations. A proinflammatory microenvironment that develops, possibly as a result of defective intestinal barrier function and host–microbial interactions, enables tumor promotion. Several molecular pathways such as tumor necrosis factor/nuclear factor-κB or interleukin 6/signal transducer and activator of transcription 3 signaling have been identified as important contributors to CAC development and could be promising therapeutic targets for the prevention and treatment of CAC. *(Cell Mol Gastroenterol Hepatol 2015;1:6–16; http://dx.doi.org/10.1016/j.jcmgh.2014.11.006)*

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Concepts about inflammation-associated cancer development go back to Rudolph Virchow's observation of increased immune cell infiltration at tumor sites more than 150 years ago.[1] Today, inflammatory conditions including infection and immune-mediated disease increase the risk of cancer. Well-known examples in the gastrointestinal tract are the increased risk for gastric cancer and gastric lymphoma in *Helicobacter pylori* infection and the increased risk for colorectal cancer (CRC) in inflammatory bowel disease (IBD).[2]

The first reports of colorectal cancer in IBD patients occurred in the early 1900s, when Crohn and Rosenberg[3] described a case of colonic adenocarcinoma in a patient with long-term ulcerative colitis (UC). The CRC risk in IBD patients initially was attributed mostly to UC and not to Crohn's disease (CD) because epidemiologic studies in the 1960s had proposed an up to 10 times greater CRC risk in UC, but not in CD, patients in comparison with the general population.[4] Disease extent and duration are regarded as the most important parameters affecting the individual CRC risk in patients with UC. Recent data also have shown an association between the degree of inflammation and the development of colonic neoplasia.[5,6] Additional risk factors include primary sclerosing cholangitis and a family history of CRC.[7] Together, the cumulative risk for CRC in UC patients has been reported as 1.6% after 10 years, 8.3% after 20 years, and 18.4% after 30 years of disease duration.[8] Because these data are based on studies from academic centers, which frequently have patients with more severe disease, true incidence rates may be lower. For instance, Jess et al[9] reported a 2.4-fold increased risk for CRC in UC patients after 15 years of disease in a meta-analysis of population-based cohort studies.

In contrast to UC, the influence of CD on CRC risk has been under debate for many decades. Although several cases of CRC were reported in CD patients beginning in the 1950s, subsequent studies could not detect increased incidence rates in comparison with the general population.[10] Recent studies have reported that the risk for CRC in patients with CD patients depends on large-bowel involvement. Similar to UC, the extent and duration of colonic inflammation are the most important risk factors for CRC development in CD patients. In this regard, the cumulative risk for CRC in CD patients has been reported to be 2.9%, 5.6%, and 8.3% after 10, 20, and 30 years of disease, respectively, in a meta-analysis.[11] Again, these data are based on studies from academic centers and therefore may overstate the actual incidence rates in patients with CD.

Because of the availability of reasonable preclinical models, our knowledge regarding the molecular mechanisms connecting inflammation and cancer development in

Abbreviations used in this paper: **AOM-DSS, azoxymethane–dextran sulfate sodium; APC, adenomatous polyposis coli; CAC, colitis-associated cancer; CD, Crohn's disease; CRC, colorectal cancer; DDR, DNA damage response; gp, glycoprotein; IBD, inflammatory bowel disease; IKK, IκB kinase; IL, interleukin; IL6R, interleukin 6 receptor; LPS, lipopolysaccharide; Myd88, myeloid differentiation primary response gene 88; NF-κB, nuclear factor-κB; NLR, NOD- and leucine-rich repeat–containing protein; NLRP, nucleotide-binding oligomerization domain- and leucine-rich repeat–containing protein family, pyrin domain-containing; NOD, nucleotide-binding oligomerization domain; RONS, reactive oxygen and nitrogen species; STAT3, signal transducer and activator of transcription 3; Th17, T-helper 17; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; UC, ulcerative colitis.**

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colitis-associated cancer (CAC) has increased rapidly in recent years. Chronic inflammation has been linked to tumor initiation, in which normal cells acquire genomic alterations that initiate tumorigenesis, as well as promotion driven by the sustained proliferation of initiated cells.[12] This review discusses recent progress in understanding immune signaling pathways involved in these steps during colitis-associated cancer development.

Oxidative Stress–Induced DNA Damage in CAC

For tumor initiation, distinct mutations of oncogenes or tumor-suppressor genes are required to allow subsequent tumor development. These include mutations that result in resistance to apoptosis as well as acquisition of malignant potential. Mutations involved in the initiation of sporadic colorectal carcinoma have been well characterized and accumulate along the individual steps of described adenoma–carcinoma sequence pathways.[13,14] Similarly, a sequence of distinct mutations occurs during the stepwise development of colitis-associated cancer. This can be referred to as the *inflammation–dysplasia–carcinoma pathway*, which describes the development of low-grade dysplasia in a background of intestinal inflammation, with subsequent progression to high-grade dysplasia, and, finally, invasive carcinoma.[15] Notably, not all lesions follow this stepwise evolution. Because the tumorigenic pathway and individual genes affected differ between sporadic and colitis-associated CRC, it is reasonable to propose that the mechanisms inducing these mutations also differ. Mutations in sporadic CRC have been attributed to several kinds of genomic and epigenetic instability including chromosomal instability, CpG island methylator phenotype, global hypomethylation, and mutations in mismatch repair genes that lead to microsatellite instability.[16] Although these genomic and epigenetic alterations also occur in CAC,[17] growing evidence supports a central role for inflammation-dependent oxidative stress in the induction of mutations that lead to CAC.

Oxidative stress occurs as an imbalance of the generation and elimination of reactive oxygen and nitrogen species (RONS).[18] Increased oxidative stress is one of the key features of chronic inflammation because cells of the innate immune system release various kinds of RONS including superoxide, hydrogen peroxide, singlet oxygen, hydroxyl radicals, and nitric oxide into the tissue microenvironment on activation. These RONS interact with the DNA of resident cells and induce various forms of DNA damage including single- and double-strand breaks, abasic sites, and nucleotide modification, all of which contribute to tumor initiation when they affect oncogenes or tumor-suppressor genes.[19]

In human IBD, studies have shown that increased RONS correlates with disease activity, as well as reduced antioxidant levels. For instance, 8-oxo-7,8-dihydro-2,-deoxyguanosine, an oxidative stress–dependent base modification, is common in inflamed and dysplastic tissue, but not in healthy mucosa.[20] Similarly, concentrations of nitric oxide are increased and correlate with oxidative damage in tissue samples of active and even inactive IBD.[21]

To repair reactive oxygen species-induced mutations, the DNA damage response (DDR) is activated, which comprises various mechanisms including direct repair, nucleotide excision repair, and others (see the article by Curtin[22] for a review). Furthermore, the DDR can regulate cellular proliferation through activation of premature cellular senescence, an irreversible arrest of cell-cycle progression that protects against the amplification of defective DNA and proliferation of mutant clones.[23] Senescence occurs in various precancerous lesions, and evasion as a result of mutations in senescence-associated genes has been regarded as a requirement for malignant transformation.

In IBD, Sohn et al[24] found an increase of DDR (Histon gamma H2A.X, phospho-checkpoint kinase 2) and senescence (Heterochromatin protein 1 gamma) markers in inflamed tissue samples from IBD patients. In UC samples, increased DDR and senescence correlated with infiltration of macrophages as a possible source of RONS. Supporting the activation of senescence as a protective mechanism against malignant transformation in CAC, high levels of senescence markers have been reported in low-grade dysplasia in comparison with nondysplastic inflamed tissues and also high-grade dysplasia of UC patients, proposing an evasion of senescence during the progression from low- to high-grade dysplasia.[25,26]

Evidence for the pathogenic role of oxidative stress and protection afforded by the DDR is provided by preclinical studies using CAC models. Meira et al[27] showed that mice deficient in alkyladenine DNA glycosylase, an enzyme involved in base excision repair, developed more DNA base lesions and higher numbers of tumors in the widely used azoxymethane–dextran sodium sulfate (AOM-DSS) model of CAC. In the same model, mice deficient in the transcription factor nuclear factor-erythroid 2–related factor 2, which regulates genes involved in antioxidant signaling pathways, also had increased numbers of aberrant crypt foci.[28] Furthermore, mice lacking the glutathione peroxidase Glutathione peroxidase 3, which is regulated by nuclear factor-erythroid 2–related factor 2 and acts as a redox enzyme, develop more tumors with higher grades of dysplasia in the AOM-DSS model.[29] GPX3-deficient mice even developed polyps after DSS treatment without AOM, indicating that increased oxidative stress without an effective DDR is sufficient for tumor initiation. After tumor initiation, the proinflammatory microenvironment also contributes to tumor promotion.

Proinflammatory Signaling Pathways: The Role of Nuclear Factor-κB in Tumor Initiation and Promotion

Inflammation occurring as a response to infection or tissue damage removes dead cells and promotes restoration of tissue integrity via stem cell and myofibroblast activation, cell proliferation, angiogenesis, and other processes. Because of the overlap between mechanisms involved in wound healing and tumorigenesis, tumors have been described as “wounds that do not heal.”[30] In fact, chronic inflammation can result in excessive tissue regeneration and