

## Remote Control of Intestinal Tumorigenesis by Innate Immunity

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### Abstract

Chronic intestinal inflammation has been linked to the development of colorectal cancer. Recent studies suggest that during chronic inflammation, the innate immune system may facilitate colon tumorigenesis in genetically predisposed individuals in response to certain indigenous microorganisms and/or cell remnants. In these individuals, therapeutic approaches that reroute the innate immune system's inflammatory and antimicrobial activities might help prevent colon tumorigenesis and metastasis. *Cancer Res*; 70(5); 1749–52. ©2010 AACR.

### Introduction

Crohn's disease is remitting-relapsing, inflammatory bowel disease that is influenced by both environmental and genetic determinants. Approximately 2 million North Americans and Europeans suffer from inflammatory bowel disease, which primarily appears in the second or third decade of life. Colorectal carcinoma (CRC) represents the most common cause of mortality in Crohn's disease. It affects around 10% of Crohn's disease patients after 30 years of disease progression (1). Similarly, prominent inflammation correlates with tumor incidence and progression in rodent models of colitis-associated colorectal cancer (CAC). Several fundamental questions are now emerging. Could certain commensals be the culprit in predisposition to CAC? Could CAC be prevented by countering the immunodeficiencies that cause Crohn's disease? Several recent findings have provided partial answers and have paved the way for immunotherapeutic intervention in genetically predisposed Crohn's disease patients.

### Extrinsic Threats: Commensals on the Premises

From birth onwards, the mammalian gastrointestinal tract harbors (and coexists in intimate contact with) a myriad of microorganisms. The composition of this microbial consortium is influenced by both environmental and host genetic factors. Imbalances in either the diversity or magnitude of the microbiota (a phenomenon that has been referred as dys-

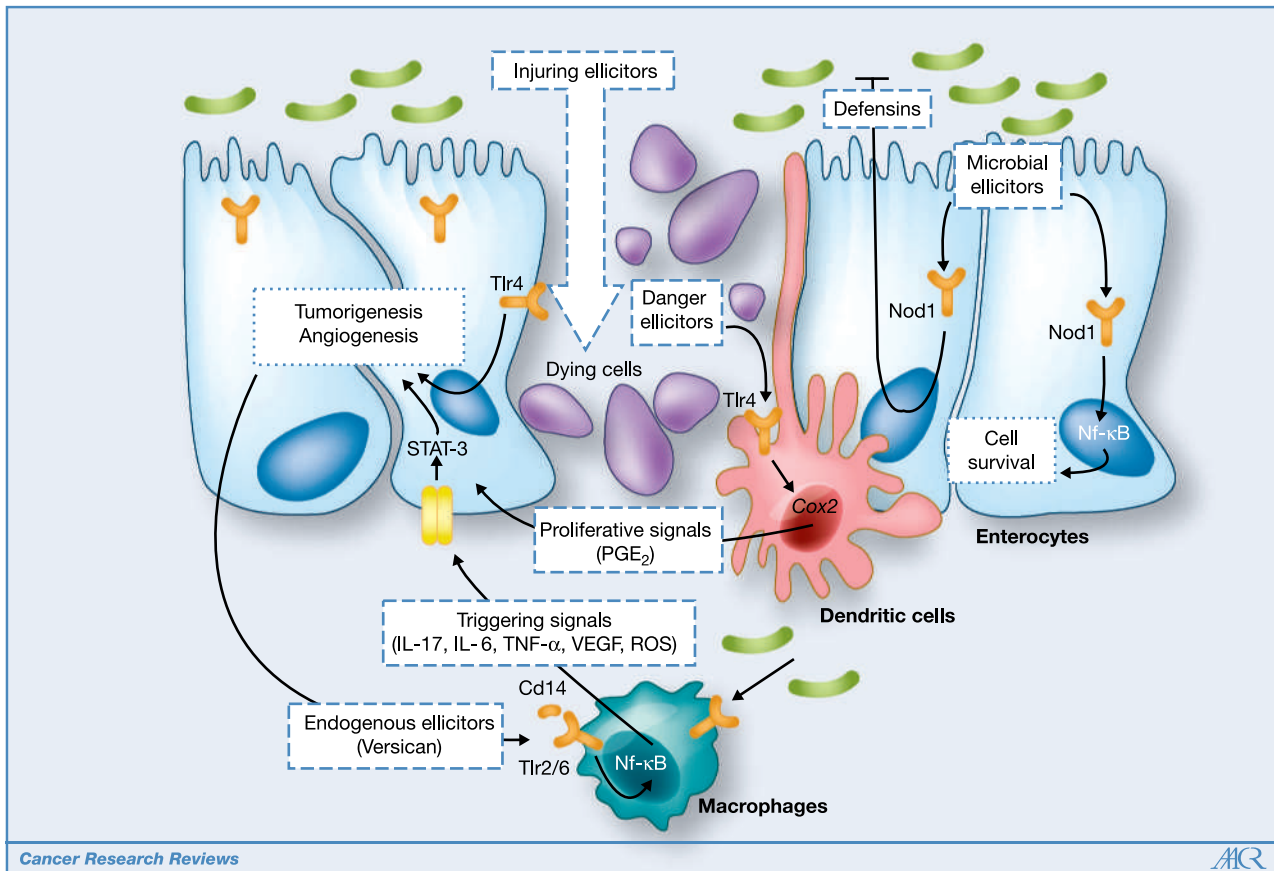
biosis) are often found within the gastrointestinal tract of Crohn's disease patients. Dysbiosis may disrupt the fragile balance between commensals and the host immune system, leading to a breakdown in mutualism and the unrestrained development of inflammatory and tumorigenic processes. In interleukin (IL)-10-deficient mice, exposure to the alkylating agent azoxymethane usually causes the development of late-stage tumors by the age of 4 months. Remarkably, this process is abolished if the intestinal microbiota is depleted (2, 3). Similarly, intestinal tumorigenesis is not observed in germ-free, transforming growth factor (*Tgf*)-beta-deficient mice after exposure to azoxymethane, when compared with animals kept in a specific pathogen-free environment (2). Concomitantly, colon injury in *Tgf*-beta-deficient mice prompts increased cell survival, enhanced activation of the transcription factor nuclear factor- $\kappa$ B (NF- $\kappa$ B), and secretion of pro-inflammatory cytokines, which may result from a host failure to sequester and/or tolerate commensals (3). Conversely, in formerly germ-free animals, the development of azoxymethane-induced colon tumors resumes after colonization by certain indigenous microorganisms, including *Bacteroides vulgatus* (3). Furthermore, *Helicobacter hepaticus* infection has been linked to enhanced tumorigenesis within the descending colon and rectum in animals intrinsically carrying a highly penetrant mutation (referred to as *Apc*<sup>Min/+</sup>) in the adenomatous polyposis coli (*Apc*) gene (4). In humans, *Apc* is a tumor suppressor gene that is highly mutated in both familial and sporadic colon cancer syndromes. More recently, a well-designed experimental study revealed that the enterotoxigenic *B. fragilis* triggers persistent colitis and accelerates tumor development in the colon of *Apc*<sup>Min/+</sup> mice by triggering activation of the signal transducer and activator of transcription-3 (STAT3)- and IL-17-mediated signaling pathway (Fig. 1; ref. 5). Conversely, the role of the anti-inflammatory colon commensal bacterium *B. thetaiotaomicron* in CRC predisposition and the potential immune escape mechanism of enterotoxigenic *B. fragilis* have yet to be explored. Taken as a whole, these studies suggest that the host innate immune system's recognition of certain components of the microbiota plays an essential role in intestinal tumorigenesis. These elements must now be characterized.

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**Figure 1.** An integrated model of the regulation of intestinal tumorigenesis through TLR4 and NOD1. In a context of intestinal injury, TLR4 is likely to favor tumor formation by triggering secretion of proinflammatory and proliferating signals by recognition of cell remnants. In contrast, NOD1 drives synthesis of defensins and antiapoptotic factors that protect against intestinal tumorigenesis in response to the microbiota.

### Remote Control of Intestinal Tumorigenesis via Toll-like Receptors

The first breakthrough in understanding the modifying role of innate immunity on CRC development came from recent work by Seth Rakoff-Nahoum and Ruslan Medzhitov. They elegantly showed that the cytosolic Toll-Like Receptor (TLR)-adaptor MyD88 is critically involved in both spontaneous and inducible development of CRC in mice (6). In *Apc<sup>Min/+</sup>* mice, genetic ablation of the MyD88-mediated signaling pathway was associated with enhanced cell death in the tumor mass and reduced expression of cytoprotective factors (include the regenerating islet-derived 3 beta and the chemokine KC) and pro-tumorigenic factors [including IL-6, cyclooxygenase-2 (Cox-2), and cytosolic phospholipase A2; Fig. 1]. Consistently, exogenous administration of PGE<sub>2</sub> restored proliferation of *Tlr4<sup>-/-</sup>* epithelial cells and the positioning of *MyD88<sup>-/-</sup>* leukocytes within the rectal crypt architecture following DSS injury (7, 8). It is noteworthy that intraperitoneal and oral administrations of exogenous PGE<sub>2</sub> gave a similar protective effect, suggesting a role for both epithelial and myeloid cells. The involvement of MyD88 in the regulation

of gut homeostasis raised the fundamental issue of the role of each TLR in the control of intestinal tumorigenesis (Fig. 1). Membrane-bound *TLR4*-deficient mice exhibited a defect in epithelial cell proliferation and abolished expression of Cox-2, when compared with controls (9). The overall vision of TLR4's role in the development of CAC has been clarified by using bone-marrow chimeric mice (10). Engagement of the TLR4-mediated signaling pathway within the injured colonic mucosa may promote stepwise progression to invasive adenocarcinoma by enhancing the secretion of PGE<sub>2</sub> by patrolling myeloid cells (10), and then by activating an epidermal growth factor receptor-mediated signaling pathway in colonocytes (9). Overall, it seems that TLR4/MyD88-mediated signaling pathway in intestinal epithelial cells is essential for recruitment of the Cox-2-producing myeloid cells that favor tumor growth and invasion (Fig. 1). Similarly, following engagement of the TLR4-mediated signaling pathway by bacterial endotoxins, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) was critically involved in the tumorigenic and metastatic properties of an epithelial colorectal cancer cell line *in vivo* (11). Recognition of myeloid cell-derived TNF- $\alpha$  was also associated with Cox-2 expression, cell proliferation,

angiogenesis, and development of CAC in a TNF receptor 1-dependent manner (12). Conversely, enhanced TLR-mediated signaling pathway correlated with a greater incidence of colorectal tumors in mice deficient for the single immunoglobulin domain-containing IL1R-related protein (SIGIRR; which is thought to negatively regulate TLR-IL1R signaling). Interestingly, restoration of SIGIRR expression in intestinal epithelial cells or depletion of commensals by antibiotic treatment were sufficient to slow progression of invasive carcinomas, suggesting that targeting the epithelial recognition of commensals by TLRs may represent a novel immunotherapy for CRC (13).

### Remote Control of Intestinal Tumorigenesis via NLRs

Unlike TLR4 and MyD88 deficiencies, the absence of Nod1 (the closest Nod2-related molecule) was recently linked to increased tumor growth in a model of CAC (14). The colon of *Nod1*-deficient mice displayed a more intense inflammatory response (as measured by NF- $\kappa$ B activation and production of inflammatory mediators) and a defective intestinal barrier function (as measured by epithelial apoptosis and intestinal permeability; Fig. 1). Interestingly, modulation of the enteric flora with a cocktail of antibiotics, (including streptomycin, gentamicin, ciprofloxacin, and bacitracin) was enough to prevent tumor formation in this CAC model, even in the absence of Nod1. Taken as a whole, these results suggest that Nod1 is involved in the surveillance of intestinal tumorigenesis by limiting microbial-induced inflammation and cell death through pathogen-recognition molecules that have yet to be identified. It is noteworthy that none of the aforementioned antibiotics is active (at least *in vitro*) against the species of the *Bacteroides fragilis* group, which includes *B. fragilis*, *B. thetaiotaomicron*, and *B. vulgatus*. This finding suggests that the development of CAC may be prevented by Nod1-independent recognition of such microorganisms, which constitute a major bacterial component within the gastrointestinal lumen (Fig. 1). In addition, the *Apc*<sup>Min/+</sup> tumor phenotype was enhanced by the absence of Nod1. Determination of the role of lymphoid tissues in intestinal tumorigenesis is now eagerly awaited, given that Nod1 is critically involved in the genesis of isolated lymphoid follicles by triggering the secretion of certain antimicrobial peptides and by activating the C-C chemokine receptor 6 (15). Overall, the differing roles of the TLR4 and NOD1 Gram-negative bacterium sensors in intestinal tumorigenesis highlight the complex physiological translation of gut-microbiota dialogue in the immune surveillance of colon cancer (Fig. 1). Additional studies on how each pattern-recognition molecule turns signals from indigenous microorganisms and dying cells into an anti- or pro-tumoral response are eagerly awaited. In particular, it will be important to determine the potential role of the Crohn's disease-predisposing gene *Nod2* in intestinal tumorigenesis, because inborn, Crohn's disease-associated *Nod2* mutations may be linked to an increased risk of developing CRC (16).

### Usurpation of the Innate Immune System by the Tumor Microenvironment

Whereas MyD88 deficiency is linked to enhanced survival of *Apc*<sup>Min/+</sup> mice, depletion of the microbiota failed to prevent intestinal tumorigenesis in these genetically predisposed animals. This finding suggests that endogenous danger elicitors (including cell remnants) may signal through TLR and/or IL1R. Recently, biochemical and genetic screening revealed that the tumor cell-derived proteoglycan versican (17) triggers the secretion of inflammatory mediators (including TNF- $\alpha$  and IL-6) by macrophages. Versican is an interstitial extracellular matrix chondroitin sulfate proteoglycan found in many tissues. In particular, the TNF- $\alpha$  production induced by versican or by the conditioned environment in carcinoma cells is abolished in *Thr2*-, *Thr6*-, and *Cd14*-deficient macrophages (Fig. 1). In contrast, Tlr1, Tlr3, Tlr4, Tlr9, and Trif were not essential for TNF- $\alpha$  production by macrophages following exposure to the carcinoma microenvironment. Consistently, metastasis was markedly reduced in *Thr2*-deficient mice and in wild-type animals reconstituted with *Thr2*-deficient macrophages. Furthermore, versican was found to bind TLR2 *in vitro* and knocking down versican expression in carcinoma was sufficient to prevent metastatic spread *in vivo*. Versican-induced TNF- $\alpha$  may create a favorable microenvironment for metastatic growth by reducing apoptosis, increasing both cell proliferation and vascular permeability (17), and thus promoting leukocyte infiltration and tumor cell extravasation. The role of hyaluronan in intestinal homeostasis and predisposition to CAC also requires further documentation because this glycosaminoglycan is thought to interact with both TLR2 and versican.

### Could Colorectal Carcinoma in Crohn's Disease Be Prevented by Redirecting the Innate Immune Response?

Current therapies for Crohn's disease are not curative and are far from optimal. Overall, refractoriness to drug therapies is observed in about 25 to 30% of Crohn's disease patients and may account for an accumulation of life-threatening events, including CRC. The intrinsic role of innate immunity in intestinal homeostasis and tumorigenesis provides support for the therapeutic manipulation of innate immunity in Crohn's disease and associated colorectal cancer. In particular, an elegant biochemical and genetic study has linked the RIGI-like receptor family member melanoma differentiation-associated gene-5 (*mda-5*) to autophagy and the autodigestion of melanoma cells (18). Given the role of Mda-5 in driving type I interferons in response to double-stranded RNA (dsRNA), this study provides also proof-of-concept for use of the dsRNA mimic polyinosine-polycytidylic acid in combating metastasis. Lastly, administration of a vitamin D analog (thought to modulate TLR2-mediated signaling pathway) is efficient in preventing the onset of colitis and thus hampering tumor growth in CAC (19). Vitamin D's antitumor effect has been linked to

decreased epithelial cell proliferation and concomitantly reduced COX2 expression.

In a complementary approach, antagonists of a large set of transcription factors (including NF- $\kappa$ B and STAT3) and inflammatory molecules (including TNF- $\alpha$  and IL-6) have been developed and evaluated in preclinical studies. Notably, the inhibition of NF- $\kappa$ B in a colorectal cancer cell line has been linked to a TRAIL-dependent, cytotoxic effect, which decreased the tumor burden and reduced the associated mortality (11). The enterocyte-specific and myeloid-specific deletion of IKK $\beta$  (a kinase enabling the release of NF- $\kappa$ B from its inhibitor I $\kappa$ B) was sufficient to protect animals from intestinal tumorigenesis through a decreased cell survival and a reduced production of inflammatory mediators, such as tumor TNF- $\alpha$  (20). In Crohn's disease, pharmacological blockade of TNF- $\alpha$  is effective in maintaining remission and promoting mucosal healing and steroid-sparing. Consistently, a reduction in inflammatory cell infiltration and tumor development was observed following curative administration of the soluble TNF- $\alpha$  receptor fusion protein etanercept in CAC (12). Whereas STAT3 is activated by engagement of TLR-mediated signaling pathways and is critically involved in tumorigenesis and metastasis (21, 22), inactivation of STAT3 improved the tumoricidal potential of a TLR9-based immunotherapeutic approach (23).

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## Concluding Remarks

Intestinal tumorigenesis seems to result from the failure to integrate several microbial threats from the microbiota and danger signals from damaged host cells with vital immunological information. Given that innate immunity plays a pivotal role in this process, gaining a better understanding of the complex regulation of the innate immune system may pave the way to immunotherapeutic interventions in Crohn's disease.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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