

The Emerging Role of NOD-like Receptors in Colorectal Cancer

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Introduction

Colorectal cancer (CRC) is the third most common cancer and fourth leading cause of cancer-related death worldwide. The major CRC susceptibility genes and pathways include WNT, RAS-MAPK, PI3K, TGF- β , P53 and DNA mismatch repair pathways. Despite our knowledge of genetic predispositions to CRC, the detailed mechanism of CRC pathogenesis is poorly defined. This is due to the heterogeneous nature of CRC and its association with multiple other factors, including inflammation, gut microbiota and diet. A growing body of evidence suggests that dysregulated immune responses in the gut orchestrate the multistep process of colorectal tumorigenesis [1-3]. Immune dysregulation in the gut is initiated by the interaction of immune cells with gut commensal bacteria when the intestinal epithelial barrier is breached, allowing commensal bacteria to invade the lamina propria. There are several dedicated pattern recognition receptors (PRRs), most notably Toll-like receptors (TLRs) and NOD-like receptors (NLRs), which continuously survey for the presence of pathogens and their products. Sensing pathogen or danger associated molecular patterns (PAMPs and DAMPs) by these PRRs leads to activation of several cell signaling pathways that participate in host defense, inflammation, cell death and proliferation. Therefore, PRRs play central roles in the pathophysiology of inflammatory diseases and tumorigenesis in gastrointestinal tract. NLR family proteins are evolutionarily conserved PRRs, which recognize PAMPs and DAMPs in the cytoplasm. Structurally, NLRs contain three major domains: a central nucleotide-binding oligomerization (NOD) region, a C-terminal leucine-rich repeat domain (LRR) and an N-terminal effector domain which could be a Pyrin domain (PYD), caspase recruitment domain (CARD), death effector domain (DED) or baculovirus inhibitor of apoptosis protein repeat (BIR) domain. At present, 22 human and 34 mice NLR members have been identified, but only a few among them have been characterized [4]. NLRs play diverse physiological functions, including the formation of the inflammasome, activation of the NF- κ B and MAPK pathways, and suppression of inflammatory signaling pathways.

Discussion

Recent studies underscore the importance of several NLR family members such as NOD1, NOD2, NLRP3, NLRC4, NLRP6 and NLRP12 in regulating intestinal inflammation and cancer. A

series of clinical studies investigated an association of NOD2 with CRC, because NOD2 is a major inflammatory bowel diseases (IBD) susceptibility gene and IBD is a risk factor for CRC. Several different mutations in the NOD2 gene have been detected in IBD. However, convincing evidence is lacking on whether any of these NOD2 mutations predisposes to CRC. In 2004, Kurawski et al. [5] first reported an association of the NOD2 3020insC single nucleotide polymorphism with the risk of CRC. This observation was later supported by other clinical studies suggesting that polymorphisms in NOD2 are linked with CRC. On the other hand, some other studies did not observe any genetic predisposition of NOD2 mutations in CRC. However, a recent meta-analysis of different SNP studies concluded that mutation in NOD2 is indeed a risk factor for CRC. This view is supported by an experimental study using azoxymethane (AOM) plus dextran sulfate sodium (DSS)-induced colorectal tumorigenesis, a widely used model for studying CRC pathogenesis. It has been shown that NOD2^{-/-} mice are susceptible to CRC with increased tumor burden. However, the precise mechanism of NOD2-mediated regulation of CRC is currently lacking [6-8]. The function of NOD2 has been implicated in activating NF- κ B and MAPK in response to bacterial cell wall component MDP. NOD1 is another NLR family protein that is structurally and functionally similar to NOD2. NOD1 activates NF- κ B and MAPK in response to peptidoglycan component iE-DAP, and experimental study demonstrated that NOD1 deficiency leads to increased tumorigenesis in mice. Given that NF- κ B and MAPK pathways regulates inflammatory and cancer-promoting genes, how reduced activation of these inflammatory signaling pathways promotes colorectal tumorigenesis is intriguing. It might be possible that defective host defense responses in the gut of NOD1- and NOD2-mutant hosts allow growth of tumor-inducing microbiota. This view is supported by a recent study showing increased growth of *Bacteroides* in NOD2-deficient mice [7]. Also, mice deficient in MyD88, an upstream mediator of NF- κ B activation, are more prone to colorectal tumorigenesis induced by AOM plus DSS. Colitis susceptibility of NOD2-deficient mice has been linked to NOD2-mediated regulation of autophagy, production of the antimicrobial peptides, and regulation of T cell responses. However, how NOD2 regulates these physiological processes and whether NOD2-mediated regulation of autophagy and T cell responses contributes to CRC pathogenesis are not clearly understood. Future study should aim to unravel the precise mechanism of NOD1 and NOD2-mediated protection against CRC.

Four NLR family members, including NLRP1, NLRP3, NLR4 and NLRP6, share a common physiological function of activation of the inflammasome, a molecular platform for the activation of caspase-1. In the inflammasome complex, an NLR interacts with pro-caspase-1 via the adapter protein ASC. Activation of the inflammasome-forming NLRs by PAMPs and DAMPs is prerequisite for scaffolding the inflammasome complex. Notably, among the 4 inflammasome-forming NLRs, NLRP1, NLRP3 and NLR4 inflammasomes have been characterized based on their ligand specificity [9]. For example, NLRP1b is activated by *Bacillus anthracis* toxin; NLRP3 is activated by a wide range of microbial and danger signal including LPS, MDP, RNA, uric acid crystals, radiation and environmental pollutants (e.g. silica, asbestos, etc.); and NLR4 is activated by bacterial flagellin. How NLRP6 inflammasome is activated is not known yet. Upon activation by the inflammasome, the cysteine protease caspase-1 plays multiple physiological functions, including induction of cell death and maturation of proinflammatory cytokines IL-1 α and IL-18. Inhibition of programmed cell death in the neoplastic cell is a hallmark feature of any cancer. Enormous effort has been paid to develop anti-cancer drugs targeting the cell death pathway. Downregulation of caspase-1 has been observed in human cancer. Although enough evidence of genetic association of caspase-1 with CRC susceptibility is lacking, animal studies in past five years provide convincing evidence that caspase-1 plays a protective function against CRC. Mice deficient in caspase-1 develop pronounced colorectal tumorigenesis induced by AOM plus DSS treatment. Activation of caspase-1 by the inflammasome is essential for the protective function of caspase-1 since mice deficient in the inflammasome components such as NLRP3, NLR4 and ASC are also susceptible to colorectal tumorigenesis. In addition to caspase-1-mediated cell death, the inflammasome downstream cytokines IL-1 β and IL-18 might play critical roles in the regulation of CRC. These two cytokines exert multiple physiological functions, including activation of immune cells, induction of inflammation, proliferation and repair [10]. However, IL-1 β production in the intestine can be regulated by other proteases, and IL-18 $^{-/-}$ and IL-18r $^{-/-}$ mice, but not IL-1r $^{-/-}$, are susceptible to colorectal tumorigenesis, suggesting that IL-18 is the critical player of anti-tumor immunity exerted by the inflammasome. This is further supported by the experiment showing IL-18 infusion reduces inflammation and hyperplasia in the colon of caspase-1-deficient mice. IL-18 is initially identified as IFN γ -inducing factors, and IFN γ is a known activator of anti-tumor signaling pathway STAT1. Consistently, IFN γ production and STAT1 activation were shown to be suppressed in caspase-1 $^{-/-}$ mouse tumors [16]. These results imply that activation of IFN γ /STAT1 signaling axis by IL-18 is an underlying mechanism of the inflammasome-mediated protection against CRC [7].

It is conceivable that multiple inflammasomes pathways are active in the gut since tumorigenesis in Nlrp3 $^{-/-}$ and Nlr4 $^{-/-}$ mice is less severe than that of caspase-1-deficient mice, and IL-18 production is not completely abrogated in Nlrp3-deficient mice. A very recent study reported that mice deficient in NLRP1b are susceptible to colitis and colitis-

associated CRC. Reduced caspase-1 activation and IL-18 production is suggestive of inflammasome-dependent protection of colon tumorigenesis in NLRP1b-deficient mice. However, how NLRP1b is activated in the gut is not clearly understood because *Bacillus anthracis* is not a commensal bacterium. This might suggest that the NLRP1b inflammasome can be activated by other unknown pattern molecules. Similarly, increased tumor burden in Nlrp6-deficient mice is associated with reduced IL-18 production. However, the involvement of NLRP6 in activating the inflammasome is still debated, since no study to date has shown direct evidence of NLRP6-mediated caspase-1 activation in an *in vitro* system. Notably, a role for NLRP6 in regulating NF- κ B and MAPK pathways has been proposed in a study done by Allen et al. [10] Supporting this finding, Normand, et al. documented increased expression of IL-17, CCL20, and MMP7, and the activation of the WNT/ β -catenin pathway, in the colons of DSS-treated Nlrp6 $^{-/-}$ mice. Notably, NF- κ B regulates several genes, whose products serve as agonists for the WNT/ β -catenin pathway in paracrine manner. Future studies should address whether NLRP6-mediated regulation of the NF- κ B and ERK signaling pathways contributes to the protection against colorectal tumorigenesis.

NLRP12 is another member of NLR family protein that has recently emerged as a critical regulator of CRC. Using AOM/DSS model of colorectal tumorigenesis, two independent studies showed that Nlrp12 $^{-/-}$ mice are hyper-susceptible to CRC with increased numbers of adenomatous polyps in the colon as compared to wild-type mice. Increased tumor burden was associated with higher proliferation, increased inflammation and induction of tumorigenic mediators such as Cox2, IL-17. At signaling level, the NF- κ B, ERK and STAT3 pathways are hyper-activated in the colons of tumor bearing Nlrp12 $^{-/-}$ mice, suggesting that NLRP12 is a negative regulator of inflammatory signaling pathways. Indeed, *in vitro* biochemical studies confirmed that NLRP12 negatively regulates the NF- κ B and ERK signaling pathways in macrophages and dendritic cells. Interestingly, NLRP12-deficiency not only leads to increased tumor burden but also promotes faster progression of tumorigenesis as more than 30% of Nlrp12 $^{-/-}$ mice developed adenocarcinoma while no wild-type mice developed adenocarcinoma at the same time [17]. It would be interesting to know whether NLRP12 regulates cancer metastasis as well. However, increased NF- κ B activation may not be the sole mechanism of pronounced colorectal tumorigenesis in Nlrp12 $^{-/-}$ mice. In fact, diverse roles for NLRP12, including participation in inflammasome formation, activation of the NF- κ B pathway, suppression of inflammation, and trafficking of dendritic cells have been described [11]. Therefore, precise mechanisms of NLRP12-mediated regulation of neoplastic transformation of epithelial cells, proliferation of tumor epithelium and cancer progression are not clearly understood. Further investigation on the role of NLRP12 in cancer signaling pathways in intestinal epithelial cells would enhance our understanding of the role of NLRP12 in CRC.

Regardless of mutations in cancer susceptibility genes, host-pathogen interaction is a critical trigger in the induction, progression, and metastasis of CRC. Our understanding of NLR biology in cancer has just begun, but the increasing evidence suggests that NLRs critically regulate CRC via diverse physiological functions. While the role of several NLRs in CRC is yet to be defined, the function of NOD2, NLRP6, and NLRP12 in CRC should be studied further to define the underlying mechanism of the regulation of CRC. A major challenge in the treatment of CRC is disease recurrence after conventional chemo and radiotherapy. Therefore, immune therapy targeting NLRs to activate anti-cancer immunity or suppress of oncogenic signaling pathways may hold greater promise in CRC treatment.

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