Editorial

Non-inflammasome forming NLRC3 suppresses tumorigenesis in the gut

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Dysfunction of intracellular nucleotide-binding domain and leucine-rich repeats (NLRs, also known as NOD-like receptors) is associated with human diseases including infections, cancer, and autoimmune disease (1,2). These innate immune pattern recognition molecules (PRRs) are essential regulators of physiological and pathological inflammation (3,4). NLRs recognize pathogen- and danger-associated molecular patterns (PAMPs and DAMPs), before they mount immune responses to both microbial pathogens and damaged self, regulate tissue repair after damage and balance gut microbiota to maintain tissue homeostasis (5,6). Defective NLR-mediated signaling in the gut contributes to colitis and tumorigenesis of colorectal cancer (CRC) by increasing the permeability of the epithelial barrier, dysregulating the proliferation of epithelial cells, and inducing oncogenic mediators (3,7-9). All NLRs contain a central NACHT domain that facilitates oligomerization, a number of N-terminal effector domains that recruit downstream signaling molecules, and multiple C-terminal leucine-rich repeats (LRRs) for ligand sensing (3). Thus far, 23 human NLRs and NLR-like proteins can be distinguished by their effector domains that match the functional properties to each NLR (10). For example, the caspase activation and recruitment domain (CARD) distinguishes the NLRC subfamily (NLRC 1–5) and permits direct interaction between members of this family and other CARD carrying adaptor proteins (9). NLRC3 (also known as CLR16.2 or NOD3) is a poorly characterized member of the NLR family (11,12). Recent studies show that NLRC3 functions as a negative regulator of Toll-like receptor (TLR)-mediated signaling pathways (13) and the DNA sensor STING in response to PAMPs or to virus infection (14). Thus far, there are few reports on the role of NLRC3 in carcinogenesis. Published in *Nature* 2016, Dr. Karki and colleagues elegantly clarified the function of NLRC3 in colitis associated tumorigenesis (11).

With wild-type and Nlrc3$^{-/-}$ mice, Karki and coauthors demonstrated a protective role of NLRC3 in tumorigenesis with two different murine models including the colitis-associated CRC model by treatment of azoxymethane and dextran sulfate sodium (AOM/DSS); and a spontaneous CRC model carrying a heterozygous mutation in the gene encoding adenomatous polyposis coli (Apc$^{Min/+}$). Compared to the wild-type mice, Nlrc3$^{-/-}$ mice suffered significantly more high-grade dysplasia in the colitis-associated CRC model, where the extent or severity of damage was mostly appeared in the middle and distal colon and rectum (11). Furthermore, significantly increased inflammatory cytokines and chemokines in the colon tissue and systemically in the sera, as well as elevated levels of pro-inflammatory mediators such as IκBα of NF-κB pathways and STAT3 in the colon tissue were prominent in Nlrc3$^{-/-}$ mice during disease transition from colitis to CRC. These data reflected the hyper-susceptibility of Nlrc3$^{-/-}$ mice to colitis. Moreover, Apc$^{Min/+}$Nlrc3$^{-/-}$ mice bore significantly higher percent of hyperplasia, exhibited increased damage in the colon where there were greatly increased number of Ki67+ proliferative cells compared to Apc$^{Min/+}$ mice as the control. Collectively, the *Nature* study from Dr Karki *et al*.

on NLRC3 supplemented new evidence and strengthened, along with data from other NLRs, such as NOD1, NOD2, NLRP3, NLRP6, NLRC4, and NLRP12, the protective role of NLRs in tumorigenesis, mostly in colon cancer (3,7-10,15).

In Karki's report, mice lacking NLRC3 especially in the intestinal epithelial cells developed the highest number of tumors, in comparison to mice lacking NLRC3 in hematopoietic cells, myeloid cells or the wild-type control (11). Further data showed NLRC3 inhibition on the hyper-proliferation of enterocytes (11). In relevant studies, NLRs act in distinct cell types to prevent from tumorigenesis (15). For example, the expression of NLRC4 (16) or NLRP6 (17) in the gut, rather than hematopoietic cells, was essential to suppress the development of colon cancer; whereas sufficient expression of NLRP3 (18), or NLRP12 (19) in hematopoietic cells was necessary to attain the same function, although conflicting data exist (20). These data reflected the fact that, as innate immune sensors to protect and maintain intestinal integrity, these NLRs not only position in the gut but also reside systemically; In addition, $Nlrc3^{-/-}$ mice had significantly higher levels of pro-inflammatory cytokines and chemokines than wild-type mice in the sera in the AOM/DSS model (11), indicating NLRC3 capability on regulating systemic inflammation that linked to CRC. Whereas it is possible that there may be a common pathway which NLRs converge on leading to protection against CRC, varied NLRs may well cooperate at different disease stages to maintain homeostasis in the gut where there is high host-microbiome interaction. Eventually, the activation of NLRs enables protective immune response against pathogens, or endogenous danger signals, while keeps tolerance to commensal microbes (1,5,15). Additional studies are warranted to elucidate these relevant mechanisms not only in animal models but also in relevant human diseases.

Previous research generally established two kinds of signaling cascades following the activation of NLRs and NLR-like proteins (9), both leading to inflammation. While NODs (including NOD1 and NOD2) form the Nodosome, other NLRs (i.e., NLRP1, NLRP3, NLRC4, etc.) assemble multi-protein inflammasome complexes of various platforms (8,15). Formation of Nodosome further activate canonical or non-canonical NF-κB signaling, as well as MAPK (p38, ERK, and JNK) pathways, leading to induction of anti-microbial peptides (AMPs), cytokines, and chemokines, and thereof inflammation. Inflammasome complexes allow cleavage and maturation of IL-1β and IL-18 and consequent inflammation and pyroptosis (6,8). In addition, activation of NF-κB, STAT3 or STAT1 signaling was involved in inflammasome-mediated inflammation (3). In Karki's study, assessment of cytokine production of IL-1 and/or IL-18 in $Nlrc3^{-/-}$ and wild-type mice revealed that NLRC3 (also known as NOD3) mediated inflammation in an inflammasome-independent manner (11). Moreover, the increased proliferation of intestinal epithelial cells and enhanced organoid formation in the absence of NLRC3 were highly related to activation of PI3K–Akt–mTOR pathways, which is commonly upregulated in human cancer (21); The treatment of a dual PI3K-mTOR inhibitor in $Apc^{Min/+}Nlrc3^{-/-}$ mice greatly reduced the tumor burden to a similar level shown in control mice (11). Further analysis showed that NLRC3 inhibited PI3K activity via breaking up the association between its p85 with p110α subunit, and thereof downstream signaling events (11). Persistence of imbalanced nutrients and cellular energy, and cellular stress may well activate PI3K-mTOR signaling, the key regulator determining the cell fate via courses of cell apoptosis and/or autophagy, leading to a status of chronic inflammation that progressively results in cancer development (22). Importantly, the effective therapy with the inhibitor on PI3K-mTOR pathways in colitis-associated CRC in Karki's study suggests future therapeutic directions to suppress the transition from chronic inflammation to cancer (11). Nonetheless, further studies are necessary to reveal specific ligands for NLRC3, and additional upstream signaling pathways in realizing NLRC3 functions during innate immune surveillance.

Compromised immune surveillance by chronic inflammation links to malignancy (6,22,23). Although a role of NLRC3 in the regulation of T-cell activation was previously indicated (24), the adaptive immunity including T and B cells seemed not involved in colitis-associated CRC in $Nlrc3^{-/-}$ mice (11). Given that many NLR family members play a protective role in tumorigenesis (3,7-10,15), current data imply that there is strictly innate-mediated immune surveillance or tumor-suppressing mechanisms operated that inhibit cancer development without priming adaptive immune responses (4,25). These mechanisms need further investigations. NLRC3 was previously characterized as a negative regulator on inflammation and NF-κB pathways (10). Whereas inflammation is often necessary for eradication of pathogens and elimination of dangerous signals to eventually restore homeostasis, persistent NLR activation can cause significant collateral damage to the host tissue that eventually results in chronic inflammation

and cancer (1,2,8). In summary, Karki and colleagues' study expanded our understanding and elucidated new mechanisms on NLRC3, whose functions are mostly undiscovered, as compared to other NLRs (11). Their data may likely encourage an area of active investigations on the mechanisms and applications related to NLRs, innate immune surveillance, tumorigenesis and related therapeutics in human disease-associated situations, not solely on cancer.

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