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NLRC3 Attenuates Colon Cancer by Down-Regulating PI3K-mTOR Signaling

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The nucleotide-binding oligomerization domain-like receptors (NOD-like receptors, NLRs) are intracellular sensors. Most of them positively affect inflammatory responses, particularly the inflammasome forming NLRs. On the other hand, several studies on gene-deficient mice have revealed that several NLRs negatively influence innate immune responses. Some recent studies have identified a novel sub-group of non-inflammasome forming NLRs that negatively influence different pathways related to inflammation and carcinogenesis. Cytosolic pattern recognition receptor NRLC3 is a negative regulator of innate immune response. In this review we will discuss finding related with NLRC3 and its mechanism by which it alter cancer pathogenesis. Recently, it has been found that mice deficient in Nlrc3 are hyper-susceptible to colitis and colitis-associated colon carcinogenesis. Oncogenic inhibitory effect of NLRC3 is more dominant in epithelial compartment than hematopoietic compartment. It down regulates mTOR signaling and reduce cell proliferation. NLRC3 interact with PI3Ks and suppress activation of PI3K dependent kinase AKT. Understanding the role of NLRC3 in cancer may facilitate the recognition of new therapeutic strategies.

Key Words: NLRC3, mTOR, Colon cancer

INTRODUCTION

Colorectal cancer is the third most common form of cancer and one of the major cause of cancer related deaths worldwide (1). Immunity and inflammation are critical determinant of carcinogenesis, influencing its initiation, promotion and progression (2). Nucleotide-binding oligomerization domain-like receptors (NOD-like receptors, NLRs) are cytosolic sensors for detection of damage-associated and pathogen-associated molecular patterns (DAMPs and PAMPs) and hence activate innate immune response to a broad range of pathogens, tissue damage and other cellular insults (3). NLR have further subfamilies called NLRA, NLRB, NLRP and NLRC. NLRC subfamily is composed of NLRC1, NLRC2, NLRC3, C2TA and NLRC5 (4). NLRC3 has the typical NOD and leucine rich repeat configuration and poorly defined CARD (5). It was originally identified as inhibitor of T cell function by slowing down the degradation of IκBα (6). NLRC3 is one of the non-inflammasome forming NLRs (6). Majority of NLRs up regulates inflammatory condition, mostly the inflammasome forming NLRs. Though, several recent studies showed that numerous NLRs down regulate innate immune responses (7). One example of such NLRs is NLRC3 that

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attenuates LPS-stimulated activation of NF-κB by down regulating TRAF6 (8). NLRC3 interact with both TBK1 and STING and impaired STING-TBK1 interaction and hence downstream type I interferon production (9). Recently, it has been reported that NLRC3 is an inhibitory sensor of PI3K mTOR pathways in cancer (8).

NLRC3 IN INFLAMMATION-ASSOCIATED COLORECTAL CANCER

Previously it has been found that mice deficient in NLRs like NLRP3 and NLRP6, exhibits increased susceptibility to colitis and colitis-associated colon tumorigenesis compared to wild-type (WT) mice in the azoxymethane-dextran sulfate sodium (AOM-DSS) model (10). Thus, Karki, *et al.* investigated the impact of NLRC3 on colorectal cancer. For this purpose they constructed colitis-associated colon cancer modal by intraperitoneally injecting a cohort of littermate WT and Nlrc3$^{-/-}$ mice with AOM followed by administration of three cycles of DSS in drinking water in repeating cycles interrupted by normal drinking water. They found more bodyweight loss in Nlrc3$^{-/-}$ mice as compared to WT mice during AOM/DSS regime. At day 80 of this regime they found high number and grade of colon tumors in the absence of NLRC3 (8).

ROLE OF NLRC3 IN INTESTINAL INFLAMMATION

Numerous studies on gene deficient mice have shown that several NLRs down regulate innate immune response. It has been previously shown that NLRC3 reduces LPS-induced NF-κB activation through inhibition of adaptor protein TNF receptor associated factor 6 (TRAF6) (9). In a single round of DSS treatment experiment NLRC3 deficiency led to sever colon length shortening and more damage to colon architecture. They found elevated protein level of IL-1β, TNF-α, IL-6 and G-CSF and chemokines KC (also known as CXCL1), MCP-1 and MIP-1α in colon tissue of Nlrc3$^{-/-}$ mice compared to wild-type mice in acute colitis modal. Consist with elevated level of pro-inflammatory cytokines they observed increased activation level of STAT3 and NF-κB pathways in colon tissue of Nlrc3 deficient mice. At day-8 and 14 of AOM-DSS treatment they found high number of neutrphils, macrophages and natural killer cells in the colons of Nlrc3 deficient mice compared to wild type mice. Such difference was not observed in untreated WT and Nlrc3 deficient mice. Previously, NLRC3 was shown to regulate T-cell activation. NLRC3 has been implicated in the regulation of T-cell activation. Although, they did not found any difference in the levels of IFNγ^{+} or TNF+ CD4^{+} T cells when wild-type and Nlrc3 deficient splenocytes were stimulated with CD3 and CD28 in the presence of IL-2 (8).

Many NLRs are also expressed in non-hematopoietic cells, such as IEC. Recently it has been shown that certain NLRs like NLRX1 act as an intrinsic tumor suppressor in IEC (11). To evaluate the contribution of hematopoietic and non-hematopoietic NLRC3 they constructed bone marrow chimera and performed AOM-DSS experiment. They observed that WT mice receiving Nlrc3 deficient bone-marrow were more susceptible to colorectal cancer than WT mice that received WT bone marrow. Similarly, Nlrc3 deficient mice that received Nlrc3 knockout bone showed more colorectal cancer than Nlrc3$^{-/-}$ mice that received WT bone marrow. Such findings were further confirmed by generating mice that specifically lack Nlrc3 in hematopoietic compartment (*Vav1creNlrc3fl/fl*) and those with specific deletion of Nlrc3 in intestinal epithelial cells (*Vil1creNlrc3fl/fl*). NLRC3 deficiency in epithelial compartment resulted in the more colorectal tumorigenesis than its deficiency in hematopoietic compartment. All these findings demonstrate that inhibitory effect of NLRC3 is more dominant in epithelial compartment than hematopoietic compartment (8).

NLRC3 CONTROLS CELL PROLEFERATION

Next they checked for cellular proliferation of Nlrc3$^{-/-}$ and WT epithelial cells through measurement of Ki67^{+} and PCNA^{+} cells. NLCR3 deficiency resulted into more proliferation than WT. Furthermore, colonic epithelial stem cell from WT developed less and small orgonoids than those from Nlrc3 deficient in an *ex-vivo* culture. Differences in orgonoids number and size were due

to differential colony forming capacity as the expression level of stem cell marker Lgr5 in colon tissue of Nlrc3$^{-/-}$ and WT mice was same. These finding were supported by reduced proliferation level of human colon cancer cell line HCT116 with over expressed NLRC3 than those expressing a control (GFP). Similarly, Nlrc3$^{-/-}$ fibroblast showed more rapid proliferation than WT fibroblasts (8).

NLRC3 REGULATES PI3K-AKT-mTOR PATHWAY

Previously it has been found that PI3K/AKT/mTOR pathway is an oncogenic driver in cancer (12). The authors sought to ascertain how NLRC3 deficiency led to excessive proliferation. So, they check for mTOR pathway in colon tissue of WT and Nlrc3$^{-/-}$ mice. They found high phosphorylation level of S6 kinase, 4E-BP1 and AKT at Ser473, which are the downstream targets of mTOR. Such increased phosphorylation level of these mTOR targets proteins were observed in Nlrc3$^{-/-}$ orgonoid than WT orgonoids. No difference was observed in Wnt signaling pathway between WT and Nlrc3$^{-/-}$ mice. Such difference in mTOR signaling pathway was observed as early as day 8 of AOM-DSS regime. No difference in cytokines level or NF-κB pathway activation was observed at this much early stage. So it can be concluded that this early stage dysregualtion of mTOR pathway may further leads to NF-κB pathway activation later on (8).

Members of the PI3K family are lipid kinases involved in multiple cellular processes, including proliferation (13). To evaluate the upstream signaling proteins of mTOR signaling pathway they checked for phosphorylation level of AKT at Thr308 in colon tissue at day 14 and found high phosphorylation level in the absence of NLRC3. Such differences in phosphorylation level were more pronounce in epithelial cell and to a lesser extent in infiltrating cells. Also, augmented phosphorylation of AKT at Thr308 was found in Nlrc3$^{-/-}$ organoids stimulated with IGF-1 compared to wild-type controls. Furthermore, they found increased activation of PDK1 in the colon tissue of Nlrc3$^{-/-}$ mice subjected to AOM-DSS regime. Hyperphosphorylation and co-localization with lysosome were found in the Nlrc3$^{-/-}$ primary fibroblasts stimulated with IGF-1 than WT primary fibroblasts treated with IGF-1. These findings were further supported by increased activation of mTOR signaling in Nlrc3$^{-/-}$ fibroblasts or WT fibroblasts treated with short interfering RNAs (siRNAs) against NLRC3 compared to their corresponding controls (8).

NLRC3 IN SPORADIC COLORECTAL CANCER

The Apc$^{Min/+}$ mouse has a point mutation in the Apc gene, which predisposes the mice to increased spontaneous intestinal tumorigenesis (14). To study the role of NLRC3 in spontaneous intestinal cancer modal the crossed Nlrc3$^{-/-}$ mice with Apc$^{Min/+}$ mice to get Apc$^{Min/+}$Nlrc3$^{-/-}$ mice. They observed higher tumore load in Apc$^{Min/+}$Nlrc3$^{-/-}$ mice than Apc$^{Min/+}$ counterparts. They found increased number of Ki67^{+} proliferative cells and cells positive for phosphorylated S6 kinase in the colon of Apc$^{Min/+}$Nlrc3$^{-/-}$ mice than Apc$^{Min/+}$ mice. Furthermore, Apc$^{Min/+}$Nlrc3$^{-/-}$ intestinal stem cells were more efficient to proliferate into organoids than Apc$^{Min/+}$ intestinal stem cells. Treatment of Apc$^{Min/+}$Nlrc3$^{-/-}$ and Apc$^{Min/+}$ mice with the NVP-BEZ235 an inhibitor of PI3K-mTOR reduced the tumor load and phosphorylation of S6 kinase in the tumors and enterocytes of Apc$^{Min/+}$Nlrc3$^{-/-}$ mice to a level observed in treated Apc$^{Min/+}$ mice. From these findings, they concluded that NLRC3 restricts cellular proliferation via the PI3K-mTOR axis during colon carcinogenesis (8).

In co-immunoprecipitation assay they found weak interaction of NLRC3 with PDK1 but no interaction with AKT. Though, they found that NLRC3 co-immunoprecipitated with p85 subunits of PI3K. In addition, they observed increased levels of interaction between the p85 and p110α subunits of PI3K in Nlrc3$^{-/-}$ primary fibroblasts or mouse bone-marrow-derived macrophages. Furthermore, they found increased level of activation and phosphorylation of p85 PI3K in the colon tissue of AOM/DSS treated Nlrc3$^{-/-}$ mice than its WT counterpart. Reconstitution of NLRC3 in Nlrc3$^{-/-}$ fibroblasts reduced phosphorylation of AKT at Thr308 and other downstream molecules to levels similar to those seen in wild-type fibroblasts upon treatment with IGF-1. So, from these results it can be demonstrated that NLRC3 disrupts an association between the PI3K p85 and p110α subunits and decreases the activity of PI3K p85 itself (8).

CLOSING REMARKS

In conclusion, this mini review demonstrates that presence of NLRC3 protect against cancer by limiting the activation of PI3k-mTOR pathway which is involved in cell proliferation. This inhibition is achieved by direct binding of NLR3 with p85 component of PI3K signaling pathway. So downstream phosphorylation and activation of mTOR signaling is inhibited and hence the cell proliferation and cancer ignition and development are abrogated. Furthermore, this inhibitory potential of NLRC3 is stronger in non-hematopoietic compartment than hematopoietic compartment. It is suggested that continuous stimulation of NLRC3 may represent a promising therapeutical strategy in cancer.

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