

NLRC4, inflammation and colorectal cancer (Review)

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Received July 9, 2024; Accepted August 19, 2024

DOI: 10.3892/ijo.2024.5687

Abstract. Chronic inflammation is recognized as a major risk factor for cancer and is involved in every phase of the disease. Inflammasomes are central to the inflammatory response and play a crucial role in cancer development. The present review summarizes the role of Nod-like receptor C4 (NLRC4) in inflammation and colorectal cancer (CRC). Reviews of the literature were conducted using Web of Science, PubMed and CNKI, with search terms including 'NLRC4', 'colorectal cancer', 'auto-inflammatory diseases' and 'prognosis'. Variants of NLRC4 can cause recessive immune dysregulation and autoinflammation or lead to ulcerative colitis as a heterozygous risk factor. Additionally, genetic mutations in inflammasome components may increase susceptibility to cancer. NLRC4 is considered a tumor suppressor in CRC. The role of NLRC4 in CRC signaling pathways is currently understood to involve five key aspects (caspase 1, NLRP3/IL-8, IL-1 β /IL-1, NAIP and p53). The mechanisms by which NLRC4 is involved in CRC are considered to be threefold (through pyroptosis, apoptosis, necroptosis and PANoptosis; regulating the immune response; and protecting intestinal epithelial cells to prevent CRC). However, the impact of NLRC4 mutations on CRC remains unclear. In conclusion, NLRC4 is a significant inflammasome that protects against CRC through various signaling pathways and mechanisms. The association between NLRC4 mutations and CRC warrants further investigation.

Contents

1. Background
2. Methods

3. NLRC4 structure and inflammation
4. NLRC4 function and CRC
5. Possible signaling pathway of NLRC4 in CRC
6. Possible mechanism of NLRC4 in CRC
7. NLRC4 GOF mutation
8. Conclusions, limitations and perspective

1. Background

Nod-like receptor C4 (NLRC4), initially characterized by Poyet *et al* (1), is known for its role in promoting apoptosis. NLRC4 is now recognized for initiating an exponential cascade of adapter recruitment and caspase (CASP)1 autoproteolysis, a process termed inflammasome activation, resulting in the cleavage of inflammatory substrates such as pro-IL-1 β , pro-IL-18 and gasdermin D (GSDMD), which are activated and inhibit the occurrence and development of numerous types of malignant tumor, including colorectal cancer (CRC) (2). Duncan and Canna (2) have examined the distinctive dual role of NLRC4 as both an initiator of inflammasome activation and an NLR protein. p53 activation, triggered by genotoxic stress and pro-inflammatory signals such as those from TNF, has been shown to increase NLRC4 levels (3,4). The first evidence that NLRC4 activation is modulated by specific ligands came from a study showing that murine macrophages deficient in NLRC4 cannot initiate CASP1 upon encountering *Salmonella typhimurium* (5). Although uncertainties remain about the specific kinases involved in the phosphorylation of NLRC4 and their functional significance, the accumulated functional insights suggest that these kinases are likely integral to the regulatory framework ensuring proper NLRC4 activation during infection (2).

Unlike other NLR proteins, comprehensive structural analyses of NLRC4 have significantly advanced the understanding of its activation triggers (2). NLRC4, as with other NLR family members, exhibits a tripartite structural arrangement: An N-terminal domain for homotypic interactions, a central domain for nucleotide binding and a series of C-terminal leucine-rich repeats (LRRs). The stability of the monomer is maintained through these interactions and the binding of ADP to the nucleotide binding site (6). Two studies (7,8) have shown that following co-expression of NLRC4 with NAIP2 and the type III secretion system (T3SS) needle protein, PrgJ,

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Key words: Nod-like receptor C4, colorectal cancer, prognosis, mechanism of action, signaling pathway

NAIP interacts with NLRC4 upon ligand binding, potentially forming disc-like structures composed of unfolded NLRC4 monomers. These monomers engage in extensive molecular interactions with adjacent NLRC4 molecules on opposing faces (2). NLRC4 is identified as a crucial component of the immune response against intestinal pathogens (2,9-11). A gene mutation in NLRC4 is suspected to be the first identified recessive mutation leading to the monogenic disorder, inflammatory bowel disease (IBD), characterized by auto-inflammation and immune system irregularities triggered by environmental stimuli (12). Inflammation plays a key role in tumorigenesis leading to oncogenic mutations, tumor promotion and angiogenesis. Tumor promoting inflammation is driven by numerous factors, including the presence of the proinflammatory cytokines, interleukin (IL)-1 β and IL-18. An important source of IL-1 β and IL-18 secretion is via activation of the inflammasome. The inflammasome is a multiprotein complex that, upon activation, leads to the processing and secretion of IL-1 β and IL-18, which is mediated by the cysteine protease, CASP1. Several inflammasomes, including NLR family pyrin domain containing 3 (NLRP3), NLRC4 and NLRP6, have been elucidated in tumorigenesis (12). Notably, inflammasomes play different roles in different types of cancer, showing the complexity of inflammation during tumorigenesis. Understanding these roles will help to identify new therapeutic targets and improve the treatment strategies of patients with cancer (12).

Inflammation plays a role in all stages of tumorigenesis. The key signaling pathway that leads to acute and chronic inflammation is via activation of the CASP1 inflammasome. Inflammasome complexes assemble upon activation of certain nucleotide-binding domains (NBDs), LRR proteins (NLRs), absent in melanoma 2 (AIM2)-like receptors or pyridines (13). The activation of inflammasome and IL-18 signaling pathways has a great protective effect in colitis-associated CRC, while excessive inflammation driven by inflammasome or IL-1 signaling pathways promotes breast cancer, fibrosarcoma, gastric cancer and lung metastasis (13). Inflammasomes are multimeric complexes consisting of NLRs, which react to a diverse array of endogenous (damage-associated molecular patterns) and exogenous (pathogen-associated molecular patterns) stimuli. Multiple lines of evidence suggest that in cancer, inflammasomes are positively correlated with features such as elevated IL-1 β and IL-18 levels, activation of NF- κ B signaling, increased mitochondrial oxidative stress and activation of the autophagy process (10,11,13,14). A number of NLRs, such as NLRP3 and NLRC4, have also been emphasized in carcinogenesis and are closely associated with activating inflammatory caspases (14). A particular genetic variant, Ala160Thr, exhibits pathogenic properties *in vitro*, amplifies NLRC4 signaling in response to stimuli (though with less potency than dominant mutations in NLRC4) and results in a slight increase in IL-18 *in vivo*. The variant form of NLRC4 (Ala160Thr) implicated in the recessive immune dysregulation was identified from intestinal epithelial cells (IECs) and colon tissue. Consequently, individuals carrying the NLRC4 (Ala160Thr) mutation are at a higher risk for developing ulcerative colitis (15). NLRC4 mutations can also cause auto-inflammatory disease (16). Additionally, a previous study has found that NLRC4 expression is upregulated in tumor tissues

compared with normal tissues and is associated with prognosis in lung adenocarcinoma (17). NLRC4 mediates the maturation and release of CASP1, further promoting the release of inflammatory factors, such as IL-1 β and IL-18, and plays a critical role in various tumors. Depending on the type of cancer, NLRC4 may act as a tumor promoter or suppressor (18). For instance, NLRC4 promotes tumor progression in breast cancer (19), glioma (20) and liver cancer (21), but functions as a tumor suppressor in melanoma (22) and CRC (23). Another study demonstrated that high expression of NLRC4 was associated with a favorable prognosis in CRC (24). The present review examines the relationship between NLRC4 and inflammation, its function, signaling pathways and the mechanisms by which NLRC4 is involved in CRC.

2. Methods

Web of Science (<https://www.webofscience.com/wos>), PubMed (<https://www.ncbi.nlm.nih.gov>) and CNKI (<https://kns.cnki.net>) were used for the present literature review, with search terms including 'NLRC4', 'colorectal cancer', 'auto-inflammatory diseases' and 'prognosis'. A total of 599 articles related to NLRC4 were retrieved and imported into EndNote X7 (Clarivate). The inclusion criteria were as follows: NLRC4 associated with signaling pathways, mechanism IBD and CRC. The exclusion criteria were as follows: Missing the full text and not associated with CRC. According to these criteria, 299 articles were excluded and 300 full text articles were included. Among these articles, 98 addressed signaling pathways, 67 focused on the mechanisms of action, 158 discussed NLRC4 and IBD and 28 explored NLRC4 and the associations with CRC (Fig. 1). The present review did not involve statistical analysis. In terms of methodology, relevant literature were collected on the structure, function, mutations and the relationship of NLRC4 with inflammation and CRC. The present review is based on the possible signaling pathways, mechanisms of action and mutations related to the structure and inflammation, function and CRC as well as the action of CRC.

3. NLRC4 and inflammation

The NLR family comprises intracellular receptors that detect bacterial molecules, with NLRC4 as one of its members (25). These NLRs are proteins characterized by LRR motifs that enable them to recognize bacterial elements within the cytoplasm of eukaryotic cells. Upon detecting these bacterial elements, the inflammasome complex, which integrates certain NLRs, is triggered. This complex is crucial for activating CASP1, an enzyme that processes pro-inflammatory cytokines, such as IL-1 β and IL-18, into their active forms (26,27). Initially, NLRC4 was primarily recognized as a sensor for flagellin in eukaryotic cells. However, research involving multiple pathogenic organisms, including *Salmonella* (18,28-30), *Legionella* (31-34), *Shigella* (25,35-38) and *Pseudomonas* (39-42), particularly at elevated pathogen concentrations, has shown that NLRC4 can initiate CASP1 activity regardless of the presence of bacterial flagellin (18,30). Additionally, emerging evidence suggests that NLRC4 can also limit bacterial infections in a CASP1-independent manner (43,44). These findings imply that NLRC4 may detect

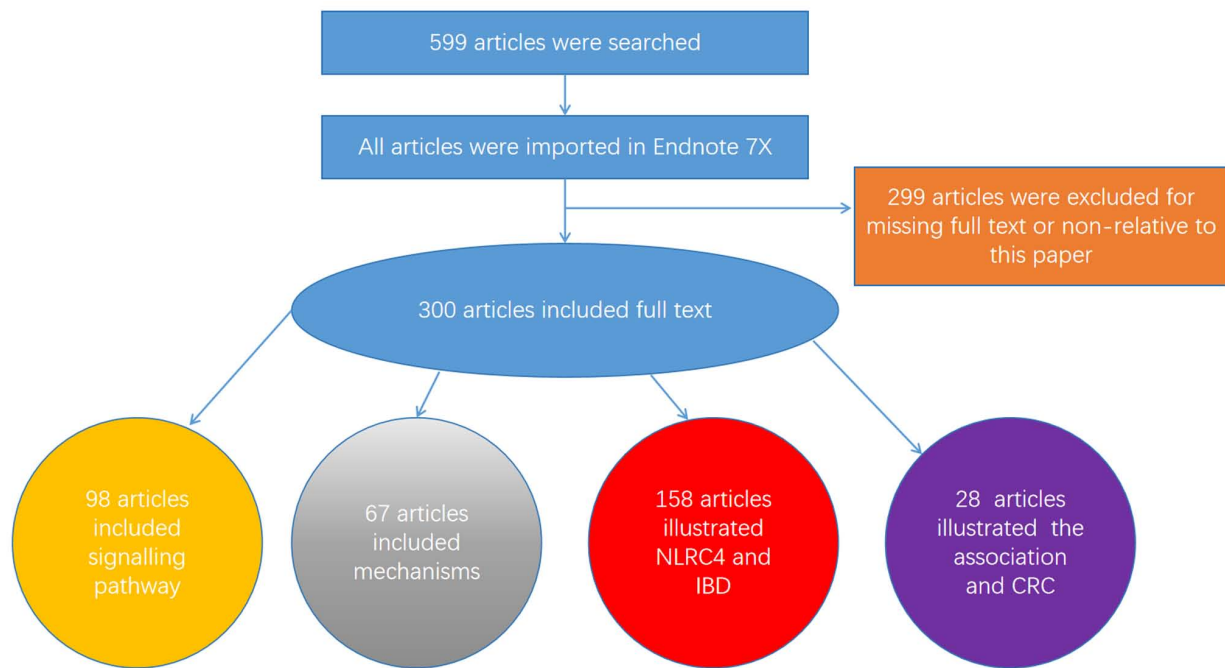


Figure 1. Literature search process. CRC, colorectal cancer; IBD, inflammatory bowel disease; NLRC4, Nod-like receptor C4.

a broader spectrum of bacterial molecules and participate in multiple immune response pathways.

The structure of NLRC4 is illustrated in Fig. 2 (25). The NLRC4 structure comprises three distinct domains: The N-terminal caspase-activating and recruitment domain (CARD; residues 1-88), which interacts with the CARDs of both ASC and CASP1; the central NACHT domain (residues 163-457), which includes ATP/GTPase activity, a characteristic P-loop and a magnesium binding site, serving as a nucleotide-binding and oligomerization platform; and the C-terminal LRR domain (residues 656-1024), which includes at least 13 LRR units and detects upstream signals to trigger NLRC4 activation (1). The NLRC4 (Ala160Thr) variant can lead to recessive immune dysregulation and autoinflammation or contribute to the development of ulcerative colitis as a heterozygous risk factor, potentially related to an increased release of IL-18 compared with IL-1 β (15,45,46).

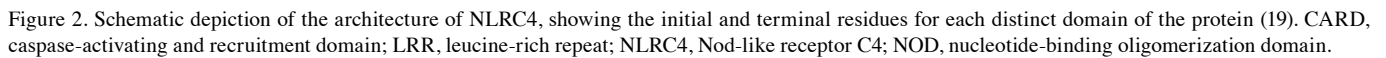
4. NLRC4 function and CRC

NLRC4 functions as a sensor within the innate immune system. A 2004 study demonstrated that bone marrow-derived macrophages (BMDMs) from NLRC4-deficient mice were unable to initiate CASP1 activation, resulting in a failure in pyroptosis when challenged with *S. Typhimurium* (5). Various pathogens possess virulence factors similar to flagellin, which are crucial for activating the NLRC4 inflammasome. These include *Pseudomonas aeruginosa* (PscI), *Shigella flexneri* (MxiI), *Escherichia coli* (EprJ and EscI), *Burkholderia pseudomallei* (BsaK) and *S. Typhimurium* (PrgI) (47). Notably, the NLRC4 inflammasome can also be activated in the absence of flagellin during certain bacterial infections (48). Thus, NLRC4 is a key modulator of the innate immune response, capable of recognizing a range of bacterial virulence factors (18). The apoptosis inhibitory proteins of the NLR family, known as NAIPs, act

as essential sensors for NLRC4 inflammasome activation. The murine genome encodes seven distinct NAIPs, while the human genome contains only one variant, hNAIP (49). Specifically, NAIP5 and NAIP6 have been shown to activate NLRC4 in response to bacterial flagellin (50). NAIP2, similar to NAIP5, serves as a receptor for the inflammasome, recognizing T3SS rod proteins such as BsaK from *B. pseudomallei* and PrgJ from *S. Typhimurium* (33). Furthermore, hNAIP can induce NLRC4 inflammasome activation in response to both flagellin and T3SS components (51-53). During bacterial invasion, the transcriptional regulation of NLRC4 and NAIPs is mediated by interferon regulatory factor 8 (IRF8) (42).

Functional redundancies between NLRP3 and NLRC4 can enhance host defense. Research indicates that mice with a dual deficiency in NLRC4 and NLRP3 due to knockout exhibit increased susceptibility to *S. Typhimurium*, with significantly higher bacterial loads in the mesenteric lymph nodes, liver and spleen compared with the healthy controls (54). Similarly, *Citrobacter rodentium*, another intestinal pathogen, can induce heightened pathology and increased susceptibility in mice lacking CASP1, NLRC4 and NLRP3, suggesting that NLRC4 is crucial for the defense against *C. rodentium* (55). In addition to its role in hematopoietic immune cell lineages during infection, NLRC4 is also activated in non-hematopoietic cell populations, notably within IECs (2). NLRC4 provides protection against certain pathogens, including *Salmonella* (28,30), *Citrobacter* (11,56) and *Legionella* (33,57). However, its activation can also be detrimental, potentially causing an excessive inflammatory response during certain infections, such as those by *Helicobacter* (18).

NLRC4 is a key factor in the pathogenesis of autoinflammatory diseases. While NLRC4 activation is crucial for initiating immune reactions and promoting inflammation in response to bacterial invasion, excessive activation can lead to unnecessary cellular death and cytokine release. Therefore, mutations causing



The functions of NLRC4 in various forms of programmed cell death, including PANoptosis, necroptosis, apoptosis and pyroptosis, have been extensively studied. During infection, activating specific programmed cell death pathways is essential for eliminating invading pathogens from the host. Necroptosis and pyroptosis are typically characterized by their lytic nature and ability to elicit an immune response, whereas apoptosis was traditionally considered immunologically silent (2). However, one previous study has suggested that apoptosis may not always be silent (63). Emerging research has demonstrated that some infectious pathogens and non-microbial stressors can induce an inflammatory form of cell death known as PANoptosis (64). PANoptosis is a distinct and physiologically relevant pathway triggered by specific stimuli and regulated by the PANoptosome, a coordinating structure that facilitates the simultaneous activation of key components associated with necroptosis, apoptosis and pyroptosis (65-74). The involvement of NLRC4 in pyroptosis has been linked to retinal ganglion cell death and has provided new insights into

the pyroptosis of microglia. This finding highlights potential therapeutic approaches for mitigating irreversible vision loss caused by glaucoma by targeting pyroptosis (75). Apoptosis is a programmed cell death process that enables the systematic and effective removal of damaged cells resulting from development or DNA damage. Apoptosis can be initiated by external signals, such as ligands binding to death receptors on the cell surface, or by internal factors such as genotoxic stress (76). The relationship between apoptosis and NLRC4 has been well established, with poly (ADP-ribose) polymerase 1 cleavage by CASPs serving as a definitive indicator of apoptosis (76). Necroptosis, a regulated form of necrotic cell death, is also crucial for the organism's defense against certain pathogens (77). During *Salmonella* infection, a recent study observed activation of mixed lineage kinase domain-like pseudokinase (necroptosis), CASP8, CASP7 and CASP3 (apoptosis), as well as CASP1 and GSDMD (pyroptosis) (62).

During various phases of cancer progression, including metastasis, angiogenesis, proliferation and immunosuppression, aberrant activation of the inflammasome plays a crucial role. Conversely, inflammasome activation can maintain the intestinal barrier and initiate tumor suppression, highlighting its complex role in tumorigenesis (78). Animal experiments involving deficiencies in key inflammasome factors such as NLRC4, Nlrp3, CASP1 and PYCARD have identified the pivotal role of NLR inflammasomes in the pathogenesis of colitis-associated cancer (CAC) (79-81). No significant differences in CAC disease outcomes or pathology were observed when comparing NLRC4^{-/-} animals with controls (wild-type animals) (82). Additionally, another study found that treating NLRC4^{-/-} mice with dextran sulfate sodium (DSS) and azoxymethane (AOM), which induce DNA damage, resulted in increased colonic epithelial cell proliferation, reduced apoptosis and larger tumor volumes (23). Additionally, NLRC4-deficient mice exhibited increased sensitivity to colitis induced by DSS compared with wild-type mice (82). Chemokines and cytokines play a crucial role in eliminating cancer cells, and NLRC4 activation is essential for the synthesis of these molecules in tumor-associated macrophages. In the B16F10 melanoma mouse model, NLRC4 is indispensable for the generation of IFN- γ by CD8⁺ and CD4⁺ T cells (83). However, a previous investigation indicated that NLRC4 does not contribute to melanoma progression, as no difference in tumor incidence was observed between NLRC4-deficient mice and wild-type littermates (84,85). Inflammation is believed to influence several phases of tumorigenesis, contributing to the host's resistance to harmful microbial infections and maintaining tissue balance. Disruptions in this process could potentially trigger inflammatory disorders and malignancies (86). NLRC4 is a pivotal component of the inflammasome complex, and its dysregulation is closely associated with the development of CRC associated with colitis (82). The role of NLRC4 in carcinogenesis varies by malignancy; it can act as either a suppressor or promoter (24). NLRC4 acts as a suppressor in CRC (23). Peng *et al* (24) found that high NLRC4 expression was associated with a favorable prognosis in CRC. However, it was not determined whether NLRC4 was an independent prognostic factor for CRC. Therefore, further investigation is needed to clarify this. The function of NLRC4 is summarized in Table I.

5. Possible signaling pathway of NLRC4 in CRC

Mutations in genes encoding inflammasome components often result in increased susceptibility to autoinflammatory diseases, infections or cancer in humans (83). NLRC4 may protect against CRC through the CASP1 signaling pathway (87). It is noteworthy that deficiencies in CASP1 and ASC render mice vulnerable to DSS-induced colitis and associated CRC (13,79-81,83,88), demonstrating that inflammasomes have a protective function in a CRC inflammatory model. NLRC4 may also protect against CRC through the NLRP3/IL-8 signaling pathway (83) (Fig. 3). NLRC4 and NLRP3 are two different inflammasomes with different expression levels that typically change consistently (75,89-91), although the precise mechanism remains unclear. One possibility is that inflammasome sensors such as NLRP3 facilitate the release of IL-18, an immune signaling protein that aids in restoring the epithelial barrier against injury. This function could explain how IL-18 exerts a protective effect against CRC associated with colitis (13,92-99).

Additionally, NLRC4 may play an anticancer role (suppressing transplantable tumors) through the IL-1 β /IL-1 receptor signaling axis in dendritic cells (100). This pathway may stimulate an effective CD8⁺ T-cell response toward transplantable tumor cells (83) (Fig. 3). NLRC4 could potentially inhibit CRC implantation through this signaling pathway, although no literature currently shows that NLRC4 directly kills CRC cells via this axis.

NLRC4 may inhibit CRC via the NAIP signaling pathway (83) (Fig. 3). Mouse NAIP1-6 proteins, integral to the NLRC4 inflammasome, have been associated with a protective effect against CRC induced by AOM-DSS (101). The specific role of NLRC4 in the AOM-DSS tumorigenesis model remains unclear; one study suggests that NLRC4 may impede colorectal tumor development by inhibiting cell proliferation and promoting apoptosis (23). However, contrasting findings from another study did not attribute a significant role to NLRC4 (79). Additionally, NLRC4 can enhance inflammatory signaling in macrophages, independent of its role in inflammasome formation, and it increases IFN- γ production in CD8⁺ and CD4⁺ T cells, thereby suppressing melanoma tumor progression in a murine model (22).

NLRC4 may also protect against CRC via the p53 signaling pathway (87). As a tumor suppressor, p53 plays an important role in regulating the cell cycle, DNA repair, apoptosis and metabolic pathways. Research shows that 60% of patients with CRC have p53 gene mutations, which are associated with increased tumor invasiveness and drug resistance. Research has also confirmed that NLRC4 is considered to be involved in the downstream apoptotic signaling pathway of p53, exerting inhibitory functions on tumor formation. p53 can activate the expression of NLRC4 mRNA and is induced by upregulation of tyrosine phosphatase, TC-45, which activates p53 (87). NLRC4 also clears damaged epithelial cells through p53 mediated apoptosis, limiting their further abnormal proliferation and transformation into malignant tumors. Therefore, an NLRC4-induced increase in tumor cell apoptosis may be achieved by mediating p53 activation (14,102,103). Further experiments are required to elucidate p53 mediated apoptosis, limiting the further abnormal proliferation and transformation of tumors into malignant tumors. For

Table I. NLRC4 function in inflammation and CRC.

NLRC4 functions ^a	Summaries of the relative factors and signaling pathways	(Refs.)
An innate cytosolic sensor (NLRC4 activated)	Caspase-1 (+), pyroptosis (+)	(5)
	NAIPs (+)	(15)
	T3SS (+)	(28,46-48)
	IRF8 (+)	(37)
Protect host defense (NLRC4 deficient mice)	<i>S. Typhimurium</i> ↑	(49)
(NLRC4 protects host against pathogens)	<i>C. Rodentium</i> ↑	(50)
	<i>Salmonella</i> (+)	(22,25)
	<i>Citrobacter</i> (+)	(11,51)
	<i>Legionella</i> (+)	(28,52)
Auto-inflammatory disease (NLRC4 over activation)	IL-1β↑, IL-18↑	(53)
(NLRC4 mutant)	HD1 (+)	(40,54)
	Neutrophils↑	(55,56)
NLRC4 in pyroptosis, apoptosis, necroptosis, and PANoptosis	IL-1β maturation, caspase-1 (+)	(70)
	PARP1 (+)	(72)
	GSDMD (+), MLKL (+), Caspase-1,3,7,8 (+)	(59)
NLRC4 and CRC	CAC (increasing or not)	(74-76)
	DSS-induced colitis (more sensitive)	(78)
	No role in the progression of melanoma	(80,81)
	Suppressor in CRC	(18,77)
	High expression associated with good prognosis of CRC	(18)

^aThe text in brackets indicates the experimental conditions. NLRC4, Nod like receptor C4; CRC, colorectal cancer; NAIPs, the NLR family apoptosis inhibitory proteins; T3SS, type III secretion system; IRF8, interferon regulatory factor 8; HD1, the helical domain 1; PARP1, poly (ADP-ribose) polymerase 1; GSDMD, gasdermin D; MLKL, mixed lineage kinase domain-like pseudokinase; CAC, colitis-associated cancer; DSS, dextran sulfate sodium.

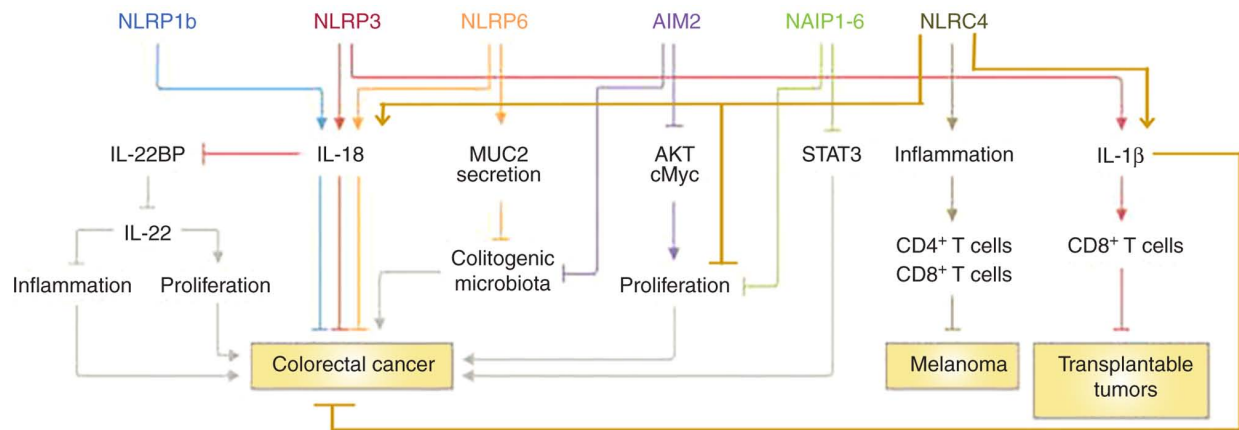


Figure 3. Inflammasome sensors have diverse roles in tumorigenesis. The NLRP1b, NLRP3 and NLRP6 inflammasomes facilitate the production of IL-18, which helps protect against colorectal cancer associated with colitis. IL-18 also triggers the anticancer activity of natural killer cells against metastatic colonic tumor cells, reduces the levels of IL-22BP and may prevent the establishment of colitis-inducing microbiota, potentially through its impact on MUC2 secretion by goblet cells. The NLRP3 inflammasome, along with the IL-1β/IL-1 receptor signaling pathway, drives a T-cell response against transplantable tumor cells. In mice, NAIP1-6 proteins regulate STAT3 phosphorylation and the transcription of genes involved in anti-apoptotic and cell proliferation mechanisms. NLRC4 inhibits melanoma growth by enhancing macrophage inflammation and increasing IFNγ production in T cells. AIM2 suppresses AKT and cMyc phosphorylation, limiting stem cell proliferation and preventing colonization by colitis-promoting microbiota (83). AIM2, absent in melanoma 2; IL-22BP, IL-22 binding protein; MUC2, mucin 2; NLRC4, Nod-like receptor C4; NOD, nucleotide-binding oligomerization domain; NLRP, NLR family pyrin domain containing.

example, the p53 pathway and NLRC4 in CRC can be further explored through *in vivo* experiments in mice or *in vitro* experiments on CRC cell lines. In clinical practice, the relationship

between NLRC4 mutations in serum, CRC adjacent tissues and tumor tissues and the prognosis of patients with CRC via the p53 pathway can be used as a research objective (104-106).

Table II. Possible signaling pathway and mechanisms of NLRC4 in CRC.

Signaling pathway	Mechanisms	(Refs.)
Caspase-1 signaling pathway	NLRC4 in pyroptosis, apoptosis, necroptosis and PANoptosis; an innate cytosolic sensor; protects intestinal epithelial cells.	(67,74-76,83-86)
NLRP3/IL-8 signaling pathway	IL-1 β (+) and IL-18 (+); caspase-1 (+); AIM2 and pyrin; ASC (+); GSDMD (+); IRF8 $\uparrow$, NAIPs helps recognize PAMPs; IFI16, DDX58 promote caspase-1; IL-1 β $\uparrow$, IL-18 $\uparrow$; NLRC4 and MCP2/CCL8 in serum $\uparrow$; epithelium-autonomous NAIP/NLRC4 $\uparrow$.	(13,70,83,88-95)
IL1 β /IL1 receptor signaling axis	Activates CD8 $^{+}$ T-cell; CD8 $^{+}$ T-cell $\uparrow$; suppress CRC cells; killing CRC cell is unknown.	(96)
NAIP1-6 signaling pathway	Inhibiting cell proliferation and promoting apoptosis; enhancing inflammatory signaling in macrophages; increases the generation of IFN γ in CD8 $^{+}$ and CD4 $^{+}$ T cells.	(74,77,79,83,97)
p53 signaling pathway	Acting as a tumor suppressor; involved in apoptosis, DNA repair and cell cycle management; inhibiting the development of tumor cells.	(97-109)

AIM2, absent in melanoma 2; ASC, apoptosis-associated speck-like protein; GSDMD, gasdermin D; IRF8, interferon regulatory factor 8; NAIPs, the NLR family apoptosis inhibitory proteins; PAMPs, pathogen-associated molecular patterns; IFI16, IFN γ -inducible protein 16; DDX58, retinoic acid-inducible gene I protein; MCP2/CCL8, monocyte chemoattractant protein 2/chemokine (C-C motif) ligand 8.

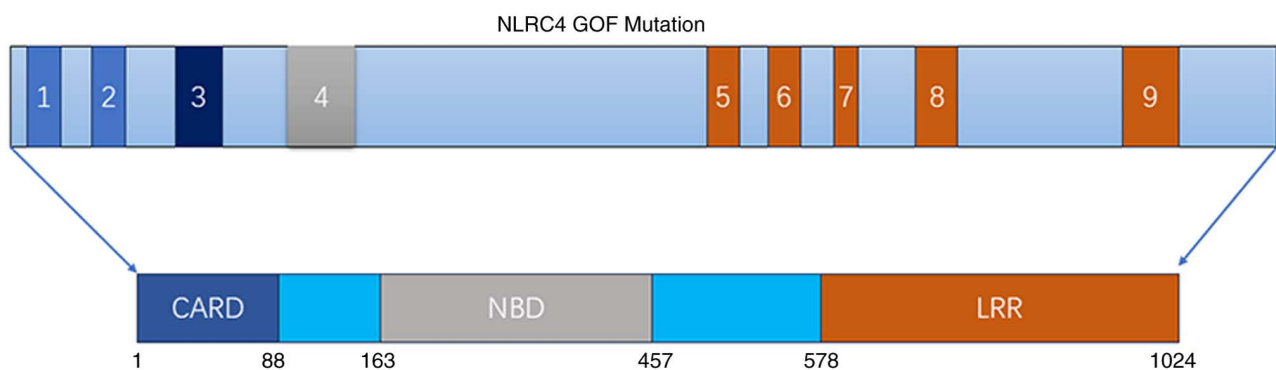


Figure 4. Diagram of NLRC4 GOF mutations (120). CARD, caspase-activating and recruitment domain; GOF, gain-of-function; LRR, leucine-rich repeat; NBD, nucleotide-binding domain; NLRC4, Nod-like receptor C4.

Bacterial flagellin, a classic pathogen-associated molecular pattern, interacts with Toll-like receptor 5 and the NAIP5 receptor (integral to the NLRC4 inflammasome), stimulating immune responses in mammals. However, the role of flagellin receptors in lower animal species is less understood (107). NLRC4 inflammasome may induce colitis inflammation and CRC via p53 signaling pathway. p53 activation is instrumental in promoting apoptosis in coelomocytes (107). Numerous studies have shown that the p53 signaling pathway is closely related to tumor cell apoptosis, thereby inhibiting tumor development (108-115). However, one study indicated that NLRC4 is not associated with the p53 signaling pathway in protecting against colonic tumorigenesis (101). Elevated pyroptosis, an indicator of a 'hot' tumor environment characterized by CD8 $^{+}$ T cells and various T cell subtypes, is influenced by oncogenic pathways including PI3K/AKT/mTOR signaling, angiogenesis, IL-2/STAT5 signaling, IL-6/Janus kinase/STAT3 signaling, epithelial-mesenchymal transition, KRAS signaling, DNA repair and the p53 pathway (116). Therefore, we suggest that

NLRC4 is involved in the p53 signaling pathway to inhibit tumor development. Mechanistically, NLRC4 is considered to play a significant role in this pathway in CRC. These potential signaling pathways are summarized in Table II.

6. Possible mechanism of NLRC4 in CRC

From the above, it can be inferred that NLRC4 suppresses and eliminates CRC cells through pyroptosis, apoptosis, necroptosis and PANoptosis. The assembly of the inflammasome complex triggers the activation of CASP1, which is responsible for the maturation of IL-1 β and IL-18 into their active forms and the cleavage of GSDMD, thereby inducing pyroptosis, a type of inflammatory cell death (117,118). Components such as NAIP-NLRC4, NLRP6, NLRP9, AIM2 and Pyrin can assemble into inflammasomes, playing a role in modulating the host's immune and inflammatory responses (119,120). NLRC4 protects against CRC as a cytosolic sensor by regulating the immune response. NLRP6, NLRP7, NLRP9, NLRP12, the

DNA sensor IFN γ -inducible protein 16 and the RNA sensor RIG-I have been associated with promoting CASP1 activation, though confirmation of their capacity to assemble into an inflammasome complex is still needed (121).

NLRC4 protects IECs to prevent CRC. IECs form a crucial barrier against pathogen invasion. The intestinal immune system's defense and its disease potential are significantly influenced by a coordinated IEC-specific response involving the CASP1 and CASP8 inflammasomes (10). Irak *et al* (122) suggested that serum levels of monocyte chemoattractant protein 2/chemokine (C-C motif) ligand 8 and NLRC4 could contribute to the development of Crohn's disease and play a protective role in maintaining intestinal homeostasis and mitigating inflammation. Another study indicated that the prompt and targeted elimination of infected enterocytes by the epithelium-autonomous NAIP/NLRC4 system is crucial to prevent an excessive TNF-induced inflammatory response that could otherwise damage the epithelial barrier (123). NAIPs protect against colonic tumors by facilitating the clearance of epithelial cells stimulated by carcinogens, likely independent of the NLRC4 inflammasome (101). The administration of AOM, a DNA-damaging substance, along with repeated DSS injections, induces CRC progression associated with colitis (124,125). In mice, oxazolone, a haptenating agent, can also trigger hemorrhagic colonic inflammation and severe submucosal edema, in addition to DSS (126). Although NLRC4 does not contribute to the rapid genetic reconfiguration of the intestine in response to flagellin, its inflammasome activation generates IL-1 β and IL-18, which protect mice from both mucosal and systemic inflammation (82). In summary, NLRC4 protects the intestinal mucosa from pathogen attack through various pathways and inflammatory protective factors, thereby preventing the occurrence of CRC.

7. NLRC4 GOF mutation

Mutations in NLRC4 resulting in GOF have been associated with several conditions, including early-onset recurrent fever, recurrent macrophage activation syndrome, enterocolitis and even cancer (15,16,18,60,120,127). The NLRC4 (Ala160Thr) variant can cause recessive immune dysregulation and auto-inflammation, or act as a heterozygous risk factor for the development of ulcerative colitis. This variant often affects epithelial cells and colon tissue (15).

The NLRC4 protein features a CARD at its N-terminus, a central NBD and a LRR domain (128). Mutations frequently occur in the NBD and LRR domains. Bardet *et al* (128) identified two mutations in the NBD: p.Arg207Lys and p.Thr337Asn (Fig. 4). Romberg *et al* (59) reported a p.Val341Ala mutation in the NBD, while Barsalou *et al* (62) identified a p.Val341Leu mutation. Additionally, the p.Ser445Pro mutation at the NBD was described by Volker-Touw *et al* (129). Other mutations in the NBD include p.Val341Ala, p.Thr337Ser, p.His443Pro and p.Ser445Pro, as noted in the literature (1,2,58,60,129). In the LRR domain, mutations such as p.Gln657Leu (61) and p.Trp655Cys (130) have been observed. Some mutations induce clinical symptoms, while others do not (16,128,131). Mice expressing a murine NLRC4 (Val341Ala) mutant showed elevated systemic IL-18 levels, indicating that the mechanisms by which this mutant induces elevated IL-18 production are conserved between humans and mice. However, while

experiments that are germfree or with infections argue against a role for commensal or pathogenic bacteria, identifying the triggers and mechanisms that synergize with IL-18 to drive NLRC4 (Val341Ala)-associated pathologies requires further research using this NLRC4 (Val341Ala) mouse model (132). The NLRC4 Val341Ala mutation is closely related to colitis, increasing IL-18 levels and potentially raising the risk of CRC from a mechanistic perspective (132). Research on the elevated expression level of Val431Ala in the tissues of patients with CRC is lacking, as is a direct link between this factor and CRC. To date, few studies have reported an association between NLRC4 GOF mutations and CRC, with only 1 study indicating that NLRC4 mutations were present in 4% of CRC cases (24). Therefore, the strategies for treating CRC that rely on NLRC4 are currently in the basic research stage, and there are not yet many clinically relevant studies. This is also one of the purposes of writing the present review.

8. Conclusions, limitations and perspective

NLRC4 is a significant inflammasome that protects against CRC through various signaling pathways and mechanisms. Mutations in NLRC4 may contribute to CRC development and could be associated with a poor prognosis. There is a lack of original studies on NLRC4 mutations and their prognosis in CRC due to some limited conditions in our institution (such as no funding support and lack of suitable patients and samples). However, the present review highlights the need to explore the relationship between NLRC4 mutations and CRC further. The clinical utility of detecting NLRC4 in the serum and tissue of patients with CRC requires additional investigation by researchers and clinicians. NLRC4 contributes to the suppression of CRC, which is the conclusion from numerous experiments and partial clinical study. However, the development of a NLRC4-dependent novel strategy to treat patients with CRC also requires further study. For instance, investigations into how to prevent NLRC4 mutations, how to block their induction of CRC and how to improve the treatment of CRC with serum or tissue NLRC4 mutations are needed.

Acknowledgments

The authors thank Mrs. Liqing Li (Central Laboratory, Huzhou Central Hospital, Huzhou, Zhejiang 313003, P.R. China) for her valuable suggestions on the manuscript.

Funding

This study was supported by the Science and Technology Project of Zhejiang Province (grant no. 2018C37090).

Availability of data and materials

Not applicable.

Authors' contributions

GT, YS, HL, HQ and ZT participated in collecting the literature; GT wrote the paper and drew the figures; YS summarized the tables. HL, HQ and ZT participated in revising the review. All

authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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