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Mini-review

NLRC5: A paradigm for NLRs in immunological and inflammatory reaction

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ABSTRACT

The nucleotide-binding domain leucine-rich repeat containing (NLR) family of proteins is mainly involved in microbial pathogen recognition, inflammatory responses, and cell death. NLRC5, the largest member of the NLR family, is currently receiving an increasing level of attention. NLRC5 has been demonstrated to be a potent negative regulator of NF-κB signaling pathway-mediated inflammatory response. Moreover, accumulating evidence has indicated that NLRC5 is closely related to pathological processes of various cancers. In this review, we present an overview on NLRC5, addressing its underlying molecular mechanisms and implications in host defense, inflammatory response, and associated cancers.

1. Introduction

The innate immune response, which is triggered by microbial infection or tissue damage, is the first line of defense against invading pathogens [1,2]. Innate immunity can be induced by diverse pathological conditions, including bacterial or viral infections, tissue injury, and metabolic disorders [2]. During these processes, pattern recognition receptors (PRRs) act as crucial sensors; they recognize the microbial pathogens, induce intracellular cytokine and chemokine secretion, trigger an inflammatory response, and finally, activate the host defense system, leading to increased phagocytosis and cell autophagy or death [3–5]. In addition to conserved molecular structures among microbial species, endogenous molecules that are released from damaged cells can also be sensed by PRRs, which have two typical recognition patterns: pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [4]. The PRRs are found in all the professional (monocytes, macrophages, and dendritic cells) or nonprofessional immune cells (neutrophils and endothelial cells) and even exist in the cells of the adaptive immune system [3,4,6]. To date, biochemical studies have identified four kinds of PRR families. These families can be classified as C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)—I-like receptors (RLRs), Toll-like receptors (TLRs), and NOD-like receptors (NLRs) [4]. However, each member of PRR families exhibits a distinct cellular location, structure, and pathogen recognition pattern, which essentially defines its immunoreaction. For instance, TLRs, one of the best-known PRRs, localize either at the cytomembrane or within endosomes. On the surface of cells, they interact with extracellular microbes and their derivatives, while in the endosomes, TLRs identify microbes that have entered the cell membrane [1,4]. Similarly, NLRs found in the cytosol have been demonstrated to recognize the extracellular and intracellular pathogens that have impaired vacuolar or phagosomal membranes [1,4,7].

The nucleotide-binding domain leucine-rich repeat containing (NLR) family of proteins, also known as CATERPILLERs, NACHT–LRRs, or NOD-like receptors, are distinguished by their tripartite domain architecture, which consists of a variable N-terminal protein-protein interaction domain, a centrally located nucleotide-binding domain (NBD) and a C-terminal leucine-rich repeats (LRRs) domain, which facilitates the recognition of and binding to PAMPs [8]. The N-terminal domain of NLRs has been verified to recruit downstream effector molecules. NLRs, in terms of their N-terminal domains, can be classified into three subfamilies: CARD (caspase recruitment domain) containing nucleotide oligomerization domain (NODs), PYD (pyrin domain) containing NALPs, and BIR (baculovirus inhibitor domain) containing NLR family

Abbreviations: NLRs, NOD-like receptors; PRRs, pattern recognition receptors; NLRC5, NLR CARD containing 5; MHC, major histocompatibility complex; CLRs, C-type lectin receptors; RIG, retinoic acid-inducible gene; NBD, nucleotide-binding domain; LRRs, leucine-rich repeats; CARD, caspase recruitment domain; PYD, pyrin domain; BIR, baculovirus inhibitor domain; NTP, nucleoside triphosphate; LMB, leptomycin B; CIITA, class II transactivator; NF-κB, Nuclear factor kappaB; HCC, hepatocellular carcinoma; HSCs, hepatic stellate cells; α-SMA, α-smooth muscle actin; COL1a, type I collagen; CD, Crohn's disease; CRC, colorectal cancer; OSCC, oral squamous cell carcinoma

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Table 1
Human NLR family (representative).

NLRs	Chromosomal location	Protein	Involvements	Author, Year
CIITA	16p13	1131aa	antigen-presentation	Choi, N.,2013
NOD1	7p14.3	953aa	NF-κB, MAPK signaling, B/T cell activation, IL-1 βsecretion.	Park, J.,2007; Mercier, B. C.,2012; Petterson, T.,2011; Kavathas, P. B.,2013
NOD2	16q12.1	273aa	NF-κB, MAPK signaling, Inflammasome, autophagy, T cell activation, viral recognition	Kobayashi, K. S.2005; Hsu, L.,2008; Travassos, L. H.,2010; Van Beelen,2007; Sabbah, A.,2009
NLRP1	17p13.2	1375aa	Inflammasome component	Masters, S. L.,2012
NLRP2	19q13.42	846aa	NF-κB signaling, Inflammasome activation, embryonic development,	Bruey, J. M.,2004; Meyer, E.,2009
NLRP3	1q44	1016aa	Inflammasome component	Hirota, S. A.,2001
NLRP4	19q13.42	994aa	NF-κB signaling, autophagy	Fiorentino, L.,2002; Jounai, N.,2011
NLRP5	19q13.42	1200aa	apoptosis, embryonic development	Liu, F.,2004; Tong, Z. B.,2000
NLRP6	11p15	892aa	Inflammasome activation, NF− κB signaling	Anand, P. K.,2012; Chen, G.,2011; Elinav, E.,2011
NLRP7	19q13.42	1037aa	Inflammasome, NF-κB signaling, pregnancy complications	Khare, S.,2012; Messaed, C.,2011
NLRP8	19q13.42	1048aa	Unknown	
NLRP9	19q13.42	991aa	systemic-onset juvenile idiopathic arthritis	Tadaki H,2011
NLRP10	11p15.4	665aa	Dendritic cell migration, NF-κB signaling, IL-1 βsecretion, antibacterial proinflammatory response	Eisenbarth, S. C.,2012; Wang, Y.,2004; Imamura, R., 2010
NLRP11	19q13.42	1033aa	systemic-onset juvenile idiopathic arthritis, Crohn's disease	Tadaki H,2011; Cummings JR,2010
NLRP12	19q13.41	1061aa	NF-κB signaling, inflammasome, dendritic cell migration, MHC	Sinem Tuncer,2014
NALP13	19q13.42	1043aa	Unknown	
NLRP14	11p15.4	1093aa	Spermatogenesis	Westerveld, G. H.,2006
NLRC3	16p13.3	1065	NF-kB signaling	Schneider, M.,2012
NLRC4	17; 17 E2	1024aa	Inflammasome component	Gong, Y. N.,2012
NLRC5	16q13	1866aa	MHC I, NF-κB signaling, type I IFN production, inflammasome activation	(details in text)
NLRX1	11q23.3	975	Mitochondrial localization, RLR signaling, immune, NF-κB signaling, autophagy	Moore, C. B.,2008; Allen, I. C.,2011; Tattoli, I.,2008; Lei, Y.,2012

apoptosis inhibitory proteins (NAIPs) [9]. NLRs are evolutionarily conserved, and their orthologs have been widely characterized in mammals, birds, and fishes. To date, 23 members of the human NLR family and at least 34 mouse NLR proteins have been identified. In mammals, NLRs are structurally and functionally related to the anti-microbial response-associated R protein family found in plants [7,10] (Table 1).

As a consequence of its unusually long C-terminal LRRs, NLRC5 (NLR family, CARD domain containing 5) is the largest member of the NLR family [8]. NLRC5 gene was first cloned and characterized in 2010 by five groups, independent of each other [8,11–14]. Meanwhile, it has been well accepted that NLRC5 can translocate to the nucleus, where it actives the transcription of MHC class I genes and antigen presentation [15]. Several types of tumor tissues exhibit decreased or no expression of MHC I genes, which is considered to be essential to circumvent (cytotoxic T lymphocytes) CTL-mediated antitumor immune responses. As the regulator of MHC class I gene expression, NLRC5 might be involved in the process of malignant transformation and could be used as a therapeutic target to enhance antitumor immunity [16,17]. Meanwhile, NLRC5 has also been confirmed to suppress both NF-κB and type I IFN signaling pathways via interaction with IKK complex and RIG-I/MDA5, respectively [13]. Specific NLRC5-siRNA promoted innate cytokine secretion and antiviral immunity as evident by the decrease in TNF-α, IL-6, and IFN-α/β production in response to LPS or poly (I:C) [13]. Moreover, increasing number of basic and clinical studies over the last few years have reported that NLRC5 is also involved in various non-immunological diseases, including cancers [18]. In this review, we focused on determining the role of NLRC5 in different physiological and pathological processes in terms of NLRC5 regulators and NLRC5 mediated-signaling pathways.

2. Biogenesis of NLRC5

NLRC5 gene originates from human chromosome 16q13, spanning a region of about 96 kbp with 55 exons, and the polypeptide encoded by it contains 1866 amino acids (aa), with a predicted size of ~200 kDa [8,13]. The homology of human NLRC5 to mouse NLRC5 is 64%. *Mus musculus* NLRC5 is located at the locus 8; 8C5 and the polypeptide encoded by it is 1915 aa long [12]. The gene structure of NLRC5 is highly conserved throughout many mammalian species and is structurally reminiscent to R proteins in plants, a protein family that is responsible for plant antimicrobial response [19]. Human NLRC5, similar to other NLRs, comprises of the typical tripartite domain structure: (1) the N terminal atypical CARD spanning 1–91 aa composed of five α-helices [20], (2) the centrally located NBD located between position 220 and 383 with all typical characteristics vital for nucleotide hydrolysis, and accompanied by winged helix and superhelical domain, and (3) the 27 LRRs at the C-terminal consisting of 713 aa, forming an LRR helix [13,14] (Fig. 1). However, the N-terminal CARD of NLRC5 has been predicted to adopt a death domain (DD) fold and exhibits no significant sequence similarity to other typical CARD domains, and may, therefore, be known as an atypical CARD [21,22]. Yet, it displays a high level of conservation among mammalian orthologs and in control of adapter protein interaction and downstream signal activation. Both Walker A motif (nucleoside triphosphate (NTP) combining site, the central NBD domain also referred to as P-loop) and Walker B motif (NTP hydrolysis site) contribute to the subcellular localization and function of NLRC5 molecule [11]. Given the unusually long stretch of C-terminal LRRs of NLRC5, it is the largest member among the NLR protein family with a molecular weight of 204 kDa, whereas other NLRs typically possess a predicted size of 80–120 kDa [8].

The location of NLRC5, identified via immunofluorescence analysis by Sven Kuenzel et al., is restricted to the cytosol. No evidence has been found regarding the lysosomal or mitochondrial localization of NLRC5 through the Lyso Tracker and MitoTracker staining methods [13]. However, Torsten and colleagues revealed that NLRC5 was located both in the cytosolic and the nuclear compartments [11]. Analysis of its cellular distribution showed that most of the cells, at baseline culture, exhibited an intermediate distribution (80%) of NLRC5, 15% of the cells displayed an exclusively cytosolic localization of NLRC5, and approximately 5% of cells displayed a nuclear localization of NLRC5. Moreover, a high accumulation of NLRC5 in the nucleus was observed in more than 75% of the cells exposed to leptomycin B (LMB), a molecule that blocks CrmA-dependent nuclear export and causes the

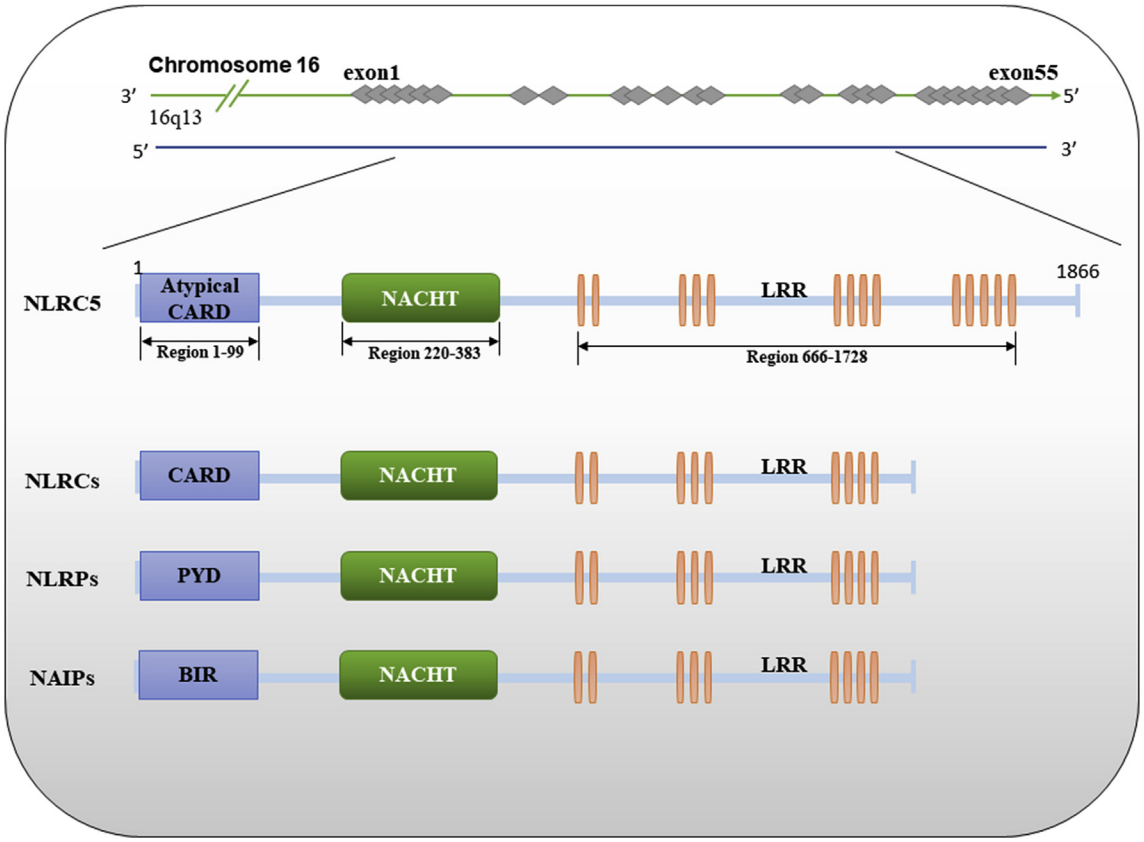

Fig. 1. Schematic representation of the structural domains of NLRC5. The NLRC5 gene is derived from human chromosome 16q13, spanning a region of about 96 kbp with 55 exons. The resulting protein is comprised of the typical tripartite domain structure, (1) the N terminal atypical CARD spanning 1–91 aa, (2) the centrally located NBD is located between position 220 and 383, and (3) the C-terminal 27 LRRs consists of 713 aa.

nuclear accumulation of proteins that shuttle between the cytosol and the nucleus [8,11]. Interestingly, the localization of NLRC5 in different cell lines is different. NLRC5 was more frequently localized to the cytosol of cells with high NLRC5 protein expression levels, whereas nuclear localization was more frequently observed for the cells that expressed lower levels of NLRC5 [11]. A similar localization pattern was observed for murine NLRC5, which could also be induced by LMB treatment [11]. Furthermore, the CARD and NBD domains of NLRC5 are also involved in its nuclear import due to the nuclear localization signals (NLSs) present within them. NLRC5 possesses an NLS between the N and C terminals in duplicate, and locus mutations within the NLS blocked the translocation of NLRC5 from cytosol to the nucleus [11]. In addition, the accumulation of NLRC5 in the cytosol could be observed when it contained mutations in the Walker A motif [11]. These findings suggested that NLRC5 is a cytosol-nuclear shuttle molecule that translocates in a CrmA-dependent manner and that the interaction of NTP with the Walker A motif of the NBD is probably necessary for the conformational transition that facilitates the nucleus import of NLRC5.

3. Expression of NLRC5

Although NLRC5 is expressed ubiquitously in many species, its expression varies across different organs and cells. Murine NLRC5 mRNAs were strongly expressed in thymus, spleen, and lung, indicating that this molecule is biologically conserved in these tissues. According to the RT-PCR results obtained by Sven Kuenzel et al., the highest expression levels of human NLRC5 mRNA were in the tissues of brain, lung, and prostate, followed by the heart, digestive tract, and thymus. The lowest NLRC5 mRNA expressions were observed in spleen, lymph nodes, and leukocytes [13]. On the contrary, Andreas Neerincx and colleagues identified that human NLRC5 mRNA was predominantly expressed in cells or tissues of the hematopoietic system that were involved in immunity [14]. Consistent with available microarray data (BioGPS), they observed highest expression of human NLRC5 mRNA in $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B cells, $CD14^+$ macrophages, and some immune tissues, such as spleen, lymph node, and leukocytes. Similarly, murine NLRC5 could be easily detected in mouse RAW 264.7 cells. On the contrary, NLRC5 was hardly detectable in brain, which was shown to exhibit the highest mRNA level in a previous study [23]. More specifically, NLRC5 mRNA was expressed in the cell lines derived from bone marrow, for instance, thymoid (Jurkat-T), myeloid (THP-1), and Raji B cells [8,12,14]. Andreas Neerincx et al. showed that except for the cervix carcinoma cell line, HeLa, epithelial cell lines, such as human embryonic kidney cell line HEK293T and human colon cell line CaCo2, exhibited marginal NLRC5 mRNA expression [14]. As controversial as the varied mRNA expression patterns of NLRC5 in different organs or cell lines are, the expression profiles of the NLRC5 protein, which directly mediate biological functions, remain largely unclear. Hence, future studies to characterize the expression of NLRC5 protein are encouraged which will be tremendously helpful in elucidating its function.

4. NLRC5-mediated pathways in immunological and inflammatory reactions

4.1. NLRC5 transactivates MHC class I genes

MHC Class I/II molecules and their accessory molecules play crucial roles in the development and activation of human adaptive immune responses by presenting antigens to T lymphocytes. Both MHC class I and class II expression levels are highly inducible and antigen presentation by them is indispensable for the activation of $CD8^+$ T cells

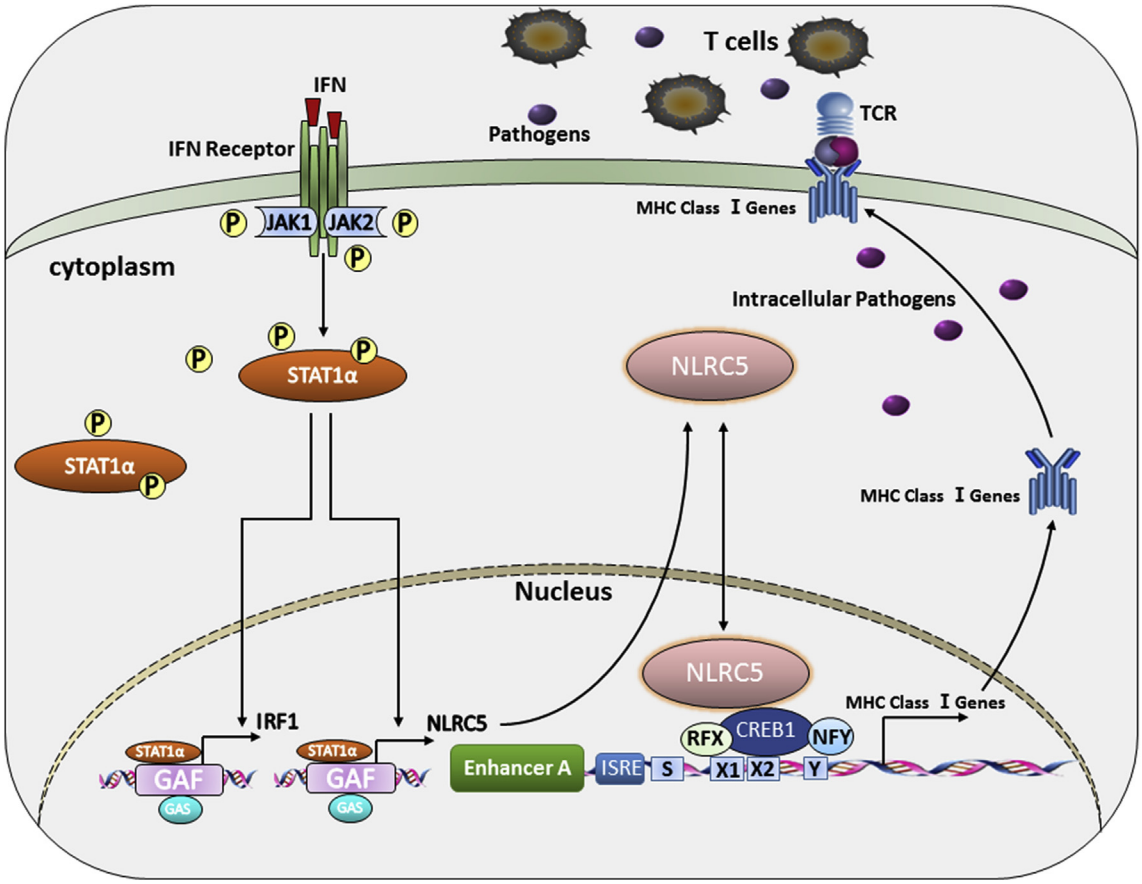

Fig. 2. Regulatory role of NLRC5 in MHC class I associated antigen presentation in host defense. Type I interferon activate the transcription factor STAT1 by phosphorylation, result in up-regulation of NLRC5 expression. NLRC5 can shuttle from cytosol to nucleus and forms a potent enhanceosome with transcription factors (such as the RFX component RFXANK/B) on the MHC class I promoter region to induce MHC class I gene expression, finally lead to the activation of antigen specific $CD8^+$ T cells.

and $CD4^+$ T cells, respectively [11]. Through the activation of T cells, MHC-dependent immune responses trigger and maintain significant inflammation during intracellular bacterial or extracellular viral infections, autoimmune diseases, and cancers. CIITA (class II transactivator), one of the NLRs identified by Steimle et al., in 1993, has been widely recognized as a master regulator for the elementary and inductive expression of all MHC II members and their accessory molecules (HLA-DO and HLA-DM) via association with the MHC enhanceosome [24]. Meanwhile, it has been shown that CIITA could also regulate the transcription of MHC class I genes [24]. Growing evidence shows that the expression levels of MHC class I genes and NLRC5 were correlated in mouse and human cell lines and tissue. The role of NLRC5 as a transactivator of MHC class I genes was initially identified in 2010 via the Gene-chip analysis of generated human JurkatT cell lines, which stably expressed wild-type or mutant-type NLRC5 with mutations in the NBD: Walker A, Walker B, and combined Walker AB [11]. Compared to cells expressing the mutant-Walker A and mutant-Walker AB NLRC5, clustering analysis presented a significantly higher difference in the gene expression profiles of WT and mutant-Walker B cell lines, which expressed the active forms of NLRC5. Both the MHC class I genes (HLA-A, −B, −C, and -E) and the molecules associated with the antigen presentation and processing of MHC class I genes (β2M, LMP2, and TAP1) were induced by active NLRC5. In addition, the increase in MHC class I proteins and their accessory molecules in cells expressing WT or the Walker B mutant NLRC5 was further confirmed through quantitative real-time PCR, western blot analysis, flow-cytometry analysis, and the luciferase assay [11,25]. Moreover, the enforced expression of NLRC5 in HEK293T cells also resulted in the upregulation of MHC class I genes; however, CIITA only marginally induced MHC class I genes, consistent with previous studies [26,27]. On the contrary, the therapeutic NLRC5 siRNA exhibited an inhibitory effect on the cell surface expression of HLA class I proteins in non-transformed primary human dermal fibroblast cell lines, THP1 and HeLa [25]. In addition, transfection of HEK293T cells with NLRC5 with mutant-Walker A or with mutant-Walker B domain confirmed that instead of NTP hydrolysis, NTP binding was responsible for the NLRC5 activation and MHC class I induction by NLRC5 [25]. Subsequently, emerging evidence has revealed the inducible role of NLRC5 in the MHC class I gene expression *in vivo* [28,29]. Splenocyte analysis in NLRC5-deficient mice showed a significant decrease in the constitutive expression of the classical murine MHC class I genes, H2−K and H2-D, in $NLRC5^{-/-}$ T, NK, and NKT cells; however, an intermediate suppression and a marginal reduction were observed in B cells and conventional dendritic cells, respectively [29]. Moreover, the expression levels of non-classical MHC class I genes, such as H2M3 and H2Qa1, and the molecules related to MHC class I antigen presentation and processing (B2m, Lmp2, and Tap1) were also impaired in the NLRC5-deficient spleen and thymus [30]. In contrast, the expression levels of MHC class II genes in T/B cells, spleen, and thymus, derived from NLRC5-deficient mice, were comparable [30,31]. More importantly, a direct association between NLRC5 and MHC class I genes has been shown by Kristina Ludigs et al. using ChIP-seq analysis in 2015 [32]. These findings together confirmed that NLRC5 specifically contributes to the expression of MHC class I members and that of the accessory genes that are involved in antigen presentation.

Given that the process of antigen presentation meditated by MHC

class I genes is pivotal for $CD8^+$ T cell development and activation, T cell proportions and development in NLRC5-deficient mice were also evaluated. The percentage of $CD8^+$ T cells was reduced in the spleen of NLRC5-deficient mice, whereas development of CD4 + T, NKT, and B cells was normal; however, NK cells showed a tendency to increase in number [29]. Interestingly, the cell number of $CD8^+$ T cells did not change significantly in the NLRC5-deficient thymus, suggesting that $CD8^+$ T cells in thymus are not affected by the lack of NLRC5 and they may play a compensatory role in MHC class I antigen presentation [30]. Moreover, as compared to WT mice, *Listeria* induced antigen-specific $CD8^+$ T cell activation was also impaired in the spleen and liver of NLRC5-deficient mice and was accompanied by an increased abundance of the bacteria in both tissues [28]. However, cell proliferation of OT-I T (OVA-specific T-cell receptor transgenic $CD8^+$ T) cells, which were adoptively transferred into NLRC5-deficient mice, was mildly affected by dendritic cells (DCs), when the mice were subjected to antigenic challenge [30], indicating that NLRC5 was not required for the T cell–priming ability of DCs, which might directly affect antigen presentation by promoting the activation of age-specific $CD8^+$ T cells (Fig. 2).

4.2. Negative regulation of NLRC5 in type I interferon (IFN) producing pathway

While the regulatory role of NLRC5 in the expression of MHC class I genes has been well recognized through several studies, several studies have suggested that NLRC5 may be also involved in type I interferon (IFN)-producing pathways during the immune response. Although, type I IFN signaling could be induced by VSV, intracellular poly (I:C)/LyoVec, poly (I:C), and LPS, the increased IFN-β secretion was only observed in $NLRC5^{-/-}$ macrophages exposed to RIG-I/MDA5 ligands (VSV or intracellular poly (I:C)), suggesting that NLRC5 might primarily interact with RIG-I/MDA5 (the dsRNA sensors) to inhibit RNA virus induced type I IFN signaling [12,31]. Specifically, phosphorylation of IRF3, a downstream target gene of type I IFN, was observed in VSV-eGFP infected macrophages. Unlike the barely detectable level of *p*-IRF3 in WT macrophages after 8 h postinfection, IRF3 phosphorylation level progressively increased with time in $NLRC5^{-/-}$ macrophages [31]. To further elucidate, the proportion of VSV-eGFP-infected cells among $NLRC5^{-/-}$macrophages was lower than that among WT macrophages, which indicated the inhibitory function of NLRC5 in type I IFN signaling-meditated antiviral immunity of macrophages [31]. In addition, this negative regulation of NLRC5 in type I IFN pathway was also confirmed in *in vivo*. Compared to WT mice, IFN-β level in sera was significantly higher in $NLRC5^{-/-}$ mice at an early time point (6 h after infection with VSV-eGFP) [31].

Although these results suggest a possible role of NLRC5 in type I IFN signaling, several other studies present contradictory results too. Neerincx et al. reported that the RNAi-mediated suppression of NLRC5, as compared with scrambled siRNA, reduced the induction of IFN-β after SeV and poly (I:C) infection in THP-1 cells [14]. Consistent with this result, silencing of NLRC5 significantly suppressed CMV-induced IFN-α secretion in human epithelial cells, while upregulation of IFN-α and prolonged phosphorylation of STAT1, downstream of IFN-γ, were observed in NLRC5 overexpressed cells [13]. More interestingly, as demonstrated by Kuenzel et al. and Guo et al., IFNβ and IFN-γ enhanced NLRC5 promoter transactivation in human A549 and HeLaS3 cells, respectively [13,33]. This feedback mechanism might affect the specific role of NLRC5 in innate immunity. Therefore, the association of NLRC5 with type I interferon signaling pathway seems to be highly cell-specific and context-dependent. Further investigation is needed to clarify the discrepancies observed by different researchers and define the exact functional role of NLRC5 in type I IFN signaling-meditated innate immune response.

4.3. NLRC5 was a potent negative regulator of NF-κB activation

Playing a pivotal role in innate and adaptive immunity, nuclear factor kappa B (NF-κB) is exceedingly indispensable for the development and survival of lymphoid cell and organs [34]. NF-κB comprises of a family of transcription factors, which are ubiquitously expressed in mammalian cells and form heterodimers or homodimers, whose activity is directly regulated by inhibitory IκB proteins. Dysregulation of NF-κB activity has been confirmed to be associated with the pathogenesis of various diseases, including immunodeficiency, virus infective diseases, inflammation, and cancers [35,36]. The IKK complex, composed of the kinase heterodimer IKKα/IKKβ and the regulatory subunit IKKγ (NEMO), mediates the phosphorylation of IκB in response to upstream activation signals [37]. This phosphorylation effect induces IκB degradation via the ubiquitin-proteasome pathway, leading to the translocation of free NF-κB from cytoplasmic compartment to the nucleus and its activation to regulate transcription downstream target molecules [34]. NLRC5 was initially demonstrated as a potent negative regulator of NF-κB activation by Cui et al. via luciferase assay [12]. NF-κB luciferase reporter plasmid significantly inhibited NF-κB activation induced by IL-1β, TNF-α, or LPS in 293 T cells. Further co-immunoprecipitation and western blot analyses revealed that NLRC5 competed with NEMO to directly interact with IKKα/IKKβ subunits, and block their phosphorylation and kinase activity [12]. Conversely, loss-of-function analyses suggested that specific knockdown of NLRC5 released IKKα/IKKβ for phosphorylation and significantly promoted production of TNF-α, IL-6, and IL-1β, NF-κB-responsive cytokines, in human THP-1, mice RAW264.7 cells, and MEFs [8,12,31]. In addition, using different NLRC5 mutants, Cui and colleagues revealed that LRR region, rather than CARD or NBD region, of NLRC5 was responsible for its inhibitory effect on IKK phosphorylation, and the size of full-length NLRC5 contributed to physically inhibit the interaction between NEMO and IKKα/IKKβ [12]. Meanwhile, the exact mechanism by which NLRC5 regulates IKK and NF-κB activation was investigated by Meng et al. through experimental and mathematical analyses [38]. Co-immunoprecipitation analyses revealed that NLRC5 could be ubiquitinated with K63 linkage in different dynamic patterns after treatment of RAW264.7 cells and MEFs with LPS. Co-localization of Ub and NLRC5 was also observed in BMMs after LPS treatment. More importantly, *in silico* analyses showed that the ubiquitination of NLRC5 influenced its binding capacity to IKKs, resulting in a decreased inhibitory effect of NLRC5 on IKK activation. Simultaneously, TRAF2 and TRAF6, which served as adapters in NF-κB signaling, were confirmed to mediate the ubiquitination of NLRC5. Hence, the suppression of IKKs-NLRC5 interaction, which was induced by NLRC5 after LPS treatment, was rescued by the genetic silencing of TRAF2/6 through a coherent feed-forward loop [38] (Fig. 3).

4.4. NLRC5 is suggested to be an activator and synergetic component of the inflammasome

Inflammasomes are large multi-molecular complexes that are widely known for their ability to mediate the activation of the proteolytic enzyme caspase-1 [39]. Subsequently, caspase-1 triggers pyroptotic cell death along with the proteolytic maturation of the pro-inflammatory cytokines, interleukin-1β (IL-1β) and IL-18 [40]. Assembly of inflammasome complexes heavily relies on the cytosolic sensing of pathogen-associated molecular patterns which enables their translocation to the cytosol during infection of pathogenic microorganisms [41]. Currently, NLRC5 is also suggested to be an activator and synergetic component of the inflammasome. Kumar et al. showed that the co-transfection of pro–IL-1β or procaspase-1 with NLRC5 in HEK293 cells resulted in the increased production of IL-1β and cleavage of caspase-1 [42]. On the contrary, as compared to scrambled si/shRNA, RNAi-mediated suppression of NLRC5 significantly blocked IL-1β secretion in *Escherichia coli*-infected human monocytic cell line, THP-1 [43]. IL-1β

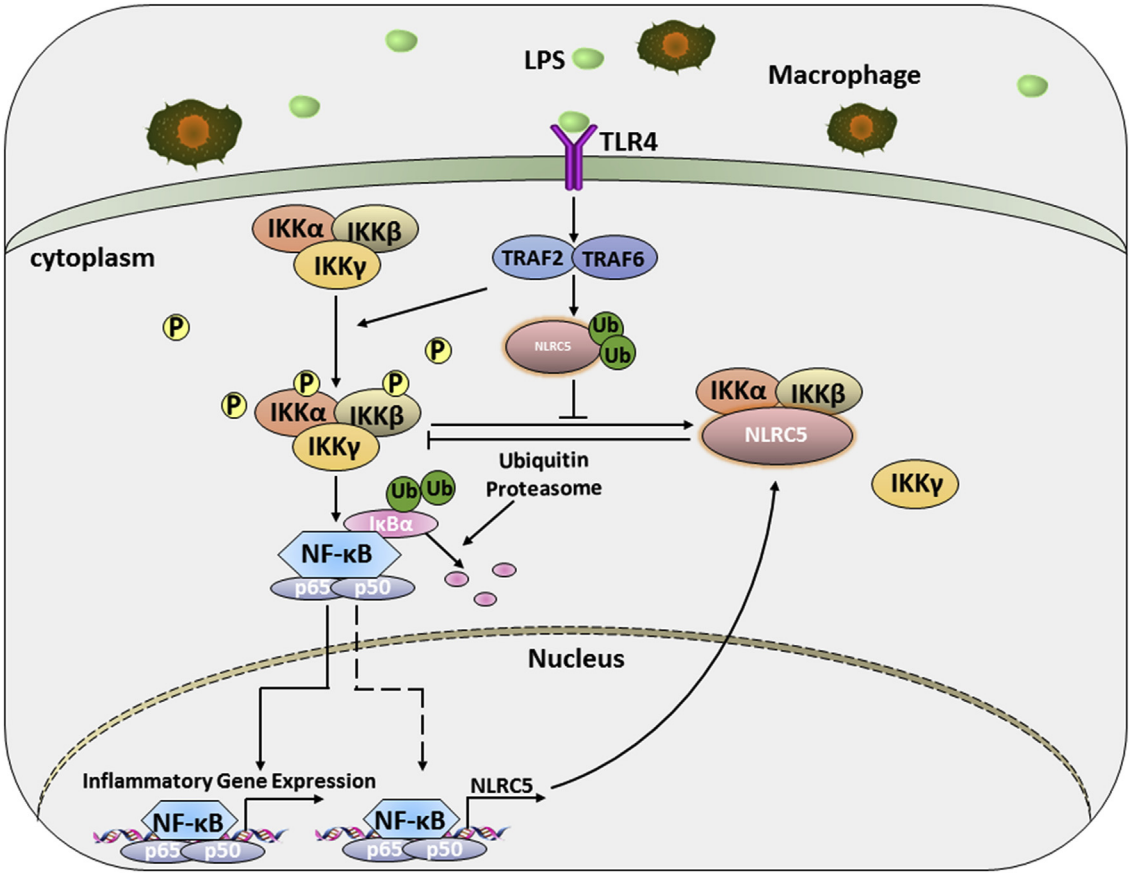

Fig. 3. NLRC5 negatively regulates the NF-κB signaling pathways. NF-κB signaling was triggered by LPS or other inflammatory factors. Phosphorylation effect induces IκB degradation via the ubiquitin-proteasome machinery, leading to the translocation of free NF-κB from cytoplasmic compartment to the nucleus and its activation to regulate downstream targets transcription. NLRC5 could compete with IKKγ to directly interact with IKKα/IKKβ subunits, block their phosphorylation and kinase activity. However, TRAF2 and TRAF6, which serve as adapters in NF-κB signaling, mediated NLRC5 ubiquitination, blocked the IKKs-NLRC5 interaction, rescued NF-κB activation.

inhibition was also observed in THP-1 cells transfected with siNLRP3 [43]. Similarly, human primary monocytes, transfected with specific NLRC5-siRNA also failed to evoke significant IL-1β production in response to *E. coli* infection [43]. Although levels of inflammasome components, NLRP3 and ASC, were not affected by the absence of NLRC5, IL-1β processing and caspase-1 maturation were suppressed by NLRC5-siRNA, strongly suggesting that NLRC5 was involved in IL-1β inflammasome formation. Extensively, candidate pathogens, *E. coli*, *S. flexneri*, and *S. aureus*, all representing bacteria that require NLRP3 and ASC to activate the inflammasome, induced significantly lower level of IL-1β in NLRC5 knockdown THP-1 cells relative to those with normal NLRC5 expression [43], suggesting that NLRC5 modulates the activation of inflammasome in response to a broad spectrum of bacterial pathogens. More intuitively, co-immunoprecipitation experiments revealed that NLRC5 directly binds with NLRP3 and ASC, and the NBD appears to be necessary and sufficient for interaction of NLRC5 with NLRP3, while the association with LRR is inhibitory [43]. However, Kumar et al. did not find significant difference in IL-1β production between cells derived from WT and NLRC5-deficient mice, both of which were exposed to activators of NLRP3 inflammasome, such as monosodium urate (MSU), LPS plus ATP nigericin, zymosan, and curdlan [35]. Similarly, the secretory level of IL-1β was also comparable between WT and NLRC5-deficient dendritic cells (GMDCs) in response to poly (dA:dT) or *Francisella tularensis*, both of which induce activation of AIM2 inflammasome. In addition, the degree of caspase-1 activation was similar in WT and NLRC5-deficient BMDMs infected with *S. typhimurium*, which induces NLRC4 and NLRP3 inflammasomes [42]. These *in vivo* observations challenged the previous *in vitro* results of NLRC5 in inflammasome activation, and suggested that NLRC5 is not responsible for IL-1β secretion during the activation of NLRP3, NLRC4, and AIM2 inflammasomes in macrophages. Hence, inflammasomes might be induced by some yet unidentified endogenous or exogenous pathogens or their activation may be cell-type specific. Future studies on NLRC5-mediated inflammasome activation and its association with other inflammasomes are required to elucidate the physiological role of NLRC5.

5. Function of NLRC5 in cancers

To date, the application of T cell-based cancer immunotherapies, including adoptive T-cell transfer and checkpoint blockade, has increased clinical benefits for patients with different types of cancers [44–47]. However, the loss of expression of MHC class I genes is a common mechanism exploited by transformed cells to evade $CD8^+$ T cell-mediated antitumor immune responses, attenuating the effectiveness of immunotherapies [48]. Given the role of NLRC5 as a transactivator of MHC class I genes, an increasing number of studies are focusing on studying the characteristics of NLRC5 in cancers. The involvement of NLRC5 in cancers was identified by Staehli and colleagues, when they found that NLRC5 expression was lower in lymphoid tumor cell lines than in primary splenocytes or B cells [29]. Subsequently, the regulatory role of NLRC5 in tumor formation was investigated by Rodriguez et al. *in vivo* [45]. As compared to wild type B16 melanoma cells, NLRC5-expressing B16 cells significantly inhibited tumor growth following subcutaneous implantation, and the number of lung tumor foci following intravenous injection into C57BL/6 hosts.

Table 2
Implications (regulators, targets and indications) of NLRC5 in cancers.

Cancer Types	Upstream Regulators	Downstream Targets	Indications	References
Prostate Cancer	HLA class I	Unknown	Progression, resistance to therapy	[44]
Melanoma	Unknown	MHC	Tumor cell immunogenicity	[45]
Hepatocellular Carcinoma	Unknown	Wnt/β-catenin; Akt	Progression, cell proliferation, migration, invasion	[46]
Clear Cell Renal Cell Carcinoma	Unknown	Wnt/β-catenin	Progression, cell proliferation, migration, invasion	[47]

However, the antitumor activity of NLRC5 was not observed in immunodeficient or $CD8^+$ T cell-depleted hosts, indicating that the protective antitumoral effect of NLRC5 was dependent on $CD8^+$ T-cell-mediated immunity. Moreover, the same group immunized C57BL/6 mice with irradiated NLRC5-expressing B16 cells, and found that this prophylactic setting effectively protected mice against B16-Wt cell engraftment [17,45]. Recently, the suppression of NLRC5 through promoter methylation was revealed during the analysis of biopsy samples obtained from thousands of solid cancer patients. In addition, 5-azacitidine (5-Aza), a DNA methylation inhibitor, restored the reduced expression of NLRC5 *in vitro* [17]. The authors suggested that the methylation of NLRC5 in cancers induced the transcriptional suppression of NLRC5, resulting in the decreased expression of MHC class I genes and evasion of $CD8^+$ T cell-mediated antitumor immune responses. Intriguingly, the effects of NLRC5 methylation could not be observed in hepatocellular carcinoma (HCC), and the methylation rate of NLRC5 in HCC tumor tissues was insignificantly lower than that in normal tissues. Coincidentally, elevated expression levels of NLRC5 were observed in human HCC tissues *in vivo* and in the three HCC cell lines (HepG2, SMMC-7721, and BEL-7402) *in vitro* [39]. Both soft agar assay *in vitro* and in a nude mouse tumorigenicity assay *in vivo* demonstrated the antitumor effect of NLRC5-shRNA. Furthermore, the suppression of the Wnt/β-catenin signaling pathway might contribute to the inhibitory role of NLRC5-shRNA in tumor formation [39,46]. Similarly, NLRC5 expression was also found to be aberrantly increased in renal cell carcinoma (ccRCC) tissue samples and ccRCC cell lines [47]. High NLRC5 expression is associated with advanced stage and poor prognosis of ccRCC patients [47]. Overexpression of NLRC5 significantly promoted proliferation, migration, and invasion of ccRCC cells *in vitro*. In addition, the loss of NLRC5 significantly decreased the growth and weight of xenografts in nude mice [47]. According to these results, the function of NLRC5 in cancers might be tissue-specific.

6. Concluding remarks

NLRs represent a pattern recognition receptor family that participates in numerous pathological and physiological processes, ranging from pathogen recognition to the modulation of antigen presentation and inflammatory signaling. NLRC5, the largest member of the NLR protein family, specifically enhances the expression levels of MHC class I genes and genes involved in antigen presentation, and consequently, induces the activation of antigen-specific $CD8^+$ T cells for host immune response against intracellular bacterial infections. However, despite the increasing number of studies, the contradictory role of NLRC5 in type I IFN signaling during immune response still remains to be explored. These conflicting results might arise from the fact that the regulatory role of NLRC5 could be pathogen-, time-, and cell-type-dependent. Moreover, NLRC5 is widely regarded as a potent negative regulator of NF-κB activation during microbial infection. In addition, the physiological role of NLRC5 in the modulation of innate immunity and inflammatory signaling has also been verified to influence the progression of numerous cancers (Table 2). Thus, an important role for NLRC5 has been established in human immune-related disorders, highlighting the importance of determining the molecular mechanisms underlying the transcriptional regulation and physiological function of NLRC5.

Conflict of interest statement

The authors declared that they have no conflicts of interest to this work.

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled “NLRC5:A Paradigm for NLRs in Immunological and Inflammatory Reaction”.

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