

Mechanisms and pathways of innate immune activation and regulation in health and cancer

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Abbreviations: PRRs, pathogen recognition receptors; PAMPs, pathogen-associated molecular patterns; DAMPs, danger-associated molecular patterns; TLRs, Toll-like receptors; RLRs, RIG-I-like receptors; NLRs, Nod-like receptors; ALRs, AIM2-like receptors; CLRs, C-type lectin receptors; IFN, interferon; DCs, dendritic cells; NOD, nucleotide-binding oligomerization domain; LRR, leucine-rich repeat; RIG-I, retinoic acid-inducible gene 1; LGP 2, laboratory of genetics and physiology 2; MDA5, melanoma differentiation-associated protein 5; AIM2, absent in melanoma 2; IFI16, interferon, gamma-inducible protein 16; DDX41, DEAD (Asp-Glu-Ala-Asp) box polypeptide 41; cGAS, cyclic GMP-AMP synthase; TIR, Toll IL-1 receptor; LPS, lipopolysaccharide; MyD88, myeloid differentiation factor 88; TIRAP, TIR domain-containing adapter protein; TRIF, TIR domain-containing adaptor inducing IFN- β ; TRAM, TRIF-related adaptor molecule; SARM, sterile α - and armadillo motif-containing protein; IRAK, interleukin-1 receptor-associated kinase; TRAF, TNFR-associated factor; NEMO, NF- κ B essential modulator; IKK, I κ B kinase; TAK1, TGF- β -activating kinase 1; IRF, interferon regulatory factor; RIP, receptor-interacting protein; TANK, TRAF family-member-associated NF- κ B activator; TBK1, TANK binding kinase 1; IKKi, inducible I κ B kinase; iE-DAP, γ -D-glutamyl-meso-diaminopimelic acid; MDP, muramyl dipeptide; Atg16L, autophagy related 16-like; MAVS, mitochondrial antiviral signaling protein; ASC, apoptosis-associated speck-like protein containing a CARD; CARD, caspase recruitment domain; LT, lethal toxin; NAIPs, neuronal apoptosis inhibitory proteins; STING, stimulator of interferon gene; CMV, cytomegalovirus; ER, endoplasmic reticulum; CDNs, cyclic dinucleotides; CYLD, the familial cylindromatosis tumor suppressor gene; Nrdp1, neuregulin receptor degradation protein 1; TRIMs, tripartite motif containing proteins; LBP, LPS-binding protein; SIGIRR, single Ig IL-1-related receptor; TRAILR, tumor-necrosis factor-related apoptosis-inducing ligand receptor; cIAP, cellular inhibitor of apoptosis protein; ROS, reactive oxygen species; BMM, bone marrow-derived macrophage; SOCS, suppressor of cytokine signaling; GBP5, guanylate-binding protein 5; PKC- δ , protein kinase C delta; PKR, dsRNA-dependent protein kinase; ULK1, autophagy related serine threonine UNC-51-like kinase; AMPK, AMP activated protein kinase; LUBAC, linear ubiquitin assembly complex; GSK3 β , Glycogen synthase kinase 3 β ; MIB, mind bomb; TRIP, TRAF-interacting protein; cDC, conventional dendritic cell; pDC, plasmacytoid dendritic cell; KSHV, Kaposi's sarcoma-associated herpesvirus; RAUL, RTA-associated E3 ligase; RACK1, receptor for activated C kinase 1; HCC, hepatocellular carcinoma

Research on innate immune signaling and regulation has recently focused on pathogen recognition receptors (PRRs) and their signaling pathways. Members of PRRs sense diverse microbial invasions or danger signals, and initiate innate immune signaling pathways, leading to proinflammatory cytokines production, which, in turn, instructs adaptive immune response development. Despite the diverse functions employed by innate immune signaling to respond to a variety of different pathogens, the innate immune response must be tightly regulated. Otherwise, aberrant, uncontrolled immune responses will lead to harmful, or even fatal, consequences. Therefore, it is essential to better discern innate immune signaling and many regulators, controlling various signaling pathways, have been identified. In this review, we focus on the recent advances in our understanding of the activation and regulation of innate immune signaling in the host response to pathogens and cancer.

Introduction

The innate immune system plays an important role as the first line of defense against invading microbes by sensing pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). The initiation and regulation of innate immune responses are orchestrated by several classes of germline-encoded pattern-recognition receptors (PRRs), including Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), Nod-like receptors (NLRs), AIM2-like receptors (ALRs), C-type lectin receptors (CLRs) and other DNA sensors.^{1–3} Upon pathogen invasion, these PRRs trigger the activation of NF- κ B, type I interferon (IFN), or other inflammasome signaling pathways, which, in turn, leads to the production of a variety of proinflammatory and antiviral cytokines and chemokines, subsequently inducing adaptive immune responses.^{4,5}

TLRs belong to a class of type I integral membrane glycoproteins that play an important role in host defense against pathogens by recognizing a wide variety of PAMPs. To date, 10 and 12 functional TLRs have been identified in humans and mice, respectively. These receptors are expressed on the cell surface (TLR1, TLR2, TLR4, TLR5, and TLR6) or in the endosome (TLR3, TLR4, TLR7, TLR8 and TLR9). TLRs are expressed on

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various immune cells including macrophages, monocytes, neutrophils, master cells, eosinophils, dendritic cells (DCs), and T cells.⁴ Since first being described in the fruit fly, TLRs have been identified as a major component in inflammatory conditions, antiviral responses, and several immune-related diseases.⁶

CLRs comprise a large family of receptors, consisting of one or more carbohydrate recognition domains (CRDs), which recognize various carbohydrate ligands. CLRs play a major role in shaping immune responses to microbial pathogens, especially fungi.⁷ They can function directly as PRRs to induce specific gene expression in macrophages and DCs, or to modulate the functions of TLRs through signaling crosstalk.^{8,9}

NLRs constitute a large protein family of intracellular sensors, the members of which share a conserved central nucleotide-binding, oligomerization domain (NOD), a leucine-rich repeat (LRR) region and a variable N-terminal effector domain.¹⁰ NLRs can be classified as receptors (Nod1 and Nod2), adaptors (NLRP3, NLRC4, and NLRP6) and regulators (NLRX1, NLRC3, NLRC5 and NLRP4) by their diverse functions. Much work has been done to reveal the functions of NLRs as receptors of PAMPs to sense microbial structures (e.g. peptidoglycan and muramyl dipeptide for Nod1 and Nod2), and activate NF- κ B signaling, or as the adaptors to form different types of inflammasomes to regulate IL-1 β and IL-18 secretion.¹¹ However, accumulating evidence suggests that NLRs can also function as the negative regulators of innate immune responses, including inflammation, antiviral immunity and autophagy.

Besides TLRs and NLRs, RLRs including retinoic acid-inducible gene 1 (RIG-I), laboratory of genetics and physiology 2 (LGP2) and melanoma differentiation-associated protein 5 (MDA5) function as cytoplasmic receptors for RNA viruses.¹² Recently, several DNA sensors, including absent in melanoma 2 (AIM2), interferon, gamma-inducible protein 16 (IFI16), DNA-dependent activator of IRFs/Z-DNA binding protein 1 (DAI/ZBP1), DEAD (Asp-Glu-Ala-Asp) box polypeptide 41 (DDX41), RNA polymerase III and the cyclic GMP-AMP synthase (cGAS) have been identified to recognize viral DNA and activate inflammasome or type I IFN signaling pathways to establish the antiviral state in the cell.³ Thus, TLRs, CLRs, NLRs, RLRs, ALRs and other DNA sensors are critical in bridging innate and adaptive immune responses by activating several key signaling pathways and producing many important cytokines and chemokines to mediate the immunological state of the host.

Innate Immune Signaling Pathways

Toll like receptor signaling pathways

TLRs are conserved membrane-associated receptors, which contain a variable number of LRR motifs, a transmembrane region and a Toll/IL-1 receptor (TIR) domain.¹³ Their LRR region may recognize and bind to PAMPs as foreign ligands. TLR4, the first identified TLR, recognizes lipopolysaccharide (LPS), a unique cell wall component of gram-negative bacteria. Specific members of the TLR family, including TLR1, TLR2, TLR5 and TLR6, sense the protein, flagellin, or lipid

components on infectious microbes.¹⁴ On the other hand, TLR3, TLR7, TLR8 and TLR9 detect single or double-strand RNA (ssRNA or dsRNA) as well as unmethylated CpG DNA.^{5,15-17}

Upon ligand binding, TLRs have the ability to dimerize and to undergo conformation changes, which lead to recruitment of TLR domain-containing adaptor proteins. To date, 5 adaptors with TLR domains have been identified, including myeloid differentiation factor 88 (MyD88), TIR domain-containing adapter protein (TIRAP)/MyD88 adaptor-like (Mal), TIR domain-containing adaptor inducing interferon (IFN)- β (TRIF), TRIF-related adaptor molecule (TRAM), and sterile α - and armadillo motif-containing protein (SARM).¹⁸ Most TLRs, including TLR1, 2, 4, 5, 7, 8 and 9 and excluding TLR3, use MyD88 as the adaptor protein to activate the canonical NF- κ B pathway.^{19,20} Once the MyD88-interleukin-1 receptor-associated kinase (IRAK) 1-IRAK4 complex is activated, it will recruit and activate TNFR-associated factor (TRAF) 6. Next, TRAF6, together with 2 ubiquitination E2 enzymes, UBC13 and URV1A, catalyze the formation of a K63-linked polyubiquitin chain on TRAF6, itself, and on NF- κ B essential modulator (NEMO), a regulatory subunit of I κ B kinase (IKK) complex. The polyubiquitin chain acts as a scaffold, recruiting TGF- β -activating kinase 1 (TAK1) and its binding proteins, TAB1, TAB2, TAB3, which leads to the phosphorylation and activation of IKK α / β .²¹⁻²³ Activated IKK α / β specifically phosphorylates the inhibitory I κ B protein, which, in turn, directly interacts with and inactivates NF- κ B. This phosphorylation results in the degradation of I κ B, allowing NF- κ B nuclear localization, and subsequent synthesis of proinflammatory cytokines, such as TNF- α , IL-1 β and IL-6, which are critical mediators of inflammatory responses.²⁴ Interestingly, MyD88 may also directly interact with interferon regulatory factor (IRF) 5 and IRF7 to induce IFN- α production through TLR7/9 signaling.^{25,26} In addition, TLR4 and TLR3 can use another adaptor protein TRIF, to recruit TRAF3, TRAF6 and receptor-interacting protein (RIP) 1, which activates IKK α / β complex and TRAF family-member-associated NF- κ B activator (TANK) binding kinase 1 (TBK1)/inducible I κ B kinase (IKKi), respectively. TBK1/IKKi directly phosphorylates IRF3 and IRF7 to activate type I IFN signaling pathways.^{14,27} These TLRs and their signaling pathways are extremely important for initiating and propagating innate immune responses, and for maintaining organism homeostasis.

Signaling pathways of Nod1 and Nod2

Nod1 and Nod2, the 2 most widely studied members of NLRs, recognize bacteria-derived *g*-D-glutamyl-meso-diaminopimelic acid (iE-DAP) and muramyl dipeptide (MDP), respectively.²⁸ Upon ligand recognition, Nod1 and Nod2 oligomerize and interact with RIP2, subsequently leading to the activation of the canonical NF- κ B and MAPK pathways.^{29,30} Nod1 and Nod2 are also involved in the autophagic response to invasive bacteria. Upon bacteria invasion, Nod1 and Nod2 recruit the autophagy related 16-like (Atg16L) to induce autophagy in a RIP2/NF- κ B independent manner.³¹ Furthermore, Nod2 also regulates antibacterial immunity through its interaction with

Atg16L, Atg5 and Atg7 to induce autophagy and to generate MHC class II antigen-specific CD4 T cell responses.³² In addition to their role in antibacterial immunity, a recent study showed that Nod2 senses viral ssRNA, triggering the activation of type I IFN signaling pathway through interaction with mitochondrial antiviral signaling protein (MAVS). However, it is not known whether Nod2 directly binds to viral ssRNA or acts as an adaptor protein.³³ Regardless, it is clear that Nod1 and Nod2 function in response to microbial structures, and lead to the activation of NF- κ B signaling for cytokine production.

Inflammasome activation by NLRs and ALRs

Several NLRs and ALRs, including NLRP1, NLRP3, NLRP6, NLRP7, NLRC4 and AIM2, function as sensors or adaptors, forming the 'inflammasome', which regulates interleukin-1 β (IL-1 β) and IL-18 production and induces cell death via pyroptosis in response to diverse microbial and endogenous danger signals.^{11,34} Inflammasomes are large protein complexes, consisting of the central NLR or ALR protein, the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC) and caspase-1. Activation of the inflammasome usually requires 2 signals: (1) transcriptional induction of pro-IL-1 β , and NLRs or ALRs; (2) specific, agonist-driven oligomerization of NLR/ALRs to initiate inflammasome assembly. Recent studies demonstrated that NLRP3 and AIM2 activation induces prion-like polymerization of the adaptor, ASC, to activate caspase-1. This self-activation modality of ASC, initiated by NLRP3 or AIM2, may represent a unified mechanism for inflammasome assembly.^{35,36}

NLRP1 inflammasome is the first complex identified in the activation of caspase-1. Human NLRP1 contains a C-terminal caspase recruitment domain (CARD) domain, which is thought to be the key component interacting with the CARD domain of procaspase-1.³⁷ Although several studies have shown that MDP activates human NLRP1 inflammasome, the direct activator of human NLRP1 is still unknown.^{38,39} There are highly polymorphic paralogs (Nlrp1a, Nlrp1b, and Nlrp1c) for mouse NLRP1, which do not have a PYD domain. Mouse Nlrp1b mediates the response to the lethal toxin (LT) of *Bacillus anthracis*,⁴⁰ whereas human NLRP1 does not. Like NLRP1, NLRC4 binds to caspase-1 with its own CARD domain. NLRC4 may act as the adaptor protein, downstream of neuronal apoptosis inhibitory proteins (NAIPs) receptor. NAIPs appear to directly bind to flagellin, T3SS rod, or T3SS needle, thereby, activating NLRC4.⁴¹⁻⁴⁴ NLRP3 is the most extensively studied inflammasome and is composed of NLRP3, ASC, and caspase-1. Human, but not mouse, NLRP3 inflammasome also contains the cardinal protein, whose function remains unclear.⁴⁵ Notably, NLRP3 inflammasome is responsive to a wide array of stimuli, ranging from microorganisms of both endogenous and exogenous origin to inorganic entities including alum asbestos and silica.⁴⁶ Whereas specific ligands have been identified for NLRP1, NLRC4, and AIM2, little is known about the direct ligands of NLRP3.⁴⁷ A recent study reveals that a RNA helicase, DHX33, senses cytosolic RNA and interacts with NLRP3 to trigger NLRP3 inflammasome activation.⁴⁸ This result indicates that

NLRP3 may act as an adaptor protein rather than a direct sensor in innate immune signaling.

AIM2 is the most recently identified DNA sensor that interacts with ASC and caspase-1 to regulate IL-1 β and IL-18 maturation upon synthetic dsDNA stimulation or DNA virus infection in a NLRP3-independent manner.⁴⁹⁻⁵² Maturation of IL-1 β and IL-18 is essentially absent in AIM2-deficient macrophages in response to Poly(dA:dT) or *F. tularensis* infection.^{53,54} Collectively, these data demonstrate that these receptors act as nucleotide sensors, mediating the innate immune response in an inflammasome-dependent or -independent manner.

Type I interferon signaling pathways induced by RNA and DNA viruses

Upon RNA virus infection, RLRs, including RIG-I (also known as DDX58) and MDA5 (also known as IFIH1), recognize cytoplasmic viral components and activate the mitochondrial signaling adaptor, MAVS (also known as VISA, IPS-1 or Cardif).⁵⁵ MAVS is a mitochondrial-associated adaptor protein, which provides the platform to recruit downstream kinases, such as TBK1 and IKKi, and leads to IRF3/IRF7 phosphorylation and to activation of type I IFN signaling pathways.⁵⁶⁻⁵⁹ Recent evidence suggests that viral infection induces the formation of very large MAVS aggregates, which are capable of activating IRF3. The MAVS fibrils operate like prions, converting inactive MAVS into its functional aggregates.⁶⁰ These results suggest that a prion-like conformational switch of MAVS may be a critical check-point in antiviral signaling cascade initiation. Another member of RLRs, LGP2, was originally believed to negatively regulate RIG-I and MDA5, due to its lack of a CARD domain.⁶¹ However, a recent study, using LGP2 knockout mice, indicates that LGP2 positively regulates type I IFN signaling in response to a variety of RNA viruses,⁶² suggesting that LGP2 may enhance RIG-I/MDA5-mediated recognition of viral RNA.

DAI (also known as ZBP1) was the first viral DNA sensor identified. It interacts with dsDNA and activates TBK1/IRF3 pathway.⁶³ Subsequent work has confirmed a role for DAI in type I IFN activation against cytomegalovirus (CMV) infection in human foreskin fibroblasts.⁶⁴ However, it has been difficult to discern whether DAI is essential for DNA sensing in vivo.⁶⁵ Recent studies have shown that RNA polymerase III can also serve as an intracellular sensor of viral DNA by transcribing AT-rich double-stranded DNA into double-stranded RNA, which, in turn, activates RIG-I and initiates the MAVS-dependent signaling cascade.^{66,67} Furthermore, IFI16 and DDX41 function as cytosolic DNA sensors and interact with another adaptor protein, stimulator of interferon gene (STING, also known as TMEM173 or MITA), to activate TBK1 and downstream signaling.^{68,69}

STING is a newly discovered adaptor protein, which plays a central role in viral dsDNA responses by mediating TBK1-dependent IRF3 activation.^{70,71} Inactive STING resides in the endoplasmic reticulum (ER), but forms discrete punctate foci with TBK1 in the perinuclear region of the cytosol after dsDNA stimulation.⁷¹ Because several DNA sensors, such as DDX41 and IFI16, have been demonstrated to activate STING, the STING-TBK1-IRF3 signaling axis is now known as the major

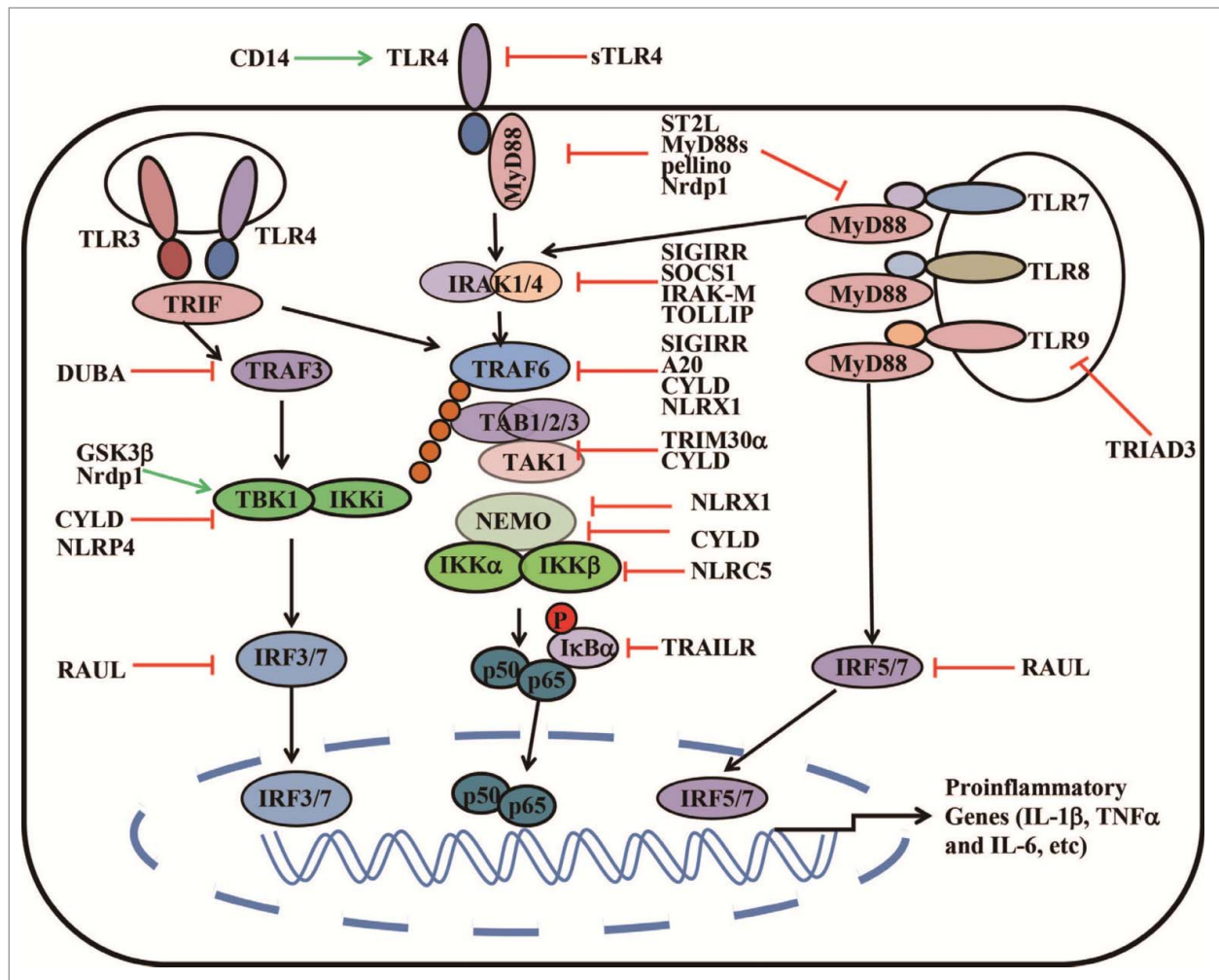


Figure 1. A schematic representation of TLR signaling pathways. TLRs are activated by ligand binding, which leads to dimerization of TLRs and to recruitment of TLR domain-containing adaptor proteins. Next, MyD88/IRAK1/IRAK4 or TRIF activates TRAF6, which, in turn, catalyzes the formation of a K63-linked polyubiquitin chain on TRAF6, itself. The polyubiquitin chain acts as the scaffold, recruiting TAK1 and its binding proteins, which leads to IKK- α/β activation. Activated IKK α/β specifically phosphorylates I κ B α , resulting in I κ B α degradation and NF- κ B translocation into the nucleus. TRIF can also recruit TRAF3 to activate TBK1 and IKKi. TBK1/IKKi directly phosphorylates IRF3/7 to activate type I IFN signaling pathways. Various molecules positively (green arrow) or negatively (red blunt arrow) regulate TLR-induced signaling pathways.

pathway of cytosolic DNA-induced type I IFN signaling. In addition, STING, itself, may work as a sensor, directly recognizing bacterial second messenger molecules called cyclic dinucleotides (CDNs), such as cyclic di-GMP (c-di-GMP), and activating type I IFN signaling.^{72,73} Notably, Z. Chen's group demonstrated that STING also senses cyclic-di-GMP-AMP (cGAMP), leading to cytosolic DNA-induced type I IFN signaling. Furthermore, cGAMP can be generated in the presence of cytosolic DNA by a cyclase enzyme called cGAS.^{74,75} The identification of cGAS may provide the missing link between DNA sensing and STING activation. Despite the fact that the cGAS-cGAMP system is essential for type I IFN induction by DNA viruses in fibroblasts and monocytes,^{74,75} whether the cGAS-cGAMP system operates similarly in other cell types, as well as in vivo, needs urgent resolution.

Regulation of Innate Immune Signaling Pathways by Diverse Regulators

Because uncontrolled innate immune responses can be harmful, even fatal, to the host,⁷⁶ stringent regulation of these signaling pathways is essential to maintain immune balance in the host. In the last few years, many positive and negative regulators have been identified to control innate immune signaling pathways at multiple levels through different mechanisms.

Regulation of TLR signaling pathways

Many molecules, ranging from membrane to cytosol, such as helper cofactors, decoy receptors, or specific enzymes, regulate TLR signaling. These molecules include co-receptors (CD14), soluble receptors (sTLR), transmembrane proteins (ST2L,

SIGIRR, TRAILR), and intracellular regulators (SOCS-1, MyD88s, TOLLIP, IRAK-M, A20, CYLD (the familial cylindromatosis tumor suppressor gene), Nrdp1, regulatory Nod proteins, TRIAD3A, tripartite motif containing proteins (TRIMs)). These molecules maintain the balance between activation and inhibition of TLR signaling in response to diverse PAMPs (Fig. 1 and Table S1).^{76,77}

Co-receptors

Several co-receptors have been identified to modulate TLR function. For example, CD14 is a glycosylphosphatidylinositol-anchored protein, which forms a horseshoe-shaped structure similar to TLR ectodomains. CD14 chaperones LPS from the LPS-binding protein (LBP) to the TLR4–MD2 complex at the cell surface and mediates TNF- α and IL-6 production in response to TLR4 and TLR2–TLR6 ligands.⁷⁸ Furthermore, CD14 is also necessary for induction of proinflammatory cytokines by interacting with TLR7 and TLR9.⁷⁹

Decoy inhibitors

Some transmembrane proteins serve as the negative regulators of TLR signaling. It is well established that soluble decoy receptors negatively regulate cytokine and chemokine production. In TLR signaling, soluble TLR4 (sTLR4) blocks the interaction between TLR4 and MD2,⁸⁰ whereas soluble TLR2 (sTLR2) acts as an agonist of TLR2 by binding to CD14.⁸¹ Since MyD88 is the major adaptor protein for most TLRs, MyD88s (a short form of MyD88) antagonizes TLR signaling by inhibiting LPS- and IL-1 β -induced, but not TNF-induced, NF- κ B activation.⁸² Although MyD88s strongly interacts with IRAK1, it does not directly interact with IRAK4. MyD88s prevents the IRAK4-mediated phosphorylation of IRAK1, which, in turn, inhibits downstream NF- κ B activation.⁸³

Transmembrane proteins

Single Ig IL-1-related receptor (SIGIRR) is a membrane protein that is highly expressed in immature DCs and epithelial cells, but not in macrophages. It interacts with IRAK and TRAF6 to negatively regulate TLR4 and TLR9 pathway. SIGIRR^{-/-} mice are more susceptible to LPS-induced septic shock.^{84,85} ST2L is a type I transmembrane protein that inhibits TLR4-mediated activation and attenuates MyD88 and Mal functions. Macrophages from ST2^{-/-} mice produce higher levels of proinflammatory cytokines in response to a variety of TLR ligands, indicating that ST2L is a potent negative regulator of TLR signaling.⁸⁶ Another receptor that inhibits TLR signaling is tumor-necrosis factor-related apoptosis-inducing ligand receptor (TRAILR). TRAILR does not have a TIR domain, but belongs to the TNF superfamily. It negatively regulates NF- κ B activation by stabilizing I κ B α , preventing its degradation.⁸⁷

Ubiquitin E3 ligases

Protein ubiquitination is a reversible covalent modification, regulating the stability, activity and localization of target proteins. Recent work has shown that many ubiquitin ligases as well as deubiquitinases regulate TLR signaling. In *Drosophila*, the

ubiquitin E3 ligase, Pellino, targets MyD88 for ubiquitination and degradation.⁸⁸ TRIAD3A is a member of the TRIAD3 family of RING-finger ubiquitin E3 ligase. It binds to the cytoplasmic domain of TLR9 and TLR4, but not to TLR2, to promote the ubiquitination and degradation of TLR4 and TLR9.⁸⁹ Another RING-finger E3 ligase, TRIM30 α , is also a negative regulator of TLR-induced NF- κ B signaling. TRIM30 α is induced by TLR agonists and interacts with the TAB2-TAB3-TAK1 complex, mediating TAB2 and TAB3 degradation.⁹⁰ Nrdp1 acts as an E3 ligase and directly binds and polyubiquitinates MyD88, leading to the degradation of MyD88 and subsequent inhibition of NF- κ B activation and proinflammatory cytokine production. Interestingly, Nrdp1 also promotes the activation of transcription factor, IRF3, and the production of IFN- β by interacting with TBK1. These results indicate that Nrdp1 may function as a bio-switch to 'preferentially' promote the TLR-type I IFN axis.⁹¹

Deubiquitinases

A20 was initially identified as a TNF-induced zinc-finger protein that is rapidly induced by both TNF- α and LPS, and is expressed in many cell types.^{92,93} A20 has been identified as a key negative regulator, controlling both MyD88-dependent and MyD88-independent TLR-signaling pathways. It acts as a deubiquitinating enzyme, removing K63-specific polyubiquitin chains from TRAF6, thus, blocking NF- κ B signaling.⁹⁴ Recently, it is reported that A20 inhibits the E3 ligase activities of TRAF6, TRAF2, and cellular inhibitor of apoptosis protein 1 (cIAP1) by disrupting their interactions with key E2 enzymes, either Ubc13 or UbcH5c.⁹⁵ In addition, A20 removes the K63-linked ubiquitin chains on RIP1 (an essential mediator of TNF signaling) and supports E3 ligase activity by facilitating K48-linked ubiquitination of RIP1 for subsequent proteasomal degradation.⁹⁶ Several A20-interacting proteins, including TAX1BP1, RNF11 and Itch, have been identified to regulate the diverse functions of A20.^{95,97,98} A20^{-/-} mice are hypersensitive to both TNF- α and LPS, develop severe inflammation, and die prematurely.⁹⁹ Another deubiquitinase, CYLD, is also important for its inhibitory function in TLR signaling. CYLD removes K63-linked ubiquitin chains from TRAF2, and thus, inhibits NF- κ B activation.^{100,101} It has been demonstrated that CYLD negatively regulates TNF- α or TLR induced-NF- κ B activation and type I IFN signaling by targeting a variety of signaling proteins, including TRAF2, TRAF6, RIP1, TAK1, NEMO, RIG-I, and TBK1, in T cells and other immune cells.¹⁰²⁻¹⁰⁵

Regulatory NLRs

Although NLRs were originally believed to function as the sensors of pathogens and cellular danger signals, recent evidence suggests that several NLRs, known as regulatory NLRs, negatively regulate TLR and RLR signaling. NLRX1 is the first NLR family member shown to be targeted to the mitochondria. It is ubiquitously expressed in a variety of tissues and cells.¹⁰⁶ To date, at least 4 distinct functions of NLRX1 have been identified: (1) to negatively modulate RIG-I-mediated antiviral responses by binding to MAVS and disrupting RIG-I-MAVS

interaction;^{106,107} (2) to negatively regulate TLR-induced NF- κ B signaling by targeting TRAF6 and IKK α / β -NEMO complex;^{107,108} (3) to potentiate TNF- α , poly (I:C) and Shigella infection-induced reactive oxygen species (ROS) production, which can further amplify NF- κ B and JNK signaling as well as proinflammatory responses;¹⁰⁹ (4) to promote virus induced-autophagy in cooperation with mitochondrial Tu translation elongation factor (TUFM).¹¹⁰ NLRC5 is another member of the NLR protein family, recognized as a novel regulator of both adaptive and innate immune responses.¹¹¹ Unlike NLRX1, NLRC5 expression is upregulated by many cytokines, such as IFNs and LPS.¹¹²⁻¹¹⁴ We, and others, have identified NLRC5 as a negative regulator of both NF- κ B and type I IFN signaling. NLRC5 inhibits IKK phosphorylation and NF- κ B signaling by interacting with IKK α / β but not NEMO. Whereas, NLRC5 inhibits type I IFN signaling by targeting RIG-I/MDA5 after viral infection and blocking the RIG-I-MAVS interaction. Accordingly, knockdown of NLRC5 by siRNA enhanced the secretion of proinflammatory cytokines (IL-6, TNF- α), and TLR-stimulated antiviral responses in a variety of cell types.^{112,113} Recently, we, and other groups, generated several lines of NLRC5^{-/-} mice and found that NLRC5 deficiency markedly enhanced proinflammatory and antiviral responses in MEFs and peritoneal macrophages, but not in bone marrow-derived macrophages (BMMs) or BMDCs. This suggests that NLRC5 may regulate innate immune responses in a cell type-specific manner in vivo.¹¹⁴⁻¹¹⁶ Other regulatory NLRs, such as

NLRC3 and NLRP6, have also been identified as negative regulators of TLR-induced NF- κ B and MAPK signaling.^{117,118}

Other intracellular inhibitors

SOCS1 belongs to the suppressor of cytokine signaling (SOCS) family and inhibits TLR-induced NF- κ B signaling by targeting IRAK1. In accordance, SOCS1^{-/-} mice have increased susceptibility to LPS-induced septic shock.^{119,120} IRAKM is a member of IRAK kinase family and is predominantly expressed in peripheral blood cells. IRAKM^{-/-} mice produce markedly enhanced levels of proinflammatory cytokines in response to LPS and CpG DNA, suggesting that IRAKM is a negative regulator of TLR-induced NF- κ B activation.¹²¹ In addition to IRAKM, Toll-interacting protein (TOLLIP) also targets IRAK1 to block TLR2 and TLR4 signaling by inhibiting IRAK1 autophosphorylation.¹²²

Regulation of inflammasomes

Recent studies have reported guanylate-binding protein 5 (GBP5) as a positive regulator of NLRP3 inflammasome. GBP5 stimulates NLRP3-ASC oligomerization by interacting with the pyrin domain of NLRP3 via its GTPase domain, and GBP5 promotes NLRP3 inflammasome activation induced by ATP, nigericin, and bacteria, but not crystalline agents.¹²³ In addition, MAVS, the key regulator of RLR signaling, has also been identified to facilitate NLRP3 inflammasome activation. Similar to GBP5, MAVS selectively promotes NLRP3 inflammasome activation in response to ATP and nigericin, but not to crystalline reagents such as silica and alum.¹²⁴ The mitochondria-dependent NLRP3 inflammasome activation also requires the functional microtubule system and metabolic signals.¹²⁵

Numerous studies have focused on the role of post-translational modification of inflammasomes. Several reports reveal that activation of NLRP3 inflammasome is strongly inhibited when NLRP3 is ubiquitinated, indicating that NLRP3 deubiquitination is an essential posttranslational protein modification for NLRP3 activation.¹²⁶⁻¹²⁸ To date, BRCC3, a member of JAMM domain-containing Zn²⁺ metalloproteases, is the only deubiquitinating enzyme identified to activate

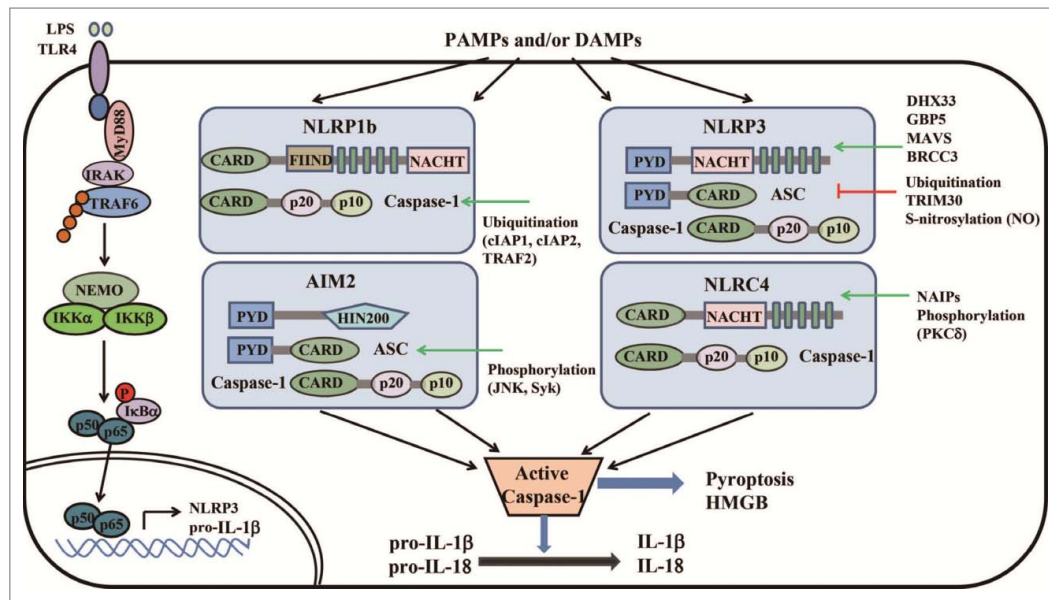


Figure 2. Activation and regulation of inflammasomes. The identified core components of inflammasomes belong to 2 families: (1) the NLR family (such as NLRP1b, NLRP3 and NLRC4); (2) the PYHIN (PYD and HIN200 domain-containing protein) family (such as AIM2). NLRs or AIM2, together with ASC, activate caspase-1, leading to downstream effector functions such as pyroptosis and processing of pro-IL-1 β and pro-IL-18. Multiple regulators exert rigorous control on these pathways through their positive (green arrow) or negative (red blunt arrow) regulation. CARD, caspase recruitment domain; FIIND, domain with function to find; LRR, leucine-rich repeat; NACHT, nucleotide binding and oligomerization domain; NLR, Nod-like receptor; PYD, pyrin; HIN200, haematopoietic interferon-inducible nuclear antigens with 200 amino-acid repeats.

NLRP3 inflammasome by deubiquitination.¹²⁸ Another report demonstrates that an E3 ligase, TRIM30, negatively regulates NLRP3 inflammasome activation by modulating reactive oxygen species production.¹²⁹ Whether other E3 ligases or deubiquitinases are required for regulating the NLRP3 inflammasome remains to be investigated. Furthermore, the AIM2 or NLRP3 inflammasome in macrophages triggers activation of the G protein, RalB, and formation of autophagosomes to eliminate active, ubiquitinated inflammasomes through autophagy.¹³⁰ Recently, a study shows that NLRP3, but not AIM2, is negatively regulated by NO-mediated S-nitrosylation.¹³¹ Besides NLRP3, the ubiquitination of caspase-1 is also required for optimized inflammasome activity. cIAP1, cIAP2, and the adaptor protein, TRAF2, interact with caspase-1-containing complexes and mediate K63-linked polyubiquitination of caspase-1, thus, activating inflammasomes.¹³²

Phosphorylation is also a critical modification regulating inflammasome activity. Phosphorylation of NLRC4 is critical for the activity of NLRC4 inflammasome, and protein kinase C delta (PKC- δ) phosphorylates NLRC4.¹³³ However, a recent study resolved the structure of NLRC4 and found that inactive NLRC4 is also phosphorylated.¹³⁴ Thus, the relationship between NLRC4 phosphorylation state and function needs further investigation. The kinase, Syk, controls both pro-IL-1 β synthesis and inflammasome activation after cell stimulation with *Candida albicans*.¹³⁵ Lyn and Syk kinases also positively regulate malarial hemozoin-induced NLRP3 inflammasome activation.¹³⁶ In addition, Syk and JNK are required for the activation of caspase-1 via the NLRP3 and AIM2 inflammasomes by phosphorylating ASC.¹³⁷ Double-stranded, RNA-dependent protein kinase (PKR) has recently been shown to contribute to the activation of the NLRP1, NLRP3, NLRC4, and AIM2 inflammasomes through direct interaction with NLRs and AIM2. Loss of PKR in macrophages results in attenuated IL-1 β and IL-18 cleavage and HMGB release in response to various stimuli.^{138,139} However, the exact role for phosphorylation in PKR-regulated inflammasome activation still needs further clarification (Fig. 2 and Table S1).

Regulation of type I IFN signaling pathway

Regulation of RLRs

RIG-I and MDA5 are key receptors for detecting viral RNAs and inducing type I IFN signaling to establish the antiviral state of the cell. Upon ligand binding, RIG-I undergoes a series of posttranslational modifications, including de-phosphorylation and ubiquitination, to form tetramers and, in turn, to activate MAVS.¹² The activity of RIG-I is tightly regulated by many positive and negative regulators. As an IFN stimulator, ZAPs associates with RIG-I to promote the oligomerization and ATPase activity of RIG-I, which leads to robust activation of type I IFN activation.¹⁴⁰ K63-linked ubiquitination of the RIG-I CARDs, as well as its CTD, is critical for RIG-I to elicit antiviral activity.^{141,142} The E3 ligase, TRIM25, interacts with the CARDs of RIG-I and delivers Lys63-linked polyubiquitin chain at Lys172 of the RIG-I CARDs to enhance the interaction between RIG-I and MAVS.¹⁴¹ Moreover, Riplet/RNF135/REUL promotes

RIG-I activation, independent of TRIM25, by catalyzing K63-linked polyubiquitin chains of RIG-I on its CTD domain.¹⁴² A recent study shows that ubiquitin-specific protease 15 (USP15) targets TRIM25 to prevent the LUBAC-dependent degradation of TRIM25 and to promote RIG-I-mediated antiviral signaling.¹⁴³ It is also reported that an unanchored polyubiquitin chain can also facilitate RIG-I-mediated type I IFN signaling.¹⁴⁴ Most recently, a structural-based study demonstrates that both TRIM25-mediated ubiquitination of RIG-I CARD and non-covalent binding of K63-linked polyubiquitin chains induce its tetramer formation, and hence, activate MAVS.¹⁴⁵ Ubiquitination is a reversible process. We, and others, have identified 2 deubiquitinases, USP3 and USP21, which negatively regulate RIG-I activity by specifically removing the K63-linked ubiquitin chains on RIG-I.^{146,147} RIG-I activity is also negatively regulated by RNF125 and Siglec-G-/SHP2/c-Cbl-mediated K48-linked ubiquitination and protein degradation.^{148,149} Besides ubiquitination, RIG-I is also negatively regulated by Ser-Thr phosphorylation on CARD domain. In unstimulated cells, RIG-I is robustly phosphorylated at S8 and T170 by protein kinase C- α/β (PKC α/β),¹⁵⁰⁻¹⁵² whereas the phosphatases, PP1 α and PP1 γ activate both RIG-I and MDA5 signal pathways by dephosphorylating RIG-I or MDA5 upon viral infection.¹⁵³ Furthermore, virus-induced upregulation of a short RIG-I splice variant (RIG-I SV) negatively regulates RIG-I signaling in a dominant-negative manner.¹⁵⁴ In contrast to the regulatory mechanisms controlling RIG-I signaling activity, mechanisms controlling MDA5 signaling activity are less known. DAK is a dihydroacetone kinase, which associates with MDA5, but not RIG-I, and specifically inhibits MDA5-mediated IFN- β induction.¹⁵⁵ MDA5 can also be activated by the phosphatases, PP1 α and PP1 γ .¹⁵³ Our group has identified NLRC5 as a negative regulator for both RIG-I and MDA5 via direct interaction upon viral infection.¹¹² Recent evidence shows that K63-linked ubiquitin chains trigger MDA5 oligomerization and activate type I IFN signaling.¹⁵⁶ We found that the deubiquitinase, USP3, negatively regulates MDA5-mediated signaling by deubiquitinating its CARD domain.¹⁴⁶

Regulation of MAVS

MAVS is a critical adaptor protein for viral RNA-mediated antiviral immunity. NLRX1 was reported to negatively regulate type I IFN signaling by blocking RIG-I-MAVS interaction.¹⁰⁶ The autophagic proteins, Atg5 and Atg12, also negatively regulate type I IFN signaling by interacting with RIG-I and MAVS.¹⁵⁷ The stability of MAVS protein is regulated by several E3 ligases for 'fine tuning' of antiviral innate immunity. PCBP2 was identified to degrade MAVS in cooperation with a HECT domain-containing E3 ligase AIP4.¹⁵⁸ Smurf2 and TRIM25 are also negative regulators of type I IFN signaling by targeting MAVS for K48-linked ubiquitination and degradation.^{159,160}

Regulation of STING

STING has been identified as the most important adaptor protein for DNA-mediated type I IFN signaling. STING is regulated by both K48- and K63-linked ubiquitination. RNF5

ubiquitinates STING with K48 linkage for protein degradation.¹⁶¹ By contrast, TRIM56 and TRIM32 positively regulate STING function by interacting with STING and targeting it for K63-linked ubiquitination. The K63-linked ubiquitination of STING may prompt the interaction between STING and TBK1, and thus, activate type I IFN signaling.^{162,163} On the contrary, NLRC3 reduces STING-dependent innate immune activation in response to cyclic di-GMP and DNA viruses by impeding STING-TBK1 interaction.¹⁶⁴ Recently, the autophagy related serine/threonine UNC-51-like kinase (ULK1/Atg1) has been identified as a novel, negative regulator of STING. After autophagy-dependent delivery of STING to endosomal/lysosomal compartments and activation of IRF3 and NF- κ B, ULK1 is free from its repressor, AMP activated protein kinase (AMPK), and subsequently, phosphorylates STING to suppress its function.¹⁶⁵

Regulation of TRAF3

The tyrosine kinase, Syk, positively regulates type I IFN production, in response to TLR4 ligand, by interacting with TRAF3 and TBK1. Syk-deficient macrophages exhibited decreased production of type I IFNs.¹⁶⁶ A recent study shows that linear, ubiquitinated NEMO, mediated by the linear ubiquitin assembly complex (LUBAC), interacts with TRAF3 and disrupts the MAVS-TRAF3 complex to block type I IFN signaling.¹⁶⁷ Deubiquitinating enzyme A (DUBA, also known as OTUD5) was the first identified deubiquitinase to remove K63-linked polyubiquitin chains of TRAF3 and negatively regulate type I IFN signaling.¹⁶⁸ Recently, HSCARG was found to inhibit TRAF3 ubiquitination via recruiting deubiquitinase OTUB1, therefore negatively regulating RIG-I signaling.¹⁶⁹ The protein stability of TRAF3 is controlled by the E3 ubiquitin ligase, TRIAD3A, which leads to K48-linked ubiquitination of TRAF3, and subsequent proteasomal degradation.¹⁷⁰ In contrast, USP25 positively regulates TLR4-dependent innate immune responses through deubiquitinating TRAF3. USP25 specifically removes the K48-linked ubiquitination of TRAF3, which is mediated by the E3 ubiquitin ligase, cIAP2, and enables a balanced innate immune response.¹⁷¹

Regulation of TBK1

It is known that TBK1 has several binding partners, including TRAF family member-associated NF- κ B activator (TANK), NAK-associated protein (NAP1), and similar to NAP1 TBK1 adaptor (SINTBAD), essential for its activation.¹⁷² Recently, GSK3 β was shown to interact with TBK1 during virus infection. GSK3 enhances TBK1 self-association and autophosphorylation at Ser 172, which is critical for IRF3 activation.¹⁷³ The activity of TBK1 is also regulated by a robust, reversible ubiquitination process. TBK1 undergoes ubiquitination with K63 linkage by mind bomb (MIB) or Ndrp1 to promote interferon production in response to RLR or TLR ligands, respectively.^{91,174} However, ABIN1, TAX1BP1 and A20 blocks antiviral signaling by disrupting K63-linked polyubiquitination of TBK1/IKKi.^{175,176} Recently, we have found that NLRP4 serves as a negative regulator of type I IFN signaling by targeting active TBK1 for

degradation. NLRP4-mediated type I IFN signaling is regulated in a TBK1-IRF3-dependent manner, but has no effect on the MyD88-IRF7-dependent pathway. After viral infection or TLR stimulation, NLRP4 specifically interacts with TBK1 and recruits the E3 ubiquitin ligase, DTX4, to promote K48-linked polyubiquitination and degradation of TBK1.¹⁷⁷ Another group found the E3 ligase, TRAF-interacting protein (TRIP), also promotes proteasomal degradation of TBK1.¹⁷⁸ Interestingly, we observed that TRIP negatively regulates TBK1 in a NLRP4-dependent manner, suggesting that NLRP4 is paramount in mediating TBK1 stability (unpublished data).

Regulation of IRF3/IRF7

IRF3 and IRF7 are master transcriptional factors of the type I IFN pathway. IRF3 and IRF7 can be activated by TLR3, TLR4 or RLRs ligands in macrophages, MEFs and conventional DCs (cDCs). Notably, IRF7 can also be activated by TLR7 and TLR9 ligands through MyD88 signaling, and IRF7 is essential for type I IFN production in plasmacytoid dendritic cells (pDCs).¹⁷⁹ Thus, the activity of IRF3/IRF7 must be tightly regulated. It has been reported that IRF7 is targeted for degradation by binding to the RTA immediate-early nuclear transcription factor encoded by Kaposi's sarcoma-associated herpesvirus (KSHV).¹⁸⁰ In addition, an RTA-associated E3 ligase RAUL (also known as KIAA10 or UBE3C) negatively regulates type I IFN by targeting both IRF3 and IRF7 for K48-linked ubiquitination and degradation.¹⁸¹ The N-Myc and STAT's interactor (Nmi), a Sendai virus-inducible protein, was also found to promote the K48-linked ubiquitination and the proteasome-dependent degradation of IRF7.¹⁸² Similarly, the E3 ligase, Ro52 (TRIM21), targets IRF3 for degradation.¹⁸³ IRF3 can also be deactivated via dephosphorylation mediated by the serine and threonine phosphatase, PP2A, and its adaptor protein, RACK1. PP2A-deficient macrophages show enhanced type I IFN signaling with diverse TLR ligands and viral infections (Fig. 3 and Table S1).¹⁸⁴

Double-edged Sword Roles of PRRs and Innate Immune Regulators in Cancer

Activation of the innate immune response through TLR- and NLR- signaling pathways serves as a link between chronic inflammation and cancer.¹⁸⁵⁻¹⁸⁸ It is well-demonstrated that chronic inflammation is a major driving force in cancer development.^{189,190} Chronic infection or chronic inflammation causes about 20% of human cancers.^{189,191} Thus, chronic inflammation, primarily caused by abnormal NF- κ B or inflammasome activation, is tightly linked to cancer through PRRs-mediated cytokine production.^{186,192-194} Emerging evidence suggests that PRRs and their regulators have both favorable and unfavorable consequences on cancer cells. On the one hand, certain PRRs induce an anti-tumor immune response to inhibit tumor progression. Conversely, uncontrolled innate immune signaling may provide a microenvironment for cancer cell proliferation and immune surveillance evasion.

The role of TLRs and their regulators in cancer

Several studies have demonstrated a significant association between prostate cancer and TLR sequence variants, including TLR4, TLR1, TLR6 and TLR10. These studies suggest that TLR sequence polymorphisms are an important risk factor for prostate cancer.^{190,195,196}

However, this may be dependent on the specific TLR, the tumor type, and the types of cells expressing the TLR. Functional analyses show that the loss of TLR4, due to polymorphisms, in human prostate and breast tumor cell lines, inhibits tumor cell proliferation and invasion, and induces tumor cell apoptosis.^{197,198} In contrast, TLR2^{-/-} mice develop significant larger and more colorectal tumors than wild-type mice, suggesting a protective role of TLR2 in colorectal tumors.¹⁹⁹

In addition, activation of TLR3 signaling by poly (I: C) inhibits tumor cell proliferation and induces apoptosis.^{200,201} Wang et al. reported TLR9 activation in non-small cell lung cancer cells sensitizes tumor cells to apoptosis, which, in turn, leads to tumor growth arrest.²⁰² Collectively, these results reveal anti-tumor functions of certain TLRs. Alternatively, another report demonstrates that specific ligand activation of TLR2, 3 and 4 on human melanoma cells induces cell migration and promotes metastatic events, suggesting that the function of TLR signaling may be tumor specific.²⁰³ Another possible interpretation of these results is that TLRs are sensors of many immune cells, and the activation of TLRs by PAMPs can stimulate diverse innate immune pathways (e.g., the NF-κB pathway) to secrete proinflammatory cytokines.

Recent studies identify an important role for MyD88, a molecule downstream in TLR-signaling pathways, in tumor development. MyD88 deficiency reduces the incidence of cancer in mice in an AOM-DSS-induced colon cancer model.²⁰⁴ Loss of MyD88 also reduces chemically induced skin cancer or liver cancer development.^{205,206} These studies

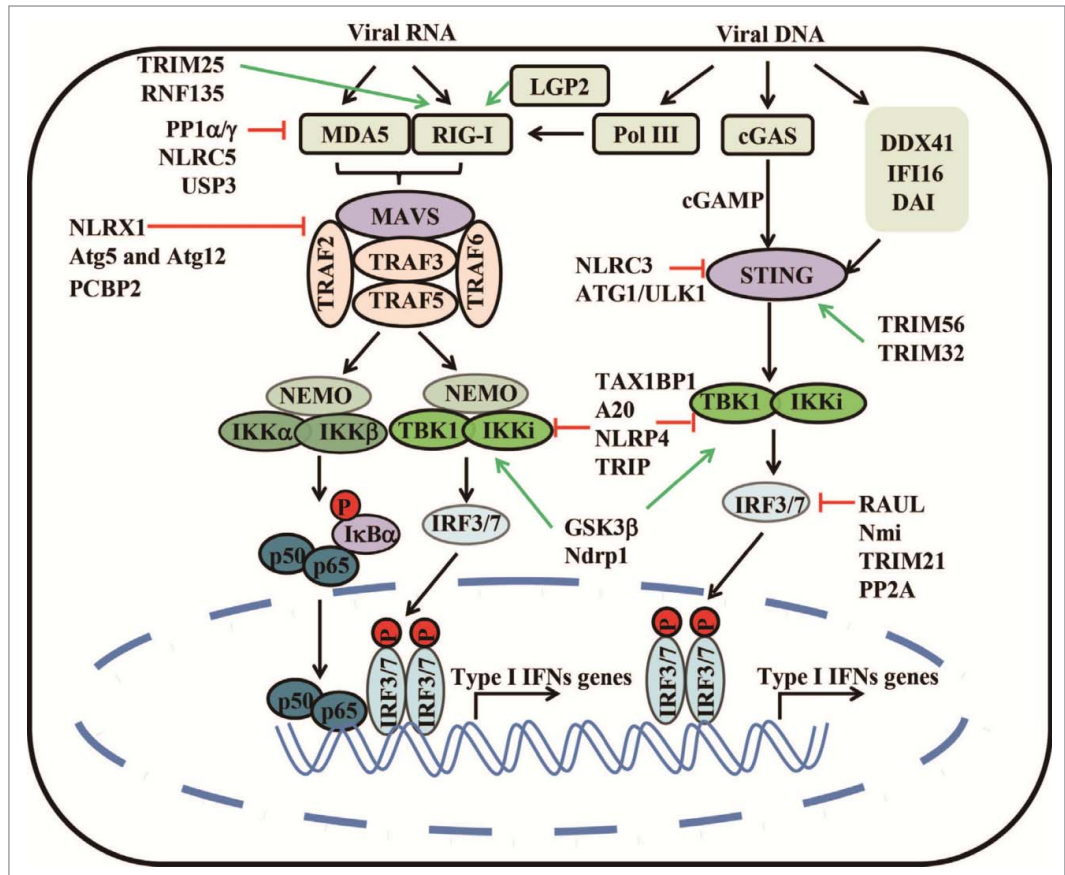


Figure 3. A schematic representation of cytosolic RNA and DNA-induced type I IFN signaling pathway and its regulators. RIG-I and MDA5 recognize different groups of viral RNAs and initiate signaling cascades that begin with prion-like polymerization of MAVS. MAVS recruits and activates TRAF2, TRAF3, TRAF5, and TRAF6. Polyubiquitin chains on these ligases are sensed by NEMO through its ubiquitin-binding domains, in turn, recruiting IKK and TBK1 complexes to phosphorylate IκBα and IRF3, respectively. LGP2 may function as a regulator to modulate the activity of RIG-I and MDA5. Viral DNA could activate cGAS and other DNA sensors, which are all proposed to transduce signals to the ER-localized adaptor protein STING. STING triggers TBK1-dependent type I IFN response. RNA polymerase III transcribes the DNA into 5'ppp-RNA, which triggers the RIG-I pathway. Most of the key molecules involved in the RNA- and DNA-sensing pathways can be targeted by E3 ligases, deubiquitinases or regulatory NLRs, which negatively (red blunt arrow) or positively (green arrow) regulate type I IFN responses.

suggest that MyD88-mediated signaling plays a key role in promoting tumor development and progression. However, another study shows that loss of MyD88 increases tumor incidence in AOM-DSS-induced model, due to the reduced ability to heal ulcers and repair DNA damage.²⁰⁷ This further reiterates the dual roles of TLR signaling in cancer.

The role of NLRs in cancer

Nod2 has been the focus of many studies because of its implication in Crohn's disease.²⁰⁸ Notably, inactivating polymorphisms of Nod2 have been associated with increased susceptibility to colorectal cancer in several patient cohorts.²⁰⁹ Similar to that observed in patients with Crohn's disease, Nod2 deficiency leads to increased colorectal cancer development in mice. Both Nod2-deficient mice and patients with Nod2 mutations have intestinal dysbiosis.²¹⁰ A recent report shows that Nod2-deficient mice are susceptible to dysbiosis, and the

susceptibility to inflammation-induced colorectal cancer is transmissible to wild-type mice by co-housing.²¹¹

Emerging evidence implicates a pivotal role for NLR inflammasomes in tumor development. NLRP3 is highly expressed in haematopoietic cells but not intestinal epithelial cells. NLRP3^{-/-} mice have increased colitis-associated cancer development.²¹² Similarly, ASC and caspase-1 deficient mice are susceptible to DSS-induced tissue injury.^{213,214} Further, another study demonstrates that NLRP3-dependent IL-18 production exerts a protective role against colorectal tumorigenesis through IFN- γ production and STAT1 signaling.²¹⁴ NLRP6 is highly expressed in intestinal tissue and has an important role in maintaining intestinal homeostasis. NLRP6 inflammasome deficiency leads to aberrant inflammation within the colon and to colitis-induced tumorigenesis.²¹⁵ NLRP6 may function as a negative regulator of colorectal tumorigenesis, partly through controlling epithelial cell self-renewal upon injury.²¹⁶ Flavell and his colleagues found that the NLRP6 inflammasome in intestinal epithelial cells may play a role in maintaining healthy gut flora.²¹⁷ In addition, the NLRC4 inflammasome is also related to inflammation-induced colorectal tumor formation in mice. Loss of the NLRC4 inflammasome causes enhanced epithelial cell proliferation and reduced apoptosis.²¹⁸ Therefore, NLRs and NLR inflammasomes are promising therapeutic targets in inflammation-induced cancers.

Type I IFN regulators and cancer

A recent study demonstrated significant downregulation of RIG-I expression in human hepatocellular carcinoma (HCC) tissues, which was correlated with poorer patient prognosis and diminished response to IFN α therapy. Mechanistically, RIG-I enhances IFN α production by amplifying IFN-JAK-STAT signaling via promoting STAT1 activation.²¹⁹ It has also been demonstrated that TBK1 plays a critical role in *Kras*-mediated tumor development. Suppression of TBK1-induced apoptosis, specifically in human lung cancer cell lines, depends on oncogenic KRAS expression.²²⁰ In addition, many negative regulators of type I IFN signaling have been shown to be involved in cancer. For example, A20 and CYLD have been identified as tumor suppressors, and mutations in A20 and CYLD have been identified in different tumor types.^{100,221,222}

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Conclusions

Since their discovery 20 y ago, TLRs and RLRs have been shown to be essential for efficient innate and adaptive immunity, and the mechanisms regulating TLR-mediated signaling pathways have been extensively studied. In contrast, for many NLRs and other viral DNA sensors, their recognized ligands and their signaling pathways remain to be determined. This review provides an updated overview of the rapidly evolving field of PRR biology and PRR signaling pathways. These innate immune receptors and their regulators are clearly important in host defense against infectious diseases, in autoimmune diseases as well as in cancer. However, challenges still remain in the understanding of how the different activation pathways of PRRs converge or diverge, and how the different innate immune signaling pathways are regulated at the molecular level. The exact roles of these PRRs in cancer immunosurveillance also remain to be determined. Understanding the regulation of innate immune signaling, cytokine production and their relationship to adaptive immune responses in cancer will be critically important for the development of cancer diagnostics and prognostics as well as effective cancer therapeutics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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