

Structural insights into the distinctive RNA recognition and therapeutic potentials of RIG-I-like receptors

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Abstract

RNA viruses, including the coronavirus, develop a unique strategy to evade the host immune response by interrupting the normal function of cytosolic retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs). RLRs rapidly detect atypical nucleic acids, thereby triggering the antiviral innate immune signaling cascade and subsequently activates the interferons transcription and induction of other proinflammatory cytokines and chemokines. Nonetheless, these receptors are manipulated by viral proteins to subvert the host immune system and sustain the infectivity and replication potential of the virus. RIG-I senses the single-stranded, double-stranded, and short double-stranded RNAs and recognizes the key signature, a 5'-triphosphate moiety, at the blunt end of the viral RNA. Meanwhile, the melanoma differentiation-associated gene 5 (MDA5) is triggered by longer double stranded RNAs, messenger RNAs lacking 2'-O-methylation in their 5'-cap, and RNA aggregates. Therefore, structural insights into the nucleic-acid-sensing and downstream signaling mechanisms of these receptors hold great promise for developing effective antiviral therapeutic interventions. This review highlights the critical roles played by RLRs in viral infections as well as their ligand recognition mechanisms. In addition, we highlight the crosstalk between the toll-like receptors and RLRs and provide a comprehensive overview of RLR-associated diseases as well as the therapeutic potential of RLRs for the

development of antiviral-drugs. Moreover, we believe that these RLR-based antivirals will serve as a step toward countering the recent coronavirus disease 2019 pandemic.

KEY WORDS

antivirals, coronavirus, COVID-19, MDA5, RIG-I, RIG-I-like receptor

1 | INTRODUCTION

Cells are equipped with specific cytosolic receptors, such as the retinoic acid-inducible gene-I (RIG-I)-like receptors (RLRs), which are associated with the host defense system as they rapidly sense the atypical nucleic acids and subsequently trigger the antiviral innate immune signaling cascade, which includes the induction of type I interferons and the expression of proinflammatory cytokines and chemokines. Type I interferons are essential cytokines that play a role in mediating antiviral immune response and are a part of both innate and adaptive immunity.¹

The recent pandemic of the coronavirus disease-2019 (COVID-19), which is characterized by fever, respiratory illness, and pneumonia, has been declared as a global health emergency. According to the World Health Organization, as of June 11, 2021, the number of confirmed cases worldwide is ~174.8 million, while the death toll has reached up to 3.7 million. Before 2019, two pandemics were caused by the coronaviruses, the severe acute respiratory syndrome coronavirus (SARS-CoV) in November 2002 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in June 2012.^{2–4} These viral outbreaks indicate that the coronaviruses pose a continuous threat to human life. Hence, it is crucial to develop efficient antivirals and vaccines against such viruses.

Several RNA viruses, including the coronavirus, contain nonstructural and accessory proteins, which block the normal functions of RLRs and facilitate the evasion of the virus from the host innate immune system.⁵ Host immune response play vital role in the control and elimination of viral infections. Coronaviruses are positive-sense enveloped RNA viruses with a genome size of 26–32 kb.⁴ Genomic analysis revealed that SARS-CoV-2 shares high sequence similarity with the causative agents of SARS and MERS. In our previous study on MERS, we elucidated the entry of the virus into the host cells, replication of the viral genome, and infectivity of the virus.⁶ In addition to structural proteins, coronaviruses also contain nonstructural and accessory proteins that are known to interfere with the antiviral innate immune response of the host.^{5–7} To ensure viral replication without detection by RLRs, these accessory proteins bind to the viral double stranded RNA (dsRNA) through the dsRNA-binding motif and prevent its recognition by cytosolic receptors. In MERS-CoV, the accessory protein 4a inhibits the production of interferons by hindering the detection of viral dsRNA by the RLRs.⁷ Additionally, a recent study has suggested that the open reading frame 6 (ORF6) of the SARS-CoV-2 and SARS-CoV plays a similar role to prevent the downstream signaling and production of type I interferons.⁸ The C-terminal tail of ORF6 is crucial for its antagonistic effect. Targeting this negatively charged motif may provide an interactive interface between ORF6 and the host proteins. Thus, it is necessary to obtain information about key receptors, such as the toll-like receptors (TLRs) and RLRs, which are involved in the efficient activation of the antiviral response of the host and aid in blocking the protein-protein interactions. Therefore, to cope with viral infections, including COVID-19, there is a dire need to gain a deeper understanding of the structure, ligand recognition, and activation mechanisms of RLRs. In addition, a comprehensive understanding of the therapeutic importance of RLRs is required for future development of drugs that can aid the treatment of autoimmune diseases, allergies, inflammatory diseases, infectious diseases, and cancers.

2 | RIG-I-LIKE RECEPTORS AT A GLANCE

RLRs recognize different types of replicating RNAs of the invading viruses present in the cytoplasm and trigger the host immune response.⁹ The mammalian RLR family includes RIG-I (also known as RIG1 and DExD/H-box helicase 58 [DDX58]), laboratory of genetics and physiology 2 (LGP2; also known as DExH-box helicase 58 [DHX58]), and the melanoma differentiation-associated gene 5 (MDA5; also known as the interferon induced with helicase C domain 1 [IFIH1]).¹⁰ Both RIG-I and MDA5 play specific roles to combat the viral invasion, while LGP2 exerts a regulatory impact on the RLR signaling pathway.¹¹ RLRs are expressed at low levels by the immune cells; RLR expression is upregulated in response to the detection of a non-self nucleic acid.¹⁰ RLR signaling is regulated via a complex process to prevent the uncoordinated and excessive transcription of cytokines.¹² Since the discovery of RLRs, numerous advances have been made in the understanding of the molecular mechanisms involved in the virus recognition process and onset of antiviral immune responses. With progress in the elucidation of the ligand recognition mechanism and other immune-system-related functions of RLRs, the potential of RLRs as therapeutic targets has received even more attention. RLR ligands can be used as vaccine adjuvants, pan-antiviral drugs, and antitumor agents.¹³ Polyinosinic-polycytidylc acid [poly(I:C); synthetic RNA] and its derivatives have been utilized as immunostimulants for therapeutic purposes.¹⁴ This review provides in-depth insights into the structures and ligand recognition mechanisms of RLRs as well as their importance in the design of effective antiviral and antitumor agents with adjuvant and therapeutic potentials.

3 | RLR SIGNALING PATHWAYS AND THE DOMAIN ARCHITECTURE OF RLRs

RLRs are members of the DExD/H-box RNA helicase superfamily and exhibit a high degree of similarity in the helicase domain. The domain architectures of RIG-I and MDA5 are similar and exhibit a high degree of structural homology (among the three RLRs being discussed); both receptors contain a core RNA helicase domain, two N-terminal tandem caspase recruitment domains (CARDs), and a regulatory C-terminal domain (CTD; Figure 1A and Table 1). The core region contains two helicase domains (Hel), helicase 1 (Hel1) and Hel2 and a Hel2 insertion domain (Hel2i). The CTD plays a crucial role in pathogen nucleic-acid recognition, while the core helicase domain reciprocally enhances ligand specificity via conformational changes.¹⁵ CARDs belong to a subfamily of the death domain superfamily and interact with adaptor proteins to initiate the downstream pathways involving the activation of transcription of the interferon (IFN)-regulatory factor 3 (IRF3), IFN-regulatory factor 7 (IRF7), and nuclear factor- κ B (NF- κ B). In addition to protein-protein interaction, CARDs are also involved in inflammatory and cell death mechanisms.¹⁶ The CTD maintains the receptor in a closed and repressed conformation to block the downstream signaling in the absence of a viral polynucleotide stimulus. Upon ligand detection, the receptor adopts an open conformation and exposes the CARDs to facilitate adaptor-binding, which further initiates the downstream signaling.^{15,17–19}

Unlike RIG-I and MDA5, LGP2 contains a helicase core region and a CTD but lacks the CARD. The helicase domain of LGP2 also detects RNA ligands and is capable of ATP hydrolysis but is unable to initiate downstream signaling.²⁰ The CTD ensures specificity of the RNA recognition and RNA-binding mechanism. LGP2 serves as a double-edged sword in the regulation of the signaling mechanism of RLRs.^{21–23} LGP2 has been reported as a receptor essential for the detection of non-self RNA molecules to induce the IFN production.²⁴ Nonetheless, contradictory outcomes of LGP2 activation have been reported so far. For example, LGP2-knockout mice show high resistance to viral infections, while high expression levels of IFNs have been detected in the double-stranded RNA (dsRNA)-exposed cells isolated from this animal model. A positive regulatory effect of LGP2 on RLR signaling has also been reported²²; this activity requires the ATPase domain of LGP2.²¹

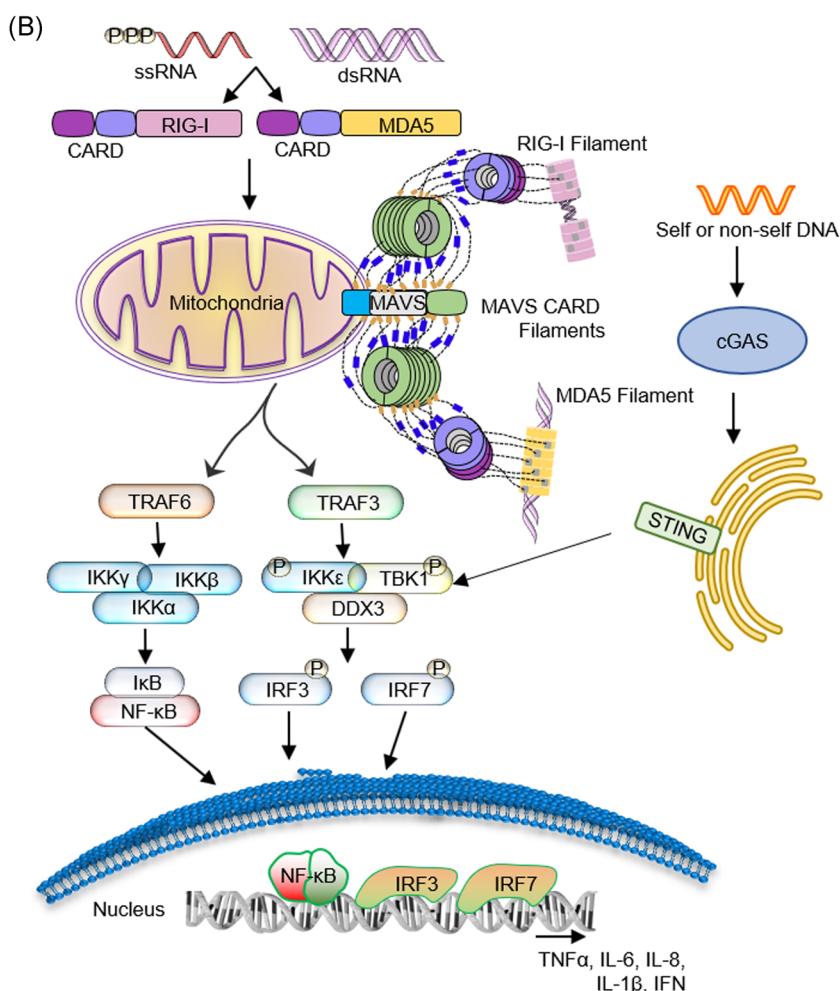
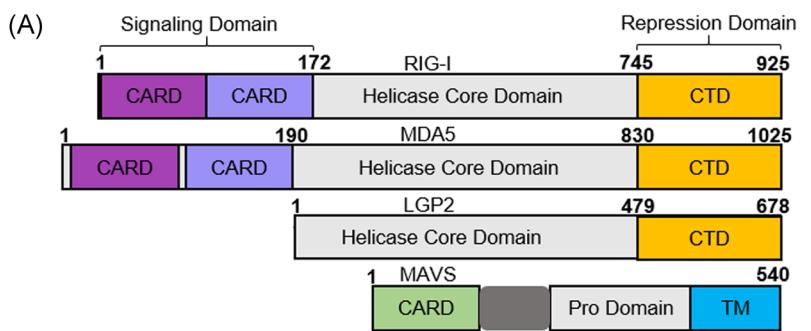


FIGURE 1 (See caption on next page)

TABLE 1 Structural properties of the RLRs

Features	RIG-I	MDA5	LGP2
Protein length	925 aa	1025 aa	678 aa
CARD1	1–87	7–97	Absent
CARD2	92–172	110–190	Absent
Helicase ATP-binding region	251–430	316–509	11–188
Helicase CTD	610–776	700–882	350–514
RLR CTD	794–925	893–1020	539–669
Family	Helicase family	Helicase family	Helicase family
Uniport ID	O95786	Q9BYX4	Q96C10
Protein Data Bank ID	5E3H; 4AY2	4GL2	3EQT

Note: The information used in the table has been retrieved from Uniport (<https://www.uniprot.org/>).

Abbreviations: LGP2, laboratory of genetics and physiology 2; MDA5, melanoma differentiation-associated gene 5; RIG-I, retinoic acid-inducible gene-I; RLR, RIG-I-like receptor.

RIG-I and MDA5 detect distinct groups of pathogens and bind to non-self RNAs that have specific features; these properties distinguish the nonredundant functions of RIG-I and MDA5.^{25,26} Upon recognition of a stimulatory RNA, these receptors go through ATP-dependent conformational alterations that initiate the downstream signaling via a signal-transducing adaptor protein. Mitochondrial antiviral signaling protein (MAVS) comprises a central proline-rich domain, a single CARD at the N terminus, and a transmembrane domain.²⁷ The oligomeric assembly of CARDs is pivotal for the RIG-I signal transduction and the MDA5 signaling pathway via MAVS. The latter forms huge oligomers via aggregation, which have the potential to activate IRF3 and the downstream signaling in virus-infected cells.²⁸ This entire process leads to signalosome formation, which triggers the downstream signaling

FIGURE 1 The domain architecture and signaling pathway of RLRs. (A) The domain arrangement of RLRs (RIG-I, MDA5, and LGP2) and the adaptor protein, MAVS. The N-terminal CARDs are present in RIG-I and MDA5, but absent in LGP2. Overall, the amino acid sequence of MDA5 is longer than that of RIG-I. The helicase core region consists of Hel1, Hel2, and an insertion domain. The CTD is crucial for RNA-sensing and binding. The adaptor protein MAVS contains a single CARD, a prodomain, and a TM domain. The CARD in MAVS is responsible for initiating the downstream signaling and interacts with CARDs of RIG-I or MDA5. (B) RLRs recognize the RNA viruses. RIG-I specifically binds to small ssRNA containing triphosphate at the 5' end (5'ppp), while MDA5 recognizes the long dsRNA. Upon RNA-binding, both receptors are activated, causing aggregation of MAVS molecules on the surface of mitochondria and formation of protein filaments (CARDs are involved in the filament formation) along with the activation of MAVS. Meanwhile, the RLR filaments establish contact with MAVS through CARDs. This event initiates the downstream signaling, where the IRF3 and IRF7 are activated via the TRAF3 and the NF-κB is activated via the TRAF6. IRF3 initiates the expression of type I IFNs, while NF-κB controls the expression of inflammatory cytokines. The self and non-self-DNA are sensed by the cGAS, which interacts with the adaptor protein, STING, present on the endoplasmic reticulum and leads to the activation of TBK-1. CARD, caspase recruitment domain; cGAMP, cyclic GMP-AMP; cGAS, cGAMP synthase; CTD, C-terminal domain; dsRNA, double-stranded RNA; Hel1, helicase 1; IFN, interferon; IRF3/7, IFN-regulatory factor 3/7; LGP2, laboratory of genetics and physiology 2; MAVS, mitochondrial antiviral signaling protein; MDA5, melanoma differentiation-associated gene 5; NF-κB, nuclear factor κB; RIG-I, retinoic acid-inducible gene-I; RLR, RIG-I-like receptor; ssRNA, single-stranded RNA; STING, stimulator of interferon genes; TBK-1, TANK-binding kinase-1; TM, transmembrane; TNF, tumor necrosis factor; TRAF3/6, TNF-receptor-associated factor 3/6 [Color figure can be viewed at wileyonlinelibrary.com]

(Figure 1B).^{29,30} The activation of two kinases, TANK-binding kinase 1 (TBK-1) and IκB kinase ε (IKKε), causes the stimulation of IRF3 and IRF7.³¹ The underlying transcription factors, IRF3 and IRF7, are phosphorylated by non-canonical IκB kinases. TBK-1 or IKKε. The phosphorylated form of IRF3 and IRF7 leads to the formation of homo- and heterodimers that are translocated to the nuclei and subsequently activate the gene transcription. Activation of NF-κB involves the IKK complex, which phosphorylates the IκBα subunit, resulting in its ubiquitin-dependent degradation. The active forms of IRF3/IRF7 and NF-κB interact with the transcription complex comprising c-Jun and the activating transcription factor-2 (ATF-2), leading to the production of type I IFN.^{27,32} IFN-α/β production further upregulates many antiviral proteins in a paracrine and autocrine manner. Other adaptor proteins have also been characterized and found to be involved in the RLR antiviral signaling cascade, including the tumor necrosis factor (TNF) receptor-associated death domain, TNF receptor-associated factor 2 (TRAF2), TNF-receptor-associated factor 3 (TRAF3), TNF-receptor-associated factor 6 (TRAF6), Fas-associated death domain, TRAF family member-associated NF-κB activator, and the receptor interacting protein 1.³³ Furthermore, the RLR signaling pathway amplifies self-signaling by inducing the transcription of RLR, thereby creating a positive feedback loop.²⁷

4 | ADVANCES IN THE STRUCTURAL UNDERSTANDING OF RLR FAMILY MEMBERS

The evolutionary history of RLRs has been studied extensively. In mammals, two major gene duplication events have yielded three RLR family members. The primary duplication has given rise to the RIG-I lineage along with the MDA5 and LGP2 branch, whereas another duplication generated two more individual lineages, that is, LGP2 and MDA5.³⁴ Phylogenetic tree constructed using the unweighted pair group method with arithmetic mean algorithm revealed that MDA5 and LGP2 are rather similar because they originated from a recent single duplication event, whereas RIG-I is located in a distant branch. The similarity in pairs of full-length proteins RIG-I:MDA5, MDA5:LGP2, and RIG-I:LGP2 is 24.5%, 36%, and 25%, respectively. Sequence alignment has revealed that RIG-I and MDA5 share 33.5% similarity in the helicase domain (Figure 2A) and 17% sequence similarity in the CARD region (Figure 2B). Additionally, LGP2 shares 30% and 50% similarity in its helicase domain with RIG-I and MDA5, respectively (Figure 2A). By contrast, the RIG-I CTD and MDA5 CTD are 21% similar, whereas the CTD of LGP2 shares 20% and 25% similarity with the CTD of RIG-I and MDA5, respectively (Figure 2C).

Just as other helicases, RLRs possess a core region, which contains a conserved motif, DExD/H, where “x” can be any amino acid residue.³⁵ The three-dimensional structure of Hel1 contains seven α-helices and seven β-sheets (Figure 3).³⁶ Hel1 and Hel2 together contribute to the open conformation; α10 and β12 of the Hel2 domain surround Hel2i, collectively forming a rigid structure.^{36,37} In an RNA–RLR complex, the helicase core region wraps the RNA molecule and creates a versatile interaction network (Figure 4). Hel1 faces a minor groove of the RNA and binds to the backbone of both strands (Figure 4B,C). Hel2i forms a helical bundle topology comprising five helices and interacts with dsRNA by establishing contacts with the minor groove. A V-shaped structure known as a pincer consisting of two long α-helices (α18, residues 746–769, and α19, residues 774–793) emerges from the Hel2 domain and connects to the CTD (Figure 3). The CTD is also known as a non-self RNA recognition domain. The residues present in the basic binding site are important for nucleic-acid sensing and downstream signaling activation.¹⁵ The α8 helix extended from Hel1 is grasped by the pincer, thus creating a strong connection among Hel domains and the CTD.³⁶

Crystal structure of the RNA-binding CTD has been proposed by Cui et al.¹⁸ to decipher the importance of its zinc-binding domain with respect to RIG-I activation. The CTD is composed of three major lobes containing antiparallel β-sheets connected through small helical turns (Figure 3). Two lobes include four strands each (β13, β14, β21, and β22 as well as β17, β18, β19, and β20, respectively), whereas the third lobe consists of two strands (β15 and β16). The β-lobes comprising the four stands are laterally linked by means of extending turns, which surround the ridge of the central β-sheet. The two loops originated the four-stranded β-sheet lobes, which contain

(A)

(B)

M5A5_CARD	TDNEFRYLISCFARVKMNYIQVEPVLWLYLT-FLPAEVEKQEIQRTVATSGNMQAVELLLS
RIG-I_CARD	MTTQEQRRSLQAFQDQYIRKLTDPTYLZSMYMPFREEEQVYQIAKEENKGPMEEATLFKK
	* : * . : * : * : * . : * : * : * . : * : * . : * : * . : * : * . : *
M5A5_CARD	LEKGVWHHLGMRFVTEALRRTGSP--LAARYMNPADHNLNLQPTLVLDLNLVRD
RIG-I_CARD	LLE-LQEEGFWRFGFLDAGHSYGLYEMDFDKKIEKLEYEVRLLKRLQPEFKTRIPTD
	* : * . : * * . : * * . : * . : * : * ; : * : * . : * : * . : *
M5A5_CARD	LDKCMEEETLTDIRETRIAAEENINGESVGRELKLKRIVO--KEMIFSAFLNVLQRQTGN
RIG-I_CARD	TCG-PLCCLGCTTGGCGTCTTGTCTTGCGGAAAGCAKUHGLLPGCAGTCAGTC

(C)

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MD5_CTD --AKHYKINPNSLTFLKNCNSVLCASGEDHIVLKEMHNWNTPEFKELY-IVRKENLA
LGP2_CTD QRENRNQFPPQVEHVHNLICLNCMWHAGHSLSGEGHVSFTHWNPINFSYNSVRDPWPVH
RIG-I_CTD -OEKPVPVKPOKEHKKLLRKCKKALACATDVRVIECTYLVQDFAKEFCVSPRHPKKP

MD5_CTD KKCADYQINGEIIICK-CGGAAGTMW/HVGLKDLPLKIRNFFVVFKNNNSKQQKKKVELI
LGP2_CTD KVFKDAPKGPKV15CNRNQFPPQVEHVHNLICLNCMWHAGHSLSGEGHVSFTHWNPINFSYNSVRDPWPVH
RIG-I_CTD FSSFEKRAKIFCARONCSHDGMGHVVKYTKTFEPVIKIESPVFVVEDIATGWQJLYSKKNDP

MD5_CTD ITFPNDLDSYESECLL
LGP2_CTD FSVPDFDFLQHCA
RIG-I_CTD FEKIPFDPQAEMSK

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(A)

Sequence 1: RIG-I_Hel_Domian 167 aa
Sequence 2: MDA5_Hel_Domian 183 aa
Sequence 3: LGP2_Hel_Domain 165 aa

Sequences (1:2) Aligned. Score: 33.5329
Sequences (1:3) Aligned. Score: 29.697
Sequences (2:3) Aligned. Score: 50.303

1-MBAS-GARD-172

(B)

Sequence 1: MDA5_CARD 172 aa
Sequence 2: RIG-I CARD 168 aa

Sequences (1:2) Aligned, Score: 16.6667

(C)

Sequence 1: RIG-I_CTD 132 aa
Sequence 2: MDA5_CTD 128 aa
Sequence 3: LGP2_CTD 131 aa

Sequences (1:2) Aligned. Score: 21.0938
Sequences (1:3) Aligned. Score: 19.8473
Sequences (2:3) Aligned. Score: 25

FIGURE 2 Pairwise sequence alignment of RLR domains. The full-length sequences of human RIG-I, MDA5, and LGP2 were downloaded from the National Center for Biotechnology Information (NCBI) protein database (accession numbers: NP_055129.2 (RIG-I), NP_071451.2 (MDA5), and NP_077024.2 (LGP2)). (A) The amino acids corresponding to the helicase domain of RLRs were aligned. The pairwise alignment score for sequences 1 and 2 is 33.5; for sequences 1 and 3 is 29.7; and for sequences 2 and 3 is 50.3. The alignment score shows that the helicase domains of MDA5 and LGP2 share more similarity than the helicase domains of either RIG-I and MDA5 or LGP2 and RIG-I. (B) Alignment of the CARDs of RIG-I and MDA5. These CARDs share 16.6% similarity as both proteins originate from separate gene duplication events. (C) The CTDs of MDA5 and LGP2 share 25% similarity. The pairwise alignment score for sequences 1 and 2 is 21 and for sequences 1 and 3, it is 19.6. CARD, caspase recruitment domain; CTD, C-terminal domain; LGP2, laboratory of genetics and physiology 2; MDA5, melanoma differentiation-associated gene 5; NCBI, National Center for Biotechnology Information; RIG-I, retinoic acid-inducible gene-I; RLR, RIG-I-like receptor

cysteine residues (C810 and C813 as well as C864 and C869) that collectively form a zinc-binding site, highly conserved among all RLRs.¹⁸ The CTDs of MDA5 and LGP2 share similar flat topology with convex and concave surfaces, containing a conserved zinc-binding motif.¹⁵ A helix α 23 present at the C-terminal of both RLRs grips the central β -sheet from the bottom. The hydrophobic residues present on the CTD surface—F838, W873, P885, and W908—are responsible for fold stabilization by engaging in hydrophobic interactions. These residues ensure CTD conservation in the core of RLR proteins.^{18,38} The electrostatic surface of the CTD features a large cleft parallel to the central lobe of the CTD. Both fringes of the cleft are covered by two helices and a loop. Residues K858 and K861 (in β 18); D872, W873, and I875 (in β 19); I889 and Q890 (in α 21); and F853 and F856 (in the β 17– β 18 extended loop) collectively form the basic cleft. K858 and K861 are not conserved among RLRs; however, the loop and residues at both fringes of the cleft are conserved. Hence, the basic cleft in RLRs' CTD is conserved and equally crucial for RNA recognition and binding. A flexible RNA-binding loop originating from β 17 to β 18 is involved in the recognition of discrete nucleic acid molecules, thereby inducing conformational changes upon ligand binding.³⁸

Even though RIG-I and MDA5, have similar domain architecture as explained above, detect nucleic acid ligands differently. The helicase domain of both RLRs similarly binds to the dsRNA but the orientation of MAD5 CTD, unlike RIG-I CTD, tilted by 20° to bring it closer to the ligand.³⁹ In MDA5, CTD also interacts with the Hel1 to wrap around the dsRNA.³⁹ This conformation assists in the filament formation that leads to oligomerization with the MAVS CARDs.

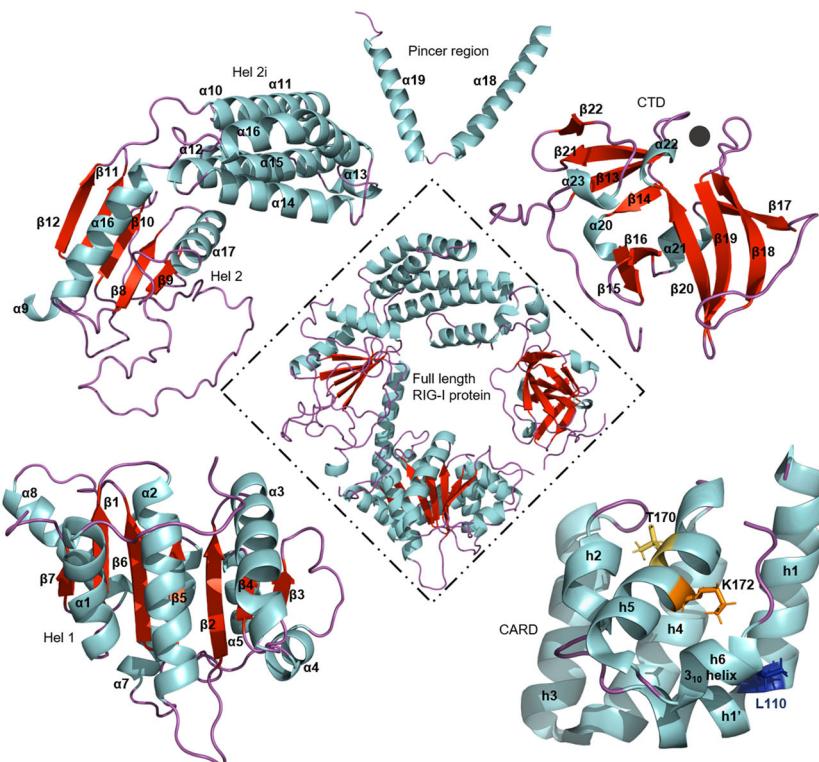


FIGURE 3 Three-dimensional structural representation of full-length RLR proteins (crystal structure of RIG-I is used in this figure; Protein Data Bank [PDB] ID: 5E3H) RLRs contain CTD, a helicase core region that include Hel1, Hel2, and a pincer domain, which connects Hel2 to the CTD. The top right panel represents the CTD containing a Zn atom (shown in gray color). Three sets of antiparallel β -sheets (red) contribute to the CTD topology. The top left panel depicts the Hel2 domain and Hel2i domain. Hel2 consists of four parallel β -sheets and three helices. In the lower left panel, a Hel1 domain is illustrated. It is composed of seven parallel β -sheets and seven α -helices (cyan). The β -sheets are surrounded by α -helices. Helicase core domains collectively wrap the RNA molecule and induce a conformational change, result in the shift from a closed to open conformation, when the CARDs are exposed and interact with MAVS (as explained in Figure 5). In the bottom left panel, the three-dimensional structure of CARDs (PDB ID: 2LWD) is shown. The ubiquitination site (K172) and phosphorylation site (T170) are highlighted in orange and yellow, respectively. CARD, caspase recruitment domain; CTD, C-terminal domain; Hel1, helicase 1; Hel2i, helicase insertion domain; MAVS, mitochondrial antiviral signaling protein; RIG-I, retinoic acid-inducible gene-I; RLR, RIG-I-like receptor [Color figure can be viewed at wileyonlinelibrary.com]

which is a key feature in MDA5 signaling.^{40,41} In a study, it has been reported that RIG-I can also form ATP-dependent filaments, but these filaments are much smaller and unstable as compared to MDA5 filaments.⁴²

Crystal structures of CARDs have not been resolved yet; however, the structural topology of a single CARD (CARD2) of RIG-I has been reported in a nuclear magnetic resonance study.⁴³ The CARD contains six helices (h1–6) just as other death domain-containing superfamily proteins do (Figure 3). The longest helix is h1 and is kinked at residue Leu110 (in RIG-I), therefore, a part of the first helix is designated as h1'. All six helices have different lengths, whereas h6 comprises the 3_{10} helix. The h5 helix is believed to undergo ubiquitination and phosphorylation at K172 and T170, respectively. The side chain of K172 at the ubiquitination site is well exposed and extends in the opposite direction from the phosphorylation site. The average distance between these two posttranslationally modified sites is 11 Å. The NH group of the ubiquitination site is covered by residues at the C terminus of the protein.⁴³ The CARDs of MDA5 share similar structural folds because of sequence homology.

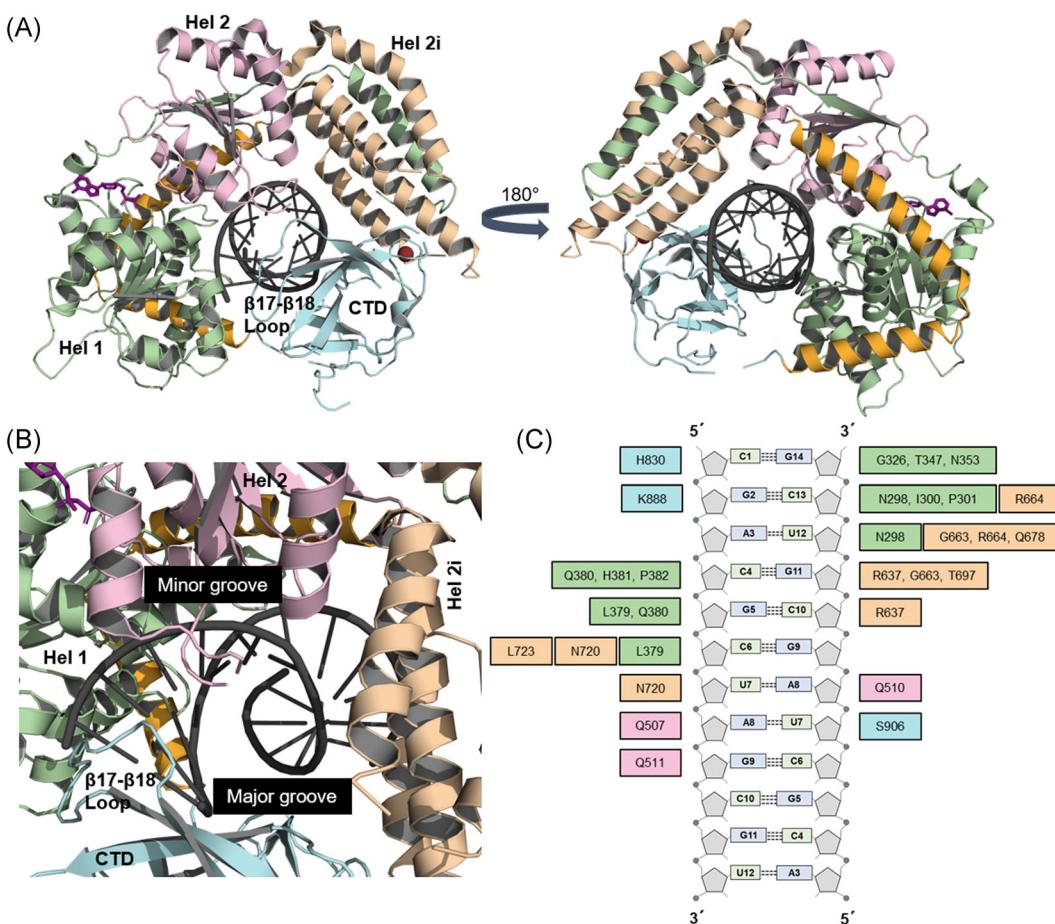


FIGURE 4 Interactions of RLRs and dsRNA (A) Top view of the RIG-I crystal structure (PDB ID: 5E3H) bound to the dsRNA reveals that the helicase core region and CTD wrap the RNA molecule, while the extended β17-β18 loop in the CTD holds the RNA terminus. (B) The helicase domain binds to a minor groove of RNA, while the CTD interacts with bases present in the major groove and the CTD loop interacts with the RNA terminus. (C) A detailed illustration of RIG-I-dsRNA interactions; residues of each domain are colored for clarity (Hel1 is green, Hel2 is pink, Hel2i is brown, and the CTD is cyan). CTD and Hel1 residues interact with the RNA terminus, while Hel2 and Hel2i engage in interactions with the central region of RNA. CTD, C-terminal domain; dsRNA, double-stranded RNA; Hel1/2, helicase 1/2; RIG-I, retinoic acid-inducible gene-I; RLR, RIG-I-like receptor [Color figure can be viewed at wileyonlinelibrary.com]

5 | STIMULUS RECOGNITION AND ACTIVATION MECHANISM OF RLRs

Deciphering how RLRs distinguish between self and foreign nucleic acids has remained a priority topic for years. RNAs synthesized by *in vitro* transcription and a dsRNA analog, poly(I:C), have been employed to gain a better understanding of the basic features of RLR agonists and the underlying signaling mechanism. Even though RLRs RIG-I and MDA5 have similar domain arrangements, they recognize distinct RNA species. Various studies have been conducted to identify a subset of viruses that are exclusively recognized by RIG-I or MDA5. Some viruses that are detected in a temporally distinct manner by the two receptors are reoviruses, flaviviruses, and

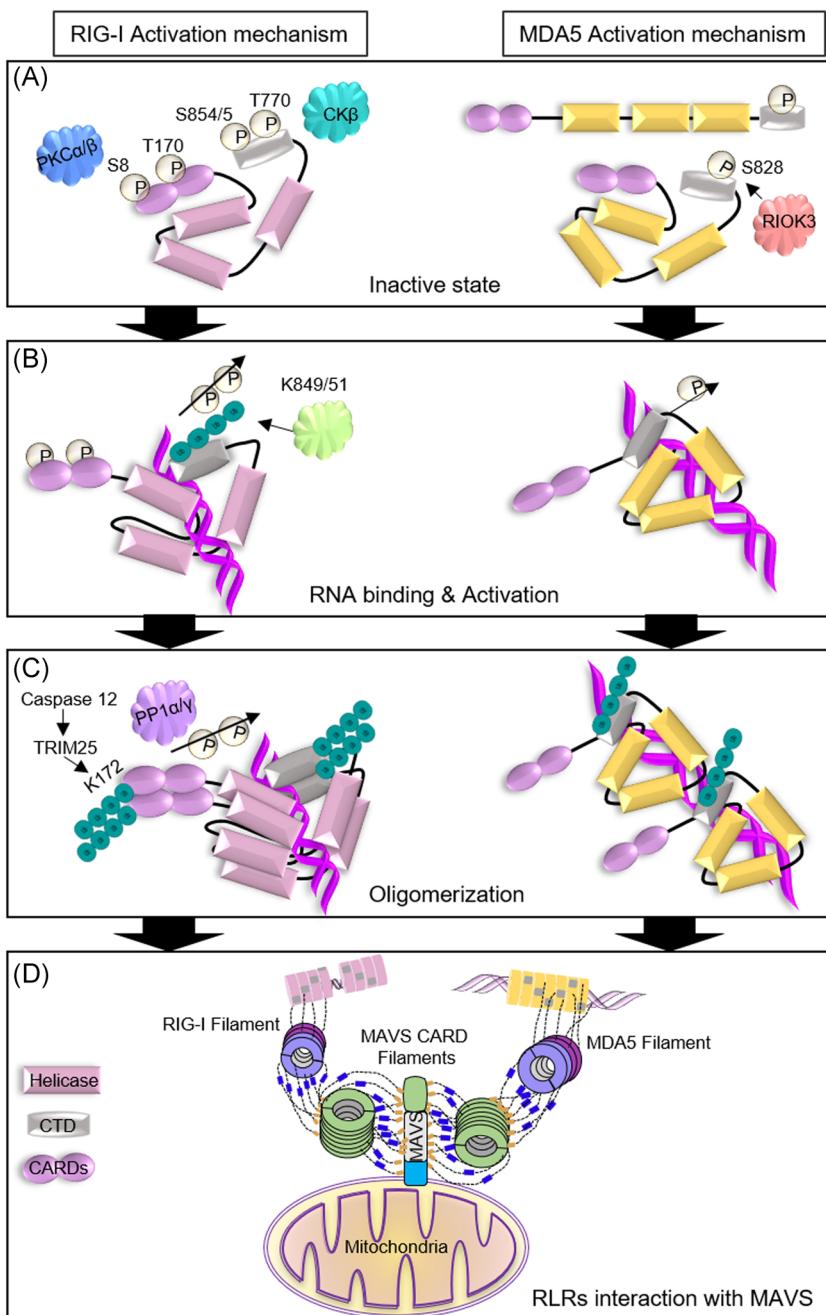


FIGURE 5 Schematic view of the activation mechanisms of RIG-I and MDA5. (A) RIG-I and MDA5 are activated upon the detection of viral dsRNA. The CTDs of both RLRs bind to the dsRNA. In the absence of ligands, both RLRs are in a suppressed state and are phosphorylated in the N-terminal domain and CTD. MDA5 may exist in either an open or closed conformation in the absence of the ligand (B) Upon sensing viral dsRNA, CTD is dephosphorylated in RIG-I and MDA5, while ubiquitination occurs only in the RIG-I CTD. (C) After that, ATP-dependent oligomerization of RIG-I occurs and filament formation occurs around dsRNA in case of MDA5, while the RIG-I N-terminal CARDs are ubiquitinated and dephosphorylated. (D) Finally, the RIG-I oligomers bind to the MAVS protein on mitochondria via CRAD-CARD interaction. CARD, caspase recruitment domain; CTD, C-terminal domain; dsRNA, double-stranded RNA; MDA5, melanoma differentiation-associated gene 5; RIG-I, retinoic acid-inducible gene-I; RLR, RIG-I-like receptor [Color figure can be viewed at wileyonlinelibrary.com]

paramyxoviruses.^{10,25} RIG-I is triggered in response to the coronavirus, vesicular stomatitis virus, influenza A virus, arenaviruses, and various paramyxoviruses, including the Newcastle disease virus, Sendai virus, and measles virus, whereas MDA5 detects picornaviruses and vaccinia virus (owing to RNA intermediates produced during the infection).^{25,44} Viruses distinctly recognized by RLRs are listed in Table 2.

TABLE 2 Viruses detected by different RLRs

Virus type	RIG-I	MDA5	LGP2	Ref.
Ebola virus	Yes	x	x	45
Coronavirus	Yes	N/A	x	46
Epstein-Barr virus	Yes	x	x	47
Encephalomyocarditis virus	x	Yes	Yes	44
Hepatitis C virus	Yes	x	x	48
Dengue virus	Yes	Yes	x	26
Influenza virus A	Yes	x	x	25
Murine hepatitis virus	x	Yes	x	49
Influenza virus B	Yes	x	x	26
Japanese encephalitis virus	Yes	x	x	25
Lassa virus	Yes	x	x	45
Lymphocytic choriomeningitis virus	Yes	x	x	50
Measles	Yes	x	x	51
Rotavirus	Yes	Yes	x	45
West Nile virus	Yes	Yes	x	52
Myxoma virus	Yes	x	x	53
Newcastle disease virus	Yes	x	Yes	25
Nipah virus	Yes	x	x	45
Rabies virus	Yes	x	x	54
Respiratory syncytial virus	Yes	x	x	26
Rift Valley fever virus	Yes	x	x	45
Sendai virus	Yes	x	Yes	25
Vesicular stomatitis virus	Yes	x	Yes	25
Murine norovirus 1	x	Yes	x	55
Mengo virus	x	Yes	Yes	25
Reovirus	x	x	Yes	26
Vaccinia virus	x	Yes	x	45
Theiler's virus	x	Yes	x	25

Abbreviations: x, NO (virus was not detected by the respective RLR); LGP2, laboratory of genetics and physiology 2; N/A, information is not available; MDA5, melanoma differentiation-associated gene 5; RIG-I, retinoic acid-inducible gene-I; RLR, RIG-I-like receptors.

5.1 | The RNA-sensing mechanism of RIG-I

RIG-I senses and binds to single-stranded RNA (ssRNA), dsRNA, and short dsRNA and recognizes the key signature, a 5' ppp moiety, at the blunt end viral RNA ~20 nucleotides long (a total of 10 bp).^{56–58} The RNA-binding loop and the basic cleft responsible for dsRNA binding are the key features shared among the CTDs of all three RLRs. To maintain the binding between dsRNA and RIG-I, the phosphate backbone of the dsRNA engages in multiple interactions with the basic residues in the cleft. The aromatic ring of phenylalanine (F853) forms a hydrophobic bond with the ribose moiety of the nucleic acid. This interaction strongly holds the dsRNA and the RNA-binding loop at their respective positions and enables lysine (K851) to bind to the dsRNA through a strong electrostatic interaction. The basic cleft of the CTD is enriched with basic amino acids that are complementary to the RNA backbone with acidic patches. This single domain is responsible for recognizing distinct RNA molecules.³⁸ RIG-I distinguishes viral RNA from host RNAs based on the presence of 5'ppp as 5'ppp is present in the host RNA at the time of transcription and is masked by capping or removed before cytoplasmic translocation.⁵⁶ ssRNA and dsRNA carrying 5'ppp recognize specific protruding motifs on RIG-I thus leading to similar structural alterations, which are significantly enhanced by ATP. A linker region connects the CTD and helicase domain; thus, the CTD retains its nucleic-acid-binding properties with higher affinity for the 5'ppp moiety as compared to monophosphate-containing dsRNA.³⁸

5.2 | The activation mechanism of the RIG-I receptor

RIG-I remains in a monomeric inactive form when an RNA ligand is absent. RIG-I can assume the closed conformation, in which the CARDs are in their inert form and cannot interact with other CARDs to initiate signaling (Figure 5). During viral infection, a 5'ppp-containing RNA or short dsRNA is produced, which binds to the CTD in the presence of ATP. Upon viral-RNA recognition, CARDs are exposed and released from autoregulation by conformational changes in RIG-I, concomitant with tripartite motif 25 (TRIM25)-dependent K63-linked polyubiquitination of a lysine residue (K172).^{59,60} RIG-I thus adopts an open conformation that favors oligomerization.⁶¹ These oligomers cooperate for the induction of downstream signaling via a CARD-dependent interaction with MAVS. This synergy drives a signaling cascade that promotes IFN production.¹⁰

5.3 | The RNA-sensing mechanism of MDA5

In contrast to RIG-I, which has been widely studied and whose nucleic-acid-binding mechanism has been adequately elucidated, there is still a gap in the understanding of the molecular determinants of viral RNAs that activate MDA5. The latter is triggered by longer dsRNA, messenger RNA (mRNA) lacking 2'-O-methylation in their 5' cap, and RNA aggregates,^{62,63} and binds to the stem-loop of RNA. In CoV infection, the 2'-O-methylation of viral nucleic acid downregulates IFN expression,⁶⁴ but the exact mechanism is not clear yet, that is, whether this posttranslational modification affects the binding of RNA to MDA5 or not.⁵⁸ As murine hepatitis virus belongs to the same family as SARS-CoV-2, therefore SARS-CoV-2 might be detected by MDA5 with similar mechanism, that is, panhandle dsRNA comprising 5'-polyuridine of murine hepatitis virus is detected by MDA5.⁶⁵ It has been reported that the endoribonuclease of coronavirus degrades poly-U sequence, that hinders the activation of the host immune system. Thus, endoribonuclease could serve as a potential target for designing effective therapeutic against COVID-19.⁶⁵ It is well known that for IFN regulation, ISGylation of RLRs is important. In the case of coronaviruses and RNA viruses, the ISGylation of MDA5 is necessary to stimulate IFN production.⁶⁶

Recently, a mechanism underlying the identification of pathogen-associated molecular patterns by MDA5 was reported, where multimerization of MDA5 and its cooperative binding along a viral RNA helix via filament formation was suggested. In addition to the involvement of the helicase domain in the MDA5–RNA interaction, the CTD also

participates in filament formation and subsequent interaction with MAVS.⁶⁷ The RNA-binding loop of MDA5 (in contrast to that of RIG-I) does not make a significant contribution to RNA binding. This is because an aromatic amino acid residue, phenylalanine, in RIG-I is replaced by a cysteine in MDA5, thereby weakening the hydrophobic interaction between the CTD and RNA. Nevertheless, a phenylalanine (F601) molecule is present at a similar position in LGP2 and interacts with RNA; this interaction is similar to the one observed in RIG-I.

5.4 | The activation mechanism of the MDA5 receptor

In an inactivated state, MDA5 exists in open or closed conformation (Figure 5). It is a well-known fact that RLR-mediated signal transduction is regulated by posttranslational modifications such as ubiquitination and phosphorylation.⁶⁰ The CTD of MDA5 is phosphorylated by RIOK3 at a serine (S828).⁶⁸ When MDA5 senses and binds to foreign dsRNA, the C terminus is dephosphorylated and ubiquitinated. Multiple MDA5 molecules combine and form a long filament, that binds to MAVS via CARD-CARD interaction. This interaction is weaker than that of a RIG-I CARD with MAVS.⁵⁹

5.5 | The RNA-sensing mechanism of LGP2

LGP2 can bind to a broad range of nucleic-acid stimuli including 5'ppp-containing ss- or dsRNA. LGP2 positively regulates IFN production via a RIG-I-dependent and MDA5-dependent antiviral response, although some researchers believe that LGP2 is involved in the autoregulation mechanism of RLRs, where it acts as a negative regulator of RIG-I.⁶⁹ Further experimental studies are required for a detailed understanding of the exact function of LGP2 and its mechanism of action in antiviral immunity.²¹ Many models have been reported that emulate the RLR-inhibitory mechanism of LGP2. The first model suggests that LGP2 prevents the binding of RNA to MDA5 or RIG-I by engaging the dsRNA.²³ The second model implies that the LGP2 CTD interferes with the multimerization of RIG-I and with the CARD-dependent interaction of RIG-I with the adaptor protein MAVS.⁴⁸ The third model is based on competitive protein–protein interaction of LGP2 with IKKi (also known as IKKe) in relation to a common binding site on MAVS. This model points to negative-feedback regulation of the antiviral host immune response governed by LGP2.⁷⁰

5.6 | The synergistic role of LGP2 in RLR signaling

LGP2 has a regulatory role in RLR signaling, however, its exact mechanism is not clear yet. LGP2 has shown a differing effect in the regulation of RIG-I and MDA5. In a study, it has been suggested that CTD of LGP2 recognizes dsRNA thus competes with RIG-I (helicase domain) to bind dsRNA patterns. Therefore, LGP2 assists MDA5 in the detection of dsRNA but might help in the RIG-I viral recognition mechanism to anticipate false positives.¹⁹ Another study described the mechanism of the LGP2 regulatory mechanism in MDA5 signaling.⁷¹ LGP2 regulates the filament assembly of MDA5 and helps in the MDA5-dsRNA interaction resulting in the upregulated MDA5-mediated signaling.^{71,72} However, detailed studies are needed to elucidate the exact regulatory mechanism of LGP2.

6 | THE SIGNATURES RESPONSIBLE FOR NON-SELF RNA RECOGNITION AND RLR STIMULATION

The specificity of RLRs reflects their significant capacity for detecting nucleic acids present in the cell during viral infection. Nonetheless, it is still debated how RLRs discriminate viral and host RNAs. Specific structural motifs and posttranslational modification in foreign RNAs have been identified as signature traits recognized by an RLR.

Besides, length and types of nucleic acids and 5' phosphorylation are believed to be crucial for potent RLR-mediated innate immune signaling.

As mentioned above, RLRs recognize distinct groups of viruses, but a few species are detected by both MDA5 and RIG-I. The 5'ppp moiety is a key structural feature for the discrimination between viral RNA and host nucleic acid, whereas the absence of 5'ppp or 5' capping can prevent the RIG-I–RNA interaction.^{18,38,73} Additionally, RIG-I can detect a diphosphate moiety-containing viral RNA.⁷⁴ Moreover, for recognition by RIG-I and its optimal activation, poly-U/UC-specific sequence motifs are required in RNA ligands.^{73,75} RNAs lacking 5'ppp or capping of the 5'ppp moiety or RNAs with inserted modified nucleotides can prevent the signaling-pathway triggering.⁵⁴ RIG-I binds to the negatively charged 5'ppp moiety via the cleft present within the CTD of this receptor,^{18,38} while the adjacent blunt-ended dsRNA nucleotide moieties help with the stabilization of this binding.^{36,76} In addition to RNA viruses, RIG-I is known to sense DNA viruses by detecting tiny RNA fragments produced during DNA replication.^{47,77} RIG-I can also detect and bind to short (25 bp) or long (>200 bp) dsRNA with monophosphate at both ends or a hydroxyl group at the 5' end. Moreover, RIG-I recognizes short dsRNA with monophosphate at the 3' end and a hydroxyl group at the 5' end, derived from cleavage by an antiviral endonuclease, ribonuclease L.⁷⁸

A study has been conducted to determine how RIG-I responds to the hepatitis C virus (HCV), where RIG-I activation is associated with the length as well as the sequence of the binding motif. RIG-I specifically responds to 50-nucleotide-long polyriboadenine and polyuridine motifs.⁷⁵ Moreover, HCV RNA, which contains a 3' untranslated region, is reported to be a strong activator as compared to viruses featuring RNA with the 5'ppp moiety.⁷⁵ In addition to this moiety, a ~20-nucleotide-long blunt-ended region base-paired intramolecularly (in ssRNA) or intermolecularly (between complementary strands of dsRNA) is another key feature for RNA detection. Thermodynamic analysis has revealed that the binding affinity of full-length RIG-I is 126-fold higher for 5'ppp-containing RNA than for other RNAs carrying a hydroxyl group.^{79,80}

Agonists of MDA5 are studied less; nonetheless, MDA5 recognizes dsRNA of various sizes, from 0.5 to 7 kb.⁶³ The term “molecular ruler” has been used for MDA5, meaning that it can respond to short dsRNA (~100 nucleotides long), but only when present in huge amounts. On the other hand, MDA5 can be efficiently activated by longer dsRNA, 1–2 kb in length. This process is mediated by the formation of filamentous oligomers due to the collaborative assembly of helicase domains by the stem of the dsRNA.^{40,67} The ability of MDA5 to detect long dsRNA might have evolved to discriminate between self and non-self RNA, because in some cases, the short base-paired regulatory RNAs are present in uninfected cells as well.⁸¹ It is possible that in infected cells, ligands recognized by MDA5 are more complex in nature rather than simply being specific. They might be in a particular conformation arising as intermediates during RNA virus replication or DNA virus transcription.^{62,82,83} The ribose 2'-O-methylation of the 5' cap of mRNA is a conserved feature of higher eukaryotes and of many viruses that replicate in a eukaryotic host. Various coronavirus mutants exist that are devoid of a 2'-O-methyltransferase activity, thus yielding higher MDA5-dependent expression of type I IFN.⁵⁸ This signature feature discriminates between self and non-self mRNA in case of coronavirus infection.

Conclusively, the promising differences between RIG-I and MDA5 in recognizing specific ligands are: ligand length (RIG-I: 25 to >200 bp; MDA5: 1–2 kb long), type of nucleic acid (RIG-I: ssRNA, short dsRNA; MDA5: long dsRNA), posttranslational modifications (RIG-I: 5'ppp moiety; MDA5: ribose 2'-O-methylation of the 5' cap), RIG-I can also detect PolyU/UC rich RNA and RNaseL RNA fragments, RNAs with panhandle structure,¹⁴ MDA5 can recognize RNaseL-cleaved self-RNAs with monophosphate ends. Upon ligand binding, in contrast to RIG-I, MDA5 assembles into a filament formation and oligomerizes with the MAVS CARD. Moreover, the role of ATP is important in binding affinities of RLRs to RNA ligands.⁴⁰ In spite of high sequence and structural similarities between RIG-I and MDA5, large differences have been identified in their regulation because of different ligands.

7 | CYTOSOLIC HOMEOSTASIS OF RLR SIGNALING

7.1 | Autoregulation

An efficient antiviral innate immune response is defined by the stimulation of a wide range of effector proteins injected by pathogens; these proteins regulate biological activities, such as protein synthesis, apoptosis, and cytokine production.⁸⁴ In the past few years, many endogenous proteins were reported to maintain an effective and balanced innate immune response; these proteins regulate the RLR signaling at multiple levels, either positively or negatively.¹⁰ In various biochemical and structural studies, an intricate multistep model has been proposed for the RIG-I activation mechanism.⁸⁵ RIG-I activation is usually regulated by conformational changes that occur due to posttranslational modifications such as ubiquitination and phosphorylation. In a study aimed to confirm that full-length RIG-I remains inactive in the absence of any relevant stimulus, overexpressed CARDs were found to be integrally active and to induce downstream signaling without any stimulus; this result suggests that full-length RIG-I remains inactive and in a signaling-repressed state in uninfected cells.¹⁷ RIG-I lacking the CTD can function normally, while an overexpressed CTD acts as a repressor and negatively regulates the RIG-I signaling by masking the CARDs.^{48,86} Once a viral pathogen-associated molecular pattern binds to the CTD, RIG-I CARDs are unmasked and interact with the MAVS CARDs that initiate the downstream signaling cascade.⁷⁶

7.2 | Endogenous cytosolic regulators of RLRs

RIG-I is regulated via ubiquitination as well, where it interacts with TRIM25, which belongs to the E3 ubiquitin ligase family and regulates the antiviral immune response.⁸⁷ TRIM25 interacts with RIG-I and facilitates K63-linked ubiquitination at a lysine residue (K172) that is crucial for the RIG-I–MAVS interaction and downstream signaling.⁸⁸ In addition to TRIM25, other regulatory E3 ligases of RIG-I are Riplet, MEX3C, and TRIM4. Among them, Riplet is an important E3 ligase for RIG-I, where it is involved in the upregulation of RIG-I-mediated signaling.^{89,90} Riplet acts as a mediator in ubiquitin conjugation with RIG-I, where ubiquitin binds to the lysine residues present at multiple sites in the CTD and CARD.⁹⁰ Moreover, E3 ligases, such as TRIM40, RNF122, and RNF125 have been reported to negatively regulate RLR signaling.^{91–93} Another important protein–zinc finger antiviral protein shorter isoform (ZAPS)—is stimulated by the 5'ppp-containing dsRNA, which positively regulates the RIG-I signaling and its ATPase activity.⁹⁴

In contrast to ubiquitination, ubiquitin-specific isopeptidases also known as deubiquitinases (DUBs; which are an important component of the ubiquitin–proteasome system) negatively regulate the RLR signaling by removing ubiquitin from the substrate. Cylindromatosis (CYLD), a DUB enzyme, engages in direct interaction with RIG-I to inhibit IFN production via the removal of K63-linked ubiquitin.⁹⁵ Additionally, CYLD performs its DUB activity while interacting with TBK-1, thereby affecting the activity of E3 ubiquitin ligase TRIM25 and E2 ubiquitin-conjugating protein UBC5. Similarly, another DUB enzyme, deubiquitinating enzyme A (DUBA), facilitates the clearance of K63-linked ubiquitin via TRAF3 interaction. This DUB activity hinders the interaction of TRAF3 with TBK-1, thus abrogating the downstream RLR signaling and IFN production.⁹⁶ Therefore, DUBs represent novel candidates for the development of targeted drugs against immune diseases.

Endogenous proteins also interact with the adaptor proteins of the RLR pathways and modulate downstream signaling. PSMA7, that is, 20S proteasomal subunit α4, interacts with MAVS to attenuate the transmission of downstream signals through proteasomal degradation of MAVS.⁹⁷ Another factor, poly(rC)-binding protein 2 (PCBP2; which is an RNA-binding protein), is switched on during viral infection and recruits AIP4 to degrade MAVS, thereby downregulating the cellular response to the viral infection.⁹⁸ ATG12 and ATG5, two autophagy-related proteins that are covalently attached via a ubiquitin like conjugation

mechanism, play a pivotal role in the regulation of RLR signaling. Molecular-interaction studies have explained the direct interaction of the ATG5-ATG12 conjugate with the CARDs of MAVS and RIG-I; this interaction subsequently downregulates the IFN promoter activity.⁹⁹ At this point, the basic understanding of the regulation of MDA5 signaling via endogenous factors is scarce. Nonetheless, the role of dihydroacetone kinase in the regulation of MDA5 activity has been studied, and it has been revealed that dihydroacetone kinase negatively affects the function of receptor MDA5 by preventing its interaction with MAVS, thereby abrogating the signaling.¹⁰⁰

8 | THE CROSSTALK BETWEEN RLRS AND OTHER IMMUNE RECEPTORS

The crosstalk among PRRs has evolved to provide robust defense against microbial infections to compensate for the diverse genetics of the host. Crosstalk may involve the induction of multiple receptors that synergistically collaborate for the enhanced immune response and specificity for microbial infection.¹⁰¹ A better understanding of RLR function in antiviral immune response and the crosstalk of RLRs with other signaling receptors can pave a new road to antiviral therapeutics. The crosstalk among PRRs is essential to enhance the antimicrobial innate immune response.¹⁰² TLRs and RLRs, being present at different locations in the cell, can respond to either different components or a single component of the same viral nucleic acid to launch the pathway of the antiviral immune response.¹⁰³ Furthermore, these receptors can regulate each other positively and negatively, in particular when various invading pathogens are involved. Nonetheless, the interference of RLRs with TLR signaling during viral infection has been proposed to increase susceptibility to bacterial infection.¹⁰⁴ RNA viruses are detected by endosomal TLRs and cytosolic RLRs, which induce IFN gene transcription in response. In an ex vivo experiment, TLR7 was shown to be involved in the induction of type I IFN by dendritic cell (DCs) in response to an RNA virus, such as the Newcastle disease virus, influenza A virus, and vesicular stomatitis virus, whereas RIG-I responds to these viruses by stimulating the production of IFN via macrophages, DCs, and fibroblasts.¹⁰⁵ Besides, TLR2/6, NALP3, and RIG-I collaborate to detect a dsDNA virus (modified vaccinia Ankara) and induce the consequent antiviral innate immune response.¹⁰⁶ Another example involves RIG-I, MDA5, and TLR3 cooperatively recognizing the rhinovirus.¹⁰⁷ TLR3, TLR7, and RIG-I collaboratively respond to West Nile virus infection and initiate a strong intracellular innate immune response.¹⁰⁸ TLR3 and RIG-I present in hepatocytes are reported to recognize HCV.¹⁰⁹ RIG-I, but not MDA5, is known for initiating signaling via activation of caspases 1 and 3 for robust induction of interleukin-1 β (IL-1 β) and IL-18.^{110,111} Activation of RIG-I by 5'ppp-containing dsRNA initiates signaling through MAVS, which induces NF- κ B for pro-IL-1 β expression. Under the influence of the same agonist, RIG-I also activates pro-IL-1 β gene transcription mediated by inflammasome signaling through an adaptor protein (apoptosis-associated speck-like protein containing a CARD [ASC]) and caspase 1. Interaction with an adaptor protein is necessary for signaling pathways and known as an important molecule governing their crosstalk. MDA5 cannot induce transcription of the pro-IL-1 β gene through the inflammasome pathway because it does not interact with ASC (adaptor protein). This scenario indicates that among RLRs, only RIG-I is capable of mediating dual signaling, that is, inflammasome and type 1 IFN pathways. In future, more investigational studies are required for a better understanding of the crosstalk between NLR family pyrin domain containing 3 (NLRP3) inflammasome activation and pro-IL-1 β production mediated by RIG-I.¹¹²

9 | RLR-RELATED DISEASES

In addition to the immediate role of dysregulated RLR-mediated signaling in autoimmune diseases, many single-nucleotide polymorphisms (SNPs) are known to be associated with the risk of developing autoimmune diseases such as psoriasis, multiple sclerosis (MS),^{113,114} systemic lupus erythematosus (SLE), dilated cardiomyopathy,¹¹⁵

and type 1 diabetes mellitus.¹¹⁶ There are no clear data on the mechanism by which MDA5 participates in autoimmunity, but recently, a research group conducted *in vivo* experiments on MDA5-mutant mice and demonstrated that MDA5 dysregulation can cause a lupus-like syndrome.¹¹⁷ The first evidence of any RLR involvement in the autoimmune phenotype was reported in 2014, by means of a missense mutation (G821S) in the *IFIH1* gene, which encodes MDA5 protein. These findings have been confirmed in a mouse model that develops lupus-like symptoms as well as nephritis and skin rash.¹¹⁷ By contrast, the role of *IFIH1* SNPs in the onset of type 1 diabetes mellitus and other autoimmune diseases is not clear yet.¹¹⁸ There are other SNPs in RLR genes that can cause immune diseases (Table 3). These mutations have been found in patients with Aicardi–Goutieres syndrome, Singleton–Merten syndrome, or SLE. The mRNA levels of RIG-I and MDA5 were analyzed in MS patients. The data revealed that the expression of both receptors was significantly reduced in patients which were upregulated by interferon- β therapy, but RIG-I expression was upregulated only. Thus, a combination therapy can be used to treat MS patients.¹¹⁹ In another recent study, the potent small molecule RIG-I antagonists have been reported.¹²⁰ They used structure–activity relationship study to design RIG-I antagonists. They used a high-throughput small-molecule screening method to identify the effective compounds against RIG-I. The derivatives of chosen compound RIG-001 were modified at R4 and R5 position and optimized. The methylation of R4 moiety improves the potency while the R5 substitution resulted in the loss of function. Therefore, suggesting the ketone group is essential for an efficient RIG-I inhibitor.¹²⁰ The direct suppression of such signaling pathways could be a possible approach to medical treatment. Nonetheless, the detailed pathogenesis and etiology of such autoimmune problems are yet to be clarified.¹¹³ This series of small molecule compounds can be used in the future to design next generation modulators of RLR using advanced drug discovery and machine learning techniques.¹²¹

TABLE 3 A list of mutations in the *IFIH1* and *DDX58* genes that are responsible for causing different diseases

Protein/gene	Mutation	Effect	Disorder
MDA5 (<i>IFIH1</i>)	G821S	Unable to respond/bind to dsRNA	Lupus-like symptoms
<i>IFIH1</i>	R822G	-	Singleton–Merten syndrome
<i>IFIH1</i>	K337G		AGS
<i>IFIH1</i>	D393V		AGS
<i>IFIH1</i>	A946T	Atypical activation of MDA5, resulting in the abnormal production of interferons	SLE
<i>IFIH1</i>	-		SLE
RIG-I (<i>DDX58</i>)	E373A	Constitutive production of type I interferons	Singleton–Merten syndrome
RIG-I	C268F	-	Congenital glaucoma
<i>IFIH1</i>	A452T		AGS
<i>IFIH1</i>	L372F		AGS
<i>IFIH1</i>	G495R		AGS
<i>IFIH1</i>	K720N		AGS
<i>IFIH1</i>	R779H/C		AGS
<i>IFIH1</i>	I923V	Loss of function of MDA5 and a gain of resistance to type 1 diabetes mellitus	Type 1 diabetes mellitus

Abbreviations: AGS, Aicardi–Goutieres syndrome; SLE, systemic lupus erythematosus.

10 | ANTICANCER PROPERTIES OF RLRS

Cancer is one of the deadliest diseases worldwide and inflammation is a crucial factor in this illness.¹²² The host immune response controls the cancer progression. Meanwhile, immunodeficiency or an underactivated immune response may be a potential risk factor for this disease.^{123–125} Due to the suppression of apoptosis and evasion of the immune system, cancer cells gain resistance to therapies. In cancer pathogenesis, apoptosis is one of the key factors; therefore, its activation may be a propitious approach to cancer treatment. Recently, it was reported that cancer cells turn on PRRs by mimicking a viral infection mechanism that results in the activation of the IFN pathway.¹²⁶ MDA5 and RIG-I triggered by a viral nucleic acid induce IFN-dependent or -independent apoptosis of cells involved in various cancers. Hence, to induce IFN-dependent apoptosis, it is necessary to initiate the RLR signaling pathway by means of oncolytic viruses or other synthetic ligands (Figure 6). Moreover, apoptosis can be induced in a caspase-3-dependent and noncanonical IFN-independent manner through MAVS or IRF3.^{127,128} The

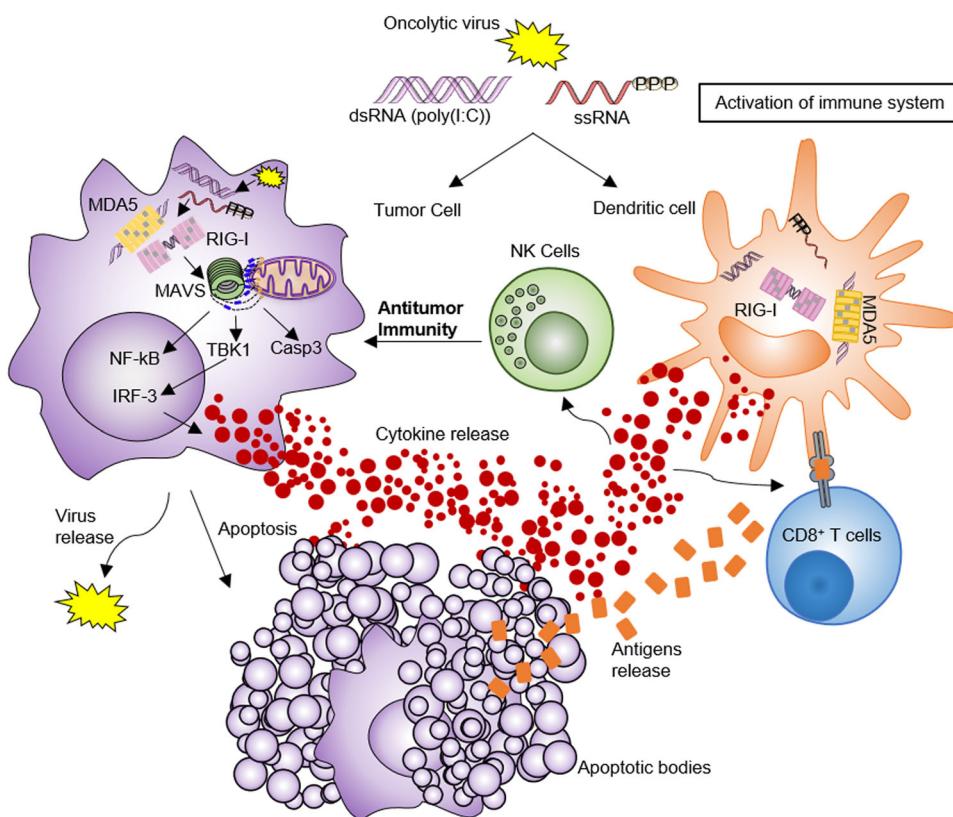


FIGURE 6 Illustration of the roles of RLRs in the tumor microenvironment during oncolytic viral therapy. Oncolytic viruses or synthetic ligands are used to activate RLRs in tumor cells and these receptors subsequently initiate the downstream signaling. This phenomenon leads to the activation of NF-κB and IRFs, which drive the release of cytokines to enhance the tumor cell apoptosis. Oncolytic viruses can also directly induce tumor cell death in an IFN-independent or -dependent manner. These synthetic ligands are recognized by immune cells, such as the DCs, which secrete cytokines to activate the cluster of differentiation 8 (CD8⁺) T lymphocytes and NK cells. Both CD8⁺ T lymphocytes and NK cells exhibit antitumor activities. DC, dendritic cell; IFN, interferon; IRF, IFN-regulatory factor; NF-κB, nuclear factor κB; NK, natural killer; RLR, RIG-I-like receptor [Color figure can be viewed at wileyonlinelibrary.com]

antitumor effect of RLRs can be enhanced by an adaptive-immunity pathway through the activation of DCs as well as the cluster of differentiation 8 (CD8⁺) cytotoxic T lymphocytes in a tumor microenvironment.

RLRs can be considered as potent targets of cancer immunotherapies for the following reasons: (1) RLRs act in an IFN-dependent or IFN-independent manner to induce the antitumor immunity and a proapoptotic state, respectively.^{129–131} (2) Cancer cells are susceptible to RLR-induced apoptosis; however, the protection of non-malignant cells is ensured by the upregulation of endogenous BCL-xL.¹³¹ (3) Apoptosis induced via RLR signaling pathways does not overlap with p53-dependent apoptosis induction and remains unaffected by p53 mutation.^{131,132} For these reasons, RLR-targeting synthetic compounds have been developed as reported in the literature and are in clinical trials. A well-known example of an immune adjuvant being evaluated in clinical trials is poly(I:C), a synthetic ligand of TLR3 as well as RIG-I or MDA5.¹³³ This ligand has been used to induce apoptosis in a mouse model of gastric adenocarcinoma. Poly(I:C)-treated cancer cells undergo apoptosis and show upregulation of RIG-I, MDA5, LGP2, and Bcl-2. Therefore, poly(I:C) may be a potent anticancer immunotherapeutic agent.¹³⁴ The synthetic compound poly-ICLC [a derivative of poly(I:C)] has been tested in combination with chemotherapy in patients with stage IV anaplastic astrocytoma.¹³⁵ Although poly-ICLC is known as an MDA5 agonist, it also activates TLR3; consequently, it may have a stronger effect by acting via both the receptors. Poly-ICLC together with temozolomide and radiation is being assessed in patients with glioblastoma.^{136–138} Additionally, poly-ICLC as a vaccine adjuvant is being tested in clinical trials against various types of cancers, such as pancreatic cancer,¹³⁹ breast cancer,¹⁴⁰ multiple myeloma,¹⁴¹ ovarian cancer,¹⁴² and gliomas.¹⁴³

Similarly, the function of RIG-I has been studied in the head and neck squamous cell carcinoma, where RIG-I is activated by 5'ppp-containing RNA, thereby inducing the IFN-independent apoptosis of cancer cells.¹⁴⁴ Besides, Bcl2-specific short interfering RNA (siRNA) with 5'ppp ends (3p-siRNA) has been directed to cancer cells, where 5'ppp activates RIG-I to overcome the immune escape. In that study, the siRNA was responsible for the silencing of Bcl2, which reversed the molecular events that govern the tumor cell survival.¹⁴⁵

Moreover, synthetic RIG-I mimetics are being investigated in preclinical experiments for their efficacy on hepatocellular carcinoma, prostate cancer, melanomas, and leukemias; these agents work via immediate activation of an immune response against cancer cells along with indirect leukocyte stimulation in the tumor microenvironment.¹⁴⁶ Testing of a RIG-I-specific compound, MK-4621 (RGT100), which has been investigated for the treatment of lymphomas and solid tumors by Merck, was terminated in a phase II trial (as of July 30th, 2019). Another compound, SB-9200 (activates RIG-I), has been investigated as an antiviral agent by Spring Bank Pharmaceuticals, Inc. and Syneos Health and is currently being evaluated in clinical trials.¹⁴⁷ Phase I is now completed (as of September 18, 2019) for the testing against HCV infection (NCT01803308) and this compound is now under investigation in a phase II trial (as of April 29, 2019) against hepatitis B (NCT02751996). Other studies have also identified these receptors as potent immunotherapeutic targets in pancreatic and colorectal cancers.^{147,148}

The therapeutic use of RLR agonists is emerging as a novel approach in the fight against cancer. RLR signaling enhances tumor cell death by pyroptosis or intrinsic or extrinsic apoptosis.¹⁴⁶ Multifunctional RLR agonists eliminate the tumor cells via multiple pathways and therefore act as multimodal weapons for the effective treatment of cancer.

11 | RECENT ADVANCES IN THE UNDERSTANDING OF THE UNDERLYING MECHANISMS OF RLRS

In recent years outstanding development has been made in the understanding of the RLR signaling mechanism that leads to the antiviral innate immune response. Much has already been studied about the positive and negative regulation of RLR signaling via various regulatory mechanisms, including posttranslational modifications, noncoding RNAs, and various interacting proteins. Identification of infection via RLR recognition of noncoding RNAs is a new technique for the detection of DNA virus infection. This method will be helpful in the detection of the human

papillomavirus and DNA viruses.¹⁴⁹ Cellular noncoding RNAs are known to regulate the transcription of RLRs as well as the signaling transduction of these receptors.¹⁴⁹ Recently, two miRNA, miR-122 and miR-3570, have been reported to regulate the MAVS expression,^{150,151} while pol-miR-731 and miR-145-5p regulate the expression of IRF7 and MDA5 respectively.^{152,153} Many NOD-like receptors have also been identified, which regulate the RLR signaling. The NLR family CARD domain containing 5 (NLRC5) competes with MAVS to hinder the interaction of CARD with RIG-I and MDA5, which results in the negative regulation of IFN- β expression.¹⁵⁴ NLR family pyrin domain containing 12 (NLRP12) interacts with TRIM25 to inhibit the RIG-I-mediated signaling. NLR family pyrin domain containing 11 (NLRP11) is known to negatively regulate RLR signaling via interaction with TRAF3.¹⁵⁵ RLR signaling is regulated by interacting with host protein and endogenous RNAs, that are responsible for post translational modifications and stress responses. Stress granules produced under stress conditions such as viral infections are involved in the upregulation of MAVS signaling by providing a platform for RLRs to sense viral RNAs.¹⁵⁶

The cytokines produced in response to RLR-mediated signaling are involved in antiviral immunity, while the dysregulation of RLR signaling leads to autoinflammatory and autoimmunity diseases. RLR signaling might also be responsible for the abnormal expression of cytokines during SARS-CoV infection.⁹⁰ IFNs have been used to treat many infectious diseases due to their antiviral effects. For the treatment of COVID-19, IFN therapy has been proposed. Some of the important interactions between RLRs and coronaviruses have been studied recently. As IFN expression is important for the host to initiate the antiviral immune response, viral proteins abolish this system by suppressing the RLR signaling. Targeting viral proteins, such as protein 4a in the Middle East respiratory syndrome virus, could be a potential therapeutic strategy.^{6,7} Similarly, other viral proteins also interact with the host proteins, such as the N protein of SARS-CoV that binds to TRIM25 resulting in the ubiquitination and inhibition of RIG-I.¹⁵⁷ Another SARS-CoV protein encoded by the ORF-9b gene blocks IFN production by binding to the translocase of outer mitochondrial membrane 70 (TOM70) (a mitochondrial protein crucial for antiviral signaling through MAVS).¹⁵⁸ SARS-CoV ORF-3b has been reported as an antagonist of IFN induction but the exact mechanism is unknown.¹⁵⁹ The membrane protein of SARS-CoV-2 interacts with RLRs and MAVS to antagonize the IFN production by restraining the formation of RLR/MAVS-mediated TRAF3/TBK-1 signaling complex.^{90,160,161} The detailed investigations of these interacting proteins may unveil potential therapeutic targets for viral diseases. In addition, the recent developments in the understanding of the identification of RNA/DNA ligands by RLRs can be used for the development of RLR-based therapies, such as the development of novel agonists and antagonists for cancers, infectious diseases, and autoimmune diseases.

12 | OUTLOOK

The homeostatic function of RLRs is indispensable for the control of viral infections via the induction of the innate and adaptive antiviral immune responses. However, unchecked inflammatory pathways may lead to fatal consequences by inducing a cytokine storm. Thus, even though the RLR pathways mediate a healthy immune response, their stimulation may lead to adverse effects. Hence, a precisely monitored immune response should be mediated through intervention of RLR-targeting immunotherapies.

Even though researchers have accumulated considerable knowledge to validate RLRs as the key factors orchestrating the antiviral immune signaling, further structural insights are needed to elucidate the complex regulatory functions of RLRs. Recent advancements in the structural studies on various domains of RLRs have improved our understanding of their virus recognition mechanism. Putting together the structural pieces of the RLR puzzle, researchers have greatly expanded the knowledge about the ligand recognition as well as the viral evasion from the RLR-mediated immune responses. Furthermore, this knowledge gives us a detailed picture of the tight regulation of RLRs in viral infections, especially during the current COVID-19 pandemic. This information combined with advanced drug discovery and machine learning methods can aid the development of efficient drugs against RLR-associated diseases.

Immune subversion strategies adopted by several viruses help them to spread infections despite the use of immunotherapies. These immune-target-masking strategies of viruses must be considered while designing novel RLR-based therapeutics to ensure the best clinical outcomes. The crosstalk between the innate and adaptive immune systems can be enhanced by using the innate immune system targeting therapies. Additionally, these treatments may have the potential to ensure long-term immunity in the patients against viruses by acting as immune adjuvants. Therefore, harnessing this immense therapeutic potential of RLRs holds promise for the development of pioneering next-generation vaccine adjuvants, which may radically transform the antiviral modalities.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Maria Batool and Sangdun Choi participated in the research design. Maria Batool performed the data analysis. Maria Batool, Moon Suk Kim, and Sangdun Choi wrote or contributed to the writing of the manuscript.

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REFERENCES

1. McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. *Nat Rev Immunol*. 2015;15(2):87-103.
2. Li Q, Guan X, Wu P, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med*. 2020;382:1199-1207.
3. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727-733.
4. Li G, Fan Y, Lai Y, et al. Coronavirus infections and immune responses. *J Med Virol*. 2020;92(4):424-432.
5. Deng X, Hackbart M, Mettelman RC, et al. Coronavirus nonstructural protein 15 mediates evasion of dsRNA sensors and limits apoptosis in macrophages. *Proc Natl Acad Sci USA*. 2017;114(21):E4251-E4260.
6. Durai P, Batool M, Shah M, Choi S. Middle East respiratory syndrome coronavirus: transmission, virology and therapeutic targeting to aid in outbreak control. *Exp Mol Med*. 2015;47:e181.
7. Batool M, Shah M, Patra MC, Yesudhas D, Choi S. Structural insights into the Middle East respiratory syndrome coronavirus 4a protein and its dsRNA binding mechanism. *Sci Rep*. 2017;7(1):11362.
8. Lei X, Dong X, Ma R, et al. Activation and evasion of type I interferon responses by SARS-CoV-2. *Nature Commun*. 2020;11(1):3810.
9. Metzger RN, Krug AB, Eisenacher K. Enteric virome sensing—its role in intestinal homeostasis and immunity. *Viruses*. 2018;10(4):146.
10. Loo YM, Gale M. Jr. Immune signaling by RIG-I-like receptors. *Immunity*. 2011;34(5):680-692.
11. Jacobs JL, Coyne CB. Mechanisms of MAVS regulation at the mitochondrial membrane. *J Mol Biol*. 2013;425(24):5009-5019.
12. Reikine S, Nguyen JB, Modis Y. Pattern recognition and signaling mechanisms of RIG-I and MDA5. *Front Immunol*. 2014;5:342.
13. Yong HY, Luo D. RIG-I-like receptors as novel targets for pan-antivirals and vaccine adjuvants against emerging and re-emerging viral infections. *Front Immunol*. 2018;9:1379.
14. Kasumba DM, Grandvaux N. Therapeutic targeting of RIG-I and MDA5 might not lead to the same Rome. *Trends Pharmacol Sci*. 2019;40(2):116-127.
15. Takahasi K, Kumeta H, Tsuduki N, et al. Solution structures of cytosolic RNA sensor MDA5 and LGP2 C-terminal domains: identification of the RNA recognition loop in RIG-I-like receptors. *J Biol Chem*. 2009;284(26):17465-17474.

16. Ferrao R, Wu H. Helical assembly in the death domain (DD) superfamily. *Curr Opin Struct Biol.* 2012;22(2):241-247.
17. Yoneyama M, Kikuchi M, Natsukawa T, et al. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol.* 2004;5(7):730-737.
18. Cui S, Eisenächer K, Kirchhofer A, et al. The C-terminal regulatory domain is the RNA 5'-triphosphate sensor of RIG-I. *Mol Cell.* 2008;29(2):169-179.
19. Pippig DA, Hellmuth JC, Cui S, et al. The regulatory domain of the RIG-I family ATPase LGP2 senses double-stranded RNA. *Nucleic Acids Res.* 2009;37(6):2014-2025.
20. Bruns AM, Pollpeter D, Hadizadeh N, Myong S, Marko JF, Horvath CM. ATP hydrolysis enhances RNA recognition and antiviral signal transduction by the innate immune sensor, laboratory of genetics and physiology 2 (LGP2). *J Biol Chem.* 2013;288(2):938-946.
21. Satoh T, Kato H, Kumagai Y, et al. LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. *Proc Natl Acad Sci USA.* 2010;107(4):1512-1517.
22. Venkataraman T, Valdes M, Elsby R, et al. Loss of DExD/H box RNA helicase LGP2 manifests disparate antiviral responses. *J Immunol.* 2007;178(10):6444-6455.
23. Rothenfusser S, Goutagny N, DiPerna G, et al. The RNA helicase Lgp2 inhibits TLR-independent sensing of viral replication by retinoic acid-inducible gene-I. *J Immunol.* 2005;175(8):5260-5268.
24. Chang M, Collet B, Nie P, et al. Expression and functional characterization of the RIG-I-like receptors MDA5 and LGP2 in Rainbow trout (*Oncorhynchus mykiss*). *J Virol.* 2011;85(16):8403-8412.
25. Kato H, Takeuchi O, Sato S, et al. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature.* 2006;441(7089):101-105.
26. Loo YM, Fornek J, Crochet N, et al. Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity. *J Virol.* 2008;82(1):335-345.
27. Chiang JJ, Davis ME, Gack MU. Regulation of RIG-I-like receptor signaling by host and viral proteins. *Cytokine Growth Factor Rev.* 2014;25(5):491-505.
28. Hou F, Sun L, Zheng H, Skaug B, Jiang QX, Chen ZJ. MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. *Cell.* 2011;146(3):448-461.
29. Seth RB, Sun L, Ea CK, Chen ZJ. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. *Cell.* 2005;122(5):669-682.
30. Dixit E, Boulant S, Zhang Y, et al. Peroxisomes are signaling platforms for antiviral innate immunity. *Cell.* 2010;141(4):668-681.
31. Liu Y, Olagnier D, Lin R. Host and viral modulation of RIG-I-mediated antiviral immunity. *Front Immunol.* 2016;7:662.
32. Panne D. The enhanceosome. *Curr Opin Struct Biol.* 2008;18(2):236-242.
33. West AP, Shadel GS, Ghosh S. Mitochondria in innate immune responses. *Nat Rev Immunol.* 2011;11(6):389-402.
34. Mukherjee K, Korithoski B, Kolaczkowski B. Ancient origins of vertebrate-specific innate antiviral immunity. *Mol Biol Evol.* 2014;31(1):140-153.
35. Luo D, Kohlway A, Pyle AM. Duplex RNA activated ATPases (DRAs): platforms for RNA sensing, signaling and processing. *RNA Biol.* 2013;10(1):111-120.
36. Luo D, Ding SC, Vela A, Kohlway A, Lindenbach BD, Pyle AM. Structural insights into RNA recognition by RIG-I. *Cell.* 2011;147(2):409-422.
37. Kolakofsky D, Kowalinski E, Cusack S. A structure-based model of RIG-I activation. *RNA.* 2012;18(12):2118-2127.
38. Takahasi K, Yoneyama M, Nishihori T, et al. Nonself RNA-sensing mechanism of RIG-I helicase and activation of antiviral immune responses. *Mol Cell.* 2008;29(4):428-440.
39. Wu B, Peisley A, Richards C, et al. Structural basis for dsRNA recognition, filament formation, and antiviral signal activation by MDA5. *Cell.* 2013;152(1-2):276-289.
40. Berke IC, Modis Y. MDA5 cooperatively forms dimers and ATP-sensitive filaments upon binding double-stranded RNA. *EMBO J.* 2012;31(7):1714-1726.
41. Peisley A, Lin C, Wu B, et al. Cooperative assembly and dynamic disassembly of MDA5 filaments for viral dsRNA recognition. *Proc Natl Acad Sci USA.* 2011;108(52):21010-21015.
42. Peisley A, Wu B, Yao H, Walz T, Hur S. RIG-I forms signaling-competent filaments in an ATP-dependent, ubiquitin-independent manner. *Mol Cell.* 2013;51(5):573-583.
43. Ferrage F, Dutta K, Nistal-Villán E, et al. Structure and dynamics of the second CARD of human RIG-I provide mechanistic insights into regulation of RIG-I activation. *Structure.* 2012;20(12):2048-2061.
44. Gitlin L, Barchet W, Gilfillan S, et al. Essential role of mda-5 in type I IFN responses to polyribonucleic:polyribocytidyl acid and encephalomyocarditis picornavirus. *Proc Natl Acad Sci USA.* 2006;103(22):8459-8464.
45. Lee HC, Chathuranga K, Lee JS. Intracellular sensing of viral genomes and viral evasion. *Exp Mol Med.* 2019;51(12):1-13.

46. Chiang C, Liu G, Gack MU. Viral evasion of RIG-I-like receptor-mediated immunity through dysregulation of ubiquitination and ISGylation. *Viruses*. 2021;13(2):182.
47. Ablasser A, Bauernfeind F, Hartmann G, Latz E, Fitzgerald KA, Hornung V. RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat Immunol*. 2009;10(10):1065-1072.
48. Saito T, Hirai R, Loo YM, et al. Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. *Proc Natl Acad Sci USA*. 2007;104(2):582-587.
49. Roth-Cross JK, Bender SJ, Weiss SR. Murine coronavirus mouse hepatitis virus is recognized by MDA5 and induces type I interferon in brain macrophages/microglia. *J Virol*. 2008;82(20):9829-9838.
50. Zhou S, Cerny AM, Zacharia A, Fitzgerald KA, Kurt-Jones EA, Finberg RW. Induction and inhibition of type I interferon responses by distinct components of lymphocytic choriomeningitis virus. *J Virol*. 2010;84(18):9452-9462.
51. Plumet S, Herschke F, Bourhis JM, Valentini H, Longhi S, Gerlier D. Cytosolic 5'-triphosphate ended viral leader transcript of measles virus as activator of the RIG-I-mediated interferon response. *PLOS One*. 2007;2(3):e279.
52. Fredericksen BL, Keller BC, Fornek J, Katze MG, Gale M Jr. Establishment and maintenance of the innate antiviral response to West Nile Virus involves both RIG-I and MDA5 signaling through IPS-1. *J Virol*. 2008;82(2):609-616.
53. Lemos de Matos A, McFadden G, Esteves PJ. Evolution of viral sensing RIG-I-like receptor genes in Leporidae genera Oryctolagus, Sylvilagus, and Lepus. *Immunogenetics*. 2014;66(1):43-52.
54. Hornung V, Ellegast J, Kim S, et al. 5'-Triphosphate RNA is the ligand for RIG-I. *Science*. 2006;314(5801):994-997.
55. McCartney SA, Thackray LB, Gitlin L, Gilfillan S, Virgin HW, Colonna M. MDA-5 recognition of a murine norovirus. *PLOS Pathog*. 2008;4(7):e1000108.
56. Goubaud D, Deddouche S, Reis e Sousa C. Cytosolic sensing of viruses. *Immunity*. 2013;38(5):855-869.
57. Schlee M. Master sensors of pathogenic RNA—RIG-I like receptors. *Immunobiology*. 2013;218(11):1322-1335.
58. Züst R, Cervantes-Barragan L, Habjan M, et al. Ribose 2'-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. *Nat Immunol*. 2011;12(2):137-143.
59. Gack MU. Mechanisms of RIG-I-like receptor activation and manipulation by viral pathogens. *J Virol*. 2014;88(10):5213-5216.
60. Brisse M, Ly H. Comparative structure and function analysis of the RIG-I-like receptors: RIG-I and MDA5. *Front Immunol*. 2019;10:1586.
61. Zerbe CM, Mousher DJ, Cole JL. Oligomerization of RIG-I and MDA5 2CARD domains. *Protein Sci*. 2020;29(2):521-526.
62. Pichlmair A, Schulz O, Tan CP, et al. Activation of MDA5 requires higher-order RNA structures generated during virus infection. *J Virol*. 2009;83(20):10761-10769.
63. Kato H, Takeuchi O, Mikamo-Satoh E, et al. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. *J Exp Med*. 2008;205(7):1601-1610.
64. Schuberth-Wagner C, Ludwig J, Bruder AK, et al. A conserved histidine in the RNA sensor RIG-I controls immune tolerance to N1-2' O-methylated self RNA. *Immunity*. 2015;43(1):41-51.
65. Hackbart M, Deng X, Baker SC. Coronavirus endoribonuclease targets viral polyuridine sequences to evade activating host sensors. *Proc Natl Acad Sci USA*. 2020;117(14):8094-8103.
66. Liu G, Lee JH, Parker ZM, et al. ISG15-dependent activation of the sensor MDA5 is antagonized by the SARS-CoV-2 papain-like protease to evade host innate immunity. *Nat Microbiol*. 2021;6(4):467-478.
67. Berke IC, Yu X, Modis Y, Egelman EH. MDA5 assembles into a polar helical filament on dsRNA. *Proc Natl Acad Sci USA*. 2012;109(45):18437-18441.
68. Takashima K, Oshiumi H, Takaki H, Matsumoto M, Seya T. RIOK3-mediated phosphorylation of MDA5 interferes with its assembly and attenuates the innate immune response. *Cell Rep*. 2015;11(2):192-200.
69. Bruns AM, Horvath CM. Activation of RIG-I-like receptor signal transduction. *Crit Rev Biochem Mol Biol*. 2012;47(2):194-206.
70. Komuro A, Horvath CM. RNA- and virus-independent inhibition of antiviral signaling by RNA helicase LGP2. *J Virol*. 2006;80(24):12332-12342.
71. Childs KS, Randall RE, Goodbourn S. LGP2 plays a critical role in sensitizing mda-5 to activation by double-stranded RNA. *PLOS One*. 2013;8(5):e64202.
72. Bruns AM, Leser GP, Lamb RA, Horvath CM. The innate immune sensor LGP2 activates antiviral signaling by regulating MDA5-RNA interaction and filament assembly. *Mol Cell*. 2014;55(5):771-781.
73. Uzri D, Gehrke L. Nucleotide sequences and modifications that determine RIG-I/RNA binding and signaling activities. *J Virol*. 2009;83(9):4174-4184.
74. Goubaud D, Schlee M, Deddouche S, et al. Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5'-diphosphates. *Nature*. 2014;514(7522):372-375.

75. Saito T, Owen DM, Jiang F, Marcotrigiano J, Gale M Jr. Innate immunity induced by composition-dependent RIG-I recognition of hepatitis C virus RNA. *Nature*. 2008;454(7203):523-527.
76. Kowalinski E, Lunardi T, McCarthy AA, et al. Structural basis for the activation of innate immune pattern-recognition receptor RIG-I by viral RNA. *Cell*. 2011;147(2):423-435.
77. Chiu YH, Macmillan JB, Chen ZJ. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell*. 2009;138(3):576-591.
78. Malathi K, Dong B, Gale M Jr., Silverman RH. Small self-RNA generated by RNase L amplifies antiviral innate immunity. *Nature*. 2007;448(7155):816-819.
79. Vela A, Fedorova O, Ding SC, Pyle AM. The thermodynamic basis for viral RNA detection by the RIG-I innate immune sensor. *J Biol Chem*. 2012;287(51):42564-42573.
80. Jiang F, Ramanathan A, Miller MT, et al. Structural basis of RNA recognition and activation by innate immune receptor RIG-I. *Nature*. 2011;479(7373):423-427.
81. Huang YH, Liu XY, Du XX, Jiang ZF, Su XD. The structural basis for the sensing and binding of cyclic di-GMP by STING. *Nat Struct Mol Biol*. 2012;19(7):728-730.
82. Feng Q, Hato SV, Langereis MA, et al. MDA5 detects the double-stranded RNA replicative form in picornavirus-infected cells. *Cell Rep*. 2012;2(5):1187-1196.
83. Triantafilou K, Vakakis E, Kar S, Richer E, Evans GL, Triantafilou M. Visualisation of direct interaction of MDA5 and the dsRNA replicative intermediate form of positive strand RNA viruses. *J Cell Sci*. 2012;125(Pt 20):4761-4769.
84. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell*. 2010;140(6):805-820.
85. Zeng W, Sun L, Jiang X, et al. Reconstitution of the RIG-I pathway reveals a signaling role of unanchored polyubiquitin chains in innate immunity. *Cell*. 2010;141(2):315-330.
86. Ramos HJ, Gale M Jr. RIG-I like receptors and their signaling crosstalk in the regulation of antiviral immunity. *Curr Opin Virol*. 2011;1(3):167-176.
87. Martin-Vicente M, Medrano LM, Resino S, Garcia-Sastre A, Martinez I. TRIM25 in the regulation of the antiviral innate immunity. *Front Immunol*. 2017;8:1187.
88. Gack MU, Shin YC, Joo CH, et al. TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature*. 2007;446(7138):916-920.
89. Oshiumi H, Miyashita M, Inoue N, Okabe M, Matsumoto M, Seya T. The ubiquitin ligase Riplet is essential for RIG-I-dependent innate immune responses to RNA virus infection. *Cell Host Microbe*. 2010;8(6):496-509.
90. Onomoto K, Onoguchi K, Yoneyama M. Regulation of RIG-I-like receptor-mediated signaling: interaction between host and viral factors. *Cell Mol Immunol*. 2021;18(3):539-555.
91. Zhao C, Jia M, Song H, et al. The E3 ubiquitin ligase TRIM40 attenuates antiviral immune responses by targeting MDA5 and RIG-I. *Cell Rep*. 2017;21(6):1613-1623.
92. Wang W, Jiang M, Liu S, et al. RNF122 suppresses antiviral type I interferon production by targeting RIG-I CARDs to mediate RIG-I degradation. *Proc Natl Acad Sci USA*. 2016;113(34):9581-9586.
93. Arimoto K, Takahashi H, Hishiki T, Konishi H, Fujita T, Shimotohno K. Negative regulation of the RIG-I signaling by the ubiquitin ligase RNF125. *Proc Natl Acad Sci USA*. 2007;104(18):7500-7505.
94. Hayakawa S, Shiratori S, Yamato H, et al. ZAPS is a potent stimulator of signaling mediated by the RNA helicase RIG-I during antiviral responses. *Nat Immunol*. 2011;12(1):37-44.
95. Friedman CS, O'Donnell MA, Legarda-Addison D, et al. The tumour suppressor CYLD is a negative regulator of RIG-I-mediated antiviral response. *EMBO Rep*. 2008;9(9):930-936.
96. Kayagaki N, Phung Q, Chan S, et al. DUBA: a deubiquitinase that regulates type I interferon production. *Science*. 2007;318(5856):1628-1632.
97. Jia Y, Song T, Wei C, et al. Negative regulation of MAVS-mediated innate immune response by PSMA7. *J Immunol*. 2009;183(7):4241-4248.
98. You F, Sun H, Zhou X, et al. PCBP2 mediates degradation of the adaptor MAVS via the HECT ubiquitin ligase AIP4. *Nat Immunol*. 2009;10(12):1300-1308.
99. Jounai N, Takeshita F, Kobiyama K, et al. The Atg5 Atg12 conjugate associates with innate antiviral immune responses. *Proc Natl Acad Sci USA*. 2007;104(35):14050-14055.
100. Diao F, Li S, Tian Y, et al. Negative regulation of MDA5- but not RIG-I-mediated innate antiviral signaling by the dihydroxyacetone kinase. *Proc Natl Acad Sci USA*. 2007;104(28):11706-11711.
101. Underhill DM. Collaboration between the innate immune receptors dectin-1, TLRs, and Nods. *Immunol Rev*. 2007;219:75-87.
102. Liu Q, Ding JL. The molecular mechanisms of TLR-signaling cooperation in cytokine regulation. *Immunol Cell Biol*. 2016;94(6):538-542.
103. Trinchieri G, Sher A. Cooperation of toll-like receptor signals in innate immune defence. *Nat Rev Immunol*. 2007;7(3):179-190.

104. Negishi H, Yanai H, Nakajima A, et al. Cross-interference of RLR and TLR signaling pathways modulates antibacterial T cell responses. *Nat Immunol*. 2012;13(7):659-666.
105. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell*. 2006;124(4):783-801.
106. Delaloye J, Roger T, Steiner-Tardivel QG, et al. Innate immune sensing of modified vaccinia virus Ankara (MVA) is mediated by TLR2-TLR6, MDA-5 and the NALP3 inflammasome. *PLOS Pathog*. 2009;5(6):e1000480.
107. Slater L, Bartlett NW, Haas JJ, et al. Co-ordinated role of TLR3, RIG-I and MDA5 in the innate response to rhinovirus in bronchial epithelium. *PLOS Pathog*. 2010;6(11):e1001178.
108. Suthar MS, Ma DY, Thomas S, et al. IPS-1 is essential for the control of West Nile virus infection and immunity. *PLOS Pathog*. 2010;6(2):e1000757.
109. Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity*. 2011;34(5):637-650.
110. Poeck H, Bscheider M, Gross O, et al. Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. *Nat Immunol*. 2010;11(1):63-69.
111. Rintahaka J, Wiik D, Kovanen PE, Alenius H, Matikainen S. Cytosolic antiviral RNA recognition pathway activates caspases 1 and 3. *J Immunol*. 2008;180(3):1749-1757.
112. Kanneganti TD. Central roles of NLRs and inflammasomes in viral infection. *Nat Rev Immunol*. 2010;10(10):688-698.
113. Kato H, Fujita T. RIG-I-like receptors and autoimmune diseases. *Curr Opin Immunol*. 2015;37:40-45.
114. Varzari A, Bruch K, Deyneko IV, Chan A, Epplen JT, Hoffjan S. Analysis of polymorphisms in RIG-I-like receptor genes in German multiple sclerosis patients. *J Neuroimmunol*. 2014;277(1-2):140-144.
115. Dou Q, Peng Y, Zhou B, et al. Association of innate immune IFIH1 gene polymorphisms with dilated cardiomyopathy in a Chinese population. *Immunol Invest*. 2014;43(7):627-637.
116. Nejentsev S, Walker N, Riches D, Egholm M, Todd JA. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. *Science*. 2009;324(5925):387-389.
117. Funabiki M, Kato H, Miyachi Y, et al. Autoimmune disorders associated with gain of function of the intracellular sensor MDA5. *Immunity*. 2014;40(2):199-212.
118. Jaidane H, Hober D. Role of coxsackievirus B4 in the pathogenesis of type 1 diabetes. *Diabetes Metab*. 2008;34(6 Pt 1):537-548.
119. Asadikaram G, Meimand HAE, Noroozi S, Sanjari M, Zainodini N, Arababadi MK. The effect of IFN-beta 1a on expression of MDA5 and RIG-1 in multiple sclerosis patients. *Biotechnol Appl Biochem*. 2020;68:267-271.
120. Rawling DC, Jagdmann GE Jr, Potapova O, Pyle AM. Small-molecule antagonists of the RIG-I innate immune receptor. *ACS Chem Biol*. 2020;15(2):311-317.
121. Batool M, Ahmad B, Choi S. A structure-based drug discovery paradigm. *Int J Mol Sci*. 2019;20(11):2783.
122. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674.
123. Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat Rev Cancer*. 2013;13(11):759-771.
124. Koebel CM, Vermi W, Swann JB, et al. Adaptive immunity maintains occult cancer in an equilibrium state. *Nature*. 2007;450(7171):903-907.
125. Kroemer G, Galluzzi L, Kepp O, Zitvogel L. Immunogenic cell death in cancer therapy. *Annu Rev Immunol*. 2013;31:51-72.
126. Wu Y, Wu X, Wu L, Wang X, Liu Z. The anticancer functions of RIG-I-like receptors, RIG-I and MDA5, and their applications in cancer therapy. *Transl Res*. 2017;190:51-60.
127. Yu CY, Chiang RL, Chang TH, Liao CL, Lin YL. The interferon stimulator mitochondrial antiviral signaling protein facilitates cell death by disrupting the mitochondrial membrane potential and by activating caspases. *J Virol*. 2010;84(5):2421-2431.
128. Chattopadhyay S, Kuzmanovic T, Zhang Y, Wetzel JL, Sen GC. Ubiquitination of the transcription factor IRF-3 activates RIPA, the apoptotic pathway that protects mice from viral pathogenesis. *Immunity*. 2016;44(5):1151-1161.
129. Duewell P, Beller E, Kirchleitner SV, et al. Targeted activation of melanoma differentiation-associated protein 5 (MDA5) for immunotherapy of pancreatic carcinoma. *Oncimmunology*. 2015;4(10):e1029698.
130. Yu X, Wang H, Li X, et al. Activation of the MDA-5-IPS-1 viral sensing pathway induces cancer cell death and type I IFN-dependent antitumor immunity. *Cancer Res*. 2016;76(8):2166-2176.
131. Besch R, Poeck H, Hohenauer T, et al. Proapoptotic signaling induced by RIG-I and MDA-5 results in type I interferon-independent apoptosis in human melanoma cells. *J Clin Invest*. 2009;119(8):2399-2411.
132. Akasaka T, Tsujii M, Kondo J, et al. 5FU resistance abrogates the amplified cytotoxic effects induced by inhibiting checkpoint kinase 1 in p53mutated colon cancer cells. *Int J Oncol*. 2015;46(1):63-70.
133. Gesuete R, Christensen SN, Bahjat FR, et al. Cytosolic receptor melanoma differentiation-associated protein 5 mediates preconditioning-induced neuroprotection against cerebral ischemic injury. *Stroke*. 2016;47(1):262-266.

134. Qu J, Hou Z, Han Q, Zhang C, Tian Z, Zhang J. Poly(I:C) exhibits an anti-cancer effect in human gastric adenocarcinoma cells which is dependent on RLRs. *Int Immunopharmacol*. 2013;17(3):814-820.
135. Salazar AM, Levy HB, Ondra S, et al. Long-term treatment of malignant gliomas with intramuscularly administered polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose: an open pilot study. *Neurosurgery*. 1996;38(6):1096-1103.
136. Rosenfeld MR, Chamberlain MC, Grossman SA, et al. A multi-institution phase II study of poly-ICLC and radiotherapy with concurrent and adjuvant temozolomide in adults with newly diagnosed glioblastoma. *Neuro Oncol*. 2010;12(10):1071-1077.
137. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*. 2009;10(5):459-466.
138. Grossman SA, Ye X, Piantadosi S, et al. Survival of patients with newly diagnosed glioblastoma treated with radiation and temozolomide in research studies in the United States. *Clin Cancer Res*. 2010;16(8):2443-2449.
139. Mehrotra S, Britten CD, Chin S, et al. Vaccination with poly(IC:LC) and peptide-pulsed autologous dendritic cells in patients with pancreatic cancer. *J Hematol Oncol*. 2017;10(1):82.
140. Dillon PM, Petroni GR, Smolkin ME, et al. A pilot study of the immunogenicity of a 9-peptide breast cancer vaccine plus poly-ICLC in early stage breast cancer. *J Immunother Cancer*. 2017;5(1):92.
141. Rapoport AP, Aqui NA, Stadtmauer EA, et al. Combination immunotherapy after ASCT for multiple myeloma using MAGE-A3/Poly-ICLC immunizations followed by adoptive transfer of vaccine-primed and costimulated autologous T cells. *Clin Cancer Res*. 2014;20(5):1355-1365.
142. Tsuji T, Sabbatini P, Jungbluth AA, et al. Effect of montanide and poly-ICLC adjuvant on human self/tumor antigen-specific CD4+ T cells in phase I overlapping long peptide vaccine trial. *Cancer Immunol Res*. 2013;1(5):340-350.
143. Okada H, Butterfield LH, Hamilton RL, et al. Induction of robust type-I CD8+ T-cell responses in WHO grade 2 low-grade glioma patients receiving peptide-based vaccines in combination with poly-ICLC. *Clin Cancer Res*. 2015;21(2):286-294.
144. Hu J, He Y, Yan M, et al. Dose dependent activation of retinoic acid-inducible gene-I promotes both proliferation and apoptosis signals in human head and neck squamous cell carcinoma. *PLOS One*. 2013;8(3):e58273.
145. Poeck H, Besch R, Maihoefer C, et al. 5'-Triphosphate-siRNA: turning gene silencing and Rig-I activation against melanoma. *Nat Med*. 2008;14(11):1256-1263.
146. Elion DL, Cook RS. Harnessing RIG-I and intrinsic immunity in the tumor microenvironment for therapeutic cancer treatment. *Oncotarget*. 2018;9(48):29007-29017.
147. Jones M, Cunningham ME, Wing P, et al. SB 9200, a novel agonist of innate immunity, shows potent antiviral activity against resistant HCV variants. *J Med Virol*. 2017;89(9):1620-1628.
148. Schnurr M, Duewell P. Induction of immunogenic cell death by targeting RIG-I-like helicases in pancreatic cancer. *Oncoimmunology*. 2014;3(9):e955687.
149. Rehwinkel J, Gack MU. RIG-I-like receptors: their regulation and roles in RNA sensing. *Nat Rev Immunol*. 2020;20(9):537-551.
150. Chu Q, Xu T, Zheng W, Chang R, Zhang L. Long noncoding RNA MARL regulates antiviral responses through suppression miR-122-dependent MAVS downregulation in lower vertebrates. *PLOS Pathog*. 2020;16(7):e1008670.
151. Xu T, Chu Q, Cui J, Bi D. Inducible microRNA-3570 feedback inhibits the RIG-I-dependent innate immune response to rhabdovirus in teleost fish by targeting MAVS/IPS-1. *J Virol*. 2018;92(2):e01594-17.
152. Han J, Sun Y, Song W, Xu T. microRNA-145 regulates the RLR signaling pathway in miiuy croaker after poly(I:C) stimulation via targeting MDA5. *Dev Comp Immunol*. 2017;68:79-86.
153. Zhang BC, Zhou ZJ, Sun L. pol-miR-731, a teleost miRNA upregulated by megalocytivirus, negatively regulates virus-induced type I interferon response, apoptosis, and cell cycle arrest. *Sci Rep*. 2016;6:28354.
154. Cui J, Zhu L, Xia X, et al. NLRC5 negatively regulates the NF-kappaB and type I interferon signaling pathways. *Cell*. 2010;141(3):483-496.
155. Qin Y, Su Z, Wu Y, et al. NLRP11 disrupts MAVS signalosome to inhibit type I interferon signaling and virus-induced apoptosis. *EMBO Rep*. 2017;18(12):2160-2171.
156. Onomoto K, Jogi M, Yoo JS, et al. Critical role of an antiviral stress granule containing RIG-I and PKR in viral detection and innate immunity. *PLOS One*. 2012;7(8):e43031.
157. Hu Y, Li W, Gao T, et al. The severe acute respiratory syndrome coronavirus nucleocapsid inhibits type I interferon production by interfering with TRIM25-mediated RIG-I ubiquitination. *J Virol*. 2017;91(8):e02143-16.
158. Jiang HW, Zhang HN, Meng QF, et al. SARS-CoV-2 Orf9b suppresses type I interferon responses by targeting TOM70. *Cell Mol Immunol*. 2020;17(9):998-1000.
159. Konno Y, Kimura I, Uriu K, et al. SARS-CoV-2 ORF3b is a potent interferon antagonist whose activity is increased by a naturally occurring elongation variant. *Cell Rep*. 2020;32(12):108185.

160. Fu YZ, Wang SY, Zheng ZQ, et al. SARS-CoV-2 membrane glycoprotein M antagonizes the MAVS-mediated innate antiviral response. *Cell Mol Immunol*. 2021;18(3):613-620.
161. Zheng Y, Zhuang MW, Han L, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) membrane (M) protein inhibits type I and III interferon production by targeting RIG-I/MDA-5 signaling. *Signal Transduct Target Ther*. 2020;5(1):299.

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