Friend or foe: RIG- I like receptors and diseases

Jie Song [a,b,c,1], Muyuan Li [a,b,c,d,1], Caiyan Li [a,b,c], Ke Liu [a,b,c], Yaxi Zhu [a,b,c,*], Huali Zhang [a,b,c,*]

[a] *Department of Rheumatology, Xiangya Hospital, Central South University, Changsha City, Hunan Province, China*
[b] *Department of Pathophysiology, Xiangya School of Medicine, Central South University, Changsha City, Hunan Province, China*
[c] *Sepsis Translational Medicine Key Lab of Hunan Province, Central South University, Changsha City, Hunan Province, China*
[d] *National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Changsha City, Hunan Province, China*

ABSTRACT

Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), which are pivotal sensors of RNA virus invasions, mediate the transcriptional induction of genes encoding type I interferons (IFNs) and proinflammatory cytokines, successfully establishing host antiviral immune response. A few excellent reviews have elaborated on the structural biology of RLRs and the antiviral mechanisms of RLR activation. In this review, we give a basic understanding of RLR biology and summarize recent findings of how RLR signaling cascade is strictly controlled by host regulatory mechanisms, which include RLR-interacting proteins, post-translational modifications and microRNAs (miRNAs). Furthermore, we pay particular attention to the relationship between RLRs and diseases, especially how RLRs participate in SARS-CoV-2, malaria or bacterial infections, how single-nucleotide polymorphisms (SNPs) or mutations in RLRs and antibodies against RLRs lead to autoinflammatory diseases and autoimmune diseases, and how RLRs are involved in anti-tumor immunity. These findings will provide insights and guidance for antiviral and immunomodulatory therapies targeting RLRs.

1. Introduction

RNA viruses compose most of the viruses related to human infectious diseases which brings forward significant public health concerns. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an RNA virus which can cause coronavirus disease 2019 (COVID-19) and has become a primary healthcare threat in recent years. The characteristic feature of SARS-CoV-2 infection is the inadequate production or low response of type-I IFNs, a class of cytokines essential for antiviral defense. Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), a typical family of cellular receptors, serve as critical cytosolic sensors of virally derived ribonucleic acids to halt viral invasion. Upon activation, these sensors participate in the composition of signaling cascades that lead to the transcription of genes encoding type I IFNs, therefore building the first line of protection against viral infections. Accordingly, viruses have evolved strategies to withstand innate defenses and promote replication, which results in virus-host conflict and causes pattern

recognition receptor (PRR)-induced systemic inflammatory disease [1].

Based on decades of overexpression or knockout studies of RLRs, it is well known that RLRs, especially RIG-I and melanoma differentiation-associated protein 5 (MDA5), are essential for antiviral defense and type I IFN production during virus invasion. Apart from this classical role, it is also found that RLRs are related to autoinflammatory and autoimmune diseases and cancers. Consequently, this made us question whether RIG-I like receptor acts as a friend or foe in diseases.

2. Basic biology of RLRs

2.1. Characterization and structure of RLRs

RLRs are extensively expressed in tissues and an abundance of cell types, including immune and nonimmune cells such as cancer cells and central nervous system cells. In contrast, they pivotally function as innate immune initiators in epithelial and myeloid cells [2,3]. As one of

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the PRRs which detect pathogens and allow eukaryotic cells to respond to infections rapidly, RLRs detect replicating viruses in the cytoplasm, particularly at the early phase of viral infection. RLRs encompass three homologous members, including RIG-I (or DEAD box polypeptide 58, DDX58), Melanoma differentiation-associated protein 5 (MDA5, also known as interferon induced with helicase C domain 1, IFIH1), and laboratory of genetics and physiology 2 (LGP2, or DExH box polypeptide 58, DHX58) [1].

Every RLR has a central conserved DExD/H-box RNA helicase domain for ATP catalysis and activation, and a C-terminal domain (CTD) for RNA binding and unwinding [4–6]. RIG-I and MDA5 have two extra tandem N-terminal caspase activation and recruitment domains (CARDs), mediating the downstream signal transduction and the transcriptional activation of type I IFNs and other immediate antiviral response genes that encode proinflammatory cytokines [7,8]. LGP2, the most mysterious RLR protein, lacks CARDs and therefore cannot convey downstream signaling, but can functionally regulate RIG-I and MDA5 signaling [9,10]. The helicase domain of LGP2 shared 30% homology with RIG-I and 40% with MDA5 [11]. In resting cells, RLRs maintain a low expression level and an auto-repressed conformation via the sequestering of the RNA helicase domain by CARD. Once the virus invades or IFN exposes, the helicase domain is pulled away from CARD, and RLRs then transform into an activated conformation. RLRs levels then rapidly increase to trigger the signal transduction pathways and elicit innate immune defense, which leads to the production of proinflammatory cytokines and an extensively effective antiviral state in the host [12] (Fig. 1).

2.2. Interactions among RLRs

Currently, LGP2 is considered to regulate the function of RIG-I and MDA5 signaling [1,13]. For effective MDA5-mediated antiviral responses against many viruses, functionally activated LGP2 is indispensable [14]. Several studies reported that LGP2$^{-/-}$ mice and LGP2$^{-/-}$ cells showed higher susceptibility and severely impaired IFN responses to certain RNA viruses (e.g., encephalomyocarditis virus) recognized by MDA5 [15,16]. Coordinated action between LGP2 and MDA5 in MDA5-dependent response was found by Annie et al., and they revealed that LGP2 stabilizes the filament assembly of MDA5 on short dsRNA and activates MDA5, amplifying the MDA5-mediated IFN response [17]. Miyamoto et al. also found that the overexpression of dsRNA-binding protein PACT stimulates the activity of the encephalomyocarditis virus (EMCV)-induced IFN-β promoter only when LGP2 and MDA5 are expressed together, rather than when MDA5 is expressed alone, suggesting that LGP2 is pivotal for triggering MDA5-dependent immune response by EMCV [18]. However, Bruns et al. reported that LGP2 is an inhibitor of MDA5-mediated IFN- β production at high concentrations but is an activator at low concentrations [19]. Furthermore, Sanchez et al. performed *In vitro* interaction analyses and discovered that the LGP2 has opposite regulatory effects on MDA5 and RIG-I. Through the

different interactions between LGP2 CTD and PACT, LGP2 specifically promotes MDA5 signaling while suppressing RIG-I signaling [20]. In SARS-CoV-2-infected cells, the separate knockdowns of only LGP2 and MDA5 can both significantly lower IFN-β mRNA level, further suggesting that MDA5 and LGP2 collaboratively detect viral RNA and trigger innate immune responses [21]. However, further analysis is required to fully elucidate the concrete interplay among these three members of RLRs.

2.3. RNA Ligands sensed by RLRs

Although RIG-I and MDA5 have many structural similarities, they detect distinct spectrums of viruses. Generally, RIG-I mainly detects RNA species from plenty of viruses belonging to Flaviviridae, Paramyxoviridae, Orthomyxoviridae, and Rhabdoviridae, whereas MDA5 detects RNA mainly from Picornaviridae [22,23]. RIG-I recognizes most of the currently studied single-stranded (ss) RNA viruses, including all negative or minus (-)-strand RNA viruses and very few positive or plus (+)-strand RNA viruses [16]. In addition, some dsDNA viruses could also be recognized by RLRs because both the positive and negative strands of dsDNA viruses are able to produce dsRNA during transcription [24]. These viral types are summarized in table 1 (Table 1).

Cellular RNA is abundant in host cells, but only virus-derived RNA can initiate the innate immune response, so there must be a molecular identification mechanism for RLRs to distinguish the molecular characteristics of self and non-self RNA. Indeed, there exists sequence-independent RNA-sensing mechanisms of RIG-I and MDA5. Whether the sensor is RIG-I or MDA5 is determined by the length and secondary structure of viral RNA [9]. RIG-I mainly binds ssRNA and 5′ mono-, di-, or tri-phosphorylated low molecular weight dsRNA. At the same time, MDA5 recognizes high molecular weight dsRNA and higher-order RNA structures with a weaker affinity than RIG-I and LGP2 [25,26–30]. More studies have shown that RIG-I recognizes 3′ poly-U/UC tract RNA of hepatitis C virus and 3′ U/A-rich RNAs of influenza virus NS1 gene [31,32]. Polyriboinosinic acid–polyribocytidylic acid is usually denoted as poly(I:C) and is a synthetic mimic of viral dsRNA polymer that simulates viral infection [33]. RIG-I and MDA5 preferentially bind to poly(I: C) smaller than 1 bp and larger than 2 kbp *in vitro*, respectively[10,34]. Additionally, mitochondrial dsRNA (mtdsRNA) is a kind of highly immunogenic RNA which is misprocessed and released into the cytoplasm and accumulates under pathological conditions, such as viral infections. MDA5 has been recently identified as the primary sensor for recognizing cytosolic mtdsRNA through MAVS-mediated downstream type I IFN signaling pathways, which may prime an antiviral immune response [35]. This finding further elucidates the importance of proper processing of self-RNA and the wide variety of ligands sensed by the RLRs family.

2.4. RLR signaling cascade

Functional studies have suggested that RNA-dependent ATP hydrolysis activity plays a critical role in distinguishing non-self RNA from self-RNA using RIG-I and MDA5 [74–76]. Upon sensing non-self RNA molecules, monomers of RIG-I or MDA5 soon form the tetrameric structures through the ATP hydrolysis-dependent interactions of N-terminal CARDs. Then the mitochondrial antiviral-signaling protein (MAVS), an adaptor molecule located on mitochondrial outer membrane or peroxisome or mitochondrial-associated membrane, is recruited via the interplay between RIG-I/MDA5 CARD and MAVS CARD [6,77–79]. MAVS then forms prion-like filaments and builds a higher-order signalosome platform for activating signalling proteins, tumor necrosis factor receptor-associated factor (TRAF), TANK-binding kinase 1 (TBK1) and I-kappa-B-kinase (IKK) kinases [80]. This results in the subsequent activation and nuclear translocation of primary transcription factors interferon regulatory factor 3/7 (IRF3/7) and transcriptional factors nuclear factor kappa-B (NF-κB), culminating in the secretion of type-I
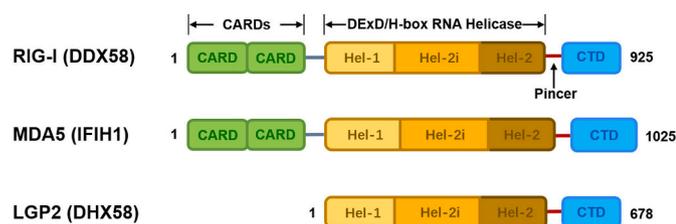

Fig. 1. Schematic representation of RLRs.
Each RLR has a conserved central DExD/H-box RNA helicase domain and a pincer domain that connects the helicase domain to the CTD domain. The helicase domain consists of Hel-1, Hel-2i and Hel-2. RIG-I and MDA5 have two CARD domains essential for the induction of downstream signaling. The amino acid sequence length of each protein is shown.

Table 1
Representative viruses sensed by RLRs following infection.

	Viruses sensed only by RIG-I	Viruses sensed only by MDA5	Viruses sensed by both RIG-I and MDA5
ssRNA (+)	**Caliciviridae** Murine norovirus-1 [36]	**Picornaviridae** Theiler's murine encephalomyocarditis virus [37] Mengo virus [37] Poliovirus [38] Saffold virus [39] **Caliciviridae** Murine norovirus-1 36]	**Picornaviridae** Encephalomyocarditis virus [40] Coxsackie B3 virus [40] Rhinovirus [41] [42] **Flaviviridae** West Nile virus [43] Dengue virus [44] Japanese encephalitis virus [37] Hepatitis C virus [45] [46] [47] **Coronaviridae** mouse hepatitis virus [48] [49] SARS-CoV-2 [50] **Flaviviridae** Zika virus [51] **Togaviridae** Sindbis virus [52]
ssRNA (-)	**Orthomyxoviridae** Influenza A virus [44] Influenza B virus [37] **Paramyxoviridae** Newcastle disease virus [53] Respiratory syncytial virus [44] Nipah [54] human parainfluenza 5 mRNA [55] **Arenaviridae** Lassa [54] Lymphocytic choriomeningitis virus [56] **Bunyaviridae** Rift Valley fever virus [54]	None	**Filoviridae** Ebola [54] [57] **Rhabdoviridae** Rabies virus [58] Vesicular stomatitis virus [59] [60] **Paramyxoviridae** Measles virus [61] [62] human Metapneumovirus [63] **Paramyxoviridae** Sendai Virus [53]
dsRNA	none	None	**Reoviridae** Reovirus [64] Rotavirus [65]
dsDNA	**Herpesviridae** Epstein-Barr virus [66] **Poxviridae** Myxoma virus [67]	**Poxviridae** Vaccina virus [68]	**Herpesviridae** Herpes simplex virus 1 [69] [70] **Hepadnaviridae** Hepatitis B virus [71] [72] [73]

Abbreviations: ssRNA(+): plus-strand RNA; ssRNA(-): minus-strand RNA; dsRNA: double-stranded RNA; dsDNA: double-stranded DNA.

IFNs and proinflammatory cytokines including IL-1β, IL-6, TNF-α, etc., respectively [2,81,82]. Thus, a potent type-I IFN antiviral response gets potently elicited (Fig. 2).

After secreted in an autocrine and paracrine manner, type I IFNs specifically bind to the IFN- α/β receptor (IFNAR), which activates the intracellular Janus kinase-signal transducer and activator of transcription (JAK–STAT) pathway, especially STAT1 and STAT2, causing downstream signal transduction [83,84]. Once phosphorylated, STAT1 and STAT2 rapidly bind to IRF9 and constitute the interferon-stimulated gene factor 3 (ISGF3) transcriptional complex 11. Next, ISGF3 binds to type I IFN-dependent gene promoters and drives the transcription and translation of IFN-stimulated genes (ISGs), which elicit both innate and adaptive immune programs within cells, therefore blocking the viral life cycle and triggering programmed cell death, especially apoptosis, in infected cells [85,86].

3. Regulation of RLR signaling cascade

Control on RLR antiviral cascade signaling is essential not only to prevent harmful and unwanted type I IFN responses in the absence of infection but also to initiate timely and sensitive pathogen recognition in the presence of pathogen invasion [87]. Moreover, the host has developed a lot of mechanisms to regulate the activity of RLRs, such as miRNAs, interacting proteins and post-translational modifications (PTMs). Through these delicate modulations, the feedback or feedforward regulatory loops of antiviral response can be successfully established.

3.1. Interacting proteins

Many dsRNA-binding proteins have been found to interact with RLRs and modulate the RLR signaling cascade, including RLR-dsRNA binding, RLR assembly, and RLR intracellular localization.

PACT, the first dsRNA-binding protein found to regulate RLR signaling cascade, directly interacts with RIG-I CTD and enhances its ATPase and helicase activities, resulting in potent initiation and maintenance of RIG-I-induced type I IFN production [88]. The overexpression of PACT stimulates about twice the production of IFN-β in SeV-infected cells [89]. PACT also facilitates RNA-induced MDA5 oligomerization and directly interacts with LGP2, thereby increasing both RIG-I- and MDA5-induced IFN production [90,91]. Interestingly, TAR-RNA-binding protein2(TRBP), the homologous protein of PACT, acts oppositely to PACT and restrains RIG-I-mediated IFN-β production induced by poly(I:C) and 5'ppp dsRNA viral mimics via specifically sequestrating RIG-I from dsRNA [92]. A coreceptor of RIG-I and MDA5 named ZCCH3 simultaneously binds with RLR-sensed RNAs and RLRs, strongly enhancing the binding activity of RLRs [93]. Nucleotide-binding oligomerization domain 1 (NOD1) [94], ribonucleoprotein PTB-binding 1 (RAVER1) [95], and DXH29 [96] can significantly and specifically amplify MDA5-dsRNA binding affinity, whereas ZFYVE1 [97] and Arf-like protein 5B (Arl5B) [98] competitively bind to virus-derived RNA and reduce MDA5-RNA binding and MDA5 oligomerization. ZFYVE1 deficiency protects the mice from death induced by MDA5-sensed EMCV, but not RIG-I-sensed VSV. Other than the combined function of ubiquitin (Ub), Ring finger protein 135 (RNF135) can additionally induce RIG-I assemblies to enhance RIG-I signaling in an RNA-length dependent manner [99]. Chaperone protein 14-3-3η promotes the antiviral immune response by facilitating the oligomerization of MDA5 and its translocation to mitochondria [100]. These findings indicate that the interacting network involved in RLR signaling is fine-tuned, complicated and comprehensive.

3.2. Post-translational modifications

Post-translational modifications (PTMs) are crucial for the activation and deactivation of RIG-I and MDA5. By regulating the activity, stability, cellular compartment, conformation and interactions with additional factors, PTMs precisely alter the functionality of RLRs. PTMs mainly include ubiquitination, phosphorylation, SUMOylation and acetylation (Table 2).

Ubiquitination of RLRs controls their signals both positively and negatively. Several E3 ubiquitin ligases, such as tripartite motif-containing protein 25(TRIM25) [101], RNF135 [99], TRIM4 [102] and MEX3C [103], augment the activation of RIG-I through Lysine (Lys, K) 63-linked polyubiquitination. On the other hand, several E3 ubiquitin ligases, such as RNF122 [104], RNF125 [105], TRIM40 [106] and carboxyl terminus of HSC70-interacting protein (CHIP) [107], negatively regulate RIG-I activity via K48-linked polyubiquitination. The activation and oligomerization of MDA5 are induced by TRIM65-mediated and K63-linked polyubiquitination [108]. Deubiquitinases (DUBs) operate as negative regulators of RLR signaling by removing the K63-linked polyubiquitin modifications. For example, A20 [109] and
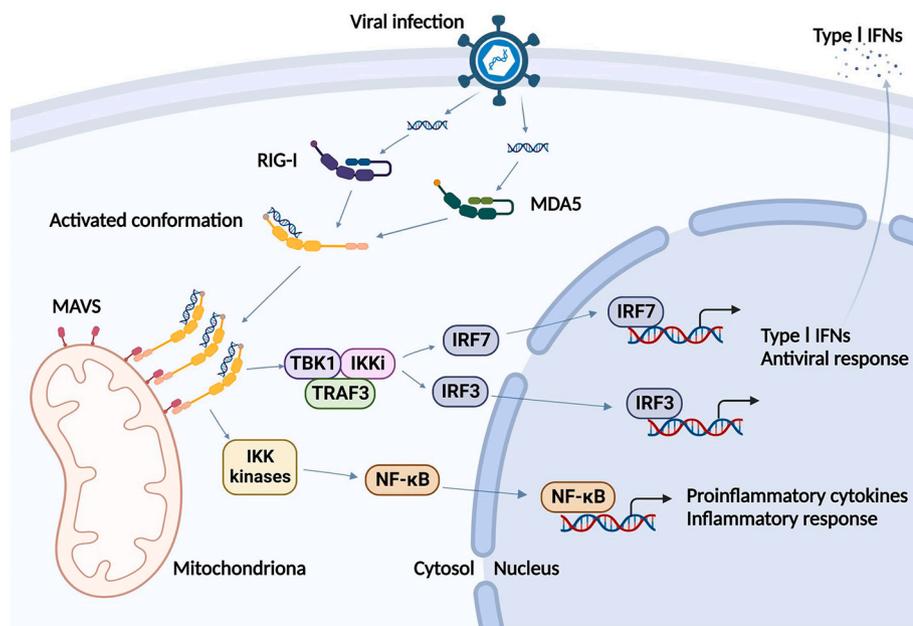
Fig. 2. The signaling pathways of the RLR. Upon viral infection, RIG-I and MDA5 recognize dsRNA and soon convert to activated conformations. Then RIG-I and MDA5 form homopolymers and recruit MAVS, which then activate TBK1/IKK kinases to generate type I IFNs for antiviral responses and activate NF-κB to transcribe the proinflammatory cytokines for inflammatory responses.
Abbreviations: RIG-I: retinoic acid-inducible gene I; MDA5: melanoma differentiation-associated protein 5; MAVS: mitochondrial antiviral-signaling protein; TBK1: TANK-binding kinase 1; IKK: I-kappa-B-kinase; IRF3/7: interferon regulatory factor 3/7; NF-κB: nuclear factor kappa-B; type I IFNs: type I interferons. [Figure was created with BioRender (https://BioRender.com).]

Table 2
Post-translational modifications of RLRs.

	Substrate	Modification	Binding site	Key Refs
TRIM25	RIG-I	K63-linked polyubiquitination	CARD (K190, K193)	[101]
RNF135			CARD, CTD (K96, K115, K788, K849, K851, K888, K907, K909)	[127] [128]
TRIM4			CARD (K164)	[102]
MEX3C			CARD (K45, K99, K169)	[103]
TRIM65	MDA5		Helicase (K743)	[108]
RNF125	RIG-I, MDA5	K48- linked polyubiquitination	CARD	[105] [106]
RNF122	RIG-I		CARD (K115, K146)	[104]
c-Cbl			CTD (K813)	[129]
CHIP			CARD	[107]
TRIM40	RIG-I, MDA5	K27/K48-linked polyubiquitination	K23, K43 and K68 in the first CARD of MDA5	[106]
TRIM13	MDA5	Unknown	upstream of IRF3	[130]
A20	RIG-I	remove K63-linked polyubiquitin	K63-linked polyubiquitin	[109]
CYLD				[110]
USP3				[131]
USP21				[132]
USP25				[133]
USP4	RIG-I	remove K48-linked polyubiquitin	K48-linked polyubiquitin	[134]
USP15	TRIM25			[135]
USP17	RIG-I, MDA5	deubiquitination	unknown	[136]
PKC-α	RIG-I	phosphorylation	CARD (Ser8)	[137] [138]
PKC-β			CARD (Thr170)	[139]
CKII			CTD (Thr770, Ser854-855)	[140]
PP1α, PP1γ	RIG-I, MDA5	dephosphorylation	CARD (RIG-I: S8, T170; MDA5: S88)	[141]
RIOK3	MDA5	dephosphorylation	CTD (S828)	[120]
TRIM38	RIG-I, MDA5	SUMOylation	K96 and K888 of RIG-I, K43 and K865 of MDA5	[123]
PIAS2 β	MDA5	SUMOylation	CARD	[142]
SENP2	RIG-I, MDA5	deSUMOylation		[123]
hdac6	RIG-I	deacetylation	CTD (K909)	[85,86]
FAT10	RIG-I	deamidation	CARD (T55)	[143]

Red represents positive regulation, and blue represents negative regulation.

cylindromatosis (CYLD) [110] both target RIG-I and efficiently inhibit RIG-I-mediated IFN-β production. Besides, ubiquitin-like (UBL) proteins play a dual-functioned regulatory role on RLRs depending on their target proteins. For instance, the UBL protein HLA-F adjacent transcription 10 (FAT10) suppresses the RIG-I-TRIM25 complex through binding and stabilizing the RIG-I protein, thereby dampening the RIG-I-mediated antiviral responses[111]. The other UBL protein, IFN-induced 15-kDa protein (ISG15), performs ISGylation (a ubiquitin-like modification) and activates MDA5 but reduces RIG-I intracellular levels [112–114]. Intriguingly, it has been recently found that SARS-CoV-2 utilizes autogenous papain-like protease to antagonize MDA5 ISGylation and evade host innate immunity, which may account for the SARS-CoV-2 immune evasion and the suppression of host IFN-I production[115]. The balance of polyubiquitination and deubiquitination regulate protein stability, oligomerization and RLR activity, also participating in innate antiviral responses.

Phosphorylation, another PTM, is regulated by the antagonism of kinase and phosphatase. In resting cells, RIG-I maintains a conformation of self-inhibition by phosphorylation of CARD and CTD to prevent prematurity of antiviral signals. After the invasion of the RNA virus, RIG-I is rapidly dephosphorylated to elicit innate antiviral response. Conventional protein kinase C-α (PKC-α) and PKC-β are mainly responsible for the phosphorylation of RIG-I CARD, which suppresses CARD ubiquitination by TRIM25 [116–118]. Similarly, the CTD domain of RIG-I and MDA5 can be phosphorylated by casein kinase II (CK II) [119] or RIO kinase 3 (RIOK3), respectively, thus disrupting their filament formation and attenuating downstream signaling [120]. In contrast, during viral infection or viral RNA binding, RIG-I and MDA5 CARD are dephosphorylated by phosphatase I α and phosphatase I γ (PP 1 α and PP 1 γ), which is crucial for their activation [121]. Based on the pivotal role of phosphorylation regulation in RLRs activation, the agonist or antagonist of kinase and phosphatase might be potential therapeutics for RLRs-related diseases.

SUMOylation is a ubiquitination-like post-translational modification that covalently attaches a small ubiquitin-like modifier (SUMO) to the Lys residues of the target protein. It also plays a vital role in protein function regulation and host antiviral defense [122]. By performing dynamic SUMOylation of RIG-I and MDA5 at K865 and K888, TRIM38 inhibits their K48-linked polyubiquitination and enhances their stability [123]. On the contrary, deSUMOylation mediated by sentrin/sumo-specific protease 2 (SENP2) prevents excessive and deleterious

immunopathological damage to the host by promoting the K48-linked polyubiquitination of RIG-I and MDA5 [123]. Like TRIM38, PIAS2β is also a SUMOylation ligase, but specifically interacts with and enhances the SUMOylation of MDA5 but not RIG-I [124].

Reversible acetylation also regulates RIG-I activation by controlling its viral RNA-binding activity. The acetylation of RIG-I CTD inhibits the recognition and binding of CTD to viral RNA, leading to the inability of RIG-I to assemble into signal-active homo-oligomers. In contrast, deacetylation of CTD by histone deacetylase 6 (HDAC6) restores RIG-I's ability to bind RNA and oligomerize [125] [126].

3.3. miRNAs

Although previous studies focusing on the role of microRNAs (miR-NAs) in immune regulation are rather limited, a growing number of studies have demonstrated that miRNAs are involved in regulating the RLR signaling pathway in mammals.

miR-146a was the first miRNA reported in 2009 to regulate the RIG-I signaling pathway. It impairs RIG-I signaling, blocks RIG-I-dependent type I IFN secretion and allows viral replication in macrophages by targeting TNFR-associated factor 6 (TRAF6) and interleukin 1 receptor-associated kinase 1/2 (IRAK1/2) during vesicular stomatitis virus (VSV) infections [144]. miR-92a also inhibits macrophage-mediated anti-VSV immune responses by directly targeting RIG-I and reducing its expression [145]. Similarly, miR-218 targets and decreases RIG-I in human and mouse macrophages while facilitating the immune evasion of VSV. Targeted inhibition of miR-218 rescues the signaling activity of RIG-I and helps macrophages defend invaded viruses [146]. miR-485 is produced by the host after viral invasion and specifically targets RIG-I mRNA for degradation and results in impaired type I IFN response and uncontrolled virus replication [147].

Unlike the previously mentioned miRNAs that negatively regulate RIG-I, Zhao et al. found that miR-136 is an agonist of RIG-I and can promote IFN-β and IL-6 secretion and accumulation, leading to the strong antagonism against H5N1 influenza A virus (IAV) replication in human lung epithelial cells (A549) [148]. Moreover, microRNA let-7b specifically augments RIG-I signaling to promote the activation and nuclear translocation of IRF3 and induces the enhanced type I IFN response during early hepatitis C virus (HCV) infection [149]. Additionally, miR-145 overexpression by liposome-mediated transient transfection led to an immune response *in vitro* via RIG-I recognition [150].

miR-125a-3p is found to reduce MDA5 expression in CD4$^+$ T lymphocytes of patients with systemic lupus erythematosus (SLE) depending on the dose [151]. In miiuy croaker, a non-mammal, miR-203 has a negative regulatory effect on MDA5 through directly targeting the MDA5 gene [152], and miR-145-5p also inhibits MDA5 transcription in a dose-and-time-dependent manner via degrading the MDA5 mRNAs [153]. In chicken spleens infected with Avian leukosis virus subgroup J (ALV-J), miR-34b-5p targets MDA5 and downregulates the genes in the MDA5-mediated signaling pathway to increase the replication of ALV-J and proliferation of ALV-J-infected cells [154].

Apart from RIG-I and MDA5, MAVS also gets regulated by miRNAs. Liu et al. found that overexpression of miR-33/33* leads to blunted MAVS activation, enhanced viral lethality, and reduced type I IFN secretion both *in vitro* and *in vivo*. The specific mechanism in which miR-33/33* prevents MAVS from forming functional homo-oligomers and activation is that miR-33/33* targets adenosine monophosphate activated protein kinase (AMPK) for posttranslational silence and disturbs mitochondrial homeostasis by suppressing mitophagy. Intriguingly, miR-33/33* transcription in macrophages is impeded by VSV stimulation in an IFNAR-dependent manner [155]. Little is known about the miRNAs that regulate LGP2.

Taken together, these studies discover a brand-new role of miRNAs in modulating host antiviral immune response and shed new insights for clinically treating not only viral infection but also autoinflammation in the years to come.

4. RLRs and infections

As cellular non-self RNA sensors, RIG-I and MDA5 are essential for interferon production and antiviral immune response in many infective diseases. RIG-I$^{-/-}$ and Mda5$^{-/-}$ mice showed high susceptibility to RNA virus infections, suggesting that RIG-I and MDA5-mediated antiviral responses are vital for eliminating RNA viruses. In addition to this, RLRs are also involved in the innate immune response against parasite and bacterium infections.

4.1. MDA5 senses and responds to SARS-CoV-2

The world is now experiencing a significant public health crisis, the COVID-19 pandemic, caused by SARS-CoV-2. Once the SARS-CoV-2 virus infects pneumocytes, cytosolic MDA5 and LGP2 predominantly mediate the delayed interferon induction and then establish an antiviral milieu through triggering ISGs. Further data demonstrated that SARS-CoV-2 intermediates also specifically trigger the interferon production via the MDA5 signaling pathway [21]. Moreover, ISGylation of MDA5 CARD domains conjugated with interferon-stimulated protein ISG15 facilitates the oligomerization of MDA5, triggering the positive feedback of innate antiviral response. However, the SARS-CoV-2 papain-like protease de-ISGylates and blocks ISG15-induced activation of MDA5, also contributing to viral evasions of host immune surveillance [115]. In a prospective study of 227 ICU patients with COVID-19, rs199076 TT variation on IFIH1, the gene coding MDA5, was found to be associated with decreased inflammation and better prognosis [156]. The single-cell sequencing analysis of upper airway tissues from normal children samples indicated that the mRNA level of MDA5 in immune cells were presented higher than that in adults, and triggered prominent antiviral immunity during SARS-CoV-2 invasion. This finding provides an explanation for why low morbidity of COVID-19 happened in children rather than in adults [157]. All of these findings contribute to our understanding of the molecular basis of SARS-CoV-2, from its innate immune recognition to the signaling response it induces.

4.2. RLRs participate in anti-malaria immune response

In addition to virus infection, MDA5 is identified as a cytosolic sensory receptor recognizing Plasmodium RNA from malaria parasites and initiated a type I IFN response controlling Plasmodium parasite replication in hepatocytes [158]. Increased phosphatidylserine on the surface of Plasmodium-infected red blood cells lead to the engulfment of Plasmodium by conventional dendritic cells (cDCs), and release Plasmodium ligand bound to MDA5, triggering an early protective IFN-I response in malaria infections [159]. Moreover, Plasmodium RNA-harboring extracellular vesicles (EVs) from Plasmodium-infected RBCs fuse with responding natural killer (NK) cells and activate the MDA5 signaling pathway, contributing to the immune response to malaria infections [160]. Recently, the internalization of Plasmodium-derived microvesicles through monocytes has been found to stimulate the RIG-I cascade and result in the binding of HUR1 to chemokine *CXCL10* mRNA 3′UTR, consequently restricting the synthesis and secretion of CXCL10. The decreased CXCL10 level is not suitable for malaria growth. However, fatal cerebral malaria grew with heightened serum CXCL10 levels, which accelerated the development of Plasmodium [161]. These data suggest that parasites have developed sophisticated mechanisms to utilize host molecules such as MDA5 and RIG-I to reprogram the immune response and control their own fate.

4.3. RLRs sense bacterial RNA

Aside from viruses and parasites, RLRs are also able to sense bacterial nucleic acids, especially those released into the cytoplasm of infected

cells by intracellular pathogenic bacteria, leading to the expression of type I IFNs. When live Listeria monocytogenes enter macrophage cytoplasm, bacterial RNA/DNA is secreted from *Listeria* and detected by RIG-I, MDA5 and STING, stimulating the release of interferon β. Meanwhile, RIG-I recognizes bacterial nucleic acids, and activated inflammasome and IL-1β release (147). A recent study showed that RIG-I recognized a complex composed of Listeria monocytogenes RNAs and a small bacterial RNA-binding protein Zea upon Listeria monocytogene infection, thereby enhancing the release of IFN-β [162]. It has been well accepted that RIG-I and MDA5, but mainly RIG-I, allow *Listeria*-infected cells to effectively sense bacterial activity, virulence, and localization in the cytosol. The intracellular *Salmonella* mRNA gets detected by RIG-I rather than MDA5 to launch the production of interferon β in nonphagocytic cells, but not in immune cells [163]. *Mycobacterium tuberculosis* RNA/DNA was released into the macrophage cytosol, inducing IFN-β production through the crosstalk between host RIG-I and STING sensing pathway [164].

These data support a broad function of RLRs in antipathogenic immunity. Furthermore, there may be redundancy or crosstalk between different DNA/RNA sensing signaling pathways mediating the antipathogenic immune response. As a result, many studies have focused on developing synthetic agonists of RLRs, especially RIG-I, to serve as potent pan-antivirals and vaccine adjuvants via the activation of IFN signaling and immune response [165–167].

5. *IFIH1* is identified as a susceptibility gene for autoimmune diseases

IFIH1 has been identified as a susceptible gene for a series of autoimmune diseases. Genome-wide association studies (GWASs) revealed that plenty of single nucleotide polymorphisms (SNPs) or single nucleotide variants (SNVs) in the *IFIH1* gene is associated with the risk for type 1 diabetes (T1D) [168], autoimmune thyroid disease [169], psoriasis [170,171], systemic lupus erythematosus (SLE) [24], and inflammatory bowel disease [172] [173], rheumatoid arthritis [174], vitiligo [175] and multiple sclerosis [176] (Table 3).

5.1. SNPs or SNVs in IFIH1 gene linked to systemic lupus erythematosus (SLE)

The rs1990760 (A946T) SNP in the IFIH1 gene is the most common coding-sequence-variant linked to autoimmune diseases. This mutation is presumed to change the expression, structure and function of MDA5. The T allele of rs1990760 is associated with anti-double-stranded DNA antibodies and low IFN-α level in serum among SLE subjects [177]. Bioinformatic analysis predicted that this variant will not change the protein structure. Indeed, it is possible that the A946T risk allele is a gain-of-function SNP and will cause augmented MDA5 expression [178]. Intriguingly, the genetic defect in a patient with rare early-onset and severe SLE is identified as de novo R779H mutation in the IFIH1 gene, which has been reported to cause Aicardi-Goutieres syndrome, a rare genetically neuroimmunological disorder [179] [180]. These data further support the role of MDA5 in the pathogenesis of SLE.

5.2. SNVs in IFIH1 gene associated with type 1 diabetes

Through association analysis, Nejentsev *et al.* found that four rare loss-of-function mutations in the *IFIH1* gene, including two mutations in the exon and two mutations in the intron, have more potent protective effects against TID risks than other non-synonymous SNPs (nsSNPs) [181]. The mechanism may be that these protective variants might disrupt the normal splicing of IFIH1 transcript, dampen the function of MDA5 and attenuate innate immune responses against viruses. In contrast, the incidence of T1D correlates with enterovirus (especially coxsackievirus B4) infection and the overall antiviral effect of MDA5 [182–184]. For the development of T1D, the interaction among

Table 3
Pathogenic SNPs and SNVs of *DDX58* (RIG-I) and *IFIH1* (MDA5).

Gene	Mutation	Type	Effect	Disorder
IFIH1	A946T	Gain-of function (GOF)	Increased expression of IFIH1	SLE
IFIH1	R779H	GOF	Increased expression of IFIH1	SLE, AGS
IFIH1	rs35337543 rs35667974 ss107794688 ss107794687	Loss of function (LOF)	Disruption of the normal splicing of IFIH1 transcript and impaired function of MDA5	T1D
IFIH1	c. 1694G>T c. C2105T c.C373A c. A1909G	LOF	Full or partial MDA5 defect	Early-onset inflammatory bowel disease
IFIH1	G821S	GOF	Spontaneous typeIIFN induction	Lupus-like nephritis
IFIH1	c. 2159G>A c. 2336G>A c. 1009A>G c. 2335C>T c. 1178A>T c. 1483G>A	GOF	Increased MDA5-dsRNA binding affinity and enhanced MDA5 filament stability	AGS
IFIH1	c. 1354G>A c. 1114C>T c.2336G>A	GOF	Increased activation of IFNB1, which encodes IFN-β	AGS
MDA5	G495R	GOF	Lost tolerance to imperfect self Alu-dsRNA duplex	AGS
IFIH1	K365E K889X c.2016delA Ile872Ter	LOF	Impaired IFN-β signalling	IMD95
DDX58	E373A C268F E383A	GOF	Constitutive activation of typeIIFNs and increased ISGs expression	SMS
IFIH1	R822Q	GOF	Increased production of typeIIFNs	SMS

Abbreviations: AGS, Aicardi-Goutieres syndrome; TID, type 1 diabetes; SLE, systemic lupus erythematosus; IMD95, immunodeficiency 95.

individual heterogeneity, constant enteroviral challenges and antiviral immune responses may play an important role [185,186]. Viral infection and type I IFN production can induce ER stress in β-cells, which leads to the generation of neoantigen epitopes. This, in turn, results in the activation of β-cell-specific autoreactive T lymphocytes and damages β-cells [184].

5.3. SNVs in IFIH1 gene involved in early-onset inflammatory bowel disease

Recently, four rare heterozygous variations in the IFIH1 gene were identified in 7 unrelated children with very early-onset inflammatory bowel disease. The variants are inherited from their parents, who have no gastrointestinal or immunological issues. These variants are believed to be loss-of-function mutations, and may lead to complete or partial MDA5 deficiency, thus impairing intestinal pathogen sensation [187]. This finding suggests the role of MDA5 in innate intestinal immunity.

5.4. IFIH1 gain-of-function mutation cause lupus-like nephritis in mice

Direct evidence linking MDA5 gain-of-function or loss-of-function variants to autoimmune phenotypes is currently limited. In mice models harboring IFIH1 G821S mutation induced by N-ethyl-N-nitrosourea (ENU), lupus-like nephritis developed with spontaneous type I IFN induction in multiple organs, which were not seen in MAVS$^{-/-}$ or IFNAR$^{-/-}$ backgrounds. And this provides the first evidence of a direct

correlation between the MDA5 gain-of-function mutation and an auto-immune phenotype [172,188]. Moreover, merely overexpression of the Ifih1 gene could not cause autoimmune symptoms, indicating that mutation-induced MDA5 dysregulation is pivotal to eliciting the auto-immune response [189]. However, it remains elusive how SNPs or SNVs in *IFIH1* cause these MDA5 structural and functional transformations, which may lead to exacerbated antiviral responses or constitutive acti-vation of the type I IFN pathway that contributes to increased suscep-tibility to these diseases. These findings are expected to deepen our understanding of the relationship between RLRs-mediated innate im-mune response and autoimmune disorders. This may assist in devel-oping the relevant therapeutics against these disorders.

6. Gain-of-function (GOF) mutations in RLRs cause auto-inflammatory diseases

Gain-of-function mutations of RLRs often make the body mistake self-derived nucleic acids for viral products and lead to aberrant type I IFN signaling production (termed *IFN-I signature*), causing extensive organ damage and many rare disorders characterized by *IFN-I signature* [190,191]. Some of these disorders are monogenic diseases, including Aicardi-Goutieres syndrome, Singleton-Merten Syndrome and Immu-nodeficiency 95, and are therefore good models for molecular studies.

6.1. IFIH1 mutations result in Aicardi-Goutieres syndrome

Aicardi-Goutieres syndrome (AGS) is a rare genetical neuro-immunological disease and type I interferonopathy characterized by impaired psychomotor development, dystonic posturing, spasticity, increased IFN-α in cerebrospinal fluid and up-regulated ISG gene expression in infancy. In 8 unrelated patients from AGS families, six heterozygous variants have been identified in the conserved helicase domains of the IFIH1 gene. *In vitro* cellular experiments demonstrated that the heterozygous IFIH1 variants led to apparent induction of interferon and ISGs genes in the absence or presence of exogenous MDA5 ligand. The biochemical assays revealed that these substitute residues increase the binding activity of MDA5 to RNA and the stability of the activated MDA5 filament [180]. However, the other three mutant MDA5 proteins encoded by novel heterozygous mutations from Japa-nese AGS patients lacked the response to specific agonists, although IFN-β was induced in hepatoma cells transfected with MDA5 mutants without agonist stimulation [192]. These data suggest that gain-of-function mutations in IFIH1 lead to constitutive MDA5 activation, interferon signaling, and eventually interferonopathy. One possibility for constitutive MDA5 activation in AGS patients is that the IFIH1var-iants alter their conformation via releasing CARD domain autor-epression, thereby eliciting interferon signaling without ligand stimulation [188]. Another possibility is that MDA5 mutants misrecog-nized self-RNA, inducing an antiviral response [180]. However, endogenous ligands for MDA5 mutants are in need to be defined. Recent evidence further revealed that MDA5 mutants from AGS patients detected and formed filaments on the self Alu-dsRNA duplex, and then turned into a constitutive activation of MDA5 signaling because these mutants lost tolerance to imperfect duplex construct [193].

6.2. IFIH1 mutations lead to Immunodeficiency 95

Immunodeficiency-95 (IMD95) is a hereditary disease characterized by severe early-onsets of viral lung infection during childhood, caused by a loss of response of the innate immune system to viral invasions. This results in a failure of interferon production and inhibition of viral replication. Four kinds of homozygous mutations in the *IFIH1* gene, including missense, nonsense, and frameshift variant, have been linked to Immunodeficiency-95 patients [187,194–196]. These MDA5 mutants impaired their activity to assemble and respond to the virus mimics, and failed to activate IFN-β pathway signaling, suggesting that they belong to loss-of-function variations.

6.3. RLRs identified as genetic defects of Singleton-Merten Syndrome

Singleton-Merten syndrome (SMS) is an unusual and hereditary disorder manifested with abnormal calcifications of the aorta and valves, dental inflammation, and osseous abnormality during childhood. The variants in DDX58 or IFIH1 have been proved to link to SMS [197]. Three heterozygous mutations in the DDX58 gene, E373A, E383A and C268F, were first identified in two SMS families characterized by glau-coma, aortic calcification, and skeletal anomalies. They result in the constitutive IFN-I production and ISGs gene expression [198]. So far, only one kind of *IFIH1* variant (R822Q) has been identified in four SMS patients from distinct families, and this mutant strengthens MDA5-mediated IFN-β production and interferon signature genes, leading to an overt inflammatory disease [180,192,199]. It will be intriguing to investigate how the distinct amino acid substitutions in MDA5 alter the conformation and function of MDA5 and lead to SMS and AGS.

Why changes in the function of one single gene can lead to so many different phenotypes of autoinflammation or autoimmune diseases re-mains to be further explored. One possible hypothesis is that these conformational changes induced by amino acid substitutions enable MDA5 and RIG-I to easily acquire activity to self dsRNA [197]. Some also consider that genetically relevant factors may play a driving role in the onset and development of diseases.

7. RLRs expression pattern in autoimmune disease

In addition to SNPs and SNVs of RLRs, a recent study showed that RIG-I expression of peripheral T lymphocyte increases in patients with DM as well as T cell lymphopenia, presenting a negative correlation between RIG-I and T cell count. Upregulated RIG-I induced apoptosis by increasing the expression of Fas and cleaved-caspase 3 protein as well as inhibiting T lymphocyte proliferation *in vitro*. RIG-I-induced apoptosis may account for the mechanism of T cell lymphopenia in DM patients [200]. Furthermore, the expression of RIG-I and MDA5 are up-regulated in the perifascicular atrophy fibers in DM biopsies, suggesting that RIG-I is involved in the endogenous production of type I IFN and the formation of perifascicular atrophy within DM patient muscle tissue [201,202]. Statistical analysis performed on 44 DM patients further confirmed that RIG-I expression in perifascicular myofibers is a reliable and promising biomarker of DM [203].

In almost half of primary Sjögren's syndrome (pSS) patients, the disease activity and autoantibody presence positively correlate with the upregulated type I IFN signature. sRIG-I and MDA5 were upregulated in plasmacytoid dendritic cells and monocytes of patients with IFN-positive pSS, contributing to the pathogenic type I IFN production [204].

8. Anti-MDA5 antibody in clinical amyopathic dermatomyositis

Dermatomyositis (DM) is a systemic autoimmune disease which in-volves muscle, skin and organs such as joints and lungs. In the serum of patients with PM/DM, myositis-specific autoantibodies (MSAs) and myositis-associated autoantibodies (MAAs) are detected. The anti-MDA5 antibody, one of the MSAs, was first identified in a Japanese cohort by Sato et al in 2005 [205]. Anti-MDA5 antibody-positive pa-tients are mainly seen in DM, especially in clinically amyopathic der-matomyositis (CADM), which is a subtype of DM, and its clinical manifestations are substantially different from other types of DM. Pa-tients with CADM display only skin or predominant skin appearance, only mild proximal or no muscle weakness and elevated serum muscle enzymes. The muscle biopsies of patients with CADM are usually normal or only contain scarce inflammation and cellular infiltrates (primarily macrophages) [206].

There are only a few data concerning the pathophysiology of CADM. Nevertheless, it is emphasized that type I IFN signaling pathway is

pivotal for DM pathogenesis [207]. Allenbach et al. found considerable upregulation of the expression of six ISGs in DM patients with positive anti-MDA5 antibodies [208]. Jiang et al. further proved that type I IFN signaling pathway is overexpressed in the skeletal muscles of anti-MDA5 DM patients [209]. These observations all account for the mild muscle phenotype of CADM.

The numbers and ratios of peripheral blood lymphocytes, especially CD4$^+$ T cells and CD8$^+$ T cells, decrease in some myositis patients with anti-MDA5 antibodies. It is associated with anaemia, thrombocytopenia, fibrinolytic abnormalities (such as increased D-dimer), liver function damage and elevated blood lipid levels. Many studies have found that the levels of TNF-α, IL-6, IL-8, IL-10, IL-18, IFN α/β/γ, IP10 and other cytokines or chemokines are increased myositis patient serum with the anti-MDA5 antibodies, and the serum ferritin concentrations were also significantly elevated. Furthermore, the levels of soluble IL-2 receptor α chain (sIL-2R or SCD25), soluble scavenger receptor CD16 (SCD163) and soluble mannose receptor CD206 (SCD206) significantly increase in serum of patients with CADM and ILD [210–213]. These inflammatory markers and proteins are essential in activating immune cells, especially T cells and macrophages. As a result, many scholars have proposed the hypothesis that macrophage activation plays a vital role in CADM associated with RP-ILD.

The incidence rate of CADM ranges from 10% to 48% in previous studies of patients with PM/DM [214]. A link is identified between anti-MDA5 antibodies and clinical characteristics such as mechanic's hand, skin ulcers, Gottron's sign or papules and V rash. For this group of patients with CADM, lung injury is the most typical and prominent feature. 80% of CADM patients are associated with interstitial lung disease (ILD), and 30-60% with rapidly progressive interstitial lung disease (RP-ILD), leading to respiratory failure and mortality as high as 50% [215,216]. Lung tissue pathology revealed diffuse alveolar damage (DAD), including hyaline membrane formation, alveolar exudation, alveolar obstruction and collapse [217,218]. Patients with CADM and RP-ILD usually have very poor prognoses. In a recent meta-analysis, anti-MDA5 autoantibody showed superior sensitivity and specificity for RP-ILD, at 77% (95% CI: 64, 87%) and 86% (95% CI:79, 90%), respectively [219] in patients with anti-MDA5 antibody-positive CADM.

However, the correlation between anti-MDA5 antibody presence and the clinical manifestations of DM patients has not been determined yet. John et al. reported that no significant association exits between anti-MDA5 autoantibody and RP-ILD [220]. The frequency of positive anti-MDA5 antibodies differs among different ethnicities, as most case reports are identified in Asian populations (especially Japanese). The prevalence percentage of ILD in typical adult-onset DM patients is only about 10% in the United States but almost 40% in Japan.

The initial reduction of anti-MDA5 antibody in most DM patients, including those in fatal cases, suggests that perhaps MDA5 is not a potential biomarker for DM patients [221]. Thus, the associated clinical phenotype in different ethnicities must be further explored.

9. Development of RLRs as therapeutic targets or prognostic indicators in cancers

More and more studies have found that tumor cells can mimic viral invasion and trigger the pathogen recognition of innate immune as well as the signal transduction of type I IFN [222]. For example, it has been proved that some damaged tumor cells leak cytosolic DNA fragments and activates the STING signaling pathway to induce an anti-tumor immune response [223]. Similarly, stromal cells within the tumor tissue can release exosomes containing nucleic acids such as small nuclear RNAs (snRNAs) that can be sensed by the RIG-I/MDA5 of adjacent tumor cells. Thus, the anti-tumor process is elicited via the production of pro-inflammatory cytokines, the activation of programmed cell death as well as enhanced cytolytic activity of NK cells and increased antigen-presenting ability of antigen presenting cells (APCs), which include macrophages and dendritic cells (DCs) [80,224,225]. When co-cultured

with pancreatic cancer cells, those dendritic cells with pre-activated RIG-I signaling can present tumor-derived antigens to T lymphocytes more effectively [225]. Consistent with this phenomenon, ovarian cancer cells are pre-stimulated with RIG-I performed apoptosis. They get easily phagocytosed by monocytes and DCs, further indicating that mimicking viral infection can elicit immunogenic cell death of tumor cells and enhance immune responses against the tumor through the activation of RLRs signaling pathway [226].

Lately, there is growing evidence that agonists of RIG-I and MDA5 can produce antiviral and anti-tumor effects in a type I IFN-dependent or -independent manner, leading to immunogenic tumor cell death and the regression of multiple types of tumors [227,228]. Treatments aimed at activating non-infectious RLRs signaling pathways are under pre-clinical investigation as an anticancer strategy, which functions through effectively initiating inflammatory responses against neoantigens of tumor cells and has received considerable attention. For instance, RLRs agonists that comprise poly (I:C), 5' -triphosphate dsRNA (ppp dsRNA), stem loop RNA (SLR) 14 and M8, may induce cancer cell apoptosis and immune responses against tumors. [229,230–235]. Through cotreatment with RLR agonist Poly(I:C)-high molecular weight (Poly(I:C)-HMW), Sato et al. finds that the anticancer effect of ionizing radiation (IR) is enhanced, and the following upregulated Fas expression is responsive to FasL-induced apoptosis. Therefore, they propose that it is promising to use a combination of RLR agonist, IR, and FasL for treating cancers [231]. SLR14 is a RIG-I agonist which has been observed to induce robust anti-tumor responses in immunogenic or non-immunogenic tumors and can serve as a powerful immune adjuvant of immune checkpoint blockade (ICB) therapy [232]. Das et al. designed a nanoparticle delivery of RIG-I's natural ligand ppp- dsRNA into pancreatic cancer and successfully seized tumor progression [234].

M8, a sequence-modified RIG-I agonist, is a uridine-rich hairpin 5'pppRNA composed of 99 nucleotides and has recently shown promising potential to induce immunogenic cell death-related and damage-associated molecular patterns (ICD-DAMP) on tumor cells and synergize with other therapies to stimulate the activation of anti-tumor immune signals [235,236]. David et al. found that intertumoral administration of the RIG-I agonist SLR20 can induce immunogenic cell death and breast cancer cell microenvironment modulation, ultimately leading to the inhibition of tumor growth and metastasis [237]. RIG-I activation has been proved to take effect in resisting a large set of human tumor cells that comprise breast cancer, cervical cancer, myeloid leukemia, prostate cancer, lung cancer, pancreatic cancer and so on [230,238]. These findings further highlight the potential of therapeutic RLRs agonists in adjuvant therapy. Newly developed RLR agonists may fill the growing need for novel therapeutics against tumors in the future. However, whether these RLRs agonists are structurally stable and whether they have corresponding, potent or specific ligands *in vivo* is a question that needs to be addressed in future studies. Furthermore, prolonged exposure to a type I IFN environment can lead to an anti-inflammatory response, and inappropriate use of RLRs agonists may also result in an out-of-control inflammatory response. Therefore, the long-term use of RLRs agonists should be fully considered, and the periodicity and dose of administration should be further studied.

Beyond the role in treating cancer, RLRs also function as prognostic indicators for specific cancers. A monocentric study by Dominik et al. prove that high RIG-I expression is associated with ovarian cancer (OC) poor outcome, including disease progression and recurrence after remission. This correlation is much more significant in p53-mutated Type-II OC. Survival analysis further disclose RIG-I as a new biomarker for OC survival with independent prognostic usages [239]. Conversely, a low level of RIG-I suggests poor prognosis in melanoma patients. As previous studies have reported, IFN-α plays a crucial role in inducing cancer cell apoptosis and performing antitumor immune responses [240,241].

Melanoma tumor-repopulating cells (TRCs) are a subpopulation of highly malignant and tumorigenic cancer cells. To counteract the IFN-

α-induced cell apoptosis and achieve immune evasion, TRCs down-regulate RIG-I expression and hamper STAT1 activation via the integrin β3 (ITGB3)-c-SRC-STAT3 signaling pathway [241]. A combination treatment of STAT3 inhibitor stattic and IFN-α could improve the therapeutic effect of melanoma, and this discovery may provide new ideas for melanoma immunotherapy. As for neuroblastoma (NB), high cytoplasmic LGP2 expression in tumor tissues is found to strongly correlate with the differentiated histological grade and higher survival rates for NB patients, suggesting that LGP2 may exert an antitumor effect and serve as a prognostic marker in NB [242]. The mechanism of LGP2's role as a tumor suppressor may be that LGP2 can enhance the sensitivity to poly (I:C)-induced apoptotic cell death and increase the sub-G1 phase of NB cells. In short, RLRs serve as crucial participants of innate immune and can influence the anti-tumor immune response of hosts through many different mechanisms, playing a potential role in tumor suppression and opening up a new way for tumor pathogenesis and immunotherapies.

10. Summary and outlook

The RLRs family and its downstream IFN signaling pathway, as one of the critical cytoplasmic RNA sensors for pathogen recognition, play an indispensable part in mediating an effective immune response of the host. However, just as the coin has both sides, the role of the RLRS family in diseases can be a double-edged sword. Moderate and controlled activation of RLRs protects the host from invading pathogens, whereas excessive and uncontrolled RLR activation may lead to unexpected pathological damage and cytokine storms, and may even cause autoimmune diseases or autoinflammatory diseases. Furthermore, the SNPs and functional activity of RLRs are associated with many monogenic inherited diseases and tumors. Therefore, understanding the role of RLRs in diseases and how to utilize it effectively has become an urgent clinical problem. Fortunately, there are currently many RLR agonists or antagonists developed and used as antiviral agents, vaccine adjuvants and antitumor drugs. With better understanding of the molecular biology of RLRs and their regulatory mechanisms, these therapies may benefit patients with a variety of treatment options in the future.

Declaration of Competing Interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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