MINIREVIEW

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Function of RIG-I-like Receptors in Antiviral Innate Immunity*

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Various cells in the body are capable of sensing infectious viruses and initiating reactions collectively known as antiviral innate responses. These responses include the production of antiviral cytokines such as type I interferon (IFN)[2] and subsequent synthesis of antiviral enzymes, which are responsible for the impairment of viral replication and promoting adaptive immune responses (1). In this minireview, we focus on a subset of molecules known as RIG-I-like receptors, which sense viral RNA molecules that trigger a danger signal.

RIG-I-like Receptors

Three genes encode RIG-I-like receptors (RLR) in human and mouse genomes (2). Three DExD/H box helicases, termed retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated antigen 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2), exhibit marked primary structure conservation in their helicase domain (Fig. 1). As the analysis of RIG-I precedes the other two, the biochemical characteristics of RIG-I will be described in this section.

As shown in Fig. 1, RIG-I contains two repeats of the caspase recruitment domain (CARD)-like motif at its N terminus. The cDNA clone was initially obtained by functional screening based on reporter gene activation, essentially consisting of tandem CARD (3). Although less efficient than the signal by full-length RIG-I activated by viral infection, overexpression of the tandem CARD alone is sufficient to generate signaling and subsequent type I IFN production (2). CARD acts as a signaling domain, which interacts with a downstream molecule (IPS-1, see below) to relay the signal. Single amino acid substitution within the first CARD (T55I) is sufficient to inactivate CARD function, and tandem CARD is necessary for its function (4). So far there is no report showing the signal-dependent proteolytic release of CARD from full-length RIG-I, suggesting that processing is unlikely in the mechanism of RIG-I activation. Full-length RIG-I exhibits undetectable or very low constitutive activity in the cell transfection assay, suggesting that the C-terminal region contains a domain for autorepression. Indeed, functional analysis revealed that the C-terminal domain (Fig. 1, *Repression Domain*) is responsible for autorepression by interacting with both CARD and helicase domains (5). Interestingly, RIG-I with loss of function of CARD, either by deletion (RIG-IC) or point mutation (T55I), is incapable of transmitting a signal upon viral infection and dominantly inhibits virus-induced signaling (3, 4). This is because of the lack of a signaling domain and the presence of a repression domain as well as RNA binding activity. RIG-I exhibits strong double-stranded RNA (dsRNA) binding activity *in vitro*. RIG-I selectively binds with poly(rI:rC), poly(rA:rU), and 5′- and 3′-untranslated regions of hepatitis C virus genomic RNA (which are predicted to form a secondary structure) but not with dsDNA, poly(rA), or yeast tRNA (3, 4). RNA binding requires intact helicase and C-terminal autorepression domains (5) (Fig. 1).

Self and Non-self RNA Discrimination by RIG-I

The above results suggest that RIG-I is a specific sensor for dsRNA, which is absent in uninfected cells but known to be accumulated in virus-infected cells; however, influenza A virus infection results in IFN gene activation without detectable dsRNA accumulation (6). In these cells, it is proposed that single-stranded RNA (ssRNA) with 5′-triphosphate functions as a ligand for RIG-I. Actually RIG-I specifically binds with RNA containing 5′-triphosphate but not with RNA containing 5′-di- or 5′-monophosphate (7). These observations led to an interesting hypothesis of how self and non-self RNA species are discriminated. As shown in Fig. 2, host RNA synthesis takes place in the nucleus. Like the viral transcript, cellular primary transcripts contain 5′-triphosphate; however, these RNAs undergo various processes; mRNA acquires a 7-methylguanosine CAP structure at its 5′-end; tRNA undergoes 5′-cleavage and a series of nucleotide base modifications; the primary transcript of ribosomal RNA readily complexes with ribosomal proteins to form ribosomal ribonucleoprotein and undergoes maturation processes, which therefore are masked from detection. Indeed, artificial capping and base modification of 5′-triphosphate ssRNA abrogated detection by RIG-I (7), whereas viral RNA, either freshly introduced by infection or produced by viral replication, contains a non-self marker, 5′-triphosphate. In this regard, 5′-triphosphate RNA generated by DNA virus may well be detected by RIG-I.

Activation Mechanism of RIG-I

It is worth noting that a single amino acid substitution K270A renders RIG-I into a dominant inhibitor (3). Lys-270 is supposed to be a critical motif for ATP binding within the helicase domain, and in the case of other DExH/D helicases, this motif is crucial for its helicase (unwinding dsRNA) activity. As proteolysis is an unlikely mechanism (above) to reverse autorepression, the current de-repression model for RIG-I is illus-

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[2] The abbreviations used are: IFN, interferon; RIG-I, retinoic acid-inducible gene I; RLR, RIG-I-like receptor(s); dsRNA, double-stranded RNA; ssRNA, single-stranded RNA; CARD, caspase recruitment domain; IPS-1, interferon promoter stimulator-1; TRAF3, TNF receptor-associating factor 3; IKK-i, IκB kinase-i; TBK-1, TANK-binding kinase-1; cDC, conventional dendritic cell; NDV, Newcastle disease virus; pDC, plasmacytoid dendritic cell; IL, interleukin; TLR, Toll-like receptor; MyD88, myeloid differentiation factor 88; IRAK1, interleukin-1 receptor-associated kinase 1; IRF, interferon regulatory factor; VSV, vesicular stomatitis virus; JEV, Japanese encephalitis virus; EMCV, encephalomyocarditis virus; RNAi, RNA interference.

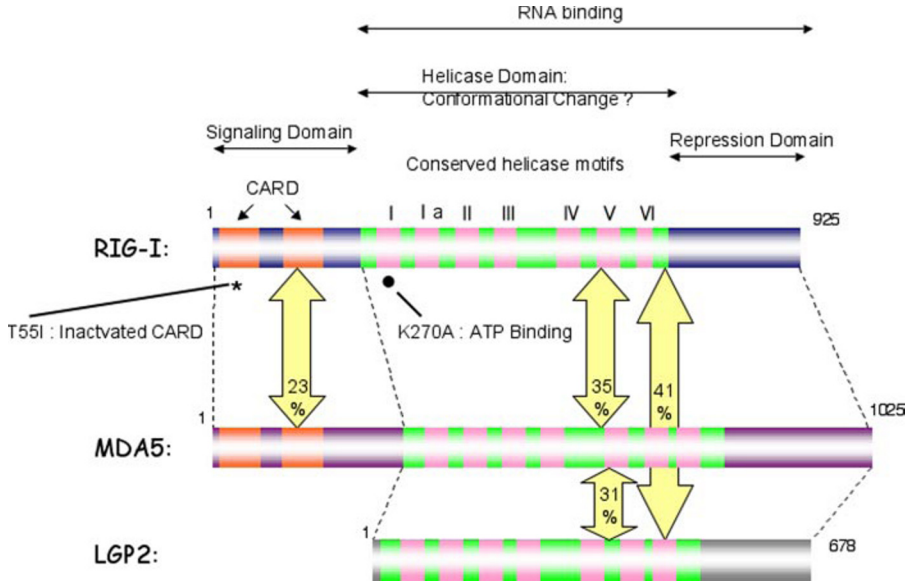

FIGURE 1. **Schematic representation of RIG-I and other RLRs.** Functional domains determined by mutagenesis are indicated. The conserved amino acid sequence of CARD and the helicase domain is indicated (percent identity, between human RLRs).

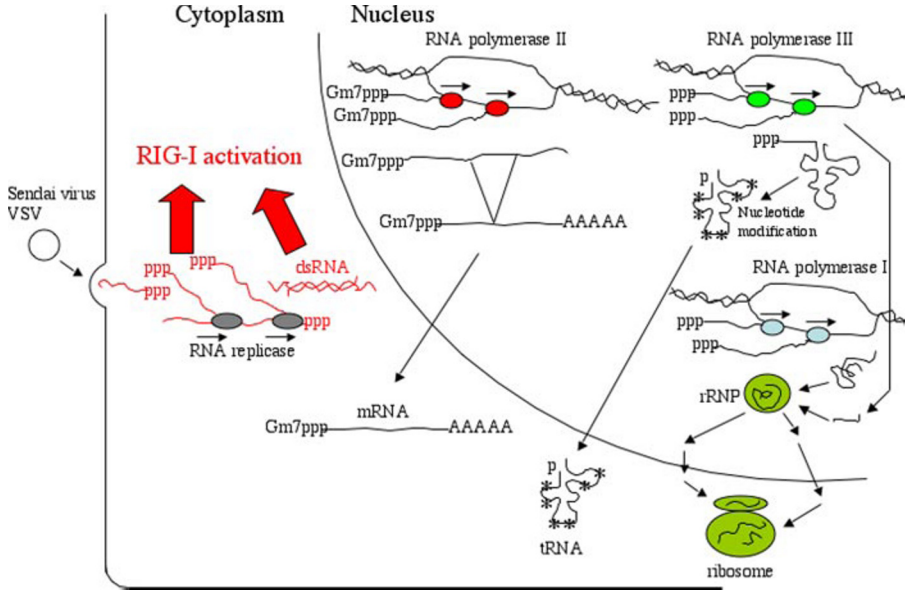

FIGURE 2. **Discrimination of self and non-self RNA by RIG-I.** Viral infection leads to the accumulation of non-self RNAs in the cytoplasm, such as dsRNA and 5′-triphosphate RNA. Cellular transcripts are modified to lack or mask these structures when transported to cytoplasm.

TABLE 1
Differential functions of RIG-I family helicases

RIG-I	Positive regulator	NDV, Sendai virus, influenza virus, VSV, JEV, *in vitro* transcribed dsRNA
MDA5	Positive regulator	Picornavirus, poly (I):poly (C)
LGP2	Negative regulator?	?

trated in Fig. 3. RIG-I exists as a "closed" structure in uninfected cells and therefore CARD is masked. The virus-specific RNA species, dsRNA or 5′-triphosphate ssRNA, specifically binds to RIG-I through its RNA binding domain. This association and ATP binding to the helicase domain change RIG-I conformation to release CARD for relaying signaling to the downstream molecule (another CARD-containing molecule, IPS-1 (alternatively termed MAVS, VISA, and Cardif)) (8–11). IPS-1 is localized on the outer membrane of mitochondria, and this localization is crucial for its function (9, 12–14) although its precise mechanism is not known. MDA5 and RIG-I, which sense a distinct set of viruses (below), commonly transmit signals to IPS-1; thus IPS-$1^{-/-}$ fibroblasts are unresponsive to either set of viruses (15, 16). The signal is branched at IPS-1, resulting in the activation of NF-κB and IRF-3 and -7. The latter involves TNF (tumor necrosis factor) receptor-associating factor 3 (TRAF3) (17) and the protein kinase, IκB kinase-i (IKK-i (ϵ)) or TANK-binding kinase-1 (TBK-1) (18, 19), which is responsible for the activation of IRF-3 and -7.

LGP2 lacks CARD, suggesting that this helicase is incapable of transmitting a positive signal. Overexpression of LGP2 in cell culture results in the dominant inhibition of virus-induced activation of IFN genes, suggesting its role as a negative regulator; however, its role *in vivo* is not established (2, 20, 21) (Table 1).

RIG-I Acts as a Major Viral Sensor in Fibroblasts and cDCs but Not in pDCs

Analyses of RIG-$I^{-/-}$ fibroblasts and conventional dendritic cells (cDCs) showed that RIG-I is essential in Newcastle disease virus (NDV)-induced IFN production; however, RIG-I is dispensable for virus-induced IFN production by plasmacytoid dendritic cells (pDCs) (22). pDCs adopt a distinct signaling cascade to produce high levels of IFN-α and sense viral infection by TLR7/8 and TLR9, activating signaling cascades MyD88, IRAK1/4, TRAF3/6, IKK-α, and IRF-7 (23, 24). It has been known that dsRNA activates TLR3 in endosome and signals through TIR domain-containing adaptor inducing IFN-β (TRIF)/TIR-containing adaptor molecule-1 (TICAM1) resulting in the activation of kinases (TBK-1 or IKK-i) and transcription factors (IRF-3 and -7 and NF-κB) (23). However, the mice defective in the TLR3-TRIF pathway exhibit normal IFN response upon viral infections (25). When poly(rI:rC) is injected into mice intravenously, IFN-β is strictly produced in a MDA5-dependent manner (below), but the TLR-TRIF pathway is dispensable. However, production of IL-8 and IL-12 p40

requires TRIF in addition to MDA5; IL-12 p40 is particularly largely TRIF-dependent (25). These observations demonstrate that although MDA5 and TLR3 signal a common pathway, different spectrums of cytokine genes are activated.

Specificity of Viral Sensing by RIG-I and MDA5

The overall structural similarity between RIG-I and MDA5 suggests the functional similarity of these proteins. Gene disruption studies revealed that these helicases sense distinct viral species (25). Cytokine production induced by the infection of Sendai virus, NDV, vesicular stomatitis virus (VSV), influenza A virus, and Japanese encephalitis virus (JEV) is markedly impaired in RIG-I$^{-/-}$ cells (Table 1). In contrast, cytokine production by encephalomyocarditis virus (EMCV), Thyler's virus and Mengo virus, all Picornaviruses (genus cardiovirus), is virtually absent in MDA5$^{-/-}$ cells (Table 1). In agreement with these observations, virus challenge experiments using knockout mice revealed that RIG-I$^{-/-}$ and MDA5$^{-/-}$ mice are selectively vulnerable to JEV and EMCV, respectively. It is remarkable that RIG-I/MDA5 deficiency exhibits a severe impact on viral infection *in vivo*, suggesting the critical function of innate immune responses in promoting adaptive immunity and virus eradication. Interestingly, genomic RNA of VSV and poly(rI:rC) selectively activates RIG-I and MDA5, respectively, suggesting that the distinct responses of RIG-I and MDA5 to different viruses are because of the distinct recognition of viral RNA by these sensors.

Virus-encoded Inhibitors of Innate Immune Responses

Viruses evolve to avoid host immune surveillance by producing inhibitors of the IFN system (Table 2). Generally, viral replication takes place in a restricted compartment where the viral genome is protected from detection by host sensors. Mouse hepatitis virus takes this strategy to avoid innate immune responses (26). Viral proteins evolve to counteract RLR functions. NS3/4A of hepatitis C virus inactivates IPS-1 by its protease activity (9, 12–14). Other viral proteins inhibit RLR signaling at various steps for their survival. It is noteworthy that many proteins encoded by DNA viruses also target RLR signaling.

RIG-I Activation by Endogenous RNA?

Although self RNA species are supposed to be tolerant to RIG-I detection (above), various RNAs with a secondary structure exist (known as non-coding and micro-RNA). Therefore, it remains to be established that RLR plays any role in physiological regulation by endogenous RNA. RIG-I$^{-/-}$ mice are embryonic lethal in certain genetic backgrounds, suggesting a role for RIG-I in development. *Caenorhabditis elegans* encodes a DExD/H box helicase, Dicer related helicase-1, which is essential for RNA interference (RNAi) in nematodes (27); however, no evidence has been reported on the role of RIG-I or MDA5 in mammalian RNAi, suggesting that structural similarity may be a consequence of evolution. Apparently, RNAi is independent of the IFN system in mammalian cells (28). It has been reported that experimental gene silencing either by transfection of 21-mer dsRNA or expression of short hairpin RNA has the potential to activate IFN gene and downstream events (28, 29). In this regard, the importance of end struc-

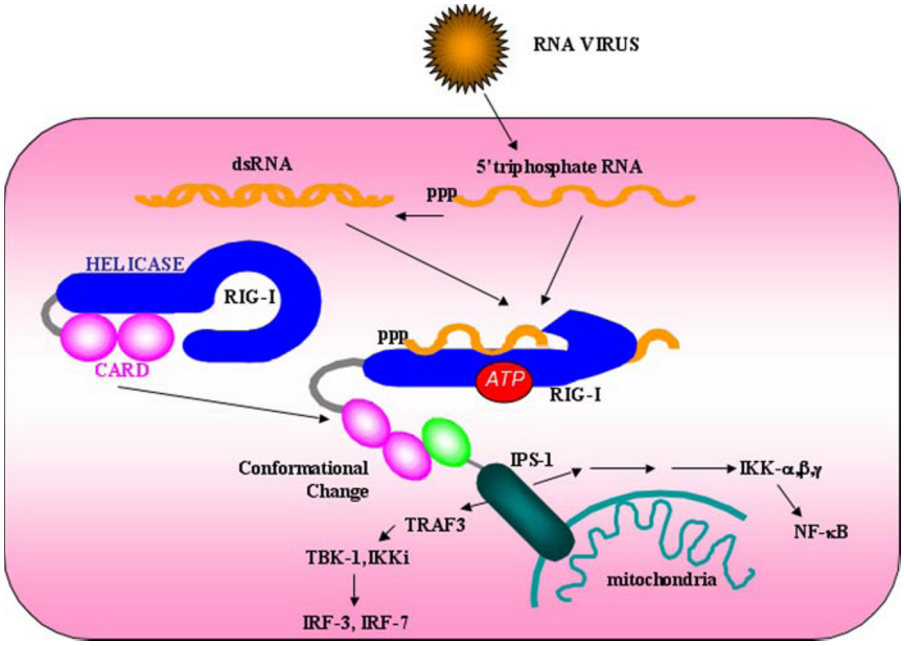

FIGURE 3. **Activation of RIG-I by viral RNA.** Model of RIG-I activation by viral RNA.

TABLE 2
Viral inhibitors of RLR signaling

Virus	Inhibitor	Mode of action	Refs.
Hepatitis C virus	NS3/4A	Cleavage of IPS-1	9, 12–14
Influenza A Virus	NS1	dsRNA binding, binding with RIG-I and IPS-1	6, 31–33
Ebola virus	VP35	dsRNA binding, inhibition of TBK-1, IKK-i	34
Paramyxo virus	V Protein	Binding to MDA5	2, 35, 36
Hanta virus (NY-1)	G1	Inhibition of TBK-1	37
West Nile virus	Unidentified	RIG-I-dependent and -independent pathway	38
Human cytomegalovirus	pp65 (ppUL83)	Inhibition of IRF-3 function	39
Herpes simplex virus	ICP0 and other	Inhibition of IRF-3 function	40
Human papilloma virus 16	E6	Inhibition of IRF-3 function	41
Vaccinia virus	E3L	Inhibition of IRF-3 function	42
Thogoto virus	ML	Inhibition of IRF-3 function	43

ture of substrate RNA is suggested (30); however, strict substrate requirements for the activation of RLR pathway and RNAi remain to be established.

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