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MINI-REVIEW

Retinoic acid inducible gene-I, more than a virus sensor

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ABSTRACT

Retinoic acid inducible gene-I (RIG-I) is a caspase recruitment domain (CARD) containing protein that acts as an intracellular RNA receptor and senses virus infection. After binding to double stranded RNA (dsRNA) or 5′-triphosphate single stranded RNA (ssRNA), RIG-I transforms into an open conformation, translocates onto mitochondria, and interacts with the downstream adaptor mitochondrial antiviral signaling (MAVS) to induce the production of type I interferon and inflammatory factors via IRF3/7 and NF-κB pathways, respectively. Recently, accumulating evidence suggests that RIG-I could function in non-viral systems and participate in a series of biological events, such as inflammation and inflammation related diseases, cell proliferation, apoptosis and even senescence. Here we review recent advances in antiviral study of RIG-I as well as the functions of RIG-I in other fields.

KEYWORDS retinoic acid inducible gene-I (RIG-I), antiviral signaling, inflammation, innate immunity

INTRODUCTION

RIG-I (retinoic acid inducible gene-I, also known as DDX58) was first discovered and cloned as an up-regulated gene in acute promyelocytic leukemia (APL) cell line NB4 upon all-*trans*-retinoic acid (ATRA) stimulation (Liu et al., 2000). It encodes a protein of 925 amino acids in human, which contains an N-terminal caspase recruitment domain (CARD), a DExD/H box helicase domain and a C-terminal repression domain (RD) (Yoneyama et al., 2004; Saito et al., 2007). RIG-I, together with MDA5 (melanoma differentiation associated gene 5) and LGP2 (laboratory of genetics and physiology 2), belong to RIG-I-like receptors (RLRs). They constitute an intracellular virus-sensing system to regulate type I interferon (IFN) production, which is independent of toll-like receptors (TLR) (Yoneyama et al., 2005).

The antiviral role of RIG-I has been well recognized, and most studies focus on its activation mechanism and downstream signaling pathway in antiviral response. However, like many other proteins, RIG-I has multiple functions that are involved in a variety of cellular and physiological processes. This review discusses the recent studies on the role of RIG-I in antiviral response as well as in inflammation, apoptosis and development.

SENSING VIRUS INFECTION

Viruses are infectious pathogens and are extremely harmful for human health. They can utilize the replication machinery of the host to replicate themselves and amplify infection. As the first line of host defense, innate immunity has several sensors to detect viruses and produce type I IFN to limit the infection. RIG-I is one of such virus sensors that recognizes double stranded RNA (dsRNA) and induces IFN-β production in a TLR-independent manner (Yoneyama et al., 2004). The different locations of TLRs and RLRs could detect different kinds of viruses. Unlike TLRs, which are membrane proteins and localized on the plasma membrane or endosome, RIG-I and other RLR family members are localized in cytoplasm and sense intracellular viral RNA (Yoneyama et al., 2005).

The structure and function of RIG-I

RIG-I contains two CARDs at N-terminus that holds the signaling activation property. Over-expression of these

tandem domains can potently drive IFN-β production in the absence of virus (Yoneyama et al., 2004; Saito et al., 2007). Recent RIG-I signaling reconstitution study shows that RIG-I CARD can also function as a receptor to bind the unanchored K63-linked unbiquitin chains in an RNA- and ATP-dependent manner, which is essential for the full activation of RIG-I (Zeng et al., 2010). In the middle of N-terminal CARD domain and C-terminal repressor domain, there is a helicase domain that contains an ATP binding motif and a TAS motif. The ATP binding motif is essential for RIG-I signaling because K270A mutation at this region disrupts IFN-β induction (Sumpter et al., 2005). The C-terminus of RIG-I contains a repression domain (Saito et al., 2007), also called regulatory domain (RD) (Cui et al., 2008). Over-expression of RD can inhibit the virus-induced IFN-β production. Moreover, the crystal structure of RD indicates that RD binds RNA and the zinc binding site in this domain is essential for RIG-I signaling (Cui et al., 2008). These results are consistent with the structural study of RIG-I C-terminal domain (CTD) by nuclear magnetic resonance (NMR) (Takahasi et al., 2008).

RIG-I is initially reported to bind dsRNA, which is considered to be the mechanism to distinguish self RNA and non-self infection (Yoneyama et al., 2004). Further study indicates that the dsRNA mimic poly (I:C) can only activate MDA5 but not RIG-I (Gitlin et al., 2006; Kato et al., 2006), while long dsRNA is the ligand for RIG-I (Kato et al., 2006). However, influenza A virus only generates single stranded RNA (ssRNA) rather than dsRNA, and it can also activate RIG-I, indicating that there should be a more accurate mechanism for RIG-I to distinguish self and non-self RNA (Pichlmair et al., 2006). Recent study also showed that RIG-I can recognize 5′-triphosphate RNA. However, if the 5′-triphosphate RNA is capped or modified by nucleoside, both types of post-transcriptional modifications fail to activate RIG-I (Hornung et al., 2006).

RIG-I functions as a virus sensor in a certain type of cells, such as fibroblasts, macrophages, conventional dendritic cells (cDC), but not in plasmacytoid dentritic cells (pDC) which use TLR system instead of the RIG-I-like receptor (RLR) system for the antiviral activity (Kato et al., 2005). As RNA binding proteins, RLRs mainly sense RNA virus. However, RIG-I and MDA5 have distinct spectra of viral detection. RIG-I mainly senses paramyxovirus, vesicular stomatitis virus (VSV) and influenza virus, while MDA5 mainly detects picornavirus (Kato et al., 2005; Kato et al., 2006).

RIG-I signaling pathway

Most knowledge of RIG-I signaling pathway comes from the related antiviral study. A CARD containing protein MAVS (also called VISA, IPS-1 or Cardif) that acts as RIG-I downstream adaptor was independently identified by four groups (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005). MAVS has an N-terminal CARD domain, a proline-rich region (PRO) and a C-terminal trans-membrane domain (TM) that is required for its mitochondrial localization and signaling. The CARD domain is essential for MAVS to initiate cellular signaling and to interact with RIG-I. Moreover, TM is also required for its signaling, and TM deletion results in the detaining of MAVS in cytosol and loss of its activity (Seth et al., 2005).

RIG-I pathway is further divided into two branches that activate IRF3 and NF-κB, respectively. MITA (also called STING) was identified as a downstream adaptor of MAVS (Ishikawa and Barber, 2008; Zhong et al., 2008). After binding MITA, MAVS recruits TBK1 and IKKε to phosphorylate IRF3 and IRF7. Activated IRF3 and IRF7 form either homo-dimers or hetero-dimers, and enter the nucleus to initiate type I IFN transcription. On the other hand, MAVS also binds TRAF6, FADD and RIP1, recruits IKKα, IKKβ and IKKγ, and finally activates NF-κB (Seth et al., 2005). Shorter form of zinc-finger CCCCH-type antiviral protein 1 (ZAPS) is a newly identified protein that associates with RIG-I and promotes RIG-I activity. Disruption of ZAPS causes impaired induction of IFNs and other cytokines, indicating that it is a key stimulatory factor of RIG-I (Hayakawa et al., 2011).

The regulation of RIG-I signaling

To avoid exaggerated IFN production, RIG-I signaling pathway is strictly regulated by several cellular mechanisms. LGP2 is the first molecule identified to suppress RIG-I signaling by competitively sequestrating dsRNA (Komuro and Horvath, 2006). However, genetic study demonstrates that LPG2 facilitates or enhances RIG-I signaling in certain virus infection (Venkataraman et al., 2007; Satoh et al., 2010). NS1 protein of influenza A virus inhibits RIG-I signaling through the interaction with RIG-I (Mibayashi et al., 2007). Autophage related protein Atg5-Atg12 complex and NLRC5 directly bind RIG-I and block the signaling (Jounai et al., 2007; Cui et al., 2010). NLRX1, gC1qR and PSMA7 are three newly identified proteins that inhibit RIG-I signaling (Moore et al., 2008; Jia et al., 2009; Xu et al., 2009). NLRX1 and gC1qR are localized on the outer membrane of mitochondria and interact with MAVS upon viral infection (Moore et al., 2008; Xu et al., 2009). NS3/4A of hepatitis C virus (HCV) cleaves MAVS at C508 and drives the export of MAVS from mitochondria, leading to inactivation of MAVS protein (Lin et al., 2006). A20, SIKE and FLN29 suppress RIG-I signaling through the interaction with other downstream molecules (Huang et al., 2005; Saitoh et al., 2005; Sanada et al., 2008).

Several post-translational modifications of RIG-I, including ubiquitylation, phosphorylation and SUMOylation, also regulate RIG-I signaling. The E3 ligase TRIM25 interacts with RIG-I and efficiently delivers the Lys63-linked ubiquitin moiety to its CARD, leading to increased activity of RIG-I signaling (Gack et al., 2007). Riplet/RNF135 ubiquitinates RIG-I and promotes IFN production both *in vitro* and *in vivo* (Oshiumi

et al., 2009, 2010). RNF125 is another ubiquitin E3 ligase that binds CARD and helicase domain of RIG-I and enhances the Lys48-linked ubiquitinization to cause RIG-I degradation (Arimoto et al., 2007). CYLD and DUBA are two RIG-I inhibitors with deubiquitinization activity, and both remove the Lys63-linked ubiquitin from RIG-I and TRAF3, respectively (Kayagaki et al., 2007; Friedman et al., 2008; Zhang et al., 2008a). A linear ubiquitin assembly complex (LUBAC) of HOIL-1L and HOIP specifically suppresses RIG-I activation by inducing TRIM25 degradation (Inn et al., 2011). Unlike most phosphorylation regulations, phosphorylated RIG-I is barely activated. Casein kinase 2 (CK2) phosphorylates RIG-I at T770, S854 and S855 in resting cells, which results in RIG-I inactivation. Once infected by RNA virus, but not DNA virus, these sites are dephosphorylated and RIG-I is consequently activated (Sun et al., 2011). Further study indicates that S8 and T170 of RIG-I are two other phosphorylation sites to keep RIG-I latent (Gack et al., 2010; NistalVillán et al., 2010). In addition to ubiquitylation and phosphorylation, recent study reports that RIG-I is also modified by small ubiquitin-like modifier-1 (SUMO-1), which enhances type I IFN production (Mi et al., 2010).

RIG-I AND INFLAMMATION

Inflammation is a defensive reaction for the host to remove harmful infection or repair damaged tissues. Acute inflammation is usually triggered by micro-organism infection and tissue injury. On the contrary, chronic inflammation is often accompanied with a variety of chronic diseases, such as type 2 diabetes and cardiovascular diseases (Medzhitov, 2008). In addition to its role in anti-viral immunity, emerged evidences also show that RIG-I participates in both acute and chronic inflammation.

Possible roles of RIG-I in inflammation and inflammation related diseases

Lipopolysaccharides (LPS) are large molecules localized at the outer membrane of Gram-negative bacteria, and they act as endotoxin to induce acute inflammation response by binding to TLR4 complex (Poltorak et al., 1998). Interestingly, RIG-I is found to be induced in LPS-stimulated endothelial cells. Moreover, over-expression of RIG-I also up-regulates cyclooxygenase-2 (COX-2) expression, indicating that RIG-I may have an important role in LPS-induced acute inflammation (Imaizumi et al., 2002). Several studies reveal that IFN-γ induces the expression of RIG-I in a variety of cell types, including human umbilical vein endothelial cells (HUVEC), vascular smooth muscle cells (SMC), urinary bladder epithelial cells, bronchial epithelial cells and pericardial mesothelial cells (Imaizumi et al., 2004a, 2004b, 2004c, 2005; Hatakeyama et al., 2007). In addition to IFN-γ, several pro-inflammatory factors are able to stimulate RIG-I expression. TNF-α and IFN-γ up-regulate RIG-I expression in keratinocytes, which is believed to be involved in psoriasis vulgaris (Kitamura et al., 2007). TNF-α alone can increase RIG-I expression in fibroblast-like synoviocytes in an IFN-β dependent manner (Imaizumi et al., 2009). Interleukin (IL)-1β drives the expression of RIG-I in human gingival fibroblasts (Sakaki et al., 2005). All these studies indicate that RIG-I is involved in the regulation of inflammation.

Higher level of RIG-I has been found in several inflammation related diseases, indicating its possible modulatory role in inflammation. Atherosclerosis is considered as an inflammatory disease, and the intimal macrophages in atherosclerotic lesions contain high level of RIG-I protein, suggesting that RIG-I may be involved in the activation of macrophage during atherosclerotic genesis (Imaizumi et al., 2007). In lupus nephritis patients, the expression level of RIG-I increases in the urinary sediment (Tsugawa et al., 2008; Imaizumi et al., 2010). Elevated RIG-I expression is also observed in synovial tissues of rheumatoid arthritis, suggesting a possible role of RIG-I in the pathogenesis of synovial inflammation (Imaizumi et al., 2008). Moreover, RIG-I knockout mice develop a colitis-like phenotype and RIG-I protein is shown to regulate T cell activation (Wang et al., 2007).

Regulatory mechanism of RIG-I in inflammation

Compared to the antiviral research, the mechanism of inflammation modulatory role of RIG-I is less studied. Until recently, several groups uncover the regulatory mechanism of RIG-I in acute and chronic inflammation. As a result of NF-κB activation, RIG-I induces the expression of inflammatory factors and chemokines, including IL-1β, IL-6, IL-8, IL-28, IL-29 and RANTES, in response to inflammatory stimulations (Kubota et al., 2006; Matikainen et al., 2006; Yoshida et al., 2007). In macrophage, RIG-I is induced by LPS through TRIF pathway, which partially results from IFN-β autocrine secretion. The induced RIG-I leads to activation of tumor necrosis factor (TNF)-α promoter and regulates LPS induced expression of TNF-α at late phase; thus, it acts as a key factor in the auto-loop cascade for the amplification of inflammatory factors (Wang et al., 2008). Our latest study reveals a novel role of RIG-I in senescence associated inflammation (Liu et al., 2011). Senescent cells secrete a spectrum of pro-inflammatory factors and chemokines, such as IL-6 and IL-8, which is called senescence associated secretory phenotype (SASP) (Coppé et al., 2008). The expression of RIG-I increases with cell passages through a mechanism relying on ATM-IRF1 activation. RIG-I mediated senescence associated inflammation requires its downstream adaptor MAVS, which activates NF-κB and AP-1 to induce the expression of inflammatory factors. An anti-ageing protein klotho suppresses senescence associated inflammation by directly interacting with RIG-I and blocking its multimer formation during senescence (Liu et al., 2011). Block of RIG-I signaling

not only inhibits senescence associated inflammation but also prolongs cell growth. It should be noted that RIG-I induced by non-viral factors only promotes the expression of inflammatory factors, but not the production of IFN-β. Therefore, the activation of NF-κB and IFN-stimulated response element (ISRE) by RIG-I should be regulated by different mechanisms.

OTHER POSSIBLE ROLES OF RIG-I

RIG-I was initially found as an inducible gene in a leukemia cell line treated with ATRA, which is an acknowledged agent to promote cell differentiation and widely used as an anti-cancer drug for leukemia (Liu et al., 2000). Therefore, it is possible that RIG-I is involved in some other cellular events such as carcinogenesis, apoptosis, senescence, cell differentiation and development.

RIG-I and cancer

It has been demonstrated that the level of RIG-I is quite low, even undetectable in most cancer cells such as prostate, breast, melanocyte and astrocyte malignant cells. However, RIG-I can be easily detected in their counterparts of normal cells (Su et al., 2007). RIG-I can be induced by IFN-γ, an immuno-modulatory factor, in human breast cancer cell MCF-7 and cervical cancer cell HeLa. Furthermore, RIG-I mediates the IFN-γ stimulation of ISG15 and CXCL11 in these cells, suggesting that RIG-I is involved in the immuno-modulatory function (Cui et al., 2004; Yuzawa et al., 2008). A micro-array screen of MDA-MB-435 human breast cancer cells showed that RIG-I is up-regulated in retinoic acid receptor β2 (RARβ2) transfected cells, indicating a possible role of RIG-I in anti-metastasis (Wallden et al., 2005). Viral infection can lead to carcinogenesis. As a viral RNA receptor, RIG-I and its signaling molecules are frequently targeted by different components of virus, which leads to virus-induced progression of malignant mesothelioma by increasing vascular endothelial growth factor (VEGF) production (Wörnle et al., 2009). These studies provide novel insight into the role of RIG-I in cancer development.

RIG-I and apoptosis

It has been reported that RIG-I is involved in the apoptosis induced by poly (I:C) or synthetic retinoid in hepatoma or melanoma cells (Pan et al., 2009; Peng et al., 2009). However, the underlying mechanism is poorly understood. IFN-β has a great potential to induce apoptosis. Although RIG-I signaling triggers the production of IFN-β, some reports show that RIG-I and MDA5 initiate a pro-apoptotic pathway independent of type I IFN. Noxa and downstream caspase-9, as well as Apaf-1, but not p53, are required for RIG-I induced apoptosis in melanoma cells, while in non-malignant cells, the RIG-I induced apoptosis can be blocked by the anti-apoptotic protein Bcl-xL (Besch et al., 2009).

RIG-I in development and proliferation

The involvement of RIG-I in development and proliferation mainly relies on the finding that RIG-I can be induced by ATRA in leukemia. However, in normal myelopoiesis without retinoic acid stimulation, RIG-I expression also increased in culture, suggesting a possible role of RIG-I in granulocytic differentiation. RIG-I deficient mice exhibit developmental disorder in myeloproliferation due to the down-regulation of IFN consensus sequence binding protein (Zhang et al., 2008b). Another recent study reveals a novel RIG-I pathway in regulating cell proliferation. Under the regulation of STAT1, RIG-I conversely augments the activation of STAT1 on ISG expression to inhibit the proliferation of leukemia cells, which is independent of MAVS signaling (Jiang et al., 2011).

RIG-I in phagocytosis

It is shown that RIG-I has a pro-phagocytosis role in bacterial infection. Knockdown of RIG-I leads to defects in phagocytosis related pathway and inhibits LPS-induced phagocytosis in macrophage. The function of RIG-I in phagocytosis is independent of MAVS, indicating the different functions of RIG-I and its signaling. RIG-I deficient mice are more susceptible to *Escherichia coli* infection, suggesting that RIG-I might also participate in the anti-bacterial immunity (Kong et al., 2009).

FUTURE PERSPECTIVES

It is suggested that RIG-I is "born" to be a stress protein induced by retinoic acid, just as described by its name. The stimulations that up-regulate RIG-I expression include double/single stranded RNA in virus infection (Kubota et al., 2006), LPS in bacterial infection (Imaizumi et al., 2002), interferons (Imaizumi et al., 2004a), TNF-α (Imaizumi et al., 2009), oxidized cholesterol (unpublished data) and even DNA damage in cell senescence (Liu et al., 2011). In addition, the expression of RIG-I is shown to be up-regulated in a series of chronic inflammatory diseases, such as atherosclerosis (Imaizumi et al., 2007) and arthritis (Imaizumi et al., 2008). To more accurately define the characteristics of RIG-I, we would rather call it a "stress" protein—it is inducible and activated under different "stresses." Nevertheless, compared to the role of RIG-I in antiviral response, the knowledge about RIG-I in other fields is limited. Extensive studies are required to answer the questions about the role of RIG-I in non-viral conditions. For example, how is RIG-I activated in non-RNA systems, such as inflammation and cell proliferation? Future studies may focus on the mechanisms of RNA-independent RIG-I activation.

ABBREVIATIONS

AP-1, activator protein 1; Apaf-1, apoptotic protease activating factor 1; ATM, ataxia telangiectasia mutated; ATP, adenosine triphosphate; Bcl-xL, B-cell lymphoma-extra large; Cardif, CARD adaptor inducing IFN-β; Caspase, cysteine-aspartic proteases; CXCL, chemokine (C-X-C motif) ligand; CYLD, cylindromatosis; DUBA, deubiquitinating enzyme A; FADD, Fas associated protein with death domain; gC1qR, receptor for global domain of complement 1 q subunit; IKK, IκB kinase; IPS-1, IFN-β promoter stimulator 1; IRF, interferon regulatory factor; ISG, interferon stimulated gene; MITA, mediator of IRF3 activation; NF-κB, nuclear factor κB; NLRC5, NOD-like receptor family CARD domain containing 5; NLRX1, NOD-like receptor family member X1; poly (I:C), polyriboinosinic:polyribocytidylic; PSMA7, proteasome subunit alpha type-7; RIP, receptor interacting protein; RNF, RING finger protein; RANTES, regulated upon activation, normal T-cell expressed, and secreted; SIKE, suppressor of IKKε; STAT, signal transducer and activator of transcription; STING, stimulator of interferon genes; TBK1, TANK binding kinase 1; TRAF, tumor necrosis factor receptor associated factor; TRIF, TIR domain containing adaptor protein inducing IFN-β; TRIM25, tripartite motif-containing protein 25; VISA, virus induced signaling adaptor

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