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REVIEW

Antiviral Signaling Through Retinoic Acid-Inducible Gene-I-Like Receptors

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Abstract The innate immune system is essential for the first line of host defense against micropathogens. In virus-infected cells, exposed viral nucleotides are sensed by pattern recognition receptors (PRRs), resulting in the induction of type I interferon. Retinoic acid-inducible gene-I-like receptors (RLRs) are a member of PRRs and are known to be crucial molecules in innate immune responses. Upon viral recognition, RLRs recruit their specific adaptor molecules, leading to the activation of antiviral signaling molecules including interferon regulatory factor-3 and nuclear factor-κB. Mitochondrial antiviral signaling (MAVS) protein is also known as one of the adaptor molecules responsible for antiviral signaling triggered by RLRs. Recent reports have identified numerous intracellular molecules involved in the antiviral responses mediated by RLRs/MAVS. Several viral proteins interfere with the RLR/MAVS signaling, allowing the virus to evade the host defense. In this review, we comprehensively update RLR-dependent antiviral signaling with special reference to the RLRs/MAVS-mediated responses.

Keywords Innate immunity · RLRs · MAVS

Introduction

The innate immune system constitutes the initial line of reactions, in mammalian cells, against invading pathogens. The micropathogens which gain entry into the cytoplasm are recognized by pattern recognition receptors (PRRs), which, in turn, activate the signal transduction cascades that lead to the expressions of a variety of cytokines (Iwasaki and Medzhitov 2004; Kawai and Akira 2006). Type I interferons (IFNs) are a group of cytokines that play a pivotal role in innate immunity with their antiviral, antiproliferative and immunomodulatory activities (Meurs and Breiman 2007). Therefore, over the past decade, many immunologists have focused their studies on the elucidation of the responses elicited through the microbes/PRRs/IFNs axis.

Toll-like receptors (TLRs) were the first PRRs to be identified and characterized on the basis of pathogen-associated molecular patterns (Akira et al. 2006). To date, ten distinct molecular species of TLRs (TLR1–TLR10) have been identified in humans. TLR3, TLR7, TLR8 and TLR9 are expressed in intracellular vesicles to sense nucleic acids from micropathogens (Trinchieri and Sher 2007). Especially, TLR3 senses double-stranded RNA (dsRNA) of viral origin; however, TLR3-null cells still have the ability to respond to polyinosinic–cytidylic acid (poly I:C), a synthetic dsRNA (Yoneyama et al. 2004), suggesting that additional cytoplasmic PRRs participate in the recognition of dsRNA.

Retinoic acid-inducible gene (RIG)-I was originally identified as one of the genes induced by *all-trans*-retinoic acid in leukemia cells (Liu et al. 2000). Our group found that RIG-I is induced by pro-inflammatory agonists including lipopolysaccharide and IFN-γ (Cui et al. 2004; Imaizumi et al. 2002), and Yoneyama et al. demonstrated that RIG-I is a sensor of intracellular dsRNA virus (Yoneyama et al. 2004). These reports prompted further studies focused on RIG-I and two other closely related molecules, melanoma differentiation-associated gene (MDA)-5 (Kovacsovics et al. 2002) and laboratory of

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genetics and physiology-2 (Cui et al. 2001), and these three molecules are now categorized as RIG-I-like receptors (RLRs). Both RIG-I and MDA-5 have a caspase recruitment domain (CARD), which is essential for the interaction with an adaptor molecule and the ensuing antiviral responses. Each member of RLRs recognizes specific viral RNA (Loo et al. 2008); so far, RIG-I appears to recognize more virus types as compared to MDA-5.

In the present review, we mainly focus on RIG-I since it is the best studied molecule of the members of RLRs.

Ligand Recognition by RLRs

Until recently, RIG-I has been thought to recognize only dsRNA; therefore, it was presumed that single-stranded (ss)RNA viruses were sensed by RIG-I during their replication whereby dsRNA is generated as a byproduct. However, Habjan et al. (2008) demonstrated that ss(−)RNA virus is unable to produce substantial amounts of dsRNA and formation of dsRNA might not be essential for the recognition of genomic RNA by RIG-I. Indeed, ssRNA harboring a 5′ppp group was demonstrated to be sufficient to activate the RIG-I-mediated type I IFN induction (Hornung et al. 2006; Pichlmair et al. 2006). Furthermore, very recently, 5′ppp-dsRNA was demonstrated to have much higher affinity to RIG-I than 5′ppp-ssRNA (Wang et al. 2010); and it seems appropriate, at present, to consider dsRNA with 5′ppp to serve as a biological target for viral recognition by RIG-I. Poly I:C, at first, was thought to activate type I IFN induction through MDA-5 (Kato et al. 2006); however, a further study demonstrated that a short length of poly I:C converts its PRRs from MDA-5 to RIG-I, suggesting that the length of dsRNA may be a critical determinant for each PRR in sensing an individual RNA virus (Kato et al. 2008). Encephalomyocarditis virus, a member of the *Picornaviridae* family, is a unique RNA virus which induces type I IFN via only the MDA-5-mediated pathway (Gitlin et al. 2006). If there is specificity of virus recognition by RLR, RIG-I and MDA-5 may recognize individual RNA viruses based on the length of replication intermediates of genomic RNA.

Based on its primary structure, RLRs are categorized into the DExH/D box RNA helicase family (Fig. 1). RIG-I contains two tandem CARDs in its N-terminal region. Introduction of RIG-I CARDs into cells results in type I IFN induction (Yoneyama et al. 2004), indicating that CARD is essential for the antiviral signaling. The middle part of RIG-I contains the DExH box-helicase domain, which is then connected to the C-terminal repressor domain. A CARD-deleted, artificial form of RIG-I exerts a dominant negative effect on the antiviral signaling (Yoneyama et al. 2004). Indeed, structural analyses of RIG-I showed that the C-terminal domain (CTD; aa 792–925), which is almost overlapping with the repressor domain, has functions as both an RNA recognition domain and a repressor domain (Cui et al. 2008; Takahasi et al. 2008). Under the resting condition, the CARDs are enveloped in other domains of the RIG-I molecule. Once a ligand is recognized by the CTD, the repressor domain induces a conformational change in the RIG-I molecule to expose CARDs on the surface of the molecule, allowing them to mediate antiviral signaling to its downstream pathway (Cui et al. 2008).

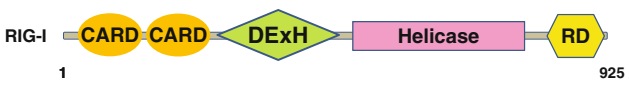

Fig. 1 Structure of RIG-I. RIG-I contains two CARDs, DExH/D box, helicase domain and C-terminal repressor domain (RD)

Modification of RIG-I by Ubiquitin and Ubiquitin-like Proteins

Protein ubiquitination has emerged as a key mechanism for the regulation of immune responses (Bhoj and Chen 2009). Recent studies have shown that RIG-I is also ubiquitinated to modulate antiviral signaling. TRIM25, a member of the tripartite motif (TRIM) protein family (Reymond et al. 2001), introduces a lysine-63-linked ubiquitin moiety to the CARD of RIG-I (Gack et al. 2007), and the lysine-63-linked ubiquitination is suggested to serve as a scaffold for the downstream signaling assembly (Wertz and Dixit 2010). Also cells lacking TRIM25 have impaired production of type I IFNs in response to viral infection, indicating that the TRIM25 ubiquitination is essential for the antiviral signaling mediated by RIG-I. Recently Riplet/RNF135 has also been demonstrated to catalyze lysine-63-linked polyubiquitination of the CTD of RIG-I (Oshiumi et al. 2009), however, it is not clear how this modification regulates the downstream signaling.

RIG-I also undergoes proteasomal degradation after conjugation with ubiquitin. A study using a yeast-two hybrid screening identified UbcH8, a ubiquitin E2 conjugating enzyme, and RNF125, a ubiquitin E3 ligase, as interacting with RIG-I (Arimoto et al. 2007). RNF125 efficiently conjugates ubiquitin to CARD of RIG-I, resulting in the degradation of RIG-I. In mass spectrometry analysis, we found that heat shock protein 90 (HSP90) interacts with RIG-I (Matsumiya et al. 2009). Interestingly, HSP90 bound to multiple sites of the RIG-I molecule except CARD. Geldanamycin, an HSP90 inhibitor, accelerated RIG-I degradation and inhibited dsRNA-induced IFN-β expression, suggesting that HSP90 protects RIG-I from protein degradation.

IFN-stimulated gene 15 (ISG15), a member of the ubiquitin-like protein family (Haas et al. 1987), is also conjugated to RIG-I (Zhao et al. 2005). Conjugation of cellular proteins with ISG15 (ISGylation) involves the coordinated activities of the enzymes in the ubiquitin system: activating enzymes E1, ubiquitin conjugating enzymes E2 and ubiquitin ligases E3 (Huang et al. 2004). Cellular levels of RIG-I protein are lowered by ISGylation with these components (Kim et al. 2008). Upon treatment with a proteasome inhibitor, however, no marked change in ISGylated protein level was observed, suggesting that ISG15 conjugation is not a specific signal for proteasome-dependent protein degradation (Malakhov et al. 2003).

Mitochondrial Antiviral Signaling Mediates RLR Signaling

In 2005, an adaptor molecule linked to RIG-I was identified independently by four groups, and the molecule was designated IPS-1 (Kawai et al. 2005), MAVS (Seth et al. 2005), VISA (Xu et al. 2005) or Cardif (Meylan et al. 2005) (referred to as MAVS in this review). Mitochondrial antiviral signaling (MAVS) is originally identified by a large-scale screening of human genes that activate the nuclear factor (NF)-κB signaling pathway (Matsuda et al. 2003). MAVS consists of an N-terminal CARD, a proline-rich region near the N-terminus, and a C-terminal transmembrane (TM) domain. The TM allows MAVS to localize onto the mitochondrial outer membrane, suggesting a crucial role for mitochondria as a platform for the signaling molecules (Seth et al. 2005). Very recently, MAVS was also identified on the peroxisome membrane (Dixit et al. 2010), and it is likely that peroxisomal MAVS is required for the rapid induction of antiviral effectors and mitochondrial MAVS for a sustained response.

The overall RIG-I/MAVS signaling pathway is schematically summarized in Fig. 2. Upon viral recognition, the RIG-I molecule changes its conformation to expose the CARD (Cui et al. 2008; Myong et al. 2009). The CARD of RIG-I interacts with the CARD of MAVS, resulting in the RIG-I-dependent induction of IFN-β (Kawai et al. 2005; Meylan et al. 2005; Seth et al. 2005; Xu et al. 2005).

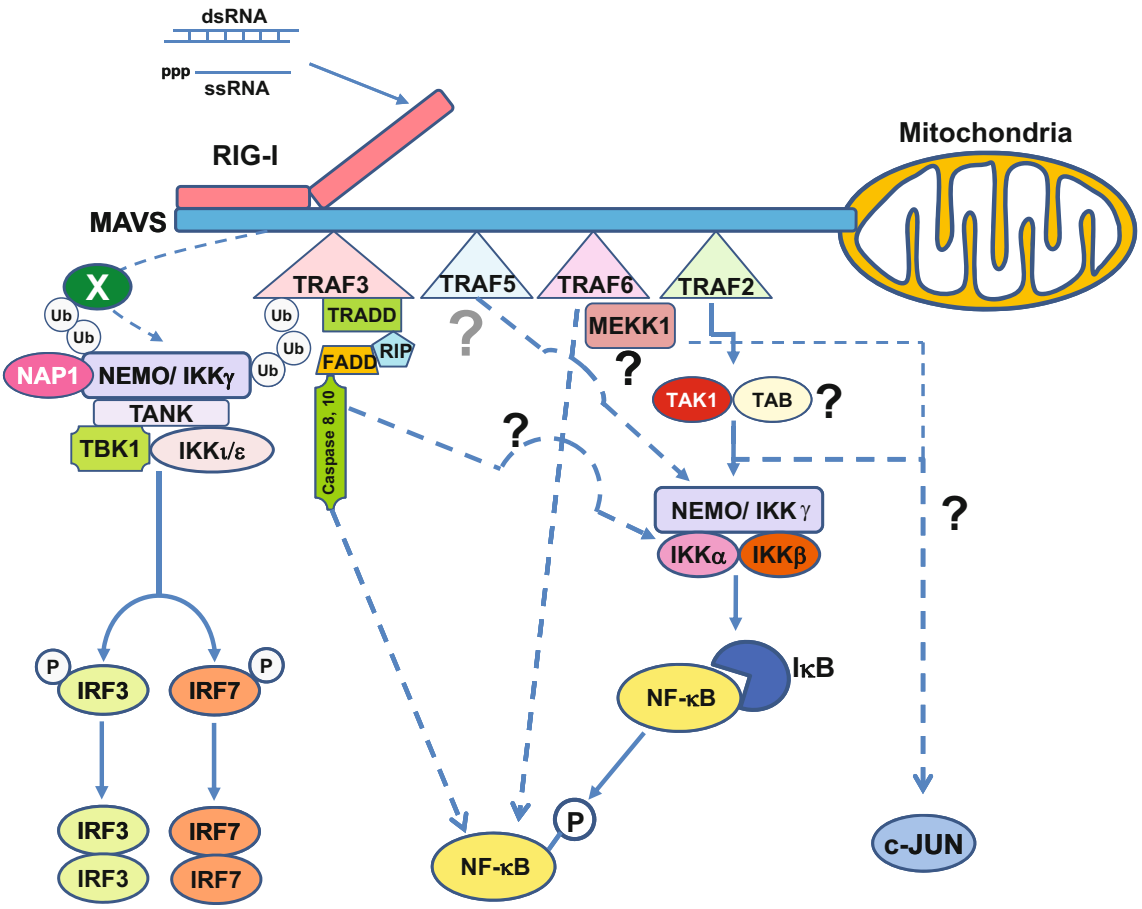

Fig. 2 Overview of the RIG-I/MAVS-mediated signaling pathway. Abbreviations are defined in the text

TRAFs Associated with MAVS

Over a decade ago (Lomaga et al. 1999; Naito et al. 1999), the family of tumor necrosis factor (TNF) receptor-associated factor (TRAF) was described as a mediator of TLR-dependent signaling, and TRAF is now considered to be involved in the innate immune system (Chung et al. 2002). As also shown in Fig. 2, MAVS was found to interact with TRAF2 and TRAF6 in a yeast two-hybrid system (Xu et al. 2005). Moreover, TRAF2-deficient mouse embryonic fibroblasts have impaired RIG-I-dependent induction of IFN-β in response to viral infection (Mikkelsen et al. 2009). However, the downstream signaling of the MAVS–TRAF2/TRAF6 pathway is still unclear.

TRAF3, another member of the TRAF family, has been recently characterized as a crucial molecule in the antiviral responses in both TLR-dependent and -independent manners (Oganesyan et al. 2006). Although TRAF3 is structurally similar to TRAF2 and TRAF5, overexpression of TRAF3 does not activate NF-κB, whereas TRAF2 does (Rothe et al. 1995). Consistent with this early observation, Saha and et al. reported that TRAF3 is not involved in the activation of NF-κB in response to viral infection (Saha et al. 2006). The C-terminal TRAF domain of TRAF3 interacts with the TRAF-interaction motif in MAVS (Saha et al. 2006). Interestingly, a reconstitution experiment between TRAF3 and TRAF5, structurally nearly identical to TRAF3 (Chung et al. 2002), showed that replacement of the C-terminal TRAF domain of TRAF3 with that of TRAF5 impaired the induction of IFN-α in response to viral infection (Saha et al. 2006). Thus, TRAF3 may be essential for the MAVS-mediated activation of IFN regulatory factor (IRF)3/7, which leads to a type I IFN response.

Lysine-63 ubiquitination was shown to be essential for viral activation of IRF3 (Zeng et al. 2009). Kayagaki et al. (2007) performed an siRNA-based screening for members of deubiquitinating enzymes that regulate type I IFN expression and found that silencing of deubiquitinating enzyme A (DUBA) augments the proline-rich region-induced type I IFN response. DUBA was further demonstrated to selectively cleave the polyubiquitin chains of the lysine-63 residue of TRAF3. Deubiquitinated TRAF3 dissociates from the downstream signaling assembly including TBK1, indicating that DUBA is a negative modulator in the MAVS-mediated signaling pathway (Kayagaki et al. 2007). A kinetic study of TRAF3 ubiquitination in cells subjected to viral infection shows biphasic polyubiquitination: early lysine-63-linked and late lysine-48-linked (Nakhaei et al. 2009). Consistent with this observation, E3 ligase Triad3A promotes lysine-48-linked polyubiquitination of TRAF3, leading to, at a later phase, its proteasomal degradation and dissociation from MAVS (Nakhaei et al. 2009).

Very recently, TRAF5 has been identified as a downstream target of MAVS (Tang and Wang 2010), of which dimerization brings about the activation of TRAF5 and TRAF3 by their auto-ubiquitination. Activation of these TRAFs, in turn, leads to the recruitment of NF-κB essential modulator (NEMO, also known as IKKγ) via its ubiquitin binding domain by two distinct pathways: ubiquitination of both TRAF3 and TRAF5 recruits NEMO/TANK/TBK1, resulting in the activation of IRF3, and ubiquitination of TRAF5 alone recruits NEMO/IKKα/IKKβ to activate NF-κB (Tang et al. 2010). From these observations, it appears that MAVS uses different members of TRAF in different types of cells, which may be regarded as one of the strategies for securing the diversity of cellular responses.

NEMO as an Adaptor Bridge

NEMO/IKKγ is a canonical IKK (IκB kinase) and forms the IKK complex (Yamaoka et al. 1998). A study with NEMO-deficient mice showed that NEMO is essential for NF-κB activation in response to viral infection (Rudolph et al. 2000). In fact, mutations in NEMO are responsible for X-linked recessive Mendelian susceptibility to mycobacterial diseases and X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency, both of which are characterized by susceptibility to multiple infections (Filipe-Santos et al. 2006; Smahi et al. 2000). NEMO, therefore, has been considered to play an important role in innate immunity; and in 2007, NEMO was demonstrated to mediate RIG-I/MAVS-dependent type I IFN induction in response to viral infection (Zhao et al. 2007). NEMO physically interacts with TANK to mediate the recruitment of IKKε/IKK$\imath$ and TBK1 to the RIG-I–MAVS complex (Zhao et al. 2007). Such a role of TANK is consistent with the previous report that overexpression of IKKε/IKK$\imath$ and TBK1 was insufficient to elicit the interaction with NEMO without coexpression of TANK (Chariot et al. 2002). Moreover, an artificial mutant of NEMO lacking the TANK-binding domain fails to recruit IKKε/IKK$\imath$ to the RIG-I/MAVS complex, resulting in inefficient type I IFN induction in response to viral infection (Zhao et al. 2007). However, according to a recent study on *Tank*$^{-/-}$ cells (Kawagoe et al. 2009), TANK is not essential for the activation of the signaling pathways by RLRs (Kawagoe et al. 2009). The most credible explanation for this discrepancy may be the difference in the types of cells studied: the former group used HEK 293 cells and the latter used conventional dendritic cells derived from bone marrow cells. NAK-associated protein (NAP-1), initially characterized as an activator of IKK-related kinases and found to interact with IKKε/IKK$\imath$ and NAK (or TBK1) (Fujita et al. 2003), also physically interacts with MAVS (Sasai et al. 2006). Structurally,

NAP-1 is 20.3% identical to NEMO and 18.2% to TANK. The conserved regions are located within the expected coiled-coil domain, and these three molecules may bind the kinase molecules to regulate the downstream signaling (Fujita et al. 2003; May et al. 2000). However, whether NAP-1 physically interacts with NEMO remains unclear.

Recently, NEMO has been found to sense lysine-63-linked polyubiquitination; furthermore, lysine-63-, but not lysine-48-linked polyubiquitination is required to activate the IKK complex in the TNF-α-mediated NF-κB signaling pathway (Ea et al. 2006; Wu et al. 2006). Likewise, NEMO also senses the lysine-63-linked polyubiquitinated TRAF3 in RIG-I/MAVS signaling, leading to the activation of IRF3, IRF7 and NF-κB (Zeng et al. 2009). This activation of IRF3 requires a ubiquitin-conjugating enzyme, Ubc5, and two ubiquitin-binding domains of NEMO (Zeng et al. 2009). Silencing of Ubc5 inhibits the activation of IRF3 in response to viral infection but has no effect on the interaction of NEMO with TANK and TBK1, suggesting that the ubiquitination is dispensable for NEMO to recruit IKKε/IKKι and TBK1 to the RIG-I–MAVS complex. Although auto-ubiquitinated TRAF3 enhances its binding to NEMO, TRAF3 deficiency does not completely block IFN-β induction or IRF3 activation (Zeng et al. 2009). This suggests that NEMO may recognize polyubiquitinated molecule(s) not yet elucidated, since lysine-63-linked ubiquitination appears to be essential for type I IFN induction.

Death Signal and RIG-I/MAVS Pathway

Members of the TNF receptor (TNFR) family, including Fas and TNFR, contain an intercellular death domain (DD) and trigger apoptosis (Strasser et al. 2009). Fas, for instance, transduces its activation to DD containing protein, namely Fas-associated death domain (FADD), and initiates apoptosis (Chinnaiyan et al. 1995). Recently, cells rendered deficient in FADD or receptor-interacting protein 1 (RIP1), another DD-containing protein, were found to lack the type I IFN response to dsRNA treatment (Balachandran et al. 2004). These findings suggest that DD-containing proteins are involved in innate immunity. Indeed, FADD and RIP1 have been demonstrated to interact with the C-terminal portion of MAVS (Kawai et al. 2005). Furthermore, dsRNA is unable to induce type I IFN or to activate NF-κB signaling in cells lacking caspase-8 or -10, both of which possess a death effector domain which interacts with FADD (Takahashi et al. 2006). Caspase-8 and -10 are cleaved and activated in the cells treated with dsRNA; however, whether the caspases link to NF-κB directly or through the IKK complex remains to be elucidated.

The TNFR-associated death domain (TRADD), a crucial adaptor of TNFRI, was also demonstrated to be essential for the RIG-I/MAVS-dependent signaling pathway. MAVS requires TRADD in the interaction with FADD. In addition, TRADD interacts with TANK and TRAF3, suggesting that TRADD serves as an adaptor molecule in the RIG–MAVS assembly as well as in the TNFRI complex (Michallet et al. 2008). Interestingly, TRADD does not interact with IKKε/IKKι; however, introduction, into cells, of exogenous TRADD enhances IRF-3- and IRF-7-induced type I IFN responses (Michallet et al. 2008). Thus TRADD is considered to mediate the proinflammatory response by forming a signaling complex (TRADDosome) with other adaptor molecules such as MAVS, TRAF (presumably TRAF3), TANK, RIP1 and FADD. Then the signal is transmitted to IRFs (IRF-3 and -7) and NF-κB through the NEMO/TBK1/IKKε/IKKι complex (Michallet et al. 2008).

Involvement of Mitogen-Activated Protein Kinase in RIG-I/MAVS Signaling

Although transcription of IFN-β requires the coordinate activation of IRF3, NF-κB and ATF-2 (Lomvardas and Thanos 2002), little is known about the role of the RIG-I/MAVS-mediated mitogen-activated protein (MAP) kinase pathway. There have been two reports focusing on the involvement of MAP kinase in the signaling through RIG-I and MAVS (Mikkelsen et al. 2009; Yoshida et al. 2008). One of these studies (Yoshida et al. 2008) reported that MEKK1, MAPKKK, and TRAF6 were required to activate Jun N-terminal kinase and p38 MAP kinase, both of which are essential for ATF-2/c-Jun activation (Junttila et al. 2008), followed by IFN-β induction through the RIG-I/MAVS signaling in response to viral infection. Furthermore, TRAF2 and TAK1 (another type of MAPKKK) were shown to be required for RIG-I-mediated p38 MAP kinase activation (Mikkelsen et al. 2009). Although both of these reports proposed models of a TRAF–MAPKKK cascade, no direct evidence has been provided for an interaction between TRAF proteins and MAPKKK in RIG-I/MAVS signaling. Studies using TRAF2- or TAK1-deficient mouse embryonic fibroblasts indicate that these molecules are likely to influence the activation of p38 MAP kinase and NF-κB followed by IFN-β induction in response to Sendai virus infection (Mikkelsen et al. 2009); however, another group demonstrated that TAK1 does not participate in the RIG-I–MAVS-dependent IFN-β induction, because TAK1-deficient cells infected with vesicular stomatitis virus are still capable of activating NF-κB and IRF3 followed by IFN-β induction (Mikkelsen et al. 2009; Yoshida et al. 2008). This inconsistency may reflect the types of virus used, which may have differentially interfered with TRAF2/6 in the RIG-I/MAVS signaling. Thus, there is still much to be elucidated on the role of MAP kinase in the RIG-I/MAVS pathway.

Escape of Viruses from RIG-I-Dependent Antiviral Responses

At first glance, it seems plausible that the RIG-I-mediated innate immune responses could overcome all RNA viruses, but in fact it is not so simple. The ways viruses evade the RIG-I-mediated innate immune responses may be divided roughly into two strategies: escape from sensing by RIG-I, and inhibition of RIG-I–MAVS signaling by viral proteins. In either case, it is very important for viruses not to let host cells upregulate type I IFN, which induces ISGs, executors of antiviral activities (de Veer et al. 2001; Sadler and Williams 2008). ISGs exert antiviral activities mainly through degradation of viral RNA or inhibition of viral protein translation; for example, 2′–5′ oligoadenylate synthetase activates the latent ribonuclease RNase L degrading viral RNA, and protein kinase R inhibits viral protein translation.

The first line of countermeasures of viruses against RIG-I-mediated innate immunity could be an attack on RLRs themselves. RIG-I has been found to be cleaved during picornavirus (Barral et al. 2009a) and cytomegalovirus (Nakhaei et al. 2009) infection. Interestingly, RIG-I senses neither picornavirus (Loo et al. 2008) nor perhaps cytomegalovirus, a DNA virus. Paramyxovirus V proteins specifically suppress activation of MDA-5, whereas paramyxovirus C proteins are a specific inhibitor for RIG-I (Childs et al. 2007; Strahle et al. 2007). Recently, V proteins were shown to block MDA-5 from dsRNA binding and consequent self-association (Childs et al. 2009). Another potential target for evading antiviral responses may be MAVS. NS3/4A protease of hepatitis C virus has been known to interfere with antiviral responses (Foy et al. 2003) since before the identification of RIG-I and MAVS as mediators of antiviral signaling. Indeed, NS3/4A suppresses RIG-I dependent type I IFN expression (Breiman et al. 2005; Foy et al. 2005) and the inhibition is due to cleavage of MAVS by NS3/4A (Loo et al. 2006). Recent studies have demonstrated that picornaviruses also cleave MAVS (Yang et al. 2007). Hepatitis A virus protease 3ABC cleaves MAVS in a manner similar to that of NS3/4A (Yang et al. 2007). Poliovirus infection triggers a caspase-dependent cleavage of MAVS (Rebsamen et al. 2008), whereas rhinovirus degrades MAVS in a caspase-independent manner (Drahos and Racaniello 2009). In fact, overexpression of caspase inhibitors or the anti-apoptotic factor Bcl-xL prevents MAVS cleavage during viral infection, suggesting that MAVS may be involved in apoptosis of virus-infected cells (Scott and Norris 2008).

The other viral countermeasures against RLRs/MAVS signaling, especially the pathways downstream to MAVS, are well illustrated in the review article by Barral et al. (2009b).

Concluding Remarks

RLR-mediated innate immune responses are currently one of the most actively studied subjects in the field of immunology. However, there are at least two things which have to be clarified. Firstly, both the RLR and TLR systems exert innate immune responses in a cell type-specific manner (Kato et al. 2005), and the balance or synergy between these pathways in antiviral signaling remains to be elucidated. It may be particularly interesting whether there is crosstalk between RLR/MAVS and TLR/Toll-interleukin-1 receptor domain-containing adaptor-inducing IFN-β. Secondly, why does antiviral signaling proceed on the surface of mitochondria and peroxisomes? Several lines of evidence indicate that the surface of these organelles is crucial in innate immune signaling; however, no clear rationale for these platforms is proposed. Ongoing research will continue to explore these questions underlying RLR/MAVS-mediated antiviral signaling.

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