Mechanisms of RIG-I-Like Receptor Activation and Manipulation by Viral Pathogens

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RIG-I-like receptors (RLRs) play important roles in the host defense to numerous viral pathogens. Since they were discovered, much light has been shed on the molecular details of how these cytoplasmic viral RNA receptors sense viral infection and orchestrate antiviral innate immunity. Intriguingly, in addition to viral RNA binding, a series of posttranslational modifications (PTMs) is required for the rapid activation of RLRs and, inversely, for the prevention of aberrant innate immune signaling. Recent discoveries have shown that viruses manipulate the PTMs of RLRs to escape innate immune detection. This article highlights some of these recent findings in this fast-evolving field.

RIG-I-LIKE RECEPTOR (RLR)-MEDIATED SENSING OF VIRAL INFECTION

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or the detection of viral pathogens, mammalian cells are equipped with a sophisticated immune surveillance apparatus comprised of a defined repertoire of molecular sensors, classically termed pattern-recognition receptors (PRRs). Upon recognition of pathogen-associated molecular patterns (PAMPs), PRRs initiate downstream signaling that results in the gene expression of antiviral molecules and many cytokines, including type I interferons (alpha/beta interferons [IFN-α/β]). Through the upregulation of interferon-stimulated genes (ISGs), IFNs then induce an antiviral state in both infected and uninfected cells, as well as tailor adaptive immune responses.

At least three main classes of PRRs have been implicated in the detection of viral nucleic acid: (i) Toll-like receptors (TLRs), which sense incoming virions in endolysosomes by binding to viral RNA (TLR3 and -7/8) or CpG-containing DNA (TLR9), (ii) the recently identified, structurally diverse group of viral DNA sensors, including cGAS, IFI16, and DAI, and (iii) RIG-I-like receptors (RLRs), identified in 2004, that are essential for the detection of viral RNA in the cytoplasm of most cell types.

RLRs, comprising RIG-I, MDA5, and LGP2, are characterized by a conserved domain structure, consisting of a central DExD/H-box helicase domain and a C-terminal domain (CTD), both of which are responsible for binding viral RNA. In addition, RIG-I and MDA5 harbor two N-terminal caspase activation and recruitment domains (CARDs) which, upon virus sensing, initiate downstream signaling, leading to type I IFN gene expression. In contrast, LGP2 lacks the CARD signaling module and has been shown to exert a regulatory role in RLR signaling; its precise action, however, is yet to be defined (reviewed in reference 1).

Virus replication studies revealed that RIG-I confers resistance to many negative-sense RNA viruses, including orthomyxoviruses, rhabdoviruses, bunyaviruses, and paramyxoviruses as well as the positive-strand hepatitis C virus (HCV); in contrast, MDA5 was shown to primarily detect members of the Picornaviridae and Caliciviridae families. Despite these early studies suggesting that RIG-I and MDA5 detect mainly nonoverlapping subsets of viral pathogens, there is new evidence that numerous viruses, including dengue virus, West Nile virus (WNV), reoviruses, and several paramyxoviruses (e.g., measles virus and Sendai virus [SeV]), are sensed by both RIG-I and MDA5 (reviewed in reference 2). Furthermore, studies using synthetic or purified viral RNA revealed important molecular signatures that are required for RLR activation. It is now well established that a 5′ triphosphate (5′ppp) moiety, present in the genomic RNA of many viruses, in concert with short blunt-end double-stranded RNA (dsRNA) stretches, such as “panhandle” structures, are critical for RIG-I’s ability to discriminate non-self from self RNA. The sequence composition of the RNA ligand also seems to play a role in RIG-I activation: for example, poly(U/UC) motifs found in the genomic RNA of HCV were shown to stimulate RIG-I when combined with a 5′ppp group. In addition to the detection of RNA viruses, RIG-I also has been shown to contribute to the detection of DNA viruses, such as Epstein-Barr virus, by recognizing 5′ppp-containing small RNA species generated through transcription of viral DNA by RNA polymerase III. In contrast to RIG-I agonists, the characteristics of the RNA ligands sensed by MDA5 are largely unknown. The current view is that MDA5 recognizes long dsRNA organized in web-like structures, as found in picornavirus-infected cells (2).

Despite these important insights into the distinct viral RNA structures that can trigger RLR activation, the important question of what the physiological ligand during an actual viral infection is has just begun to be elucidated. Next-generation sequencing of viral RNA complexed with RIG-I in cells infected with influenza A virus (IAV) or SeV confirmed that short 5′ppp-containing viral RNAs produced during replication are physiological ligands for RIG-I (2). In addition to sensing viral replication products, can RIG-I also recognize virion RNA that is tightly packed with viral proteins? This question was recently answered by Weber et al., who showed that the nucleoprotein-encapsidated 5′ppp-RNA of incoming virions triggers RIG-I activation immediately after entry into the cell (3). Together, these studies indicate that during probably most viral infections, multiple RNA species—internalized with the virion and produced during viral replication—distinctly...
trigger RIG-I and/or MDA5 activation, likely at different time points during infection. In support of this model, distinct viral RNA products generated during WNV infection were shown to sequentially stimulate RIG-I and MDA5 activation: RIG-I early during infection, and MDA5 at later time points (4). More-detailed studies are needed to identify the authentic RNA ligands for RIG-I and MDA5 and to define the contributions and dynamics of action of these two sensors during other viral infections.

**INTERPLAY OF UBQUITINATION AND PHOSPHORYLATION REGULATES RLR SIGNALING**

Upon viral RNA recognition by the CTD and helicase, RIG-I and MDA5 initiate antiviral signaling by interacting through their CARDs with the CARD of the adaptor protein MAVS (also called IPS-1, VISA, or CARDIF). MAVS contains a transmembrane domain that anchors it to both mitochondria and peroxisomes. Intriguingly, from these two organelles, MAVS was shown to induce a biphasic antiviral response (5). From peroxisomes, MAVS acti-

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MANIPULATION OF RLR’S SIGNALING ACTIVITIES BY VIRAL PATHOGENS

Coevolution with their hosts enabled successful viral pathogens to manipulate and shape innate immune responses for their own benefit. Many different viral strategies for RLR evasion have been identified, including modification of the 5’ppp signature moiety in the viral genome and inhibition of key signaling molecules downstream of RLRs, such as MAVS. Recent studies provided evidence that viruses manipulate critical PTMs of RLRs to escape innate immunity. Several viruses have been shown to specifically modulate the Lys63-linked ubiquitination of RIG-I through targeting of the E3 ligases TRIM25 and Riplet. IAV, using its NS1 protein, targets TRIM25 through a direct interaction with its coiled domain (CCD) (14). Mechanistically, NS1 binding to the CCD prevents TRIM25 from self-assembling into its oligomeric, enzymatically active form, thereby suppressing RIG-I CARD ubiquitination. Interestingly, the NS1 proteins of some strains of IAV also target Riplet, thereby blocking ubiquitination of RIG-I at the CTD (15). Moreover, it has been reported recently that the NS3-4A protease of HCV targets Riplet, but not TRIM25, for cleavage (9). Thus, HCV NS3-4A blunts RIG-I signaling at two distinct steps: by cleaving MAVS, as previously reported, and by cleaving Riplet. Furthermore, Kaposi’s sarcoma-associated herpesvirus, arteriviruses, and noroviruses encode viral deubiquitinating enzymes to actively remove Lys63-linked ubiquitin chains from the RIG-I-CARDS, thereby suppressing downstream signaling (16, 17). As phosphorylation/dephosphorylation of RLRs is critical for their immune signaling ability, it is conceivable that viruses have also evolved means of manipulating the RLR phosphorylation state. To keep RLRs in the phosphorylated, inactive state, viruses may either directly induce RLR phosphorylation or block their dephosphorylation by PP1α/γ. Indeed, while encephalomyocarditis virus and poly(I-C)-RNA efficiently triggered RLR dephosphorylation, indicating that these viruses manipulate the RLR phosphorylation state to escape immune detection (M. E. Davis, M. K. Wang, L. J. Rennick, F. Full, S. Gablese, A. W. Mesman, S. I. Gringhuis, T. B. H. Geijtenbeek, W. P. Duprex, and M. U. Gack, submitted for publication).

CONCLUSION

The recent discovery of specific PTMs that marks determine the signaling “on” or “off” state of RIG-I and MDA5 may greatly facilitate research investigating RLR activation during viral infection and pathological conditions, such as autoimmune disease. New insights into the host regulatory mechanisms of RLR signaling may stimulate drug development designed to either boost antiviral signaling or dampen it in situations where the RLR response has gone awry. Furthermore, the discovery of novel virus-host interactions to escape the RLR response may open up novel therapeutic avenues for infectious diseases.

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REFERENCES


