RESEARCH HIGHLIGHT

Long noncoding RNA: TRIMming the viral load

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Viruses are obligate intracellular entities that require a living host and its machinery for replication. During replication, viral components known as pathogen-associated molecular patterns (PAMPs) are sensed by a family of innate immune sensors or pattern recognition receptors (PRRs) and promote an antiviral state. Viral nucleic acids are one of the key PAMPs sensed by cytosolic RIG-I-like receptors (RLRs), Cyclic GMP-AMP Synthase (cGAS), Absent In Melanoma 2 (AIM2), Interferon Gamma Inducible Protein 16 (IFI16), and Z-DNA Binding Protein 1 (ZBP1 or DAI) and endosome-localized Toll-like receptors (TLR3, 7, 8, and 9).[1] Recognition of viral RNA by Retinoic acid-inducible gene I (RIG-I), Melanoma differentiation associated gene 5 (MDA5), and TLR3, 7, and 8 or viral DNA by TLR9, cGAS, AIM2, IFI16, and DAI triggers a cascade of signaling events to recruit transcription factors, interferon regulatory factors and NF-κB. Activated IRF3/7 and NF-κB translocate to the nucleus and induce expression of type I and III interferons and proinflammatory cytokines, respectively.[1] The production of these interferons is further enhanced in an autocrine and paracrine manner through the JAK-STAT signaling pathway. Type I and III interferons further induce interferon-inducible genes, and together with proinflammatory cytokines, they develop an antiviral state. The antiviral state is characterized by by apoptosis of virally infected cells and inhibition of the cellular protein synthesis machinery, making the uninfected cells resistant to viral infection and initiates virus-specific adaptive immune responses (Fig. 1a).

The cytosolic sensors RLRs consist of three members, (RIG-I, MDA5, and Laboratory of genetics and physiology 2 (LGP2), which recognize viral RNA. These sensors contain a DExD/H-box RNA helicase domain at the C-terminus with an ATP binding motif. RIG-I and MDA5 additionally possess two caspase activation and recruitment domains (CARDs) at the N-terminus, which are required for homotypic CARD-CARD interactions and downstream signaling. LGP2 lacks the CARD and therefore is considered to acts as a negative regulator of the RLR pathway by sequestering RNA away from RIG-I and MDA5.[2] However, in vivo genetic studies revealed that LGP2 potentiates RIG-I and MDA5-mediated responses under physiological conditions.[3] RIG-I and MDA5 are specialized to recognize viral RNAs with different molecular weights, and MDA5 preferentially binds to long (>1 kb) dsRNA molecules. In contrast, RIG-I participates in the recognition of short (<300 bp) RNA molecules, 5′ppp dsRNA, 5′pp dsRNA or ssRNA. In the inactive state, RIG-I and MDA5 adopt a closed structure with the CARDs concealed by its C-terminal domain (CTD). The interaction of RNA with CTD activates RIG-I by exposing the CARDs, promoting oligomerization and finally interacting with a mitochondrial membrane-associated CARD containing adapter protein interferon-β promoter stimulator-1 (IPS-1), also known as mitochondrial antiviral signaling protein, virus-induced signaling adapter or CARD adapter inducing interferon-β.[4] Activation of IPS-1 leads to the recruitment of signaling proteins such as TRAF2, TRAF6, and TRADD. These changes recruit TRAF3 and TANK to induce the activation of TBK1 and IKKε and finally leads to the production of type I and III interferons and proinflammatory cytokines through the transcription factors IRF3/IRF7 and NF-κB, respectively (Fig. 1a).

The RLR pathway is vital for viral defense, and its dysregulation can cause autoimmune diseases;[5] therefore, the RLR signaling pathway is tightly regulated at the posttranslational level. Regulation of the RLR pathway by ubiquitination is pivotal for antiviral responses and immune homeostasis and occurs at various levels, such as sensing, signaling and transcription factor activation. Ubiquitin is a small peptide consisting of 76 amino acids, and multiple ubiquitin molecules can be sequentially added to one of the seven lysine (K) residues of ubiquitin (K6, K11, K27, K29, K33, K48, and K63),[6] resulting in a variety of ubiquitin chains that can then be covalently linked to a target protein by E3 ubiquitin ligase. This posttranslational modification called ubiquitination can alter the function or stability of a protein depending on the type of ubiquitination. RIG-I is regulated by a number of E3 ubiquitin ligases, K27 and K48-linked ubiquitination by TRIM40[7] and K48-linked ubiquitination by RNF122,[8] and STUB1[9] promotes proteasomal degradation, while K63-linked ubiquitination promotes oligomerization and RIG-I stabilization. In addition, multiple ubiquitin chains can be added to different lysine residues of RIG-I, and this multisite RIG-I ubiquitination is essential for its activation and helps in fine-tuning of type-I interferon production during virus infection.[10] Binding of RNA ligands by RIG-I CTD exposes its CARDs for K63-linked ubiquitination at distinct sites by TRIM25, TRIM4, MEX3c and Riplet.[10–14] Ubiquitination stabilizes RIG-I oligomerization and promotes the interaction with the CARD of IPS-1, resulting in IPS-1 aggregation and dimerization in the mitochondrial membrane, required for downstream signaling.

TRIM25 is an interferon-inducible E3 ubiquitin ligase that interacts with RIG-I through its C-terminal SPla and the RYanodine receptor (SPRY) domain and ubiquitinates the second CARD of RIG-I at the lysine residues K99, K169, K172, K181, K190, and K193.[15] The K172R mutation severely reduced RIG-I ubiquitination, suggesting that K172 is essential for TRIM25-mediated RIG-I ubiquitination and subsequent binding to IPS-1. Being a pivotal molecule in antiviral signaling, TRIM25 is tightly regulated through several different mechanisms. Linear ubiquitin assembly complex adds K48-linked ubiquitin molecules and marks TRIM25 for degradation, while USP15 removes these marks and prevents proteasomal degradation during viral infection.[16] Noncoding RNAs

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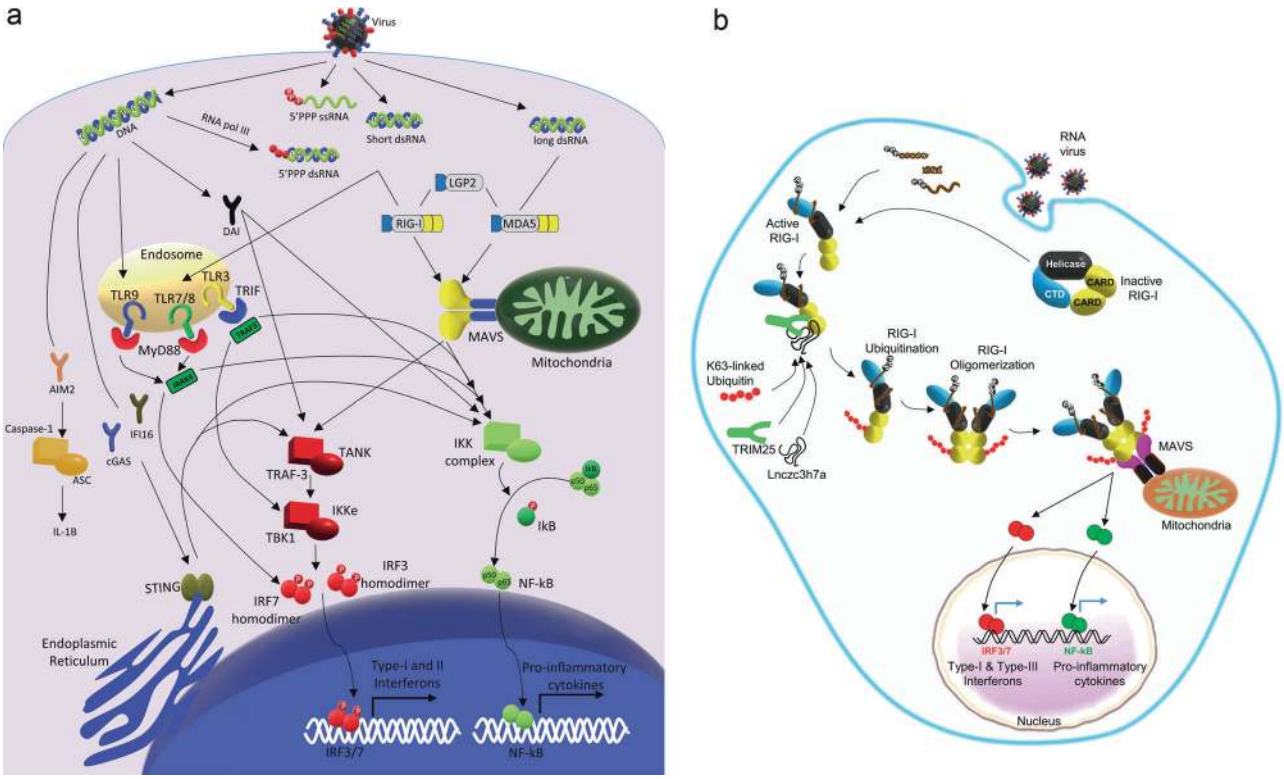

Fig. 1 Role of long noncoding RNA in the RIG-I-mediated antiviral signaling pathway: **a** Viral PAMPs are recognized by intracellular PRRs followed by recruitment of different adapter proteins, including TIR-domain-containing adapter-inducing interferon-β (TRIF), Myeloid Differentiation Primary Response 88 (MyD88), mitochondria localized IPS-1, and endoplasmic reticulum-localized stimulator of interferon genes (STING) to activate TBK1/IKKε and the IKK complex. Activated TBK1 and IKK phosphorylate IRF3/7 or IκB, leading to activation and nuclear translocation of IRF3/7 or NF-κB, consequently increasing the transcription of Type-I interferons and proinflammatory cytokines. After sensing viral DNA, AIM2 also induces the conversion of the inactive form of proIL-1β to its bioactive form IL-1β through an inflammasome complex that consists of ASC and caspase-1. Interferons and proinflammatory cytokines collectively help resolve viral infection. **b** Inactive RIG-I exists in a closed conformation with its CTD masking the CARDs. RNA binding to the helicase domain unmasks the CARD for downstream signaling and allows binding of the TRIM25 interaction to host long noncoding RNA lnczc3h7a to the helicase domain of RIG-I. Lnczc3h7a acts as a scaffold to facilitate the RIG-I-TRIM25 interaction so that TRIM25 can add K63-linked ubiquitin chains to the CARD of RIG-I. Ubiquitination results in RIG-I oligomerization and interaction with IPS-1. IPS-1 then recruits several signaling proteins to finally activate IRF3/7 or NF-kB, which upon nuclear translocation induce Type-I interferons and proinflammatory cytokines

are also involved in the regulation of TRIM25; for instance, miRNA-873 and miRNA-3614-3p target the 3′-UTR of TRIM25 mRNA, leading to its downregulation.[17,18] Interestingly, recent studies have shown that TRIM25 protein can also bind with cellular RNA.[19] Although this opened new opportunities by which TRIM25 can regulate the RLR pathway, the exact role of the RNA binding activity of TRIM25 is not yet clear.

Recently, Cao and colleagues have shed light on a new mechanism in which TRIM25 can bind to a long noncoding RNA (lncRNA) to modulate RLR pathway activation.[20] LncRNAs are >200 nucleotide transcripts transcribed by RNA polymerase II and are capped, spliced, and polyadenylated like mRNAs but do not encode proteins.[21] LncRNAs have been shown to function in a variety of ways; they can serve as a signal for transcription, a decoy for regulatory factors, a scaffold for assembly of multimolecular complexes, a guide for RNPs, or enhancers (enhancer RNAs) to modulate the three-dimensional organization of DNA at the promoter.[22] The group discovered a novel virus-inducible lncRNA, lnczc3h7a, that is encoded by introns of the gene encoding the Zc3h7a protein and serves as a molecular scaffold facilitating the TRIM25–RIG-I interaction. The authors demonstrated that TRIM25 binds lnczc3h7a with its SPRY domain but not with the coiled-coil domain, as reported previously. Initiation of viral infection triggers binding of lnczc3h7a with the helicase domain of RIG-I. These interactions bring TRIM25 and RIG-I in close proximity as demonstrated by a proximity ligation assay to promote ubiquitination of RIG-I by TRIM25, an essential step for RIG-I oligomerization and subsequent activation of the downstream signaling pathway. Through individual-nucleotide-resolution cross-linking and immunoprecipitation experiments and mutation studies, the authors showed that nucleotides 308, 311, and 332, located within two nearby stem-loops in the predicted RNA secondary structure, are critical for lnczc3h7a binding to TRIM25 and RIG-I. Knockdown of lnczc3h7a showed a significant reduction of type I interferons and proinflammatory cytokines after challenge with the RIG-I-responsive vesicular stomatitis virus or Sendai virus but not with the MDA5-responsive encephalomyocarditis virus, a DNA virus, herpes simplex virus-1 or stimulation with a TLR4 ligand, lipopolysaccharide, indicating that lnczc3h7a specifically modulates RIG-I signaling. Mice lacking lnczc3h7a showed decreased production of type I interferons and proinflammatory cytokines in the sera, liver, spleen, and lungs and consequently a high viral load in these vital organs, further indicating an indispensable role of lnczc3h7a in the RIG-I pathway. Lnczc3h7a failed to bind RIG-I in vesicular stomatitis virus-infected TRIM25 knockout cells, indicating that the lnczc3h7a-RIG-I interaction is TRIM25 dependent. The authors also demonstrated by competition assays that lnczc3h7a and viral RNA appear to have separate binding sites inside the helicase domain of RIG-I, and therefore, lnczc3h7a does not compete with viral RNA analogs, poly (I:C) or 5′ppp-dsRNA for binding. Furthermore, in vitro transcribed 5′-OH lnczc3h7a failed to induce the ATPase activity of RIG-I, indicating that it does not trigger RIG-I activation on its own. Overall, lnczc3h7a

acts as a scaffold to facilitate the interaction between TRIM25 and RIG-I during the initiation of viral infection and therefore acts as a positive regulator of the RLR pathway in response to RNA virus infection (Fig. 1b).

Although role of small noncoding RNAs, such as microRNA-485-5p, has been demonstrated previously in the regulation of RIG-I,[23] this recent study by Cao and colleagues has revealed the role of host long noncoding RNAs in the regulation of innate immunity and also provided new mechanistic insights into the complexity of virus sensing by RIG-I. The study showed how inducible noncoding RNAs can facilitate sensing by RIG-I and induction of antiviral innate immune responses. These results also raise several new questions: First, does a similar mechanism for virus sensing exist in humans? If not, why was such an important mechanism lost during evolution, considering that RLR signaling is under a positive selection pressure.[24] Second, TRIM25 also ubiquitinates several other molecules in the RLR pathway, does lnczc3h7a facilitates the interaction of TRIM25 with these molecules in a similar manner as RIG-I? Answering these questions may provide new insights for the development of new methods as therapeutic interventions during RNA virus infection.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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