




# The crosstalk between viral RNA- and DNA-sensing mechanisms

Chunmei Cai<sup>1,4</sup> · Yan-Dong Tang<sup>2,3</sup> · Guocai Xu<sup>1,4</sup> · Chunfu Zheng<sup>2,5</sup> 

Received: 26 July 2021 / Revised: 17 October 2021 / Accepted: 18 October 2021 / Published online: 29 October 2021  
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2021

## Abstract

Viral infections pose a severe threat to humans by causing many infectious, even fatal, diseases, such as the current pandemic disease (COVID-19) since 2019, and understanding how the host innate immune system recognizes viruses has become more important. Endosomal and cytosolic sensors can detect viral nucleic acids to induce type I interferon and proinflammatory cytokines, subsequently inducing interferon-stimulated genes for restricting viral infection. Although viral RNA and DNA sensing generally rely on diverse receptors and adaptors, the crosstalk between DNA and RNA sensing is gradually appreciated. This minireview highlights the overlap between the RNA- and DNA-sensing mechanisms in antiviral innate immunity, which significantly amplifies the antiviral innate responses to restrict viral infection and might be a potential novel target for preventing and treating viral diseases.

**Keywords** DNA sensing · RNA sensing · Crosstalk · Viral infection · Type I interferon

## Introduction

The innate immune response serves as the first-line defense against invading pathogens, including viruses [1]. The initiation of innate immune response depends on the recognition of pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) by pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), NOD-like receptors (NLRs), and several other

nucleic acid sensors [2, 3]. The PRR signaling pathways generally converge on triggering type I interferon (IFN-I) and proinflammatory cytokines production, resulting in the induction of hundreds of interferon-stimulated genes (ISGs) for viral clearance [4, 5]. Since viruses have few unique characteristics that may be utilized as PAMPs, the innate immune system depends mostly on PRRs detecting virus-derived nucleic acid to stimulate IFN-I production [6]. PRRs' various subcellular sites allow the host to detect infection throughout the viral life cycle [7].

Viral nucleic acid-sensing TLRs all localize to the endosome. TLR3, TLR7, and TLR8 are RNA sensors; however, TLR9 is a DNA sensor [8, 9]. TLR3 recognizes double-stranded (ds) RNA in a sequence-independent manner [10]. Highly homologous TLR7 and TLR8 contain one binding site with specificity for guanosine and uridine, respectively, and additional binding sites for detecting single-stranded (ss) RNA [11–13]. TLR9 is the first identified DNA sensor, recognizing the unmethylated cytosine-phosphate-guanosine (CpG) DNA of viral genomes [14, 15]. When the endosomal TLRs are activated, TLR3 utilizes TIR-domain-containing adapter-inducing interferon (TRIF) to induce IFN-I production via both IKK $\alpha$ / $\beta$ -NF- $\kappa$ B and TBK1-IRF3/7 axes, while TLR7/8/9 recruit myeloid differentiation primary response 88 (MyD88) to trigger IKK $\alpha$ / $\beta$ -NF- $\kappa$ B-axis-mediated IFN-I production [8, 15, 16].

✉ Chunfu Zheng  
zheng.alan@hotmail.com

<sup>1</sup> Research Center for High Altitude Medicine, School of Medical, Qinghai University, Xining 810016, Qinghai, China

<sup>2</sup> Department of Immunology, School of Basic Medical Sciences, Fujian Medical University, Fuzhou 350108, Fujian, China

<sup>3</sup> State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute of Chinese Academy of Agricultural Sciences, Harbin, China

<sup>4</sup> Key Laboratory of Application and Foundation for High Altitude Medicine Research in Qinghai Province, Qinghai University, Xining 810016, Qinghai, China

<sup>5</sup> Department of Microbiology, Immunology and Infectious Diseases, University of Calgary, Calgary, AB T2N 4N1, Canada

The RLRs are responsible for sensing viral RNA in the cytosol, while sensors for detecting cytosolic DNA are more complicated. The RLRs, which include RIG-I, melanoma-differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2), are characterized by a C-terminal dsRNA-binding domain and a DEAD-box helicase domain [17]. RIG-I preferentially recognizes short dsRNA (< 300 bp) with 5' triphosphate (5'-ppp) moiety and also detects short fragments of poly (I:C), 5' diphosphate, as well as circular RNA [18, 19]. MDA5, on the other hand, preferentially binds lengthy and irregular dsRNA (> 300 bp), implying a complicated higher order structure [20]. Following the recognition of distinct dsRNA features, the distinctive caspase-recruitment domains (CARDs) of RIG-I and MDA5 but not LGP2 recruit and activate the downstream adaptor proteins, such as mitochondrial antiviral signaling protein (MAVS), ultimately triggering IFN-I production through both IKK $\alpha$ / $\beta$ -NF- $\kappa$ B and TBK1-IRF3/7 pathways [8, 16].

The cyclic GMP-AMP synthase (cGAS) is a primary indispensable cytosolic sensor for detecting long dsDNA with bounded protein and short dsDNA by low and high concentrations of cGAS, respectively [21]. IFN- $\gamma$ -inducible protein-16 (IFI16), a member of the pyrin and HIN domain (PYHIN)-containing protein family, is located in both the cytoplasm and the nucleus to recognize the DNA of a few viruses [22–25]. Cytoplasmic IFI16 merely recognizes dsDNA of herpes simplex virus type I (HSV-1) and vaccinia virus (VV), as well as senses ssDNA from human immunodeficiency virus type 1 (HIV-1)-infected CD4+ T cells, to produce IFN-I. After detecting the dsDNA of HSV-1, sarcoma-associated herpesvirus (KSHV), and Epstein–Barr virus (EBV), the nuclear IFI16 oligomer translocates to the cytoplasm via unknown mechanisms to ultimately trigger STING-mediated IFN-I production and/or inflammasome-mediated IL-1 $\beta$  production. The DEAD-box helicases 41 (DDX41) has been identified as a dsDNA sensor during HSV-1 viral infection and B-form DNA transfection [26]. The recognition of cytosolic DNA subsequently initiates a Stimulator of Interferon Genes (STING)-mediated IFN-I production via both IKK $\alpha$ / $\beta$ -NF- $\kappa$ B and TBK1-IRF3/7 signaling pathways [15, 16].

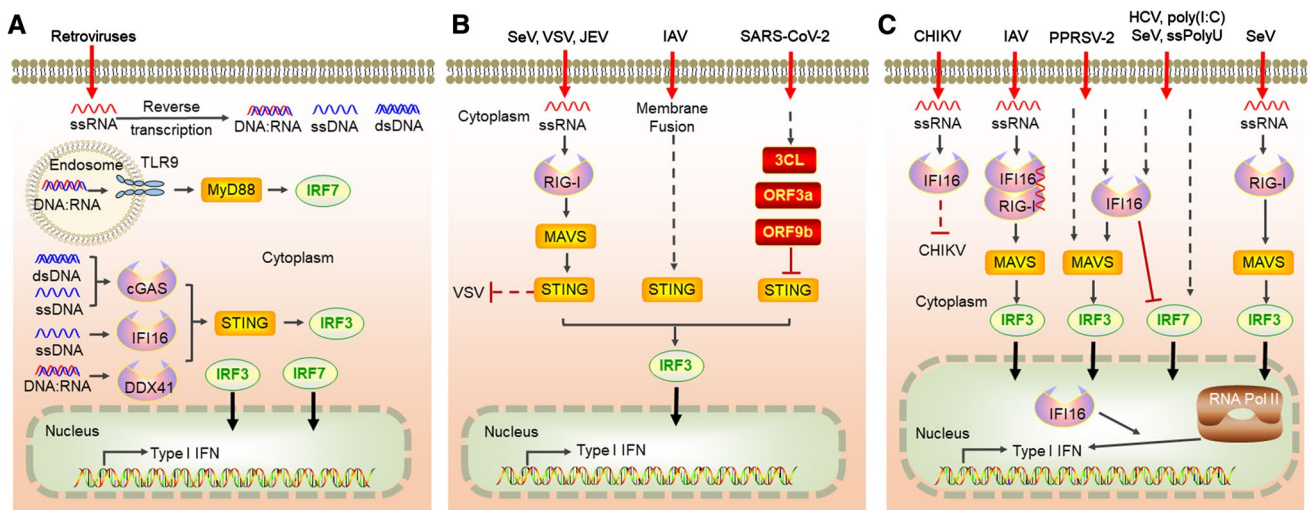
Although viral RNA and DNA sensing generally rely on diverse receptors and adaptor proteins, their downstream signaling is similar. Remarkably, accumulating evidence suggests the high degree of crosstalk between viral DNA- and RNA-sensing mechanisms in antiviral innate immunity, which amplify the antiviral innate response against both RNA and DNA viruses via detecting infection throughout the viral life cycle.

## Detection of RNA virus by DNA sensors

### Sensing of retroviruses

Retroviruses are enveloped, linear, non-segmented ssRNA viruses [27]. Retrovirus replication produces intermediate nucleic acids, including viral ssRNA, RNA:DNA hybrids, ssDNA, and dsDNA. Studies on human and murine retroviruses, HIV-1, and murine leukemia virus (MLV), indicated significant crosstalk between DNA and RNA sensing (Fig. 1A). cGAS is a primary retroviral DNA sensor that detects dsDNA in a sequence-independent way while preferentially recognizing ssDNA stem-loop structures [28–30]. During HIV-1 or MLV infection, cGAS plays a crucial role in triggering IFN-I production via the cGAMP-STING axis in murine and human cells [28], which is the first study identifying cGAS as a general innate immune sensor of retroviral DNA to trigger innate immune responses against retroviruses infection. Ross's and Schlee's groups further revealed that cGAS preferentially recognizes retroviral dsDNA and ssDNA with partially mismatched stem-loop structures [29, 30]. Furthermore, HIV-1-induced host DNA damage also can stimulate cGAS-mediated IFN-I production [31].

IFI16 also recognizes retroviral ssDNA stem-loop structures to induce IFN-I production, detects retroviral dsDNA to activate the inflammasome, and directly limits retroviral infection [25, 32, 33]. The activation of IFI16 by HIV-1 ssDNA results in the production of IFN-I through a STING–TBK1–IRF3/7 pathway and the subsequent ISGs production to suppress HIV-1 replication in macrophages [25], suggesting the important role of IFI16 in restricting HIV-1 replication in macrophages via stimulating IFN-I production. On the contrary, the recognition of HIV-1 dsDNA by IFI16 activates the inflammasome-mediated pyroptosis of host CD4+ T cells, contributing to progression to AIDS [32]. However, the role of IFI16 in sensing retroviral DNA as well as inducing IFN-I production remains controversial. Gray et al. revealed that all mouse AIM2-like receptors (ALR, including IFI204, the IFI16 homolog) are dispensable for the IFN-I production responding to transfected DNA ligands (calf thymus DNA, CT-DNA), DNA virus (mouse cytomegalovirus, MCMV) infection, and retrovirus (VSV-G pseudotyped, self-inactivating lentivirus) infection in mouse models lacking all 13 ALR sensors [34]. In addition, knockout of IFI16 in human primary fibroblasts does not affect IFN-I production during human cytomegalovirus (HCMV) infection. As we have described above, several groups have suggested that IFI16 confers resistance to HSV-1, KSHV, VV, or HIV-1 via detecting viral genomic or derived DNA to induce IFN-I-mediated antiviral immune responses



**Fig. 1** DNA sensor-mediated detection of RNA viruses. **A** DNA sensors mediated the detection of retroviruses. Both retroviral dsDNA and ssDNA activate cGAS-STING-mediated IFN-I response. The HIV-1 ssDNA initiates IFI16-mediated IFN-I production. Both TLR9 and DDX41 sense DNA:RNA hybrids of synthesis and MLV, respectively. **B** STING-mediated recognition and restriction of RNA viruses. STING interacts with RIG-I and MAVS to promote IFN-I response against RNA viruses, such as SeV, VSV, and JEV. IAV initiates cGAS-independent STING activation via the membrane fusion

process. Early pharmacological activation of STING restricts SARS-CoV-2, which in turn encodes several proteins to inhibit STING function. **C** The emerging antiviral function of IFI16 in RNA virus infection. IFI16 directly interacts with the genomic RNA of both CHIKV and IAV to suppress CHIKV infection and enhance RIG-I-mediated IFN-I production. IFI16 also directly binds MAVS and IRF7 to promote and inhibit IFN-I response to PPRSV-2 and HCV et al., respectively. Besides, IFI16 promotes the RNA Pol II recruitment to IFN- $\alpha$  promoter to enhance IFN- $\alpha$  expression

[22–25], indicating that IFI16 may participate in sensing and IFN-I production in specific cell types and/or in response to certain pathogens. IFI16, the IFN-inducible factor, directly inhibits viral transcription, LINE-1 retrotransposition, and viral reactivation from latency during HIV-1 infection in primary CD4<sup>+</sup> T cells by targeting the transcription factor Sp1 [33], suggesting the important role of IFI16 in restricting HIV-1 in an IFN-I-independent manner and the potential role in suppressing the spread of HIV/AIDS. The in-depth understanding of the roles of Sp1 and its inhibitor, IFI16, in HIV-1 infection is imperative for the development of effective vaccines and therapies.

Ross's group revealed that DDX41 preferentially detects the RNA:DNA intermediate generated by MLV reverse transcription, and the dendritic cells (DCs) but not myeloid-derived cells are responsible for in vivo control of virus infection through effectively initiating the antiviral immune responses [29], indicating that DDX41 and cGAS recognize RNA:DNA hybrid and dsDNA produced at reverse transcription's early and late stages, respectively. Both DDX41 and cGAS are required for STING-dependent IFN-I production during MLV infection. However, since DDX41 lacks enzymatic activity to produce cGAMP and activate STING, the underlying mechanisms of how nucleic acid-bound DDX41 activates the STING-mediated signaling pathway need further investigation. Interestingly, a recent study showed that virus-derived synthetic RNA:DNA hybrids may trigger

TLR9-dependent IFN-I production in human macrophage cells and murine DCs [35], indicating that the retroviruses, a family of RNA viruses, can be detected by a few DNA sensors to enhance the host antiviral innate immunity through their DNA intermediates.

### Noncanonical role of STING-mediated signaling in sensing and controlling RNA viruses

STING, as previously stated, is a key adaptor protein for detecting cytosolic DNA. In pioneering studies, STING has also been discovered as a key player in the crosstalk of viral DNA and RNA sensing (Fig. 1B). Several RNA viruses, including vesicular stomatitis virus (VSV), Sendai virus (SeV), influenza A virus (IAV), Japanese encephalitis virus (JEV), and retroviruses, could be detected via STING signaling pathways [27, 36–39]. Previous studies have demonstrated that the STING localizes to the outer membrane of mitochondria and can be activated via interacting with the RLR signaling pathway downstream adaptor MAVS [39, 40]. Accumulating evidence showed that STING could directly transmit RIG-I-MAVS-mediated signals, activated by dsRNA or ssRNA of VSV/SeV/JEV, to promote IFN-I production [37–39]. Surprisingly, AT-rich dsDNA of HSV-1 or EBV is transcribed into 5'-ppp dsRNA to initiate

RIG-I-MAVS-STING-mediated IFN-I production [41, 42]. However, the underlying mechanism by which STING participates in this process remains unknown, and several DNA viruses with AT-rich genome can activate RNA-mediated signaling pathways in a RIG-I-MAVS-STING-independent manner, which will be discussed later. Strikingly, the membranes fusion between IAV and host cell can initiate cGAS-independent STING activation to trigger IFN-I production, which can be suppressed by IAV hemagglutinin fusion peptide (FP) via directly interacting with STING to inhibit its dimerization and TBK1 activation [36]. How innate immune systems detect the membrane fusion of the virus with the host to trigger STING-dependent signaling is not yet understood.

Nonetheless, the role of STING in IFN-I production during RNA virus infections is still controversial. Several studies have found that the lack of STING significantly reduces IFN-I production induced by VSV or SeV infection in humans and murine [40, 43]. On the other hand, some investigations have found that during SeV or VSV infection, STING impairment did not affect IFN-I production [44, 45]. Hence, the role of STING in RNA virus-mediated IFN-I production might be a virus- or cell-type-dependent manner. Furthermore, the activation of RIG-I by SeV or 5' pppRNA promotes STING expression [46]. These results suggested that the STING may play a pivotal role in antagonizing RNA viruses.

A recent study showed that STING plays an indispensable role in inhibiting the replication of several RNA viruses in murine fibroblasts [44]. The function of STING in establishing an antiviral state in the VSV infection model is strongly connected to its capacity to influence the translation initiation of both host and viral genes but not its ability to modulate the basal production of IFNs in uninfected cells or autophagy. As a result, STING inhibits the translation machinery from restricting viral protein synthesis during RNA virus infections, resulting in decreased intracellular viral load. This effect may attribute to the inhibition of protein translocation by STING via interacting with the translocon complex. It is worth noting that the STING-mediated signaling pathway also plays a crucial role in controlling severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a novel RNA virus causing pandemic disease COVID-19 since 2019. STING is primarily expressed in lung alveolar epithelial cells, endothelial cells, and spleen cells, the primary targets in COVID-19 pathogenesis [47]. Recent studies showed that the early activation of STING by diABZI, diABZI-4, or CDG<sup>SF</sup> restricts SARS-CoV-2 infection in human cells and mice in vivo through both IFN-dependent and -independent mechanisms [48–50], suggesting that the early activation of STING induces downstream IFN-I and ISGs that protect the host from SARS-CoV-2 infection.

Interestingly, several groups demonstrated that coronaviruses could encode viral proteins to impair STING function. Chen's group has shown that the SARS-CoV papain-like protease suppressed the IFN-I production via binding and disrupting the STING-TRAF3-TBK1 complex to inhibit IRF3 activation [51]. Wang's and Yu's groups have reported recently that the 3CL, ORF3a, and ORF9b of SARS-CoV-2 can antagonize the STING function [52, 53]. As a result, to evade antiviral immunity, SARS-CoV-2 suppresses the early activation of STING by encoding numerous viral proteins. STING activation prevented SARS-CoV-2 infection, suggesting that RNA viruses might activate STING-mediated antiviral innate immunity. We hypothesized that the STING might detect the SARS-CoV-2 through the unknown mechanisms to initiate antiviral immunity, which would be substantially inhibited by numerous SARS-CoV-2 proteins to allow viral replication and infection. To reveal the underlying mechanisms of activated STING-mediated inhibition of SARS-CoV-2 will help develop novel therapeutics against SARS-CoV-2.

### The emerging crucial function of IFI16 in innate immune responses to RNA viruses

Besides sensing the DNA intermediates generated by retroviral replication to activate STING-mediated IFN-I response, IFI16 plays a crucial role in detecting other RNA virus infections (Fig. 1C). A recent study implicated novel mechanisms for sensing RNA viruses by IFI16 (identified as a DNA sensor). Jiang et al. proposed that upon IAV infection, IFI16 directly interacts with both IAV RNA and host RIG-I to magnify RIG-I-mediated IFN-I production through promoting K63-linked polyubiquitination of RIG-I [54], suggesting that IFI16 is an RNA-binding protein (RBP) to sense viral RNA during IAV infection. Furthermore, IFI16 substantially enhances the RIG-I transcription by binding and recruiting RNA Pol II to the RIG-I promoter [54]. They revealed that the IFI16 enhances the sensitivity of RIG-I signaling via multiple regulatory mechanisms. Remarkably, IFI16 also plays a pivotal role in the tight regulation of the IFN/ISG signaling pathway. Chang et al. recently demonstrated that IFI16 directly binds MAVS to promote MAVS-mediated IFN-I production and efficiently restricts the replication of porcine reproductive and respiratory syndrome virus 2 (PPRSV-2) in MARC-145 cells [55]. It has been reported that in SeV-infected THP1 cells, IFI16 directly affects the transcription of IFN- $\alpha$  through promoting the RNA polymerase (Pol) II basal recruitment to the promoter of IFN- $\alpha$  [56]. It remains to be determined how IFI16 increases RNA Pol II loading on IFN- $\alpha$  promoter. However, recent studies have revealed that in mouse hepatitis coronavirus (HCV) or SeV infected, poly(I:C) or ssPolyU transfected NIH3T3 cells or marrow-derived DCs, the murine orthologue of



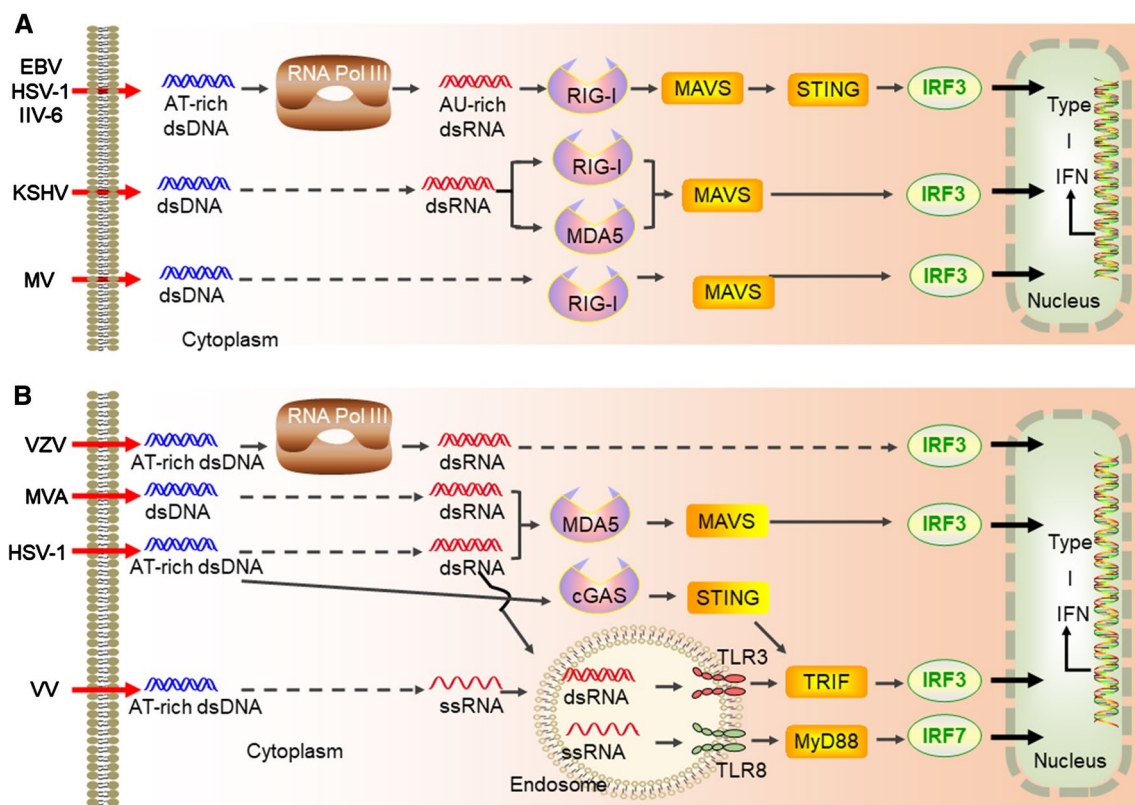
IFI16 (IFI204) directly associates with the DNA binding domain (DBD) of IRF7 and thus prevents IRF7 from promoter binding to inhibit the IRF7-mediated IFN-I production substantially [57], indicating a novel negative regulation of IFN-I by IFI16 during viral infection. Interestingly, Kim et al. found that IFI16 directly interacted with chikungunya virus (CHIKV) genomic RNA via the VIRAL Cross-Linking And Solid-phase Purification (VIRAL Cross-Linking And Solid-phase Purification) and inhibited viral replication and maturation [58]. However, the underlying mechanisms of how IFI16 bound viral RNA and inhibited IFN-I production are still elusive.

## RNA sensor-mediated DNA virus recognition

### RIG-I-mediated sensing of DNA viruses

It has been reported that RIG-I-mediated detection of DNA viruses is RNA Pol III dependent and independent (Fig. 2A).

As described above, AT-rich dsDNA from HSV-1 or EBV may be transcribed to dsRNA with a 5' triphosphate end group to trigger RIG-I-MAVS-STING-axis-mediated IFN-I production, which is RNA Pol III-dependent. Chiu et al. treated the Raw264.7 cells or B95-8 cells (human B cell line) with ML-60218 to inhibit RNA Pol III before infecting them with adenovirus/HSV-1 or EBV [41]. They found that the inhibition of RNA Pol III significantly reduced IFN-I induction during adenovirus, HSV-1, or EBV infection. They further employed radiolabeled poly(A-U) RNA probe to identify the poly(A-U) RNA in the RIG-I complex isolated from poly(dA-dT) but not mock-transfected cells, suggesting that poly(dA-dT) directs the synthesis of poly(A-U) RNA, subsequently recognized by RIG-I to induce IFN-I production via RIG-I-MAVS-STING-axis. This report first delineated a mechanism linking DNA sensing with the RIG-I pathway. Besides, Invertebrate Iridescent Virus 6 (IIV-6) is a DNA virus of the *Iridoviridae* family with a genome that is 71% AT [59]. Several studies have indicated that the



**Fig. 2** Sensing DNA viruses by RNA sensors. **A** RIG-I-mediated detection of DNA viruses. After HSV-1, EBV, or IIV-6 infection, the RNA Pol III-mediated transcription of AT-rich dsDNA to AU-rich dsRNA further triggers RIG-I-MAVS-STING-axis-mediated IFN-I production. Both RIG-I and MDA5 recognized host-derived dsRNA directed by KSHV dsDNA to trigger IFN-I response. MV activates RIG-I-mediated IFN-I production through unrevealed mechanisms. **B** Other RNA-sensing machinery for identifying DNA viruses. RNA Pol III is responsible for converting AT-rich DNA into RNA PAMP

to trigger proper IFN-I production during VZV infection through an unrevealed RNA-sensing mechanism. The MDA5 and TLR3 are also responsible for detecting the intermediate RNA directed by the HSV-1 dsDNA to initiate IFN-I response. Besides, the engagement of cGAS-STING and TLR3 pathways by TRIF amplifies the antiviral responses against HIV-1. The TLR8 identifies the ssRNA directed by VV dsDNA to trigger MyD88-dependent IFN-I induction. The MDA5 also senses the dsRNA directed by MVA dsDNA to induce IFN-I production

IIV-6 could be sensed by RIG-I (the orthologue in *Drosophila* is Dicer-2) and elicit a powerful antiviral response [60, 61]. Goodman's group suggested that IIV-6-induced IFN-I production in mammalian cells was also RNA Pol III dependent [61].

Accumulating studies have shown that RIG-I and MAVS are required for KSHV, a large double-stranded DNA virus, to induce IFN-I [62]. A recent study on RLR-dependent sensing of KSHV has revealed that either MDA5 and RIG-I or RIG-I alone are responsible for detecting host-derived RNAs directed by the KSHV genome [63, 64]. Karijolich's group showed that MDA5 and RIG-I recognized distinct features of RNAs, and MDA5 induced more potent antiviral immunity via canonical and noncanonical signaling pathways [63]. Notably, the RIG-I-mediated IFN-I production during KSHV infection is RNA Pol III independent [64]. In KSHV-infected 293T cells, inhibiting or depleting RNA Pol III did not affect IFN-I production. Furthermore, Wang et al. previously demonstrated that the myxoma virus (MV), a large cytoplasmic DNA virus, could be mainly detected by RIG-I to initiate IFN-I production in primary human macrophages (pHMs) [65]. However, they did not reveal the underlying mechanisms by which RIG-I detects signals from the MV genome.

### Other RNA-sensing machinery detects DNA viruses

Except for RIG-I, the host bears other RNA sensors recognizing DNA viruses (Fig. 2B). Employing a whole-exome sequencing approach, Mogenson's group has shown that pediatric patients with mutations in the subunits of RNA Pol III (POLR3A and/or POLR3C) are predisposed to severe varicella-zoster virus (VZV) infection [66]. The AT content of the VZV genomic sequence ranges from 70 to 80%. They further demonstrated that mutations of RNA Pol III reduced the capacity to convert AT-rich DNA into RNA PAMP to trigger proper IFN-I production during VZV infection, although which RNA-sensing mechanism was involved in this process remains unknown. Subsequent studies on adult patients revealed that mutations in the RNA Pol III machinery significantly affected VZV infection control [67, 68]. Their findings provided genetic and immunologic evidence of a role for RNA sensors in controlling DNA virus in humans.

Furthermore, the intermediate RNA directed by the HSV-1 genome can also be detected in RIG-I-independent manners. Previous studies have shown that upon HSV-1/2 infection, MDA5 is required for IFN-I production in human but not murine macrophages and that the MDA5 and RIG-I work synergistically in detecting viral RNA to trigger IFN-I production [69–71]. Recent findings showed that the function of TLR3 on sensing HSV-1 dsRNA to elicit antiviral immunity remains disputed in various cell types and species

[72]. Strikingly, the Sen's group recently revealed that the TRIF could combine cGAS-STING and TLR3 pathways by engaging with both STING and TLR3 to establish a powerfully antiviral state to suppress HSV-1 replication [73]. The contribution of these two pathways in controlling HSV-1 infection is also cell types and species specific.

The VV dsDNA genome is likewise abundant in poly(A)/T sequences. When plasmacytoid dendritic cells (pDCs) were stimulated with VV or VV DNA, the IFN-I production and pDCs activation were mediated by TLR8 but not TLR7/9 via recognition of poly(A)/T-rich motifs to restrict viral infection efficiently [74]. This work identified a previously unappreciated role for TLR8 in detecting AT-rich DNA virus and revealed a unique strategy of TLR8-mediated pDC activation for restricting VV infection. The MDA5 is also responsible for sensing the dsRNA generated from the genome of Modified VV Ankara (MVA), an attenuated double-stranded DNA poxvirus, to initiate IFN-I production in macrophage, which is beneficial for designing MVA vaccine vectors with improved immunogenicity [75].

### Concluding remarks

Viruses remain a major danger to world health, particularly the SARS-CoV-2 strain that has been circulating in recent years. Despite the incremental progress made in understanding the multiple DNA/RNA sensor-mediated innate immune responses to viral infection, many critical unknowns remain about the overlap between DNA- and RNA-sensing mechanisms. The crosstalk between DNA- and RNA-sensing mechanisms enables the host to detect infection throughout the viral life cycle to eliminate invading pathogens and effectively prevent impairment to the host. Interestingly, viruses adopt numerous strategies to evade host antiviral innate immunity via suppressing this overlap [76]. Advances in these areas would facilitate developing viral vaccines or adjuvants and therapeutics that selectively target nucleic acid sensors. This review highlights the interrelationship between viral DNA- and RNA-sensing mechanisms. Further comprehensive elucidation of this crosstalk is expected to have far-reaching consequences.

**Acknowledgements** C.C. was supported by the National Natural Science Foundation of China (No. 81960292). We apologize to investigators whose contributions were not cited due to space limitations.

**Author contributions** All the authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

## References

1. Liu J et al (2016) Post-translational modification control of innate immunity. *Immunity* 45(1):15–30
2. Brubaker SW et al (2015) Innate immune pattern recognition: a cell biological perspective. *Annu Rev Immunol* 33:257–290
3. Zheng C (2021) The emerging roles of NOD-like receptors in antiviral innate immune signaling pathways. *Int J Biol Macromol* 169:407–413
4. Chen K et al (2017) Regulation of type I interferon signaling in immunity and inflammation: a comprehensive review. *J Autoimmun* 83:1–11
5. Cai C, Yu X (2020) A mathematic model to reveal delicate cross-regulation between MAVS/STING, inflammasome and MyD88-dependent type I interferon signalling. *J Cell Mol Med* 24(19):11535–11545
6. Schlee M, Hartmann G (2016) Discriminating self from non-self in nucleic acid sensing. *Nat Rev Immunol* 16(9):566–580
7. Choi Y et al (2018) Autophagy during viral infection—a double-edged sword. *Nat Rev Microbiol* 16(6):341–354
8. Liu G, Gack MU (2020) Distinct and orchestrated functions of RNA sensors in innate immunity. *Immunity* 53(1):26–42
9. Paludan SR et al (2019) DNA-stimulated cell death: implications for host defence, inflammatory diseases and cancer. *Nat Rev Immunol* 19(3):141–153
10. Alexopoulou L et al (2001) Recognition of double-stranded RNA and activation of NF-kappaB by Toll-like receptor 3. *Nature* 413(6857):732–738
11. Heil F et al (2004) Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. *Science* 303(5663):1526–1529
12. Tanji H et al (2015) Toll-like receptor 8 senses degradation products of single-stranded RNA. *Nat Struct Mol Biol* 22(2):109–115
13. Zhang Z et al (2016) Structural analysis reveals that toll-like receptor 7 is a dual receptor for guanosine and single-stranded RNA. *Immunity* 45(4):737–748
14. Hemmi H et al (2000) A Toll-like receptor recognizes bacterial DNA. *Nature* 408(6813):740–745
15. Briard B et al (2020) DNA sensing in the innate immune response. *Physiology (Bethesda)* 35(2):112–124
16. Crowl JT et al (2017) Intracellular nucleic acid detection in autoimmunity. *Annu Rev Immunol* 35:313–336
17. Yoneyama M et al (2005) Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. *J Immunol* 175(5):2851–2858
18. Goubau D et al (2014) Antiviral immunity via RIG-I-mediated recognition of RNA bearing 5'-diphosphates. *Nature* 514(7522):372–375
19. Chen YG et al (2017) Sensing Self and Foreign Circular RNAs by Intron Identity. *Mol Cell* 67(2):228–238 e5
20. Pichlmair A et al (2009) Activation of MDA5 requires higher-order RNA structures generated during virus infection. *J Virol* 83(20):10761–10769
21. Andreeva L et al (2017) cGAS senses long and HMGB/TFAM-bound U-turn DNA by forming protein-DNA ladders. *Nature* 549(7672):394–398
22. Unterholzner L et al (2010) IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol* 11(11):997–1004
23. Kerur N et al (2011) IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma-associated herpesvirus infection. *Cell Host Microbe* 9(5):363–375
24. Ansari MA et al (2015) Herpesvirus genome recognition induced acetylation of nuclear IFI16 is essential for its cytoplasmic translocation inflammasome and IFN-beta responses. *PLoS Pathog* 11:7 e1005019
25. Jakobsen MR et al (2013) IFI16 senses DNA forms of the lentiviral replication cycle and controls HIV-1 replication. *Proc Natl Acad Sci USA* 110(48):E4571–E4580
26. Zhang Z et al (2011) The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat Immunol* 12(10):959–965
27. Saez-Cirion A, Manel N (2018) Immune responses to retroviruses. *Annu Rev Immunol* 36:193–220
28. Gao D et al (2013) Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. *Science* 341(6148):903–906
29. Stavrou S et al (2018) DDX41 recognizes RNA/DNA retroviral reverse transcripts and is critical for in vivo control of murine leukemia virus infection. *MBio* 9(3):e00923
30. Herzner AM et al (2015) Sequence-specific activation of the DNA sensor cGAS by Y-form DNA structures as found in primary HIV-1 cDNA. *Nat Immunol* 16(10):1025–1033
31. Piekna-Przybylska D et al (2017) Deficiency in DNA damage response, a new characteristic of cells infected with latent HIV-1. *Cell Cycle* 16(10):968–978
32. Monroe KM et al (2014) IFI16 DNA sensor is required for death of lymphoid CD4 T cells abortively infected with HIV. *Science* 343(6169):428–432
33. Hotter D et al (2019) IFI16 targets the transcription factor Sp1 to suppress HIV-1 transcription and latency reactivation. *Cell Host Microbe* 25(6):858–872 e13
34. Gray EE et al (2016) The AIM2-like receptors are dispensable for the interferon response to intracellular DNA. *Immunity* 45(2):255–266
35. Obermann HL et al (2019) RNA-DNA hybrids and ssDNA differ in intracellular half-life and toll-like receptor 9 activation. *Immunobiology* 224(6):843–851
36. Holm CK et al (2016) Influenza A virus targets a cGAS-independent STING pathway that controls enveloped RNA viruses. *Nat Commun* 7:10680
37. Nazmi A et al (2012) STING mediates neuronal innate immune response following Japanese encephalitis virus infection. *Sci Rep* 2:347
38. Chen LL et al (2010) Molecular basis for an attenuated cytoplasmic dsRNA response in human embryonic stem cells. *Cell Cycle* 9(17):3552–3564
39. Ishikawa H, Barber GN (2008) STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature* 455(7213):674–678
40. Zhong B et al (2008) The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. *Immunity* 29(4):538–550
41. Chiu YH et al (2009) RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell* 138(3):576–591
42. Ablasser A et al (2009) RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat Immunol* 10(10):1065–1072
43. Ishikawa H et al (2009) STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature* 461(7265):788–792
44. Franz KM et al (2018) STING-dependent translation inhibition restricts RNA virus replication. *Proc Natl Acad Sci USA* 115(9):E2058–E2067
45. Chen H et al (2011) Activation of STAT6 by STING is critical for antiviral innate immunity. *Cell* 147(2):436–446
46. Liu Y et al (2017) RIGulation of STING expression: at the crossroads of viral RNA and DNA sensing pathways. *Inflamm Cell Signal* 4(1):e1491

47. Berthelot JM, Liote F (2020) COVID-19 as a STING disorder with delayed over-secretion of interferon-beta. *EBioMedicine* 56:102801
48. Wu JJ et al (2021) A novel STING agonist for cancer immunotherapy and a SARS-CoV-2 vaccine adjuvant. *Chem Commun (Camb)* 57(4):504–507
49. Li M et al (2021) Pharmacological activation of STING blocks SARS-CoV-2 infection. *Sci Immunol* 6(59):eabi9007
50. Humphries F et al (2021) A diamidobenzimidazole STING agonist protects against SARS-CoV-2 infection. *Sci Immunol* 6(59):eabi9002
51. Chen X et al (2014) SARS coronavirus papain-like protease inhibits the type I interferon signaling pathway through interaction with the STING-TRAF3-TBK1 complex. *Protein Cell* 5(5):369–381
52. Rui Y et al (2021) Unique and complementary suppression of cGAS-STING and RNA sensing- triggered innate immune responses by SARS-CoV-2 proteins. *Signal Transduct Target Ther* 6(1):123
53. Han L et al (2021) SARS-CoV-2 ORF9b antagonizes type I and III interferons by targeting multiple components of the RIG-I/MDA-5-MAVS, TLR3-TRIF, and cGAS-STING signaling pathways. *J Med Virol* 93(9):5376–5389
54. Jiang Z et al (2021) IFI16 directly senses viral RNA and enhances RIG-I transcription and activation to restrict influenza virus infection. *Nat Microbiol* 6(7):932–945
55. Chang X et al (2019) IFI16 inhibits porcine reproductive and respiratory syndrome virus 2 replication in a MAVS-dependent manner in MARC-145 cells. *Viruses* 11(12):1160
56. Thompson MR et al (2014) Interferon gamma-inducible protein (IFI) 16 transcriptionally regulates type I interferons and other interferon-stimulated genes and controls the interferon response to both DNA and RNA viruses. *J Biol Chem* 289(34):23568–23581
57. Cao L et al (2019) P200 family protein IFI204 negatively regulates type I interferon responses by targeting IRF7 in nucleus. *PLoS Pathog* 15(10):e1008079
58. Kim B et al (2020) Discovery of widespread host protein interactions with the pre-replicated genome of CHIKV using VIR-CLASP. *Mol Cell* 78(4):624–640 e7
59. Jakob NJ et al (2001) Analysis of the first complete DNA sequence of an invertebrate iridovirus: coding strategy of the genome of Chilo iridescent virus. *Virology* 286(1):182–196
60. Bronkhorst AW et al (2012) The DNA virus Invertebrate iridescent virus 6 is a target of the *Drosophila* RNAi machinery. *Proc Natl Acad Sci USA* 109(51):E3604–E3613
61. Ahlers LR et al (2016) Invertebrate iridescent virus 6, a DNA virus, stimulates a mammalian innate immune response through RIG-I-Like receptors. *PLoS One* 11(11):e0166088
62. West JA et al (2014) An important role for mitochondrial antiviral signaling protein in the Kaposi's sarcoma-associated herpesvirus life cycle. *J Virol* 88(10):5778–5787
63. Zhao Y et al (2018) RIG-I like receptor sensing of host RNAs facilitates the cell-intrinsic immune response to KSHV infection. *Nat Commun* 9(1):4841
64. Zhang Y et al (2018) RIG-I detects Kaposi's sarcoma-associated herpesvirus transcripts in a RNA polymerase III-independent manner. *MBio* 9(4):e00823
65. Wang F et al (2008) RIG-I mediates the co-induction of tumor necrosis factor and type I interferon elicited by myxoma virus in primary human macrophages. *PLoS Pathog* 4(7):e1000099
66. Ogunjimi B et al (2017) Inborn errors in RNA polymerase III underlie severe varicella zoster virus infections. *J Clin Invest* 127(9):3543–3556
67. Carter-Timofte ME et al (2018) Varicella-zoster virus CNS vasculitis and RNA polymerase III gene mutation in identical twins. *Neurol Neuroimmunol Neuroinflamm* 5(6):e500
68. Carter-Timofte ME et al (2019) Mutations in RNA Polymerase III genes and defective DNA sensing in adults with varicella-zoster virus CNS infection. *Genes Immun* 20(3):214–223
69. Rasmussen SB et al (2009) Herpes simplex virus infection is sensed by both Toll-like receptors and retinoic acid-inducible gene-like receptors, which synergize to induce type I interferon production. *J Gen Virol* 90(Pt 1):74–78
70. Melchjorsen J et al (2010) Early innate recognition of herpes simplex virus in human primary macrophages is mediated via the MDA5/MAVS-dependent and MDA5/MAVS/RNA polymerase III-independent pathways. *J Virol* 84(21):11350–11358
71. Choi MK et al (2009) A selective contribution of the RIG-I-like receptor pathway to type I interferon responses activated by cytosolic DNA. *Proc Natl Acad Sci USA* 106(42):17870–17875
72. Danastas K et al (2020) Herpes simplex virus type 1 interactions with the interferon system. *Int J Mol Sci* 21(14):5150
73. Latif MB et al (2020) Relative contributions of the cGAS-STING and TLR3 signaling pathways to attenuation of herpes simplex Virus 1 replication. *J Virol* 94(6):e01717
74. Martinez J et al (2010) Toll-like receptor 8-mediated activation of murine plasmacytoid dendritic cells by vaccinia viral DNA. *Proc Natl Acad Sci USA* 107(14):6442–6447
75. Delaloye J et al (2009) Innate immune sensing of modified vaccinia virus Ankara (MVA) is mediated by TLR2-TLR6, MDA-5 and the NALP3 inflammasome. *Plos Pathog* 5(6):e1000480
76. Zhu H, Zheng C (2020) The race between host antiviral innate immunity and the immune evasion strategies of herpes simplex virus 1. *Microbiol Mol Biol Rev* 84(4):e00099

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.