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Review

Cytosolic DNA-sensing immune response and viral infection

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**Abbreviations:** cGAS, cyclic GMP-AMP synthase; STING; stimulator of IFN genes; cGAMP, cyclic GMP-AMP; dsDNA, double-stranded DNA; ER, endoplasmic reticulum; TBK1, TANK-binding kinase 1; IRF3; interferon regulatory factor 3; NF-κB; nuclear factor-κB; RIG-I, Retinoic acid inducible gene-I; IPS-1, IFN-β promoter stimulator-1; RT, reverse-transcription; HIV-1, human immunodeficiency virus-1; DENV; Dengue virus; KSHV, Kaposi sarcoma-associated herpesvirus; HSV-1, herpesviruses-1; HBV, hepatitis B virus; HcoV, human coronavirus; SARS, severe acute respiratory syndrome; PEDV, porcine epidemic diarrhea virus; HTLV-1, human T lymphotropic virus type-1; WNV, West Nile virus; JEV, Japanese encephalitis virus; HPV18, human papillomavirus 18; hAd5, human adenovirus type-5; IAV, influenza A virus; YFV, yellow fever virus; HCV, hepatitis C virus; MHV68, murine gammaherpesvirus 68; NDV, Newcastle disease virus; SeV; Sendai virus.
Abstract

It has not been fully elucidated how host cells recognize many kinds of RNA and DNA viruses and initiate innate antiviral responses against them. Over the past decade, investigations into the mechanisms underlying these antiviral responses have focused extensively on immune surveillance sensors that recognize virus-derived components (such as lipids, sugars, and nucleic acids). These researches have suggested that the antiviral responses are mediated by cytosolic or intracellular compartment sensors and their adaptor molecules (e.g., the TLR and MyD88, RIG-I and IPS-1 or cGAS and STING axis) for the primary sensing of virus-derived nucleic acids, and lead to the production of type I interferons (IFNs), pro-inflammatory cytokines, and chemokines by the host cells. Thus, host cells have evolved an elaborate host defense machinery to recognize and eliminate virus infection. In turn, to achieve sustained viral infection and induce pathogenesis, viruses have also evolved several counteracting strategies to realize an immune escape by targeting immune sensors, adaptor molecules, intracellular kinases, and transcription factors. In this review, we discuss recent discoveries on the role of the cytosolic nucleic acid-sensing immune response in viral recognition and the control of viral infection. In addition, we consider the regulatory machinery of the cytosolic nucleic acid-sensing immune response, because these immune surveillance systems must be tightly regulated to prevent aberrant immune responses to self and non-self-nucleic acids.
Introduction

Vertebrate cells possess pattern recognition receptors (PRRs) to enable detection of pathogens such as viruses, bacteria, fungi and parasites. Immediately after the pathogen invades the cells, the PRRs detect different combinations of pathogen-specific molecules, such as lipids, sugars, and nucleic acids, called pathogen-associated molecular patterns (PAMPs), and activate an innate antiviral response to eliminate the pathogens (1). Among the molecules composing the PAMPs, pathogen-derived nucleic acids are the most potent mediators of innate antiviral responses. The primary induction of innate antiviral responses through the detection of nucleic acids is also critical for the subsequent induction of an acquired immunity response. Over the past ten years, the responsible immune surveillance sensors, such as the Toll-like receptors (TLRs), cytosolic RNA sensors (primary retinoic acid-inducible gene-I (RIG-I)/melanoma differentiation-associated protein 5 (MDA5)), cytosolic DNA sensors (primary cyclic GMP-AMP synthase (cGAS) and others), and sensors of the inflammasome pathway (e.g., the primary NOD-like receptor (NLR) family, which consists of two major subfamilies, NLRC and NLRP), have been well characterized (2-4). These immune surveillance sensors are ubiquitously expressed, which enables the detection of pathogens invading at the cell surface and cytoplasmic or nuclear compartment in several cell types, and lead to the production of type I interferons (IFNs), pro-inflammatory cytokines, and chemokines by host cells. However, it has also been suggested that these immune surveillance systems must be tightly regulated to prevent aberrant immune responses to especially self-nucleic acids derived from damaged cells, senescent cells, apoptotic cells, and fertilization (5,6). Indeed, RIG-I, the cytosolic sensor for RNA, distinguishes self from non-self RNA through its interaction with a 5’-triphosphate or 5’-diphosphate, which are not present in the transcribed RNA species
in vertebrate cells (1). Both RIG-I and MDA5 are also known to sense the synthetic or viral double-stranded RNA (dsRNA), although MDA5 may also detect dsRNA with a high molecular weight, in contrast to the preferential detection of short dsRNA by RIG-I (7). Studies using genetically engineered mice revealed that RIG-I is crucial for the detection of several negative-stranded RNA viruses (e.g., vesicular stomatitis virus (VSV), Newcastle disease virus (NDV) and influenza A and B virus (IAV and IBV)), as well as the detection of positive-stranded RNA viruses (e.g., Japanese encephalitis virus (JEV) and hepatitis C virus (HCV)) (8). In contrast, MDA5 dominantly detects picornaviruses (e.g., encephalomyocarditis virus (EMCV)). Though exceptions to this requirement have been reported, the ability of RIG-I and MDA5 to distinguish self from non-self in this way ensures that anomalous immune responses to cellular RNA do not occur. Responses to self-DNA such as those described above are far more unclear. The cytoplasmic DNA-sensing by cGAS in collaboration with an adaptor protein, stimulator of IFN genes (STING), does not distinguish between cellular and pathogen-derived DNA (9,10). Of note, it has also been reported that the chronic inflammatory response induced by self-DNA via cGAS/STING may be responsible for the induction of aberrant inflammatory diseases such as systemic lupus erythematosus (SLE), Aicardi-Goutières syndrome (AGS), and polyarthritis (9,10). While the engulfed apoptotic cells represent a possible source of self-DNA, endogenous DNases in both the cytoplasmic (e.g., DNase-III, also referred to as TREX1) and lysosomal (e.g., DNase-II) compartments can degrade these endogenous self-DNAs and ensure that inappropriate responses are not initiated (9,10). In the case of the leaking of mitochondrial DNA (mtDNA) into the cytoplasm following mitochondrial damage, intracellular caspase activation can control the aberrant immune response (11-13). Similarly, cGAS/STING and its necessary cofactors and cellular DNA are compartmentalized such that sensing
of self-DNA is avoided; the receptor in the cytosol and the ligand (DNA) in the nucleus are sequestering (9). On the other hand, recent studies have shown that cell cycle progression during mitosis following a DNA-damage response may lead to the formation of micronuclei, thereby eliciting a cGAS/STING-mediated micronuclear DNA-sensing immune response (14,15). Then, following DNA damage and micronuclei formation, cGAS may re-localize to the micronuclei bodies and recognize micronuclear DNA, followed by the initiation of downstream signal activation. In addition the DNA-damage response discussed above, two independent research groups have also reported that an intrinsic DNA during cellular senescence were also recognized by the cGAS/STING axis, and this activation preceded the induction of an inflammatory response (this is defined as the senescence-associated secretory phenotype (SASP)) (16,17). Then, the production of SASP factors such as inflammatory cytokines and chemokines may reinforce the senescence-associated cells via autocrine and paracrine routes. Thus, remarkably, the cGAS/STING pathway appears to regulate inflammatory disorders manifested through the detection of self-DNA during DNA damage and cellular senescence. The cGAS/STING pathway may not only be important for the recognition of DNA virus infection, but may also be critical for the host defense against RNA virus infection. However, the detailed mechanism by which STING controls the RNA virus infection remains to be determined. Of note, viruses have evolved an elaborate mechanism to escape detection by the cGAS/STING axis, or to suppress activation of the cGAS/STING pathway; this escape mechanism includes the downstream signal activation of cGAS/STING axis.

In this report, we provide an overview of recent discoveries regarding the cytosolic DNA-sensing pathway, with a focus on the cGAS/STING pathway and its modulation by various host factors. We also discuss the elaborate evasion strategy of certain viruses.
that target different steps in this signaling pathway, with a focus on recently published work concerning clinically important viruses.

**The identification of putative DNA sensors**

*TLR-independent type-I IFN production upon stimulation with DNA ligands*

In contrast to the more clearly identified mechanisms of the TLR and RLR signaling pathways, there is still no universal agreement in regard to the cytosolic DNA sensors and their regulatory mechanisms (18). Following the reports that cytosolic DNA-sensing TLR-independent IFN production is implicated in the transfected synthetic double-stranded DNA (dsDNA) in murine fibroblast cells and immunocompetent cells (19,20), many studies have attempted to identify the putative DNA sensors that may activate the region downstream of the TBK1/IRF3 axis. Cumulative evidence of the involvement of these putative DNA sensors is briefly described below.

The first of the putative DNA sensors was identified by Takaoka et al., who reported that a DNA-dependent activator of IRF3 (DAI, also referred to as DLM-1/ZBP1), which is one of the IFN-inducible genes, was involved in the dsDNA recognition for the TBK1-mediated IRF3 activation (21). DAI may form a signaling complex with TBK1 and IRF3 for the production of type I IFNs in response to synthetic dsDNA or DNA virus infection (e.g., herpes simplex virus-1 (HSV-1)) in L929 murine fibroblast cells. However, Ishii and colleagues reported that IFN production was not impaired in response to synthetic B-form dsDNA and HSV-1 infection in several types of cells lacking DAI function, in addition to a normal response to plasmid-based DNA immunization in DAI-deficient mice (22). These results indicate that the function of DAI may be cell-type specific, or may redundantly replicate the DNA-sensing innate immune response.
Critical role of the cytosolic DNA-sensing innate immune response via the cGAS/STING axis

Prior to the consecutive introduction of several DNA sensors (described below), Dr. Barber and colleagues introduced the stimulator of IFN genes (STING; also referred to as MITA, MPYS or ERIS), which is encoded by the TMEM173 gene, as a molecule enabling the activation of the IFNβ promoter. STING is a 379 amino acid protein consisting of multiple transmembrane regions; it is localized at the endoplasmic reticulum (ER) and plays an essential role in the cytosolic DNA-mediated innate immune response and the response of DNA-based immunization (23-25). Several cytosolic DNA species derived from microbial pathogens can trigger STING-dependent signal activation via the TBK1-mediated IRF3 axis and the IKKs-mediated NF-κB axis, respectively. In response to being stimulated by a DNA ligand, STING may dynamically translocate from the ER to the perinuclear-Golgi region, and form a signaling complex with kinase TBK1 in order to induce IRF3 activation. STING may also lead to the protein degradation via a ubiquitin-mediated proteasome pathway to terminate the signal activation near the perinuclear-Golgi region. STING has also been suggested to associate with dsDNA directly, although the physiological relevance of this significance remains to be clarified (26). A later study indicated that STING binds directly to cyclic dinucleotides (CDNs), which are known as bacterially derived second messenger molecules, at a high affinity level via its dimer formation (27). Around the same time, it was confirmed that genetically engineered mice possessing a single point mutation in the STING gene (T596A, referred to as Goldenticket) failed to associate with CDNs and produce type I IFN in response to bacterially produced CDNs (28), suggesting that STING is a direct innate immune sensor for the production of CDN-
mediated type I IFNs. In addition, several groups have determined the crystal structure of complexes formed by binding between CDNs and STING via the cytoplasmic C-terminal region (29). Through all these points of enquiry, the discovery of STING should promote our understanding of the molecular mechanisms of cytosolic DNA-mediated innate immune responses, although it is still suggested that a cytosolic DNA sensor located in the region upstream of STING will be identified in a universally accepted manner.

Such a cytosolic DNA sensor might be implicated in the generation of an endogenous catalytic enzyme that enabled the production of CDNs in response to DNA pathogens for production of type I IFNs. In 2013, Dr. Chen and colleagues reported a major breakthrough in this line of research. Namely, they discovered cyclic GMP-AMP (cGAMP), a type of CND consisting of different phosphodiester linkages, and its catalytic enzyme (cyclic GMP-AMP synthase (cGAS), which is encoded by MB21D1 or C6orf150 gene) (30,31). In the cytoplasm, cGAS may directly bind to viral DNA derived from not only several species of DNA viruses but also reverse-transcribed DNA that is produced by retroviruses through the reverse transcription of the RNA genome. Following the binding of DNA, cGAS may induce the conformational rearrangement and catalyze the synthesis of cGAMP using the cellular ATP and GTP. In fact, it was demonstrated that cGAMP may induce STING-dependent signal activation and this induction would be occurred via the direct association of cGAMP with bacterially derived CDNs (25). This detailed signaling transduction via the cGAS/STING axis is summarized in Fig. 1. It is interesting to note that the cGAS/STING pathway did not require the protein-protein interaction between sensor and adaptor for the signal transduction, like the pathways of other PRRs (e.g., the TLR, RLR and inflammasome pathways), but rather it could be mediated by cGAMP or CDNs. Another interesting
point is that cGAMP may be transferred from virus-infected cells to neighboring uninfected cells via gap-junction channels, and thereby promote STING activation independently of type I IFN signaling mediated by the IFN-receptor/JAK-STAT axis (32). This may be considered a novel strategy for the rapid conveyance of anti-viral signals in a horizontal manner. Importantly, it has been confirmed that the cytosolic DNA-mediated innate immune response and the response of DNA-based immunization play non-redundant roles of in mice and several cGAS-lacking mouse cell types. The cGAS/STING pathway also plays crucial roles in not only host defense against DNA pathogens but also the induction of autoimmune and inflammatory diseases through the sensing of self-DNA. Thus, the cGAS/STING pathway must be properly regulated for the maintenance of cellular homeostasis and immune response. However, these detailed subjects are beyond the scope of this review and have been considered extensively elsewhere.

**The role of putative DNA sensors in type-I IFN production**

Another DNA sensor candidate gene, IFN-γ-inducible protein 16 (IFI16), which is a PYHIN family member protein, has also been shown play a role in the recognition of synthetic dsDNA and viral DNA derived from viruses that are replicated in the nucleus (e.g., HSV-1, KSHV, HCMV, and EBV) (33). Although IFI16 is predominantly expressed in the nucleus in the steady state, it may re-localize from the nucleus to cytosol in a shuttling manner for the sensing of viral DNA (33). In addition, it has been reported that IFI16 may be involved in not only DNA-sensing inflammasome activation but also the DNA damage response in apoptotic cells (34). Similar to IFI16, the cytosolic DNA sensor Absent in melanoma 2 (AIM2), which may induce IL-1β and IL-18 rather than type-I IFNs production through the inflammasome pathway after DNA
sensing in the cytosol has been proposed (35-37). Recently, AIM2-like receptors (ALRs), which consist of 13 members possessing a Pyrin-signaling domain and a DNA-binding HIN domain (e.g., p204, which is referred to as a mouse ortholog of human IFI16), have been shown not to contribute to the DNA sensing for type-I IFN production and the induction of self-DNA-mediated autoimmune diseases such as AGS (38). However, most recently, the establishment of human IFI16-deficient cells on the basis of macrophages and keratinocytes using CRISPR/Cas9 or a TALEN approach indicated that the production of type-I IFNs and IFN-stimulated genes (ISGs) in response to synthetic dsDNA and HSV-1 infection are impaired (39,40). Interestingly, these reports also showed that IFI16 is prerequisite for the full-activation of cGAS/STING function, although an in vivo evaluation will be needed to fully clarify the physiological relevance of IFI16.

Subsequent researches identified the helicase DDX41 as a putative DNA sensor by using sub-siRNA libraries targeted to the 59 members of the DExD/H helicase, and also showed that DDX41 is involved in DNA recognition and the regulation of DNA virus infection in immunocompetent cells rather than epithelial cells (41). Surprisingly, DDX41 has also been shown to be involved in the recognition of bacterial-derived second messenger molecules (such as cyclic dinucleotides (CDNs), which include cyclic di-AMP and cyclic di-GMP) for the type-I IFN production (42). The findings from crystal structure analyses revealed that the binding regions for dsDNA and CDNs overlapped, suggesting that DDX41 has the potential to recognize different ligands via the DEAD domain (43). Of note, IFI16 and DDX41, but not DAI, have been proposed to function as upstream molecules of STING through their physical interaction. While DDX41 is suggested to play a role in cytosolic DNA-mediated immune response in collaboration with the adaptor STING, a detailed investigation using genetically
engineered mice will be needed to clarify the role of the physiological relevance of STING for DDX41.

A correlation between the DNA damage response and innate immune response mediated by virus infection has been suggested, although conflicting observations were also reported by Stetson et al. (20). Most recently, several DNA damage-inducible host factors, such as the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), and its binding co-factors Ku70/80, or meiotic recombination 11 (Mre11), have also been suggested to be involved in the cytosolic DNA-sensing immune response through the direct interaction with DNA ligands (44,45). Upon simulation with synthetic DNA ligands or infection with DNA virus, only partial redundancy of ISG expression was observed in DNA-PKcs-deficient murine cells, suggesting the high potential for a cytosolic DNA-sensing innate immune response. On the other hand, it is interesting to note that Mre11 may be specifically involved in the response to synthetic dsDNA, but not involved in the response to DNA virus infection or to treatment with DMXAA, which is known as a STING-specific agonist. Additionally, these observations confirmed that type-I IFNs are produced in response to stimulation with DNA damage-inducing chemical agents (e.g., etoposide or cisplatin) (46). Recent studies using DNA damage-inducing agents such as 7,12-dimethylbenz-α-anthracene (DMBA) have suggested that the following pathway underlies the DNA damage-induced immune response via the cytosolic DNA-sensing pathway: DMBA-induced DNA damage results in nucleosome leakage into the cytosol, and then elicits cGAS/STING-dependent signal activation via self-DNA recognition (9). In addition to the direct sensing of DNA, it has been proposed that the indirect machinery of the cytosolic DNA-sensing innate immune response is involved. The leucine-rich repeat (in Flightless 1) interacting protein 1 (LRRFIP1) has been shown to function as an amplifier of cytosolic
nucleic acid-sensing immune responses for the production of type-I IFNs via the transcriptional co-activator β-catenin but not via IRF3 activation (47). Thus, accumulating evidence indicates that various genes function in the cytosolic nucleic acid-sensing immune responses in a ligand-specific or cell type-specific manner.

Taken together, these results show that, although the functional relevance of some of the DNA sensor candidates (e.g., DDX41, IFI16, DNA-PKcs, Mre11, and others) still needs to be fully investigated, there is consensus that cGAS and STING are bona fide cytosolic DNA-mediated regulators.

Modulation of the cGAS/STING pathway by Post-Translational Modifications (PTMs)

Recent investigations have demonstrated that intracellular post-translational modification (PTM) systems, especially phosphorylation and ubiquitination, participate in the cGAS/STING pathways that positively or negatively modulate enzymatic activity, subcellular distribution, protein stabilization and degradation, conformational rearrangement, and signal transduction. Here, we provide an update on the role of PTMs in regulation of the cGAS/STING pathway (Table 1).

Involvement of intracellular kinases for cGAS/STING function

Upon DNA ligand stimulation, it has been shown that STING may be phosphorylated at amino acid position Serine-366 (S366) as a primary acceptor site of phosphorylation through a couple of intracellular kinases (48,49). First, the kinase TBK1 was proposed to be involved in the positive regulation of STING phosphorylation to promote IRF3 activation. A subsequent study showed that the autophagy-related serine/threonine protein kinases ULK1 and ULK2 were involved in
the process of STING phosphorylation that enabled the termination of signal activation. These results indicated that each of these distinct kinases, TBK1 and ULK1/2, may possess opposite functions via the same residue S366 for STING phosphorylation. Further studies will be needed to resolve these conflicting observations and to prove the physiological relevance of ULK1/2 using genetically engineered mice. On the other hand, the presence of an additional residue at position S358 of human STING also has the potential to affect STING phosphorylation, although the impact of this phosphorylation remains to be clarified (50). Most recently, it was shown that ribosomal protein S6 kinase 1 (S6K1) may also participate in the positive regulation of STING-dependent signal activation, although the kinase activity of S6K1 was found to be dispensable (51). In contrast to the observation of STING-mediated phosphorylation, only one intracellular kinase that is involved in the modulation of cGAS function has been reported. A DNA virus (like HSV-1)-inducible Akt (also referred to as protein kinase B (PKB)) may be involved in the phosphorylation of cGAS at S305 (at S291 in murine cGAS) within the enzymatic domain of cGAS, thereby suppressing the enzymatic activity of cGAS (52). This may be considered one of the strategies by which HSV-1 escapes the DNA-sensing immune response and achieves a sustained infection. Further studies will be needed to determine whether there is an intracellular phosphorylation kinase that can positively regulate cGAS function.

**Involvement of intracellular ubiquitin ligases for cGAS/STING function**

Attempts to understand the detailed mechanism of cGAS/STING function have indicated that STING may also accept an intracellular ubiquitination process at the multiple lysine (K) residues through the recruitment of distinct ubiquitin E3 ligases. First, several members of the Tripartite interaction motif (TRIM) family of RING E3
ligases, such as TRIM56 and TRIM32, have been proposed to play a role in the STING ubiquitination via conjugation of K63-linked poly-ubiquitination in a manner that positively impacts STING function (53,54). Conversely, in addition to TRIM29 and TRIM30α, E3 ubiquitin ligase RING finger protein 5 (RNF5) may induce the degradation of STING via conjugation of K48-linked poly-ubiquitination for the termination of signal activation (55-57). Additionally, each of two distinct molecules—the autocrine motility factor receptor (AMFR)-insulin-induced gene 1 (GP78/INSIG1) E3 ubiquitin ligase complex and mitochondrial E3 ubiquitin protein ligase 1 (MUL1)—have also been shown to be involved in the STING ubiquitination via conjugation of K27- and K63-linked poly-ubiquitination, respectively, to facilitate STING-dependent signal activation (58,59). Most recently, it has been reported that RNF26 may catalyze STING ubiquitination via conjugation of K11-linked poly-ubiquitination at the same conjugating site of RNF5. This may be considered to contribute positively to STING function through the competition with RNF5-mediated poly-ubiquitination of STING (60). In contrast to the accumulated evidence regarding STING ubiquitination, little is known about cGAS function. The E3 ubiquitin ligase RNF185 may be involved in the cGAS ubiquitination via conjugation of K27-linked poly-ubiquitination for the promotion of an enzymatic activity of cGAS (61). Moreover, it has been shown that TRIM56 may also play a role in cGAS-mediated mono-ubiquitination for the facilitation of cGAMP production (62). At the present time, however, an intracellular E3 ubiquitin ligase that negatively regulates cGAS function remains to be identified. Given the involvement of multiple E3 ubiquitin ligases in modulation of the cGAS/STING pathway, further investigations will be needed to determine the elaborate interactions.
The PTMs system must be reversible in order to alternatively regulate the PRR-mediated signal transduction via de-ubiquitination or via de-phosphorylation. For instance, the protein phosphatase, Mg\(^{2+}\)/Mn\(^{2+}\) dependent 1A (PPM1A), has been shown to be involved in the de-phosphorylation of S358 of STING to prevent STING-dependent signal activation (63), while the kinase responsible for the de-phosphorylation of S366 is currently unknown. Recently, it was reported that inactive rhomboid protein 2 (iRhom2) may facilitate de-conjugation of RNF5-mediated K48-linked poly-ubiquitination of STING through the recruitment of a de-ubiquitination enzyme, eukaryotic translation initiation factor 3 subunit 5 (EIF3S5) (64). Another PTM-independent function of iRhom2 may be to regulate STING translocation through the recruitment of the ER translocon-associated protein TRAPβ (23). On the other hand, it was also shown that some kinds of ubiquitin-specific proteases (USPs) are involved in the modulation of STING function. USP18 and USP20 may facilitate de-conjugation of RNF5-mediated K48-linked poly-ubiquitination of STING in addition to the K33-linked poly-ubiquitination (65). Moreover, USP13 has also been shown to act as a de-conjugated enzyme to STING-mediated K27-linked and K33-linked poly-ubiquitination, respectively (66). Although the impact of K33-linked poly-ubiquitination of STING has still not been defined, distinct USPs may manipulate STING function via de-conjugation at multiple lysine residues.

While the PTMs that control cGAS/STING function remain to be fully defined (e.g., the physiological role of noncanonical polyubiquitin linkage types such as K11 and K33 on the STING ubiquitination), elucidation of the mechanism underlying this signal modification could help to establish a potent therapeutic approach against auto-inflammatory diseases that are mediated by the cGAS/STING axis.
Viral strategy for evasion of the cGAS/STING pathway

Upon DNA virus infection, host cells may initiate the induction of various effector anti-viral genes, such as type-I IFNs, ISGs, and pro-inflammatory cytokines or chemokines, through the cGAS/STING pathway. However, it has been widely reported that a number of viruses are equipped with mechanisms to counteract the cGAS/STING pathway during both acute and persistent viral infection, and these activities may be the means by which the viruses escape the host immune surveillance system. So far, many different immune evasion strategies employed by several viruses have been elucidated, including i) interference with the functions of host innate immune response via physical interactions with viral antagonistic proteins targeted to sensors, adaptors, related intracellular kinases, and transcription factors, ii) inducing degradation or specific cleavage at the protein levels, and iii) the sequestration of signal transduction molecules targeting the PTM systems. Here, we summarize these strategies with a focus on recently published studies (Fig. 2).

Manipulation of cGAS/STING function by DNA viruses

Chronic hepatitis B virus (HBV) infection is a major cause of chronic liver diseases such as hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). A recent study has shown that the HBV polymerase (pol) gene prevents STING-dependent signal activation through a blockade of STING-mediated K63-linked poly-ubiquitination, leading to chronic persistent infection of HBV in hepatocyte cells (67). On the other hand, the packaging of relaxed-circular HBV DNA into the viral capsid protein was able to block direct recognition from the cGAS-mediated sensing process, which was recently reported as an alternative evasion strategy of HBV (68). On the other hand, it has also been suggested that hepatocytes do not produce the type-I IFNs in response to
synthetic dsDNA or HBV infection due to a lack of the cGAS/STING pathway in hepatocyte cells (69,70). This may also be considered the reasonable explanation why HBV has been able to specifically adapt in hepatocyte cells and could contribute to weak capacity of this cell type to eliminate HBV infection. Further investigation will be necessary to clarify the interaction between HBV infection and STING-dependent signal activation in hepatocyte cells.

Members of the herpesvirus family are widely used as ligands for the induction of DNA-mediated immune responses both in vitro and in vivo, and there is accumulated evidence that these viruses have antagonistic effects on the cGAS/STING pathway. The HSV-1-encoded tegument protein UL41 was shown to induce cGAS degradation via the proteasomal pathway, while the HSV-1 immediate early (IE) protein ICP0 may affect the stability and function of STING in certain cell types (71,72). Other tegument proteins such as UL46 interfered with the function of cGAMP-mediated STING by physically interacting with STING and its downstream kinase TBK1 (73). HSV-1 ICP27, an IE protein conserved among all herpesviruses, may also interact with STING and TBK1 in order to interfere with STING-dependent signal activation (74). HSV-1 VP22, encoded by the UL49 gene, may also possess an antagonistic function on the cytosolic DNA-sensing immune response through modulation of the enzymatic activity of cGAS (75). While deamidation impairs the ability of cGAS to catalyze cGAMP synthesis, it has been shown that the HSV-1 UL37 tegument protein may promote cGAS deamidation for the attenuation of cGAMP-mediated anti-viral activity (76).

Recent studies have demonstrated that HSV-1 may target transcription factors that are located downstream of the STING/TBK1 axis as part of its immune evasion strategy. Some of the viral tegument proteins, such as UL24, and UL36, in addition to the viral serine protease of HSV-1, VP24, have evolved certain strategies to target IRF3 and NF-
kB by negatively regulating them (77-79). Interestingly, it has been demonstrated that the γ34.5 gene of HSV-1 encodes a virulence factor for HSV-1-mediated pathogenesis that may also act as an antagonistic factor on the cGAS/STING pathway (80). This result appears to agree with the finding that an HSV-1 mutant in which the γ34.5 gene was deleted no longer exhibited an antagonistic function in infected cells, and thereby facilitated IFN production in a STING-dependent manner (26). On the other hand, an oncogenic herpesvirus, Kaposi sarcoma-associated herpesvirus (KHSV), encoding the viral interferon regulatory factor 1 (vIRF1) gene has been shown to prevent the association between STING and TBK1, thereby inhibiting the initiation of IRF3-mediated signal activation (81). The authors in this paper also identified five other KSHV-encoded proteins that could suppress the STING-dependent signal activation. The latency-associated nuclear antigen (LANA) of KSHV has been shown to play a pivotal role for viral replication. Zhang and colleagues also reported that the N-terminal truncated cytoplasmic isoforms of LANA may associate with cGAS directly in order to interfere with the cGAS-dependent signal activation (82). Moreover, the murine gammaherpesvirus 68 (MHV68) encoding de-ubiquitination (DUB) enzyme ORF64 (also referred to as the KSHV ORF52 homolog) has also been shown to suppress STING-dependent signal activation (83). Mechanistically, this occurred as a DNA-sensing process in a DUB activity-dependent manner. Additionally, one of the KSHV-encoded tegument proteins, ORF52, was also shown to antagonize cGAS function through its direct association with both cGAS and viral DNA (84). Furthermore, the authors in this paper showed that the homologues of ORF52 genes derived from Epstein-barr virus (EBV) and Rhesus monkey rhadinovirus (RRV), in addition to MHV68, exhibited similar inhibitory functions targeting cGAS, suggesting that the antagonistic function of gammaherpesviruses is evolutionally conserved. Overall, these
results indicated that herpesviruses may modulate the cytosolic DNA-mediated immune response via an elaborate mechanism that involves the viral encoding of several antagonistic genes.

Moreover, a viral oncoprotein containing the LXCXE motif, which is also conserved among a small number of DNA tumor viruses, was recently shown to suppress the cytosolic DNA-sensing immune response. For instance, viral encoding oncoproteins such as E7 and E1A derived from human papillomavirus 18 (HPV18) and human adenovirus type-5 (hAd5) exhibited suppression of the cytosolic DNA-sensing immune response through the direct association with STING (85). Interestingly, it has been shown that the LXCXE-containing tumor proteins were also expressed in many kinds of immortalized cells and permanently impaired the cytosolic DNA-sensing immune response via cGAS/STING. This might be part of the cytotoxic mechanism of the viral oncoprotein that exhibits LXCXE-mediated antagonism of the STING pathway.

**Manipulation of cGAS/STING function by RNA viruses**

Although the role of cGAS/STING in the recognition and counteraction of DNA viruses has been well described as detailed above, recent studies have also reported on a number of RNA viruses, particularly positive-stranded RNA viruses, that are targeted by this pathway.

The non-structural protein 4B (NS4B) of yellow fever virus (YFV), which belongs to the *Flaviviridae* family, was first reported as a viral protein interacting with STING. Analysis of the sequence alignment revealed that STING possesses a highly structural homology domain with NS4B of dengue virus (DENV) and hepatitis C virus (HCV) in addition to YFV (23). Subsequent studies indicated that HCV NS4B suppresses
STING-dependent signal activation via its direct interaction with STING at the near the viral replication complex on the ER (86-88). Although the detailed machinery of the NS4B-mediated counteraction of STING are not fully understood, it will be necessary to verify the functional evidence of the cGAS/STING pathway in hepatocyte cells, just as described above for HBV infection.

Another elegant viral immune evasion strategy is disruption of the dsRNA-mediated innate immune response via proteolysis of mitochondrial adaptor IPS-1 (also known as MAVS, VISA, and CARDIF) by hepatitis C virus (HCV) NS3/4A serine protease (88). By means of this strategy, some RNA viruses encoding viral proteases (e.g., hepatitis A virus (HAV) 3C protease, enterovirus 71 (EV71) 2A protease, rhinovirus 2A and 3C protease, and coxsackievirus B3 (CVB3) 3C protease) can directly cleave IPS-1 (88). With regard to the cGAS/STING pathway, NS2B/NS3 protease of dengue virus (DENV) first exhibited a similar inhibitory evasion mechanism via the proteolysis of human but not murine STING (89,90). This result may support the observation that DENV replication is restricted in mice due to the inability of DENV protease to cleave the murine STING. Most recently, this conserved strategy to cleave STING was also observed in other flavivirus NS2B/NS3 proteases derived from Zika virus, West Nile virus (WNV), and Japanese encephalitis virus (JEV), but not YFV (91). Accordingly, there may be a non-proteolytic mechanism to counteract STING function by YFV-encoded viral protease, as described above. On the other hand, it has also been proposed that the Zika virus may possess an alternative strategy for immune escape. The Zika virus-encoded NS1 protein has been shown to promote the proteolysis of cGAS rather than STING through the recruitment of caspase-1 activation, suggesting that Zika virus infection manipulates the interplay between the inflammasome and cGAS/STING pathways (92). These results indicate
that the Flaviviridae family has evolved to escape from innate immune responses in humans by targeting distinct signal adaptor molecules of cytosolic nucleic acid sensors.

Some RNA viruses have also evolved a mechanism to evade innate immune responses targeting the PTM systems involved in the cGAS/STING pathway. Virus-encoded deubiquitylating enzymes (DUBs) such as papain-like proteases (PLPs) derived from human coronavirus (HCoV)-NL63, severe acute respiratory syndrome (SARS) CoV and porcine epidemic diarrhea virus (PEDV) were also shown to suppress the STING-dependent signal activation in a manner similar to the HBV polymerase gene (93-95). Similarly, the Tax protein of human T lymphotropic virus type-1 (HTLV-1), which plays a critical role in promoting viral replication and T cell transformation, was also shown to exhibit interference in STING-mediated K63-linked poly-ubiquitination via physical interaction (96). As described in the section above, conjugation of K63-linked poly-ubiquitination has been shown to be involved in the positive regulation of PRRs-mediated signal activation. Thus, a reasonable strategy for viral-mediated immune evasion could be to counteract the STING-mediated K63-linked poly-ubiquitination.

While counteraction of the cGAS/STING pathway by RNA viruses classified as positive-sense RNA viruses has been reasonably well elucidated, much less is known about the actions of negative-sense RNA viruses. The influenza A virus (IAV) belongs to the Orthomyxoviridae family, whose members possess negative-sense RNA, and IAV was recently reported to elicit IFN production through a viral envelope-mediated fusion process in a STING-dependent but cGAS-independent manner (97). Similarly, the other enveloped RNA viruses, such as Newcastle disease virus (NDV) and Sendai virus (SeV) also exhibited the same roles for the induction of innate immune response, although it still remains to be clarified the molecular mechanism of signal transduction.
Interestingly, it was shown that the hemagglutinin (HA) fusion peptide (FP) of IAV may associate with STING via its dimerization interphase domain, thereby inhibiting the STING-mediated dimer formation for the initiation of STING-dependent signal activation. Thus, this is the first evidence of a negative-sense RNA virus exerting an agonistic effect on STING.

In contrast to the well-studied viral escape strategies that directly target the functioning of STING, less is known about the function of cGAS during RNA virus infection. Recently, it has been reported that the viral capsid proteins of HIV-1 and HIV-2 may suppress the cGAS-mediated DNA-sensing process by recruiting host factors for HIV replication, such as cofactors cleavage and polyadenylation specificity factor subunit 6 (CPSF6) and cyclophilin-A (Cyp-A), respectively (98,99). More specifically, the mutated HIV capsids with an impairment of Cyp-A association could be stimulated by the cGAS-mediated immune activation, suggesting that an intact HIV capsid is a determinant factor for the immune evasion strategy of HIV-1.

Despite the fact that the counterpart to STING functions via proteolysis by DENV protease, it has been shown that the release of mitochondrial DNA via induction of cellular damage in DENV-infected cells may have the potential to stimulate a cGAS/STING-mediated immune response (Fig.4). To avoid such signal activation, an NS2B protease co-factor derived from DENV may directly target cGAS to lead to proteasomal degradation (100). It remains unclear whether species-specific effects are involved in this NS2B protease co-factor-dependent immune evasion strategy—e.g., the proteolysis of human but not murine STING in a protease-dependent manner.

**Concluding remarks**
The past decade has seen a rapid advance in our understanding of the cytosolic DNA-sensing pathways, especially the cGAS/STING axis, including their regulatory mechanisms and the development of their related diseases such as SLE, AGS, and polyarthritis. It remains to be determined whether other types of inflammatory diseases may also be manifested through a defect in cGAS/STING function (e.g., its mediated by gain- or loss-of-function mutations). Thus, an improved understanding of the mechanisms underlying overactivity of inflammation via the cGAS/STING pathway could lead to the design of potent therapeutic agents and strategies to overcome undesirable inflammatory diseases and certain types of cancers. In addition, although the cGAS/STING pathway may contribute to detection and elimination of infection by both DNA and RNA viruses, these viruses have also evolved over time to escape or manipulate this signal activation through several evasion strategies as described above. Indeed, such manipulation is considered a critical ability of viruses for the establishment of both lytic and persistent infection and elicitation of characteristic pathogenesis. Further knowledge on the non-canonical mechanism of cGAS-independent STING activation and its evasion strategy by RNA virus infection will be highly informative for understanding the host-pathogen interaction. The cGAS/STING pathway may function in a variety of cell types, but the details of its functioning in hepatocyte cells that enable the persistent infection of hepatotropic viruses remain to be elucidated. Similarly, an understanding of virus-mediated immune evasion strategies could provide novel insights into viral evolution and the potential design of novel anti-viral agents. Finally, along with the accumulating insights regarding the cGAS/STING pathway and its involvement in the virus-host interaction, it will also be important to understand the loss of cGAS/STING function in several cancer cells or transformed cells by the viral encoding oncoprotein. This loss might involve signal manipulation
via epigenetic silencing and the insertion of missense mutations into the cGAS/STING locus. Further elucidation of this point may provide important information for the treatment of several viral oncogeneses.

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Conflict of interest

The authors declare no conflict of interest.

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**Figure legends**

**Fig. 1 Cytosolic DNA-sensing immune response via cGAS/STING**

A schematic of the cytosolic DNA-sensing immune response via the cGAS/STING pathway. Upon DNA virus infection, the cytosolic DNA sensor cGAS directly recognizes viral DNA and catalyzes cGAMP, which utilizes the cellular GTP/ATP, thereby triggering activation of the signal adaptor STING via a direct interaction with cGAMP. cGAS may also recognize the transfected dsDNA or viral DNA that is produced by HIV-1 through the reverse-transcription of viral RNA. Following the binding of cGAMP, STING is translocated from the ER to perinuclear-Golgi, and may form a signaling complex with kinase TBK1 (the phosphorylation of STING at
Serine(S)-366 occurs here after translocation) to induce the production of IRF3-mediated type I IFNs. STING may also activate the production of NF-κB(p65)-mediated pro-inflammatory cytokines. cGAS may also recognize self-DNAs, such as the released nucleosome and micronuclear DNA in the cytoplasm during DNA damage or cellular senescence, promoting STING-dependent signal activation. The missense mutation of a number of cellular DNases may induce the aberrant inflammatory response via the cGAS/STING axis due to a failure of self-DNA digestion in necrotic or inappropriately apoptotic cells. Abbreviations: cGAS, cyclic GMP-AMP synthase; STING; stimulator of IFN genes; cGAMP, cyclic GMP-AMP; dsDNA, double-stranded DNA; ER, endoplasmic reticulum; TBK1, TANK-binding kinase 1; IRF3; interferon regulatory factor 3; NF-κB; nuclear factor-κB; RT, reverse-transcription; P; phosphorylation.

**Fig. 2 Viral strategies for evading the cGAS/STING pathway**

A schematic summarizing the virus-mediated immune strategies for evasion of the cGAS/STING pathway. To escape from the cytosolic DNA-sensing immune response via the cGAS/STING pathway, several viruses may manipulate this signal activation through several evasion strategies, including i) inhibition of the function of cGAS or STING via physical interaction, ii) manipulation of the PTMs system involved in the cGAS/STING function, iii) induction of the proteolysis and degradation of cGAS or STING, iv) sequestration of the DNA-sensing process mediated by cGAS, v) inhibition of STING-dependent signal activation at the level of transcription factors such as IRF3 and NF-κB. Abbreviations: HIV-1, human immunodeficiency virus-1; DENV; Dengue virus; KSHV, Kaposi sarcoma-associated herpesvirus; HSV-1, herpesviruses-1; HBV, hepatitis B virus; HcoV, human coronavirus; SARS, severe acute respiratory syndrome;
PEDV, porcine epidemic diarrhea virus; HTLV-1, human T lymphotropic virus type-1; WNV, West Nile virus; JEV, Japanese encephalitis virus; HPV18, human papillomavirus 18; hAd5, human adenovirus type-5; IAV, influenza A virus; YFV, yellow fever virus; HCV, hepatitis C virus; MHV68, murine gammaherpesvirus 68; 63, K63-linked ubiquitin.

Fig. 3 Crosstalk between enveloped RNA virus infection and STING dependent signal activation

A schematic of the viral RNA-sensing immune response via the cytosolic DNA-sensing pathway. Upon RNA virus infection, the RNA helicase RIG-I directly recognizes viral RNA and activates the IFN production through the interaction with mitochondrial adaptor IPS-1. (A) STING may also associate with signaling complex of RIG-I and IPS-1 to promote the triggering of the antiviral response in cells upon RNA virus infection (23). (B) Enveloped RNA viruses such as IAV, NDV, and SeV activate IFN production through a viral envelope-mediated fusion process in a STING-dependent but cGAS-independent manner, although it still remains to be clarified the molecular mechanism of signal transduction (97). As shown in Fig.2, the hemagglutinin (HA) fusion peptide (FP) of IAV may also associate with STING via its dimerization interphase domain, thereby inhibiting the STING-dependent signal activation.

Abbreviations: NDV, Newcastle disease virus; SeV; Sendai virus; RIG-I, Retinoic acid inducible gene-I; IPS-1, IFN-β promoter stimulator-1.
Fig. 4 A model of DENV-mediated activation of the cytosolic DNA-sensing pathway in dendritic cells (DCs)

A schematic of DENV-mediated activation of the cytosolic DNA-sensing pathway through the induction of mitochondrial dysfunction. Upon DENV infection in DCs, RIG-I directly recognizes viral RNA and activates the IFN production through the interaction with mitochondrial adaptor IPS-1. DENV infection also triggers the mitochondrial damage response via the induction of ROS, resulting in mitochondrial DNA release in the cytoplasm that in turn stimulates the signal activation via cGAS/STING axis. As shown in Fig.2, DENV NS2B/NS3 protease and NS2B protease co-factor alone may directly target STING and cGAS for the suppression of their signal activation. Abbreviations: ROS, reactive oxygen species; DCs, dendritic cells.

Table 1 Overview of the post-translational modifications (PTMs) involved in the cGAS/STING pathway

This table summarizes the several kinases, phosphatase, ubiquitin ligases and de-ubiquitin ligases that modulate cGAS/STING-dependent signal activation. These enzymes may be catalyzed or remove specific amino acid residues or specific linkage types of ubiquitin to the target substrate. iRhom2 may function to recruit EIF3S5, which acts as a de-ubiquitin ligase to remove K48-linked poly-ubiquitin from STING.
Table 1: Overview of the post-translational modifications (PTMs) involved in the cGAS/STING pathway

| Enzyme   | Substrate | Proposed function                                      | Reference |
|----------|-----------|--------------------------------------------------------|-----------|
| **Kinase/Phosphatase** |           |                                                        |           |
| TBK1     | STING     | phosphorylation of Serine-366                          | (48)      |
| ULK1/2   | STING     | phosphorylation of Serine-358                          | (50)      |
| S6K1     | STING     | STING interaction in kinase-independent manner         | (51)      |
| Akt (PKB)| cGAS      | phosphorylation of Serine-305                          | (52)      |
| PPM1A    | STING     | De-phosphorylation of Serine-358                        | (63)      |
| **UB-ligase** |       |                                                        |           |
| TRIM56   | STING     | K63-linked poly-ubiquitation                            | (53)      |
|          | cGAS      | mono-ubiquitination                                     | (62)      |
| TRIM32   | STING     | K63-linked poly-ubiquitination                          | (54)      |
| TRIM29   | STING     | K48-linked poly-ubiquitination                          | (57)      |
| TRIM30α  | STING     | K48-linked poly-ubiquitination                          | (56)      |
| RNF5     | STING     | K48-linked poly-ubiquitination                          | (55)      |
| AMFR/INSIG1 | STING  | K27-linked poly-ubiquitination                          | (58)      |
| MUL1     | STING     | K63-linked poly-ubiquitination                          | (59)      |
| RNF26    | STING     | K11-linked poly-ubiquitination                          | (60)      |
| RNF185   | cGAS      | K27-linked poly-ubiquitination                          | (61)      |
| **DUB-ligase** |      |                                                        |           |
| iRhom2   | STING     | De-conjugation of K48-linked poly-ubiquitination        | (64)      |
| EIF3SS   | STING     | De-conjugation of K48-linked poly-ubiquitination        | (64)      |
| USP18    | STING     | De-conjugation of K33/K48-linked poly-ubiquitination    | (65)      |
| USP20    | STING     | De-conjugation of K33/K48-linked poly-ubiquitination    | (65)      |
| USP13    | STING     | De-conjugation of K27/K33-linked poly-ubiquitination    | (66)      |

This table summarizes the several kinases, phosphatase, ubiquitin (UB) ligases and de-ubiquitin (DUB) ligases that modulate cGAS/STING-dependent signal activation. These enzymes may be catalyzed or remove specific amino acid residues or specific linkage types of ubiquitin to the target substrate.
Fig. 3

Enveloped RNA viruses (IAV, NDV, SeV)

Fusion of viral membranes

Viral RNA

IAV-HA(PP)

RIG-I

STING

TBK1

IRF3

Type I IFNs

IAV-HA(PP)

STING

TBK1

IRF3

Type I IFNs

Mitochondria

TBK1

IRF3

Type I IFNs

IAV-HA(PP)

STING

TBK1

IRF3

Type I IFNs

Fig. 4

DENV

Viral RNA

ER

DENV-NS2B/NS3

STING

GTP/ATP

CGAMP

Mitochondrial DNA

ROS

IPS-1

TBK1

IRF3

Type I IFNs

Nuclear-
Golgi

IRF3

p65

p65

Type I IFNs

Inflammatory cytokines

Cytoplasm

Type I IFNs