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REVIEW
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Cytosolic DNA-sensing immune response and viral infection

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Abstract

How host cells recognize many kinds of RNA and DNA viruses and initiate innate antiviral responses against them has not yet been fully elucidated. 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). The findings of these studies have suggested that antiviral responses are mediated by cytosolic or intracellular compartment sensors and their adaptor molecules (e.g., TLR, myeloid differentiation primary response 88, retinoic acid inducible gene-I, IFN-β promoter stimulator-1, cyclic GMP-AMP synthase and stimulator of IFN genes axis) for the primary sensing of virus-derived nucleic acids, leading to production of type I 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 infections. In turn, to achieve sustained viral infection and induce pathogenesis, viruses have also evolved several counteracting strategies for achieving immune escape by targeting immune sensors, adaptor molecules, intracellular kinases and transcription factors. In this review, we discuss recent discoveries concerning the role of the cytosolic nucleic acid-sensing immune response in viral recognition and 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.

KEYWORDS
cyclic GMP-AMP synthase, stimulator of IFN genes, interferon, viral immune evasion

Abbreviations: AGS, Aicardi-Goutières syndrome; AIM2, absent in melanoma 2; AMFR, autocrine motility factor receptor; CDN, cyclic dinucleotide; cGAS, cyclic GMP-AMP synthase; cGAMP, cyclic GMP-AMP; DAI, DNA-dependent activator of IRF3; DENV, dengue virus; DNA-PKcs, DNA-dependent protein kinase; dsDNA, double-stranded DNA; dsRNA, double-stranded RNA; DUB, de-ubiquitination; EIF3S5, eukaryotic translation initiation factor 3 subunit 5; ER, endoplasmic reticulum; HBV, hepatitis B virus; HCV, hepatitis C virus; HSV-1, herpes simplex virus-1; IAV, influenza A virus; IFI16, IFN-γ-inducible protein 16; IPS-1, IFN-β promoter stimulator-1; IRF3, interferon regulatory factor 3; iRhom2, inactive rhomboid protein 2; ISG, IFN-stimulated gene; JEV, Japanese encephalitis virus; KSHV, Kaposi sarcoma-associated herpesvirus; LANA, latency-associated nuclear antigen; MDA5, melanoma differentiation-associated protein 5; MHV68, murine gamma-herpesvirus 68; Mre11, meiotic recombination 11; NDV, Newcastle disease virus; NF-κB, nuclear factor-κB; NLR, NOD-like receptor; PPM1A, protein phosphatase, Mg^{2+}/Mn^{2+} dependent 1A; PRR, pattern recognition receptor; PTM, post-translational modification; RIG-I, retinoic acid inducible gene-I; RNF5, RING finger protein 5; S6K1, S6 kinase 1; STING, stimulator of IFN genes; TBK1, TANK-binding kinase 1; TRIM, tripartite interaction motif; USP, ubiquitin-specific protease; YFV, yellow fever virus.

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1 | INTRODUCTION

Vertebrate cells possess PRRs to enable detection of pathogens such as viruses, bacteria, fungi and parasites. Immediately after a 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, and activate an innate antiviral response to eliminate the pathogens [1]. Among the molecules composing the pathogen-associated molecular patterns, pathogen-derived nucleic acids are the most potent mediators of innate antiviral responses. The primary induction of innate antiviral responses through detection of nucleic acids is also critical for subsequent induction of an acquired immunity response. Over the past ten years, the responsible immune surveillance sensors, such as the TLRs, cytosolic RNA sensors (primary RIG-I/MDA5), cytosolic DNA sensors (primary cGAS and others), and sensors of the inflammasome pathway (e.g., the primary NLR family, which consists of two major subfamilies, NLRC and NLRP), have been well characterized [2–4]. These immune surveillance sensors are ubiquitously expressed, enabling detection of pathogens invading at the cell surface and cytoplasmic or nuclear compartment in several cell types and leading to production of type I IFNs, proinflammatory 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 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 dsRNA; additionally, 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 have revealed that RIG-I is crucial for detection of several negative-stranded RNA viruses (e.g., vesicular stomatitis virus, NDV and influenza A and B virus), as well as detection of positive-stranded RNA viruses (e.g., JEV and HCV) [8]. In contrast, MDA5 dominantly detects picornaviruses (e.g., encephalomyocarditis virus). 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 less clear. Cytoplasmic DNA-sensing by cGAS in collaboration with an adaptor protein, 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 induction of aberrant inflammatory diseases such as systemic lupus erythematosus, AGS and polyarthritis [9,10]. While 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 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 sequestered [9]. Additionally, recent studies have shown that cell cycle progression during mitosis following a DNA-damage response may lead to 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 initiation of downstream signal activation. In addition to the DNA-damage response discussed above, two independent research groups have also reported that the cGAS/STING axis also recognizes an intrinsic DNA during cellular senescence and that this activation precedes induction of an inflammatory response (this is defined as the senescence-associated secretory phenotype) [16,17]. Subsequently, production of senescence-associated secretory phenotype factors such as inflammatory cytokines and chemokines may reinforce senescence-associated cells via autocrine and paracrine routes. Thus, remarkably, the cGAS/STING pathway appears to regulate inflammatory disorders manifested through detection of self-DNA during DNA damage and cellular senescence. The cGAS/STING pathway may not only be important for recognition of DNA virus infection, but also be critical for the host defense against RNA virus infection. However, the detailed mechanism by which STING controls RNA virus infection remains to be determined. Of note, viruses have evolved an elaborate mechanism for escaping detection by the cGAS/STING axis or for suppressing activation of that pathway; this escape mechanism includes downstream signal activation of the 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 strategies of certain viruses that target different steps in this signaling pathway, with a focus on recently published work concerning clinically important viruses.

2 | IDENTIFICATION OF PUTATIVE DNA SENSORS

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

In contrast to the more clearly identified mechanisms of the TLR and RIG-I-like receptor signaling pathways, there is still no universal agreement regarding the cytosolic DNA sensors and their regulatory mechanisms [18]. Following reports that cytosolic DNA-sensing TLR-independent IFN production is implicated in the transfected synthetic dsDNA in murine fibroblast 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. Accumulated evidence for 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 DAI (also referred to as DLM-1/ZBP1), which is one of the IFN-inducible genes, is involved in dsDNA recognition for TBK1-mediated IRF3 activation [21]. DAI may form a signaling complex with TBK1 and IRF3 for production of type I IFNs in response to synthetic dsDNA or DNA virus infection (e.g., HSV-1) in L929 murine fibroblast cells. However, Ishii and colleagues reported that IFN production is not impaired in response to synthetic B-form dsDNA and HSV-1 infection in several types of cells that lack DAI function; additionally, DAI-deficient mice have a normal response to plasmid-based DNA immunization [22]. These findings indicate that the function of DAI may be cell-type specific or redundantly replicate the DNA-sensing innate immune response.

2.2 | 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), Barber and colleagues introduced STING (also referred to as MITA, MPYS or ERIS), which is encoded by the *TMEM173* gene and enables activation of the IFNβ promoter. STING is a 379 amino acid protein consisting of multiple transmembrane regions; it is localized in the ER and plays an essential role in cytosolic DNA-mediated innate immune responses and responses to DNA-based immunization [23–25]. Several cytosolic DNA species derived from microbial pathogens can trigger STING-dependent signal activation via either the TBK1-mediated IRF3 axis or IKKs-mediated NF-κB axis. 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 protein degradation via a ubiquitin-mediated proteasome pathway to terminate signal activation near the perinuclear-Golgi region. It has also been suggested that STING associates with dsDNA directly; however, the physiological relevance such an association remains to be clarified [26]. In a later study, it was found that STING binds directly to CDNs, which are known to be bacterially derived second messenger molecules, with high affinity via formation of dimers [27]. Around the same time, it was also found that genetically engineered mice with a single point mutation in the STING gene (*T596A*, referred to as *Goldenticket*) failed to associate with CDNs or produce type I IFN in response to bacterially produced CDNs [28], suggesting that STING is a direct innate immune sensor for 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 improve our understanding of the molecular mechanisms of cytosolic DNA-mediated innate immune responses; however, a cytosolic DNA sensor located in the region upstream of STING may be identified in the future in a universally accepted manner.

Such a cytosolic DNA sensor may be implicated in generation of an endogenous catalytic enzyme that enables production of CDNs in response to DNA pathogens for production of type I IFNs. In 2013, Chen and colleagues reported a major breakthrough in this line of research. Namely, they discovered cGAMP, a type of CND consisting of different phosphodiester linkages, and its catalytic enzyme (cGAS), which is encoded by *MB21D1* or *C6orf150* gene [30,31]. In the cytoplasm, cGAS may bind directly to viral DNA derived from not only several species of DNA viruses, but also from reverse-transcribed DNA that is produced by retroviruses through reverse transcription of the RNA genome. Following binding of DNA, cGAS may induce conformational rearrangement and catalyze synthesis of cGAMP using cellular adenosine triphosphate and guanosine-5′-triphosphate. Indeed, it has been demonstrated that cGAMP may induce STING-dependent signal activation and that this induction would occur via direct association of cGAMP with bacterially-derived CDNs [25]. Details of this signaling transduction via the cGAS/STING axis are summarized in Figure 1. Of note, the cGAS/STING-mediated signal transduction does not require protein–protein interactions between sensor and adaptor that characterizes pathways of other PRRs (e.g., the TLR, RIG-I-like receptor and inflammasome pathways); rather, it may 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, thereby promoting 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 rapid conveyance of anti-viral signals in a horizontal manner. Importantly, it has been confirmed that cytosolic DNA-mediated innate immune responses and responses to DNA-based immunization play non-redundant roles in mice, including several cGAS-lacking mouse cell types. The cGAS/STING pathway not only plays crucial roles in host defense against DNA pathogens, but also in induction of autoimmune and inflammatory diseases through sensing of self-DNA. Thus, the cGAS/STING pathway must be properly regulated for maintenance of cellular homeostasis and immune responses. However, details of these subjects are beyond the scope of this review; they have been reported extensively elsewhere.

2.3 | Role of putative DNA sensors in type-I IFN production

Another DNA sensor candidate gene, IFI16, which is a member of the PYHIN family of proteins, has also been shown to play a role in recognition of synthetic dsDNA and viral DNA derived from viruses that are replicated in the nucleus (e.g., HSV-1, KSHV, human cytomegalovirus and Epstein–Barr virus) [33]. Although IFI16 is predominantly expressed in the nucleus in the steady state, it may shuttle between the nucleus and cytosol for sensing of viral DNA [33]. In addition, IFI16 may reportedly be involved in both DNA-sensing inflammasome activation and DNA damage responses in apoptotic cells [34]. Similar to IFI16, a cytosolic DNA sensor 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, of which there are 13 members, all of which possess a pyrin-signaling domain and a DNA-binding HIN domain (e.g., p204, which is seen as a mouse ortholog of human IFI16), have been shown not to contribute to DNA sensing for type-I IFN production or 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 indicates that production of type-I IFNs

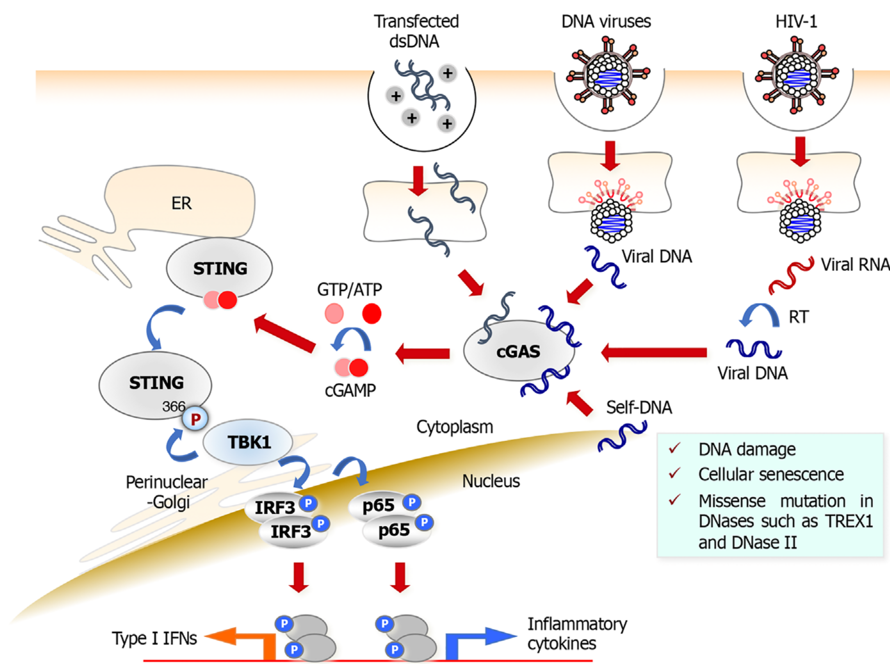

FIGURE 1 Cytosolic DNA-sensing immune response via cGAS/STING. A schematic of the cytosolic DNA-sensing immune response via the cGAS/STING pathway. Upon DNA virus infection, the cytosolic DNA sensor cGAS directly recognizes viral DNA and catalyzes cGAMP, which utilizes cellular GTP/adenosine triphosphate, thereby triggering activation of the signal adaptor STING via direct interaction with cGAMP. cGAS may also recognize the transfected dsDNA or viral DNA that is produced by HIV-1 through 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 (phosphorylation of STING at Serine[S]-366 occurs here after translocation), thus inducing production of IRF3-mediated type I IFNs. STING may also activate 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. Missense mutation of a number of cellular DNases may induce aberrant inflammatory responses via the cGAS/STING axis as a result of failure of self-DNA digestion in necrotic or inappropriately apoptotic cells. P; phosphorylation

and *ISGs* in response to synthetic dsDNA and HSV-1 infection is impaired [39,40]. Interestingly, these studies also showed that IFI16 is a prerequisite for full-activation of cGAS/STING function; however, *in vivo* evaluation is needed to fully clarify the physiological relevance of IFI16.

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

There may be a correlation between the DNA damage response and innate immune responses mediated by virus infection; however, Stetson *et al*. have reported conflicting findings [20]. Most recently, it has been suggested that several DNA damage-inducible host factors, such as the catalytic subunit of DNA-PKcs, and its binding co-factors Ku70/80, or Mre11, are involved in the cytosolic DNA-sensing immune response through 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 a high potential for a cytosolic DNA-sensing innate immune response. However, 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 indicate 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 have suggested that the following pathway underlies the DNA damage-induced immune response via the cytosolic DNA-sensing pathway: 7,12-dimethylbenz-α-anthracene-induced DNA damage results in nucleosome leakage into the cytosol and then elicits cGAS/STING-dependent signal activation via self-DNA recognition [9]. It has been proposed that, in addition to direct sensing of DNA, the indirect machinery of the cytosolic DNA-sensing innate immune response is also involved. The leucine-rich repeat Flightless-interacting protein 1 has been shown to function as an amplifier of cytosolic nucleic acid-sensing immune responses for 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 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) still needs to be fully investigated, there is consensus that cGAS and STING are *bona fide* cytosolic DNA-mediated regulators.

3 | MODULATION OF THE cGAS/STING PATHWAY BY PTMS

Recent investigations have demonstrated that intracellular PTM systems, particularly 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).

3.1 | Involvement of intracellular kinases in cGAS/STING function

It has been shown that, upon DNA ligand stimulation, STING may be phosphorylated at amino acid position Serine-366 (S366) as a primary acceptor site of phosphorylation through several intracellular kinases [48,49]. The kinase TBK1 was the first to be proposed to be involved in positive regulation of STING phosphorylation to promote IRF3 activation. A subsequent study showed that the autophagy-related serine/threonine protein kinases ULK1 and ULK2 are involved in the process of STING phosphorylation that enables termination of signal activation. These results indicate that these distinct kinases, TBK1 and ULK1/2, may possess opposite functions via the same residue S366 for STING phosphorylation. Further studies using genetically engineered mice are needed to resolve these conflicting observations and determine the physiological relevance of ULK1/2. The presence of an additional residue at position S358 of human STING also has the potential to affect STING phosphorylation; however, the impact of this phosphorylation remains to be clarified [50]. Most recently, it was shown that ribosomal protein S6K1 may also participate in the positive regulation of STING-dependent signal activation; however, the kinase activity of

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]
		Phosphorylation of serine-358	[50]
ULK1/2	STING	Phosphorylation of serine-366	[49]
S6K1	STING	STING interaction in kinase-independent manner	[51]
Akt (protein kinase B)	cGAS	Phosphorylation of serine-305	[52]
PPM1A	STING	De-phosphorylation of serine-358	[63]
UB-ligase			
TRIM56	STING	K63-linked poly-ubiquitination	[53]
	cGAS	Mono-ubiquitination	[62]
TRIM32	STING	K63-linked poly-ubiquitination	[54]
TRIM29	STING	K48-linked poly-ubiquitination	[57]
TRIM30a	STING	K48-linked poly-ubiquitination	[56]
RNF5	STING	K48-linked poly-ubiquitination	[55]
AMFR/INSIG1	STING	K27-linked poly-ubiquitination	[58]
Mitochondrial E3 ubiquitin protein ligase 1	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]
EIF3S5	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 various 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 recruit EIF3S5, which acts as a de-ubiquitin ligase to remove K48-linked poly-ubiquitin from STING.

S6K1 has been found to be dispensable [51]. In contrast to the observation of STING-mediated phosphorylation, only one intracellular kinase that is involved in modulation of cGAS function has been reported. A DNA virus (like HSV-1)-inducible Akt (also referred to as protein kinase B) may be involved in 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 one of the strategies by which HSV-1 escapes the DNA-sensing immune response and achieves sustained infection. Further studies are needed to determine whether there is an intracellular phosphorylation kinase that can positively regulate cGAS function.

3.2 | Involvement of intracellular ubiquitin ligases in cGAS/STING function

Attempts to determine the detailed mechanism of cGAS/STING function have indicated that STING may also accept an intracellular ubiquitination process at multiple lysine (K) residues through recruitment of distinct ubiquitin E3 ligases. First, it has been proposed that several members of the TRIM family of RING E3 ligases, such as TRIM56 and TRIM32, play a role in 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 RNF5 may induce degradation of STING via conjugation of K48-linked poly-ubiquitination for termination of signal activation [55–57]. Additionally, each of two distinct molecules—the AMFR-insulin-induced gene 1 (GP78/INSIG1) E3 ubiquitin ligase complex and mitochondrial E3 ubiquitin protein ligase 1—have also been shown to be involved in 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 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 cGAS ubiquitination via conjugation of K27-linked poly-ubiquitination for 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 facilitation of cGAMP production [62]. Thus far, however, an intracellular E3 ubiquitin ligase that negatively regulates cGAS function has not yet been identified. Given the involvement of multiple E3 ubiquitin ligases in modulation of the cGAS/STING pathway, further investigations are needed to clarify the elaborate interactions.

The PTMs system must be reversible in order to alternatively regulate PRR-mediated signal transduction via de-ubiquitination or de-phosphorylation. For instance, PPM1A has been shown to be involved in de-phosphorylation of S358 of STING to prevent STING-dependent signal activation [63], whereas the kinase that is responsible for de-phosphorylation of S366 has not yet been identified. Recently, it was reported that iRhom2 may facilitate de-conjugation of RNF5-mediated K48-linked poly-ubiquitination of STING through recruitment of a de-ubiquitination enzyme, EIF3S5 [64]. Another possible PTM-independent function of iRhom2 is regulation of STING translocation through recruitment of the ER translocon-associated protein TRAPβ [23]. On the other hand, it has also been shown that some types of USP are involved in modulation of STING function. USP18 and USP20 may facilitate de-conjugation of RNF5-mediated K48-linked poly-ubiquitination of STING in addition to K33-linked poly-ubiquitination [65]. USP13 has also been shown to act as a de-conjugated enzyme to STING-mediated K27-linked and K33-linked poly-ubiquitination [66]. Although the impact of K33-linked poly-ubiquitination of STING has not yet been determined, distinct USPs may manipulate STING function via de-conjugation at multiple lysine residues.

While the PTMs that control cGAS/STING function are yet to be fully defined (e.g., the physiological role of noncanonical polyubiquitin linkage types such as K11 and K33 on 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.

4 | VIRAL STRATEGIES FOR EVASION OF THE cGAS/STING PATHWAY

Upon DNA virus infection, host cells may initiate induction of various effector anti-viral genes, such as type-I IFNs, *ISG*s 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 for counteracting the cGAS/STING pathway during both acute and persistent viral infection and that these activities may be the means by which these viruses escape the host immune surveillance system. Thus far, many different immune evasion strategies employed by various viruses have been identified, including: (i) interference with the functions of the 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 level; and (iii) sequestration of signal transduction molecules targeting the PTM systems. Here, we summarize these strategies with a focus on recently published studies (Figure 2).

4.1 | Manipulation of cGAS/STING function by DNA viruses

Chronic HBV infection is a major cause of chronic liver diseases such as hepatitis, cirrhosis and hepatocellular carcinoma. A recent study has shown that the HBV polymerase (*pol*) gene prevents STING-dependent signal activation by blocking STING-mediated K63-linked poly-ubiquitination, leading to chronic persistent infection of hepatocytes by HBV [67]. Additionally, packaging of relaxed-circular HBV DNA into the viral capsid protein has been shown to block direct recognition by the cGAS-mediated sensing process; this was recently reported to be an alternative evasion strategy of HBV [68]. It has also been suggested that hepatocytes do not produce type-I IFNs in response to synthetic dsDNA or HBV infection because hepatocytes lack the cGAS/STING pathway [69,70]. This may also be considered a reasonable explanation for HBV's ability to specifically adapt in hepatocyte cells and may contribute to these cells' weak capacity to eliminate HBV infection. Further investigation of the interaction between HBV infection and STING-dependent signal activation in hepatocytes is necessary.

Members of the herpesvirus family are widely used as ligands for 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 has been shown to induce cGAS degradation via the proteasomal pathway; additionally, HSV-1 immediate early protein ICP0 may affect the stability and function of STING in certain cell types [71,72]. Other tegument proteins such as UL46 reportedly interfere with the function of cGAMP-mediated STING by physically interacting with STING and its downstream kinase TBK1 [73]. HSV-1 ICP27, an

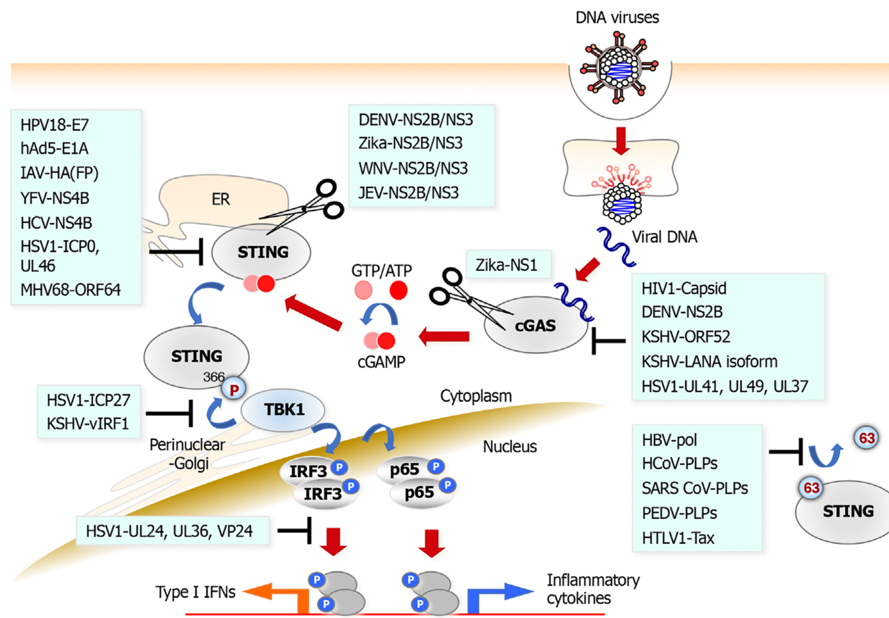

FIGURE 2 Viral strategies for evading the cGAS/STING pathway. Schema summarizing virus-mediated immune strategies for evasion of the cGAS/STING pathway. To escape from the cytosolic DNA-sensing immune response via the cGAS/STING pathway, several viruses may manipulate this signal activation through various 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 proteolysis and degradation of cGAS or STING; (iv) sequestration of the DNA-sensing process mediated by cGAS; and (v) inhibition of STING-dependent signal activation at the level of transcription factors such as IRF3 and NF-κB. HcoV, human coronavirus; SARS, severe acute respiratory syndrome; PEDV, porcine epidemic diarrhea virus; HTLV-1, human T lymphotropic virus type-1; 63, K63-linked ubiquitin.

immediate early protein that is conserved in all herpesviruses, may also interact with STING and TBK1, thus interfering with STING-dependent signal activation [74]. HSV-1 VP22, which is encoded by the *UL49* gene, may also possess an antagonistic function against the cytosolic DNA-sensing immune response through modulation of the enzymatic activity of cGAS [75]. Deamidation impairs the ability of cGAS to catalyze cGAMP synthesis; additionally, it has been shown that HSV-1 UL37 tegument protein may promote cGAS deamidation, thus attenuating 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. In addition to the viral serine protease of HSV-1, VP24, some viral tegument proteins, such as UL24 and UL36, have evolved certain strategies to target IRF3 and NF-κB 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 against the cGAS/STING pathway [80], which is consistent with the finding that an HSV-1 mutant in which the *γ34.5* gene has been deleted no longer exhibits an antagonistic function in infected cells, thereby facilitating IFN production in a STING-dependent manner [26]. On the other hand, an oncogenic herpesvirus, KHSV, which encodes *viral interferon regulatory factor 1* gene, has been shown to prevent an association between STING and TBK1, thereby inhibiting initiation of IRF3-mediated signal activation [81]. The authors of this paper also identified five other KSHV-encoded proteins that can suppress STING-dependent signal activation. The LANA of KSHV has been shown to play a pivotal role in viral replication. Zhang and colleagues have also reported that N-terminal truncated cytoplasmic isoforms of LANA may associate with cGAS directly, thus interfering with cGAS-dependent signal activation [82]. Moreover, MHV68 encoding DUB enzyme ORF64 (also referred to as a KSHV ORF52 homolog) has also been shown to suppress STING-dependent signal activation [83]. The mechanism responsible for this involves a DNA-sensing process in a DUB activity-dependent manner. Additionally, a KSHV-encoded tegument protein, ORF52, has also been shown to antagonize cGAS function through its direct association with both cGAS and viral DNA [84]. Furthermore, the authors of this paper showed that, in addition to MHV68, the homologs of *ORF52* genes derived from Epstein–Barr virus and Rhesus monkey rhadinovirus exhibit similar inhibitory functions targeting cGAS, suggesting that the antagonistic function of gamma herpesviruses is evolutionally conserved. Overall, these results indicated that herpesviruses may modulate the cytosolic DNA-mediated immune response via an elaborate mechanism that involves viral encoding of several antagonistic genes.

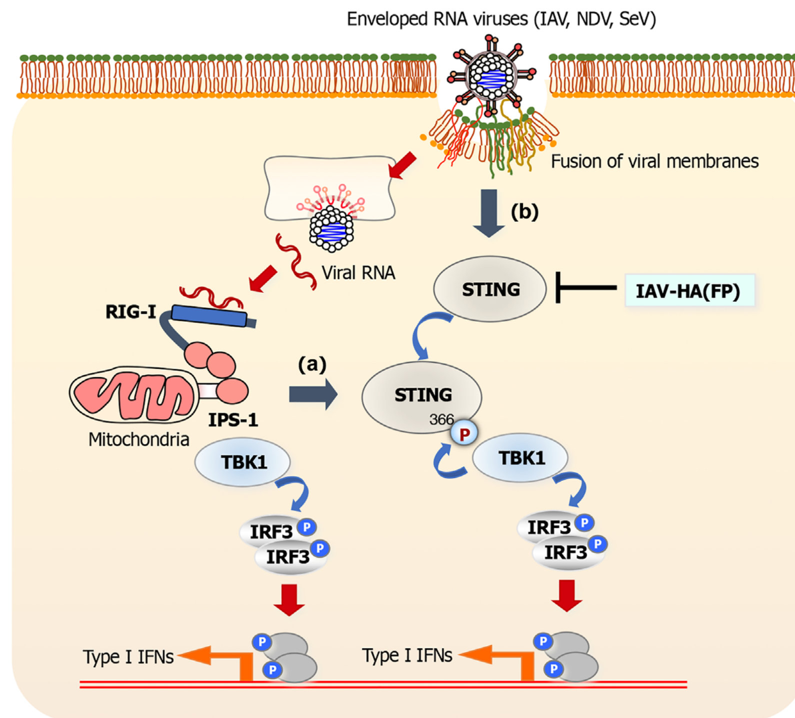

FIGURE 3 Crosstalk between enveloped RNA virus infection and STING dependent signal activation. Schema showing the viral RNA-sensing immune response via the cytosolic DNA-sensing pathway. Upon RNA virus infection, RNA helicase RIG-I directly recognizes viral RNA and activates IFN production through interaction with mitochondrial adaptor IPS-1. (a) STING may also associate with signaling complex of RIG-I and IPS-1, promoting triggering of the antiviral response in cells upon RNA virus infection (23). (b) Enveloped RNA viruses such as IAV, NDV and Sendai virus activate IFN production through a viral envelope-mediated fusion process in a STING-dependent but cGAS-independent manner; however, the molecular mechanism of signal transduction is yet to be clarified (97). As shown in Figure 2, the hemagglutinin fusion peptide of IAV may also associate with STING via its dimerization interphase domain, thereby inhibiting STING-dependent signal activation. Abbreviations: FP, fusion peptide

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 and human adenovirus type-5 suppress the cytosolic DNA-sensing immune response through direct association with STING [85]. Interestingly, it has been shown that LXCXE-containing tumor proteins are also expressed in many types of immortalized cells and permanently impair the cytosolic DNA-sensing immune response via cGAS/STING. This may be a component of the cytotoxic mechanism of the viral oncoprotein that exhibits LXCXE-mediated antagonism of the STING pathway.

4.2 | Manipulation of cGAS/STING function by RNA viruses

Although the role of cGAS/STING in 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 YFV, which belongs to the *Flaviviridae* family, was first reported as a viral protein that interacts with STING. Analysis of the sequence alignment revealed that STING possesses a highly structural homology domain with NS4B of DENV and HCV in addition to YFV [23]. Subsequent studies have found that HCV NS4B suppresses STING-dependent signal activation via its direct interaction with STING near the viral replication complex on the ER [86–88]. Although details of the mechanism of the NS4B-mediated counteraction of STING are not fully understood, it is necessary to obtain functional evidence of the cGAS/STING pathway in hepatocytes, as described above for HBV infection.

Another elegant viral immune evasion strategy is disruption of dsRNA-mediated innate immune responses via proteolysis of mitochondrial adaptor IPS-1 (also known as MAVS, VISA and CARDIF) by HCV NS3/4A serine protease [88]. Some RNA viruses that encode viral proteases (e.g., hepatitis A virus 3C protease, enterovirus 71 2A protease, rhinovirus 2A and 3C protease and coxsackievirus

FIGURE 4 A model of DENV-mediated activation of the cytosolic DNA-sensing pathway in DCs. Schema showing DENV-mediated activation of the cytosolic DNA-sensing pathway through induction of mitochondrial dysfunction. Upon DENV infection in DCs, RIG-I directly recognizes viral RNA and activates IFN production through interaction with mitochondrial adaptor IPS-1. DENV infection also triggers a mitochondrial damage response via induction of reactive oxygen species, resulting in mitochondrial DNA release in the cytoplasm that in turn stimulates signal activation via the cGAS/STING axis. As shown in Figure 2, DENV NS2B/NS3 protease and NS2B protease co-factor alone may directly target STING and cGAS, thus suppressing their signal activation. Abbreviations: ROS, reactive oxygen species.

B3 3C protease) can directly cleave IPS-1 by means of this strategy [88]. With regard to the cGAS/STING pathway, NS2B/NS3 protease of DENV was initially found to have a similar inhibitory evasion mechanism via proteolysis of human, but not murine, STING [89,90], which is consistent with the observation that DENV replication is restricted in mice because DENV protease cannot cleave murine STING. Most recently, this conserved strategy for cleaving STING was also observed in other flavivirus NS2B/NS3 proteases derived from Zika virus, West Nile virus and JEV, but not YFV [91]. Accordingly, there may be a non-proteolytic mechanism for counteracting STING function by YFV-encoded viral protease, as described above. It has also been proposed that the Zika virus may possess an alternative strategy for immune escape. Zika virus-encoded NS1 protein has been shown to promote proteolysis of cGAS rather than STING through 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 the mechanism of targeting distinct signal adaptor molecules of cytosolic nucleic acid sensors to escape from innate immune responses in humans.

Some RNA viruses have also evolved a mechanism for evading innate immune responses that targets the PTM systems involved in the cGAS/STING pathway. Virus-encoded DUB enzymes, such as papain-like proteases derived from human coronavirus-NL63, severe acute respiratory syndrome CoV and porcine epidemic diarrhea virus, have also been shown to suppress STING-dependent signal activation in a manner similar to that of the *HBV polymerase* gene [93–95]. Similarly, the Tax protein of human T lymphotropic virus type-1, which plays a critical role in promoting viral replication and T cell transformation, has also been shown to interfere with 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 positive regulation of PRR-mediated signal activation. Thus, one strategy for viral-mediated immune evasion could be to counteract STING-mediated K63-linked poly-ubiquitination.

Counteraction of the cGAS/STING pathway by RNA viruses classified as positive-sense RNA viruses has been reasonably well elucidated; however, much less is known about the actions of negative-sense RNA viruses. IAV belongs to the *Orthomyxoviridae* family, the members of which possess negative-sense RNA. It was recently reported that IAV elicits IFN production through a viral envelope-mediated fusion process in a STING-dependent, but cGAS-independent, manner [97]. Similarly, other enveloped RNA viruses, such as NDV and Sendai virus also play the same roles in inducing innate immune response; however, the molecular mechanism of signal transduction is yet to be clarified (Figure 3). Interestingly, it has been shown that the hemagglutinin fusion peptide of IAV may associate with STING via its dimerization interphase domain, thereby

inhibiting the STING-mediated dimer formation that initiates STING-dependent signal activation. Thus, this is the first evidence for 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. It has recently 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 and cyclophilin-A, respectively [98,99]. More specifically, mutated HIV capsids in which cyclophilin-A association is impaired can be stimulated by 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 release of mitochondrial DNA via induction of damage to DENV-infected cells may have the potential to stimulate a cGAS/STING-mediated immune response (Figure 4). To avoid such signal activation, an NS2B protease co-factor derived from DENV may directly target cGAS, thus leading to proteasomal degradation [100]. It remains unclear whether species-specific effects are involved in this NS2B protease co-factor-dependent immune evasion strategy, for example, proteolysis of human, but not murine, STING in a protease-dependent manner.

5 | 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 development of diseases associated with them, such as systemic lupus erythematosus, AGS and polyarthritis. It remains to be determined whether other types of inflammatory diseases may also be associated with defects in cGAS/STING function (e.g., mediated by gain- or loss-of-function mutations). Thus, an improved understanding of the mechanisms underlying overactivity of inflammation mediated via the cGAS/STING pathway could lead to design of potent therapeutic agents and strategies for overcoming undesirable inflammatory diseases and certain types of cancer. In addition, although the cGAS/STING pathway may contribute to detection and elimination of infection by both DNA and RNA viruses, over time these viruses have also evolved the ability to escape or manipulate this signal activation through several evasion strategies, as described above. Indeed, such manipulation is considered critical to the ability of viruses to establish both lytic and persistent infection and elicit characteristic pathogenesis. Further information on the non-canonical mechanism of cGAS-independent STING activation and the evasion strategies of RNA viruses will strongly facilitate understanding of the host–pathogen interaction. The cGAS/STING pathway may function in a variety of cell types; however, details of the mechanisms by which hepatotropic viruses achieve persistent infection of hepatocytes have yet 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 virus–host interactions, it is also important to better understand the loss of cGAS/STING function in some types of cancer or transformed cells by viral encoding oncoprotein. This loss may involve signal manipulation via epigenetic silencing and 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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