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Innate Immune Response to Cytoplasmic DNA: Mechanisms and Diseases

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Abstract

DNA has been known to be a potent immune stimulus for more than half a century. However, the underlying molecular mechanisms of DNA-triggered immune response have remained elusive until recent years. Cyclic GMP-AMP synthase (cGAS) is a major cytoplasmic DNA sensor in various types of cells that detect either invaded foreign DNA or aberrantly located self-DNA. Upon sensing of DNA, cGAS catalyzes the formation of cyclic GMP-AMP (cGAMP), which in turn activates the ER-localized adaptor protein MITA (also named STING) to elicit the innate immune response. The cGAS-MITA axis not only plays a central role in host defense against pathogen-derived DNA but also acts as a cellular stress response pathway by sensing aberrantly located self-DNA, which is linked to the pathogenesis of various human diseases. In this review, we summarize the spatial and temporal mechanisms of host defense to cytoplasmic DNA mediated by the cGAS-MITA axis and discuss the association of malfunctions of this axis with autoimmune and other diseases.

INTRODUCTION

Mammalian cells utilize pattern recognition receptors (PRRs) to surveil the existence of pathogen- or danger-associated molecular patterns (PAMPs or DAMPs) in the extracellular environments and intracellular compartments (1, 2). Microbial DNA, which is easily exposed to the cytoplasm during microbial infection, is a typical PAMP that is sensed by host cells (1, 3). In addition, endocytosed foreign DNA from the dying cells or damaged tissues, as well as self-DNA leaking from either mitochondria or the nucleus, can act as an intracellular DAMP to trigger the innate immune response (1, 4). In addition to the membrane-bound Toll-like receptor 9 (TLR9), which recognizes extracellular DNA translocated in the endolysosomes (1, 5, 6), a dozen candidate sensors for intracellular DNA have been reported in the last few years, including cyclic GMP-AMP synthase (cGAS), IFI16, DDX41, AIM2, LSM14A, DNA-PKcs, MRE11, DAI, and RNA polymerase III (7–15). Although these candidate sensors have been reported to be involved in DNA sensing in distinct cellular compartments of certain cell types, both in vitro and in vivo evidence demonstrate that cGAS is the most used cytoplasmic DNA sensor in a variety of cell types (8, 16).

After recognition of DNA, cGAS catalyzes the synthesis of cyclic GMP-AMP (cGAMP) from ATP and GTP (8), which in turn binds to and activates an endoplasmic reticulum (ER)-located protein called mediator of IRF3 activation (MITA) or stimulator of interferon genes (STING), leading to transcription of effector genes involved in innate immune and inflammatory responses (17, 18). The cGAS-MITA axis is tightly and delicately regulated in a spatial and temporal manner to ensure proper immune response to clear invading pathogens or aberrant cells, and to avoid insufficient immune response or excessive immune damage (1). It has been demonstrated that deregulation of the cGAS-MITA axis is associated with inflammatory/autoimmune diseases and tumorigenesis (19).

In this review, we summarize the molecular mechanisms of cGAS-mediated innate immune responses, strategies to counteract the cGAS-MITA axis for immune escape by DNA viruses, and strategies utilized by cells to prevent aberrant self-DNA sensing. Finally, we discuss how deregulation of the cGAS-MITA pathways is associated with severe human diseases.

MOLECULAR MECHANISMS OF DNA SENSING BY cGAS

Structural Properties of cGAS

cGAS is a 522–amino acid protein that contains an N-terminal unstructured and highly positively charged domain and a C-terminal nucleotidyltransferase (NTase) domain. Both the N- and C-terminal domains of cGAS bind to DNA. Although the N-terminal domain of cGAS has low amino acid sequence homology among different species, some critical and function-determining residues are conserved (20–22). Recently, it was demonstrated that the N-terminal domain of cGAS drives liquid-liquid phase separation of the cGAS/DNA complex, which is critical for cGAS activation upon its binding to double-stranded DNA (dsDNA) (23). The C-terminal domain of cGAS consists of a central catalytic pocket and two separate surfaces with positive charges, through which cGAS interacts with the sugar phosphate backbone of the DNA duplex (24–28). Upon binding to dsDNA, cGAS assembles into a 2:2 cGAS-dsDNA oligomeric complex with two molecules of dsDNA embedded in two cGAS molecules (27, 28). cGAS dimers form ladder-like networks between two separate stretches of dsDNA or on one long crooked dsDNA helix, which markedly enhances the stability of each individual cGAS-dsDNA complex along the dsDNA (29).

Once bound to dsDNA, the catalytic pocket of cGAS undergoes a structural rearrangement, leading to the synthesis of 2′3′-cGAMP from ATP and GTP with one phosphodiester bond between the 2′-hydroxyl of GMP and 5′-phosphate of AMP and another between the 3′-hydroxyl of AMP and 5′-phosphate of GMP (25, 30–32). Notably, it has been observed that double-stranded RNA (dsRNA) or single-stranded DNA (ssDNA) can also bind to cGAS, but neither of them causes a rearrangement of the catalytic pocket of cGAS (24, 26), which gives an explanation for specific activation of cGAS by dsDNA.

Formation of cGAS-DNA Liquid Droplets

Recently, it was proposed that cGAS and dsDNA interaction in the cytosol would cause formation of micrometer-sized biomolecule-condensed liquid droplets through liquid phase separation (**Figure 1**) in which cGAS and reactants are concentrated to promote the synthesis of cGAMP (23). In addition to reactant concentration, another important function of liquid droplets under physiological conditions might be to enrich the invaded microbial DNA. Consistent with this hypothesis, the formation of cGAS-dsDNA foci is observed within dsDNA-transfected cells (8). Further studies suggest that the liquid droplets of cGAS-dsDNA are dynamic granules, implying that the cGAS-dsDNA liquid phase separation is a dynamic and reversible process under physiological conditions, which is critical for timely termination of the innate immune response to DNA (23). The formation of cGAS-dsDNA liquid-like granules has been reported to be regulated by several factors, including the concentrations of cGAS and dsDNA, cytoplasmic salt concentrations, and free zinc ions, suggesting that cGAS is activated only when the concentrations of cytosolic dsDNA and ions reach certain levels, such as in the case of microbial infection (23). Additionally, G3BP1 has been reported to constitutively interact with cGAS, which is required for formation of foci (liquid droplets) of cGAS upon DNA stimulation (33) (**Figure 1**). Interestingly, cGAS-dsRNA also forms liquid droplets but does not produce cGAMP, which suggests that liquid phase separation is insufficient for cGAS activation (23).

Coreceptors for cGAS

Although structural studies on cGAS alone or in complex with dsDNA provide insights into the general modes of its interaction with dsDNA and activation, these events are more complicated under physiological conditions. Since cGAS is expressed at low levels in cells and binds to a broad set of DNA ligands with low affinities, it has been speculated that there are coreceptors to promote a cGAS-mediated innate immune response. It has been demonstrated that polyglutamine binding protein 1 (PQBP1) binds to reverse-transcribed HIV-1 DNA and interacts with cGAS to initiate an IRF3-dependent innate immune response (34). Interestingly, PQBP1 specifically binds to DNA derived from retroviral infection but no other cytosolic DNA (34). The properties that make retroviral DNA specific to PQBP1 are unknown. Recently, ZCCHC3 was identified as a more general coreceptor for cGAS (35) (**Figure 1**). ZCCHC3 deficiency impairs an innate antiviral response induced by various transfected dsDNA as well as different types of DNA viruses, including herpes simplex virus 1 (HSV-1), vaccinia virus, human cytomegalovirus (HCMV), and mouse cytomegalovirus in vitro and in vivo (35). In vitro experiments suggest that ZCCHC3 dramatically increases the affinity of cGAS to dsDNA as well as oligomerization of cGAS in response to dsDNA (35). It has been shown that ZCCHC3 can also act as a coreceptor for RIG-I and MDA5, two sensors for viral RNA (36), suggesting that ZCCHC3 plays a general role in antiviral innate immunity. Since the formation of liquid droplets of cGAS-DNA is important for an efficient innate immune response to DNA, it would be interesting to determine

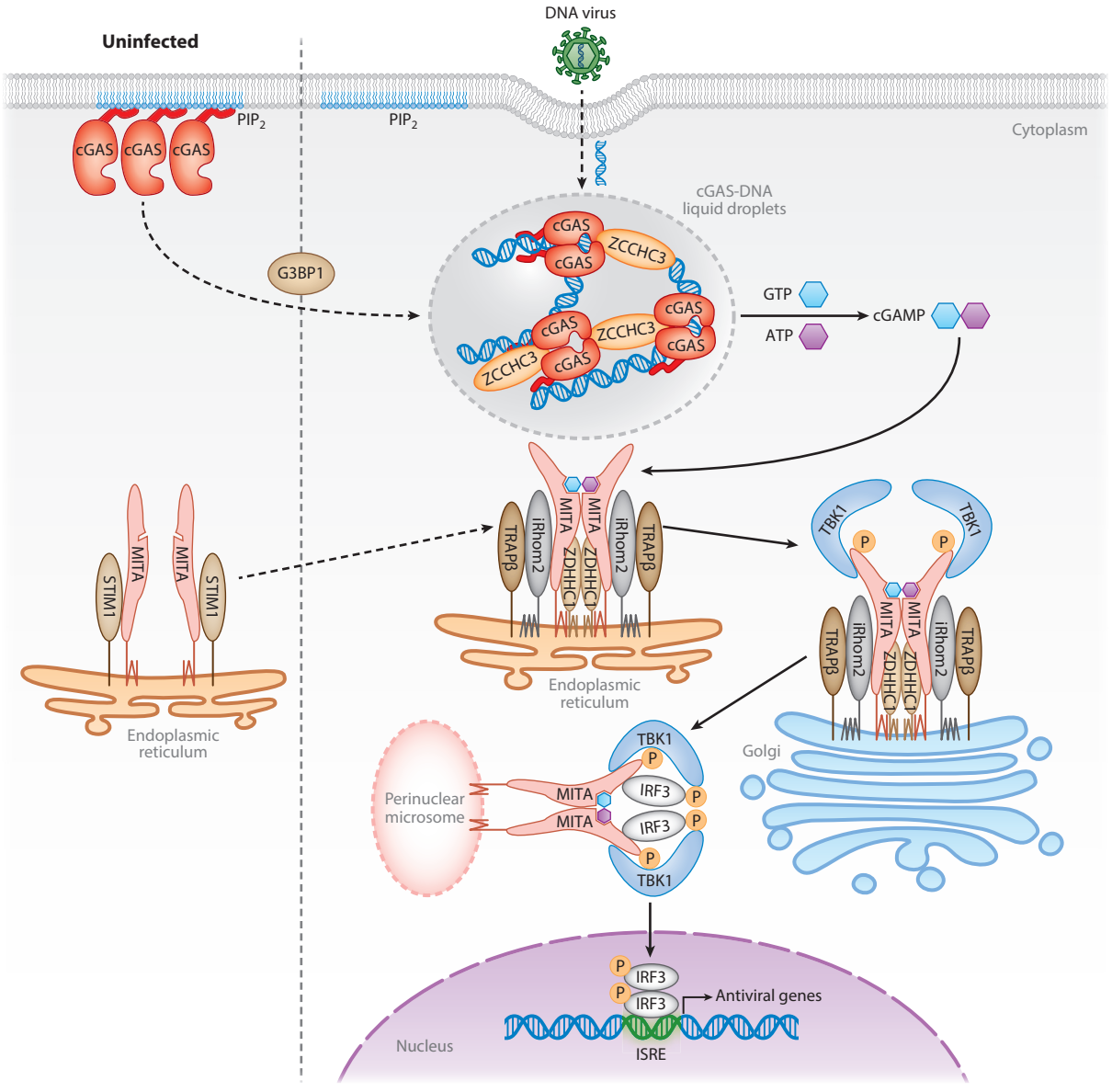

Figure 1

Innate immune response to viral DNA mediated by the cGAS-MITA axis. The plasma membrane–bounded cGAS exists as a monomer in uninfected cells. After binding to microbial or self-DNA with the help of G3BP1 and the coreceptor ZCCHC3, cGAS-dsDNA forms liquid droplets in which ATP and GTP are utilized as substrates to synthesize cGAMP. cGAMP binds to the ER-located MITA, which is then translocated by the iRhom2-TRAPβ translocon to the Golgi apparatus, and then further to perinuclear microsomes. During the translocation process, TBK1 and IRF3 are recruited to the MITA complexes, leading to the phosphorylation and activation of IRF3 as well as the eventual induction of antiviral genes. Abbreviations: cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP synthase; MITA, mediator of IRF3 activation; PIP_2, phosphatidylinositol 4,5-bisphosphate.

whether and how these coreceptors are related to the formation and function of cGAS-DNA liquid droplets.

SIGNALING OF THE cGAS-MITA AXIS

Subcellular Distribution of cGAS

Although it is well established that cGAS plays an essential role in sensing cytoplasmic DNA, the subcellular compartments where cGAS acts are less clear. This is further complicated because of the nonuniform and case-dependent subcellular location of microbial and self-DNA. It has been shown that cGAS is a cytosolic DNA sensor (8). However, subcellular fractionation and biochemical analysis suggest that cGAS is predominantly located at the plasma membrane and not in the cytosol, especially in phagocytes. The plasma membrane localization of cGAS is mediated by the binding of its N-terminal unstructured domain to the plasma membrane lipid rafts via phosphatidylinositol 4,5-bisphosphate (PIP_2) (21). Surprisingly, during mitosis, a major fraction if not all of cGAS is associated with chromosomes after breakdown of the nuclear envelope (37), which raises an important question as to how cells maintain immune silence during mitosis. Interestingly, a small fraction of cGAS is located in the nucleus, especially in certain epithelial cells (21). The functional significance of the nuclear localization of cGAS is unclear. Since cGAS forms large foci (probably liquid droplets of cGAS-DNA complex) in the cytoplasm after DNA transfection (8, 23), it is possible that DNA transfection triggers translocation of cGAS from the plasma membrane to the cytoplasm to form liquid droplets with DNA for cGAMP production (**Figure 1**). It is also possible that distinctly located cGAS detects DNA derived from various sources and located in the same cellular compartments.

Trafficking and Activation of the Adaptor Protein MITA

Sensing of DNA by cGAS causes synthesis of cGAMP, which acts as a second messenger molecule and binds to the ER-associated transmembrane adaptor protein MITA (also known as STING) (17, 18). In uninfected cells, MITA is retained at the ER by the Ca^{2+} sensor STIM1 via direct interaction (38). STIM1 deficiency causes spontaneous activation of MITA and enhanced expression of type I interferons under resting conditions in cells and mice (38). After binding to cGAMP, MITA undergoes structural rearrangement and oligomerization (39, 40) that is modulated by another ER-associated protein called ZDHHC1 (41). MITA then interacts with iRhom2, which recruits the Sec5/TRAPβ/Sec61β translocon complex to MITA, resulting in translocation of the MITA-associated complex to ER-Golgi intermediate compartments (ERGICs) (41–43). At the ERGIC, the TBK1 kinase and the transcriptional factor IRF3 are recruited to MITA via two distinct motifs (40). In this complex, TBK1 firstly catalyzes phosphorylation of MITA at Ser^{366} and then IRF3 at Ser^{396}, leading to IRF3 dimerization and translocation into the nucleus for induction of downstream effector genes (42, 44). Furthermore, MITA is translocated to perinuclear punctate structures, where it undergoes lysosome-dependent degradation, which contributes to the termination of the innate immune response (18, 45).

Interestingly, upon DNA virus infection, the synthesized cGAMP from the infected cells can be transferred to neighboring cells through gap junctions or to newly infected cells through incorporation into viral particles, leading to amplification and spreading of the innate antiviral response (46–48). In addition to cGAMP produced by host cells, cyclic dinucleotides derived from living bacteria, such as cyclic di-GMP (cGMP) and cyclic di-AMP (cAMP), can also bind to MITA and trigger production of type I interferons, activation of Nod-like receptor family pyrin

domain–containing 3 (NLRP3) inflammasomes, and autophagy to limit bacterial replication and facilitate host cell survival (49, 50).

POSTTRANSLATIONAL REGULATION OF THE cGAS-MITA PATHWAYS

The cGAS-MITA pathways have to be delicately regulated in a spatial and temporal manner to efficiently mount an innate immune response to clear DNA pathogens and to terminate it in a timely way to avoid immune damage. Numerous studies have demonstrated that posttranslational modifications of cGAS, MITA, and their downstream components are crucial for proper controls of innate immune response to DNA. These posttranslational modifications include phosphorylation, ubiquitination, sumoylation, glutamylation, and acetylation (1). Below we summarize the posttranslational modifications of cGAS and MITA (**Figure 2**). Regulation of other downstream components such as TBK1 and IRF3 is discussed in previous reviews (1).

Posttranslational Regulation of cGAS

Phosphorylation is one of the most widely used strategies to alter the properties and functions of substrates, such as activity, subcellular location, protein stability, and protein interaction. Through a series of proteomics analyses, dozens of serine and threonine residues of both the N- and C-terminal domains of cGAS have been identified to be modified by phosphorylation, suggesting that cGAS is probably regulated by multiple phosphorylation events on different residues. However, the functions and molecular mechanisms of most of these phosphorylation events on cGAS are still unclear, except that phosphorylation of cGAS at Ser^{305} by AKT was reported to inhibit the catalytic activity of cGAS (51). Thus, more studies on the functions and mechanisms of cGAS phosphorylation and dephosphorylation are needed.

Ubiquitination is one type of common and important posttranslational modification in cells and is catalyzed by ubiquitin ligases and deconjugated by deubiquitinating enzymes. There are seven lysine residues (Lys^6, Lys^{11}, Lys^{27}, Lys^{29}, Lys^{33}, Lys^{48}, and Lys^{63}) in the molecule of ubiquitin, and any of these lysine residues can be ligated by another ubiquitin molecule, which contributes to the diversity of linkage types (K6-, K11-, K27-, K29-, K33-, K48- and K63-linked) of polyubiquitin chains as well as their distinct functions (52). Several distinct types of polyubiquitination of cGAS have been identified. It has been demonstrated that K27-linked polyubiquitination of cGAS at Lys^{173} and Lys^{384} by RNF185 after DNA virus infection promotes its enzymatic activity (53), while K48-linked polyubiquitination of cGAS at Lys^{414} by an unknown E3 ligase causes its p62-dependent autophagic degradation at the late phase of viral infection (54). It has also been reported that cGAS is modified by K48-linked polyubiquitination at Lys^{271} in uninfected cells and at Lys^{464} at the late phase of infection by unknown E3 ligases, which promotes its proteasomal degradation and contributes to homeostasis of cGAS for proper initiation and attenuation of an innate immune response (55). How cGAS is deubiquitinated in uninfected and infected cells is unknown.

It has been reported that sumoylation plays critical roles in regulation of the host response to viral infection (55–57). cGAS is sumoylated at Lys^{217} in uninfected cells and further sumoylated at Lys^{464} in the early phase of DNA viral infection by TRIM38. Sumoylation of cGAS at Lys^{217} suppresses its K48-linked polyubiquitination at Lys^{271} by physical hindrance to maintain the stability of cGAS in uninfected cells, while sumoylation of cGAS at Lys^{464} prevents its K48-linked polyubiquitination at the same residue to prevent its degradation by the ubiquitin-proteasomal pathway in the early phase of viral infection (55). The maintenance of cGAS stability in uninfected and early-infected cells is important for efficient mounting of an innate antiviral response

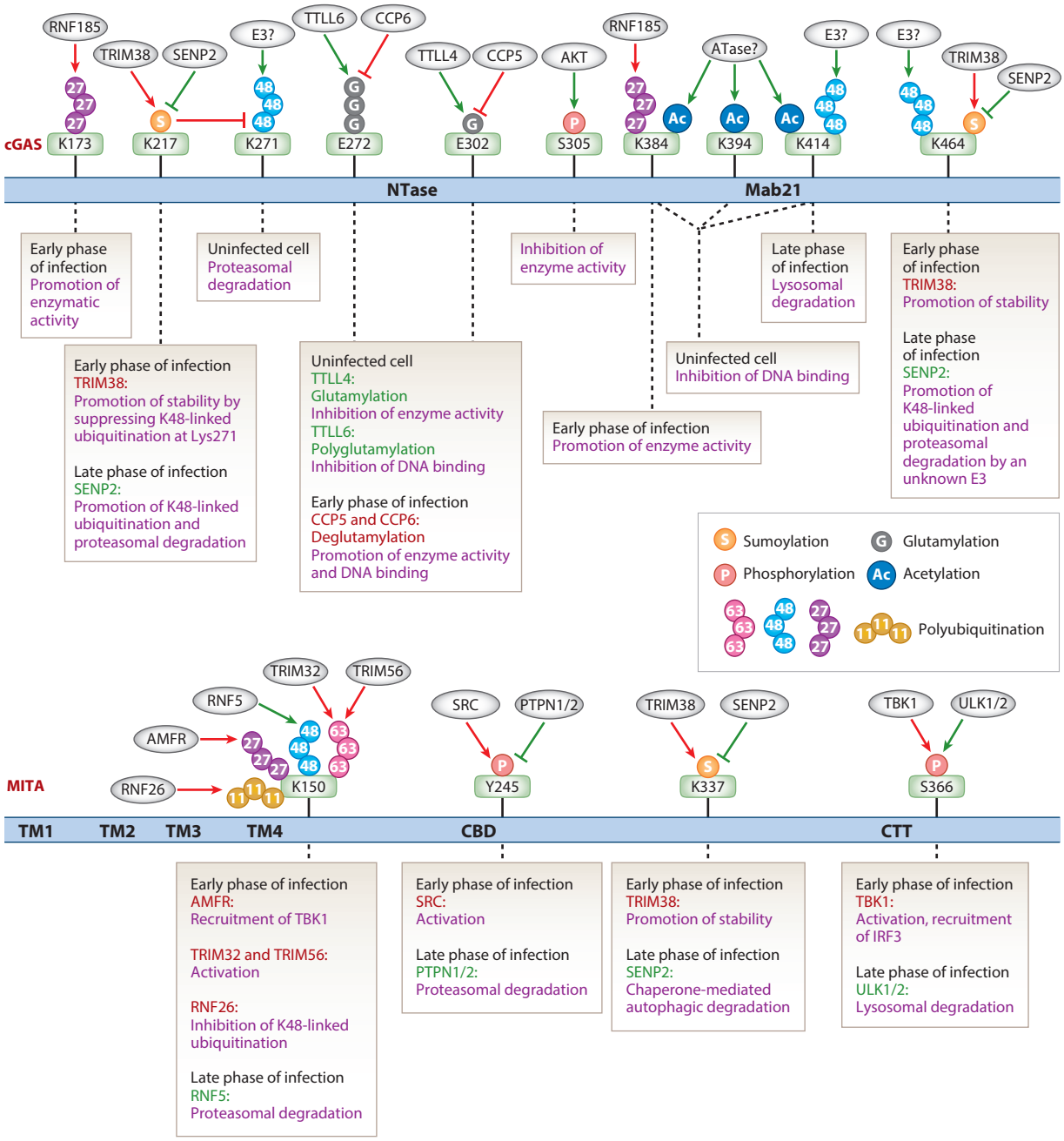

Figure 2

Regulation of cGAS and MITA activities by different posttranslational modifications. The cGAS-MITA axis is heavily and dynamically regulated by various posttranslational modifications, such as polyubiquitination, sumoylation, glutamylation, acetylation, and phosphorylation in uninfected and/or infected cells. These posttranslational modifications are important for regulating the stability and activation of components involved in the cGAS-MITA axis, ensuring efficient onset of innate immune response to cytoplasmic DNA and its timely termination to avoid host damage. Abbreviations: CBD, C-terminal-binding domain; cGAS, cyclic GMP-AMP synthase; CTT, C-terminal tail; MITA, mediator of IRF3 activation.

in the early phase of infection. In the late phase of infection, the SUMO moieties of cGAS at Lys^{217} and Lys^{464} are removed by the desumoylating enzyme SENP2, leading to K48-linked polyubiquitination and proteasomal degradation of cGAS and thereby attenuation of an innate antiviral response (55).

Dynamic glutamylation and deglutamylation of cGAS regulate activation of cGAS (58). Genetic studies suggest that mice deficient in the carboxypeptidase CCP5 or CCP6 are more susceptible to DNA viruses. Biochemical analyses suggest that cGAS undergoes dynamic glutamylation and deglutamylation before and after viral infection. Polyglutamylation of cGAS at Glu^{272} by the enzyme TTLL6 impairs its DNA-binding ability, whereas TTLL4-mediated monoglutamylation of cGAS at Glu^{302} suppresses its synthase activity in uninfected cells. Upon viral infection, CCP6 and CCP5 hydrolyze the polyglutamate and monoglutamate moieties of cGAS, respectively, which promotes cGAS activation (58).

Very recently, it was shown that cGAS is acetylated by an unknown acetyltransferase at multiple residues, including Lys^{384}, Lys^{394}, and Lys^{414}, which inhibits the activation of cGAS in uninfected cells (59). Upon viral infection, cGAS undergoes deacetylation. Interestingly, aspirin can acetylate cGAS at these residues and efficiently inhibit a cGAS-mediated innate immune response in vitro and in vivo. Aspirin also effectively suppresses self-DNA-induced autoimmunity in Aicardi-Goutières syndrome (AGS) patient cells and in an AGS mouse model, which provides a potential therapy for treating DNA-mediated autoimmune diseases (59). As we mention above, Lys^{384} of cGAS can also be conjugated by RNF185 for K27-linked polyubiquitination at the early phase of viral infection, while Lys^{414} of cGAS can be catalyzed for K48-linked polyubiquitination in uninfected cells, indicating that divergent and dynamic posttranslational modifications of cGAS at these residues may occur at different phases of viral infection, though the underlying regulatory mechanisms remain elusive.

Posttranslational Regulation of MITA

Posttranslational regulation of MITA has been heavily investigated. It has been demonstrated that phosphorylation of MITA at Ser^{358} primes for its recruitment of TBK1 (17), while TBK1-mediated phosphorylation of MITA at Ser^{366} is critical for the recruitment of IRF3 to MITA upon viral infection (44). Conversely, the phosphatase PPMIA dephosphorylates MITA to suppress its activation in response to viral infection (60). ULK1/2 has also been reported to catalyze the phosphorylation of MITA at Ser^{366}, leading to suppression of MITA activity (45). Mutation of Ser^{366} to either alanine or aspartate abolishes MITA-mediated induction of type I interferons (45). It is possible that MITA is phosphorylated by TBK1 or ULK1/2 at different phases of viral infection, and the same phosphorylation of MITA in different contexts results in different functional outcomes. Recent studies suggest that MITA is also phosphorylated at Tyr^{245} by the tyrosine kinase SRC, which is critical for its dimerization and stability (61). It is possible that in the late phase of DNA virus infection, tyrosine phosphatases dephosphorylate MITA Tyr^{245}, resulting in attenuation of an innate immune response to DNA.

Several different types of polyubiquitin modifications, including K63-, K48-, K11-, and K27-linked polyubiquitination, regulate MITA protein level and activity before and/or after viral infection (62). Interestingly, Lys^{150} of MITA has been reported to be modified by all these different types of polyubiquitination, probably at different phases of viral infection. For example, after viral infection, TRIM32 and TRIM56 catalyze K63-linked polyubiquitination of MITA, whereas AMFR promotes its K27-linked polyubiquitination of MITA (63). These types of polyubiquitination are important for its recruitment of TBK1 and therefore contribute to the onset of an innate antiviral response. The E3 ligase RNF5 catalyzes K48-linked polyubiquitination and proteasomal

degradation of MITA to negatively regulate the innate antiviral response at the late phase of infection (64). It has also been shown that the E3 ligase RNF26 promotes K11-linked polyubiquitination of MITA at Lys^{150}, a residue also targeted by RNF5 for K48-linked polyubiquitination. Therefore, RNF26 protects MITA from RNF5-mediated K48-linked polyubiquitination and degradation, which contributes to quick and efficient type I interferon and proinflammatory cytokine induction after viral infection (65). Recently, several deubiquitination enzymes have been reported to deconjugate the polyubiquitin moieties of MITA. USP13 and USP49 were reported to remove K27/33- and K63-linked polyubiquitin chains of MITA, respectively, to inhibit its activation (66, 67), while USP20 removes K48-linked polyubiquitin chains from MITA to promote its stability and activation (68, 69). How these polyubiquitination and deubiquitination processes are spatially and temporally coordinated or regulated needs further investigation.

Viral infection also induces sumoylation of MITA at K337 by TRIM38, which is crucial for its oligomerization and activation as well as for maintenance of stability by inhibiting chaperone-mediated autophagy, leading to an optimal innate antiviral response (55). Phosphorylation of MITA at Ser^{366} after viral infection facilitates recruitment of SENP2 for its desumoylation in the late phase of viral infection, leading to lysosomal degradation of MITA and negative regulation of the innate antiviral response (55).

VIRAL EVASION OF cGAS-MITA-MEDIATED INNATE IMMUNE RESPONSE

Since the cGAS-MITA axis plays critical roles in the innate immune response to DNA viruses, various DNA viruses have evolved strategies to counteract this axis for evading host immunity (70). The evading strategies employed by herpesviruses have been extensively investigated and are excellent examples for understanding how the cGAS-MITA axis is targeted by viruses (**Figure 3**).

Suppression of cGAS Activity

The enzymatic activity of cGAS is indispensable for exerting its antiviral function. Thus, targeting cGAS to block its enzymatic activity by viral proteins is a death blow for the innate immune

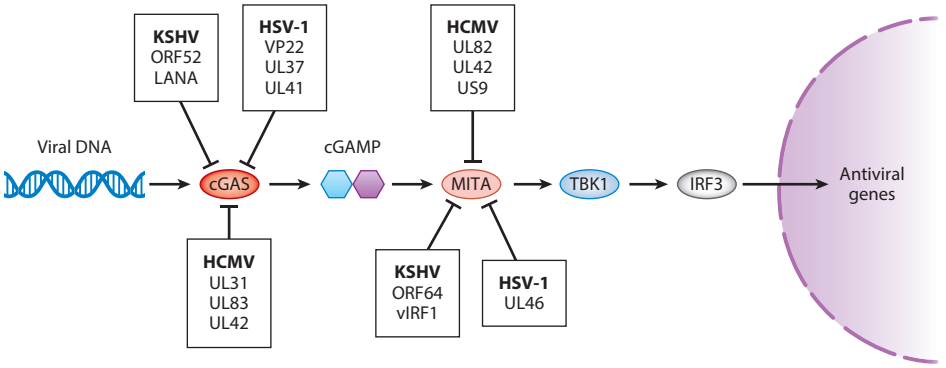

Figure 3

Strategies of immune escape of herpesviruses by antagonizing the cGAS-MITA axis. The activities of cGAS and MITA are suppressed by various viral proteins of herpesviruses during viral infection, leading to the inhibition of DNA binding, oligomerization, and/or enzymatic activity of cGAS, and blockage of translocation and/or oligomerization of MITA, or its recruitment of TBK1 and IRF3. These cause the ultimate suppression of transcriptional induction of antiviral genes. Abbreviations: cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP synthase; HCMV, human cytomegalovirus; HSV-1, herpes simplex virus 1; KSHV, Kaposi sarcoma herpesvirus; MITA, mediator of IRF3 activation.

system. The cytoplasmic isoforms of Kaposi sarcoma herpesvirus (KSHV) LANA antagonize the innate immune DNA sensor cGAS to suppress expression of type I interferons (71). The tegument protein ORF52 of KSHV inhibits the enzymatic activity of cGAS, which blocks the production of cGAMP and the innate antiviral response (72). Similarly, several different HCMV proteins, including UL31, UL83, and UL42, have also been reported to interact with cGAS and inhibit its DNA binding, oligomerization, or enzymatic activity (73–75). Whether these viral proteins function independently or cooperatively, or target distinct steps from DNA binding to enzymatic activation of cGAS, is unknown. HSV-1 also evolves strategies to inhibit cGAS. HSV-1 VP22 binds to cGAS and inhibits its enzymatic activity (76). HSV-1 tegument protein UL37 deamidates cGAS, resulting in impairment of cGAMP synthesis by cGAS (77). HSV-1 UL41 causes degradation of cGAS in the cytosol to abrogate host recognition of viral DNA (78).

Suppression of MITA Activity

Activation of MITA in response to cytoplasmic DNA involves several critical steps, such as cGAMP binding, phosphorylation, dimerization, and trafficking, any of which might be targeted by viruses for immune escape. For example, KSHV vIRF1 antagonizes MITA activity by preventing it from interacting with TBK1, thereby inhibiting the phosphorylation of MITA at Ser^{366} (79). KSHV ORF64 antagonizes MITA activity via its deubiquitinating enzyme activity (80). HCMV tegument protein UL82 impairs MITA-mediated signaling via two mechanisms (81). On one hand, UL82 inhibits the translocation of MITA from the ER to perinuclear punctate structures by disrupting the MITA-iRhom2-TRAPβ translocon complex. On the other hand, UL82 impairs the recruitment of TBK1 and IRF3 to the MITA complex (81). HCMV UL42 inhibits the trafficking and activation of MITA by facilitating p62/LC3B-mediated degradation of TRAPβ (74). HCMV US9 interacts with MITA and suppresses its dimerization and activation (82). HSV-1 UL46 interacts with MITA and TBK1 to suppress the production of type I interferons (83). It has been found that the cGAS-MITA axis mediates the initial burst of type I interferon production upon murine cytomegalovirus (MCMV) infection (84). As expected, MCMV also evolves strategies to antagonize the cGAS-MITA axis. It has been revealed that the MCMV-encoded m152 protein selectively dampens MITA-mediated IRF3 but not NF-κB activation by antagonizing MITA trafficking, resulting in a delayed type I interferon response to MCMV infection (85). Collectively, the herpesviruses utilize these distinct strategies to suppress the host innate immune response and establish successful infection.

CELLULAR MECHANISMS FOR INNATE IMMUNE RESPONSE TO SELF-DNA

Extensive studies have demonstrated that cGAS recognizes DNA from various sources, including microbial, synthesized, and cellular DNA. The lack of a pathogen-specific property of cGAS in DNA sensing raises an important question as to how cGAS remains immune-silent in uninfected cells. In addition to the negative regulatory mechanisms of the cGAS-MITA axis mentioned above, other mechanisms may also be utilized by the host cells to avoid an innate immune response to self-DNA, and deregulation of these mechanisms results in an innate immune response to self-DNA.

Mislocation of Self-DNA

Intracellular self-DNA is mainly located in the nucleus and mitochondria in interphase, whereas the DNA sensor cGAS is mostly located on the plasma membrane and in the cytosol (21).

Compartmentalization of intracellular DNA is an important strategy utilized by cells to keep cGAS isolated from self-DNA to avoid self-response, which guarantees an accurate and orderly innate immune response to pathogens. If the integrity of these compartments were disrupted with self-DNA leaking into the cytosol, a strong inflammatory response would be triggered (21). For example, cells treated with DNA damage inducers form fragile micronuclei in which the fragmented genomic DNA can be recognized by cGAS, leading to activation of an innate immune response (86). During infection, certain viral proteins cause alteration of mitochondrial membrane potential and leakage of mitochondrial DNA into the cytosol, which subsequently induces a strong innate immune response (87). Chronic changes in lipid metabolism may also interfere with the proper localization of mitochondrial DNA, thereby initiating an innate immune response (88, 89).

Interestingly, there are some physiological processes in which the integrity of the intracellular compartments is changed but without triggering an immune response. For instance, when apoptosis occurs, mitochondrial DNA is released into the cytoplasm through BAX/BAK-assembling pores at the mitochondrial outer membrane, which would trigger an innate immune response via the cGAS-MITA axis (90, 91). Simultaneously, mitochondrial cytochrome *c*–activated caspases cleave cGAS and IRF3 to suppress the immune response in a timely way (90, 91). During mitosis, a major fraction of cGAS binds to chromatin DNA after nuclear envelope breakdown, but no obvious innate immune response is induced (37, 86, 92). Therefore, there are mechanisms to ensure the cGAS-MITA axis is not functional during mitosis.

Deregulation of Self-DNA in the Cytosol

Studies with mouse models and human genetic disorders with defects in specific nucleases reveal that the deoxyribonucleases (DNases) in host cells play a critical role in avoiding innate immune response to self-DNA. Intracellular nucleases maintain homeostasis of cellular DNA by continuously degrading obsolete DNA exposed in the cytosol to avoid detrimental DNA accumulation as a consequence of normal cellular function (93, 94). There are at least three nucleases that participate in the degradation of obsolete DNA materials. The 3′-5′ exonuclease TREX1 is responsible for clearing micronuclei or DNA fragments from DNA damage responses, as well as suppressing retroelement transposition (95, 96). The lysosomal endonuclease DNase II degrades endocytosed DNA from apoptotic cells and damaged DNA from the nucleus (97–99). SAMHD1, through its deoxynucleotide triphosphate phosphohydrolase activity, regulates intracellular nucleotide levels, which are not only critical for reverse transcription of retroviral DNA but also important to maintaining genome integrity (100, 101). Additionally, SAMHD1 has been reported to regulate DNA replication fork resection (102). All of these nucleases are required to prevent induction of type I interferons and chronic inflammation under physiological conditions, and loss of any of them triggers lethal autoimmune diseases, which can be rescued by blockage of cGAS-MITA signaling in mouse models (see below) (103, 104).

ASSOCIATION OF SELF-DNA SENSING WITH HUMAN DISEASES

The coordination of various regulatory mechanisms maintains the homeostasis of the innate immune response to self-DNA in the cells. Deregulation of these mechanisms may cause excessive or continuous sterile inflammation and promote development or deterioration of human diseases.

Self-DNA Sensing in Host Defense Against Microbes

In addition to microbial DNA, sensing of cellular mitochondrial DNA (mtDNA) by cGAS also contributes to host defense against microbial infection. For example, after HSV-1 infection, the

virus-encoded protein UL12 induces mitochondrial stress and subsequent release of mtDNA into the cytosol for cGAS activation (87). Other pathogens, including certain virulent strains of *Mycobacterium tuberculosis* and the RNA flavivirus dengue virus, have also been reported to cause mitochondrial damage and mtDNA release and thereby activate the cGAS-MITA-mediated innate immune response (105, 106). Another RNA virus, West Nile virus (WNV), has also been reported to activate the cGAS-MITA axis, and cGAS- or MITA-deficient mice are more susceptible to WNV infection (107). Whether WNV activates the cGAS-MITA axis through mtDNA or other unidentified cellular mechanisms remains to be investigated.

Self-DNA Sensing in Autoimmune Diseases

Several studies have demonstrated that some severe autoimmune diseases are caused by genetic mutation of several nuclease-encoding genes, such as TREX1, SAMHD1, and RNase H2. Since these nucleases are responsible for degrading nucleic acids in cells, the accumulation of endogenous DNA or RNA material is believed to be a common pathogenic factor in certain autoimmune diseases (104). This notion has been confirmed by mouse gene knockout studies (94, 103).

AGS is a type of systemic inflammatory disease that is characterized by various clinical manifestations in newborn infants, such as severe mental and physical handicap, chronic sterile lymphocytosis, and systemically increased levels of type I interferons (108). In humans, *TREX1* is the first gene reported to be associated with the rare genetic disease. Mice with mutation or deletion of *Trex1* develop fatal autoinflammatory phenotypes in multiple organs and die within one month after birth, which is quite similar to the pathogenesis of AGS in humans (109). Importantly, all these abnormalities can be rescued by further deletion of *cGas* or *Mita* genes in mice (109). Mutations of other genes, including those encoding SAMHD1 and all components of the RNase H2 endonuclease complex, trigger cGAS-MITA activation and are also associated with occurrence of AGS (110).

Systemic lupus erythematosus (SLE) is a severe chronic inflammatory disease in humans that can affect almost all organs and tissues, including the skin, joints, kidneys, blood cells, and nervous system. Though the presentation and course of SLE are highly variable, ranging from indolent to fulminant, it is generally associated with systemically increased levels of type I interferons and defective clearance of apoptotic cells (104). Recently, it was demonstrated that cGAMP levels in the peripheral blood are elevated in approximately 15% of SLE patients (111). A genetic study suggests that a polymorphism in the *TREX1* gene is a marker of SLE susceptibility (112). Similarly, RNase H2 mutation is also associated with SLE in addition to AGS (113). These studies provide evidence that cGAS sensing of DNA derived from apoptotic cells is an important trigger for the manifestations of SLE and other autoinflammatory diseases.

Self-DNA Sensing in Anticancer Immunity

Spontaneous anticancer immunity plays a key role in surveilling and suppressing cancer development. The anticancer immune response is thought to be mediated mainly by immune cells, including natural killer and T cells (114). However, how the immune system traces cancerous cells remains poorly understood. Recent studies have shown that the cGAS-MITA pathway plays an important role in both autonomous and nonautonomous anticancer immune surveillance and response (115–119).

During cell division, cancer cells with abnormal chromosomes or genomic DNA damage usually produce micronuclei or chromatin fragments, which would translocate to the cytoplasm to activate the cGAS-MITA pathway in a cell-autonomous manner, leading to the production of

immune-stimulatory molecules and cytokines, which trigger an immune response to suppress or clear cancer cells. In addition, cancer cells can be phagocytosed by antigen-presenting cells (APCs), such as dendritic cells and macrophages, in a non-cell-autonomous manner. The DNA of cancer cells may be detected by cGAS in APCs, leading to production of type I interferons and other immune-stimulatory molecules, which are important for activation and proliferation of tumor-specific T cells as well as their recruitment to tumors (115, 116). Based on the antitumor functions of the cGAS-MITA axis, cGAMP and its analogs or small-molecule agonists of MITA (diABZIs) exhibit strong anticancer effects in mouse models, especially when these reagents are used in combination with PD-1 or PD-L1 antibodies, two reagents used in anticancer immunotherapy (117, 118, 120).

Cancer cells also evolve strategies to modulate the cGAS-MITA axis for evasion of immune surveillance. Various cancer cells utilize different strategies to downregulate expression of cGAS and/or MITA, allowing them to evade immune surveillance of hosts (119). Some cancer cells can utilize the cGAS pathway to their own advantage. It has been shown that brain metastatic cancer cells can transfer cGAMP to adjacent astrocytes to activate a MITA-mediated inflammatory response, leading to metastasis of brain cancer cells in mice (121). cGAS may also promote tumorigenesis by inhibiting intracellular homologous recombination (122). These findings suggest that activation of the cGAS-MITA pathway leads to different outcomes in tumorigenesis and metastasis, and more caution needs to be taken in utilizing MITA agonists as cancer therapeutics.

Self-DNA Sensing in Other Pathologic Processes

There is growing evidence that self-DNA-induced immune and inflammatory responses also play critical roles in the development and/or deterioration of several common diseases. Recently, it was shown that sensing of self-DNA derived from dying cells causes a myocardial infarction–related inflammatory response. Suppression of cGAS or MITA activity in these mice is able to increase the survival rate of mice after myocardial infarction (123, 124). Recent studies have demonstrated that the pathogenesis of Parkinson disease is also related to the cGAS-MITA pathway (125). In familial Parkinson disease, loss-of-function mutations have been found in genes encoding the E3 ubiquitin ligase Parkin and protein kinase Pink1 (126). Both Parkin and Pink1 have been shown to play a key role in autophagy of damaged mitochondria, a process known as mitophagy (127). Interestingly, mice deficient for Parkin or Pink1 accumulate more damaged mitochondria and mtDNA under stress and produce more inflammatory cytokines and type I interferons in the brain (125). Knockout of MITA reduces inflammatory phenotypes and rescues the loss of dopaminergic neurons and motor defects observed in mice with Parkinson disease mutations (125). Therefore, damaged mtDNA seems to lead to inflammatory responses in patients with Parkinson disease by activating the cGAS-MITA pathway. In recent years, there is accumulating evidence that suggests an important role of the cGAS-MITA axis in regulation of obesity-induced inflammation and other metabolic diseases, which has been reviewed elsewhere (128). These studies underline the importance of the cGAS-MITA axis in pathogenesis of certain human diseases, and interference of this axis may provide promising strategies for treatment of these diseases.

FUTURE PERSPECTIVES

Studies in the last decade have established a key role of the cGAS-MITA pathway in cytoplasmic sensing and defense of microbial DNA or mislocated self-DNA. Although some key mechanisms for the signaling and regulation of the cGAS-MITA axis have been resolved, many outstanding questions remain unanswered. For example, the subcellular locations and related functions

of cGAS are not well understood. How is cell membrane–bound cGAS released into the cytosol for detection of cytosolic DNA? How are liquid droplets of cGAS-dsDNA formed and regulated in vivo? In the nucleus and during mitosis, cGAS is associated with cellular DNA but fails to induce an innate immune response. The mechanisms behind these phenomena are unknown. It has been reported that MITA is involved in an immune response triggered by virus–cell membrane fusion (129); whether cGAS is involved in this process is unclear. Furthermore, though sensing of mtDNA by cGAS has been reported to contribute to host defense against microbial infection, the underlying mechanisms as well as the universal application to microbial defense call for further investigation. As we discussed above, the cGAS-MITA pathway is heavily regulated by various types of posttranslational modifications, including phosphorylation, ubiquitination, sumoylation, glutamylation, and acetylation, and many of these modifications are regulated by different enzymes. How these mechanisms of posttranslational regulation are spatially and temporally coordinated and what their relative contributions are to the eventual outcomes of innate immune responses are unknown. The answers to these questions need an integrated and systematic approach in future studies. Lastly, several recent studies have demonstrated that the innate immune response to viral infection is regulated by metabolites via metabolic pathways or direct interaction of metabolites with immune signaling molecules (61, 89, 130). Therefore, a more comprehensive network of metabolism and the innate immune response to cytoplasmic DNA needs to be further investigated. Answering these and other outstanding questions will not only help us understand the complicated and precise regulatory mechanisms of the innate immune response to cytoplasmic DNA but will also certainly promote the development of therapeutics for severe human diseases, such as infectious diseases, autoimmunity, neurodegenerative diseases, and cancer.

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