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Review

Regulation and function of the cGAS-MITA/STING axis in health and disease

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ABSTRACT

The innate immune systems detect pathogens via pattern-recognition receptors including nucleic acid sensors and non-nucleic acid sensors. Cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS, also known as MB21D1) is a cytosolic DNA sensor that recognizes double-stranded DNA (dsDNA) and catalyzes the synthesis of 2′,3′-cGAMP. Subsequently, 2′,3′-cGAMP binds to the adaptor protein mediator of IRF3 activation (MITA, also known as STING, MPYS, ERIS, and TMEM173) to activate downstream signaling cascades. The cGAS-MITA/STING signaling critically mediates immune responses against DNA viruses, retroviruses, bacteria, and protozoan parasites. In addition, recent discoveries have extended our understanding of the roles of the cGAS-MITA/STING pathway in autoimmune diseases and cancers. Here, we summarize the identification and activation of cGAS and MITA/STING, present the updated functions and regulatory mechanisms of cGAS-MITA/STING signaling and provide a comprehensive understanding of the cGAS-MITA/STING axis in autoimmune diseases and cancers.

Introduction

The innate immunity plays a vital role in defense against the invasion of pathogenic micro-organisms and the maintenance of immune homeostasis. Innate immune cells recognize the structurally conserved components of pathogenic microorganisms called pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs). Subsequently, PRRs activate downstream signaling pathways and elicit anti-microbial immune responses or pro-inflammatory responses. For example, pathogen-derived RNA is detected by the cytosolic RNA sensors retinoic acid-inducible gene (RIG-I)-like receptors (RLRs) (Hu & Shu, 2018). Upon binding to dsRNA or 5′ triphosphorylated ssRNA, RIG-I undergoes conformational change to recruit and elicit the oligomerization and activation of the mitochondrial antiviral signaling protein (MAVS, also known as VISA, IPS-1, and Cardif) (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005). Activated MAVS further induces the expression of type I IFNs and pro-inflammatory cytokines via the activation of transcription factors IRF3 and NF-κB. To date, multiple cytosolic DNA sensors detecting pathogen DNA have been identified (Chiu et al., 2009; Li, Shu, et al., 2013; Sun et al., 2013; Takaoka et al., 2007; Unterholzner et al., 2010; Zhang et al., 2011). Specifically, the cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) synthase (cGAS) detects dsDNA and catalyzes the synthesis of 2′,3′-cGAMP which binds to the adaptor protein mediator of IRF3 activation (MITA, also known as STING, MPYS, ERIS, and TMEM173) and activates signaling cascades leading to the induction of type I IFNs and pro-inflammatory cytokines (Li, Wu, et al., 2013; Sun et al., 2013). Accordingly, knockout of cGAS or MITA significantly inhibits the production of type I IFNs after HSV-1 infection, and mice deficient in cGAS or MITA are hypersensitive to HSV-1 infection (Ishikawa et al., 2009; Li, Wu, et al., 2013). In addition to the antimicrobial functions of cGAS and MITA, emerging studies have broadened their roles in autoimmune diseases and cancers. Here, we review the recent advances in activation and regulation of the cGAS-MITA signaling pathway and summarize the updated roles of the cGAS-MITA signaling axis in autoimmune diseases and cancers.

An overview of the cGAS-MITA/STING signaling pathway

Structure, localization, and activation of MITA/STING

Because the MAVS-deficient MEFs respond normally to cytoplasmic

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DNA challenge (Sun et al., 2006), it is believed that there exist other adaptor proteins mediating intracellular DNA- and DNA virus-triggered signaling. In 2008–2009, four groups have independently identified a key adaptor protein that mediates DNA virus-triggered and cytoplasmic DNA-induced signal transduction and has been named as MITA (Zhong et al., 2008), STING (Ishikawa & Barber, 2008), MPYS (Jin et al., 2008), and ERIS (Sun et al., 2009). The phosphorylation of IRF3 and the production of type I IFNs are almost completely inhibited after DNA virus infection or DNA transfection in MITA-knockout cells and the MITA-deficient mice exhibit hypersensitivity to HSV-1 infection compared to the wild-type controls, indicating that MITA plays an indispensable role in cytosolic DNA-induced immune responses (Ishikawa et al., 2009).

The human and the murine MITA consist of 379 or 378 amino acid residues respectively and share with 81% homology. MITA consists of 4 transmembrane (TM) domains at the N-terminus responsible for its membrane localization, a cytoplasmic ligand-binding domain (LBD), and a flexible C-terminal domain (CTD) that is responsible for downstream TBK1 interaction (Burdette & Vance, 2013; Yin et al., 2012). The LBD domain exists as a dimer that displays the butterfly shape and is capable of binding cyclic di-nucleotide (CDN) such as s c-di-GMP, c-di-AMP, and 2′,3′-cGAMP (Burdette et al., 2011; Huang et al., 2012; Whiteley et al., 2019; Yin et al., 2012; Zhang, Bai, & Chen, 2020). The binding of CDN in the LBD domain induces the conformational changes of the LBD domain and a 180° rotation in relation to the TM domain for the ordering of the CTD of MITA and the oligomerization of MITA, which promotes the recruitment of TBK1 to activate IRF3 and NF-κB (de Oliveira Mann et al., 2019; Morehouse et al., 2020; Shang et al., 2019; Wu et al., 2014; Zhang et al., 2019, 2020c; Zhao et al., 2019).

MITA is strictly regulated in uninfected cells. On one hand, the Ca^{2+} sensor stromal interaction molecule 1 (STIM1) and TOLLIP interact with MITA to retain and stabilize it in the ER membrane, respectively (Pokatayev et al., 2020; Srikanth et al., 2019). On the other hand, the unfolded protein response (UPR) effector IRE1α promotes MITA trafficking from ER to lysosomes for degradation, which is partially mediated by the SREBP2-SCAP complex and the lysosomal membrane protein Niemann–Pick type C1 (NPC1) (Chu et al., 2021; Pokatayev et al., 2020). The binding of CDNs to MITA disrupts the STIM1-MITA interactions and initiates the translocation of MITA from ER to the Golgi apparatus via the ER-Golgi intermediate compartment (ERGIC). In such a process, iRhom2 interacts with MITA and recruits the Sec5/TRAPβ/Sec61β complexes to facilitate the translocation to ERGIC (Luo et al., 2016) and STEEP promotes ER membrane curvature to reinforce SEC24C-mediated MITA ER exit via COPII vesicles (Gui et al., 2019; Zhang, Nandakumar, et al., 2020). Meanwhile, the Surface 4 integral membrane protein (SURF4) interacts with sectional MITA at the Golgi apparatus to promote the encapsulation of MITA into COPI vesicles, which retrieves MITA from the Golgi apparatus back to the ER to counteract the activation process of MITA (Deng et al., 2020). At the Golgi apparatus, MITA undergoes palmitoylation, which promotes the recruitment of TANK binding kinase 1 (TBK1) (Kwon & Bakhoum, 2020). Fang and colleagues have recently reported a mechanistic understanding of MITA activation at the Golgi apparatus (Fang et al., 2021). Sulfated Glycosaminoglycans (sGAGs), a kind of linear acidic polysaccharides, can bind to MITA at the Golgi apparatus, leading to the polymerization of MITA and the recruitment of TBK1. Afterward, TBK1 phosphorylates the C-terminal domains of MITA to recruit IRF3 for activation. Phosphorylated IRF3 binds to the nuclear shuttle protein Karyopherin alpha 2 (KPNA2) and is translocated to the nucleus to induce transcription of an array of downstream genes (Cai et al., 2020). In parallel, MITA also activates the IKK complex to mediate the activation of NF-κB and the induction of NF-κB-driven inflammatory genes (Chen, Sun, & Chen, 2016). In addition, the active MITA-containing ERGIC serves as a membrane source for LC3 lipidation, which is a key step for autophagosome biogenesis dependent on WIPI2 and ATG5 but independent of the ULK and VPS34-Beclin kinase complexes (Gui et al., 2019). MITA-mediated activation of autophagy is important for the clearance of DNA and viruses in the cytosol and such a process is independent of the recruitment of TBK1. Afterward, MITA is transported to perinuclear compartments and undergoes lysosome-mediated degradation to terminate hyperimmune responses (Kwon & Bakhoum, 2020). Alternatively, the overactivated MITA by excessive CDNs forms a puzzle-shaped membranous structure (known as MITA-TBK1-CDN sponge) to constrain MITA and TBK1 and prevent innate immunity from overactivation in a manner dependent on microtubules (Yu et al., 2021). Consistently, $MITA^{E336G/E337G}$ loses the condensation ability and thereby exhibits enhanced antiviral activity in cells. Therefore, the location of MITA is tightly controlled temporally and spatially which balances the quality and quantity of innate immune signaling in the absence and presence of viral infections.

Structure and activation of cGAS

Multiple cytosolic DNA sensors have been identified including DAI, RNA polymerase III, IFI16, and DDX41 (Chiu et al., 2009; Takaoka et al., 2007; Unterholzner et al., 2010; Zhang et al., 2011). However, it is still elusive how cytosolic DNA induces the expression of type I IFNs through distinct but functionally overlapped sensors. In 2013, by biochemical fractionation from cytosolic extracts of the murine fibrosarcoma cell line L929 cells and quantitative mass spectrometry assays, Dr. Zhijian Chen's group has identified a cytosolic DNA sensor cGAS (Sun et al., 2013) which directly binds to DNA and catalyzes the synthesis of cGAMP to induce type I IFNs in a manner dependent on MITA.

As a nucleotidyltransferase (NTase), human cGAS consists of 507 amino acids and is composed of a poorly conserved N-terminal domain (amino acid residues 1–160), a highly conserved C-terminal NTase (160–330) domain, and a Mab21 (213–513) domain (Li, Zhang, et al., 2013; Wu et al., 2014). cGAS contains three DNA-binding sites (site A, B and C) at its C-terminus that are important for its activation (Li, Shu, et al., 2013; Xie et al., 2019; Zhang, Wu, et al., 2014). The positively charged patches (such as K407 and K411) in these sites of cGAS mediate the cGAS-DNA binding and mutations of the key residues in any of the three DNA-binding sites of cGAS dampen the enzymatic activity of cGAS and the induction of type I IFNs in cells (Li, Shu, et al., 2013; Xie et al., 2019; Zhang, Wu, et al., 2014). Structural analysis of human cGAS demonstrates that the K187 and L195 of hcGAS in site A interfere with the binding of DNA to cGAS (Zhou et al., 2018). Interestingly, murine cGAS has much higher enzymatic activity than hcGAS and mcGAS contains N172 and R180 that correspond to K187 and L195 of hcGAS (Zhou et al., 2018). Furthermore, mutation of these two sites ($hcGAS^{K187N/L195R}$) significantly increases the enzymatic activity of hcGAS to a comparable level of the activity of mcGAS. These biochemical and structural studies have elegantly revealed the mechanism of DNA binding to cGAS.

The binding of DNA to cGAS results in the formation of a 2 cGAS:2 DNA complex, which efficiently catalyzes ATP and GTP into 2′,3′-cGAMP (Fig. 1). The length of DNA for sufficient cGAS activation is ~45 bp and longer DNA would recruit cGAS dimers to form ladder-like networks with DNA and efficiently promote the synthesis of 2′,3′-cGAMP by cGAS (Andreeva et al., 2017; Kranzusch et al., 2013). Consistent with this notion, short DNA (20 bp) barely activates cGAS *in vivo* and *in vitro*, though it binds to cGAS and induces the formation of cGAS dimers (Andreeva et al., 2017). The binding of DNA induces liquid phase separation of cGAS and thereby the cGAS-DNA complex is encapsulated in liquid droplets (Fig. 1), in which the activated cGAS catalyzes the synthesis of 2′,3′-cGAMP in a manner dependently on Zn^{2+} and the concentration of cGAS and DNA (Du & Chen, 2018). Recently, Mn^{2+} is reported to be indispensable for the activation of cGAS (Wang et al., 2018). Structural evidence shows that Mn^{2+} activates monomeric cGAS in the absence of DNA and also synergizes with dsDNA for the activation of cGAS (Hooy et al., 2020; Zhao, Ma, et al., 2020). Collectively, these available data have clearly demonstrated the step-wise and ion-mediated activation of cGAS.

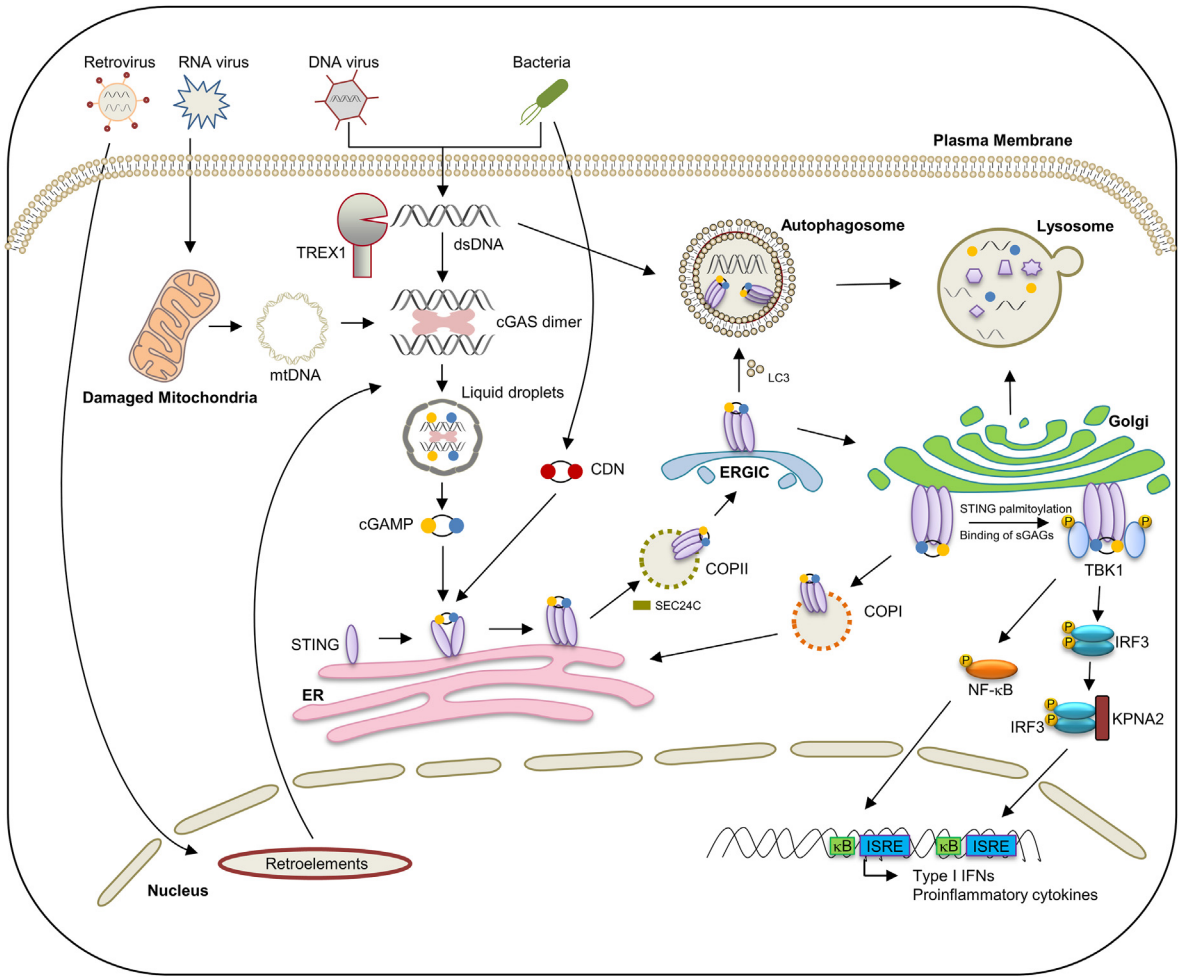

Fig. 1. The cGAS-MITA/STING pathway. The accumulation of cytosolic DNA is a mark of pathogen invasion. Cytosolic DNA sourced from DNA virus, damaged mitochondria, or reverse transcription of retroelements is detected by cGAS, leading to the formation of cGAS-DNA liquid droplets, in which cGAS, ATP, and GTP are concentrated to initiate the synthesis of cGAMP. MITA subsequently binds to cGAMP and undergoes dimerization and polymerization. Polymerized MITA encapsulated in SEC24C- mediated COPII vesicles translocates from the ER to Golgi via ERGIC, where MITA triggers autophagosome formation via LC3 lipidation, which leads to the clearance of cytosolic DNA and pathogens. At the Golgi apparatus, MITA undergoes palmitoylation and binds to sGAGs, which contributes to the recruitment of TBK1 and autophosphorylation of TBK1. TBK1 phosphorylates the C-terminal domains of MITA to recruit IRF3, after that IRF3 also is phosphorylated by TBK1 and translocates to the nucleus in a KPNA2-dependent manner, mediating the expression of type I IFNs. Moreover, MITA also activates NF-κB to mediate the expression of inflammatory genes. After the translocation process, MITA would be targeted to the lysosome for degradation and retrieve from Golgi back to the ER to avoid hyperactivation. cGAMP, 2′,3′-cyclic GMP-AMP; sGAG, sulfated glycosaminoglycans; IFN, interferon.

Localization of cGAS

It has been proposed that cGAS is a cytoplasmic protein to sense cytosolic DNA by biochemical and confocal immunofluorescent assays (Sun et al., 2013). However, recent studies have suggested that cGAS also localizes in the plasma membrane and the nucleus. cGAS interacts with phosphoinositide through its N terminal domain and thereby resides on the plasma membrane to distinct self- and viral DNA in resting murine and human phagocytes (Barnett et al., 2019). Upon binding to DNA, cGAS disassociates from phosphoinositide and translocates from the cell surface to cytoplasm, which facilitates liquid droplet formation as well as signaling transduction. cGAS has been observed in the nucleus when overexpressed in normal human fibroblasts and keratinocytes (Orzalli et al., 2015). Treatment of genotoxic agents, including etoposide, H_2O_2, and camptothecin, induces DNA damage, which leads to the translocation of cGAS from the cytosol to the nucleus (Liu, Zhang, Wu, et al., 2018). In the nucleus, cGAS is recruited to the double-stranded breaks and interacts with PARP1 to suppress homologous recombination, thereby promoting the accumulation of DNA damages and cell death (Liu, Zhang, Wu, et al., 2018; Ray Chaudhuri & Nussenzweig, 2017). During mitosis, cGAS is post-translationally regulated and rapidly translocates from the cytoplasm to chromosomes where cGAS is sequestered and inhibited (discussed below). These studies collectively demonstrate that cGAS is localized in the nucleus and plasma membrane under specific conditions. More quantitative studies of the cellular distribution of cGAS are required in future investigations.

cGAS-mediated sensing of pathogenic microorganisms

Because cGAS binds to DNA irrespective of its sequences, cGAS could be activated by all pathogenic microorganisms that produce dsDNA or ssDNA during infections and replication. Multiple studies showed that DNA viruses, including herpes simplex virus 1 (HSV-1) and HSV-2, adenoviruses, murine gammaherpesvirus 68, vaccinia virus, human papillomavirus (HPV), cytomegalovirus, and papillomavirus, are sensed by cytoplasmic cGAS to initiate immune responses (Fig. 1) (Lam et al., 2014; Schoggins et al., 2014; Sun et al., 2013; Tan et al., 2018). Consistently, cGAS knockout mice are unable to produce type I interferons and inflammatory cytokines and, thus, are susceptible to these viral pathogens. In addition, retroviruses including HIV-1 and HIV-2, murine leukemia

virus (MLV), and simian immunodeficiency virus (SIV) can activate cGAS-mediated immune responses in a manner dependently on the reverse-transcribed viral cDNA (Gao et al., 2013; Lahaye et al., 2013; Sumner et al., 2020) (Fig. 1). Several RNA viruses also indirectly stimulate the cGAS-MITA pathway by inducing mitochondrial DNA release (Fig. 1) (Aguirre et al., 2017; Moriyama et al., 2019). Compared to the wild-type mice or cells, the cGAS knockout mice or cells exhibit impaired production of type I IFNs after infections of *Plasmodium* (Gallego-Marin et al., 2018; Hahn et al., 2018) and various intracellular bacteria, including *Listeria monocytogenes* (Nandakumar et al., 2019), *Chlamydia trachomatis* (Zhang, Yeruva, et al., 2014), and *Francisella tularensis* (*F. novicida*) (Man et al., 2015) (Fig. 1). These findings together suggest that cGAS senses a broad spectrum of invading microorganisms.

Regulation of the cGAS-MITA/STING signaling pathway

To avoid harmful pathology caused by aberrant cGAS-MITA-mediated innate inflammatory responses, the immune system has evolved comprehensive and multi-level mechanisms to tightly regulate the cGAS-MITA signaling pathway, including (1) homeostatic regulation of cGAS activation; (2) transcriptional regulation of cGAS and MITA; and (3) post-translational modifications (PTMs) of cGAS and MITA.

Homeostatic regulation of cGAS activation by controlling the availability of self-DNA

Under normal conditions, mitochondrial DNA (mtDNA) is encased within the outer and inner mitochondrial membrane and thus inaccessible to cGAS (Zierhut & Funabiki, 2020). Intrinsic apoptosis in a manner of activation of Bak and Bax leads to mitochondrial damage, ultimately resulting in the release of mtDNA. Yet, this cytoplasmic mtDNA does not lead to the activation of cGAS, as apoptosome complex blocks the cGAS-MITA pathway via proteolytic cleavage of cGAS (White et al., 2014). Alternatively, damaged mitochondria are immediately cleared in selective degradation of a process termed mitophagy that avoids the activation of cGAS (Sliter et al., 2018). Recently, one report demonstrates that apoptotic cells generate extrachromosomal circular DNA (eccDNA) that could trigger MITA-mediated immune responses (Wang et al., 2021). Whether this process involves cGAS-mediated cGAMP production is unknown.

The nuclear envelope (NE) provides a physical barrier that separates cytosolic cGAS from chromosomal DNA. However, the NE is disassembled during cell division, thereby making chromosomal DNA accessible to cGAS. Some key mechanisms to ensure the non-responsiveness of cGAS to chromosomal DNA have been characterized. First, binding to the nucleosome of cGAS during cell mitosis locks cGAS into a monomeric state, and such a steric hindrance inhibits spurious activation of cGAS by chromosomal DNA (Boyer et al., 2020; Cao et al., 2020; Kujirai et al., 2020; Michalski et al., 2020; Pathare et al., 2020; Zhao, Xu, et al., 2020). Second, barrier-to-autointegration factor 1 (BAF) outcompetes cGAS for DNA binding during mitosis, which prohibits the formation of cGAS-DNA complexes (Guey et al., 2020). Third, circular RNA antagonist for cGAS (cia-cGAS) harbors a stronger binding affinity to nuclear cGAS than chromosomal-DNA, consequently restraining cGAS-mediated production of type I IFNs in long-term hematopoietic stem cells (LT-HSCs) (Xia et al., 2018). A fourth mechanism has been proposed by two recent reports (Li et al., 2021; Zhong et al., 2020). During mitosis, Aurora kinase B and cyclin-dependent kinase 1 (CDK1) selectively phosphorylate cGAS, which significantly prohibits its activity. Together, these findings suggest that cGAS activation by chromosomal or mitochondrial self-DNA is limited under unperturbed conditions.

The endogenous retroelements-derived DNA also activates cGAS-mediated immune responses (Zierhut & Funabiki, 2020). Human endogenous retroviruses (HERVs) sourcing from exogenous retroviruses that successfully integrate themselves into the host genome are inherited by successive generations (Ishak et al., 2018). Multiple reverse transcription events of HERVs make reverse-transcribed cDNA accessible to cGAS. DNase enzymes, including DNase I (Ahn & Barber, 2014), DNase II (Rodero et al., 2017), and exonuclease 1(Trex1) (Stetson et al., 2008), are responsible for clearing retroelements-derived DNA, thereby limiting the engagement between self-DNA and cGAS.

Moreover, the binding of cGAS to DNA involves extensive ionic interactions between the positively charged surface of cGAS and the negatively charged DNA (Du & Chen, 2018). Such interactions are susceptible to the cytosolic salt concentration, which may be responsible for limiting the spurious activation of cGAS by self-DNA below a certain threshold. Overall, it appears that vertebrates have evolved a series of precise regulatory elements to prevent self-DNA-mediated spontaneous activation of cGAS. Future studies are necessary to establish the mechanistic basis of homeostatic cGAS regulation by self-DNA and the signaling consequences.

Transcriptional regulation of cGAS and MITA/STING

It has been reported that cGAS is induced by type I IFNs through JAK-STAT-axis in both human and mouse macrophages (Ma et al., 2015). However, this positive feedback induction of cGAS by type I IFNs is contrary to the observations that MITA inhibits the activation of JAK1-STAT1 signaling cascades by inducing phosphorylation of SHP1 and SHP2 (Dong et al., 2015). Moreover, NCOA3 is an epigenetic factor maintaining the transcriptional levels of *cGAS* expression, and miR25/93 targets NCOA3 to repression of cGAS in hypoxic tumor cells (Wu et al., 2017). In several human colorectal cancer and melanoma cell lines, *cGAS* expression is also silenced via the epigenetic hypermethylation process, which could be overturned by treating with the demethylation agent (Xia et al., 2016b, 2016c).

Similar to cGAS expression, MITA expression at the transcriptional level is also inhibited in many cancer cell lines (Xia et al., 2016b, 2016c). In addition, a recent study shows that mutation or loss of LKB1, an omnipresently expressed master serine/threonine kinase, results in significant silencing of MITA expression, which is partially mediated by epigenetic silencing enzymes such as DNMT1 and EZH2 (Kitajima et al., 2019). Human and murine primary hepatocytes do not express MITA, which might be responsible for the impaired immune responses to Hepatitis B virus (HBV) infection (Thomsen et al., 2016). In addition, the P-body protein LSm14A was reported to regulate pre-mRNA processing of *MITA* (Liu et al., 2016). In *Lsm14a*$^{-/-}$ DCs, mRNA of *MITA* but not *cGAS*, *TBK1*, or *IRF3* is remarkably reduced, suggesting that LSm14A is indispensable for maintaining the mRNA level of *MITA*. These studies have demonstrated epigenetic and post-transcriptional regulation of cGAS and MITA.

Post-translational modification of cGAS

PTMs of cGAS and MITA, including polyubiquitination, phosphorylation, acetylation, SUMOylation, glutamylation, and palmitoylation have been shown to extensively regulate the cGAS-MITA signaling pathway during the resting state and viral infection (Table 1).

In resting cells, tubulin tyrosine ligase-like 6 (TTLL6) and TTLL4 catalyze polyglutamylation and monoglutamylation of cGAS, resulting in inhibition of its DNA-binding ability and synthase activity, respectively (Xia, Ye, et al., 2016). cGAS also undergoes acetylation at K384, K394, or K414, which keeps cGAS inactive without DNA challenges, though the enzyme responsible for this process is unknown (Dai et al., 2019). During mitosis (as described above), cyclin-dependent kinase 1 (CDK1) and mitotic kinases, including Aurora kinase B (AurB), phosphorylates cGAS to block its ability of self-DNA detection, which helps to prevent autoimmunity (Li et al., 2021; Zhong et al., 2020). PPP6C constitutively dephosphorylates mcGAS in un-infected cells at S420, impairing its ability to bind to GTP and thereby leading to its inactivation (Li & Shu, 2020). Moreover, cGAS is constitutively ubiquitinated at K271 and undergoes degradation in resting cells. TRIM38 catalyzes SUMOylation of cGAS at

Table 1
Post-translational modifications of cGAS and MITA/STING.

Protein	Occurring contexts	Enzyme	Modification	Residues	Refs
Signaling activation					
cGAS	Resting cells	TRIM38	SUMOylation	K231 (mouse K217)	Hu et al. (2016)
		USP29	Removal of K48-linked polyubiquitination	Mouse K271	Zhang et al. (2020)
	Stimulated cells	TRIM56	Monoubiquitination	K335	Seo et al. (2018)
		RINCK	Monoubiquitination	N.D.	Seo et al. (2018)
		TRIM38	SUMOylation	K479 (mouse K464)	Hu et al. (2016)
		RNF185	K27-linked polyubiquitination	K173/K384	Wang et al. (2017)
		USP29	Removal of K48-linked polyubiquitination	Mouse K271	Zhang, Bai, and Chen (2020)
		USP14	Removal of K48-linked polyubiquitination	K414	Chen, Meng, et al. (2016)
		CCP5	Removal of monoglutamylation	E302	Xia, Ye, et al. (2016)
		CCP6	Removal of polyglutamylation	E272	Xia, Ye, et al. (2016)
		KAT5	Acetylation	K47/K56/K62/K83	Song et al. (2020)
		HDAC3	deacetylation	N.D.	Dai et al. (2019)
MITA	Resting cells	EIF3S5	Removal of K48-linked polyubiquitination	N.D.	Luo et al. (2016)
	Stimulated cells	RNF26	K11-linked polyubiquitination	K150	(Qin et al., 2014)
		AMFR	K27-linked polyubiquitination	K137/K150/K224/K236	Wang et al. (2014)
		MUL1	K63-linked polyubiquitination	K224/K236/K289/K338	Ni et al. (2017)
		TRIM56	K63-linked polyubiquitination	K150	Tsuchida et al. (2010)
		TRIM32	K63-linked polyubiquitination	K20/K150/K224/K236	Zhang et al. (2012)
		RNF115	K63-linked polyubiquitination	K20/K224/K289	Zhong et al. (2020e)
		TRIM38	SUMOylation	K338	Hu et al. (2016)
		USP20	Removal of K48-linked polyubiquitination	N.D.	Zhang et al. (2016)
		CYLD	Removal of K48-linked polyubiquitination	N.D.	Zhang et al. (2018)
		EIF3S5	Removal of K48-linked polyubiquitination	N.D.	Luo et al. (2016)
		USP44	Removal of K48-linked polyubiquitination	K236	Zhang, Tang, et al. (2020)
		OTUD5	Removal of K48-linked polyubiquitination	K347	Guo et al. (2021)
		CSK	Phosphorylation	Y240/Y245	Gao et al. (2020)
		EGFR	Phosphorylation	Y245	Wang et al. (2020)
		TBK1	Phosphorylation	S366	Zhang et al. (2019)
		N.D.	Palmitoylation	C88/C91	Mukai et al. (2016)
Signaling inhibition					
cGAS	Resting cells	CDK1	Phosphorylation	S305 (mouse S291)	Zhong et al. (2020)
		AurB	Phosphorylation	N.D.	Li et al. (2021)
		TTLL4	monoglutamylation	E302	Xia, Ye, et al. (2016)
		TTLL6	polyglutamylation	E272	Xia, Ye, et al. (2016)
		PPP6C	Dephosphorylation	S435 (mouse S420)	Li and Shu (2020)
		N.D	Acetylation	K384/K394/K414	Dai et al. (2019)
	Stimulated cells	AKT	Phosphorylation	S305 (mouse S291)	Seo et al. (2015)
		DNA-PK	Phosphorylation	T68/S213	Sun et al. (2020)
MITA	Resting cells	USP13	Removal of K27-linked polyubiquitination	N.D.	Sun et al. (2017)
	Stimulated cells	USP13	Removal of K27-linked polyubiquitination	N.D.	Sun et al. (2017)
		USP49	Removal of K63-linked polyubiquitination	N.D.	Ye et al. (2019)
		USP21	Removal of K27/K63-linked polyubiquitination	N.D.	(Chen et al., 2017)
		N.D.	Nitro-alkylation	C88/C91/H16	Hansen et al. (2018)
		PPM1A	Dephosphorylation	S358	Li et al. (2015)
Degradation					
cGAS	Stimulated cells	SENP2	DeSUMOylation	K479 (mouse K464)	Hu et al. (2016)
MITA	Stimulated cells	SENP2	DeSUMOylation	K338	Hu et al. (2016)
		TRIM30α	K48-linked polyubiquitination	K275	Wang et al. (2015)
		RNF5	K48-linked polyubiquitination	K150	Zhong et al. (2009)
		TRIM29	K48-linked polyubiquitination	K370	Xing et al. (2017)
		ULK1	Phosphorylation	S366	Konno et al. (2013)
		PTPN1/2	Dephosphorylation	Y245	Xia et al. (2019)

K217, which inhibits K48-linked polyubiquitination of cGAS at K271 through the physical/spatial hindrance, and ultimately stabilizes cGAS (Hu et al., 2016). The deubiquitinase ubiquitin Specific Protease 29 (USP29) constitutively targets cGAS for deubiquitination at K271, thereby stabilizing cGAS in uninfected cells (Zhang, Tang, et al., 2020). However, the E3 ligases that regulate the ubiquitination of homeostatic cGAS remain to be identified.

Viral infection induces a vast number of post-translational modifications of cGAS (Table 1). Cytosolic carboxypeptidase 5 (CCP5) removes the monoglutamylation of cGAS, whereas CCP6 hydrolyzes the polyglutamylation of cGAS, both of which lead to activation of cGAS in response to DNA challenges (Xia, Ye, et al., 2016). The acetylation exerted by lysine acetyltransferase 5 (KAT5) at the N-terminal domain of cGAS is indispensable to initiate the immune responses in stimulated cells, whereas the acetylation of the C-terminal of cGAS blocks the activity of cGAS (Dai et al., 2019; Song et al., 2020), suggesting that the acetylation at different domains of cGAS may lead to a different function of cGAS. Besides, the E3 ubiquitin ligase TRIM14 recruits deubiquitinase USP14 to remove K48-linked ubiquitin chains and thereby inhibits degradation of cGAS by autophagic pathway in HSV-1 infected cells (Chen, Meng, et al., 2016). Interestingly, we have recently reported that HSV-1 infection induces the degradation of cGAS via the proteasomal pathway, which is counteracted by the deubiquitinase USP29 (Zhang, Tang, et al., 2020). It is thus likely that the stability of cGAS after viral infection is controlled by multiple mechanisms.

Two studies revealed that the E3 ubiquitin ligase TRIM56 and RINCK target cGAS for monoubiquitination, thereby positively regulating the synthesis of cGAMP (Liu, Zhang, Cai, et al., 2018; Seo et al., 2018). TRIM38 also specifically catalyzes the SUMOylation of cGAS to prevent the proteasomal degradation of cGAS at the early phase of viral infection. Subsequently, SENP2 de-SUMOylates cGAS at the late phase of infection to shut down the immune responses (Hu et al., 2016), demonstrating an elegant temporal step-wise control of cGAS activity and stability. The kinases AKT and DNA-PK phosphorylate cGAS during infection of DNA

virus, which robustly suppresses its enzymatic activity and fine-tunes immune responses to DNA stimulation (Seo et al., 2015; Sun et al., 2020). Future investigations are required to elucidate whether and how these PTMs function cooperatively or redundantly to regulate the activity and stability of cGAS in a cell-type or stimuli-dependent manner.

Post-translational modification of MITA/STING

Resting-state MITA is regulated by ubiquitination and deubiquitination. The K27 linked-polyubiquitin chains are removed by the deubiquitinating enzyme (DUB) ubiquitin-specific protease 13 (USP13), which inhibits the basal activity of MITA in a manner dependent on blocking its recruitment of TBK1 (Sun et al., 2017). Moreover, inactive rhomboid protein 2 (iRhom2) recruits the deubiquitination enzyme EIF3S5 to constitutively deconjugate K48-linked polyubiquitin chains from MITA and prevent its degradation through the proteasomal pathways (Luo et al., 2016). Recently, one report has shown that death-associated protein kinase 3 (DAPK3) stabilizes MITA in resting cells via suppressing its K48-linked poly-ubiquitination and degradation of proteasome pathway (Takahashi et al., 2021). However, the E3 ligases that regulate the ubiquitination of homeostatic MITA have not been identified yet.

Though the binding of cGAMP leads to dimerization of MITA, the full activation of MITA requires various PTMs accompanied by budding off the ER to ERGIC. HSV-1-infection induces K27-linked polyubiquitin of MITA and such a PTM is mediated by AMFR, which provides an anchoring platform for recruiting TBK1 (Wang et al., 2014). It has been shown that TRIM56, TRIM32, and MUL1 catalyze K63-linked ubiquitination of MITA, which promotes the oligomerization and full activation of MITA (Ni et al., 2017; Tsuchida et al., 2010; Zhang et al., 2012). However, studies with TRIM56 and TRIM32 knockout mice suggest that deletion either of them has minimal effect on ubiquitination of MITA after HSV-1 infection (Seo et al., 2018; Yang, Liu, et al., 2017). Whether these two enzymes function redundantly for the ubiquitination of MITA is unknown. More recently, we have demonstrated that knockout of RNF115 impairs HSV-1-induced K63-linked ubiquitination of MITA and the oligomerization of MITA, suggesting that RNF115 is a bona fide E3 ligase for the K63-linked ubiquitination of MITA after HSV-1 infection (Zhang, Xiong, et al., 2020). In contrast, cGAMP- or HSV-1-induced dimerization of MITA is not affected by the knockout of RNF115, indicating that the oligomerization of MITA is followed by cGAMP-mediated dimerization and requires 63-mediated ubiquitination.

The MITA dimers or oligomers bud off the ER and are trafficking to the Golgi apparatus where it undergoes palmitoylation to activate downstream signaling cascades (Mukai et al., 2016). Such a PTM is suppressed by nitro-alkylation modified by endogenously formed nitro-fatty acids in the late phase of viral infection (Hansen et al., 2018; Mukai et al., 2016). Afterward, TBK1 is recruited to and phosphorylates MITA at S366, thereby promoting the recruitment and phosphorylation of IRF3 (Zhang et al., 2019). Consistently, the S366A mutation in MITA was found to be unable to interact with and activate IRF3 in infected cells. However, the MITA^{S365A} (an ortholog of hMITA^{S366A}) mice do not show hypersensitivity to HSV-1 infection compared to the wild-type mice (Yamashiro et al., 2020; Yum et al., 2021), indicating a TBK1-independent role of MITA for anti-viral immune responses. In this context, MITA also promotes autophagy to clear viral DNA which is believed as a primordial function of MITA (Gui et al., 2019). Other kinases including DAPK3 and CSK are also involved in the phosphorylation and activation of MITA (Gao et al., 2020; Takahashi et al., 2021; Wang et al., 2020). Collectively, these data indicate a sequential multi-step activation of MITA after viral infection, i.e., cGAMP-mediated dimerization, ubiquitination-mediated oligomerization, palmitoylation- and phosphorylation-mediated recruitment of TBK1 and IRF3.

To avoid overwhelming activation of MITA and harmful immune responses, suitable PTMs are essential to tune down the activation of MITA. The E3 ligases RNF5, TRIM30α, and TRIM29 catalyze K48-linked ubiquitination and proteasomal degradation of MITA at the late stage of viral infection, which is counteracted by USP20, USP44, CYLD, EIF3S5, and OTUD5 (Guo et al., 2021; Luo et al., 2016; Wang et al., 2015; Xing et al., 2017; Xu et al., 2020; Zhang et al., 2016, 2018; Zhong et al., 2009). USP49 removes K63-linked polyubiquitination of MITA, thereby negatively regulating the signaling pathway (Ye et al., 2019). Besides, serine/threonine UNC-51-like kinase (ULK1) phosphorylates MITA after the activation of IRF3 and NF-κB during the infection of HSV-1, which finally avoids sustained production of inflammatory cytokines (Konno et al., 2013). Phosphatase PPM1A and PTPN1/2 also act as negative regulators by dephosphorylation of MITA to avert hyperactivation of the MITA-mediated immune responses (Li et al., 2015; Xia et al., 2019). Together, although a mass of enzymes and their corresponding PTMs have been identified to covalently modify MITA (Table 1) during different phases of viral infection, further work in this field is needed to elucidate how different PTMs crosstalk and dynamically regulate the activity and stability of MITA.

cGAS-MITA/STING pathway in autoimmune disease

As described above, cGAS is inactive or kept away from self-DNA under homeostatic conditions. Accordingly, dysregulation of such a process would result in constitutively and spontaneously activation of cGAS, which promotes the upregulation of proinflammatory cytokines and induces autoimmune disorders. MITA is kept in ER as monomer under homeostatic conditions. Removal of the ER-retention factors or gain-of-function mutations of MITA would lead to constitutive activation of MITA and auto-inflammatory diseases.

DNase and RNase mutations

To identify endogenous DNA sensors, Stetson et al. have identified TREX1 as a sensor of cytosolic DNA (Stetson et al., 2008). However, TREX1 does not trigger activation of IRF3 and the induction of type I IFNs. Rather, the knockout of TREX1 leads to the accumulation of cytosolic DNA and sustained autoantibody production and inflammation. Trex1 is a 3′→5′ DNA exonuclease and consists of the N-terminal DNase domain necessary for exonuclease activity and the C-terminal transmembrane helix necessary for ER localization. Accumulative DNA in the cytoplasm from various sources, including occasional leakage of nuclear DNA, the release of mtDNA, and DNA from reverse-transcribed endogenous RNA, can be recognized and digested by TREX1 (Fig. 1). A number of clinical studies reveal that mutations in TREX1 have been identified in autoimmune diseases including Aicardi-Goutieres syndrome (AGS), systemic lupus erythematosus (SLE), and retinal vasculopathy with cerebral leukodystrophy (RVCL) (Crow & Manel, 2015; Grieves et al., 2015; Hasan et al., 2015; Hemphill et al., 2021; Stetson et al., 2008). These mutations in both N-terminal and C-terminal domain result in loss-of-function of TREX1 and the abnormal accumulation of cytosolic DNA and ultimately constant activation of the cGAS-MITA pathway. Similarly, Trex1-deficient or -mutated (*Trex1*$^{D18N/D18N}$) mice exhibit the lethal autoimmune phenotype, which can be genetically rescued by depletion of cGAS or MITA (Gao et al., 2015; Morita et al., 2004; Simpson et al., 2020; Stetson et al., 2008; Xiao et al., 2019). These observations suggest that the activation of the cGAS-MITA pathway mediates the autoimmune phenotypes in *Trex1*$^{-/-}$ or *Trex1*$^{D18N/D18N}$ mice.

Deoxyribonuclease (DNase) II encoded by *DNASE2*, an endonuclease that digests ssDNA and dsDNA in the lysosome, is also related to autoimmune diseases in humans (Hong et al., 2020; Rodero et al., 2017). Biallelic mutations in *DNASE2* result in deficiency of Dnase II endonuclease activity, consequently leading to overproduction of interferons and ISGs in the lymphocytes and monocytes. Similarly, deletion of *cGas*, *Mita,* or *Ifnar1* rescues the lethal autoimmune phenotypes of *DNaseII*$^{-/-}$ mice (Ahn et al., 2012; Gao et al., 2015).

Several mutations in ribonuclease H2 (RNase H2) are also identified in patients with AGS syndrome (Gunther et al., 2015; Nishimura et al., 2019). RNase H2 removes RNA/DNA hetero-stranded nucleic acids, such

as RNA embedded in double-stranded DNA during DNA replication and R loops formed during transcription structure. Rnaseh2a^{G37S} and Rnaseh2a^{A174T} are activity-attenuated mutants. The Rnaseh2a^{G37S} and Rnaseh2a^{A174T} knock-in mice exhibit increased expression of interferons and ISGs and autoimmunity, which can be rescued by deletion of MITA (Mackenzie et al., 2016; Pokatayev et al., 2016). A subset of AGS patients carry mutations in three other genes including SAMHD1, ADAR1, and IFIH1, and exhibits the phenotypes of upregulation of ISGs (Coggins et al., 2020; Oda et al., 2014; Rice et al., 2009, 2012). However, whether such symptom is dependent on cGAS-MITA is unknown. In addition, the gain-of-function mutations of cGAS have not been identified.

MITA/STING gain-of-function mutations

In 2014, a clinical study reported the discovery of MITA gain-of-function mutations (MITA N154S and V155M) in six patients exhibiting systemic inflammation, scaling lesions of extremities and cheeks, and interstitial lung disease, which is termed STING-associated vasculopathy with onset in infancy (SAVI) (Liu et al., 2014). Over the past 7 years, other gain-of-function mutations of MITA have been identified, including S102P/F279L, V147L, G166E, C206Y, R281Q, R284Q, R284G, and R284S (Chia et al., 2016; Konig et al., 2017; Konno et al., 2018; Melki et al., 2017; Motwani et al., 2019a, 2019b; Munoz et al., 2015; Saldanha et al., 2018; Seo et al., 2017). All of the mutant MITA spontaneously dimerize and bud off from the ER to the Golgi apparatus even in the absence of cGAMP. Continuous accumulation of mutant MITA in the Golgi apparatus leads to activation of TBK1 and IRF3, which results in the production of interferons and ISGs. Furthermore, homozygous knock-in mice carrying MITA^{N153S} mutation (human MITA N154S ortholog) can not survive gestation, whereas heterozygous MITA^{N153S} and MITA^{V154M} (human MITA V155M ortholog) mice develop T cell cytopenia, myeloid cell expansion, spontaneous pulmonary and renal inflammation, similar to SAVI patients (Bennion et al., 2019; Bouis et al., 2019; Warner et al., 2017). MITA^{N153S} mice also exhibit defects in lymph-node organogenesis and innate lymphoid cell development (Bennion et al., 2020). However, the overwhelming type I IFN gene expression is not observed in the MITA^{N153S} mice, which has been reported in patients with SAVI. In contrast, activation of the functional NF-κB pathway plays a dominant role in the SAVI-like disease of MITA^{N153S} mice. The different phenotypes between the SAVI patients and the gain-of-function MITA mutated mice indicate different regulatory mechanisms of human and mouse MITA. One recent study shows that MITA^{N153S} mice develop intestinal inflammation in the colon in a manner dependent on the abnormal accumulation of MITA in myeloid cells, suggesting that MITA^{N153S} could be the potential target for patients with inflammatory bowel disease (IBD) (Shmuel-Galia et al., 2021). Collectively, these studies indicate that abnormal activation of the MITA pathway contributes to various autoimmune diseases, suggesting that therapeutic targeting of these responses could be beneficial for the treatment of the related diseases.

cGAS-MITA/STING pathway in cancer

In recent years, emerging studies have implied the important regulatory function of the cGAS-MITA signaling pathway in tumors. Several small-molecule compounds as agonists for MITA have been developed for clinical trials to test their effects on cancer prevention.

Tumor suppressive role

Accumulating evidence has shown that the cGAS-MITA pathway

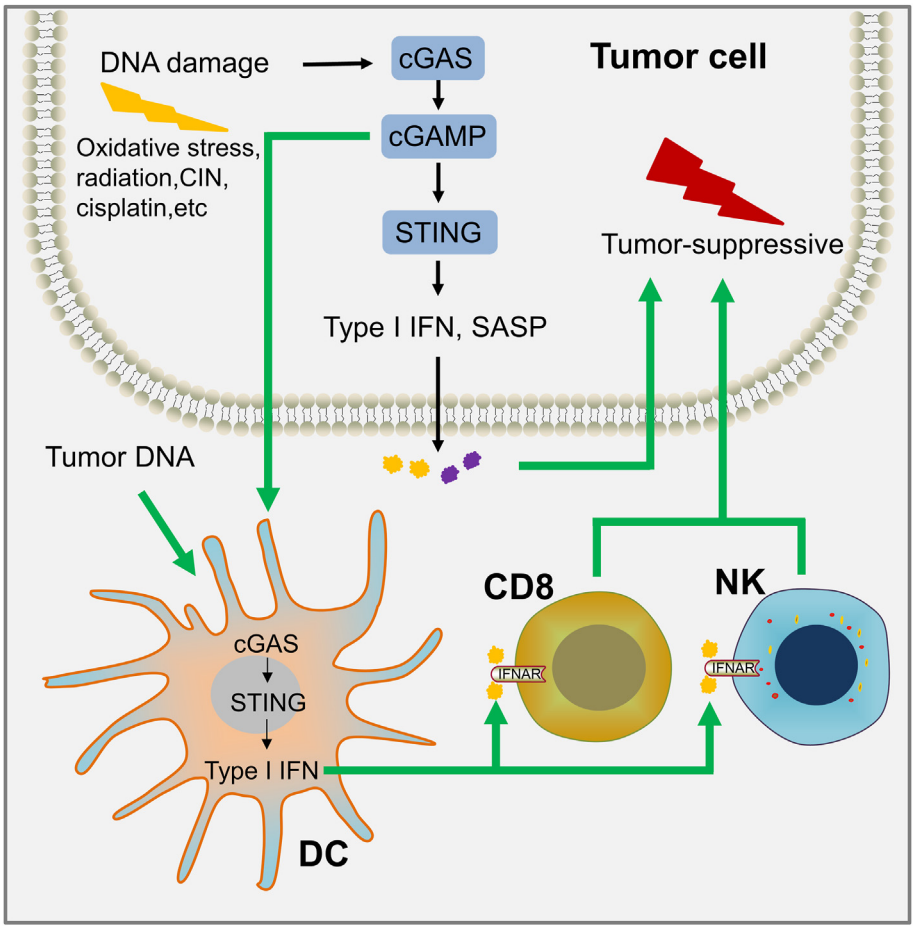

Fig. 2. Regulation of cGAS-MITA/STING pathway in tumor suppression. In early neoplastic cells, the cGAS-MITA pathway plays a tumor-suppressive role. DNA damage sourced from such as oxidative stress, radiation, CIN, or cisplatin leads to DNA accumulate in the cytoplasm, which activates the cGAS and promotes the synthesis of cGAMP. On one hand, cGAMP binds to MITA to upregulate the expression of type I IFNs and SASP genes in tumors, which in turn mediates tumor-suppressive effects. On the other hand, the cGAS-MITA pathway could mediate the crosstalk between the tumor cells and DCs nearby. Tumor DNA or tumor-derived cGAMP could activate the cGAS-MITA signaling in DCs, finally initiating tumor clearance mediated by immune cells such as CD8$^+$ T cells or NK cells. CIN, chromosomal instability; cGAMP, 2′,3′-cyclic GMP-AMP; IFN, interferon; SASP, senescence-associated secretory phenotype; DC, dendritic cell; NK cells, natural killer cells. IFN-R, IFN receptor.

plays a tumor-suppressive role (Fig. 2). One of the most important features of cancer cells is chromosomal instability (CIN) that leads to chromosomal missegregation during mitosis, which ultimately promotes the formation of micronuclei (Crasta et al., 2012). The micronuclear envelopes are subject to break up, which causes the accumulation of genomic DNA in the cytoplasm (Harding et al., 2017; Mackenzie et al., 2017). Alternatively, radiotherapies, and chemo-reagents such as cisplatin, and intrinsic DNA damage also lead to the production of cytoplasmic DNA (Ahn et al., 2014; Dou et al., 2017; Harding et al., 2017; Mackenzie et al., 2017). Besides, oxidative stress in cancer cells causes mitochondrial damage by permeabilization of the inner and outer mitochondrial membranes, consequently leading to the release of mtDNA in the cytoplasm (Sansone et al., 2017; Tan et al., 2015). When the DNA in the cytoplasm accumulates above the threshold, it would activate the cGAS-MITA pathway that upregulates the production of type I IFNs to promote the infiltration and activation of immune cells, such as T cells and nature killer (NK) cells to elicit host immune responses (Harlin et al., 2009; Marcus et al., 2018). Moreover, the cGAS-MITA signaling upregulates the senescence-associated secretory phenotype (SASP) genes including inflammatory cytokines, chemokines, growth factors, and proteases, thereby facilitating senescence of cancer cells and tumor clearance (Dou et al., 2017; Kwon & Bakhoum, 2020; Yang, Wang, et al., 2017). Alternatively, tumor-derived DNA or cGAMP that is engulfed by surrounding DCs activates cGAS-MITA signaling and promotes IFN-β production. Subsequently, IFN-β promotes the cross-presentation of $CD8\alpha^+$ DC subset for activation of tumor antigen-specific $CD8^+$ T cells (Woo et al., 2014). Consistently, mice that lack IFNAR1 in DCs display significant defects in antigen cross-presentation to $CD8^+$ T cells (Diamond et al., 2011). DC or tumor-derived IFN-β also activates the NK cells in a manner dependent on IFNAR signaling in NK cells (Marcus et al., 2018). Ultimately, activated T cells and NK cells in turn traffic to tumor sites and promote tumor suppression.

Under selective pressure, some surviving cancer cells are prone to harbor cGAS or MITA deficiency. MITA expression has been reported to be undetectable in several cancerous melanomas and colorectal adenocarcinoma cell lines (Xia et al., 2016b, 2016c). Moreover, *KRAS*-driven lung cancers markedly silence MITA expression owing to deficiency of LKB1 (Kitajima et al., 2019). The deficiency of MLH1 that is responsible for regulating exonuclease 1 nuclease activity results in DNA accumulating in the cytosol and activating the cGAS-MITA pathway (Guan et al., 2021). One study demonstrates that cGAS expression is deficient in human defective MMR gene *Mlh1* (dMLH1) cancer lines (Lu et al., 2021), and loss of or impaired cGAS-MITA responses confers defective MMR (dMMR) tumors resistance to immune-checkpoint blockade (ICB) therapy, which suggests that the cGAS-MITA pathway is a potential biomarker for immunotherapy in patients with dMMR cancers.

Considering MITA suppresses tumorigenesis, multiple agonists of MITA have been developed to mimic the activation of the cGAS-MITA pathway for tumor suppression. The agonist of MITA, 5,6-dimethylxanthenone-4 acetic acid (DMXAA), promotes the activation of MITA and destroys the blood vessel walls in tumor tissues, which significantly facilitates the fade of solid tumors in mice (Gao et al., 2014; Zhao et al., 2002). However, clinical trials of treatment DMXAA (also known as ASA404) with non-small cell lung cancer patients are failed in phase III due to tiny therapeutic effects (Lara et al., 2011). It is now clear that DMXAA only binds and activates mouse MITA but not human MITA (Conlon et al., 2013). CDN 3′3′-cGAMP induces the expression of type I interferon in mouse melanoma, liver cancer, and lung cancer cells by activating MITA (Tang et al., 2016). Intraperitoneal injection of 3′3′-cGAMP into Eμ-TCL1 transgenic mice can significantly inhibit the process of chronic lymphocytic leukemia (CLL). In addition, 2′,3′-cGAMP also presents an anti-cancer effect on mouse colon cancer, reducing the size of mouse tumors and prolonging the survival of mice (Yang et al., 2019). Expect for endogenous CDNs, synthetic modified CDNs are well developed. ML RR-S2 CDN (also known as ADU-S100 or MIW815) exhibits significant antitumor efficiency in multiple mouse cancer models and binds human MITA (Corrales et al., 2015). Follow-up clinical trials of ADU-S100 or in combination with checkpoint (CTLA-4 or PD-1) inhibitors are now ongoing to phase I or phase II (Table 2). Other drugs including E7766, BMS-986301, IMSA101, GSK3745417, IMSA101, MK-1454, and SB 11285 are currently undergoing clinical trials and are present in Table 2. Recently, MSA-2 and SR-717, non-nucleotide MITA agonists, were reported to display anti-tumor activity (Chin et al., 2020; Pan et al., 2020). MSA-2 amenable to oral administration induces elevations of IFN-β and almost complete tumor regression in MC38 tumor-bearing mice. Intraperitoneal injection of SR-717 exhibits anti-tumor activity in a manner dependent on activating of $CD8^+$ T cells, NK cells, dendritic cells, and the cross-priming of antigens. However, MSA-2 and SR-717 have not entered into clinical trials yet.

Tumor promoting role

Though cGAS and MITA are lowly expressed in tumor cells, high expression of cGAS and MITA has already been reported to be positively correlated with poor prognosis in a small part of patients with colorectal cancer (An et al., 2019). Chronic inflammation induced by MITA-associated SASP may facilitate oncogene-driven senescence suppression (Fig. 3) (Dou et al., 2017; Toso et al., 2014). Moreover, activation of cGAS-MITA, triggered by DNA derived from CIN-generated micronuclei, induces noncanonical NF-κB signaling responses and metastasis in a tumor cell-autonomous manner (Bakhoum et al., 2018). Alternatively, Brain metastatic cancer cells can utilize gap-junctions to transfer cGAMP to astrocytes, activating MITA and downstream inflammatory genes such as IFN-α and TNF, which, in turn, elicits the STAT1 and NF-κB pathways in brain metastatic cancer cells, ultimately promoting tumor growth and chemoresistance (Chen, Boire, et al., 2016). In addition, activation of MITA enhances infiltration of regulatory T cells and enzyme indoleamine 2,3-dioxygenase (IOD), consequently mediating tolerance of immune response and inhibition of T cells proliferation (Liang et al., 2015; Munn & Mellor, 2016). MITA agonist is able to upregulate the expression of PD-L1, a protein that inhibits the immune response, which promotes tumor progression (Corrales et al., 2016; Fu et al., 2015). Autophagy mediated by MITA is another inducement of immune evasion and tumor promotion (Pommier et al., 2018; Terai et al., 2018). T cells expressing activated MITA exhibit cytopenia phenotype, indicating that constitutive activation of MITA in T cells leads to dysfunction or exhaustion of T cells (Cerboni et al., 2017; Wu et al., 2019). In this context, one recent report has revealed that MITA possesses IFN-independent activities in T cells, which is responsible for tumor immune evasion by inducing T cell death (Wu et al., 2020), though the mechanism by which MITA induces T cell death is unknown now. It is thus likely that the stage and types of tumors are associated with the tumor-suppressive or promoting roles of the cGAS-MITA pathway. Therefore, several agonists showed impressive potential in antitumor immunity due to the suppressive role of MITA in early tumorigenesis. However, prolonged activation of the cGAS-MITA signaling may negligently inhibit antitumor immunity and drive tumor metastasis, which makes the application of MITA agonists more challenging in the clinic. To conquer this challenge, further work is required to unveil the molecular requirements and regulations that function in tumor progression or suppression via identifying the downstream cascade of the cGAS-MITA pathway.

Concluding remarks

The discovery of cGAS and MITA has fundamentally advanced the understanding of immune responses mediated by immune-stimulatory DNA. The activation of the cGAS-MITA pathway ensures the sufficient expression of downstream anti-pathogenic genes, whereas excess immune responses lead to tissue damage and immune pathology (Crowl et al., 2017). Thus, it is reasonable that a series of regulatory elements sequentially functions to fine-tune the immune responses in different

Table 2
Summary of MITA/STING agonists in clinical trials.

Agent	Cancer type	Phase	Target	NCT number
ASA404	Adult Solid Tumor	I	MITA	NCT00003697
ASA404	Hormone Refractory Metastatic Prostate Cancer	II	MITA	NCT00111618
ASA404 + Paclitaxel + Carboplatin	Non-small Cell Lung Cance	I	MITA + microtubule + DNA synthesis	NCT00674102
ASA404 + Paclitaxel + Carboplatin	Locally Advanced and Metastatic NSCLC	I/II	MITA + microtubule + DNA synthesis	NCT00832494
ASA404	Refractory Tumors	I	MITA	NCT00856336
ASA404	Solid Tumors	I	MITA	NCT00863733
ASA404	Advanced or Recurrent Solid Tumors	I	MITA	NCT01285453
ASA404 + Paclitaxel + Carboplatin	Small Cell Lung Cancer	II	MITA + microtubule + DNA synthesis	NCT01057342
ADU-S100	Head and neck cancer	II	MITA	NCT03937141
ADU-S100 ± ipilimumab	Solid tumors/lymphomas	I	MITA ± CTLA-4	NCT02675439
ADU-S100 + PDR001	Solid tumors/lymphomas	I	MITA + PD1	NCT03172936
E7766	Urinary bladder neoplasms	I	MITA	NCT04109092
E7766	Lymphoma/advanced solid tumors	I	MITA	NCT04144140
GSK3745417	Neoplasms	I	MITA	NCT03843359
MK-1454	Solid tumors/lymphomas	I	MITA	NCT03010176
MK-1454 + pembrolizumab	Head and neck squamous cell carcinoma	II	MITA + PD1	NCT04220866
BMS-986301	Solid cancers	I	MITA	NCT03956680
SB 11285	Solid tumor	I	MITA	NCT04096638

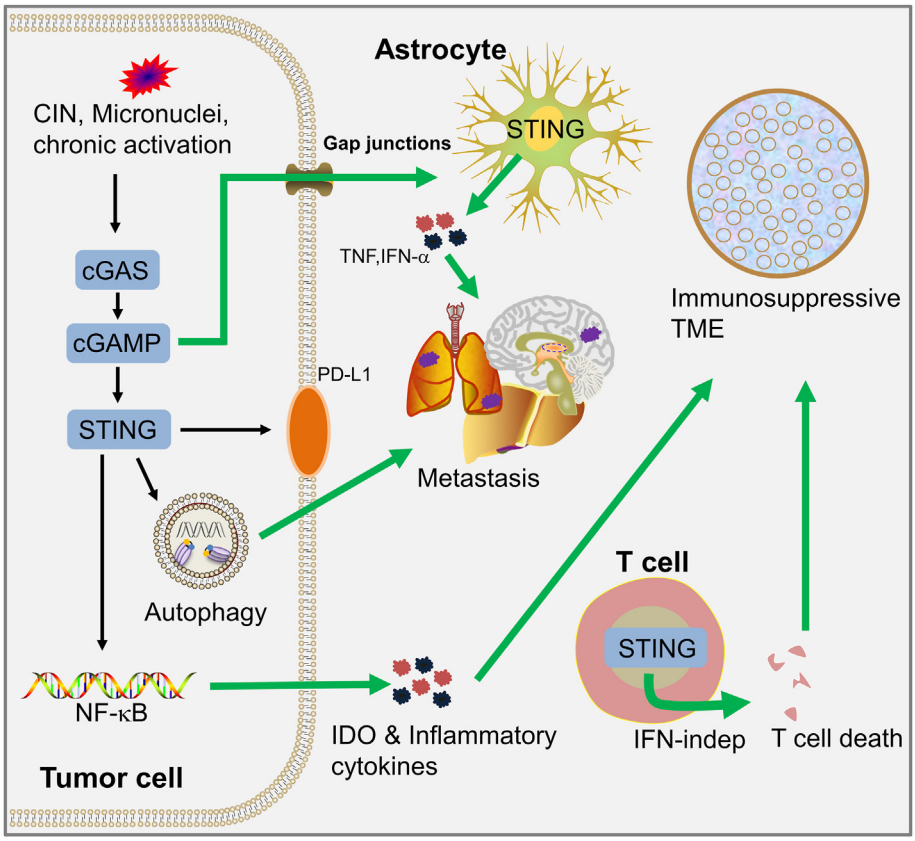

Fig. 3. Regulation of cGAS-MITA/STING pathway in tumor promotion. The cGAS-MITA pathway exerts its function as a tumor promoter in metastatic tumor cells. Tumors carrying high chromosome instability could promote the formation of micronuclei, which ruptures and release DNA to the cytosol, triggering the activation of the cGAS-MITA signaling. Chronic activation of the pathway promotes the suppression of type I IFNs expression and initiates the upregulation of noncanonical NF-κB signaling, facilitating tumor metastasis. The IDO and proinflammatory cytokines together induce the formation of immunosuppressive TME. Moreover, MITA activation could lead to T cell exhaustion in a manner independently of IFN, which also contributes to the maintenance of immunosuppressive TME. MITA may also promote tumor metastasis via PD-L1 upregulation and autophagy process. In addition, tumors can directly transfer the cGAMP to neighboring cells such as astrocytes by gap junctions, ultimately accelerating the progression of tumor metastasis.

phases of immunostimulatory DNA stimulation or viral infection. Recent studies showed that cGAS and MITA undergo phase condensation during viral infection, which provides deeper insights into the regulation of their activities (Du & Chen, 2018; Minhas & Holehouse, 2021; Xu et al., 2021; Yu et al., 2021). In this context, studies are required to explore how the regulators crosstalk to each other to dynamically adjust the activities of the cGAS-MITA pathway.

The cGAS-MITA pathway plays a vital role in autoimmune diseases and cancers. TREX1 deficiency and MITA mutations, as we know, lead to autoimmune diseases, such as AGS, SLE, RVCL, and SAVI, suggesting that the cGAS-MITA axis can be targeted for therapeutic intervention of these diseases in the future. However, the activation of the cGAS-MITA pathway in cancers is a double-edged sword. Acute immune responses induced by the cGAS-MITA signaling may function as a barrier to early neoplastic progression, while chronic activation of the pathway may induce the formation of an immunosuppressive tumor environment (TME), eventually leading to tumor growth and metastasis. MITA agonists have been developed to enhance tumor immunogenicity. However, it should be cautious that hyperactivation of MITA signaling may inadvertently worsen the pathology of patients with cancers. Thus, further work is necessary to understand the different regulations behind two different outcomes induced by the cGAS-MITA signaling, to ensure careful selection of patients to proceed personalized therapeutic regimen.

Declaration of competing interest

The authors declare no conflict of interests.

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