DNA sensing by the cGAS–STING pathway in health and disease

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Abstract | The detection of pathogens through nucleic acid sensors is a defining principle of innate immunity. RNA-sensing and DNA-sensing receptors sample subcellular compartments for foreign nucleic acids and, upon recognition, trigger immune signalling pathways for host defence. Over the past decade, our understanding of how the recognition of nucleic acids is coupled to immune gene expression has advanced considerably, particularly for the DNA-sensing receptor cyclic GMP–AMP synthase (cGAS) and its downstream signalling effector stimulator of interferon genes (STING), as well as the molecular components and regulation of this pathway. Moreover, the ability of self-DNA to engage cGAS has emerged as an important mechanism fuelling the development of inflammation and implicating the cGAS–STING pathway in human inflammatory diseases and cancer. This detailed mechanistic and biological understanding is paving the way for the development and clinical application of pharmacological agonists and antagonists in the treatment of chronic inflammation and cancer.

Pattern recognition receptors
Germline-encoded receptors that detect pathogen-associated molecular patterns (microbial products).

Cyclic dinucleotides
A class of nucleic-acid second messenger molecules that typically contain two mononucleotides connected by a unique phosphodiester linkage, such as cyclic 2′-5′,3′-5′ adenine monophosphate guanine monophosphate linkages in human cyclic GMP–AMP.

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Protection from infection relies on pattern recognition receptors, which recognize microbial products and trigger signalling pathways that orchestrate antimicrobial defences and activate adaptive immunity[1]. Over the past two decades, the ability to sense microbial pathogens through recognition of their nucleic acids has emerged as a key feature of innate immunity in mammalian cells. Aberrant (5′ triphosphorylated or 5′ diphosphorylated) single-stranded and double-stranded RNAs or DNAs, RNA–DNA hybrids and cyclic dinucleotides are all recognized as foreign nucleic acids[1–3]. The profound immune-stimulatory capacity of nucleic acids has been appreciated for decades, leading to intense efforts to identify the receptors and pathways engaged by these nucleic acid molecules.

The pathways involved in RNA recognition have been reviewed elsewhere[1] and will therefore not be covered herein. In the case of DNA, three major receptors have been described in mammalian cells that collectively account for most of our current understanding of DNA-driven immune responses — Toll-like receptor 9 (TLR9), absent in melanoma 2 (AIM2) and cyclic GMP–AMP synthase (cGAS) (FIG. 1). TLR9 is expressed on the endosomal membrane, where it samples extracellular DNA, specifically CpG hypomethylated DNA that enters the cell through the phagolysosomal system[4]. The importance of TLR9 in protection from viruses and other pathogens has been clearly established (reviewed in REF.[5]). Double-stranded DNA (dsDNA) that gains access to the cytosolic compartment triggers the formation of the AIM2 inflammasome, a caspase 1-activating complex that controls the proteolytic maturation of the proinflammatory cytokines IL-1β and IL-18, as well as activation of the pore-forming protein gasdermin D, an executioner of pyroptosis[6–10]. AIM2 is crucial for protection from DNA viruses and some cytosolic bacterial pathogens. However, the most pronounced output following cytosolic DNA accumulation is induction of a broad transcriptional programme that includes genes encoding the type I interferons and, in turn, an extensive array of interferon-stimulated genes (ISGs)[11,12]; cGAS is the DNA-binding protein that represents the initiator of this response, which is mediated by the downstream adaptor molecule stimulator of interferon genes (STING). Since its discovery in 2013, our understanding of cGAS has rapidly advanced in terms of its role in host defence, autoimmune disorders and cancer biology.

In this Review, we focus on the current understanding of DNA sensing through the cGAS–STING pathway, describing how the cytoplasmic cGAS receptor is activated, how downstream signalling is coordinated and detailing the plethora of regulatory mechanisms that keep this pathway in check. We also discuss how human genetics has informed our understanding of this pathway through the identification of rare monogenic diseases resulting from mutations in genes that normally restrain the cGAS–STING pathway. Furthermore, we describe new studies linking the cGAS–STING pathway to the development of a broad array of sterile inflammatory conditions and detail its emerging role in cancer. Lastly, we highlight how knowledge of this pathway is

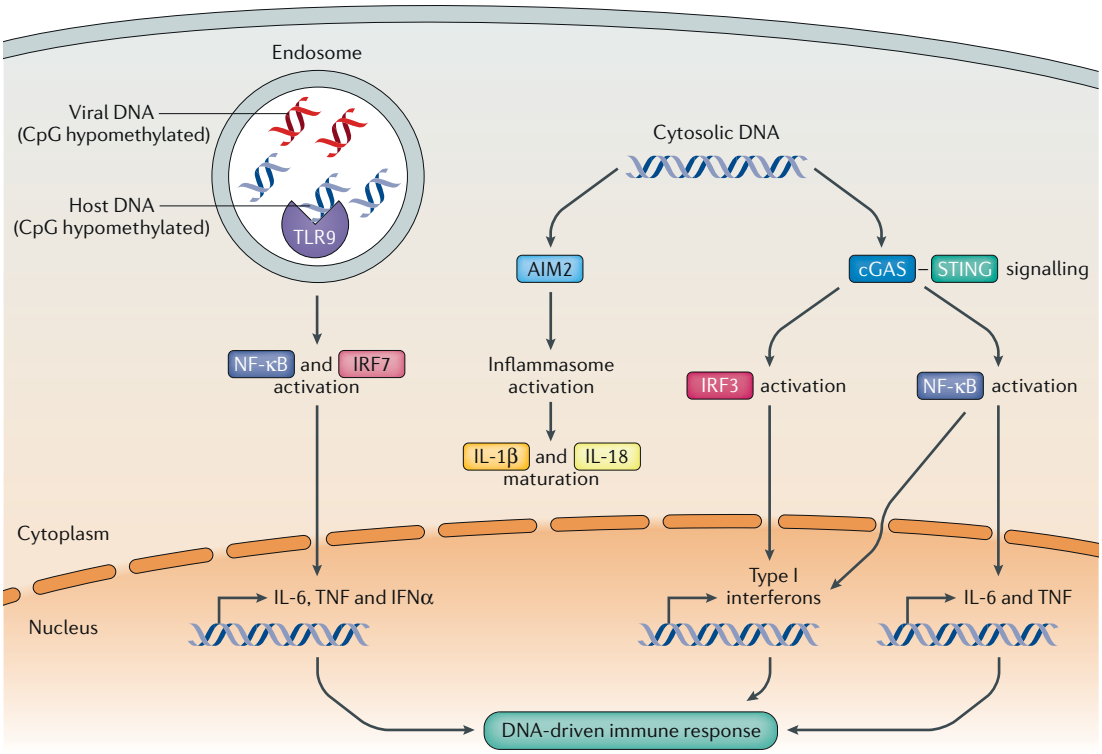


Fig. 1 | **DNA-sensing receptors.** In mammalian cells, the three major DNA-sensing receptors that drive immune responses to foreign DNA are Toll-like receptor 9 (TLR9), absent in melanoma 2 (AIM2) and cyclic GMP–AMP synthase (cGAS). TLR9, which is localized to the endosomal membrane, senses CpG hypomethylated DNA and, in turn, activates the transcription factors nuclear factor-κB (NF-κB) and interferon regulatory factor 7 (IRF7), leading to expression of genes encoding proinflammatory cytokines and interferons, respectively[4,5]. In the cytosol, AIM2 binds to double-stranded DNA, leading to the formation of a multimeric protein complex called the AIM2 inflammasome, which leads to the activation of caspase 1 and the maturation of the proinflammatory cytokines IL-1β and IL-18, and, ultimately, pyroptotic cell death[6–10]. Finally, cGAS activation by cytosolic DNA leads to endogenous generation of cyclic GMP–AMP, a unique second messenger, which binds to stimulator of interferon genes (STING), leading to activation of TANK-binding kinase 1 (TBK1) and IRF3, resulting in the transcription of genes encoding type I interferons[24]. DNA sensing through the cGAS–STING pathway also results in activation of NF-κB and transcription of proinflammatory cytokines such as IL-6 and tumour necrosis factor (TNF)[28]. IFNα, interferon-α.

being leveraged to treat inflammatory diseases and to enhance the cancer immunotherapy armamentarium.

The cGAS–STING pathway

The DNA-sensing nucleotidyl transferase enzyme cGAS, its second-messenger product cyclic GMP–AMP (cGAMP) and the cGAMP sensor STING (also known as MITA[13], ERIS[14] or MPYS[15,16]) form a major DNA-sensing mechanism in the cytoplasm of mammalian cells. The engagement of this pathway during infection with cytosolic bacterial pathogens and some DNA viruses leads to the transcriptional induction of type I interferons and the nuclear factor-κB (NF-κB)-dependent expression of proinflammatory cytokines. STING activation also results in activation of additional cellular processes. Genetic evidence from mice genetically engineered to lack these molecules indicates clear susceptibility to bacterial and viral infection[1].

cGAS–STING-mediated DNA-sensing

The DNA sensor cGAS is activated by DNA through direct binding (FIG. 2), which triggers conformational changes that induce enzymatic activity[17–21]. Although any DNA, foreign or self, can cause cGAS activation, the length of the DNA is important. Short DNAs of ~20 bp can bind to cGAS, but longer dsDNAs of >45 bp can form more stable ladder-like networks of cGAS dimers, leading to stronger enzymatic activity[21,22]. Human cGAS prefers longer DNAs than its mouse counterpart owing to two amino acid substitutions in the DNA-binding domain[23]; these human-specific substitutions remodel DNA interactions to favour higher-order cGAS–DNA oligomers.

Active cGAS converts GTP and ATP into cGAMP[24] (FIG. 2). This endogenous second messenger is unique as it contains unusual mixed phosphodiester linkages between the 2′-hydroxyl group of GMP and the 5′-phosphate of AMP, and also between the 3′-hydroxyl group of AMP and the 5′-phosphate of GMP, forming a novel 2′3′-cGAMP isomer[20,25,26]. DNA binding to cGAS also induces liquid–liquid phase separation and the formation of liquid-like droplets, in which cGAS is activated. These lipid droplets seem to function as 'microreactors' by increasing the concentrations of the enzyme and reactants to enhance cGAMP production[27].

The 2′3′-cGAMP product binds to STING, an endoplasmic reticulum (ER)-localized adaptor[24,28,29], to form dimers, tetramers and higher-order oligomers[30,31]

Pyroptosis
A caspase 1/11-dependent inflammatory cell death process.

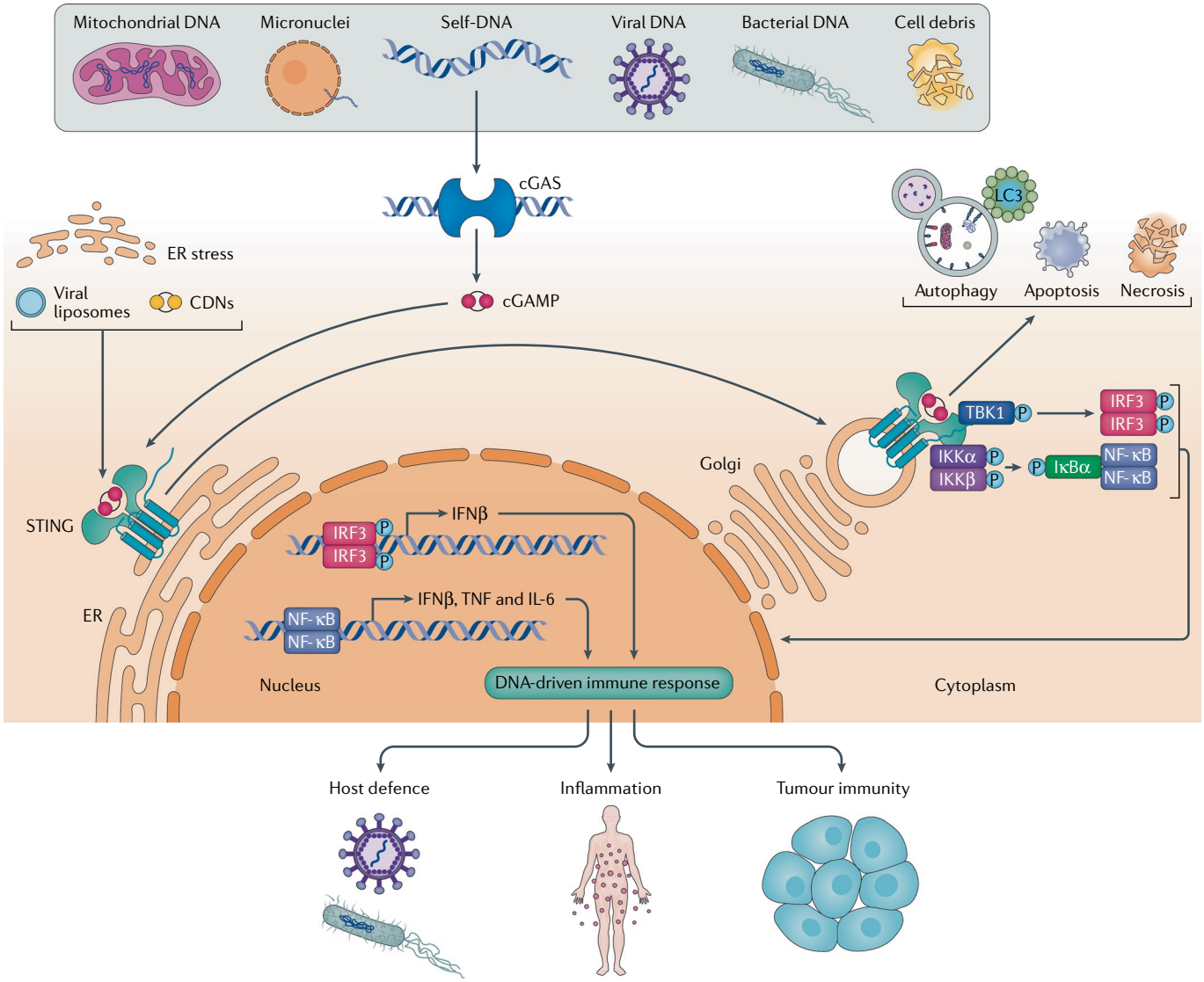


Fig. 2 | **The cGAS–STING DNA-sensing pathway.** Cytosolic DNA sensing by the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway is implicated in a variety of biological processes. The prime function of this pathway is in host defence, but new studies have established a link between this pathway and the development of a variety of inflammatory conditions and have revealed a role in tumour immunity[202]. DNA from various sources can enter the cytosol and engage the cGAS–STING pathway. DNA binding activates cGAS to generate the second messenger cyclic GMP–AMP (cGAMP), which binds to the endoplasmic reticulum (ER)-localized adaptor protein STING. Stimuli other than cGAMP, including ER stress, viral liposomes and cyclic dinucleotides (CDNs), can also activate STING[35,36,203]. Upon activation, STING translocates from the ER to the Golgi, where it recruits kinases such as TANK-binding kinase 1 (TBK1) and IκB kinase (IKK), which phosphorylate interferon regulatory factor 3 (IRF3) and the nuclear factor-κB (NF-κB) inhibitor IκBα, respectively. Phosphorylated IRF3 dimerizes and translocates to the nucleus to activate transcription of genes encoding type I interferons such as interferon-β (IFNβ). IκBα phosphorylation results in translocation of NF-κB to the nucleus, where it activates transcription of genes encoding proinflammatory cytokines such as IL-6 and tumour necrosis factor (TNF). STING signalling also results in activation of other cellular process such as microtubule-associated protein 1A/1B-light chain 3 (LC3)-mediated autophagy, apoptosis and necroptosis[39,57,61]. Phosphorylation is indicated by P.

(FIG. 2). STING also binds to cyclic dinucleotides produced by bacteria directly, including cyclic diGMP, cyclic diAMP and bacterial cGAMP, all of which have conventional (3′5′) phosphodiester linkages[32]. Notably, in humans, allelic variation in *STING* can result in altered sensitivity to bacterial cyclic dinucleotides. The wild-type STING-R232 variant is activated by both bacterial cyclic dinucleotides and 2′3′-cGAMP, whereas a STING-H232 variant is compromised for activation by bacterial cyclic dinucleotides[26]. In general, the 2′3′-cGAMP is thought to bind with higher affinity to STING than to bacterial cyclic dinucleotides, which suggests that STING is more strongly activated when the cGAS receptor is engaged[20].

STING undergoes a conformational change upon cGAMP binding. Two wings of the protein are brought into juxtaposition with the ligand situated deep inside the binding pocket, and a lid consisting of four anti-parallel β-sheet strands is rearranged on top of the binding pocket, resulting in a closed conformation. This conformational change leads to a 180° rotation of the ligand-binding domain, leading to formation of STING

oligomers through side-by-side packing of dimeric STING molecules[18,20,33]. Several stimuli other than cGAMP and bacterial cyclic dinucleotides can also activate STING (FIG. 2). For example, ER stress induced by thapsigargin or generated during infection with Gram-positive bacteria can activate STING[34,35]. In addition, viral membrane fusion has been shown to trigger STING activation, independent of DNA sensing[36].

In the resting state, STING is retained in the ER through interactions with the Ca^{2+} sensor stromal interaction molecule 1 (STIM1)[37]. Following cGAMP-driven conformational changes, STING traffics through the ER–Golgi intermediate compartment (ERGIC) and the Golgi apparatus in a process that is dependent on the cytoplasmic coat protein complex II (COPII) and ADP-ribosylation factor (ARF) GTPases[38,39] (FIG. 2). STING is also palmitoylated at the Golgi, and this post-translational modification is essential for its activation[40]. Following its translocation to the Golgi, STING interacts with TANK-binding kinase 1 (TBK1), an IκB kinase (IKK)-related kinase that controls the activation of the transcription factor interferon regulatory factor 3 (IRF3)[29,41]. Following its direct phosphorylation by TBK1, the C-terminal tail region of STING serves as a docking site for IRF3, which is then phosphorylated and activated by TBK1 (REF.[42]). Activated IRF3 dimerizes and translocates to the nucleus to regulate the transcription of interferon-β (IFNβ), a type I interferon[43]. Newly produced IFNβ then functions through autocrine and paracrine signalling by binding to and activating a heterodimeric receptor complex consisting of IFNα receptor 1 (IFNAR1) and IFNAR2. The IFNAR1–IFNAR2 complex engages the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) signalling pathway to induce the transcription of several ISGs, the protein products of which prevent viral replication, assembly and release[44].

NF-κB is also activated and controls the transcription of proinflammatory cytokines and chemokines (FIG. 2). TNF receptor-associated factor 6 (TRAF6), NF-κB essential modulator (NEMO), IKKβ and TBK1 have all been implicated in the STING-dependent regulation of NF-κB[45–47]. Tripartite motif-containing protein 32 (TRIM32) and TRIM56 have been shown to synthesize ubiquitin chains that bind to NEMO and presumably mediate the ubiquitination of NEMO to activate IKKβ, leading to NF-κB activation[47]. Lastly, besides NF-κB and IRF3, STAT6 is another transcription factor activated by the STING pathway; STAT6 interacts with STING and is phosphorylated by TBK1, resulting in its dimerization and translocation to the nucleus to induce expression of a specific set of chemokines, including CCL2, CCL20 and CCL26, which are important in eliciting antiviral responses[48].

Additional functions of STING

Homologues of the cGAS–STING pathway proteins are found in evolutionarily distant organisms that lack IRFs or NF-κB such as *Nematostella vectensis*, an anemone species that is >500 million years diverged from humans[49]. The C-terminal tail that controls IRF and NF-κB signalling in mammalian STING is lacking in the *N. vectensis* STING homologue, suggesting that this ancient form of STING — and perhaps its mammalian counterpart — has additional functions beyond DNA sensing (FIG. 2).

Autophagy is one such function of primitive STING. In mammalian cells, the STING-containing ERGIC serves as a membrane source for microtubule-associated protein 1A/1B-light chain 3 (LC3) lipidation, a key step in autophagosome formation that requires WD repeat domain phosphoinositide-interacting protein 2 (WIPI2) and autophagy protein 5 (ATG5)[39]. This STING-dependent autophagy response is important for the clearance of DNA in the cytosol. STING-induced autophagy is important for protection from *Mycobacterium tuberculosis*[50,51] and Gram-positive bacteria[35], and for restricting Zika virus infection in *Drosophila melanogaster*[52,53]. In non-phagocytic cells, autophagy contributes to the clearance of cytosolic DNA, but impaired autophagy can also induce activation of the STING pathway[39,54,55].

The cGAS–STING pathway is also linked to other cell death pathways, including apoptosis, pyroptosis and necroptosis. In humans, cytosolic DNA triggers a STING-dependent NOD-, LRR- and pyrin domain-containing 3 (NLRP3) pathway controlling cell death[56], whereas ligation of STING induces apoptosis in T cells, naive B cells or malignant B cells[57–59]. Sensing of DNA by the cGAS–STING pathway also activates receptor-interacting serine/threonine-protein kinase 3 (RIPK3) and elicits necroptosis in bone marrow-derived macrophages[60]. This pathway requires signalling through both type I interferon receptors and tumour necrosis factor receptors (TNFRs), revealing synergy between these pathways regarding their ability to induce cell death[60]. In addition, the executioner of necroptotic cell death mixed lineage kinase domain-like protein (MLKL) is also regulated via STING-dependent type I interferon to elicit necroptosis[61].

cGAS–STING activation by pathogens

Responses to DNA viruses, including herpes simplex virus 1 (HSV1) and HSV2, adenoviruses, murine gammaherpesvirus 68, vaccinia virus, cytomegalovirus and papillomavirus, are controlled by type I interferons downstream of the cGAS–STING pathway (reviewed in REF.[1]). Mice deficient for cGAS or STING are unable to induce type I interferon responses and, as such, are susceptible to these viral pathogens. Besides DNA viruses, retroviruses such as HIV, simian immunodeficiency virus (SIV) and murine leukaemia virus also activate cGAS. Some other RNA viruses are also influenced by the cGAS–STING pathway. For instance, cGAS is protective against West Nile virus and Dengue virus infection in mice, the mechanisms for which are still poorly understood. In the case of Dengue virus, mitochondrial DNA is released into the cytosol of infected cells to activate cGAS and elicit protective type I interferon responses[62]. Some other RNA viruses such as vesicular stomatitis virus (VSV) activate STING; however, unlike other viral pathogens, STING activation during VSV infection leads to a block in protein translation, restricting viral replication[63].

In addition to viruses, both Gram-positive and Gram-negative bacteria activate the cGAS–STING pathway.

Necroptosis
A receptor-interacting serine/threonine-protein kinase (RIPK)-dependent programmed cell death pathway.

Listeria monocytogenes produces cyclic diAMP, which directly binds to STING, leading to its activation without engaging cGAS, whereas for other bacteria such as *Mycobacterium tuberculosis*, group B streptococcus and bacteria of the genera *Legionella*, *Listeria*, *Shigella*, *Francisella*, *Chlamydia* and *Neisseria*, microbial DNA is detected by cGAS and STING is subsequently activated via cGAMP generation (reviewed in REF.[1])

Regulation of the cGAS–STING pathway

Type I interferons and inflammatory cytokines induced downstream of cGAS–STING signalling are crucial for protection of the host from infection. However, these cytokines are also dangerous if produced in excess or for too long, leading to tissue damage and immune pathology[64]. Unsurprisingly, a multitude of regulatory mechanisms, including ligand availability and post-translational modifications or protein–protein interactions that modify cGAS or STING stability, activity and trafficking, keep the cGAS–STING pathway in check to maintain immunological balance (TABLE 1).

Ligand availability

As cGAS binds to dsDNA in a sequence-independent manner, both foreign and host DNA can activate the cGAS–STING pathway. Thus, controlling the availability of host dsDNA is essential for prevention of erroneous pathway activation. Two key mechanisms ensure that cGAS does not encounter host dsDNA.

The first mechanism involves DNase enzymes that digest dsDNA, thereby limiting cGAS ligand availability (FIG. 3a). DNA accrual in the cytosol of healthy cells is limited by the action of DNase enzymes localized in distinct subcellular compartments. DNases are present extracellularly (DNase I and DNase I-like 3 (DNase IL3)), within the phagolysosomal compartment (DNase II) or within the cytosol (three-prime repair exonuclease 1 (TREX1; also known as DNase III)), collectively ensuring that self-DNA cannot accumulate to engage cGAS or other sensors[65].

The second mechanism is compartmentalization; DNA is sequestered in the nucleus or mitochondria of healthy cells and is therefore inaccessible to cGAS, which is primarily localized in the cytosol. However, new evidence now suggests that cGAS can be localized on the plasma membrane or in the nucleus (BOX 1). Although DNA ligands and their cytosolic receptors are separated in healthy cells, under certain conditions, this compartmentalization is compromised. For example, processes such as cell division, during which the nuclear membrane is transiently dissolved, pose a conundrum to the concept of compartmentalization[66,67]. Likewise, mitochondrial DNA can access the cytosol if mitophagy is impaired or when cells undergo apoptosis[68]. Thus, additional mechanisms must exist to limit cGAS–DNA encounters under these types of situations.

Pathway activation status

A wide range of regulatory mechanisms function on the cGAS–cGAMP–STING pathway to positively and negatively regulate its activation status, specifically by modifying cGAS or STING stability, activity and trafficking via post-translational modifications or protein–protein interactions (TABLE 1).

Negative regulators. Numerous layers of negative regulation exist that limit cGAS–STING signalling (TABLE 1). In resting cells, cGAS expression levels are post-translationally regulated through autophagy in order to restrict cGAS protein levels in the absence of infection[69]. Beclin 1, an autophagy protein, interacts with cGAS in a DNA-dependent manner, blocking its enzymatic activity during viral infection, suggesting that the autophagy machinery can regulate both cGAS activity and expression levels[70]. Additional post-translational modifications also negatively regulate cGAS, including phosphorylation, acetylation, monoglutamylation and polyglutamylation[71–73]. cGAS is also cleaved through the action of inflammatory and apoptotic caspases[74], and its activation is limited by gasdermin D (downstream of the AIM2 inflammasome)[75], indicating that crosstalk occurs between several pathways to keep the activity of this key sensor in check.

The second messenger cGAMP is also negatively regulated. In humans, phosphodiesterases such as ectonucleotide pyrophosphatase/phosphodiesterase family member 1 (ENPP1) regulate cGAMP levels by limiting its stability[76,77]. Unsurprisingly, emerging evidence suggests that pathogens also manipulate cGAS activity and cGAMP generation in order to limit, impede or evade cGAS-triggered host defences. Multiple pathogens, including *M. tuberculosis* and Group B streptococcus, encode phosphodiesterases that target cGAMP for destruction[78]. This strategy is not unique to bacterial pathogens, as a 2019 study identified poxvirus immune nucleases (poxins), which are conserved in mammalian poxviruses, as cGAMP-degrading enzymes[79].

In addition to cGAS, STING is also regulated at multiple levels. STING traffics from the ER to the ERGIC and then to the Golgi and post-Golgi vesicles before eventually being degraded by the lysosome[80]. STING trafficking and degradation are also regulated by a variety of mechanisms; for example, NLR family CARD domain-containing protein 3 (NLRC3) interacts with STING to inhibit its trafficking[81], and STING ubiquitination by the E3 ubiquitin-protein ligase RNF5 marks STING for degradation[82]. In addition, endogenously formed nitro-fatty acids generated during viral infection covalently modify STING by nitro-alkylation and block STING palmitoylation, which is required for signalling[83].

Positive regulators. In addition to the numerous layers of negative regulation, mechanisms that promote or enhance cGAS–STING signalling have also been reported (TABLE 1). Ras GTPase-activating protein-binding protein 1 (G3BP1) has been identified as a cGAS accessory protein that promotes cGAS oligomerization and DNA binding[84]. In addition, several proteins that enhance STING trafficking, expression and signalling have been identified. Inactive rhomboid protein 2 (IR-HOM2) has been reported to recruit translocon-associated protein subunit-β (TRAPβ; also known as SSR2), which facilitates trafficking of STING from

Mitophagy
An autophagic process in response to cellular damage or stress during which mitochondria are degraded.

Phosphodiesterases
Enzymes that break phosphodiester bonds in cyclic dinucleotides.

Table 1 | **Regulators of the cGAS–STING pathway**

Pathway component regulated	Mode of action	Molecules involved	Mechanism	Residues and/or domains involved[a]	Refs
Regulation of ligand availability					
cGAS	DNA digestion in lysosome	DNase II	Hydrolysis of DNA phosphodiester linkages	NA	97,207
	Intracellular DNA digestion	DNase III	Digestion of DNA with mismatched 3′ end	NA	97,208
Signalling activation					
cGAS	Deglutamylation	CCP5 and CCP6	Enhanced enzymatic activity	Removal of glutamate chain at E302 and E272	73
	SUMOylation	TRIM38	Increased stability	NA	209
	DeSUMOylation	SENP7	Enhanced activation	NA	210
	Ubiquitination	RINCK (also known as TRIM41)	Increased cGAMP synthesis	NA	211
		RNF185	Enhanced enzymatic activity	K27-linked polyubiquitination at K173 and K384	212
		TRIM56	Enhanced cGAMP production	Monoubiquitination at K335	213
	Deubiquitination	TRIM14	Enhanced stability	Recruits USP18 to cleave K48-linked ubiquitination at K414	69
		USP14	Enhanced stability	K48-linked ubiquitination at K414	69
	Protein–protein binding	G3BP1	Increased DNA-binding affinity	Binds to N terminus of cGAS	84
		PQBP1	Enhanced cGAMP production	Binds to cGAS via PQBP1 WW domain	214
		ZCCHC3	Enhanced oligomerization	Binds to NTase and the C-terminal fragment of cGAS	215
STING	Palmitoylation	NR	STING activation	Palmitoylation at C88 and C91 of STING	40
	Ubiquitination	AMFR	Translocation from the ER via Golgi	K27-linked polyubiquitination	86
		TRIM32	Enhanced interaction with TBK1	K63-linked ubiquitination at K20, K150, K224 and K236	216
		TRIM56	Enhanced interaction with TBK1	K63-linked ubiquitination at K150	217
		RNF26	Prevention of degradation	K11-linked polyubiquitination at K150	218
		UBXN3B	Facilitates STING–TRIM56 interaction	TRIM56-mediated K63-linked ubiquitination via UAS domain of UBXN3B	219
		MUL1 (also known as RNF218)	Facilitates STING trafficking	K63-linked polyubiquitination at K224	220
	Deubiquitination	ZDHHC1	Maintenance of stability and STING aggregation	K48-linked polyubiquitination; N terminus of STING interacts with the N terminus of ZDHHC1	85,87
		CYLD	STING stabilization	K48-linked polyubiquitination via CYLD USP domain	89
	Protein–protein binding	SAR1A and SEC24C	Facilitates STING trafficking	NA	39
		DDX41	Enhanced STING signalling	DDX41 binds to transmembrane domains	221,222
		IFI16	Enhanced STING activation	IFI16 interacts with STING via PYRIN domain	223,224
		IRHOM2	Recruitment of TRAPβ for STING trafficking	Interaction via transmembrane domains	28,85
Signalling inhibition					
cGAMP	cGAMP hydrolysis	ENPP1	Hydrolysis of cGAMP	NA	77
cGAS	Phosphorylation	AKT	Inhibition of enzyme activity	S291 in mouse cGAS; S305 in human cGAS	72
	Glutamylation	TTLL4/TTLL6	Inhibition of enzyme activity	Monoglutamylation at E302 and polyglutamylation at E272	73
	Protein–protein binding	Beclin 1	Suppressed cGAMP synthesis	Interaction between the coiled-coil domain of Beclin 1 and the central NTase domain of cGAS	70
	DeSUMOylation	SENP2	Degradation via the proteasome pathway	NA	209

Table 1 (cont.) | **Regulators of the cGAS–STING pathway**

Pathway component regulated	Mode of action	Molecules involved	Mechanism	Residues and/or domains involved[a]	Refs
Signalling inhibition (cont.)					
STING	Protein–protein binding	NLRX1	Disrupts STING–TBK1 interaction	Interacts with STING through its NBD domain	225
		STIM1	Retains STING in the ER	Interaction via transmembrane domains	37
	Dephosphorylation	PPM1A	Prevents STING dimerization and aggregation	STING dephosphorylation at S358	226
	Ubiquitination	RNF5	STING degradation	STING ubiquitination at K150	82
		NLRC3	Blocks STING trafficking	Interacts with membrane-bound STING at C terminus	81
	Enhanced ubiquitination	TRIM30a	STING degradation via proteasome-dependent pathway	K48-linked ubiquitination at K275	227
	Deubiquitination	USP13	Prevents TBK1 recruitment to STING	K27-linked ubiquitination	228
		USP21	Prevents TBK1 and IRF3 recruitment to STING	K27/K63-linked polyubiquitination	229
	Nitro-alkylation	Nitro-fatty acids	Inhibit palmitoylation and cause suppression of TBK1 phosphorylation	C88 and C91 STING residues	83
Degradation					
cGAS	Cleavage	Caspase 1/ caspase 4/ caspase 5/ caspase 11	Degradation	Caspase 1 cleaves cGAS at D140/D157	230
STING	Phosphorylation	ULK1	Degradation via autophagy	ULK1/2 phosphorylate STINGat S366	231
		p62/SQSTM1	Direct transfer of ubiquitinated STING to autophagosome	NA	232
		ATG9a	Degradation via autophagy	Binds to STING	233

ATG9a, autophagy protein 9a; cGAMP, cyclic GMP–AMP; cGAS, cyclic GMP–AMP synthase; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase family member 1; ER, endoplasmic reticulum; G3BP1, Ras GTPase-activating protein-binding protein 1; IFI16, gamma-interferon-inducible protein 16; IRF3, interferon regulatory factor 3; IRHOM2, inactive rhomboid protein 2; MUL1, mitochondrial ubiquitin ligase activator of NFKB 1; NA, not applicable; NBD, nucleotide-binding domain; NLRC3, NLR family CARD domain-containing protein 3; NLRX1, NLR family member X1; NR, not reported; PPM1A, protein phosphatase 1A; PQBP1, polyglutamine-binding protein 1; RINCK, RING finger-interacting protein with C kinase; SENP, sentrin-specific protease; SQSTM1, sequestosome 1; STIM1, stromal interaction molecule 1; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; TRAPβ, translocon-associated protein subunit-β; TRIM, tripartite motif-containing protein; USP, ubiquitin carboxyl-terminal hydrolase; ZCCHC3, zinc finger CCHC domain-containing protein 3. [a]Residues and/or domains are unknown wherever they are not mentioned.

the ER to the ERGIC[85]. In addition, the E3 ubiquitin ligase complex of AMFR and insulin-induced gene 1 protein (INSIG1) lead to the polyubiquitination of STING and recruitment of TBK1 (REF.[86]). ZDHHC1, an ER-associated palmitoyltransferase, enhances dimerization of STING[87], whereas sterol regulatory element-binding protein cleavage-activating protein (SCAP) traffics with STING and facilitates IRF3 recruitment[88]. In addition, the deubiquitinase enzyme CYLD removes K48-linked ubiquitin chains to stabilize STING protein expression[89].

Exactly how and when these post-translational modifications come into play is, as yet, unclear. Furthermore, additional work in this area is needed to determine the cellular context in which these mechanisms operate.

cGAS–STING in inflammatory disease

DNA sensing through cGAS–STING has emerged as a key pathway in the initiation and pathogenesis of autoimmune and autoinflammatory diseases. Self-DNA from various sources can aberrantly accumulate in the cytosol, gaining access to the cGAS receptor to trigger pathway activation in several disease contexts.

Type I interferonopathies

Studies over the past few years have highlighted the importance of DNases in limiting the cytosolic accumulation of DNA and cGAS activation. A growing number of monogenic diseases characterized by mutations in one or more DNases, as well as in STING, have been identified (FIG. 3b; TABLE 2). These inflammatory diseases are characterized by excessive production of type I interferons and are collectively termed type I interferonopathies[90].

DNase mutations. Aicardi–Goutieres syndrome (AGS) is one such disease that resembles congenital virus infection and is characterized by high levels of circulating type I interferons in patients, leading to neuronal inflammation[90]. A subset of patients with AGS have mutations in TREX1, a 3′–5′ exonuclease found on the ER that digests endogenous DNA that accumulates within cells[91]. Studies in *Trex1*-null mice have provided crucial insights into the pathophysiological mechanisms underpinning the development of AGS. Although *TREX1* mutations lead to high levels of type I interferons and central nervous system inflammation in humans, *Trex1*-null mice

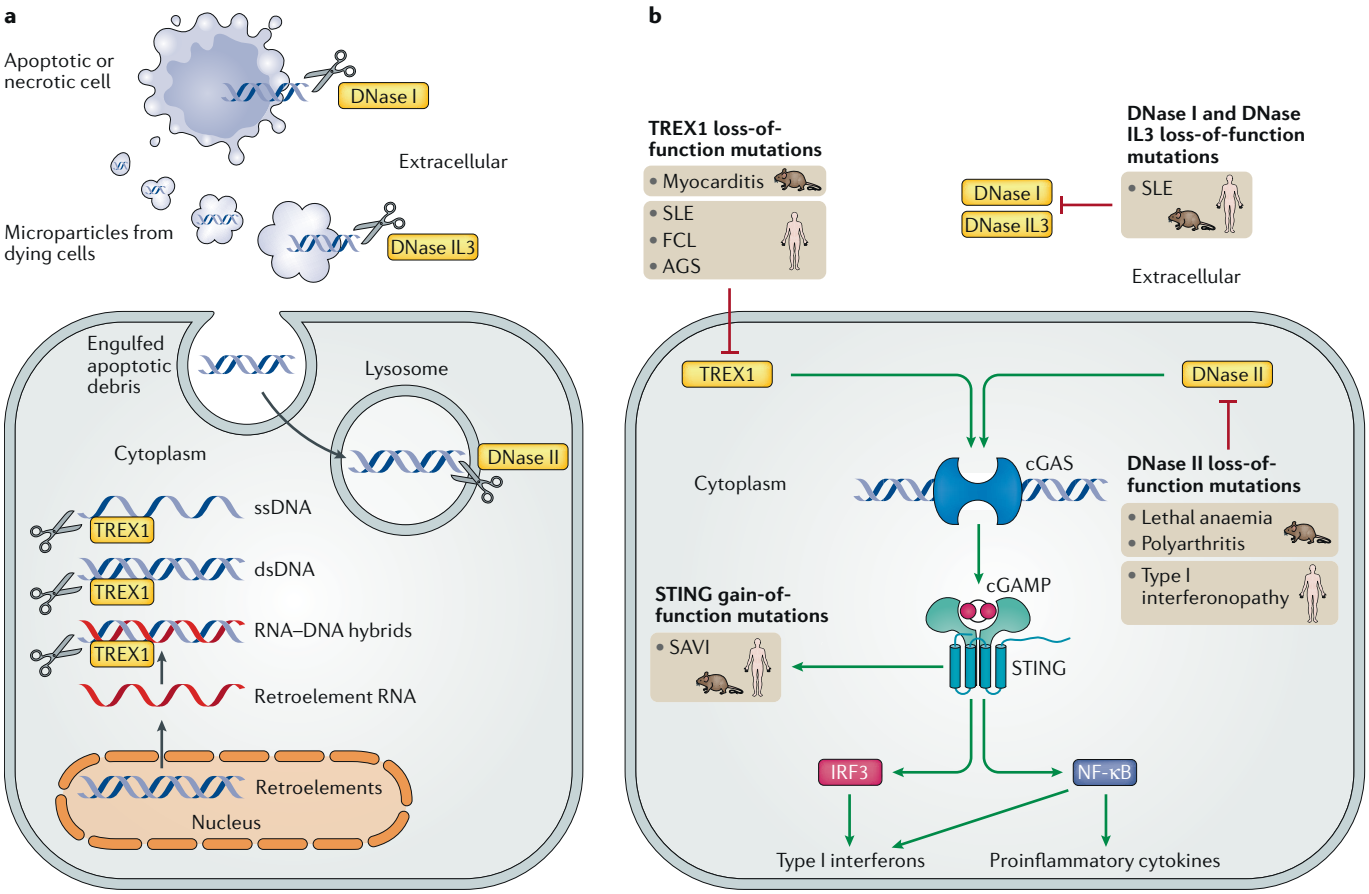


Fig. 3 | **cGAS–STING-mediated autoimmunity. a** | DNases function across various cellular compartments to digest DNA and avoid immune activation[64]. DNase I is a secreted extracellular DNase that can cleave both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) in a non-specific manner. DNase I-like 3 (DNase IL3), a member of the DNase I family, digests chromatin present in extracellular microparticles derived from apoptotic cells. DNase II is expressed in different tissues and is localized in phagolysosomes, where it hydrolyses phosphodiester linkages in DNA. Three-prime repair exonuclease 1 (TREX1; also known as DNase III) is a DNA exonuclease localized in the cytoplasm, where it digests ssDNA or nicked dsDNA. TREX1 also digests reverse-transcribed DNA or RNA–DNA hybrids derived from endogenous retroelements. **b** | Impaired function of DNases can cause the accumulation of DNA in the cytoplasm and, consequently, activation of the cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway. In humans, loss-of-function mutations in DNase II or TREX1 (depicted as red inhibitory arrows) result in monogenic diseases, including type I interferonopathies and Aicardi–Goutieres syndrome (AGS; a class of type I interferonopathy), respectively[204]. Human TREX1 mutations also lead to heterogeneous autoimmune disorders such as systemic lupus erythematosus (SLE) and familial chilblain lupus erythematosus (FCL)[94]. However, loss-of-function mutations in mouse models manifest differently compared with those in humans, including lethal anaemia and polyarthritis due to loss-of-function mutations in DNase II or myocarditis due to loss-of-function mutations in TREX1 (REFS[93,99]). Loss of activity of DNase I and DNase IL3 has also been associated with SLE in both humans and mice[102–105]. Gain-of-function mutations (depicted as green arrows) in STING have been identified as a new class of type I interferonopathy termed STING-associated vasculopathy with onset in infancy (SAVI), characterized by constitutive STING activation and interferon production in humans as well as in mice[107,114]. cGAMP, cyclic GMP–AMP; IRF3, interferon regulatory factor 3; NF-κB, nuclear factor-κB.

develop high levels of interferons in the heart, leading to myocarditis. In *Trex1*-null mice, these abnormalities are fully rescued by deletion of *Cgas*, *Sting* or the gene encoding the receptor for type I interferons (*Ifnar1*)[92,93]. The source of the cGAS ligand in AGS is not entirely clear, although endogenous retroelements and by-products of DNA replication and repair are both candidates[90]. Multiple distinct disease-associated mutations in *TREX1* have been identified (reviewed in REFS[94,95]).

A related disease caused by biallelic mutations in *DNASE2*, which encodes DNase II, has also been described in humans[96]. These mutations lead to loss of DNase II endonuclease activity, resulting in high levels of interferons and increased ISG expression in the patient's lymphocytes and monocytes, as well as other defects in haematopoiesis. DNase II is confined to the phagolysosomal compartment and functions in the degradation of DNA from engulfed apoptotic cells. In mice, *Dnase2* deletion is embryonic lethal owing to development of anaemia and the overproduction of type I interferons, a phenotype that is reversed on concomitant deletion of *Cgas*, *Sting* or *Ifnar1* (REFS[97–99]). Interestingly, *Dnase2*$^{-/-}$ mice also develop interferon-independent inflammation that manifests as a polyarthritis, highlighting the importance of interferon-independent pathways downstream of STING in disease[100]. The AIM2 receptor also contributes to the arthritic phenotypes in these animals through regulation of IL-1 expression[100]. Beyond arthritis, clinical manifestations also include massive splenomegaly and the production of antinuclear antibodies,

Endogenous retroelements
Genes that can integrate anywhere into the human genome, often referred to as mobile genetic elements; endogenous retroelements arise from integration of retroviruses into human genomes.

Antinuclear antibodies
During autoimmunity, these antibodies are made against self-proteins such as histones, nucleosomes or DNA.

Box 1 | **cGAS localization**

Cyclic GMP–AMP synthase (cGAS) has been proposed to function in the cytosol, where it is activated by foreign or self-DNA that gains access to this compartment. However, some studies from the past year have suggested that cGAS can be localized in both the nucleus and at the plasma membrane.

Initial studies using fluorophore-tagged cGAS variants defined cGAS as a cytosolic protein[196]. However, studies have now demonstrated specific conditions in which cGAS is localized to the nucleus. For instance, in non-dividing mouse embryonic fibroblasts, cGAS translocates to the nucleus during mitosis or cell division[138]. In mitotic cells, cGAS is localized with the chromatin DNA, suggesting its role in cell cycle regulation. In another study, DNA damage induced shuttling of cGAS from the cytosol to the nucleus[140]; following DNA damage, kinases, such as B-lymphoid tyrosine kinase (BLK), phosphorylated cGAS, enabling its nuclear translocation. DNA damage and increased genomic instability in tumour cell lines result in erroneous segregation of chromosomes, leading to the formation of secondary nuclear structures such as micronuclei. Breakdown of micronuclei membranes during mitosis or genotoxic stress resulted in recruitment of cGAS to the chromatin fragments localized in these micronuclei[132,133]. In addition, in mouse haematopoietic stem cells, cGAS was found in the nucleus, where its enzymatic activity was blocked to suppress expression of type I interferons and support the long-term survival of haematopoietic stem cells in the bone marrow of mice[197]. Furthermore, Volkman et al.[198] tested several cell lines and also found that inactive cGAS is a nuclear protein tethered tightly to the nuclear membrane, an interaction that requires intact chromatin. This nuclear localization of cGAS was independent of cell type, stage of the cell cycle or DNA damage. The authors hypothesized that the majority of cGAS is nuclear and inactive and that only a small proportion of cGAS is cytosolic and active in terms of its DNA-sensing and cyclic GMP–AMP (cGAMP)-generating functions. Taken together, these studies indicate that, under specific circumstances, cGAS resides in the nucleus and associates with chromatin.

Surprisingly, cGAS was also reported to synthesize cGAMP in the nucleus and induce innate immune activation of dendritic cells[199]; however, nuclear localization of cGAS leads to reduced cGAMP levels as compared to cytosolic cGAS. The N terminus of cGAS was shown to determine its nuclear localization and promote cGAS activation[200] and to be crucial for the DNA-induced liquid-phase condensation of cGAS[27].

Adding to the controversy of cGAS localization, a study from 2019 revealed that cGAS localizes to the plasma membrane in mouse and human phagocytes through a phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2; also known as PIP_2)-binding domain located at its N terminus[201]. Deletion of the N-terminal domain of cGAS resulted in its mislocalization from the plasma membrane to the cytoplasm and decreased its ability to detect viral DNA. By contrast, N-terminal-deleted cGAS mutants responded better to DNA damage.

Overall, these observations suggest that the subcellular localization of cGAS provides a unique opportunity for it to discriminate between self-DNA and non-self-DNA. In light of these findings, cGAS localization, activity and transport could conceivably be differentially regulated in certain contexts. Perhaps new functions of cGAS could be uncovered based on where it is located.

which are absent in mice expressing a functionally inactive form of the TLR chaperone protein unc-93 homologue B1 (UNC93B1)[101]. In DNase II-deficient cells, DNA in phagolysosomal compartments would presumably engage TLR9 and might gain access to the cytosol, where it might engage cGAS and AIM2, driving inflammatory responses. These phenotypes reveal a complex interplay of cytosolic and endosomal DNA sensors in the context of inflammatory diseases.

Heterozygous mutations in *DNASE1* (which encodes DNase I, an enzyme that digests extracellular DNA at sites of high cell turnover) have also been identified in patients with systemic autoimmunity such as systemic lupus erythematosus (SLE)[102]. Mutations in *DNASE1L3* (which encodes DNase IL3, a DNase I-related enzyme involved in the degradation of DNA microparticles from apoptotic debris) are also associated with SLE in humans[103]. Mouse models with deletion of one or both alleles of *Dnase1* or *Dnase1l3* also develop SLE-like disease similar to that observed in humans[104,105]. However, the role of cGAS in sensing these DNase I or DNase IL3 substrates is not yet known, and these diseases are probably more complex than AGS and other type I interferonopathies given that the extracellular DNA that accumulates would engage other sensors in the phagolysosomal compartment (for example, TLR9).

STING gain-of-function mutations. Over the past 5 years, a spectrum of autosomal dominant gain-of-function mutations in STING have been identified in >25 patients with an autoinflammatory disease called STING-associated vasculopathy with onset in infancy (SAVI), a new class of type I interferonopathy[106–109]. Patients with SAVI exhibit early-onset systemic inflammation, severe skin vasculopathy and interstitial lung disease resulting in pulmonary fibrosis and respiratory failure. The SAVI-associated STING mutations lead to spontaneous dimerization and activation of STING in the absence of cGAMP[107]. These gain-of-function STING mutants are thought to traffic from the ER in the absence of cGAMP and transit through the ERGIC and Golgi[38]. Despite the severe phenotypes of many patients with SAVI, mutations leading to constitutive STING activation in different individuals can have very different clinical manifestations[110,111]. Mouse models of SAVI show that hyperactivation of STING causes sterile inflammation, immune abnormalities and lung inflammation similar to that seen in human patients, although upregulation of ISG expression in the SAVI mice is minimal compared to what is observed in humans[112–114]. Interestingly, a direct comparison of murine models of the two most common SAVI-associated STING mutations revealed mutation-specific outcomes, whereby the mutation with the strongest capacity for STING activation (STING-V154M) resulted in lung fibrosis, whereas mice with a weaker mutation (STING-N153S) developed lung inflammation that failed to progress to fibrosis[114]. One of the striking features of SAVI mice is T cell cytopenia, and T cell dysfunction is thought to mediate lung inflammation in these animals[112–114]. However, although several reports have linked STING activation to programmed necrosis[60,61,115], the T cell death observed in SAVI mice (STING-V154M) was independent of MLKL-driven necroptosis[114]. Unexpectedly, and in contrast to the phenotypes observed in *Trex1*-null and *Dnase2*-null mice, the immune abnormalities and mortality of SAVI mice were not rescued by deletion of *Ifnar1* or *Irf3* (REFS[112–114]). However, lung inflammation in SAVI mice was shown to be mediated by $CD8^+$ T cells[116]. Constitutive STING activation in T cells was shown to impair Ca^{2+} flux and increase ER stress upon T cell receptor activation, which led to decreased T cell viability[117]. These observations suggest that dysregulation of STING in T cells is important in the pathogenesis of SAVI. Investigation of the extent to which these findings in mouse models are relevant to human disease will be an important next step.

Systemic lupus erythematosus
An autoimmune disorder in which the immune system aberrantly attacks host tissues.

Table 2 | **cGAS–STING pathway mutations linked to human disease**

Protein and function	Mutations in patients	Domain	Effect of mutation	Human phenotype	Mouse phenotype	Refs
DNase II; lysosomal endonuclease	G347C, A362T	N-terminal phospholipase D domain	Loss of catalytic activity	Type I interferonopathy	Lethal anaemia (*Dnase2*$^{-/-}$*Ifnar1*$^{-/-}$ mice)	96,99
TREX1; cytosolic exonuclease	T138N, D18N, T32R, K66R, L92Q	N-terminal exonuclease domain	Loss of exonuclease function	AGS	Myocarditis (*Trex1*$^{-/-}$ mice)	90,93, 234,235
	T303P, P290fs	C-terminal transmembrane helix			SLE-like disease (TREX1-D138N mutant mice)	
	D18N, N125fs	N-terminal exonuclease domain		FCL	Production of autoantibodies (TREX-V235fs mutant mice)	
	R114H, A158V	N-terminal exonuclease domain		SLE	–	
	Y305C, G306A	C-terminal transmembrane helix				
	V249fs, L28fs	C-terminal transmembrane helix	Disruption of intracellular localization	RVCL		
STING; adaptor protein	V147L	C terminus of cyclic dinucleotide-binding domain	Gain-of-function	SAVI (cutaneous and pulmonary)	SAVI, pulmonary manifestations (STING-N153S and STING-V154M mutant mice)	108–110, 114,236
	N154S, V155M	N-terminal dimerization region	Constitutive STING activation		–	
	R281Q	C-terminal tail domain				
	R284G, C206Y	C-terminal tail domain		Cutaneous SAVI		
	G166E	N-terminal dimerization region				
	R284S	Outside dimerization domain		Pulmonary SAVI		

AGS, Aicardi–Goutieres syndrome; cGAS, cyclic GMP–AMP synthase; FCL, familial chilblain lupus; RVCL, retinal vasculopathy with cerebral leukodystrophy; SAVI, STING-associated vasculopathy with onset in infancy; SLE, systemic lupus erythematosus; STING, stimulator of interferon genes; TREX1, three-prime repair exonuclease 1.

Self-DNA and inflammatory diseases

An emerging theme in the literature is the ability of self-DNA to promote a broader spectrum of human disease beyond the aforementioned diseases with a genetic component.

Self-DNA-driven inflammation contributes to the inflammatory response and tissue damage observed following myocardial infarction. In a mouse model, the uptake of cellular debris by macrophages following myocardial infarction led to the cGAS–STING-dependent production of proinflammatory cytokines and chemokines, increased inflammation in the heart, and decreased ventricular function and survival[118]. Genetic or pharmacological blockade of the cGAS–STING pathway ameliorated all of these myocardial infarction-induced disease features.

In addition, cGAS has been linked to macular degeneration, whereby mitochondrial DNA was reported to engage cGAS to elicit noncanonical inflammasome activation in human cell culture and mouse models[119]. Degeneration of the retinal pigment epithelium was a result of caspase activation, which required both cGAS-driven type I interferon responses and gasdermin D-dependent IL-18 production.

Self-DNA-driven inflammation has also been linked to Parkinson disease. Indeed, mitochondrial DNA released in cells from *Prkn*-null or *Pink1*-null mice exposed to mitochondrial stress was reported to engage the STING pathway[120]. Deletion of *Sting* in *Prkn*-null and *Pink1*-null mice resolved the inflammation, motor defects and neurodegeneration observed in their *Sting*-proficient counterparts.

Type I interferons are also important for maintenance of intestinal homeostasis through regulation of the development and function of regulatory T cells in the gut[121–123]. Under homeostatic conditions, the intestinal flora activates STING and induces expression of type I interferons[124]. In line with this functionality, *Sting*-null mice have an increased sensitivity to experimentally induced colitis and enteric infections[124]. A causal role for STING in the development of nonalcoholic fatty liver disease has also been demonstrated. STING activation in hepatic macrophages led to the production of proinflammatory cytokines, leading to nonalcoholic steatohepatitis, which is characterized by hepatic steatosis and fibrosis[125].

The cGAS–STING pathway has also been implicated in Hutchinson–Gilford progeria syndrome, a premature ageing disease resulting from the production of progerin, a truncated variant of lamin A. Studies in fibroblasts derived from patients with Hutchinson–Gilford progeria syndrome show that progerin leads

to unchecked replication stress and induction of the cGAS–STING pathway. Treatment of these cells with calcitriol, an inhibitor of STAT1, reduced both replication stress and an interferon-induced gene signature, ameliorating progerin-dependent phenotypes in cells[126].

A study published in 2019 reported the role of cGAS activation in Bloom syndrome, a rare genetic disease caused by a deficiency for Bloom syndrome protein (BLM), which maintains DNA integrity upon genomic insult[127,128]. BLM-deficient cells show accumulation of DNA damage and formation of micronuclei. The marked genetic instability observed in patients with Bloom syndrome predisposes them to various cancers and immunodeficiency. Peripheral blood from these patients revealed increased expression of inflammation-associated genes and ISGs. Studies in *Blm*-null cells reported that the accumulated micronuclei contained cGAS and the ISG signature was driven by the cGAS–STING pathway[129]. This study linked the genomic instability observed in patient populations with cytosolic innate immune sensing.

Collectively, these observations indicate that activation of the cGAS–STING pathway contributes to various inflammtory diseases and chronic inflammation in different tissues, suggesting that therapeutic targeting of these responses could be beneficial in a broad array of human diseases.

cGAS–STING and DNA damage

Emerging data indicate that both cGAS and STING have a key role in modulating the DNA damage response and in the surveillance of micronuclei, findings that could have important implications for cancer.

Micronuclei and DNA damage response

As the biology describing DNA damage and its direct interactions with cGAS advance, it is becoming clear that DNA does not simply 'leak' out of the nucleus but rather forms micronuclei following DNA damage that activate cGAS–STING signalling[130–133]. DNA damage is a broad term that encompasses a multitude of distinct mechanisms of structural damage to DNA that can occur at distinct stages of the cell cycle. The formation of micronuclei is a consequence of nuclear DNA damage and occurs following progression through mitosis, which creates a membrane-enclosed perinuclear package of damaged DNA[134]. DNA damage caused by merotelic kinetochores, lagging chromosomes or through a dysfunction in chromosomal breakage–fusion–bridge cycle generates an imbalance in the chromosome copy number (>2N chromosomes) and leads to chromosome instability (CIN)[131,135]. Although other forms of DNA damage, such as DNA damage during its replication, are linked to micronuclei formation, a progression through mitosis seems to be a key requirement for its formation[136,137].

Several reports have demonstrated that cGAS mediates a cellular surveillance mechanism for detection of DNA localized to micronuclei. cGAS colocalizes with markers of DNA damage, such as phosphorylated histone H2AX (γH2AX), in micronuclei in a process that is dependent on cellular mitosis[132,133,138]. The mitotic dependence of micronuclei formation and colocalization with cGAS was observed in a study showing that irradiation of tumour cells arrested in the G0 phase did not lead to formation of micronuclei or generate cGAS-dependent secretion of cytokines[133]. Similar interactions between cGAS and micronuclei were noted upon oxidative stress, irradiation and etoposide treatment, reiterating a role for cGAS in the cellular response to DNA damage in micronuclei[138,139].

The cGAS response to DNA damage does not seem to be limited to micronuclei, as observed in a study that reported cGAS translocation into the nucleus following DNA damage, where it bound to locations of DNA double-stranded breaks[140]. Furthermore, nuclear cGAS colocalized with γH2AX and DNA repair machinery proteins such as poly(ADP-ribose) polymerase 1 (PARP1). Surprisingly, instead of activating enzymatic catalysis and cGAMP production, nuclear cGAS associated with sites of DNA damage and inhibited the DNA repair process. In this study, inhibition of cGAS transport and localization of cGAS to DNA double-stranded breaks was proposed to minimize DNA damage and enhance DNA repair mechanisms. However, this new role for cGAS might simply be an early step in the colocalization of cGAS with sites of DNA damage in micronuclei. For instance, perhaps transport of cGAS into the nucleus and detection of sites of DNA damage restricts nuclear repair and promotes formation of the organelle-like membrane-encapsulated micronuclear particle in which cGAS signalling commences. However, this mechanism, although feasible, is just one potential explanation of how cGAS might end up inside micronuclear structures. Thus, studies measuring the time course of events that link cGAS-mediated nuclear DNA damage response to that of the micronuclei might shed light on whether these two mechanisms of DNA damage response are connected.

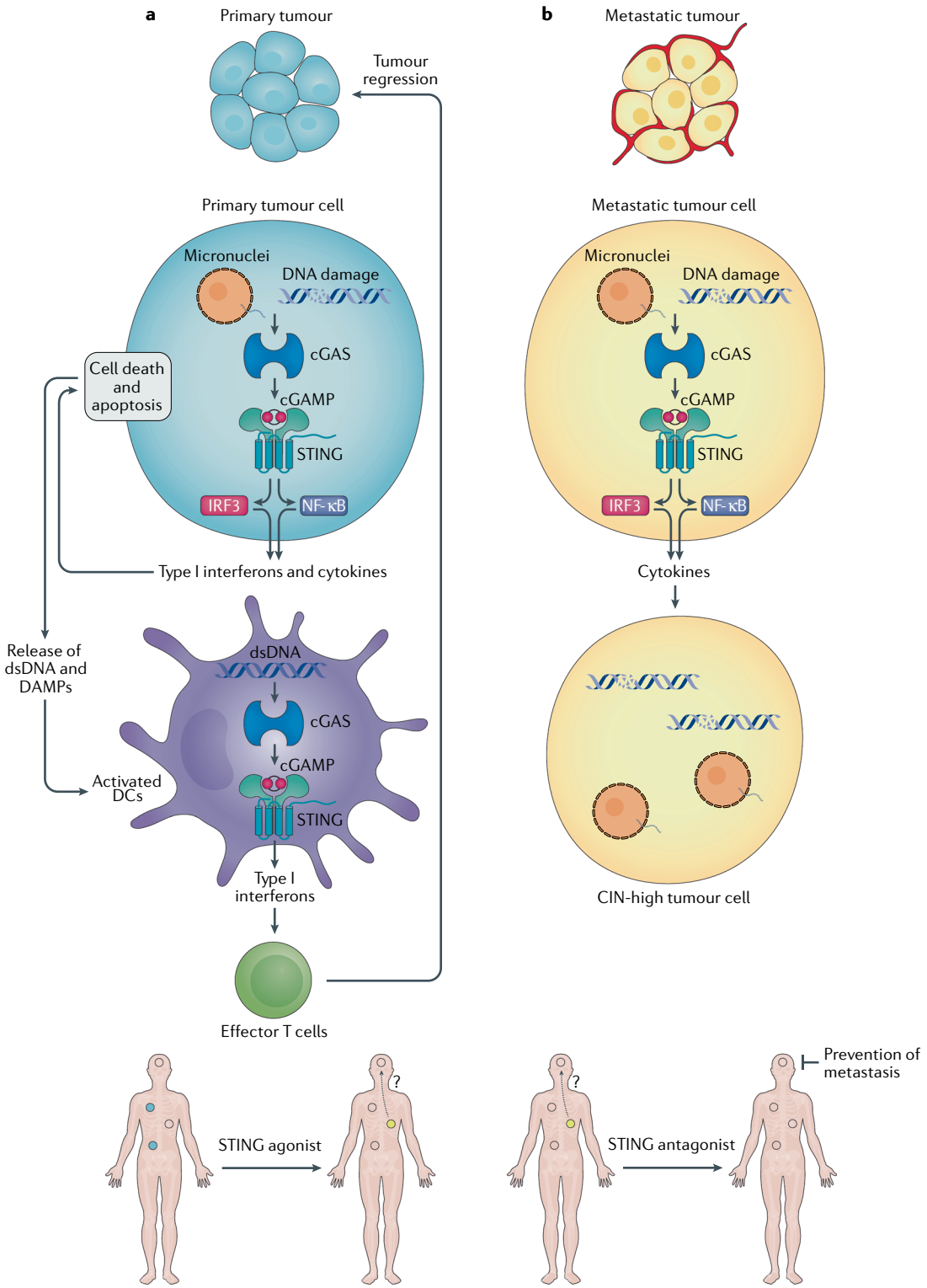
The mechanism by which detection of damaged DNA and how the balance of cytokines secreted downstream of the cGAS–STING pathway contribute to the fate of damaged DNA localized in micronuclei is unclear. DNA encapsulated in micronuclei can be extruded from the cells, degraded by nucleases (such as TREX1) or maintained in a persistent micronuclei state, which is a predominant source of propagating genome instability[134]. Other studies have shown that micronuclear DNA is reincorporated into genomic DNA at subsequent mitotic events[141]. In some instances, the fate of micronucleated cells is a determining feature that leads to apoptosis, which has a vital role in the elimination of cells that become dysfunctional or tumorigenic. Finally, detection of micronuclei by cGAS and consequent activation of STING signalling is probably a determining factor in how the cell responds to chromothripsis. Overall, a DNA damage response to micronuclei that is dependent on cGAS and STING could plausibly direct how the cell chooses to manage DNA damage and dysfunction. Irreparable levels of DNA damage might potentiate proapoptotic signalling and clearance[57] of the cell, whereas dysfunction in the DNA damage response process might result in cellular transformation and, subsequently, tumour cell proliferation.

Merotelic kinetochores
Kinetochores arranged in a merotelic orientation, whereby one kinetochore is attached to opposing spindle poles.

Breakage–fusion–bridge cycle
A process whereby broken chromosomes fuse with other broken chromosomes that segregate to opposite spindle poles during mitosis, forming the chromosome bridge; the bridge is consequently broken during mitosis, instigating a cyclic pattern of chromosomal breakage and fusion that propagates chromosome instability.

Chromosome instability
A type of genomic instability in which chromosomes are unstable, such that either whole chromosomes or parts of chromosomes are duplicated or deleted.

Chromothripsis
A cellular description of asynchronous chromosome condensation coincident with a high frequency of chromosomal rearrangements condensed to a specific region of a chromosome.



Implications for cancer

Emerging roles for cGAS and STING in modulating the DNA damage response in tumours and in control of CIN creates a paradox in how modulation of the cGAS–STING pathway towards inhibition or activation could lead to distinct outcomes in cancer (FIG. 4).

Damage of genomic DNA induced by ionizing radiation or chemotherapy agents (such cisplatin and etoposide) causes cGAS–STING-dependent cytokine production, which, notably, has a beneficial role in defining the therapeutic effects of these cancer treatments[139,142–144] (FIG. 4a). It is worth reflecting on the notion that the historical success of radiotherapy and chemotherapy in cancer are due, in part, to innate immune signalling by the cGAS–STING pathway. Furthermore, PARP1 inhibition in certain tumour settings blocks DNA damage repair mechanisms, causing cytosolic accumulation of genomic DNA in micronuclei

◀ Fig. 4 | **Balancing the roles of the cGAS–STING pathway activation and inhibition in cancer. a** | During tumorigenesis, and in tumour cells, pathological changes occur in genomic DNA, including DNA damage, chromosome instability (CIN) and formation of micronuclei. DNA derived from DNA damage or micronuclei stimulate cyclic GMP–AMP synthase (cGAS)-mediated production of cyclic GMP–AMP (cGAMP), activation of stimulator of interferon genes (STING), and the interferon regulatory factor 3 (IRF3)-dependent and nuclear factor-κB (NF-κB)-dependent transcription of cytokines and type I interferons. These tumour cell-dependent cellular responses to DNA damage and micronuclei instigate tumour cell death and apoptosis, leading to release of double-stranded DNA (dsDNA) and other tumour-derived antigens (DAMPs). Uptake of dsDNA by tumour-resident dendritic cells (DCs) elicits a complimentary cGAS–STING-dependent type I interferon-mediated activation of a therapeutically beneficial antitumour immune response, for example, through activation of effector T cells such as tumour-associated, antigen-specific $CD8^+$ T cells, which destroy tumour cells[149,151,205,206]. In a hypothetical scenario, a patient with non-malignant primary tumours (blue circles) treated with a STING agonist might develop antitumour immunity, leading to the elimination of tumours that express a specific repertoire of tumour-associated antigens. However, tumour cells that transform to a metastatic state (yellow circles) might lead to metastasis. **b** | The persistence of CIN-high tumour cells creates a highly mutagenic environment, which might enable tumours to evade immune-mediated elimination and potentiate tumour metastasis[130,153]. cGAS and STING activation lead to maintenance of CIN through expression of a distinct, and currently unknown, profile of cytokines that might lead to proliferation of tumour cells with micronuclei and maintenance of CIN. In a hypothetical scenario, pharmacological inhibition of cGAS–STING-dependent maintenance of CIN and tumour cell proliferation might protect patients from tumour metastasis.

that activate a cGAS–STING-dependent antitumour immune response[145–148]. In dendritic cells, detection of this tumour-derived DNA by cGAS stimulates the cGAMP-dependent activation of STING and, consequently, secretion of type I interferons[149,150]. These innate immune signals enhance tumour antigen presentation, potentiating a tumour-associated, antigen-specific $CD8^+$ T cell response linked to tumour regression[150–152].

However, in a surprising twist, cGAS–STING-dependent DNA-sensing of micronuclei in tumour cells can promote cytokine signalling, which maintains CIN and leads to metastasis[130,153] (FIG. 4b). The involvement of cGAS and STING in regulation and maintenance of CIN in tumour cells implies a seemingly contradictory role for the pathway in cancer. A study comparing human brain metastases with primary tumours from the same patients showed that metastatic tumour cells possess a higher frequency of CIN, as measured by chromosome copy number and genome integrity[130]. This observation led scientists to the hypothesis that high levels of CIN promote metastasis. Indeed, mice injected with CIN-high tumour cells demonstrated a markedly greater burden of tumour metastasis and lower survival than mice injected with CIN-low tumour cells[130]. Further characterization of the underlying mechanism showed that CIN-high cells had a higher frequency of micronuclei that colocalize with cGAS and instigate STING-dependent noncanonical signalling to the NF-κB p100 subunit (NFKB2) and the transcription factor ReLB[130]. In a separate study, tumour metastasis in mouse brain was shown to be dependent on production of cGAMP by cGAS in tumour cells[154]. Interestingly, transport of cGAMP through gap junctions from tumour cells to astrocytes stimulated canonical STING signalling and activation of IRF3 and NF-κB target gene transcription, promoting brain metastasis. In both studies, modelling of pathway inhibition by short hairpin RNA-mediated reduction of cGAS or STING expression in tumour cells prevented metastasis[130,154]. In one example, DNA sensing through STING promoted tolerogenic immune regulatory expression of indoleamine 2,3-dioxygenase (IDO), which exacerbated tumour growth in the Lewis lung carcinoma mouse model of tumour metastasis[155]. Although the mechanism by which cGAS–STING-dependent cytokine production maintains CIN in micronuclei is unclear, activation of tumour cell-autonomous transcriptional programmes might promote metastasis.

Targeting the cGAS–STING pathway

Medicines that target the cGAS–STING pathway are intensely pursued by the pharmaceutical industry and small biotechnology firms alike. The range of clinical applications for therapeutics targeting the cGAS–STING pathway are expanding, with evidence from murine models bolstering the proposition that both agonists and antagonists of the pathway can influence patient management across a wide spectrum of diseases such as cancer, neurodegeneration, chronic viral infections, metabolic diseases, and autoinflammatory or autoimmune diseases.

Cancer

Advances in cancer immunotherapy have transformed the treatment of patients with cancer. However, despite the tremendous success of immuno-oncology agents such as the immune checkpoint inhibitors (anti-cytotoxic T lymphocyte antigen 4 (CTLA-4), anti-programmed cell death 1 ligand 1 (PD-L1) and its receptor anti-programmed cell death 1 (PD-1) antibodies), only a small subset of patients have a durable response[156,157]. Most patients have tumours that are either completely resistant at treatment onset or demonstrate an initial tumour response but relapse over time. This relapse is caused, in part, by the lack of antitumour T cell response and loss of tumour-associated antigen expression, which could potentially be overcome by stimulation of innate immune cells within the tumour microenvironment.

Among the different medical treatments under development to stimulate the innate immune response in tumours, STING agonists are particularly attractive. STING is widely expressed in multiple cell types in the tumour microenvironment, has a unique ability to elicit the balanced secretion of type I interferons and pro-inflammatory cytokines, and is an important pathway responsible for tumour-associated antigen presentation and regression in mouse tumour models[150,152]. Direct pharmacological activation of STING induced remarkable tumour regression in mouse models, reinforcing the role of the cGAS–STING pathway in cancer[150,151,158–160]. Beyond stimulating T cell proliferation, activation of STING causes tumour vascular collapse and contributes to tumour cell death and apoptosis, enhancing release of tumour-associated antigens[59,145,159,161,162]. However, the greatest promise of STING agonists is their potential combination with other immuno-oncology agents. STING agonists increase immunogenicity in non-immunogenic tumours and are expected to improve the efficacy of immunotherapy[163,164]. Furthermore, STING agonists can reverse resistance to anti-PD-1 agents in mouse tumour models[143,165–168]. Immuno-oncology

combinations might be inherently important for the therapeutic benefit of STING agonists, as activation of STING causes expression of immunosuppressive proteins such as PD-L1 and CCR2 (REFS[147,165,167,169]). For instance, STING-dependent expression of CCR2 on myeloid-derived suppressor cells causes recruitment of protumorigenic monocytes to inflamed tumour tissue, which is prevented by combination treatment with CCR2 antagonists[169]. Thus, STING agonists represent an immune-oncology modality that anchors innate immunity to combinations with a variety of complimentary immunotherapies.

The first wave of STING agonists in clinical development are structurally derived from the natural STING ligand cGAMP, with modifications that improve stability and extend pharmacokinetics[145,165,170,171]. The majority of published reports focus on models that apply intratumoural delivery to maximize tumour cytokine response while minimizing the possibility of systemic cytokine toxicity linked to serious adverse events in humans known as cytokine release syndrome[172]. Two such STING agonists have been evaluated in phase I human studies (ADU-S100 and MK-1454)[173,174] and are limited to patients with accessible solid tumours amenable to intratumoural delivery. Both studies reported comparable results, demonstrating that escalating doses of STING agonists were tolerated with measurable target engagement in treated tumours and biological signs of $CD8^+$ T cell infiltration in tumours. However, both studies were notably limited with regard to response in non-injected tumours, underscoring a shift towards development of STING agonists designed for systemic delivery that can simultaneously target multiple tumour sites in patients.

The recent discovery of the amidobenzimidazole class of small-molecule STING agonists establishes a new type of agents that cause tumour regression in mouse models[175]. However, unlike the intratumoural approach, the amidobenzimidazoles were developed for intravenous delivery. Importantly, a systemic STING agonist opens the possibility for treating patients with inaccessible solid tumours and overcomes the unique antigenic differences among tumours from a given patient (that is, different tumours might possess different antigenic peptides). As a proof of concept, intravenous systemic delivery of an amidobenzimidazole STING agonist demonstrated remarkable single-agent activity and recapitulated $CD8^+$ T cell-dependent antitumour immunity[175]. The rapid pharmacokinetic clearance of amidobenzimidazoles coupled with their high potency leads to a spike in systemic exposure, a so-called hit-and-run approach to priming STING function without prolonged systemic exposures[175]. While intratumourally administered STING agonists elicit antitumour immune responses derived from a single tumour, systemic delivery of a STING agonist might induce responses to a wider repertoire of tumour-associated antigens represented in distinct tumour sites.

As therapeutic approaches that directly and indirectly modulate STING signalling emerge, a balance between cytokine signalling and timing might play into the therapeutic effects in primary and metastatic tumour settings. Important differences in the frequency of CIN in primary versus metastatic tumours highlight a potential basis for therapeutic selection between cGAS–STING pathway agonists and antagonists in cancer (FIG. 4b). In orthotopic patient-derived xenograft mouse models, tumours derived from patients with metastatic tumours were enriched with cells characterized by high CIN, whereas cells derived from the primary tumours were characterized by low CIN[130]. The researchers did not explicitly segregate the unique contributions of the STING pathway in primary and metastatic tumours, but this distinction might support the bifurcating roles of modulating the cGAS–STING pathway. While agonists of STING in primary or non-metastatic tumours promote tumour immunity and regression, cGAS–STING pathway inhibition might prevent progression and metastasis (FIG. 4b). Whether CIN-high tumour cells evade clearance by the immune system is unclear but, given the highly unstable and mutagenic nature of CIN-high tumours, perhaps these cells evolve to lose antigenic peptides associated with the $CD8^+$ T cell antitumour response. Thus, future therapies modulating the cGAS–STING pathway might be tailored to the cancer type, stage of disease at treatment onset and propensity for metastasis.

Viral or bacterial infection

Beyond cancer, the development of STING agonists has been proposed for a number of different therapeutic purposes, including use as a vaccine adjuvant and for chronic viral or bacterial infections. When used as an adjuvant, bacterial cyclic dinucleotides as well as human cGAMP have demonstrated improved efficacy of vaccination[29,171,176–181]. Importantly, STING agonists as vaccine adjuvants improve antigen-specific IgG response through a mechanism dependent on a T helper 1 (T_H1), T_H2 and T_H17 cell response[176,179,182]. In addition, despite the lack of STING expression in hepatocytes[183], STING agonist treatment in mice infected with hepatitis B virus reduced viral load and weakened susceptibility to hepatitis B virus[184]. Similarly, modulation of the cGAS–STING pathway was proposed to have a potential therapeutic benefit in *M. tuberculosis* infection[185]. A 2019 study reported a unique therapeutic application of a STING agonist in a model of SIV-infected *Cynomolgus macaques*, demonstrating a potential method to achieve a functional cure for HIV infection in human patients[186]. In this study, the STING agonists (cyclic diGMP and cGAMP) and a TLR7/8 agonist (R848) caused reactivation of latent SIV, the first step in purging latently infected T cells from tissues[186].

Inflammatory diseases

Development of medicines that inhibit the cGAS–STING pathway are also supported by the aforementioned strong genetic evidence defining the importance of STING in SAVI[107,110] and AGS[187] and even in patients diagnosed with SLE[188–191]. These rare monogenic diseases share clinical manifestations with SLE, supporting the hypothesis that the cGAS–STING pathway has a broader role in driving inflammation and pathogenesis beyond the rare monogenic diseases described herein. Whether or not inhibition of the cGAS–STING pathway

will influence patient management in the clinical setting remains to be seen, but studies in preclinical animal models treated with TBK1 inhibitors or genetic knockout of the cGAS–STING pathway in inflammatory models support the notion that pathway inhibitors could rescue aspects of the inflammatory phenotypes[91,97,192–194].

Conclusions

The discovery and characterization of the cGAS–STING pathway has provided a new framework for understanding the immune-stimulatory capacity of dsDNA. The exquisite sensitivity of the pathway to foreign nucleic acids enables the generation of robust antiviral responses to protect the host from viruses and other pathogens. This feature can also be leveraged for antiviral therapy or vaccine adjuvancy. However, the sensitivity of this pathway to foreign or self-DNA alike is a double-edged sword. Maintaining 'ignorance' to self-DNA when cells that express cGAS are replete with self-DNA is a major challenge. In this Review, we have described how the nucleus and mitochondria represent a major source of immunostimulatory self-DNA. The breakdown in the mechanisms that ensure discrimination of self from non-self-DNA and prevent erroneous ligation of cGAS by nuclear or mitochondrial DNA is emerging as a common feature in diverse diseases characterized by inflammation as an underlying mechanism. In the future, determining the exact DNA ligands that instigate cGAS-driven inflammation in different disease contexts will be important. Further elucidation of the counter-regulatory mechanisms that help maintain ignorance to these sources of self-DNA, especially during cell division, will probably emerge as intense efforts over the next few years continue to focus on these questions.

The exciting discoveries in this field have provided new opportunities for therapeutic intervention in a growing number of diseases. In time, clinical studies will probably reveal whether targeting the cGAS–STING pathway will be beneficial for the treatment of patients with AGS, SAVI, Bloom syndrome or mutations in *DNASE2*. Perhaps, in time, this pathway will also represent a common therapeutic target for more complex inflammatory diseases such as SLE, macular degeneration, neurodegeneration, cardiovascular disease and beyond, in which the cGAS–STING pathway serves as a more general response to cellular stress and tissue damage. Understanding whether or not type I interferons induced downstream of STING represent the sole driver of disease pathogenesis will also be informative. The emerging studies in mouse models, particularly those of DNase II deficiency and SAVI, are revealing that interferons are not the only driver of pathological inflammation in the STING pathway[101,114]. How these models are reflective of the human conditions will be addressed in clinical trials evaluating inhibitors of interferons and JAK–STAT signalling, which are well underway.

There is also great excitement about the potential of STING agonists in cancer, alone or, more importantly, together with other modalities such as immune checkpoint inhibitors, to improve patient outcomes. A therapeutic paradigm in which both activation and inhibition of the cGAS–STING pathway might have distinct roles in the treatment of patients with cancer is starting to emerge. On the one hand, activation of STING might enhance tumour immunogenicity through a mechanism that requires cGAS–STING pathway activation in tumour-resident dendritic cells[149]. Conversely, inhibition of the cGAS–STING pathway in metastatic tumour cells containing micronuclei might prevent their survival, proliferation and spread to new sites. The mechanism by which the secretion of cytokines promotes a metastatic state as well as whether cGAS–STING signalling has a role in maintaining high CIN and retention of micronuclei are still unclear, but genetic deletion of *Cgas* and/or *Sting* in metastatic tumour models seems to be protective. Pharmacological modulation of the cGAS–STING pathway will undoubtedly have important roles in promoting antitumour effects and tumour immunogenicity in some settings, while inhibiting metastatic tumour growth in others[195]. With continued investigation into this pathway, the future will probably hold new discoveries relevant to both human health and disease.

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