InvivoGen infocus

Follow the path to STING

STING (STimulator of INterferon Genes) has become a focal point in immunology research as well as a target in drug discovery. As a signaling hub in innate immunity, STING is a pattern recognition receptor (PRR) of paramount importance in orchestrating the body's response to pathogenic, tumor, or self DNA in the cytoplasm. InvivoGen offers a growing family of products to help you explore STING, its signaling partners, cytokine induction activity and therapeutic potential.

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Introduction

Entry of self or foreign nucleic acids into the cytoplasm can signal various problems, including pathogenic infection by incoming microbes, aberrant apoptosis of neighboring cells, mitochondrial or nuclear damage, and the presence of tumors. Until the discovery of STING in 2008, detection of nucleic acids as Pathogen-associated Molecular Patterns (PAMPs) had been largely imputed to Toll Like Receptors (TLRs), a family of PRRs sensing the extracellular milieu or the endosomal lumen[1]. STING was first identified as a cytosolic nucleic acid sensor playing an essential role in the induction of type I interferon (IFN) responses and the control of certain viral infections[2,3]. It was proposed to be an adaptor-like molecule which integrates sensing/downstream signaling of both viral RNA and double-stranded DNA (dsDNA), but its positioning remained unclear for a few years. Indeed, although it was shown that STING is a direct sensor of cytosolic cyclic-dinucleotides (CDNs) commonly produced by invading bacteria, its direct interaction with dsDNA could not be demonstrated, suggesting the intervention of at least one additional protein[4]. The identity of the major dsDNA cytosolic sensor was resolved in 2013: the cyclic GMP-AMP synthase (cGAS) is activated upon direct DNA binding and subsequently catalyzes the production of a non-canonical CDN, which in turn, activates STING[5,6]. STING activation results in a signaling cascade which ultimately leads to recruitment and activation of innate and adaptive immune cells. Briefly, upon binding to a single CDN molecule, activated STING and TANK-binding-kinase-I (TBK1) interact to induce an active interferon regulatory factor (IRF3) dimer which then binds to interferon-stimulated responsive elements (ISRE) in the nucleus and leads to IFN-α/β production[7]. The production of NF-κB-dependent inflammatory cytokines is also observed downstream of STING activation but the underlying mechanisms remain opaque[8] (Fig.1). This review addresses different aspects of STING activity and regulation, notably through interaction with other PRRs including DNA sensors, RNA sensors, inflammasomes and TLR7. Finally, various disease conditions favoring a therapeutic targeting of STING are discussed.

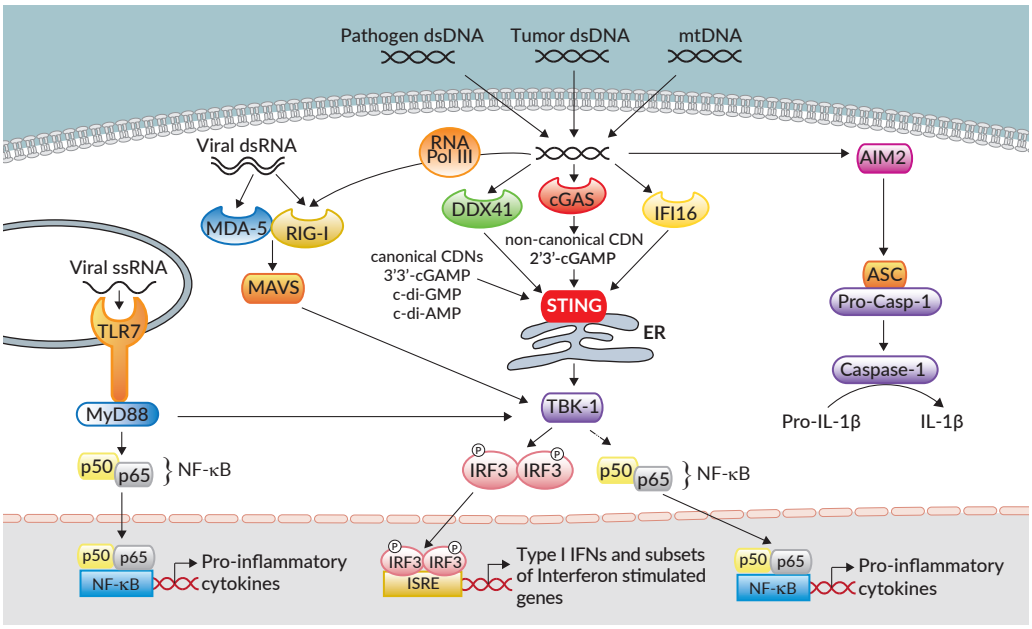

Figure 1: The STING signaling pathway

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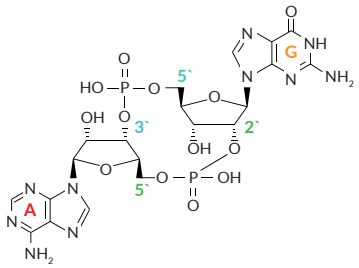
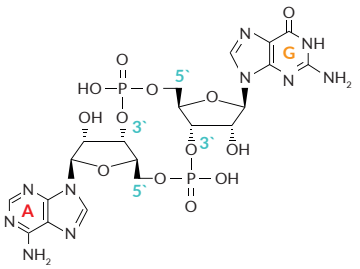
STING activation

Cyclic dinucleotides

The known natural STING agonists correspond to the four naturally occurring CDNs, all of which are based on the nucleosides guanosine (G) and/or adenosine (A). Before the discovery of STING, CDNs had already been reported as bacterial messenger molecules and shown to exhibit anti-microbial[9], adjuvant[10], pro-DNA-replicative[11], anti-cancer[12] and cell cycle-modulatory activities[13]. Among the naturally occurring CDNs, c-di-GMP, c-di-AMP and 3'3'-cGAMP are classified as canonical CDNs and are released into host cells during infection. However, the fourth CDN, 2'3'-cGAMP, is produced by the DNA sensor cyclic GMP-AMP synthase (cGAS) in mammalian cells and is referred to as a non-canonical CDN because of the position of the phosphodiester bonds between the guanosine and adenosine nucleosides[5,6]. Microbial CDNs contain a (3',5')(3',5') phosphodiester linkage (denoted as 3'3'), whereas the mammalian CDN contains a (2',5')(3',5') linkage (denoted as 2'3').



Chemical structure of 2'3'-cGAMP

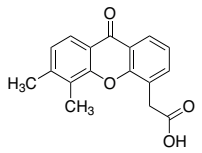


Chemical structure of 3'3'-cGAMP

In addition to their utility as research reagents, CDN STING agonists are being pursued as immunotherapy agents. InvivoGen scientists recently developed a novel series of potent, STING-activating CDNs based on the adenosine (A) and inosine (I) nucleosides, the latter of which is not found in natural CDNs[14]. The synthetic cAIMP and its difluoro derivatives are analogs of the bacterial 3'3'-cGAMP. The difluoro cAIMP compounds are not only more resistant to enzymatic cleavage but also more potently induce IRF3 and NF-κB pathways (Fig. 2). Of note, STING activity can be negatively regulated during bacterial infection following binding of bacterial CDNs to other DNA sensors such as DDX41 and the oxidoreductase RECON[15-17].

DMXAA

Discovered in 1991, 5,6-dimethylxanthenone-4-acetic acid (DMXAA; also known as ASA404 or vadimezan) is a synthetic compound and vascular disrupting agent that showed great promise as an oncology drug candidate in murine experiments[18]. However, it ultimately failed Phase III clinical trials for non small-cell lung cancer (NSCLC)[19]. Interestingly, DMXAA was reported to be a STING agonist in 2012[20], but was later revealed to be a potent agonist of murine STING that is totally inactive towards human STING[21]. This species-specific difference accounted for the efficacy of DMXAA in murine models as well as for its clinical failure. Efforts are now underway in both industry and academia to create DMXAA analogs that activate human STING. Nevertheless, DMXAA remains a useful research ligand for inducing the STING pathway in murine cell lines and in mice.



Chemical structure of DMXAA

Table 1: Reporter cell lines related to cGAS/STING signaling

CELL LINE	PRODUCTS	DESCRIPTION	UNIT SIZE	CAT.CODE
B16	**B16-Blue™ ISG Cells**	IRF-SEAP reporter mouse melanoma cells	3-7 x 10^6 cells	bb-ifnabg
	B16-Blue™ ISG-KO-STING Cells	IRF-SEAP reporter STING knockout cells	3-7 x 10^6 cells	bb-kostg
HEK293	**HEK-Blue™ ISG Cells**	IRF-SEAP reporter human embryonic kidney cells	3-7 x 10^6 cells	hkb-isg
	HEK-Blue™ ISG-KO-STING Cells	IRF-SEAP reporter STING knockout cells	3-7 x 10^6 cells	hkb-kostg
HEK293T	**293T-Dual™ hSTING-A162 Cells**	IRF-SEAP and IFN-β-Lucia reporter cells with A162 human STING	3-7 x 10^6 cells	293d-a162
	293T-Dual™ hSTING-H232 Cells	IRF-SEAP and IFN-β-Lucia reporter cells with H232 human STING	3-7 x 10^6 cells	293d-h232
	293T-Dual™ hSTING-R232 Cells	IRF-SEAP and IFN-β-Lucia reporter cells with R232 human STING	3-7 x 10^6 cells	293d-r232
	293T-Dual™ mSTING Cells	IRF-SEAP and IFN-β-Lucia reporter cells with murine STING	3-7 x 10^6 cells	293d-mstg
RAW 264.7	**RAW-Lucia™ ISG Cells**	IRF-Lucia reporter cells	3-7 x 10^6 cells	rawl-isg
	RAW-Lucia™ ISG-KO-cGAS Cells	IRF-Lucia reporter cGAS knockout cells	3-7 x 10^6 cells	rawl-kocgas
	RAW-Lucia™ ISG-KO-IRF3 Cells	IRF-Lucia reporter IRF3 knockout cells	3-7 x 10^6 cells	rawl-koirf3
	RAW-Lucia™ ISG-KO-STING Cells	IRF-Lucia reporter STING knockout cells	3-7 x 10^6 cells	rawl-kostg
THP-1	**THP1-Dual™ Cells**	NF-κB-SEAP and IRF-Lucia reporter human monocyte cells	3-7 x 10^6 cells	thpd-nfis
	THP1-Dual™ KO-cGAS Cells	NF-κB-SEAP and IRF-Lucia reporter cGAS knockout cells	3-7 x 10^6 cells	thpd-kocgas
	THP1-Dual™ KO-STING Cells	NF-κB-SEAP and IRF-Lucia reporter STING knockout cells	3-7 x 10^6 cells	thpd-kostg
	THP1-Dual™ KI-hSTING-A162 Cells	NF-κB-SEAP and IRF-Lucia reporter A162 human STING knockin cells	3-7 x 10^6 cells	thpd-a162
	THP1-Dual™ KI-hSTING-H232 Cells	NF-κB-SEAP and IRF-Lucia reporter H232 human STING knockin cells	3-7 x 10^6 cells	thpd-h232
	THP1-Dual™ KI-hSTING-M155 Cells	NF-κB-SEAP and IRF-Lucia reporter M155 human STING knockin cells	3-7 x 10^6 cells	thpd-m155
	THP1-Dual™ KI-hSTING-R232 Cells	NF-κB-SEAP and IRF-Lucia reporter R232 human STING knockin cells	3-7 x 10^6 cells	thpd-r232
	THP1-Dual™ KI-hSTING-S154 Cells	NF-κB-SEAP and IRF-Lucia reporter S154 human STING knockin cells	3-7 x 10^6 cells	thpd-s154

STING activation and signaling

In agreement with its central implication in the induction of innate immune responses, STING is found throughout the body, notably in the barrier organs[3]. It is expressed most strongly in skin endothelial cells, alveolar type 2 pneumocytes, bronchial epithelium and alveolar macrophages[2,22]. STING-dependent cytokine induction has been evaluated in diverse cell types either *ex vivo* (in whole blood[14] and in primary cells such as peripheral blood mononuclear cells[23]) or *in vitro* in cell lines (human THP-1 monocytes[14,23-25], HEK293 human embryonic kidney cells[2], RAW murine macrophages[14,25,26] and B16 murine melanoma[25]). This is typically done by treating cells with STING agonists and then assaying for production of type I IFNs, TNF-α or other cytokines. STING-dependent cytokine induction can then be confirmed by using STING-KO cells, STING pathway inhibitors, siRNAs or other tools. Notably, STING is either deactivated, undetectable or not expressed in certain cell lines, such as HEK293T and HeLa human cervical cancer[27,28]. InvivoGen provides numerous human and murine cell lines where the wild-type STING gene has been knocked out or replaced by a STING variant.

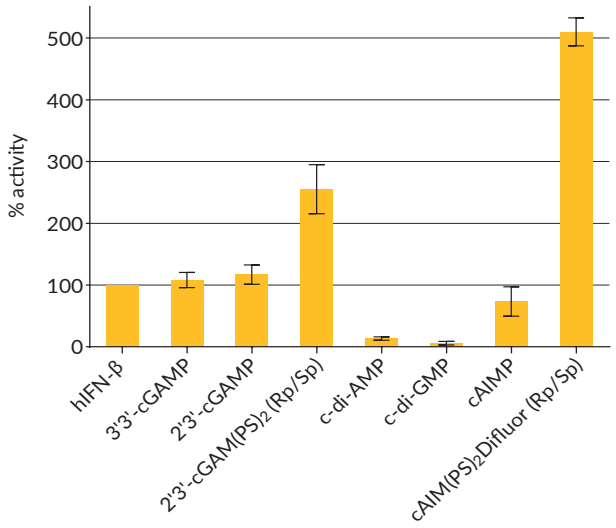


Figure 2: Induction of the interferon regulatory factor pathway by various STING ligands in THP1-Dual™ cells. IRF induction was determined by measuring the relative light units (RLUs) in a luminometer using QUANTI-Luc™, a Lucia luciferase detection reagent. The IRF induction of each ligand is expressed relative to that of hIFN-β at 1 x 10^4 U/ml (taken as 100%).

Table 2: Cyclic dinucleotides and DMXAA

CATEGORY	PRODUCTS	DESCRIPTION	UNIT SIZE	CAT.CODE
cGAMP	**3'3'-cGAMP**	Cyclic [G(3',5')pA(3',5')p]	500 µg	tlrl-nacga
	3'3'-cGAMP VacciGrade™	Preclinical grade of cyclic [G(3',5')pA(3',5')p]	1 mg	vac-nacga
	2'3'-cGAMP	Cyclic [G(2',5')pA(3',5')p]	500 µg	tlrl-nacga23
	2'3'-cGAMP VacciGrade™	Preclinical grade of cyclic [G(2',5')pA(3',5')p]	1 mg	vac-nacga23
	2'3'-cGAM(PS)2 (Rp/Sp)	Bisphosphorothioate analog of 2'3'-cGAMP	250 µg	tlrl-nacga2srs
c-di-GMP	**c-di-GMP**	Cyclic [G(3',5')pG(3',5')p]	1 mg	tlrl-nacdg
	c-di-GMP VacciGrade™	Preclinical grade of cyclic [G(3',5')pG(3',5')p]	1 mg	vac-nacdg
	2'3'-c-di-GMP	Analog of c-di-GMP	500 µg	tlrl-nacdg23
c-di-AMP	**c-di-AMP**	Cyclic [A(3',5')pA(3',5')p]	1 mg	tlrl-nacda
	c-di-AMP VacciGrade™	Preclinical grade of cyclic [A(3',5')pA(3',5')p]	1 mg	vac-nacda
	2'3'-c-di-AMP	Analog of c-di-AMP	500 µg	tlrl-nacda23
	2'3'-c-di-AM(PS)2 (Rp,Rp)	Bisphosphorothioate analog of 2'3'-c-di-AMP	100 µg	tlrl-nacda2r-01
	2'3'-c-di-AM(PS)2 (Rp,Rp) VacciGrade™	Preclinical grade of bisphosphorothioate analog of 2'3'-c-di-AMP	500 µg	vac-nacda2r
c-AIMP	**cAIMP**	Cyclic [A(3',5')pI(3',5')p]	500 µg	tlrl-nacai
	cAIMP Difluor	Difluor cyclic [A(3',5')pI(3',5')p]	250 µg	tlrl-nacaidf
	cAIM(PS)2 Difluor (Rp/Sp)	Difluor and bisphosphorothioate analog of cAIMP	100 µg	tlrl-nacairs
Non-CDN	**DMXAA**	5,6-dimethyl-xanthenone-4-acetic acid	5 mg	tlrl-dmx

Inhibition of cGAS/STING signaling

cGAS/STING signaling can be blocked directly or indirectly by endogenous, exogenous and synthetic molecules. Several biotech and pharma companies are developing cGAS or STING antagonists for therapeutic applications, notably in autoimmune disorders associated with type I interferonopathy (excessive production of type I IFNs). Such molecules could mimic pathogenic proteins which impair the STING pathway in order to subvert the immune system and facilitate infection. Examples include dengue virus NS2B protein[29], hepatitis B virus polymerase[30], herpes simplex virus 1 (HSV-1) ICP27[31], human cytomegalovirus tegument protein UL82[32], influenza A virus fusion protein[33] and Shigella protein IpaJ[34]. It was shown that STING is also inhibited via direct binding to E1A and E7 viral oncogenes[28].

Moreover, numerous synthetic molecules can inhibit the cGAS/STING pathway at different points upstream or downstream of STING. For instance, Steinhagen *et al.* showed that oligonucleotides (ODN) containing repetitive TTAGGG motifs, such as ODN A151, act as cGAS competitive inhibitors[35]. Mukai *et al.* used 2-bromopalmitate, an inhibitor of STING palmitoylation, to block IFN production in HEK293 cells expressing constitutively active STING mutants[36]. Alternatively, Pokatayev *et al.* used the TBK1 inhibitor BX795 to attenuate cytokine production in mutant mouse embryonic fibroblasts that they employed to model STING dependent autoinflammation[37]. Chen *et al.* employed SB202190, a p38 MAPK inhibitor, to block STING deubiquitination during HSV-1 viral escape[38]. Finally, McFarland *et al.* separately tested the NF-κB inhibitors Celastrol, Bay 11-7082 and MG-132 in cellular assays of STING signaling in response to bacterial infection[17].

Table 3: Synthetic inhibitors of the cGAS/STING pathway

PRODUCTS	DESCRIPTION	UNIT SIZE	CAT.CODE
Amlexanox	TBK1/IKKε inhibitor	50 mg	inh-amx
Bay 11-7082	IκB-α inhibitor - NLRP3 inflammasome inhibitor	10 mg	tlrl-b82
BX795	TBK1/IKKε inhibitor - TLR signaling inhibitor - RLR inhibitor	5 mg	tlrl-bx7
Celastrol	NF-κB inhibitor	1 mg	ant-cls
MG-132	26S proteasome inhibitor - Autophagy activator	5 mg	tlrl-mg132
ODN TTAGGG (A151)	cGAS inhibitor - TLR9 inhibitor - AIM2 inhibitor	1 mg	tlrl-ttag151-1
SB202190	MAP kinase inhibitor - Autophagy inducer	5 mg	tlrl-sb90

STING regulation

Although STING is best known for its role in immune responses to cytoplasmic DNA sensed by cGAS, it is also implicated in signaling pathways for other DNA or RNA sensors, autophagy, ER stress and metabolism. Furthermore, STING, like other PRRs, is also regulated through numerous post-translational modifications that ensure its proper location, timing and function (Fig. 3).

DNA pathways

STING has been linked to DNA sensors other than cGAS. There are reports of STING signaling following detection of viral[39] or bacterial[40] DNA by IFI16, and after detection of viral DNA by DDX41[41]. Interestingly, STING appears to be regulated by the cytoplasmic DNA sensor AIM2. Corrales *et al.* reported that in murine macrophages and dendritic cells, the AIM2 inflammasome antagonizes the STING pathway during the response to cytoplasmic DNA, by inducing pyroptosis via caspase 1[23]. Along these lines, Liu *et al.* found that during infection of murine macrophages with *Mycobacterium bovis*, AIM2 conjugates to bacterial DNA in the cytoplasm to inhibit STING-dependent induction of IFN-β and autophagy[42]. STING also seems to be connected to genomic structure and repair mechanisms. For example, Malik *et al.* claimed that STING can induce chromatin compaction, and they suggested that this effect might be linked to immune responses (e.g. antiviral response) or to nuclear envelope-linked diseases[43]. Interestingly, constitutive activation of the cGAS/STING pathway in a DNA damage response deficient subtype of breast cancer in a cell cycle-specific manner has been described[44]. These findings are consistent with other reports, which linked STING signaling to the DNA-damage sensor MRE11, and to the DNA replication and repair enzyme RAD51 (via MRE11)[45,46]. Further research revealed that the Cre/LoxP system of gene recombination leads to accumulation of damaged DNA and ultimately, to STING activation[47]. Other evidence suggests that STING signaling following DNA viral infection is dampened by caspase 1-mediated cleavage of cGAS[48]. Last, cGAS/STING signaling has also been widely described as a response to mitochondrial DNA (mtDNA) that enters the cytoplasm, as occurs during oxidative stress[49].

RNA pathways

STING appears to interact with RNA sensing pathways at different levels. These interactions can occur directly via RNA sensors such as MDA-5 and RIG-I[50], and via MAVS[51] a RIG-I activated adaptor protein. STING may also take part in RNA pathways indirectly, after enzymatic conversion of cytoplasmic DNA into RNA (or vice versa)[52]. Indeed, the efficacy of the RIG-I ligand 5'ppp-dsRNA as protection against HSV-1 infection correlates directly to STING expression levels, indicating the importance of STING in anti-viral RIG-I signaling[53]. Remarkably, this trend extends to murine models: STING-KO mice treated with 5'ppp-dsRNA are highly susceptible to HSV-1, whereas WT mice receiving the same treatment are protected[53]. Other work reported that activation of RIG-I induces expression of STING[54], suggesting a functional link between the two. Likewise, it has been observed that DNA damage in bone marrow-derived macrophages leads to activation of the cGAS/STING pathway, which in turn induces type I IFNs and upregulation of immune genes, including RIG-I[55]. Recent findings highlighted an important link between cytoplasmic nucleic acid pathways (cGAS/STING or MDA5/MAVS) and the endosomal RNA sensor TLR7 in a model of malaria infection[56]. Indeed, activation of the cytoplasmic nucleic acid pathway induces SOCS1, which in turn inhibits MyD88, the adaptor protein for TLR7[56]. Finally, it was revealed that the dimerization, translocation and activation of STING during pathogenic infection requires the adaptor protein TRIF, an adaptor for the RNA sensor TLR3[57].

Autophagy

cGAS/STING signaling has been linked to autophagy on various levels. For example, cGAS has been reported to be degraded by p62-dependent selective autophagy after it senses cytoplasmic DNA[58]. During infection with live Gram-positive bacteria such as *Listeria innocua*, phagocytes undergo autophagy following STING-dependent sensing of c-di-AMP[59]. During *Mycobacterium tuberculosis* infection, the cGAS/STING axis triggers both type I IFN production and autophagy[60], including selective autophagy of this pathogen[61]. On the contrary, during *Mycobacterium bovis* infection, the cytoplasmic DNA sensor AIM2 has been reported to inhibit STING induced autophagy[42]. There have been reports that cGAS and STING each interacts with autophagy proteins in other contexts, although the nature of these interactions remains unclear. For instance, direct interaction between cGAS and Beclin-1 halts production of 2'3'-cGAMP by the former[62], thus preventing constitutive activation of STING and consequently, hyperproduction of type I IFNs. Furthermore, following activation, STING seems to be trafficked from the endoplasmic reticulum (ER) to the Golgi by an autophagy-like process that depends on Atg9a[63].

ER-stress and apoptosis

A role for the STING/IRF3 axis in alcoholic liver disease has been described, whereby ethanol-induced ER stress leads to activation and phosphorylation of IRF3 via STING. This drives the interaction of p-IRF3 with the pro-apoptotic protein Bax, and eventually to apoptosis of hepatocytes[64], which was subsequently reported to be STING- and IRF3-dependent[65]. Further research has found that the Bax/Bak-mediated apoptosis generates DNA that activates the cGAS/STING pathway to induce type I IFNs and that this response is blocked by apoptotic caspases such as Apaf 1, caspase-3/7 and caspase-9[66]. Studies in B cells revealed that STING function depends on ER stress responses conveyed through the IRE 1/XBP-1 pathway and that STING agonists provoke mitochondria-mediated apoptosis[67]. Finally, ER stress-induced apoptosis during *Mycobacterium bovis* infection has been linked to the STING/IRF3 axis[68].

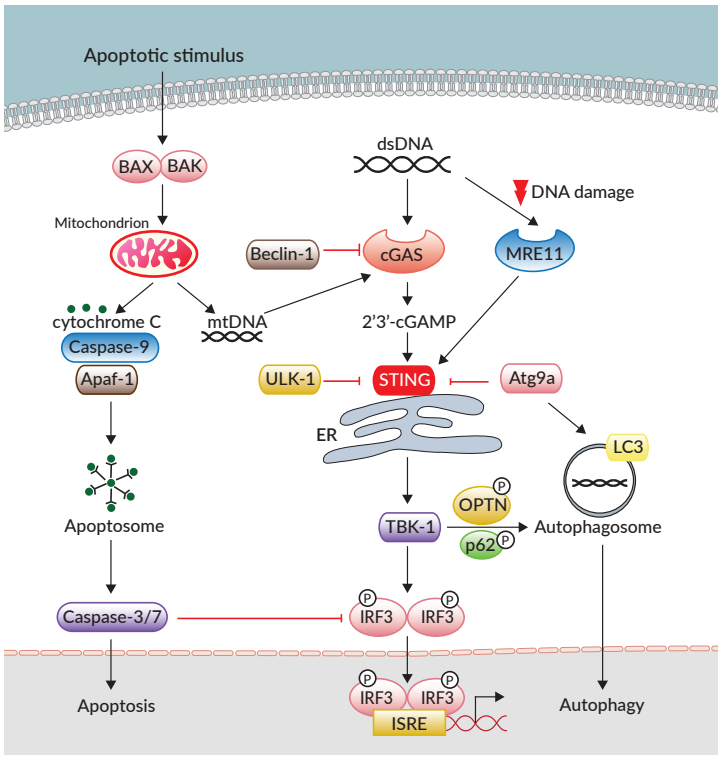


Figure 3: Regulation of STING signaling.

Post-translational regulation

Various post-translational modifications have been described for STING, spanning its activation up to its degradation. However, the spatiotemporal sequence of these modifications remains poorly understood. Both STING and cGAS appear to be stabilized upon sumoylation by TRIM38 relatively early in signaling[69]. Activation of STING seems to require palmitoylation, presumably by DHHC proteins[36]. Phosphorylation of STING by TBK1 enables its binding to IRF3, whereas phosphorylation by ULK1 appears necessary for its degradation[70]. Likewise, ubiquitination of STING by ubiquitin ligases can serve different purposes such as promoting TBK1 recruitment (TRIM32, TRIM56 or AMFR); enabling degradation (RNF5); or preventing degradation (RNF26)[70]. STING-β, a human alternative STING transcript isoform which lacks the transmembrane domains, has been shown to antagonize STING function through 2'3'-cGAMP and other transducer molecules (e.g. TBK1) sequestration[71].

Genetic variation of STING

Human STING is encoded by the gene Tmem173, which appears in several variants within the population. Recent work has revealed that the sequence differences among these variants can markedly affect STING function and consequently, impact human health (Fig. 4).

Early work on human STING variants suggested that they vary widely in their responsiveness to microbial CDNs. For instance, the variants R232H and HAQ (R71H-G230A-R293Q) are drastically less sensitive to c-di-GMP and c-di-AMP than their most prevalent variant, 232R-RGR (71R-230G-293R), the latter of which accounts for roughly 60% of the human population[72]. Importantly, the HAQ variant has proven to be a null allele that does not respond to 2'3'-cGAMP or the potent synthetic CDN STING agonist 2'3'-c-di-AM(PS)2(Rp,Rp)[73]. The open questions on the functionality of STING-HAQ are especially relevant to THP1 cells, which carry this variant.

Other genetic variations leading to loss or gain of STING functionality have been revealed. An alternative splicing isoform of STING has been identified: it lacks exon 7 and acts as a dominant negative regulator of type I IFN production as it cannot bind to TBK1[74]. This STING variant, however, does not impair the NF-κB pathway. On the contrary, other mutations in STING can lead to excessive activation of interferon stimulated genes (ISGs). This is the case in patients suffering from STING-Associated Vasculopathy with Onset in Infancy (SAVI), a pediatric condition characterized by excessive inflammation, dermatologic and pulmonary tissue damage, as well as abnormal antibody production. SAVI-patients carry one of four mutations in exon 5 (V147L, N154S, V155M or V147M) leading to constitutive type I IFN production, probably due to constitutive dimerization of STING, although this is still unclear[22,75,76,77]. Recently, three more gain-of-function mutations in SAVI-like patients have been uncovered: C206Y, R281Q and R284G[78]. These findings suggest that more pathogenic STING alleles may be soon discovered. Last, another case of dominant STING gain-of-function mutation (G166E) has been identified in several family members with chilblain lupus[79].

Table 4: STING gene variants

STING VARIANT	DESCRIPTION	CAT.CODE*
STING-WT	R232 isoform	puno1-hstingwt
STING-WT-HA	HA-tagged coding sequence	puno1ha-hsting
hSTING-A162	A162 isoform (S162A)	puno1-hsting-a162
hSTING-A230	A230 isoform (G230A)	puno1-hsting-a230
hSTING-H232	H232 isoform (R232H)	puno1-hsting-h232
hSTING-HAQ	HAQ (R71H-G230A-R293Q) isoform	puno1-hsting-haq
hSTING-M155	M155 isoform (V155M)	puno1-hsting-m155
hSTING-MRP	Isoform lacking exon 7	puno1-hsting-mrp
hSTING-N200	N200 isoform (I200N)	puno1-hsting-n200
hSTING-S154	S154 isoform (N154S)	puno1-hsting-s154

* All plasmids are provided as 20 µg of lyophilized DNA.

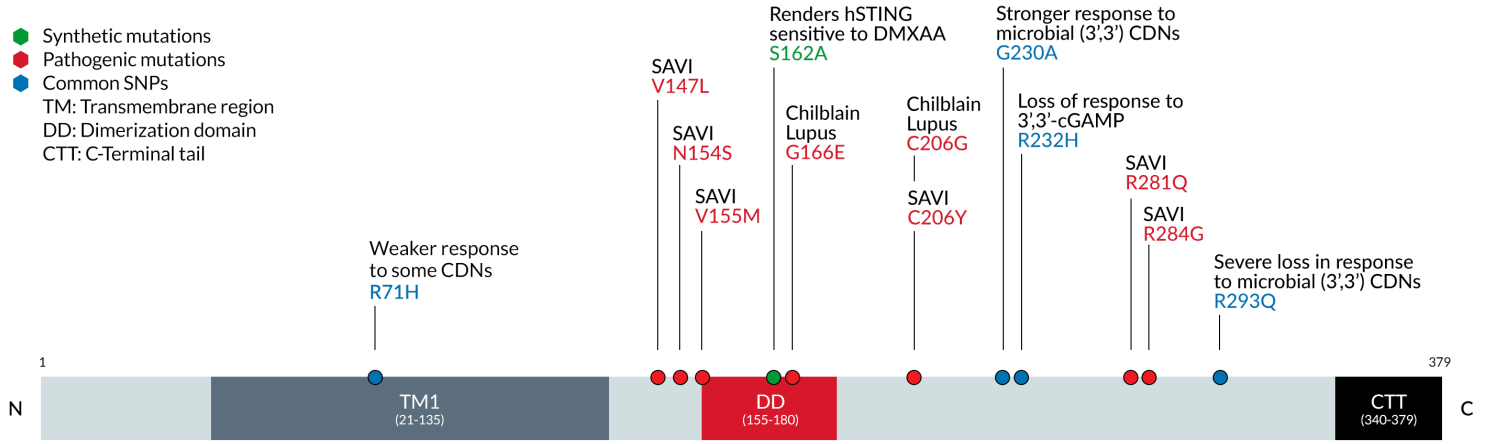


Figure 4: Schematic of human STING genetic variants and their functional effect or disease association.

Therapeutic targeting of STING

The cGAS/STING axis interacts with other innate immune pathways, such as DNA sensors (AIM2 and IFI16) and RNA sensors (RIG-I and MDA5). Therefore, STING is implicated in many health disorders such as infectious diseases, cancer, and autoimmunity.

Viral infections

Researchers have been evaluating CDN STING agonists as vaccine adjuvants long before the discovery of STING[80]. Detection of viral DNA (e.g. herpes virus) or RNA (e.g. coronavirus) by cGAS and/or other sensors leads to activation of STING, which induces a robust anti-viral response through type I IFNs[81]. Also, a cGAS-independent activation of STING has been reported: enveloped-RNA viruses fuse to the host cell membrane, and then directly interact with and activate STING[33]. Interestingly, the 2'3'-cGAMP produced by cGAS in infected cells can travel within viral particles[82], or via gap junctions[83], to reach neighboring cells, thus propagating a local anti-viral response. Of note, many viruses emit peptides and proteins that directly or indirectly inhibit host STING signaling. Examples include dengue virus, whose protease complex NS2B/3 binds to and cleaves STING, and hepatitis C virus, whose protein NS4B also binds to and deactivates STING[50]. Moreover, certain host endogenous factors can block STING signaling upon viral attack, such as the mitochondrial-localized protein NLRX1, which is required for HIV-1 infection[84]. Together, these findings underscore the evolutionary tit-for-tat between viral pathogens and host immunity.

Bacterial infections

Although bacteria produce CDNs, STING signaling upon bacterial infection is principally dependent of 2'3'-cGAMP production by cGAS. Bacterial species known to activate STING via cGAS include Mycobacteria, Legionella, Listeria, Shigella, Francisella, Chlamydia, Neisseria and group B Streptococcus[49]. Thus, STING-dependent production of type I IFNs has been reported in diverse cellular and animal models of bacterial infection such as *Streptococcus pneumoniae* in mice[85]. Among examples of direct activation of STING by bacterial CDNs, *Mycobacterium tuberculosis* releases c-di-AMP into the cytoplasm[24]. Furthermore, c-di-AMP has been imputed in infection by *Listeria monocytogenes*, although its importance relative to that of bacterial DNA, and the resulting STING-induction remains unclear[86,87].

Cancer

Tumor DNA can induce antigen-presenting cell activation through the cGAS/STING axis and thus contribute to anti-tumor immunity through priming of antigen-specific cytotoxic CD8+ T cells. This immunosurveillance mechanism has been reported in models of breast cancer[88], colorectal cancer[89] and melanoma[90], among others. In fact, it has also been shown to underpin the efficacy of radiation therapy, through immunostimulatory DNA release by dying irradiated tumor cells[91]. Based on this, the synthetic CDN STING agonist 2'3'-c-di AM(PS)2 (Rp/Rp) is being evaluated in a Phase I clinical trial for solid tumors and blood cancers[92].

Perplexingly, deficient or excessive STING activities have each been imputed in cancer. The former involves tumor cell survival enabled by a lack of tumor-suppressive interleukine-22 binding protein induction[93]— indeed, many tumors lack active cGAS or STING[89]— whereas the latter involves inflammatory tumorigenesis caused by excessive cytokine production, as reported in models of colitis[94] and brain metastasis[51]. Also supporting a pernicious role for STING in certain cancers are findings that STING activation can induce expression of factors that inhibit effector T cells, such as IDO[95] and PD-L1[44]. Cancer therapies related to the cGAS/STING pathway must therefore account for the functional activity of this axis in tumor and healthy cells.

Autoimmunity

Accumulation of self-nucleic acids (DNA, RNA, or DNA/RNA hybrids) in the cytoplasm leads to constitutive activation of cGAS/STING signaling and production of inflammatory cytokines that, when chronic, can cause autoimmune diseases. A common pathologic trait in these cases is dysregulated enzymatic processing of DNA or RNA, as with mutated Trex1 or RNAse H2, in Aicardi-Goutières Syndrome (AGS)[49], or mutated POLA1, in X-Linked Reticulate Pigmentary Disorder (XLRPD)[96]. However, such autoinflammation can also derive from mutations in STING itself, leading to its constitutive activation, as in a lupus-like syndrome[75] or in SAVI[22]. To date, at least eight STING mutations separately leading to autoinflammation have been identified, some of which (e.g. V147L, N154S and V155M) seem to induce constitutive exit of STING from the ER[34]. Alternatively, significantly elevated cGAS expression has been reported in lupus patients relative to control subjects, those with detectable 2'3'-cGAMP in their peripheral blood exhibited worse symptoms[97]. Given all

these findings, inhibitors of cGAS/STING signaling, including cGAS or STING antagonists, are now being pursued as possible therapies for autoimmune diseases.

Conclusion

A decade of research has brought to light STING as a key adaptor in the immune response to cytosolic nucleic acids in numerous situations. The activation or repression of STING and its signaling is of great interest in many therapeutic fields including microbial infections, cancer, and autoimmune disorders (Fig. 5). Although major advances have been made through crystallization, genetic and functional characterization of STING variants, much more needs to be unraveled as more sophisticated levels of STING regulation are being uncovered. As an example, an intriguing regulation loop has been discovered between type I IFN production and lipid metabolism in order to induce DNA-independent STING activation and to counter viral infectivity[98]. Moreover, because STING and other nucleic sensors pathways (RIG-I/MDA-5, AIM2, and TLRs) converge to the same downstream signaling, better comprehension of their complex interplay is required to develop therapeutic drugs that regulate the inflammatory innate and adaptive immune responses. Last, the next challenge will be to establish suitable *in vivo* delivery of such molecules.

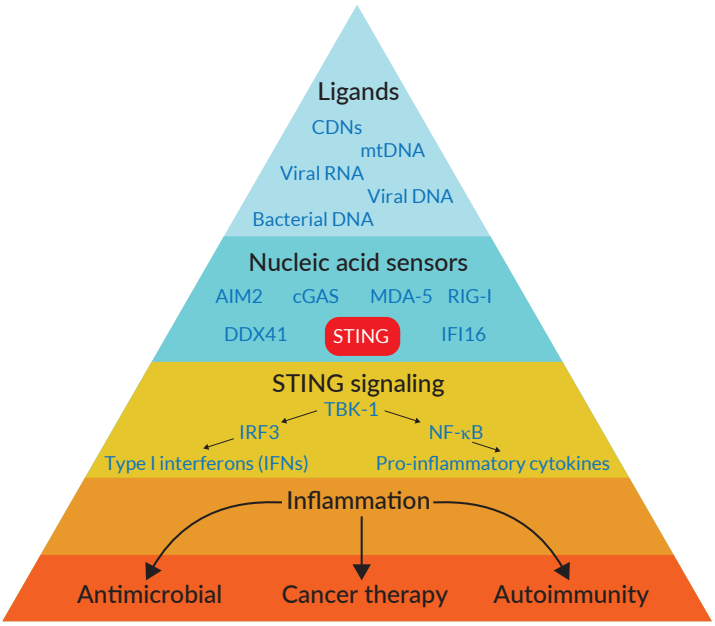


Figure 5: Central role of STING in sensing nucleic acids and in inflammation.

Abbreviations

CDNs: cyclic dinucleotides
cGAS: cyclic GMP-AMP synthase
ER: endoplasmic reticulum
IFNs: interferons
IRF3: interferon regulatory factor 3
ISGs: interferon-stimulated genes
ISRE: interferon-responsive element
NF-κB: nuclear factor kappa light-chain-enhancer of activated B cells
PRR: pattern recognition receptor
SAVI: STING-Associated Vasculopathy with Onset in Infancy
SNP: single nucleotide polymorphism
STING: stimulator of interferon genes
TBK1: TANK-binding-kinase-I
TNF-α: tumor necrosis factor alpha

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