Trends in Biochemical Sciences

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Review

The Evolution of STING Signaling and Its Involvement in Cancer

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The cyclic GMP-AMP synthase (cGAS)–stimulator of interferon genes (STING) pathway has been primarily characterized as an inflammatory mechanism in higher eukaryotes in response to cytosolic double-stranded DNA (dsDNA). Since its initial discovery, detailed mechanisms delineating the dynamic subcellular localization of its different components and downstream signaling have been uncovered, leading to attempts to harness its proinflammatory properties for therapeutic benefit in cancer. Emerging evidence, however, indicates that a crucial primordial function of STING is to promote autophagy, and that downstream interferon (IFN) signaling emerged recently in its evolutionary history. Furthermore, studies suggest that this pathway is a crucial regulator of cellular metabolism that potentially couples inflammation to nutrient availability. We focus on the evolutionarily conserved functions of STING, and we discuss how a broader understanding of this pathway can help us to better appreciate its potential role in cancer and harness it for therapeutic benefit.

Highlights

The cGAS–STING pathway is a crucial antiviral defense mechanism in mammalian cells.

The role of cGAS–STING in cancer is complex because this pathway has been shown to exhibit both tumor-suppressive and tumor-promoting effects.

Although STING was initially identified as a mediator of IFN signaling, recent studies have revealed that autophagy, and not IFN signaling, is a more highly conserved function in evolution.

Mounting evidence suggests that STING signaling is complex and is dependent on interacting partners at the endoplasmic reticulum (ER).

Disruption of STING signaling impacts on ER stress and unfolded protein responses, NF-κB signaling, and cellular metabolism.

The IFN-independent functions of STING explain its tumor-promoting and prometastatic effects.

The cGAS–STING Pathway: A Brief History

The importance of cytosolic double-stranded (ds) nucleic acid signaling in higher eukaryotes has been the subject of renewed focus over the past two decades following the discovery of the **cGAS–STING pathway** (see Glossary) and the **RIG-I pathway** that sense and respond to cytosolic dsDNA and dsRNA, respectively [1–3]. Cytosolic dsDNA promotes a robust type I IFN response downstream of cGAS dsDNA sensing and the generation of the cyclic dinucleotide cGAMP (cyclic GMP-AMP) [4]. cGAMP subsequently activates the effector protein, STING, that promotes downstream inflammatory signaling defined by the induction of interferon-stimulated genes (ISGs) [5,6]. Following its discovery in 2008, many studies centered on the proinflammatory nature of STING signaling and its regulation, focusing primarily on its role as an antiviral defense mechanism [7]. Later, the majority of studies in cancer research depicted STING activation as tumor-suppressive [8], which motivated the development of STING agonists. The rationale behind the development of STING agonists is to reactivate IFN signaling and antitumor immunity through direct STING engagement in the tumor microenvironment [9].

Recent analysis of STING protein sequences, however, demonstrated that the IFN signaling-related function of this protein, that is mediated by its C-terminal tail (CTT), is a relatively recent addition, and that its conserved role is the induction of cellular autophagy in response to cytosolic dsDNA [10]. Furthermore, the identification of crosstalk between **mammalian target of rapamycin complex 1** (mTORC1) signaling and the cGAS–STING pathway uncovered a crucial role for this signaling axis in insulin resistance, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD) [11–14]. Even the role of dsDNA as a sole activator of STING has been challenged by studies reporting that viral particle fusion with the plasma membrane is sufficient to promote STING-dependent IFN signaling in a dsDNA- and cGAS-independent manner [15,16]. Similarly, in cancer, the tumor-suppressive role of cGAS–STING signaling has been challenged by evidence demonstrating that it can contribute to tumorigenesis as well as to cancer

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progression and metastasis [17,18]. Therefore, the cellular and systemic functions of cGAS–STING signaling extend well beyond its role as an IFN-inducing pattern recognition receptor, and appear to be highly context-dependent.

We discuss here the diverse roles of the cGAS–STING pathway, covering the spectrum of its evolutionarily conserved functions. We propose that a more comprehensive understanding of how STING impacts on cellular homeostasis and stress responses will enable a more rigorous understanding of its context-dependent role in cancer, and allow it to be successfully harnessed for therapeutic benefit.

The Evolution of Cytosolic Nucleic Acid Sensing and Signaling

Sensing of cytosolic ds nucleic acids is one of the crucial components of pattern recognition and pathogen evasion. It is not surprising, therefore, that nucleic acid sensors are conserved across most forms of life. Multiple studies have explored the evolutionary origins and conservation of cGAS across the Kingdom of Life [19,20]. An enzyme called DncV in *Vibrio cholera* was shown to synthesize cyclic dinucleotide second messengers. In *Escherichia coli*, DncV can synthesize c-AMP-GMP and c-di-AMP [21]. Later, an enzyme synthesizing cUMP-AMP was characterized from *E. coli* and, because of its structural homology to DncV, it was termed DncV-like nucleotidyltransferase (encoded by *cdnE*) [22]. Interestingly, these early cGAS proteins in both *E. coli* and *V. cholera* activate phospholipase A2, resulting in pores in the inner membrane of bacteria and eventual cell death during bacteriophage infection, thus preventing the spread of infection to neighboring cells [23,24].

Similarly to cGAS, bioinformatic analyses have identified candidate STING homologs in bacteria and diverse metazoans, and suggest that most homologs have a conserved ability to bind **cyclic dinucleotides** (CDNs) [25,26]. Recently, Morehouse *et al*. reported the presence of bacterial STING homologs that primarily occur as fusions to the Toll/interleukin-1 receptor (TIR) adaptor domain [27]. The bacterial STING was also observed to drive oligomerization of TIR effector domains and rapid NAD^+ cleavage during bacteriophage infection, enabling host survival. The best-characterized functional eukaryotic STING homolog was identified in the sea anemone, *Nematostella vectensis* (nvSTING). Interestingly, and despite only 29% sequence homology between nvSTING and human STING (hSTING), their 3D structures are superimposable. Both proteins crystallize in distinct open and closed conformations, revealing their dynamic nature. Further, nvSTING has a higher affinity for 2′,3′ cGAMP than the bacterial 3′,3′ cGAMP, similarly to hSTING (<1 nM) [19,20,28].

The recent evolutionary addition to STING, the CTT, is present in most vertebrates, but with some exceptions including amphibians such as *Xenopus tropicalis* and *X. laevis* [20,26]. The STING CTT is crucial for type I IFN induction and for the recruitment of TBK-1 and IRF-3. The STING protein in *X. tropicalis* and *X. laevis* is able to bind 2′-3′ cGAMP, as assayed by electrophoretic mobility shift assay, but its signaling function and role in immunity to viral infections remain to be tested. nvSTING, that also lacks the CTT, is unable to induce IFN-β; however, the chimeric STING produced by fusion with the human CTT produces low levels of IFN-β [19]. Therefore, the ability of STING to induce type I IFN signaling through IRF3 phosphorylation is an evolutionarily recent event.

Despite its wide conservation, there is evidence that STING has also undergone evolutionary divergence within the animal kingdom. For instance, all Nematoda lack STING, and some insect STING proteins do not bind CDNs [26]. It remains unclear whether insect STING can still signal in a CDN-independent manner, as has been shown with human STING.

Glossary

cGAS–STING pathway: a pattern-recognition pathway that senses cytosolic double-stranded DNA via cGAS (cyclic GMP-AMP synthase), followed by activation of STING (stimulator of interferon genes) leading to the activation of inflammatory pathways.
Chromosomal instability (CIN): a phenomenon in which cells exhibit increased rates of chromosome-segregation errors during cell division that lead to the formation of daughter cells abnormal karyotypes; CIN leads to genomic copy-number alterations and the presence of DNA in the cytosol.
Cyclic dinucleotides (CDNs): crucial signaling molecules/secondary messengers composed two nucleotides arranged in a cyclic configuration; 2′3′-cGAMP is produced by mammalian cGAS, whereas bacteria produce 3′3′-cGAMP, cyclic di-GMP, and cyclic di-AMP, thus acting as ligands for pattern-recognition receptors.
ERphagy: the degradation of endoplasmic reticulum (ER) via canonical autophagy pathway; usually reported to occur upon induction of ER stress during bacterial infection and release of bacterial cyclic-di-GMP.
Exosomes: endosomes budding intraluminally create a multivesicular body structure; this ultimately fuses with the plasma membrane and releases multiple membrane-bound vesicles that are defined as exosomes.
Lipolysis: the hydrolysis of lipids that primarily occurs in adipose tissue; breakdown of triglycerides leads to the production of glycerol and fatty acids.
Major histocompatibility complex (MHC): a protein complex present in all jawed vertebrates that is responsible for antigen presentation on the cell surface that leads to activation of T cell responses. The MHC-I complex is present on all cell types, whereas MHC-II is present only on antigen-presenting cells.
Mammalian target of rapamycin complex 1 (mTORC1): one of the two complexes of mTOR; mTORC1 is one of the central regulators of autophagy, senses nutrient availability, and governs macromolecular synthesis, among other functions.
NF-κB pathway: nuclear factor κ light-chain enhancer of activated B cells is a family of transcription factors that regulate inflammation and are crucial for T cell activation during an immune

Nonetheless, these organisms instead have robust RNA interference-based defense mechanisms to combat viral infection, and perhaps these and other alternative mechanisms predispose to loss of STING [20]. This type of evolutionary analysis might shed light on the essentiality of the various STING functions and potential redundancies with other nucleic acid sensors.

dsDNA- and cGAS-Independent Activation of STING

STING, also known as *TMEM173*, *ERIS*, *MITA*, or *MPYS*, was initially discovered as an endoplasmic reticulum (ER)-resident sensor of CDNs with varying orders of affinity [29–33]. It has four transmembrane helices that anchor it to the ER membrane, a cytoplasmic domain that binds the ligand, and the two domains are connected by a short linker [34,35]. Upon cGAMP binding, STING undergoes a conformational change that promotes higher-order oligomerization, followed by its transport via COPII vesicles from the ER via the ER–Golgi intermediate compartment (ERGIC) to the Golgi apparatus (GA), and finally to **perinuclear microsomes** where it recruits TBK1 and IKKα/β to induce proinflammatory transcriptional signaling [36]. Although the cellular compartment where STING associates with TBK1 remains controversial, ranging between ERGIC and GA, the association of STING and TBK1 is crucial for its downstream IFN signaling (Figure 1) [36–38]. Although the detailed stepwise mechanism for STING translocation is unknown, several effector proteins have been identified, namely the translocon proteins Trapβ, Sec61β, the transmembrane emp24 protein transport domain-containing 2 (TMED2), sorting nexin 8 (SNX8), inactive rhomboid protein 2 (iRhom2), the autophagy-associated phosphatidylinositol 3-kinase VPS34, and the ubiquitin regulatory X domain-containing protein 3B (UBXN3B) [3,39–43]. iRhom2 recruits EIF3s5, a deubiquitination enzyme that is involved in removal of its K48-linked polyubiquitin chains, thereby maintaining its stability as well as mediating recruitment of Trapβ for its downstream translocation [40]. SNX8 is crucial for the recruitment of VPS34 [42]. In addition, YIPF5, a member of the Yip family of proteins involved in ER–Golgi transport, was recently found to be crucial for STING translocation COPII vesicles, through which STING is transported to multiple membrane compartments [44]. Abrogation of COPII via deletion of SAR1A or SEC24 or downregulation of YIPF5 leads to defects in autophagy as well as in the IFN signaling function of STING [44]. Further, the STING ER exit protein (STEEP/C_XORF56) has been identified to increase phosphatidylinositol 3-phosphate (PI3P) production that is crucial for the ER membrane curvature that enables COPII-mediated exit [45]. Thus, trafficking of STING is dependent on the assembly of a complex machinery that governs its translocation from the ER, a crucial feature that regulates its multiple functions (Figure 1). Some studies have also highlighted that STING can be activated even in the absence of the ligand cGAMP (Box 1).

Retention of STING in the ER requires binding to other ER proteins (Figure 2). In a recent study, Srikanth *et al.* reported that the Ca^{2+} sensor STIM1 interacts with, and is responsible for, STING retention in the ER. *STIM1*$^{-/-}$ THP1 cells exhibited increased STING dimerization and phosphorylation of TBK1 and IRF3, resulting in the activation of type I IFNs and inflammatory cytokines [46]. It remains unclear whether STING activation in *STIM1*$^{-/-}$ cells requires cGAMP. However, the clinical features of patients with inactivating mutations in *STIM1* are similar to those with STING-associated vasculopathy with onset in infancy (SAVI), wherein mutant STING is constitutively active. In related work, it was found that Toll-interacting protein (TOLLIP) is also a crucial stabilizer of STING that retains it at the ER in fibroblasts [47]. Destabilization of this interaction led to enhanced lysosomal degradation of STING mediated by the ER stress-response regulator, IRE-1α. Thus, STING acts a versatile signaling hub to integrate multiple stimuli, highlighting the need to understand the distinct roles of its independent domains in its overall function.

response; this pathway is further divided into canonical and noncanonical pathways in which the canonical pathway is crucial for immune responses to cancers and the noncanonical pathway activation leads to metastasis.
Perinuclear microsomes: a vesicular component that consists of part of the ER membrane bound by ribosomes and is found in the vicinity of the nucleus.
RIG-I pathway: a pattern-recognition receptor that is responsible for detecting cytosolic double-stranded RNA including viral RNA and promotes type I interferon production.

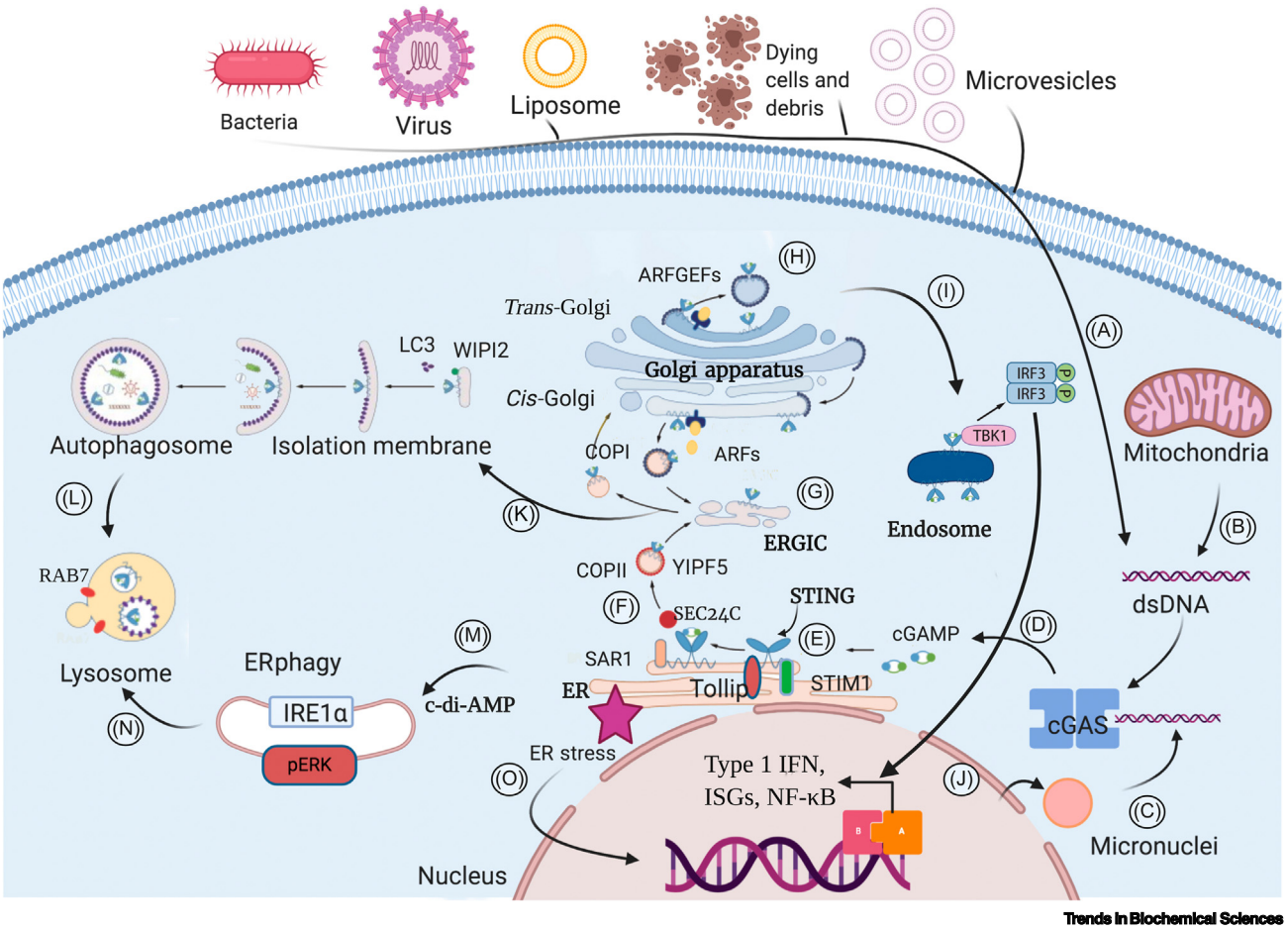

Figure 1. The cGAS–STING Signaling Cascade. Double-stranded (ds) DNA from multiple extracellular sources, such as bacteria, viruses, dying cells, and microvesicles (A), as well as intracellular sources such as mitochondria (B) and micronuclei generated from primary nucleus (C), is sensed by cGAS that synthesizes cyclic GMP-AMP (cGAMP) (D). cGAMP activates STING, that is resident in the endoplasmic reticulum (ER), via an interaction with proteins such as STIM and TOLLIP (E). Upon cGAMP binding, STING is transported via COPII vesicles (F) from the ER to the ER–Golgi intermediate compartment (ERGIC) (G) to the Golgi apparatus in a process that is dependent on SAR1, Sec24C, YIPF5, and ARF family members (H). STING is finally trafficked to endosomes (I) where it also interacts with TBK1 and IRF3, culminating in type I interferon (IFN) production (J). From the ERGIC, STING also provides a double-membrane scaffold for the formation of autophagosomes formation (K) that ultimately fuse with lysosomes (L). In addition, bacterial c-di-AMP leads to ER stress, and this can initiate STING-mediated ERphagy (M) culminating in lysosomal fusion (N) as well as in type I IFN production (O). Figure created via Biorender.com. Abbreviation: P, phosphorylation.

STING and Autophagy

Earlier studies recognized that induction of cellular autophagy is a hallmark of viral infection. STING trafficking together with TBK1 into autophagosomes represents a crucial negative feedback mechanism that limits unchecked inflammation in response to cytosolic dsDNA [39,48,49]. Using STING orthologs from multiple organisms, Gui *et al.* demonstrated that transfection of dsDNA into BJ immortalized fibroblasts led to LC3 lipidation and the formation of double-membrane, LC3-II-bearing autophagosomes in addition to the widely reported IFN induction [10]. The specificity of this pathway for cytosolic dsDNA sensing was established because only dsDNA, and not dsRNA, resulted in autophagy induction accompanied by TBK1 and IRF3 phosphorylation. Importantly, expression of STING from *N. vectensis* or *X. tropicalis*, neither of which contains the CTT responsible for downstream activation of TBK1 and IRF3, or deletion of CTT from human STING, still led to autophagosome formation, indicating that autophagy is a primordial function of STING protein, which later on acquired the ability to induce IFN signaling [10]. It further suggests that the merger of

Box 1. Ligand-Independent Noncanonical Functions of STING

Although STING engagement by cGAMP is considered to be the canonical activation mechanism, emerging evidence suggests that STING can be activated in a cGAS- and cGAMP-independent manner. Binding of cationic liposomes or virus-like particles (VLPS) devoid of DNA to the plasma membrane can directly activate STING (see Figure 2 in main text) [15,16]. Thus, STING activation might confer cross-resistance to RNA viruses. Indeed, HEK293 cells transformed with HIV fusion protein ENV and cocultured with THP1 macrophages exhibited increased IFN production [15]. Furthermore, STING was found to be activated in a cGAS-independent manner upon infection by influenza A virus, an enveloped RNA virus [16]. This noncanonical mode of STING activation is mediated by a highly conserved arginine residue that is located away from the cGAMP-binding pocket, adjacent to the STING dimerization surface; the exact mechanism remains to be characterized [15]. Collectively, this supports the role of STING as a sensor of unscheduled membrane-fusion events. It is important to note that the magnitude of ISG induction in response to membrane fusion is significantly lower than that seen during cGAMP-mediated STING activation, suggesting that addition of the CTT of STING might have been the result of an evolutionary pressure to maximize ISG induction in response to viral infection, especially in multicellular organisms that have adaptive cell-mediated immunity [15].

STING can also function independently of cGAS in different protein complexes induced upon DNA damage by etoposide. IFI16, ATM, PARP-1, TP53, and TRAF6 form a complex that includes STING. TRAF6-mediated K63-linked ubiquitination of STING leads predominantly to NF-κB activation rather than to type I IFN signaling. Further, the formation of perinuclear punctate structures, a hallmark of ligand-dependent activation of STING, was absent in this process [104].

Naturally occurring gain-of-function mutations in STING have been found to promote ligand-independent activation, leading to diseases such as STING-associated vasculopathy with onset in infancy (SAVI), familial chilblain lupus, or systemic lupus erythematosus-like syndrome [105–108]. Point mutations in STING in patients suffering from these diseases show a constitutive presence of STING in ERGIC and Golgi membranes. Although some constitutively active mutations are thought to mimic ligand binding, some have postulated that other mutations might also prevent interaction with unidentified proteins that are responsible for its retention within the ER [36,107]. In a related study, Melki *et al.* identified three novel mutations on the surface of STING that lead to constitutive STING activation [108]. Therefore, it appears

these two functions is likely one of the key milestones in the evolution of a complex and robust immune response in multicellular organisms. Interestingly, evidence for **major histocompatibility complex** (MHC) conservation extends to jawed fish species, and it is possible that the gain of the STING CTT coincided with the emergence of cell-mediated immunity ensuring robust antigen presentation [50].

ERGIC membranes have been reported to be a membrane source for autophagosome formation, mediated by PI3 kinase and COPII signaling [51,52]. However, STING-mediated autophagy differs from conventional autophagy because it is independent of beclin 1, ULK1, and ULK2, but regulated by WIPI2 and ATG5. Furthermore, DNA degradation in these autophagosomes is crucial for reducing viral titers and for removal of cytosolic dsDNA following DNA damage [10,49]. This is supported by other studies showing that, although STING is not essential for rapamycin-dependent autophagy, it is required for cytosolic dsDNA-mediated autophagy [39]. It is possible that STING might have a more active and direct structural role in membrane trafficking than merely being passively trafficked as part of its regulation. The specificity of cargo loaded into STING-dependent autophagosomes remains to be explored.

The ability of STING to traffic through multiple membrane compartments upon activation (i.e., from ER to ERGIC to GA) makes it a good candidate to have evolved to enable the formation of double-membrane structures. Exploring this possibility will require advanced microscopic techniques and ideally mutations that specifically impact on STING trafficking-specific functions during each step of the pathway.

STING at the Nexus of Metabolism and Inflammation

Emerging evidence points towards an important role for cGAS–STING in cellular metabolic rewiring, suggesting that this pathway can couple inflammation to metabolism (Figure 3). In a

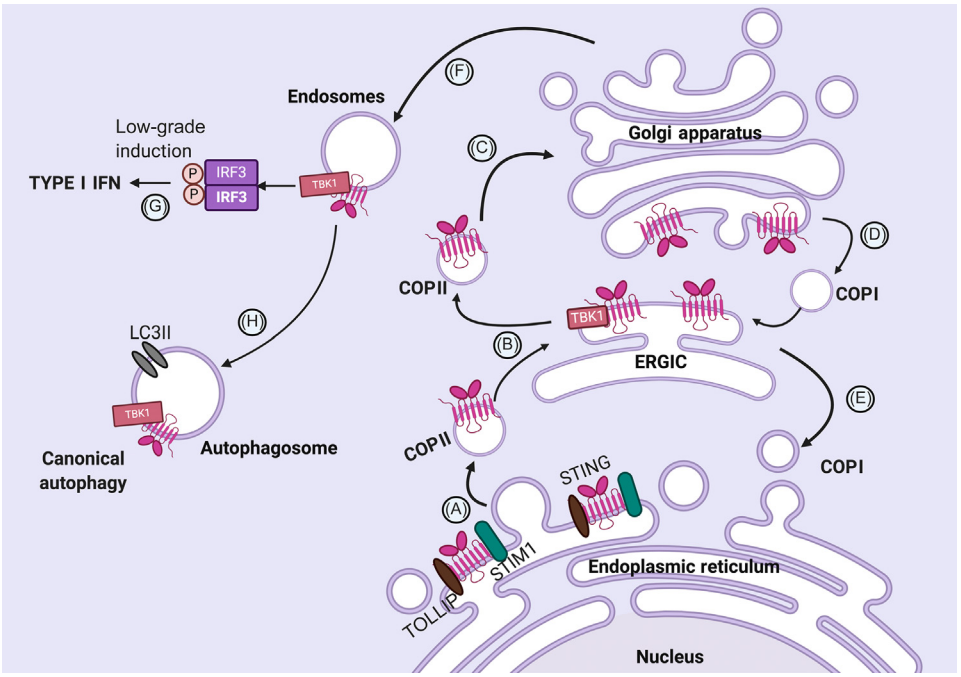

Figure 2. Ligand-Independent STING Activation. (A) STING (in purple) is anchored in the endoplasmic reticulum (ER) by multiple proteins such as TOLLIP and STIM1. (B,C) STING, when activated by liposomal fusion at the plasma membrane, triggers ligand-independent activation (in the absence of cyclic GMP-AMP, cGAMP), wherein it traverses from the ER to the ER–Golgi intermediate compartment (ERGIC) and from the ERGIC to the Golgi apparatus (GA) on COPII vesicles. (D,E) Retrograde GA to ERGIC to ER transport occurs via COPI vesicles. (F) It finally reaches the endosomes. STING putatively interacts with TBK1 in the ERGIC or GA. (G) The STING–TBK1 complex phosphorylates IRF3, resulting in type I IFN production but to a lower extent than that observed upon ligand-dependent activation. (H) STING is eventually degraded via canonical autophagy.

recent study by Bai *et al.*, induction of obesity in mice maintained on a high-fat diet (HFD) was found to promote the release of mitochondrial DNA (mtDNA) into the cytosol of adipocytes, leading to IFN signaling and the production of inflammatory cytokines [11]. The release of mtDNA was prevented by increased mitochondrial integrity via overexpression of mitochondrial disulphide-bond A oxidoreductase-like protein (DsbA-L), that rescued chronic inflammation and restored insulin sensitivity (Figure 3A). Conversely, adipose tissue-specific knockout of DsbA-L in mice led to severe inflammation triggered by mtDNA release and cGAS–STING pathway activation. It also promoted insulin resistance and glucose intolerance, irrespective of the diet they were fed [11]. The downstream effectors of metabolic rewiring by STING remain poorly understood, and some appear to play opposing roles. For instance, TBK1 was found to directly phosphorylate and deactivate a central energy homeostasis regulator, AMPK, at Thr172 [53]. Adipose tissue-specific *Tbk1*$^{-/-}$ mice maintained on a HFD showed increased secretion of CCL2, leading to increased macrophage infiltration and inflammation. Further loss of TBK1 increased TNF-α production, resulting in increased insulin resistance [53–55]. One study also established that TNF-α production can lead to a further increase in inflammation via induction of the noncanonical **NF-κB pathway**, which was abrogated upon NIK deletion [53]. TBK1 is known to attenuate noncanonical NF-κB through phosphorylation and degradation of NF-κB-inducing kinase (NIK) (Figure 3A). In separate studies, however, pharmacological inhibition of the downstream effector TBK1/IKKε by amlexanox or knockout of IRF3 improved glucose tolerance, reduced body weight, and enhanced insulin sensitivity [12,14,56]. Taken together,

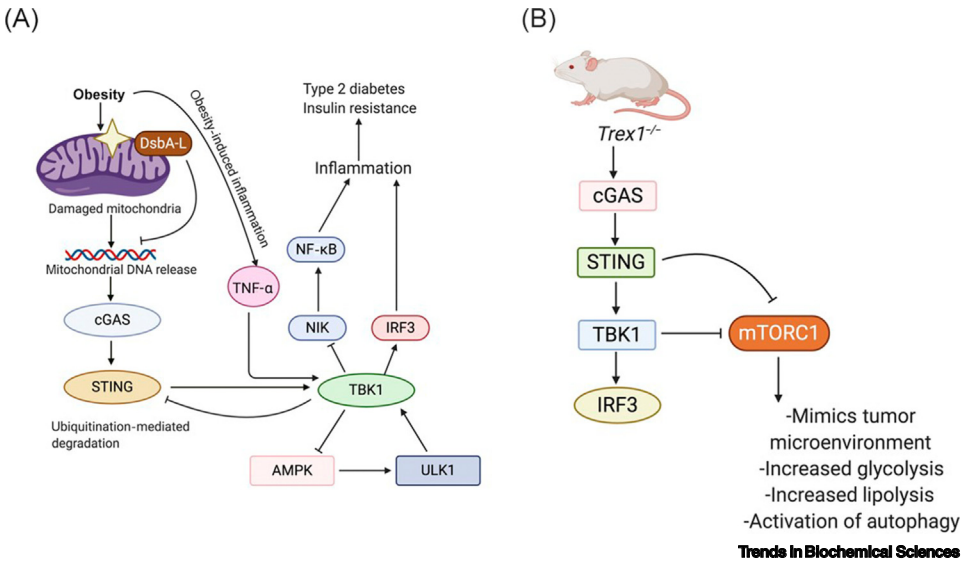

Figure 3. cGAS–STING Signaling as a Regulator of Cellular Metabolism. (A) Mitochondrial oxidation and resulting damage during obesity can lead to leakage of oxidized mitochondrial double-stranded DNA (dsDNA) that activates the cGAS–STING–TBK1 signaling axis. This leakage is prevented by overexpression of mitochondrial disulphide-bond A oxidoreductase-like protein (Dsb A-L). TBK1 is also activated upon macrophage infiltration into adipose tissue, leading to TNF-α secretion. Collectively, this leads to NF-κB and IRF3 activation, resulting in inflammation, insulin resistance, and type 2 diabetes. AMPK activation also leads to TBK1 activation via ULK1, and TBK1 in turn inhibits AMPK in a feedback loop. Conversely, TBK1 has also been found to inhibit NIK and prevent NF-κB mediated inflammation. (B) In *Trex1*$^{-/-}$ mice, that contain increased amounts of cytosolic dsDNA, STING activation was found to suppress mTORC1. This regulation was reported to be due to TBK1-mediated inhibition, although a TKB1-independent effect was also observed. mTOR inhibition leads to increased catabolic processes such as autophagy, lipolysis, and glycolysis. DNA damage and resulting cGAS–STING-dependent inflammatory signaling is thus a crucial regulator of cellular metabolism and energy homeostasis.

these studies indicate that STING, and its downstream effector TBK1, can play multiple and perhaps opposing roles depending on its mode of activation (chronic versus acute) in obesity and metabolic regulation.

A more direct link between STING activation and metabolic dysregulation was shown in patients with NAFLD, as well as in mouse models maintained on a HFD, where STING levels were found to be increased. STING$^{gt/gt}$ mice (carrying a point mutation in STING T596A leading to no detectable protein) maintained on HFD showed a rescue of hepatic steatosis (accumulation of fat in the liver), inflammation, and fibrosis [13,57,58]. It is unclear whether this effect is specific to inhibition of STING function in hepatocytes or other cells. Luo *et al.* demonstrated that transfer of bone marrow-derived macrophages (BMDMs) from STING wild-type mice to STING$^{gt/gt}$ mice maintained on HFD restored the inflammatory phenotype, steatosis, and fibrosis, suggesting that STING in macrophages plays a direct role in the onset of NAFLD. Further, in the presence of the STING agonist, DMXAA, hepatocytes and stellate cells incubated with wild-type BMDMs showed increased lipid accumulation and inflammation compared to their STING-knockout counterparts [13].

Hasan *et al.* demonstrated a direct link between the master regulator mTORC1 and cGAS–STING signaling [59]. mTORC1 activation leads to inhibition of autophagy and **lipolysis**, and activation of anabolic processes and macromolecular biosynthesis [60]. In *Trex1*$^{-/-}$ mice, that exhibit an overactive cGAS–STING signaling axis because of the persistence of undigested

cytosolic dsDNA, mTORC1 activity was found to be downregulated. The resulting increase in inflammation in *Trex1*$^{-/-}$ mice was restored upon loss of either STING or IRF3, but the metabolic changes were restored only in *Trex1*$^{-/-}$ STING$^{gt/gt}$ mice, suggesting that some of the metabolic effects of STING might be independent of its downstream effectors such as IRF3, potentially suggesting that the noncanonical NF-κB pathway is involved [59]. Interestingly, pulldown of TBK1 revealed a physical interaction with mTORC1 and its substrates Raptor, 4EBP, and S6P only in *TREX1*$^{-/-}$ cells and not in their wild-type counterparts. In another study, TBK1 was found to inhibit mTORC1 activity via direct phosphorylation of Raptor at Ser877 [61]. In addition, pharmacological inhibition of TBK1 rescued the adverse inflammation seen upon loss of TREX1, and this has also been validated in prostate cancers where TBK1 was reported to inhibit mTORC1 activity [57,62].

The role of STING in metabolic disorders cannot be solely attributed to its role in transcriptional inflammatory signaling, and emerging studies on its interactions, trafficking, and crosstalk with different organelles suggest alternative mechanisms by which it might impact on cellular metabolism. Collectively, these findings establish a link between metabolism and STING.

Role of STING in Ca^{2+} Homeostasis and the ER Stress Response

STIM1, the ER Ca^{2+}-sensor discussed above, is crucial not only for STING signaling but also for Ca^{2+} homeostasis in the ER, thereby regulating ER stress. Loss of STING or its translocation from the ER in response to cGAMP activation leads to depletion of ER Ca^{2+} stores, followed by STIM1 movement and refill of stores from extracellular Ca^{2+} via association with plasma-membrane sensor Orai1 [46]. In another study by Wu *et al.* it was observed that the N145S SAVI STING mutant led to disrupted Ca^{2+} homeostasis, causing ER stress in T cells and cell death upon T cell receptor (TCR) activation. The authors further identified the widely conserved domain of STING (amino acids 322–343) is responsible for modulating the unfolded protein response (UPR) independently of type I IFN induction. STIM1 overexpression reversed STING N145S-mediated ER stress and resulting cell death [63].

In macrophages, cyclic-di-AMP secreted by Gram-negative bacteria is sensed by STING in the ER through an unknown mechanism. However, this process leads to ER stress and mTORC1 inactivation, leading to the induction of canonical autophagy and **ERphagy**. Activation of ER stress responses in this setting prevents infection and results in cell death and IFN production by localization of STING on autophagosomal membranes, a dual edged strategy that protects the host from active bacterial infection [64]. Maintenance of ER homeostasis is crucial for protein synthesis, post-translational modifications, and vesicular trafficking. The role of STING in the maintenance of these functions suggests bidirectional interplay between STING and ER-resident proteins that couple the ER stress response to inflammation and cellular metabolic regulation.

The Role of the cGAS–STING Pathway in Cancer

The well-documented role of type I IFN signaling in promoting proinflammatory immune infiltration has led to significant interest in the potential role of cGAS–STING as a mediator of successful antitumor immunity [65]. However, emerging data suggest that, like its multifaceted role in normal cells, STING signaling in cancer supports diverse functions, some of which appear to be dichotomous unless taken in the proper context. A distinctive feature of cancer cells is **chromosomal instability** (CIN), defined as an increased frequency of chromosome mis-segregation during mitosis [66]. This leads to generation of lagging chromosomes and aberrantly segregating chromosome fragments during anaphase, followed by the formation of micronuclei after mitotic

exit. A conspicuous feature of micronuclei is their frequent rupture during S-phase that exposes the cytoplasm to genomic dsDNA [67]. Because cGAS recognizes both self and non-self dsDNA, this pathway is chronically activated in cancers in which CIN is rampant [1,17,68]. In addition to inherent CIN, radiation therapy and many DNA-damaging chemotherapeutic agents have been found to induce DNA damage and micronuclei formation, leading to cGAS–STING pathway activation and inflammation [69,70]. Another putative mechanism of DNA spillage into the cytosol is oxidative stress that promotes mtDNA leakage [11,71,72]. Notably, oxidized DNA is relatively more resistant to TREX1-mediated degradation and therefore it is a potent source of STING activation [73]. DNA has also been found in the cytosol of cells in the tumor microenvironment, such as phagocytic cells and endothelial cells, and is believed to have entered these cells when apoptotic tumor cells were engulfed [74,75]. How this DNA escapes from the phagosomes and into the cytosol is unknown.

The expression of cGAS and STING varies greatly across human cancers. In an analysis of RNA-seq datasets for over 1000 cell lines of the Cell Line Encyclopedia (CCLE), Dou *et al.* revealed no correlation between *STING* mRNA levels and induction of ISGs; however, there was a strong correlation between its expression and the induction of other proinflammatory genes associated with the senescence-associated secretory phenotype (SASP), and this favors NF-κB as the predominant pathway downstream of STING in cancer [76]. A putative mechanism for IFN-less inflammatory signaling is via induction of the p38 pathway, which is activated in response to stress originating from DNA damage and chromosome mis-segregation and has been shown to selectively inhibit IFN signaling downstream of STING without impacting on NF-κB activity. Notably, p38 activates a STING-deubiquitinating enzyme, USP 21, that can modulate STING signaling output [77].

In virtue of their persistent exposure to cytosolic dsDNA arising from CIN and mitochondrial dysfunction, tumors have also evolved to modulate the expression of cGAS–STING pathway genes so as to avoid its antitumor proinflammatory effect. However, this leads to chronic inflammation and immune infiltration, which can further promote tumor growth. Understanding the mechanisms by which tumors evade chronic cGAS–STING signaling might shed light on their evolutionary trajectory and offer opportunities for therapeutic intervention. For instance, loss of chromosome 9p, which harbors the IFN gene cluster, is a frequent event in colorectal and pancreatic cancers [78]. Thus, tumors lacking 9p would be able to signal through NF-κB downstream of STING without eliciting an IFN response. Alternative mechanisms of immune evasion can involve the downregulation of STING levels, which was described in gastric and colorectal cancers as well as in advanced stage tumors [79,80]. However, a pan-cancer analysis of >10 000 samples from the TCGA (The Cancer Genome Atlas) database revealed that inactivating mutations and genomic loss of *CGAS* or *STING* occur in <1% of tumor types. Furthermore, many tumor types such as breast, head and neck, lung, and pancreatic cancers exhibit increased expression of *CGAS* and *TMEM173* mRNAs compared to their normal tissue counterparts [81]. Thus, the majority of tumors appear to preserve cGAS and STING proteins and instead modulate downstream IFN signaling.

Tumor-derived cGAMP can be exported from cancer cells and transferred to neighboring cells, resulting in direct activation of STING in the tumor microenvironment [82]. Recently, SLC19A1 has been shown to be an importer of cGAMP from the extracellular milieu, and also of other CDNs with varying affinities [83,84]. cGAMP can also be transferred through astrocyte–cancer cell gap junctions [85]. Further, connexins are reported to transfer cGAMP from epithelial cells to macrophages during viral infection, to bystander cells in cancer, and are upregulated in melanoma cells upon bacterial infection [86–88]. Finally, dsDNA and cGAMP from tumor cells are also

transferred to neighboring cells via **exosomes** where they activate the cGAS–STING signaling axis [89,90]. The transfer of dsDNA or cGAMP to non-tumor, bystander, and phagocytic cells has been shown to promote antitumor effects as well as facilitating tumor progression such as metastasis [91]. The conserved mechanisms of cGAMP transfer draw their roots to eukaryotic antiviral defense mechanisms that appear to be often hijacked, rather than suppressed, in advanced tumors.

Tumor cGAMP is an important factor in the activation of natural killer (NK) cells. In melanoma and lymphoma mouse models, steady-state activation of cGAS in tumor cells, followed by paracrine cGAMP uptake by host NK cells, promotes type I IFN signaling leading to antitumor immunity [92]. Earlier studies linking cGAS–STING activation in cancer to type I IFN led to the development of an array of STING agonists for anticancer therapy (Box 2) [93]. However, despite robust immunological infiltration upon type I IFN production following agonist administration to mouse models, their efficacy in humans has been limited either as single agents or in conjunction with immune checkpoint blockade [92,94–96]. This suggests that tumors have evolved mechanisms to dampen type I IFN signaling downstream of STING. Furthermore, in some instances, depletion of Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs), but not STING deletion, led to defects in $CD8^+$ T cell priming, suggesting that the role of pattern recognition receptors in mediating antitumor immunity is a redundant process [97]. These observations, together with emerging evidence suggesting that chromosomally unstable tumors exhibit intrinsic resistance to cGAS–STING activation, complicate efforts to effectively target this pathway in cancer [98].

STING and Cancer Metastasis

The majority of cancer patients succumb to metastatic progression [91]. Many metastatic cancers exhibit increased CIN, and recent work by our group has revealed a link between CIN-induced chronic cGAS–STING activation and the ability of cancer cells to migrate and spread to distant organs. To elucidate the role of ongoing chromosome mis-segregation during metastatic progression, isogenic breast and lung cancer cells were generated that have varying rates of CIN [17]. This isogenic system of (CIN^{high} and CIN^{low}) cells, which were equally aneuploid but

Box 2. Targeting STING for Cancer Therapy

The discovery that STING is a key mediator of the type I IFN response that enables a robust T cell response catapulted the development of STING agonists [109]. Because 2′,3′-cGAMP was found to have high affinity for STING, the first line of drugs synthesized to activate this pathway were hydrolyzable cyclic dinucleotides (CDNs). DMXAA (5.6 dimethylxanthanone-4-acetic acid) is the most widely studied STING agonist [110,111]. It was found to be efficacious in various combinations with radiotherapy and chemotherapy in mouse models of breast, prostate, and colorectal carcinoma, as well as melanoma [110,112,113]. Multiple human trials of the compound as a stand-alone intravenous drug, as well in combination with docetaxel, carboplatin, and paclitaxel have been conducted [114–117]. Several of these prematurely terminated owing to poor efficacy and adverse events. However, detailed structural analysis recently revealed that DMXAA binds to mouse STING, but not to human STING, resolving the controversy regarding failure of this potent therapeutic [118,119]. Multiple STING agonists were since developed to overcome the deficiencies of DMXAA. E7766 is currently in a Phase I trial as a standalone drug in multiple indications including solid tumors, lymphomas, and non-muscle invasive bladder cancer [120]. GSK3745417 is being tried as a standalone as well as in combination with the PD1 inhibitor pembrolizumab for advanced refractory/relapsed solid tumors and lymphomas [121,122]. ADU S-100 (also known as MIW815) is currently being tested with multiple immunotherapy combinations such as with pembrolizumab for recurrent and metastatic head and neck cancer, and with ipilimumab (anti-CTLA-4 antibody) and PDR001 (anti-PD1 antibody) for advanced metastatic solid tumors and lymphomas. Preliminary results have shown modest responses in refractory melanoma and triple-negative breast cancer [95,123–125]. Other STING agonists being investigated are SB11285 with nivolumab and BMS 986301 with ipilimumab and nivolumab [126–130]. However, the discovery of ENPP1 (ectonucleotide nucleoside pyrophosphatase/phosphodiesterase) suggests that hydrolyzable CDN analogs face a threat of limited efficacy in patients with higher expression of ENPP1 [131]. Therefore, the two recently discovered non-nucleotide analogs MSA-2 and SR-717 may hold greater promise [132,133]. Because prolonged activation of this pathway poses the threat of triggering chronic inflammation and metastasis, patient selection as well as the development of targeted delivery methods are likely to become quintessential for the success of STING agonists [17].

differed primarily in their CIN rates, enabled the exploration of the role of CIN in metastasis. Interestingly, despite the presence of chronic cGAS–STING signaling in CIN^{high} cancer cells, there was no evidence of an IFN response or of ISG expression. Instead, cells with high levels of CIN exhibited non-canonical NF-κB pathway activation, expression of mesenchymal traits, and an increased predilection for metastasis [17]. The role of cGAS–STING activation in promoting noncanonical NF-κB signaling has been reported before by Abe *et al.*, and specific channeling of cGAS–STING signaling into noncanonical NF-κB production provides a direct link between genomic instability, inflammation, and metastasis [17,98,99].

The mechanisms by which STING activates noncanonical NF-κB are poorly understood. It is possible that STING traverses through different compartments following association with TBK1, thus enabling differential induction of non-canonical NF-κB; indeed, knockdown of YIPF5 led to reduced TBK1 recruitment and downstream IFN-β production, but NF-κB signaling was not affected [44]. Notably, non-canonical NF-κB activation is independent of TBK1, suggesting that STING can signal through NIK, a key regulator of this alternative pathway. NIK activation is highly dependent on its stability, and it is plausible that STING might alter the stability or internalization rates of plasma membrane-bound NIK inhibitors such as TRAF2/3 and c-IAP1/2 [100]. A better understanding of how STING might influence NIK could offer an opportunity for therapeutic intervention to disrupt this metastasis-enabling signaling axis.

A broader understanding of how cancer cells uncouple IFN signaling from NF-κB activation will be crucial for targeting adaptive mechanisms that are hijacked by tumor cells to spread to distant organs. To this end, K63-linked polyubiquitination of STING by mitochondrial E3 ubiquitin protein ligase 1 (MUL1) was shown to be essential for inducing an IFN response to cytosolic dsDNA [101]. This process was crucial for STING trafficking, ER translocation, and IRF3 activation; however, it did not affect the NF-κB pathway. Interestingly, loss of K63-linked polyubiquitination did not impact on STING binding to cGAMP or on STING dimerization, raising a question regarding the widely reported crucial role STING trafficking from the ER in the induction of NF-κB pathway, or if this is an upstream function of STING before its translocation from the ER [101].

STING can also promote metastatic progression through paracrine signaling between cells in the tumor microenvironment (Figure 4) [102]. cGAS–STING activation in mesenchymal stem cells after exposure to ionizing radiation promotes the secretion of CCL5, leading to macrophage infiltration that promotes the formation of distant metastases [103]. Furthermore, GAP junctions formed by breast and lung cancer cells expressing protocadherin 7 (PCDH7), in association with connexin 43 in astrocytes, enable cGAMP transfer to astrocytes where STING activation promotes the secretion of TNF-α and IFN-α that enable the outgrowth of brain metastases [85]. The authors posit that paracrine signaling by these molecules activates NF-κB and STAT1 signaling in cancer cells, thus promoting metastatic cancer cell survival in the brain, proliferation, and chemoresistance. GAP junction inhibitors, such as tonabersat and meclofenamate, inhibited brain metastasis in an immunocompetent mouse model [85]. Given that membrane-fusion events lead to STING activation, it is also possible that a surge in membrane fusion upon exosome shedding in cancer can in fact also lead to STING activation and promote metastasis [15]. In summary, dissecting the role cGAS–STING pathway in both antitumor immunity and cancer progression will enable us to target them for therapeutic benefit.

Concluding Remarks

Since its discovery, STING has been recognized as a crucial sensor of dsDNA viruses and for inducing downstream inflammation that is crucial to resolution of these infections. Nevertheless, recent studies have revealed its evolutionarily conserved function in promoting autophagy,

Outstanding Questions

Given the role of STING in multiple fundamental cellular processes, including autophagy, ER stress, and the UPR, as well as in cellular metabolism, what is the central evolutionarily conserved role of STING?

Did the IFN-related function of STING coevolve with an adaptive immune system?

Can the IFN-independent functions of STING explain its dichotomous role in cancer?

How does the signaling downstream of STING depend on the nature of pathway activation? Specifically, does chronic activation, as seen in cancer, promote cellular survival by upregulating stress-response pathways?

Is STING a passive cargo or does it play a central role in membrane trafficking?

What determines the ultimate outcome of STING signaling? Can these be modulated in cancer to maximize the antitumor immune responses while minimizing the prometastatic effects?

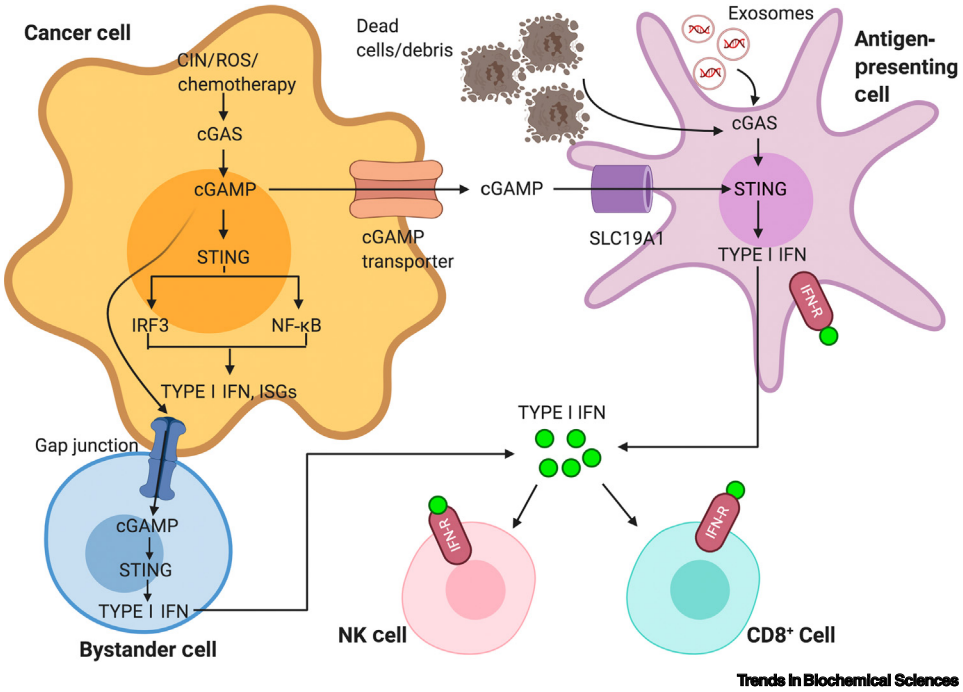

Figure 4. Effects of cGAMP Transport in the Tumor Microenvironment. In addition to being active in cancer cells, the cGAS–STING pathway can also be activated in other cells in the tumor microenvironment through paracrine mechanisms. These include transfer of dsDNA or cGAMP from tumor cells to host cells. cGAMP can be transferred from the tumor cells via exosomes or through gap junctions to adjacent bystander cells, or may be directly exported to the extracellular space through a so far uncharacterized transporter, where it can be taken up by other cell types such as antigen-presenting cells through the SLC19A1 folate transporter. Paracrine cGAS–STING activation can lead to type I IFN production which in turn is crucial for the infiltration and activation of NK and $CD8^+$ T cells in the tumor microenvironment. However, paracrine cGAS–STING activation was also found to promote brain metastasis through an unknown mechanism. Abbreviations: dsDNA, double-stranded DNA; cGAMP, cyclic GMP-AMP; CIN, chromosomal instability; NK, natural killer; ROS, reactive oxygen species.

another essential process during clearance of cytoplasmic dsDNA. The more recent acquisition of IFN signaling via its CTT suggests it might have coevolved with the MHC peptide-presentation machinery and the development of cell-mediated immunity in multicellular organisms. These multifaceted functions of STING place this pathway at the nexus between inflammation, cellular stress responses, and metabolism (see Outstanding Questions). It is through this lens that its role in cancer should be investigated, a disease in which this pathway is chronically activated in response to endogenous and exogenous genotoxic stressors. The discovery of the role of STING in tumor progression and metastasis highlights the complexity of its activation in cancer, and suggests that a more nuanced approach might be warranted to target cytosolic nucleic acid sensing in cancer. This approach will necessitate the stratification of patients based on their STING activation status, the IFN responsiveness of the tumor, and whether or not tumor cells have already acquired the necessary changes to overcome sensitivity to STING signaling.

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