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The STING controlled cytosolic-DNA activated innate immune pathway and microbial disease

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Abstract

The innate immune system is critically important for the primary sensing of invading pathogens. Over the past decade, the cellular sensors important for recognizing microbial entry into the host cell have been largely elucidated. These sensors, some of which are evolutionarily conserved, include the Toll-like receptor (TLR) and RIG-I-like helicase family (RLH) pathway that can recognize bacterial and viral non-self nucleic acid. In addition, a cellular sensor referred to as STING (for stimulator of interferon genes) has been shown to be critical for triggering host defense countermeasures, including stimulation of the adaptive immune response, following the detection of cytosolic DNA species. The STING pathway has now been shown to be critical for activating innate immune gene transcription in response to infection by DNA pathogens such as herpes simplex virus 1 (HSV1) as well as retroviruses. In addition, it is clear that chronic STING activation can also cause autoinflammatory disease manifested by self-DNA. Here we review recent developments in our understanding of STING function, including importance in the control of microbial disease.

Keywords

STING; DNA sensor; Interferon; Host defense

The Stimulator of interferon genes (STING) protein was discovered through its ability to trigger innate immune gene transcription including the production of type I interferon (IFN) in response to microbial infection [1]. STING, also known as MITA, ERIS, MPYS, and TMEM173, is a 379 amino acid protein in human cells, comprising several transembrane regions in its N-terminal region, which exists as a dimer in the endoplasmic reticulum (ER) [2]. STING exerts broad anti-pathogen functions, is independent of the Toll-like receptor (TLR) pathway and may be evolutionarily conserved, with homologs existing in Drosophila. For example, following DNA virus, retrovirus or bacterial infection, STING-dependent signaling is activated culminating in the nuclear translocation of the transcription factors interferon regulatory factor 3 (IRF3), nuclear factor-kB (NF-κB) and Jun N-terminal protein

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Conflict of interest

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kinase/stress-activated protein kinase (JNK/SAPK) pathway Which facilitates the production of dozens of innate and adaptive immune modulatory proteins including proinflammatory cytokines such as CXCL10 and TNFα [2–4]. Mice lacking STING are viable, although extremely sensitive to infection by a variety of DNA and RNA viruses [1,2,5]. Considerable efforts have been undertaken in the past few years to unravel the mechanisms of STING activation and determine its ability to potently switch on the production of host defense proteins. What has been discovered is that STING is effectively activated in the presence of cytosolic DNA species generated from DNA viruses such as herpes simplex virus 1 or even bacteria such as tuberculosis and Listeria [2–4,6]. In addition, STING is stimulated by transfected DNA including purified viral DNA, bacterial genomic DNA, calf thymus DNA, plasmid DNA, and synthetic double-stranded DNA (dsDNA) complexed with cationic liposomes [5]. Such ligands completely fail to induce type I IFN production, as well as other innate immune proteins in STING deficient fibroblasts, conventional dendritic cell, and macrophages [1,5].

Evidence indicates that STING can directly bind to dsDNA species, although in mammalian cells the affinity of association is low [7]. The search for DNA sensors that may facilitate STING activity led to the discovery of cyclic GMP-AMP synthase (cGAS) that generates the cyclic dinucleotide (CDN), cyclic GMP-AMP (cGAMP) from GTP and ATP in a DNA-dependent manner [8,9]. When inactive, cGAS exists as a monomer but forms a 2:2 complex in the presence of dsDNA [4,10]. In human cells, sequence non-specific dsDNA greater than 45 base pairs is able to stimulate cGAS catalytic activity. Bioinformatics analysis indicates that cGAS has a conserved motif generally found in the nucleotidyltransferase (NTase) family which is highly similar to that of 2′-5′-oligoadenylate synthase (OAS1), a dsRNA binding protein that generates 2′-5′-oligoadenylates [11]. These nucleotides induce dimerization and activation of RNase L enabling this protein to degrade viral RNA to inhibit viral replication. cGAS is found in metazoans but not in bacteria or in insects such as Drosophila [11]. In mammals, cGAS synthesized cGAMP binds to STING dimers in the ER to initiate innate immune gene production, by mechanisms that remain to be fully clarified [4]. In bacteria, cyclic di-AMP and cyclic di-GMP are secreted and function as ubiquitous second messengers to trigger a spectrum of responses to internal and external stimuli [12]. These CDNs also can activate STING directly in same way as cGAMP. cGAS deficient mice showed similar phenotypes to STING deficient mice in susceptibility to DNA pathogen infection and essentials for IFN production upon dsDNA treatment [13].

Activation of STING by DNA viruses induces trafficking from the ER area through the Golgi apparatus to perinuclear regions via a mechanism resembling non-canonical autophagy [5]. This trafficking involves STING escorting TBK1 to endosomal/lysosomal regions, which likely harbor the transcription factors IRF3 and NF-kB [5]. In the ER, STING associates with the translocon associated protein (TRAP) complex, formed of the four TRAP subunits (α–Δ) linked to a trimer comprising SEC61α, SEC61β and SEC61γ [1]. The translocon complex is critical for protein folding, N-linked glyosylation and secretion, although it is not clear if STING plays a role in these processes [14]. A hallmark of autophagy involves microtubule-associated protein 1A/1B-light chain 3 LC3 (LC3-I) being converted to a phosphatidylethanolamine-conjugated form (LC3-II) before being recruited to pre autophagosomal (PAS) membranes arising from the ER [15]. Conversion of LC3-I to

LC3-II is observed following STING activation, and not in STING deficient cells [16]. Normally, autophagy is induced under starvation conditions and autophagosomes engulf cellular organelles such as mitochondria to generate required nutrients such as amino acids [15]. Such autophagosomes manifest as double-membraned vesicles that fuse to lysosomes responsible for degrading the organelles. However, STING-related autophagosomes do not have doublemembrane structures, but rather single-membrane ones similar to those formed by TLR9-interacting DNA-immune complexes [16,17]. In addition, autophagy-related gene 7 (ATG7), which is an essential molecule for autophagosome formation under starvation condition, is not essential for STING trafficking and innate immune gene activation in response to dsDNA [16]. However, several genes involved in canonical autophagy are known to contribute to DNA-induced autophagy such as ATG9 [18]. In ATG9 deficient cells, vesicle formation harboring STING and TBK1 is dramatically enhanced following dsDNA treatment. Consequently, IRF3 is more activated and type I IFN production increases. In addition, data indicates a requirement for vacuolar protein sorting 34 (Vps34) in STING-dependent autophagy [16]. Vps34 is class III phosphatylinositol 3-kinase (PI3K), which phosphorylates phosphatidylinositol to form phosphatidylinositol (3)-phosphate (PtdIns(3)P) under starvation conditions as well as in the presence of cytosolic dsDNA [19–21]. Future studies will involve attempts to understand how STING, following association with CDN's, triggers autophagosome formation and undergoes trafficking. Identifying the responsible regulatory molecules that facilitate this process will not only shed light into the mechanisms of STING function, but perhaps into the processes controlling cellular autophagy processes.

As emphasized, STING is essential for protection against DNA-based microbes such as HSV-1 [5]. However, it remains to be seen whether STING plays a major role in host defense against other DNA-based pathogens such as fungi or parasites such as Malaria [22–26]. It is further noteworthy that STING is also known to plays an important role in defense against negative-stranded RNA viruses such as VSV (Vesicular stomatitis virus), Sendai virus (SeV) or positive-stranded RNA viruses such as Dengue virus [1,5,27]. MEF's or mice lacking STING are very sensitive to negative-stranded RNA virus replication and lethal infection respectively [5]. A number of viruses, such as Dengue Virus, are now known to target STING for inhibition, presumably to escape deleterious consequences that would affect their survival [27]. However, a noted ambiguity is that RNA viruses and synthetic dsRNA such as poly I:C do not induce STING trafficking or STING-dependent innate immune gene transcription [1]. Moreover, STING deficient cells are able to normally produce type I IFN following Poly I:C treatment. Thus, how STING prevents RNA virus infection remains to be determined though speculatively may involve its role in the ER and plausibly translocon-associated function. Further investigation will be entailed for understanding how STING prevents RNA virus replication.

Observing that activation of STING can induce potent innate immune transcriptional events has led to evaluating whether ligands that activate STING signaling could be useful as adjuvants in anti-pathogen and vaccine related strategies [28–30]. Certainly, CDN's are now being evaluated in this way. It also worth noting that STING signaling has been shown to be important for the adjuvant effects of plasmidbased DNA vaccines [5]. For example, mice lacking STING did not generate robust adaptive immune responses to DNA vaccines.

Therefore, understanding the STING pathway may also lead to the new generation of safer and more effective DNA-based plasmid immunization regimes.

Stimulation of the STING pathway may afford new opportunities to develop vaccines and anti-pathogens therapeutic. However, chronic STING activation is now known to be harmful to the host and can lead to lethal inflammatory disease [31–33]. For example, while cytokines and Type I IFN are required for protection of the host and for stimulating adaptive immunity, overproduction of these cytokines can lead to autoinflammatory disease. In one case, the engulfment of apoptotic cells and degradation of apoptotic DNA by phagocytes requires the exonuclease DNaseII. Loss of this DNaseII inhibits the ability of the phagocytes to digest apoptotic DNA [34,35]. Such DNA leaks from lysosomal compartments within the phagocyte and triggers the activation of STING leading to chronic pro-inflammatory cytokine production. Mice lacking DNaseII die before birth due to high levels of type I IFN being produced. However, DNaseII$^{-/-}$/STING$^{-/-}$ deficient mice are completely viable since loss of STING prevents apoptotic DNA-driven cytokine production [31]. Similar mutations have not been found in humans likely lethal. However, other STING-dependent autoinflammatory diseases have been found to occur within the human population. For example, another exonuclease, referred to as Trex1, 3′-repair exonuclease 1, is almost certainly involved in severe cases of systemic lupus erythematosus (SLE) and AGS (Aicardi-Goutieres syndrome) characterized by patients harboring high levels of cytokines [36,37]. Indeed, a number of mutations in Trex1 have now been cataloged in patients with SLE and AGS. Recent data has suggested that Trex1 is responsible for degrading self DNA that may leak out into the cytosol and activate cytosolic DNA sensors such as cGAS/STING. Trex1$^{-/-}$ mice usually die within 10 weeks of birth since they suffer from inflammatory myocarditis caused by chronic cytokine production [38]. However, similar to the situation with DNaseII, Trex1$^{-/-}$STING$^{-/-}$ mice are completely viable [32]. The key cellular producers of such cytokines were shown to be derived from hematopoetic cells such as macrophages and dendritic cells [33]. Evidence indicates that Trex1 could conceivably be responsible for degrading aberrant genomic DNA left over from the cell division process, which otherwise may escape into the cytoplasm. Thus, Trex1$^{-/-}$ macrophages undergoing differentiation may be able to generate high levels of cytokine production through the STING pathway. Conversely, mutations in STING itself have been found to occur. In this instance, the mutations rendered STING highly activate and able to stimulate high levels of cytokine production. The mutations lead to a vascular and pulmonary syndrome (VAPS) referred to as STING-associated vasculopathy with onset of infancy (SAVI) [39]. Finally, chronic STING activation may be involved in the generation of inflammation driven malignant disease, since STING-deficient mice have been shown to be resistant to carcinogen-mediated cancer of the skin (Ahn et al., Nature Communications, 2014;5:5166). Thus, therapeutically targeting the STING pathway may provide a new way to treat inflammatory disease and plausibly cancer.

It is therefore apparent that the host has devised a number of strategies designed to negatively control STING activity, to avoid chronic pro-inflammatory cytokine induction. For example, following activation by cytosolic dsDNA, STING is phosphorylated at several serine sites and becomes rapidly degraded after trafficking and the activation of transcription factors IRF3 and NF-kB [16]. One phosphorylation site identified, serine 366 (S366) is highly conserved from avian to humans. A phospho-mimetic mutant of S366 (S366D) failed

to stimulate activation of IRF3 but not NF-kB. Thus, phosphorylation of S366 inhibits STING's ability to drive IRF3dependent gene transcription, including type I IFN. Unc-51 like autophagy activating kinase 1 (ULK1), also known as ATG1, was identified as the kinase responsible for S366 phsophorylation. Suppression of ULK1 expression lead to sustained IRF3 activity and increased IFN production. Surprisingly, ULK1 was not found to be required for cytosolic DNA-induced autophagy, as it is in yeast and mammalian cells following starvation. Thus, STING activation leads to a non-canonical autophagy related process, independent of ULK1/ATG1, thought dependent on Vps34 [16]. A second method of STING regulation has recently been reported and involves microRNA's. For example, miR-576-3p expression was found to suppress select RNA and DNA virus infection [40]. miR-576-3p was found to be IRF3-inducible and to target STING as well as, MAVS and TRAF3. Feedback mechanism to reduce interferon expression and set an antiviral response threshold to likely avoid excessive inflammation.

Collectively, understanding the STING pathway may lead to a better understanding of microbial pathogenesis and cellular biological processes such as autophagy and host defense. In addition, unraveling STING function will promote the discovery of new therapies to treat inflammatory diseases and inflammation-associated cancer. Conversely, developing new methods to transiently trigger STING signaling may further spearhead the creation of novel adjuvants that may revolutionize vaccine development.

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