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Molecular Pathways: Targeting the Stimulator of Interferon Genes (STING) in the Immunotherapy of Cancer

Leticia Corrales¹ and Thomas F. Gajewski^{1,2}

¹Department of Pathology, The University of Chicago, Chicago, Illinois

²Department of Medicine, Section of Hematology/Oncology, The University of Chicago, Chicago, Illinois

Abstract

Novel immunotherapy approaches are transforming the treatment of cancer, yet many patients remain refractory to these agents. One hypothesis is that immunotherapy fails because of a tumor microenvironment that fails to support recruitment of immune cells including CD8⁺ T cells. Therefore, new approaches designed to initiate a de novo anti-tumor immune response from within the tumor microenvironment are being pursued. Recent evidence has indicated that spontaneous activation of the Stimulator of Interferon Genes (STING) pathway within tumor-resident dendritic cells leads to type I interferon (IFN) production and adaptive immune responses against tumors. This pathway is activated in the presence of cytosolic DNA, that is detected by the sensor cyclic-GMP-AMP synthase (cGAS), and generates cyclic GMP-AMP (cGAMP), which binds and activates STING. As a therapeutic approach, intratumoral injection of STING agonists has demonstrated profound therapeutic effects in multiple mouse tumor models, including melanoma, colon, breast, prostate, and fibrosarcoma. Better characterization of the STING pathway in human tumor recognition, and the development of new pharmacologic approaches to engage this pathway within the tumor microenvironment in patients, are important areas for clinical translation.

Background

The STING pathway

STING (Stimulator of **I**nterferon **G**enes, also known as TMEM173, MITA, ERIS, and MPYS) is an adapter transmembrane protein that resides in the endoplasmic reticulum (ER). In eukaryotic cells, activation of STING occurs when double stranded DNA gains access to the cytosol. This pathway was originally uncovered in search for a mechanism by which DNA viruses could be sensed by the host immune system. However, STING pathway activation also can occur with certain bacterial and parasitic infections (1), and more recently has been described to occur under conditions when mammalian DNA itself can attain access to the cytosol (2), (3). Cytosolic DNA is detected upon binding to the sensor

Corresponding Author: Thomas F. Gajewski, The University of Chicago, 5841 S. Maryland Avenue, MC2115, Chicago, IL 60637. Phone: 773-702-4601; Fax: 773-702-3163; tgajewsk@medicine.bsd.uchicago.edu.

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cyclic-GMP-AMP synthase (cGAS, MB21D1), which catalyzes the synthesis of cyclic GMP-AMP (cGAMP) from guanosine triphosphate (GTP) and adenosine triphosphate (ATP). cGAMP functions as a second messenger that binds and activates STING (4, 5). Upon binding of cGAMP, STING undergoes conformational changes that trigger its trafficking from the ER to the Golgi to perinuclear endosomes (6). Consequently, STING recruits tank-binding kinase 1 (TBK1) and is, in turn, phosphorylated by TBK1, which renders it accessible for the binding of the transcription factor interferon regulatory factor 3 (IRF3) (7). TBK1 then phosphorylates IRF3 which translocates to the nucleus to drive transcription of IFN- β and other genes (8–10) (Fig. 1).

The functional relevance of cGAS to the STING pathway has been demonstrated in cGAS-deficient cells. Production of the cytokines IFN- α and IFN- β , collectively referred to as type I IFNs, is impaired in cGAS^{-/-} macrophages, fibroblasts and dendritic cells that have been transfected with DNA, or infected with DNA viruses including vaccinia virus, HSV-1 or MHV68 (11, 12). In addition, cGAS detects HIV and other retroviruses, since they generate intermediate DNA in their replication cycles (13). Interestingly, it has been demonstrated that cGAMP, as a small molecule second messenger, can be transferred through gap junctions from cGAMP-producing cells to neighboring cells (14), thus comprising a mechanism that enables infected cells to spread innate immune activation to non-infected cells.

Beyond its role in sensing the presence of infectious agents, the STING pathway also is involved with sensing mammalian DNA directly. Pathological accumulation of cytosolic DNA leads to autoimmune diseases such as Aicardi-Goutières syndrome (15) or systemic lupus erythematosus (SLE) (16). This pathological accumulation of cytosolic DNA can be mimicked using DNase II-deficient mice, which are defective in degradation of DNA within lysosomes thereby leading to escape into the cytosol. Intercrossing of STING-deficient mice with DNase II^{-/-} mice rescues the inflammation-related embryonic lethality normally seen in these animals (3). These data imply that activation of the STING pathway is involved in the pathologic consequences of DNA-mediated inflammatory disorders. In further support of this notion, gain-of-function mutations in TMEM173 (the gene encoding STING) have been identified in patients with an inflammatory vascular-pulmonary syndrome, characterized by overproduction of type I IFNs (17).

Type I IFNs and the STING pathway in cancer

Spontaneous T cell responses against tumors in vivo have been observed, both in human cancer patients and in murine models (18). The presence of activated CD8⁺ T cells in solid tumors correlates with better prognosis in colorectal cancer (19), ovarian cancer (20), breast cancer (21), melanoma (22), gastrointestinal stromal tumors (23), and others. The presence of a T cell response against tumors reflects successful T cell priming by adequate activated antigen presenting cells (APC) in the tumor microenvironment. Taking into account the sterile tumor setting that lacks microbial-derived triggers, activation signals in APCs must come from endogenous adjuvants generated within the tumor (24). Gene expression analysis of human melanoma metastases revealed that tumor infiltration of CD8⁺ T cells correlates with the expression of genes that are known to be induced by type I IFNs (25). The type I

IFN profile has also been shown to predict favorable clinical responses to therapeutic cancer vaccines (26) and to anthracycline-based chemotherapy in patients with breast carcinoma characterized by poor prognosis (27). Infiltration of plasmacytoid dendritic cells (pDCs; a subpopulation of DCs that produces high amounts of type I IFNs) into the skin lesions of vitiligo patients generates type I IFN and thus drives the activation and recruitment of autoimmune T cells (28). Overall, the link between a type I IFN profile and T cell responses suggests that these cytokines might be involved in the generation of an adaptive T cell response against tumor antigens. Indeed, endogenous type I IFN was shown to be required for the prevention of methylcholanthrene-induced and transplantable tumors (29). Mechanistically, animals deficient in the type I IFN signaling pathway, such as deficiency in the Interferon-alpha/beta receptor alpha chain (IFNAR) or in the Signal Transducer and Activator of Transcription 1 (STAT1), showed reduced priming of T cells against tumor-associated antigens. This defect was mapped to the level of the APC compartment, in particular to the basic leucine zipper transcription factor ATF-like 3 (BATF3)-driven lineage of DCs, characterized by expression of CD8 α or CD103 in mice (30, 31). Absence of host type I IFN signaling was associated with reduced accumulation of CD8 α ⁺ DCs within the tumor. Mixed bone marrow chimeras confirmed that type I IFN signaling in the CD8 α ⁺ DC lineage was necessary for maximal T cell priming against tumors in vivo (31). Conditional deletion of the type I IFNR in the CD11c (integrin alpha X chain, a pan-marker of DCs) compartment, also led to poor spontaneous T cell priming against tumors (30). These data suggest that recognition of cancer cells in vivo involves the activation of a pathway within DCs that leads to production of type I IFNs, which in turn drives effective processing of antigens by CD8 α ⁺/CD103⁺ DCs and subsequent presentation of antigenic peptides on major histocompatibility complex (MHC) class I molecules to cytotoxic CD8⁺ T cells, a process that is known as cross-priming (Fig. 1).

These data provided a clue regarding the innate immune signaling pathways that might be involved in anti-tumor adaptive immune responses, as it must be an innate immune pathway that induces type I IFN production. Mechanistic studies using mouse transplantable tumor models revealed that the role of two of the major sensing pathways, TLR signaling via myeloid differentiation primary response gene 88 (MyD88) and/or TIR-domain-containing adapter-inducing interferon- β (TRIF) (32), and the purinergic receptor P2X, ligand-gated ion channel, 7 (P2X7R) signaling due to extracellular ATP binding (33), were dispensable in the generation of spontaneous T cell priming against tumor antigens. However, animals deficient in STING or IRF3 showed a defect in T cell priming and failed to reject immunogenic tumors (34). Ex vivo analysis demonstrated the presence of tumor-derived DNA within the cytosol of tumor-infiltrating DCs, and this correlated with translocation of IRF3 to the nucleus and expression of IFN- β . Therefore, these data strongly suggest that the host STING pathway is the main innate immune sensing pathway for detection of tumors in vivo, and that the activation of this pathway in APCs within the tumor microenvironment drives the subsequent T cell priming against tumor-associated antigens.

A protective role for the type I IFN and the STING pathways has also been reported in various additional in vivo tumor models. Melanoma and lymphoma cell lines expressing OVA peptide (B16.OVA and EL4.OVA, respectively) generated an adaptive immune response against tumor-associated antigens after cryoablation treatment in an IFNAR-

dependent manner (35). This study also demonstrated that the CD11c⁺ subset is the main source of type I IFNs after sensing DNA released by dying cells. The molecular mechanism governing this effect involved activation of the STING/TBK1/IRF3 pathway. Interestingly, STING deficiency in DCs also impaired the generation of anti-nuclear antibodies in an inducible model of SLE. These data imply that the generation of an anti-tumor response by dying cells and the generation of autoimmunity share the same molecular mechanism of DNA sensing. Expression of type I IFNs was also detected to be induced in a model of glioma generated using a sleeping beauty transposon system (36). In this model, CD11b⁺ (integrin alpha M chain) brain-infiltrating leukocytes were the main type I IFN producers in a partially STING-dependent manner. Thus, mouse survival and production of type I IFNs were reduced in glioma-bearing mice having a non-functional mutation (I199N) in STING (37). Two independent studies have demonstrated the protective role of STING in an inducible colon cancer model using azoxymethane/dextran sodium sulfate (AOM/DSS) (38, 39). STING-deficient hosts were more susceptible to colitis and displayed markedly increased tumor formation with accelerated kinetics. One of these studies showed that this protective role of the STING pathway must be explained by the activation of inflammatory wound repair initiating cytokines and the suppression of growth inhibitory IL-22 binding protein (IL-22BP) by IL-18 (38). The other study found increased levels of the proinflammatory cytokines IL-6 and keratinocyte chemoattractant (KC) in STING-deficient mice, due to the impaired regulation of the NF- κ B and STAT3-signaling pathways (39). Thus, STING-dependent innate immunity appears to control tumorigenesis in this model. However, contrary to the protective role of STING and the above models, it has been also shown that STING^{-/-} animals are resistant to 7,12-dimethylbenz[a]anthracene (DMBA)-induced skin cancer (40). In this model, STING activation by DNA leaked from the nucleus of carcinogen-damaged cells in the dermis led to cytokine production and recruitment of infiltrating phagocytes that, in turn, drove inflammatory processes, thereby promoting tumor development. Tumor development was ablated in STING-deficient mice, indicating that activation of this pathway is a necessary component of inflammation-induced carcinogenesis in some settings.

Clinical-Translational Advances

Development of STING agonists as a cancer therapeutic

The discovery that STING is a crucial component of the innate immune sensing of tumors has generated two main clinical implications. First, as a type I IFN signature is linked to tumor T cell infiltration, and since this phenotype correlates with better outcomes, it is plausible that STING activation in the tumor microenvironment and subsequent production of type I IFNs could be used as a prognostic/predictive biomarker. Second, induction of STING activation or direct release of STING-derived cytokines in the tumor microenvironment might have immunotherapeutic potential in the clinic. One reasonable strategy is to deliberately activate host STING in the tumor microenvironment, in order to activate efficient cross-priming of tumor specific antigens to CD8⁺ T cells and facilitate the trafficking of effector T cells by inducing the production of key chemokines. This rationale has motivated the development of direct agonists of STING as a potential cancer therapeutic (Fig. 1).

Interestingly, a previous anti-cancer drug that had been in development has recently been discovered to be an agonist of mouse STING. Flavone acetic acid (FAA) showed substantial activity against murine colon tumors (41) through a novel mechanism of hemorrhagic necrosis. These encouraging data led to clinical translation, with this new class of agents being described as vascular disrupting agents. However this agent failed in a Phase I clinical trial and showed no activity in rat tumor models (42), raising the question of possible species-specificity. In an attempt to obtain similar drugs that produced tumor hemorrhagic necrosis, the molecular structure of FAA was modified, generating several compounds; 5,6-dimethylxanthene-4-acetic acid (DMXAA) was the compound with the highest potency and it also showed activity against a rat mammary carcinoma (43). Similar to FAA, DMXAA showed anti-tumor activity in different mouse models (44). However, this agent also failed in the clinic when combined with chemotherapy in a Phase III trial in non-small cell lung cancer (45). It is noteworthy that the molecular target of DMXAA was not known at that time, which hampered further development. Interestingly, recent structure-function studies of mouse and human STING demonstrated that DMXAA is a direct ligand for mouse STING (46–49), but not for human STING. This difference likely explains the lack of clinical activity of this compound in humans. These observations re-ignited enthusiasm for developing agonists of human STING that might recapitulate the potent anti-tumor activity observed with mouse STING agonists in vivo.

The discovery of cyclic-dinucleotides (CDNs), bacterial second messengers with a variety of physiological effects (50), as natural ligands of STING (8, 51), combined with the identification of cGAMP as a key cyclic dinucleotide in metazoa (5), provided a framework for pursuing STING-activating therapeutics. Of note, CDNs had been used as effective vaccine adjuvants even before their role as STING ligands was discovered (52). However, the therapeutic anti-tumor effect of CDNs has only recently been tested. Intraperitoneal injection of cyclic-GMP (cGMP) has been shown to inhibit the growth of pre-established 4T1 breast tumors (53). In this study, the bacterial-derived canonical cGMP, which contains two 3'-5' linkages, was used. This molecule may not be suitable for clinical development, since single nucleotide polymorphisms in the human STING (hSTING) gene have been shown to affect responsiveness to canonical CDNs (54, 55). Non-canonical cGAMP, generated by the activity of mammalian cGAS, contains a single 3'-5' and a single 2'-5' phosphodiester bond, and activates all hSTING variants (14, 54–56). Rational modifications of CDNs led to synthetic dithio mixed-linkage CDNs that were tested in vitro and in vivo for their capacity to activate all hSTING variants in addition to mSTING (57). The lead molecule ML RR-S2 CDA showed several features that improved both stability and lipophilicity, promoting significantly increased STING signaling as compared with endogenous and pathogen-derived CDNs. Similarly to DMXAA, intratumoral injection of ML RR-S2 CDA into pre-established B16 melanoma tumors caused complete tumor elimination in most of treated mice, induced lasting systemic antigen-specific CD8⁺ T cell immunity. Around 50% of treated animals were free of tumors and survived more than 150 days after intratumoral injection. Furthermore, they were completely protected against a second tumor re-challenge. Similar results were seen in the 4T-1 breast cancer and MC26 colon cancer models.

These preclinical studies suggest that intratumoral injection of ML RR-S2 CDA is necessary to achieve a maximal therapeutic effect. While this may limit the application of this compound to the treatment of directly accessible tumors, it has been shown that local treatment of one tumor induces systemic immunity that effectively induces regression of distant tumors. Thus, an abscopal effect may facilitate systemic anti-tumor activity. These principles are similar to those involved with the therapeutic activity of the oncolytic virus T-VEC for patients with melanoma (58), or the TLR9 agonist CpG along with local low-dose radiation therapy in patients with non-Hodgkin's lymphoma (59). Both of these approaches induce regression of non-treated tumors upon intratumoral application to a single lesion.

Additional strategies to bring type I IFNs to the tumor microenvironment

Due to the benefit of type I IFN induction in innate immune activation in the tumor microenvironment, alternative approaches also have been investigated to transform the tumor microenvironment favorably for T cell-mediated regression. These include intratumoral injection of TLR ligands (60), introduction of tumor necrosis factor (TNF) ligand superfamily member 14 (LIGHT) (61), and injection of oncolytic viruses (62). In addition, strategies to directly deliver type I IFN into the tumor microenvironment using tumor-targeting mAbs coupled to IFN- β have been investigated (63). The therapeutic effect of low doses of type I IFNs to the tumor microenvironment was shown to be T cell-dependent and mediated through type I IFN signaling on host DCs. However, high doses of intratumoral type I IFN might have mainly an anti-angiogenic effect, which was mediated through the IFNAR on endothelial cells (64, 65). Finally, directed radiation to the tumor site also induces type I IFN production, thereby augmenting T cell priming (66). Mechanistically, the induction of type I IFNs by local radiation also appears to depend on the host STING pathway (67), and cGAMP treatment of tumors potentiates the therapeutic effect of radiation by enhancing tumor-specific CD8⁺ T cell functions.

Conclusions

The STING pathway of cytosolic DNA sensing is an important innate immune sensing mechanism, driving type I IFN production in the tumor context. Intratumoral STING agonists hold promise as a cancer therapeutic. Numerous questions remain unanswered, and a deeper biologic understanding of the pathway is still needed. First, the mechanism by which DNA derived from tumor cells gains access to host APCs is not yet known. Second, the activation of STING, and its functional consequences, in different cell subsets within the tumor microenvironment need to be addressed. Third, as radiation induces type I IFN by activation of the STING pathway, the role of STING in the efficacy of other cancer therapeutics, including chemotherapy and kinase inhibitors, also should be explored. Finally, there is little known about the negative and positive regulators of the STING pathway that could be relevant for the cancer context. A deeper understanding of feedback mechanisms will facilitate continued development of alternative strategies to favorably regulate the STING pathway as a therapeutic.

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Figure 1.

Working model of the innate immune sensing of tumors leading to spontaneous T cell responses in vivo. In the tumor microenvironment, tumor-derived DNA (likely released by dead cells, or via acquisition of DNA-containing vesicles) can gain access to the cytosol of intratumoral dendritic cells (DCs). Recognition of cytosolic DNA by cyclic GMP-AMP (cGAMP) synthase (cGAS), and generation of cGAMP, leads to the activation of STING (stimulator of interferon genes). This results in the phosphorylation of tank-binding kinase 1 (TBK1) and subsequent activation, which in turn phosphorylates the transcription factor

interferon regulatory factor 3 (IRF3). This activates the transcription of type I interferon (IFN) genes. The STING pathway can be also deliberately stimulated by the use of direct STING agonists, when the compounds are therapeutically administered into the tumor microenvironment. In vivo studies using gene-targeted mice demonstrated a crucial role of STING pathway activation, type I IFN production, and its signaling on the BATF3 (basic leucine zipper transcription factor ATF-like 3) lineage of DCs for spontaneous antitumor T cell responses in vivo and recruitment of effector T cells into the tumor microenvironment. dsDNA, double-stranded DNA.

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