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

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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 antitumor 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 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 Interferon Genes; 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 the 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 cyclic GMP-AMP synthase (cGAS, MB21D1), which catalyzes the synthesis of cyclic GMP-AMP (cGAMP) from guanosine triphosphate and adenosine triphosphate (ATP). cGAMP functions as a second messenger, can be transferred through gap junctions as a macromolecular 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 noninfected cells.

Beyond its role in sensing the presence of infectious agents, the STING pathway also is involved with sensing mammalian DNA directly. Pathologic accumulation of cytosolic DNA leads to autoimmune diseases such as Aicardi-Goutieres syndrome (15) or systemic lupus erythematosus (SLE; ref. 16). This pathologic 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 (APCs) 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 (pDC, 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, for example, with a deficiency in the IFN\(\alpha/\beta\) receptor alpha chain (IFNAR) or in the STAT1, showed reduced priming of T cells against tumor-associated antigens. This defect was mapped to the

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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 (DC). Recognition of cytosolic DNA by cyclic GMP-AMP (cGAMP) synthase (cGAS), and generation of cGAMP, leads to the activation of STING. This results in the phosphorylation of tank-binding kinase 1 (TBK1) and subsequent activation, which in turn phosphorylates the transcription factor IRF3. This activates the transcription of type I IFN genes. The STING pathway can also be 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.
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 CD8α or CD103 in mice (30, 31). Absence of host type I IFN signaling was associated with reduced accumulation of CD8α+ DCs within the tumor. Mixed bone marrow chimeras confirmed that type I IFN signaling in the CD8α+ DC lineage was necessary for maximal T-cell priming against tumors in vivo (31). Conditional deletion of the type I IFN receptor in the CD11c+ (integrin α 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 CD8α+/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 sensing pathways that might be involved in antitumor 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 IFNβ (TRIF; ref. 32), and the purinergic receptor P2X, ligand-gated ion channel, 7 (PX72R) signaling due to extracellular ATP binding (33), were dispensable in the generation of spontaneous T-cell priming against tumor antigens (34, 35). However, animals deficient in TRIF 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 IFNβ. Therefore, these data strongly support 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 a protective 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 antinuclear antibodies in an inducible model of SLE. These data imply that the generation of an antitumor response by dying cells and the generation of autoimmunity share the same molecular mechanism of DNA sensing. Expression of type I IFNs was also shown to be induced in a model of glioma generated using a sleeping beauty transposon system (36). In this model, CD11b+ (integrin α 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 nonfunctional mutation (1199N) 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, refs. 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 IL22-binding protein (IL22BP) by IL18 (38). The other study found increased levels of the proinflammatory cytokines IL6 and keratinocyte chemoattractant (KC) in STING-deficient mice due to the impaired regulation of the NF-kB and STAT3-signaling pathways (39). Thus, STING-deficient innate immunity appears to control tumorigenesis in this model. However, contrary to the protective role of STING and the above models, it has also been 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, 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 anticancer 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 single 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-dimethylxanthone-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 antitumor 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 reignited enthusiasm for developing agonists of human STING that might recapitulate the potent antitumor activity observed with mouse STING agonists in vivo.

The discovery of cyclic dinucleotides (CDN), bacterial second messengers with a variety of physiologic effects (50), as natural ligands of STING (8, 51), combined with the identification of cGAMP as a key cyclic dinucleotide in metazoans (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 antitumor effect of CDNs has only recently been tested. Intrapерitoneal injection of cyclic GMP (cGMP) has been shown to inhibit the growth of preestablished 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 SNPs in the human STING (hSTING) gene have been shown to affect responsiveness to canonical CDNs (54, 55). Noncanonical 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 thio 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 CDN 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 CDN into preestablished B16 melanoma tumors caused complete tumor elimination in most of the treated mice and 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 rechallenge. Similar results were seen in the 4T1 breast cancer and MC26 colon cancer models.

These preclinical studies suggest that intratumoral injection of ML RR-S2 CDN is necessary to achieve a maximal therapeutic effect. Although 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 antitumor 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 radiotherapy in patients with non-Hodgkin lymphoma (59). Both of these approaches induce regression of nontreated tumors upon intratumoral application to a single lesion.

Additional strategies to bring type I IFNs to the tumor microenvironment

Because of 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 approaches include intratumoral injection of TLR ligands (50), introduction of TNF ligand superfamilly member 14 (LIGIT; ref. 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 IFNβ 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 antiangiogenic 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 IFNs by local radiation also appears to depend on the host STING pathway (67). And cGAMP treatment of tumors potentiated 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, little is 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.

Disclosure of Potential Conflicts of Interest

L. Corrales and T.F. Gajewski are listed as co-inventors on a patent-pending application related to the use of STING agonist as cancer treatment, which is owned by the University of Chicago. No other potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: L. Corrales, T.F. Gajewski

Development of methodology: T.F. Gajewski

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T.F. Gajewski

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Study supervision: T.F. Gajewski

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