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STING, DCs and the link between innate and adaptive tumor immunity

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Abstract

Cancer and the immune system are intimately related. Much of the bulk of tumors is comprised of stromal leukocytes with immune functions, which serve to both promote and inhibit tumor growth, invasion and metastasis. The T lymphocytes of the adaptive immune system are essential for tumor immunity, and these T cells are generated by cross-priming against tumor associated antigens. Dendritic cells (DCs) are essential in this process, serving as the cellular link between innate and adaptive immunity. As a prerequisite for priming of adaptive immune responses, DCs must take up tumor antigens, process them and present them in the context of the major histocompatibility complex (MHC). DCs also serve as sensors of innate activation signals from cancer that are necessary for their activation and effective priming of cancer specific T cells. Here we discuss the role of DCs in the sensing of cancer and in priming the adaptive response against tumors. Furthermore, we present the essential role of the Stimulator of Interferon Genes (STING) signaling pathway in producing type I interferons (IFNs) that are essential in this process.

Keywords

Tumor immunity; Cancer immunotherapy; Interferon; STING; Dendritic cell; Radiation therapy

1. Introduction

Cancer and the immune system are intimately related. Much of the bulk of tumors is comprised of stromal leukocytes with immune functions, which serve to both promote and inhibit tumor growth, invasion and metastasis. The T lymphocytes of the adaptive immune system are essential for tumor immunity, and these T cells are generated by cross-priming against tumor associated antigens. Dendritic cells (DCs) are essential in this process, serving as the cellular link between innate and adaptive immunity. As a prerequisite for priming of adaptive immune responses, DCs must take up tumor antigens, process them and present them in the context of the major histocompatibility complex (MHC). DCs also serve as

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sensors of innate activation signals from cancer that are necessary for their activation and effective priming of cancer specific T cells. Here we discuss the role of DCs in the sensing of cancer and in priming the adaptive response against tumors. Furthermore, we present the essential role of the Stimulator of Interferon Genes (STING) signaling pathway in producing type I interferons (IFNs) that are essential in this process.

2. Dendritic cells: gatekeepers of adaptive immunity

Dendritic cells (DCs) are a heterogeneous population of professional antigen-presenting cells (APCs) from hematopoietic origin that serve as a key link between the innate and adaptive immune systems, and are the lynchpin in generating anti-tumor immunity (Steinman and Idoyaga, 2010). DCs initially develop in the bone marrow from a Flt3 expressing common myeloid precursor shared with monocytes and macrophages (macrophage-DC progenitor, MDP) which gives rise to DC-restricted progenitors that migrate to the lymphoid organs. Development of specific subsets is dictated by transcriptional regulatory programs, sensing of growth factors (e.g. GM-CSF and FLT3L), and cues from the local environment. Consequently, the various DC subtypes display distinct transcriptional programs, morphology, phenotype, and function in different tissues under different conditions. Here we provide a brief overview of the three main DC populations; blood borne and lymphoid DCs, tissue DCs, and monocyte-derived DCs that encompass the inflammatory DC populations involved in tumor immunity. In-depth coverage of DC development, lineages and subtypes has been excellently reviewed elsewhere (Hashimoto et al., 2011; del Rio et al., 2010; Randolph et al., 2008; Merad et al., 2013; Chopin et al., 2012; Satpathy et al., 2011; Moore et al., 2012; Moore and Anderson, 2013; Liu and Nussenzweig, 2010).

2.1. Blood borne and lymphoid DCs

DCs are generally separated into two major groups within the blood and lymphoid tissues of mice and humans; plasmacytoid DCs (pDCs) and conventional DCs (cDCs). The cDCs are further divided into subsets based on their presumed development from myeloid or lymphoid precursors and their expression levels of CD8 α , CD4 and CD11b. Due to technological advancements in omics and multi-parameter functional and phenotypic assessments, this rudimentary division has been shown to be too limited to properly capture the true variety of DC populations. Currently, there is a movement to divide the various subtypes of conventional DCs into two main categories, cDC1 and cDC2, based on a combination of lineage and expression of transcription factors and surface markers. While this approach to classification is a significant improvement, it has been primarily developed in mice and does not fully translate to the human system. However, clear genomic and phenotypic patterns exist in both mouse and human DC populations, giving confidence in these tools for identification and subdividing of the major DC populations.

2.1.1. Conventional DCs; cDC1 and cDC2—cDC1s are efficient at cross-presenting and priming CD8 $^+$ T cells against cell-associated antigens, but have relatively low CD4 $^+$ T cell activating potential (Shortman and Heath, 2010). Mouse cDC1s express high levels of CD11c, MHCII, CD8 α , CD24, and often XCR1, CD141, Clec9a, DEC-205 and CD103

and require transcription factors IRF8, Id2 and Batf3 for their development. They generally express mRNA for most Toll Like Receptors (TLRs) except TLR5 and TLR7 and are characterized by high TLR3 expression (Edwards et al., 2003). Human cDC1 also express CD11c, HLA-DR, XCR1, CD141, Clec9a, DEC-205 and TLR3, and Batf3 has been implied in human cDC1 development (Poulin et al., 2010; Poulin et al., 2012), while IRF8 might affect both cDC1 and cDC2 development (Hambleton et al., 2011; Salem et al., 2014). Like mouse cDC1, human cDC1 have the greatest capacity for cross-presentation within the human DC populations (Poulin et al., 2010; Crozat et al., 2010; Jongbloed et al., 2010).

In contrast to the cDC1 population, mouse and human cDC2 strongly promote CD4+ T cell responses and have relatively poor capacity to cross-present cell-associated antigens to CD8+ T cells. Mouse and human cDC2 express several overlapping markers, including CD11c, MHCII, CD11b and CD172a (Signal regulatory protein Sirpa). Mouse cDC2 often express CD4 while human cDC2 are further identified by CD1c/BDCA1 expression. Mouse cDC2 have been shown to require IRF4, Eb2, RelB and Notch2 for their development, the role for these factors in human cDC2 development is not known.

2.1.2. Plasmacytoid DCs—pDCs are factories for type I IFN, playing an important role in the immune response to infection due to their capacity to produce large amounts of type I IFN upon engagement of TLR7 and TLR9. Mouse pDCs express relatively low levels of CD11c and MHCII and high levels of PDCA1 (CD317), B220, CD123, and SiglecH. Human pDC also have reduced CD11c and HLA-DR levels and co-express BDCA2 (CD303), BDCA4 (CD304), and CD123. Both human and mouse pDC development requires the transcription factor E2-2 and its target gene, Spi-B (Sasaki et al., 2012; Schotte et al., 2004; Cisse et al., 2008; Reizis, 2010). Although functionally relatively similar, human pDCs are able to cross-present cell-associated antigens in contrast to mouse pDCs, which show very poor capacity to do so (Hoeffel et al., 2007; Villadangos and Young, 2008).

2.2. Tissue DCs

A large percentage of cDCs reside in peripheral tissues and non-lymphoid organs where they help maintain tissue homeostasis, promote immune tolerance, and serve as first responders to infectious and neo-plastic insults. While these cells are generally less well characterized than their blood and lymphoid associated counterparts, they play an important role in anti-tumor responses as they represent the first DC populations present on site in the tumor microenvironment.

2.2.1. Skin DC—Three main DC populations have been described in the skin (Merad et al., 2013; Haniffa et al., 2015; Kashem et al., 2017). Mouse and human Langerhans cells (LCs) reside in the epidermis and share several markers (CD11c, MHCII/HLA-DR, CD11b, CD172a, langerin, EpCAM, and E-Cadherin). Mouse LCs further express CD205, while human LCs express CD1a and CD1c. Studies in mice indicate that although LCs share many characteristics with cDC, they are resistant to ionizing radiation and self-renew in situ. LCs have been shown to cross-present cell-associated antigens in vitro, but their capacity to do so in vivo has not been established (Igyarto and Kaplan, 2013). The two other principal mouse skin DC subsets are cDC1-like CD103⁺CD11b^{lo}Langerin⁺ DCs and cDC2-

like CD103⁻CD11b^{hi}Langerin⁻ DCs, which reside in the dermis. The CD103⁺ dermal DCs have the capacity for cross-presentation of cell-associated and particulate antigens (Henri et al., 2010; Romani et al., 2010; Bedoui et al., 2009). In humans, BDCA-1 expression is found on most of the dermal DCs with the majority of cells co-expressing CD11c and CD1a. Interestingly, similarly to mice, the cDC1-like BDCA-3⁺ dermal DCs demonstrate superior cross-presentation of soluble antigens as compared with other DC populations (Haniffa et al., 2012).

2.2.2. Other tissue DCs—Similar to the skin, various DC populations have been identified in the intestine. The mouse lamina propria contains 2 major populations, CD103⁺XCR1⁺CD11b⁻CD172⁻ DCs that are related to the Batf3, IFR8 and Id2 dependent cDC1 and CD103⁺XCR1⁻CD11b⁺CD172⁺ DCs that are related to the IRF4 and Notch2 dependent cDC2. In humans, analogous populations have been identified, and are divided based on expression of CD103⁺XCR1⁺CD141⁺Clec9a⁺CD172⁻ and CD103⁻XCR1⁻CD141⁻Clec9a⁻CD172⁺. A smaller, less studied CD103⁻XCR1⁻CD11b⁺/CD172a⁺ population can also be found in both mouse and humans, but its function is poorly understood (Bekiaris et al., 2014). cDC1 and cDC2 like cells are also found in the lung, with CD103⁺langerin⁺XCR1⁺Clec9a⁺ DC displaying potent capacity for cross-presentation compared to their cDC2⁻ like CD11b⁺CD172⁺ counterparts. CD103⁺/CD141⁺ and CD11b/CD172⁺ DC populations have been identified in both human and murine kidney and liver, but there is currently limited information on their functional properties (Rogers et al., 2014; Rahman and Aloman, 2013). Several of these tissue-associated cDC can migrate to the draining lymph nodes through the lymphatic system and are called tissue-migratory cDC. The phenotype of these DC therefore strongly correlates with the phenotype of their tissue resident counterparts.

2.3. Monocyte derived DCs

In addition to these steady state DCs, there are several populations of monocyte-derived DC (moDC) which differ from cDC in that they are derived from monocytes and not pre-DCs, migrating into tissues from the periphery. Generally, inflammatory moDC arise at the site of inflammation and the local inflammatory cues prompt their development and functional capacities. Inflammatory moDCs have been found in the skin of patients with atopic dermatitis (inflammatory dendritic epithelial cells (IDEC)), psoriasis (TNF and iNOS producing, TiP DCs), and synovial fluid of arthritis patients (Wollenberg et al., 1996; Segura and Amigorena, 2013). These types of moDC express CD11c, MHCII, CD11b, Ly6C, and CD206 (mouse) and CD11c, HLA-DR, BDCA1, CD1a, FcεR1, CD206, CD172a, CD14 and CD11b (human). The development of both human and mouse inflammatory moDCs seems to depend on transcription factor Zbtb46 and growth factor M-CSF (Segura and Amigorena, 2013). While in vivo cross-presentation of cell-associated antigens has not been well established by this subset, in vitro generated human and mouse DCs can efficiently cross-present cell-associated and soluble antigens.

2.4. Tumor infiltrating DCs (TIDCs)

Most of these DC subsets have been found in tumors where they play a major role in cancer immune-surveillance by coordinating adaptive immunity against tumor antigens.

The relative composition and functionality of TIDCs is strongly dictated by the tumor microenvironment, and can be highly dynamic, depending on factors such as tumor growth rate, vascularization and hypoxia, accumulation of other leukocytes, and cytokines and other soluble factors secreted by neoplastic cells. The frequency of TIDCs correlates with favorable prognosis and is inversely correlated with tumor pathologic stage in a variety of cancers (Karthaus et al., 2012; Ladanyi et al., 2007; Reichert et al., 2001). Due to their role in initiating anti-tumor immunity, there is a negative selective pressure hampering the accumulation of DCs by tumor-secreted mediators that inhibit dendropoiesis, promote DC apoptosis, and accelerate DC turnover (Esche et al., 1999; Pirtskhalaishvili et al., 2000; Peguet-Navarro et al., 2003; Kiertscher et al., 2000). Moreover, the tumor and its microenvironment actively exploit various mechanisms to suppress proinflammatory functions of infiltrating DCs and even induce DCs to suppress immune responses, and promote immune tolerance, tumor vascularization, and metastases (Ma et al., 2012; Ma et al., 2013; Tran Janco et al., 2015; Chaput et al., 2008).

3. DCs and T cell activation

In order to generate tumor-specific T cell responses, DCs must endocytose tumor antigens, process them and present them in the context of MHC along with co-stimulatory molecules and cytokines. The MHC/peptide complex of tumor antigens provides the first of three signals needed for optimal T cell activation. The MHC/peptide complex is recognized by its cognate T cell receptor (TCR) on naïve T cells in the draining lymphoid tissue, and the strength of T cell activation correlates with the MHC/peptide complex density on the DC as well as the TCR affinity for these complexed peptide antigens. The second signal derives from interactions of membrane associated co-stimulatory molecules in the synapse of T cells and DCs. This signal is mediated by co-stimulatory molecules and their ligands, including CD28-CD80/CD86, CD40LCD40, 4-1BBL/41BB, and SLAM-SLAM associated protein, as well as molecules that provide negative signaling such as PDL1 and PDL2 (reviewed in (Chen and Flies, 2013)). In the absence of sufficient co-stimulation (signal 2), T cells become anergic and fail to respond upon subsequent encounters with their cognate MHC/peptide complex. The third signal dictates the phenotype and function of the primed T cell, and is mediated by paracrine signaling by soluble cytokines. These can be pro-inflammatory (e.g. IL-12 and type I IFNs) or anti-inflammatory (e.g. TFG- β and IL-10), and help determine expression of chemokine receptors and integrins that facilitate T cell homing to specific target tissues. By providing these three signals, TIDCs induce tumor specific immunity mediated by CD8+ T cells, which can help eliminate primary and metastatic tumors, and protect against cancer recurrence.

However, TIDCs often have functional defects which impair their ability to prime anti-tumor immune responses and can even suppress anti-tumor immunity and facilitate tolerance. These functional deficits range from decreased capacity for phagocytosis, reduced antigen processing capacity, abnormal motility and decreased expression of MHCI, MHCII, pro-inflammatory cytokines, and co-stimulatory molecules such as CD40, CD80 and CD86 (Stoitzner et al., 2008; Diao et al., 2010; Diao et al., 2011; Ataera et al., 2011; Chiba et al., 2012). TIDCs also often have increased expression of inhibitory mediators, including the

cytokines IL-10 and TGF- β , IDO, iNOS, arginase, and the inhibitory B7 family members PDL1 and PDL2.

3.1. Cross-presentation of cell-associated antigens (Signal 1)

One of the central dogmas of immunology is that endogenous antigens are efficiently presented in the context of MHCI and exogenous antigens are presented in the context of MHCII. Intriguingly, some DCs are able to take up exogenous antigens in certain contexts (e.g. cell associated or particulate), process them, load them on MHCI, and present them on the cell surface through a process called cross-presentation (Compeer et al., 2012; Burgdorf et al., 2008; Bevan, 1976; Bevan, 2006). This is the prevailing mechanistic hypothesis explaining how TIDCs present tumor antigens in the context of MHCI, providing Signal 1 for priming naïve CD8⁺ T cells in the draining lymph nodes.

Several pathways for cross-presentation have been identified. The cytosolic pathway closely resembles the classic pathway for presentation of endogenous peptides on MHCI, in which endogenous proteins are cleaved by the proteasome, generating peptides that are subsequently translocated into the ER lumen by TAP^{1/2} for further processing and loading onto newly synthesized MHCI molecules (Amigorena and Savina, 2010; Joffre et al., 2012). Antigens cross-presented by this mechanism are taken up by DCs where they escape endosomes, entering the cytosol by an unknown mechanism where they are then cleaved by the proteasome. In contrast to the cytosolic pathway, cross-presentation through the vacuolar pathway does not require TAP^{1/2} and seems to result from antigen processing and loading of peptide antigens onto MHCI in the endocytic compartments. A third mechanism involving phagosome-ER fusion has also been demonstrated in DCs (Guermónprez et al., 2003; Ackerman et al., 2003; Houde et al., 2003). This is a sort of hybrid between the cytosolic and vacuolar pathways in which phagosomes combine with the ER and acquire antigen processing proteins such as MHCI and TAP^{1/2}. Antigens escape these phagosomes where they are cleaved by the proteasome, generating peptides that are transported back into the ER-phagosome where they are further processed and loaded on MHCI.

3.1.1. DC subsets and cross-presentation—Specific subsets of DCs have been shown to be more capable to cross-present cell-associated antigens than other populations. While most DC populations can cross-present materials under targeted experimental conditions, optimal presentation of (tumor) cell-associated antigens seems to be limited to a few populations of cDC1s (Faure et al., 2009; Nierkens et al., 2013; Thacker and Janssen, 2012). Several mechanisms have been uncovered that explain the improved capacity for cross-presentation of cell-associated antigens by these cDC1s. These include increased capacity to take up cell-associated materials, alterations in trafficking and acidification of endocytosed materials, and alterations in expression of components of the antigen processing machinery or its intracellular trafficking. DCs are able to recognize and endocytose dying cells by means of receptors that discriminate between “don’t eat me” signals provided by healthy cells (including CD47 that is recognized by CD172 and CD31), “find-me” and “eat-me” signals provided by specific entities on dead and dying cells (reviewed in (Medina and Ravichandran, 2016; Elliott and Ravichandran, 2016; Bratton and Henson, 2008; Liu et al., 2015)). The “find-me” signals have been suggested to include ATP,

uridine 5' triphosphate (UTP), sphingosine 1-phosphate (S1P), lysophosphatidyl choline, and fractaline/CX3CL1. Depending on the manner of cell death, the dying cells will expose "eat-me" signals on a continuum of dynamic and distinct cellular changes. Phosphatidyl serine (PtdSer), an early "eat-me" signal, can be bound directly by receptors such as TIM-1, TIM-3, TIM-4 and BAI or indirectly, using bridging molecules MFGE8, Gas6, protein S. Additional "eat-me" signals have been shown to result from changes in expression of ICAM-3, Cq1, oxidized lipids and alterations in glycosylation patterns. In the last decade, several new receptors and pathways that promote internalization of dying cells have been identified and it has become clear that the expression of these receptors is associated with distinct DC populations. Specifically, expression of DEC205, Clec9a and Axl/Lrp1/RANBP9 by cDC1 has been linked to the uptake of dead and dying cells (Shrimpton et al., 2009; Sancho et al., 2009; Ramirez-Ortiz et al., 2013; Zelenay et al., 2012; Subramanian et al., 2014). Intriguingly, use of specific internalizing receptors has been shown to affect subsequent endosomal trafficking, with receptors that promote uptake of smaller particles associated with a slower endosomal acidification rate. The delayed endosomal acidification would reduce proteolytic degradation and preserve antigenic structures, thereby providing a longer time-span where antigens can escape into the cytosol for processing (Compeer et al., 2012; Thacker and Janssen, 2012; Ackerman and Cresswell, 2004; Basha et al., 2008). cDC1 already display an increased propensity for delayed endosomal/lysosomal fusion compared to other DC populations, and one can easily envision that different combinations of DC populations and endocytic receptor usage will greatly affect the efficacy of a specific DCs in their capacity to cross-present cell-associated antigens in the context of MHCI.

3.2. DC maturation and activation (Signals 2 and 3)

DCs form the cellular link between innate and adaptive anti-tumor immunity, but only in the context of the appropriate maturation signals. The maturation status of the DC is critically important to the induction of T cell responses as it dictates the expression levels of MHC and membrane-associated and soluble co-stimulatory molecules (Lutz and Schuler, 2002). Immature DCs reside in the periphery and have great capacity for endocytosis. They express low levels of MHC class I and II, and membrane associated co-stimulatory molecules including CD40, CD80 and CD86. Upon maturation, DCs reduce expression of CCR6 and increase expression of CCR7 which facilitates homing to the draining lymphoid tissues. During migration to the lymphatics, DCs process the endocytosed antigens for presentation in MHC class I and II molecules, and increase the expression of MHCI/II and activating co-stimulatory molecules while acquiring the capacity to produce proinflammatory cytokines and chemokines that will facilitate their interaction with antigen-specific T cells. In contrast to normal tissue DCs, these essential functions are often impaired in TIDCs, which can maintain their immature phenotype. This can contribute to tumor tolerance, and has been suggested to result from decreased capacity to respond to normal maturation signals as a result of immune suppressive mediators produced by the tumor and its microenvironment.

3.2.1. Maturation signals in the immune response to infection and cancer—

Several pathways for DC maturation have been identified, with only some of them relevant to sterile inflammation and cancer, although there is significant overlap between pathogen sensing signals and signals that mediate sterile inflammation. Most research has been

performed in the context of infectious pathogens which has led to the identification of pathogen-associated molecular patterns (PAMPs) that engage specific pattern recognition receptors (PRRs) on the DC surface and in intracellular vesicles. Each DC subset has its own unique expression pattern of PRRs, causing these DC populations to respond differently upon engagement of the same PRR, providing highly modulated nuance to the immune response (Segura et al., 2010). There are several families of PRRs, including the Toll-like receptors (TLRs) and C-type lectin receptors that mainly recognize extracellular stimuli, and cytosolic PRRs, including RIG-I-like receptors (RLRs) and NOD-like receptors (NLRs) that are critically important for the sensing of intracellular pathogens (Kawai and Akira, 2009; Loo and Gale, 2011; Elinav et al., 2011; Osorio and Reis e Sousa, 2011; Kawai and Akira, 2011; Pandey et al., 2014). As maturation of DC by PRR signaling has been covered by various other reviews, we will provide a summary of different maturation signals, with emphasis on the STING and type I IFN pathway.

3.2.1.1. Toll-like receptors (TLRs): TLRs were the first PRRs to be identified and in addition to sensing PAMPs, they can also detect certain markers of sterile, lytic cell death, implicating them in tumor immunity in addition to infectious processes. The TLRs are membrane bound receptors that include at least 12 (mouse) and 10 (human) proteins that recognize a wide range of PAMPs and even some danger-associated molecular patterns (DAMPs), a broader term including endogenous molecules secreted by cells undergoing stress, or released during tissue damage and cell death that signal inflammation. Prokaryotic products recognized include lipoproteins (TLR $\frac{1}{2}$ /6), lipopolysaccharide (TLR4), and flagellin (TLR5) on the cell surface membrane. The intracellular vesicle-associated TLRs recognize dsRNA (TLR3), single stranded RNA (ssRNA, TLR7/8) and DNA (TLR9). Depending on the type of TLR, its signaling requires one or several of the adapter molecules MyD88, TRIF, TIRAP and TRAM, followed by activation downstream mediators which ultimately induce the translocation of NF κ B or IFN-regulatory factors (IRFs), including IRF3, IRF5, and IRF7 (Kawai and Akira, 2009; Kawai and Akira, 2011). These receptors can induce the rapid transcription of inflammatory cytokines, including type I IFN, IL-6, TNF α , and IL-12.

3.2.1.2. C-type lectin receptors: Non-mammalian carbohydrates such as mannose and acetylglucosamine form one such class of PAMPs recognized by C-type lectin receptors (Osorio and Reis e Sousa, 2011). These receptors are clustered into an activating group signaling through immunoreceptor tyrosine-based activation (ITAM) like motifs and ITAM-binding FcR γ adaptor molecules, and an inhibitory group which mediate their effect through immunoreceptor tyrosine-based inhibition (ITIM)-motifs. While important in sensing many bacteria, they are less relevant to innate immune sensing of cancer.

3.2.1.3. NOD-like receptors (NLRs): NOD-like receptors (NLRs) encompass group of cytosolic innate sensors that predominantly recognize bacterial cell components. Upon engagement with their cognate ligands, NLRs oligomerize into a caspase-1-activating scaffold known as the inflammasome. Active caspase-1 subsequently functions to cleave the proinflammatory IL-1 family of cytokines into their bioactive forms. The AIM2-like receptor (ALR) or p200 family contains several sensors of cytoplasmic DNA, including

IFI16 and AIM2. Like the NLRs, AIM2 functions in an inflammasome that complexes with caspase-1 and promotes the cleaving of the proinflammatory IL-1 family of cytokines. Interestingly, while having some homology with AIM2, the IFI16 (mouse p204) binds dsDNA, but does not form an inflammasome, and signals via STING for the induction of type I IFNs and other inflammatory mediators.

3.2.1.4. Retinoic acid inducible gene (RIG)-I like receptors.: Retinoic acid inducible gene 1 (RIG-I, or DDX58) like receptors are another family of PRRs comprised of DexD/H box RNA helicases that play an important role in antiviral immunity that detect viral associated RNA (Loo and Gale, 2011). This family encompasses cytosolic proteins RIG-I that binds 5'-triphosphorylated RNA and short double stranded RNA (dsRNA), and melanoma differentiation associated protein 5 (MDA5) that recognizes long dsRNA. RIG-I and MDA5 signal via their caspase activation and recruitment domain (CARD) to the mitochondrial antiviral signaling domain (MAVS, also known as Cardif or IPS-1) which subsequently stimulates TBK1, IKK and the IRF/NF κ B pathways resulting in the induction of inflammatory cytokines. Additional studies indicate that, for specific viruses, the RIG-I pathway converges with the STING pathway potentiating the anti-viral response.

3.2.1.5. DNA sensing by STING.: Recently, STING has been identified as an important regulator of immunity by mediating type I IFN production in response to cytosolic DNA (Burdette and Vance, 2013; Ishikawa et al., 2009). STING is a protein sensor of cytosolic DNA anchored in the ER. While initial studies suggested that STING may sense DNA structures directly, it is now widely accepted that other cytosolic DNA binding molecules upstream of STING are required for the activation of the pathway. The cyclic-GMP-AMP synthase (cGAS) is considered the predominant DNA sensor in this pathway. Upon binding to DNA in the cytosol, cGAS catalyzes the synthesis of cyclic GMP-AMP (cGAMP) from guanosine triphosphate (GTP) and adenosine triphosphate (ATP) (Ablasser et al., 2013; Civril et al., 2013). cGAMP functions as a second messenger that binds to and activates STING, resulting in its trafficking from the ER to the Golgi and on to perinuclear endosomes where it mediates subsequent signaling via phosphorylation of tank-binding kinase 1 (TBK1) and IRF3, which induces transcription of type I IFNs and other inflammatory genes (Fig. 1).

While the cGAS-cGAMP pathway is considered the dominant pathway, there is evidence that other sensors recognize cytosolic DNA associated with viral infection and use the STING-TBK-IRF3 pathway to induce type I IFN responses. Among them, DNA-dependent activator of IFN-regulatory factor (DAI), DEXD/H-box RNA helicase DDX41, DNA dependent protein kinase (DNA-PK), and IFN- γ -inducible protein (IFI16 in humans and ifi204/p204 in mouse) have been implied.

3.2.2. Signals associated with sterile, lytic cell death and the release of DAMPs—As with the immune response to pathogen infections, DCs must be appropriately activated in order to prime T cell responses against cancer. However, uptake of dying cells is generally considered to be a tolerogenic event in order to maintain tissue homeostasis and prevent development of autoimmune disorders. DCs that have phagocytosed dying cells have been reported to produce the immune-suppressive cytokines TGF- β and IL-10,

and are significantly inhibited in their capacity to produce pro-inflammatory cytokines and upregulate co-stimulatory molecules.

One mechanism for sensing cancer and developing inflammation in a sterile environment involves the release of endogenous molecules that serve as DAMPs, which can signal through the same PRRs that sense pathogen derived molecules. DAMPs encompass cellular components and factors that are either present before death and released by dying cells or generated as a result of cellular stress. Examples include ATP which may engage the NOD/NLRP3 inflammasome, HMGB1 which has been suggested to signal through TLR4, filamentous actin which is recognized by CLEC9a, as well as heat shock proteins and uric acid (Zhang et al., 2012). Normally intracellular and out of the reach of DCs under physiological conditions, these DAMPs serve as a danger signal when released into the extracellular milieu by stressed or dying cancer cells. When DAMPs are accessible to DCs, their presence signifies the loss of integrity of the cell membrane, suggestive of tissue damage caused by noxious origins such as infection, mechanical injury, or hypoxia. In a cancer vaccination animal model, TLR4 and its downstream signaling adaptor MyD88 have been implicated in the immune response induced by radiation and certain chemotherapies, however in other models TLR4/MyD88 was not found to be necessary or sufficient for the development of an immune response to cancers.

More recently, type I IFN production and signaling through the STING pathway has been identified as having an essential role in the development of anti-tumor immunity in response to tumor derived DNA. Protection of the integrity of genetic information encoded in DNA has been a primary prerogative of living organisms since their inception, and sensing threats in the form of DNA damage through radiation exposure, oxidative stress, and viral infection is essential to maintaining and continuing the line of genetic information. Cells have maintained this potential, especially in APCs such as DCs, which respond to DNA damage and cytosolic DNA by producing type I IFN. In addition to the recognition of dsDNA, several reports suggest that STING can promote type I IFN production upon RNA-virus infection through its interaction with the RNA sensing pathway components RIG1 and MAVS (Nazmi et al., 2012; Sun et al., 2012). However, evidence that the STING pathway in DCs is activated by RNA upon uptake of dead or dying cells is not available.

4. STING/IFN is an essential link between DNA damage and immunity

4.1. Type I IFN and DC function

Type I IFN signaling in DCs has been shown to promote their capacity to prime and cross-prime T cells in several ways. Type I IFNs are cytokines with potent activity on DC phenotype and function through their effects on all three signals needed to generate a robust T cell response. Type I IFNs are a collection of 13 IFN α proteins, as well as IFN β , IFN κ , and IFN ω , all of which signal through the common interferon (α and β) receptor (IFNAR). Essentially all nucleated cells express IFNAR, a heterodimer including the two distinct subunits IFNAR1 and IFNAR2. Binding of IFNs to their receptors leads to dimerization of the two subunits, resulting in auto phosphorylation of tyrosine kinase 2 (TYK2) and Janus activated kinase (JAK) 2 that activate the signal transducer and activator of transcription (STAT) 1 and 2 (Theofilopoulos et al., 2005). The activated STAT proteins

dimerized and rapidly translocate to the nucleus where they bind IFN-stimulated response elements (ISREs), initiating the transcription of hundreds of IFN-sensitive genes (ISGs).

Type I IFN facilitates cross-priming by DCs through a variety of mechanisms. It promotes antigen persistence through the reduction of the endosomal-lysosomal acidification rate, thus facilitating the storage of exogenously acquired cell-associated antigens in RAB5+ and RAB11+ compartments (Thacker and Janssen, 2012; Lorenzi et al., 2011). Furthermore, type I IFN promotes transcription and translation of the immunoproteasome subunits $\beta 1i$ (LMP2), $\beta 2i$ (MECL-1), and $\beta 5i$ (LMP7) (Shin et al., 2006; Shin et al., 2007), which modifies the peptidase activity of the constitutive proteasome, changing the repertoire of peptides for binding and presentation on MHCI. In addition, IFN α treatment promotes localization of MHCI molecules to antigen storage compartments in DCs, enhancing antigen presentation (Spadaro et al., 2012). In addition to these effects on antigen persistence, processing and loading, type I IFN also increases overall surface expression levels of MHCI and MHCII, as well as the membrane associated co-stimulatory molecules CD40, CD80, and CD86. Moreover, type I IFN can serve as a signal 3 cytokine, enhancing CD8+ T cell priming through its signaling within T cells (Agarwal et al., 2009; Curtsinger and Mescher, 2010; Xiao et al., 2009).

4.1.1. Type I IFN is essential for cross-priming and generation of tumor specific immunity in murine cancer models—

In more recent years a body of research has clearly linked DCs, type I IFN and the STING pathway to anti-tumor responses (see Table 1). In murine models, type I IFN seems to be required for anti-tumor immunity. Dunn et al. demonstrated that mice that lacked IFNAR were more susceptible to carcinogen-induced tumors, and mice treated with anti-IFNAR antibodies were much more susceptible to transplanted tumor outgrowth than mice given isotype controls (Dunn et al., 2005a; Dunn et al., 2005b). Fuertes et al. discovered spontaneous antitumor CD8+ T cell responses were defective in mice lacking either IFNAR or STAT1, a transcription factor immediately downstream of IFN signaling (Fuertes et al., 2011). Studies of mice lacking IFNAR specifically on DCs or lacking Batf3-dependent DC populations demonstrated that the anti-tumor responses were dependent on cDC1-like DCs (Diamond et al., 2011; Hildner et al., 2008; Klarquist et al., 2014).

4.1.2. Type I IFN and tumor immunity in humans—

It is not possible to be certain of the role of type I IFN in the immune response to human cancers, but there is strong correlative data suggesting its importance. In human patients with metastatic melanoma, metastatic lesions infiltrated with T cells are characterized by an IFN gene signature (Fuertes et al., 2011; Harlin et al., 2009). This inflamed phenotype is associated both with inhibitory factors that suppress T cell function and with response to immunotherapy, including checkpoint inhibitors targeting CTLA-4 and PD-1 (Ji et al., 2012; Topalian et al., 2012). A type I IFN gene signature in the primary tumor at diagnosis has also been found to be associated with improved prognosis and response to anthracycline chemotherapy in women with breast cancer (Sistigu et al., 2014). These associations are supported by studies in murine models, demonstrating that breast cancer is more likely to metastasize to bone when the IRF7 pathway is blocked (Bidwell et al., 2012). This pattern also holds in

studies of cancer vaccines, where a similar gene signature has been found to correlate with improved clinical responses. In aggregate, these findings support the relevance of type I IFN responses in human patients, and in fact motivated much of the work highlighting the importance of IFN in the animal models (Ulloa-Montoya et al., 2013).

4.2. STING is essential for tumor immunity

Signaling through the STING pathway is an essential precursor to generating the type I IFN needed for both spontaneous and treatment induced cancer immune responses. DNA sensing by STING is the upstream link that triggers type I IFN production by DCs and facilitates effective cross-priming of tumor specific CD8+ T cells. This DNA is likely derived from stressed and dying cancer cells, and is introduced into the DC cytosol through a yet unknown mechanism where it binds to cGAS, which initiates STING mediated type I IFN transcription (Fig. 2). The importance of this pathway has been demonstrated in a variety of tumor models, including colon carcinoma, melanoma, and lymphoma.

4.2.1. Spontaneous tumor immunity—The STING pathway is required for the development of spontaneous tumor immunity, with evidence coming from a variety of mouse tumor models. The seminal experiments performed by Woo, et al. demonstrated that STING and IRF3 deficient mice do not develop spontaneous CD8+ T cell responses against melanoma expressing a model antigen. In contrast, MyD88 knockout mice have no defect in spontaneous cross-priming of tumor specific T cells (Woo et al., 2014). STING was also required for spontaneous rejection of an immunogenic sarcoma, and the authors nicely explore the mechanism, showing that the STING pathway is activated in host DCs as a result of sensing tumor derived DNA (Woo et al., 2014; Woo et al., 2015). These findings have been extended to other tumor models, such as colitis induced cancer, in which STING deficient mice have increased susceptibility to colon cancer oncogenesis in response to inflammation (Zhu et al., 2014; Ahn et al., 2015). In a mouse model that spontaneously develops intracranial glioma, a loss of function mutation in STING results in reduced production of type I IFN by myeloid cells with impairment of tumor control by the immune system (Ohkuri et al., 2014). DNA damage is the likely stimulus for activating STING and transcription of type I IFN, as a breast cancer model defective in DNA damage repair is found to have accumulation of DNA in the cytosol and activation of an IFN response in a STING dependent manner (Parkes et al., 2017). These tumors with impaired DNA damage repair are grown in mice, they develop a T cell infiltrate compared with the wild type breast cancer tumors.

4.2.2. Radiation induced tumor immunity—Importantly, various therapeutic approaches have now been shown to require the DC-STING-IFN pathway for their effects. Radiation therapy is a prime example of this. Ionizing radiation has long been known to selectively kill cancer cells and has been used in cancer treatment for local tumor control for over a century. Cytotoxicity through damage of genomic DNA is thought to be the principal mechanism for the effects of radiotherapy, but more recently evidence has emerged that implicates the immune system in tumor control mediated by radiation (Apetoh et al., 2007; Burnette and Weichselbaum, 2013; Burnette et al., 2011; Lee et al., 2009). Furthermore, focal irradiation of tumors activates DCs and induces cross-priming of tumor specific T cells

that mediate systemic anti-tumor immunity (Demaria et al., 2004; Gupta et al., 2012; Lim et al., 2014; Lugade et al., 2005; Weichselbaum et al., 2017). This radiation induced anti-tumor immune response is dependent on STING and its upstream mediator cGAS (Deng et al., 2014a). Tumors in mice lacking these genes are not controlled by an otherwise effective dose of radiation, their DCs do not produce type I IFN in response to radiation, and there is a defect in cross-priming of tumor specific CD8+ T cells after radiotherapy.

4.2.3. Chemotherapy induced tumor immunity—Similar to radiation therapy, many antineoplastic chemotherapy agents induce cytotoxicity by damaging chromosomal DNA. Fibroblasts treated with Ara-C undergo DNA damage with nuclear DNA transported into the cytosol which can be sensed by STING, triggering a type I interferon response (Lan et al., 2014). Chemotherapy agents such as etoposide can also have direct effects on DCs causing DNA damage and transport of DNA to the cytosol which also elicits a STING-dependent Type I interferon response.

Different classes of chemotherapy agents induce differing types of DNA damage, and one can speculate that chemotherapeutic agents that support the formation of ligands for the STING pathway would provide clinical benefits by facilitating anti-tumor immunity. However, there is currently little known on the induction of STING ligands and type I IFN inducing capacity of the main DNA damaging chemotherapy agents.

4.2.4. STING and oncogenesis and cancer progression—Intriguingly, recent studies indicate that not all of the effects of STING activation promote tumor immunity and protection. STING agonists can also promote oncogenesis, for example mice treated cutaneously with 7,12-dimethylbenz[a]anthracene (DMBA) develop inflammation and skin cancer which is mediated by STING (Ahn et al., 2014). STING can also modify the tumor microenvironment to become more tolerogenic promoting tumor progression and metastasis through induction of IDO which promotes Treg function and immune tolerance of tumors by cytokines such as IL-10 and TGF- β (Lemos et al., 2016; Huang et al., 2013). At this point it is not clear why or under which circumstances STING and type I IFN promotes or suppresses anti-tumor immunity, although chronic inflammation is a well accepted stimulus of oncogenesis, and one can speculate that the complex tumor micro-environment with its various regulatory components such as Tregs, myeloid derived suppressor cells, and macrophages may help determine the effects of IFN stimulation.

5. Harnessing IFN responses in cancer therapy: mimicking the endogenous immune response to cancer

5.1. Cancer immunotherapy using pharmacologic STING agonists

In recent years, pharmacologic agents that activation the STING pathway have been used to mimic and augment the role of STING mediated production of type I IFN in anti-tumor immunity. Administration of STING ligands (c-di-GMP, DMXAA, cGAMP, CDN) significantly improve anti-tumor responses and animal survival in a diverse array of mouse models of solid and blood borne cancers (Deng et al., 2014a; Baird et al., 2016; Chandra et al., 2014; Corrales et al., 2015; Curran et al., 2016). DMXAA is a STING agonist with a

multi-faceted effect on the tumor microenvironment, and treatment of B16 melanoma with DMXAA augments tumor-specific CD8⁺ T cell responses which effectively immunize and protect mice against future tumor challenge (Corrales et al., 2015). Similar results are seen in mouse models of prostate and breast cancer as well as sarcoma (Baguley and Ching, 1997). In light of these promising results from the animal models, DMXAA was tested in a clinical trial of patients with non-small cell lung cancer. Unfortunately, DMXAA does not bind to human STING and thus was unable to activate this pathway, resulting in a negative trial (Lara et al., 2011; Gao et al., 2013). Another STING agonist, the cyclic diguanylate nucleotide (CDN) c-di-GMP, is effective in the treatment of 4T1 mammary carcinoma. A synthetic CDN agonist MLRR-S2 CDA has high affinity for human and murine STING and stimulates type I IFN production in human PBMCs (Corrales et al., 2015). This agent induces immune mediated tumor rejection of melanoma, breast and colon carcinoma in mice (Corrales et al., 2015). CDN based STING agonists have also been used in conjunction with the GVACs cancer vaccine along with a PD1 antagonist, which resulted in an additive effect on tumor immunity (Fu et al., 2015). This opens new avenues for additional novel therapies including CD47 (“don’t eat me” signal) blockade which has been shown to be critically dependent on the IFN/STING pathway (Liu et al., 2015).

5.2. Cancer immunotherapy using ionizing radiation

As described above, radiotherapy induces DNA damage that is sensed by TIDCs through the STING pathway, and there are currently many pre-clinical and several clinical studies utilizing radiation to stimulate anti-tumor immunity. Since radiotherapy alone rarely induces an anti-tumor immune response that results in systemic control of metastatic disease, the therapeutic effects of radiation on the innate and adaptive immune system have been largely explored in combination with other immunotherapy modalities. The initial studies demonstrating the synergistic effect of radiation combined with immune modulators were performed by Demaria and Formenti. Their group observed improved tumor control within the irradiated field and objective responses in distant sites of disease using FLT3 ligand or antibody antagonists to the CTLA-4 checkpoint receptor in breast cancer and colon cancer models (Demaria et al., 2004; Demaria et al., 2005a; Dewan et al., 2009; Demaria et al., 2005b). Similar findings in other animal models combining radiation with PD-1 inhibitors, DC vaccines, and other immune modulators have been observed, and further mechanistic studies have been performed by multiple groups (Deng et al., 2014b; Filatenkov et al., 2015; Sharabi et al., 2015a; Sharabi et al., 2015b; Vatner et al., 2014; Yoshimoto et al., 2014). One study even combined radiation therapy with a pharmacological STING agonist and found improved tumor control and anti-tumor immunity, suggesting that radiation is providing a unique ingredient to the production of anti-tumor immunity above and beyond STING activation (Baird et al., 2016).

These animal studies have been translated successfully into preliminary clinical trials of patients with metastatic cancer, and there are many ongoing trials testing this in patients with a variety of malignancies. Most of these trials combine radiation with an immune checkpoint inhibitor of CTLA-4 or PD-1 with the goal of priming tumor specific T cells with radiation, and boosting that T cell response with the checkpoint inhibitors to overcome the suppressive

microenvironment. Preliminary results appear promising, but mature results from these trials are pending (Golden et al., 2015).

With radiotherapy there are always questions about the optimal dose to use and how many treatments to deliver (fractionation) for activating STING and initiating an immune response. This is an active question in the field, however there are few preclinical studies and even fewer clinical trials comparing different doses and fractionation treatment schedules. Some of the original studies by the Demaria group explored dose and fractionation, finding that 24 Gy delivered in three fractions of 8 Gy resulted in more robust systemic tumor control compared with 30 Gy in five fractions or a single fraction of 20 Gy (Dewan et al., 2009). A recent study by this group explored the mechanistic underpinnings of why a single larger fraction of 20 Gy of radiation is not as effective as three fractions of 8 Gy, and found that both treatments resulted in transport of dsDNA to the cytosol of tumor cells where it was sensed by cGAS, which activated STING and type I IFN transcription (Vanpouille-Box et al., 2017). However, the larger dose of radiation induced the three prime exonuclease 1 (TREX1), which degraded the cytosolic DNA, limiting STING activation and the IFN response. Studies by other groups comparing single fraction treatment with fractionated treatment have shown conflicting results, however most of these utilized single fraction treatment doses < 20 Gy, the apparent threshold for activation of TREX1.

Beyond dose and fractionation, there are other questions that remain about the optimal application of radiation therapy for stimulating STING mediated tumor immunity. Some of these questions include the importance of the anatomical location of the target lesion, whether it is better to treat the primary tumor or metastatic sites, the effect of radiation dose rate, and the effect of different modalities and energies of ionizing radiation (e.g. photon vs. proton vs. electron). These questions remain to be worked out.

6. Conclusion

The role of the immune system in combatting cancer has been a topic of speculation and debate over the past century, with mounting experimental evidence in support of this function of the immune system. However, it was not until recent years with the success of cancer immunotherapy applied to patient care that cancer immunity has received widespread acceptance. How the immune system is alerted to the presence of cancer has been one of the central questions in cancer immunology. DCs are the link between the innate and adaptive immune systems, only priming T cell responses against cellular antigens in the context of an inflammatory signal. There has been some debate regarding the nature of this inflammatory signal in the response to tumors, but recent evidence summarized in this review support the hypothesis that damaged DNA released by cancer cells is the stimulus for generating tumor immunity. This DNA gains access to the cytoplasm of DCs where it is recognized by cGAS which triggers STING and production of the type I IFNs necessary for effective cross-priming of CD8+ T cells against tumor antigens.

The primacy of DNA sensing as a stimulus for DC activation in response to cancer helps inform an ontological question in cancer immunology. Cancer has historically been viewed as pathogenic process separate from the host organism. Even the concept of “tumor” and

“host” suggests an otherness to cancer. This thinking naturally led to a search for the danger signals produced in response to cancer, and broadened our understanding of innate immunity as a means to detect self from non-self through PAMPs into a more generalized view as a means of detecting danger from the absence of danger through DAMPs. The fact that DCs are sensing damaged DNA rather than a DAMP associated with lytic cell death places the immune response to cancer more in the realm of assaults on the integrity of the genome rather than an exogenous pathogen.

Although much of the mechanism for sensing of cancer derived DNA via the STING pathway in DCs has been elucidated, many questions remain. One such question relates to how tumor DNA taken up by DCs gains access to the DC cytosol. It may be more than coincidence that DC populations that are efficient in cross-presentation and that exhibit delayed endosomal acidification seem to be explicitly implicated in the STING dependent induction of type I IFN. One can envision a mechanism for DNA escape from endosomal compartments analogous to the manner by which endocytosed proteins escape into the cytosol where they are processed into peptides for presentation on MHCI. Another unanswered question relates to the nature of the direct upstream signaling molecules that bind cytosolic DNA and engage STING, as these could be targeted for therapeutic benefit. It is also intriguing how STING activation promotes anti-tumor immunity in some models, while in others it suppresses the immune response to the tumor, promoting tolerance.

This is a most exciting time for tumor immunology, as we are seeing the scientific understanding of cancer translated into human clinical trials. The application of therapies targeting the STING pathway to mimic and enhance the endogenous immune response to cancer is currently underway, and results from these trials will help enhance our understanding of cancer immunity, offering a hopeful future for enlisting the immune system in the fight against cancer.

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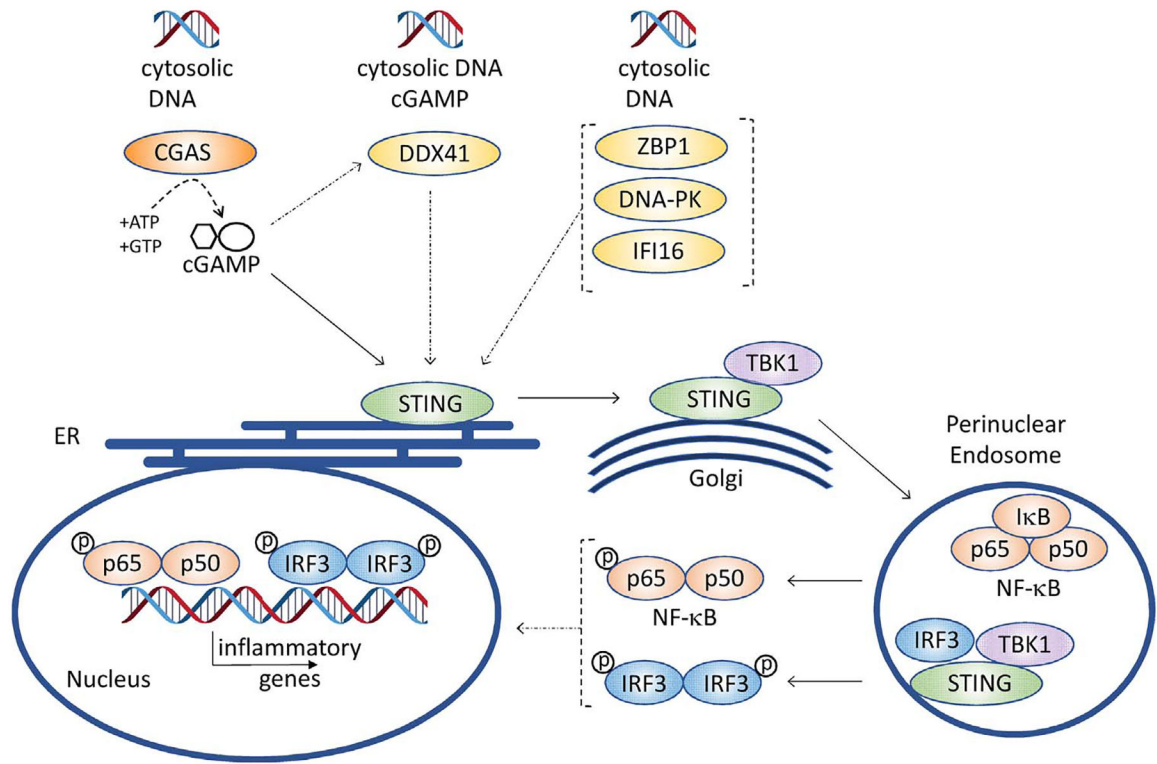


Fig. 1. The STING signaling pathway. Cytosolic DNA is primarily sensed by cyclic-GMP-AMP synthase (cGAS), which generates cyclic GMP-AMP (cGAMP) from guanosine triphosphate (GTP) and adenosine triphosphate (ATP). cGAMP functions as a second messenger that binds to and activates STING, resulting in its trafficking from the ER to the Golgi and on to perinuclear endosomes where it mediates subsequent signaling via phosphorylation of tank-binding kinase 1 (TBK1), IRF3, and NF- κ B, which induce transcription of type I IFNs and other inflammatory genes. DDX41, ZBP1, DNA-PK, and IFI16 can also act as sensors of cytosolic DNA with similar downstream effects on STING.

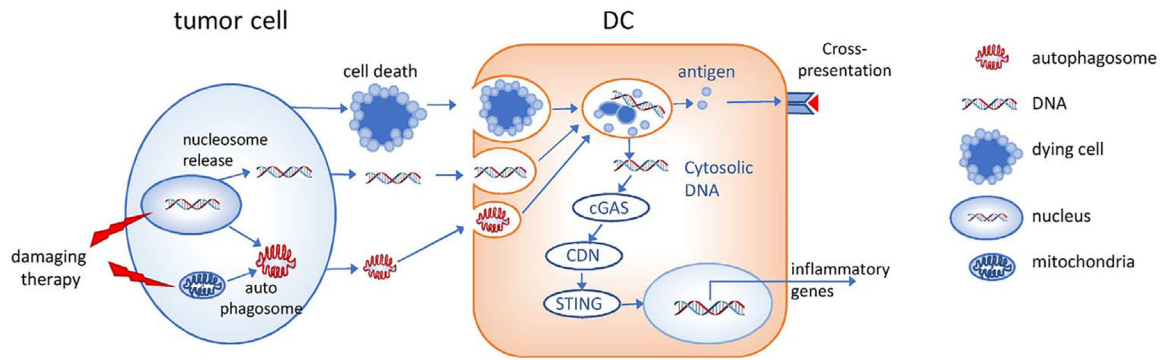


Fig. 2. STING-mediated induction of type I IFN in DCs upon sensing of tumor DNA. DNA damaging therapy results in the induction of cell death, nucleosome release, mitochondrial damage that are endocytosed by DCs. Upon escape of endosomal tumor-derived DNA into the cytosol, tumor-DNA can interact with cGAS which results in the induction of CDN and the activation of STING. Downstream signaling from STING induces pro-inflammatory genes, including type I IFNs. In parallel, protein escape from endosomal degradation promotes cross-presentation in MHC class I.

Table 1

Immune responses dependent on type I IFN, STING and DAMP (DNA).

Author	Pathway	Model	Outcome
Dunn et al. (2005a,b)	IFNAR ^{-/-}	Carcinogen induced tumors, MCA induced tumor lines	Mice lacking IFNAR were more susceptible to carcinogen induced tumors and had increased tumor outgrowth in tumor transplant settings.
Fuertes et al. (2011)	IFNAR Ab IFNAR ^{-/-}	B16-S1Y	Tumor rejection and induction of tumor-specific CD8 ⁺ T cells is impaired in KO mice. Type I IFN sensing in Batf3 dependent CD8 ⁺ DC is required for anti-tumor response.
Burnette et al. (2011)	STAT1 ^{-/-} Batf3 ^{-/-} IFNAR ^{-/-}	Radiotherapy + B16/F10	Progression free survival depends on type I IFN sensing. Administration of type I IFN improves anti-tumor response.
Hildner et al. (2008)	Batf3 ^{-/-}	H31m1 fibrosarcoma	Mice lacking Batf3-dependent CD8 ⁺ DC failed to control tumor growth.
Klarquist et al. (2014)	IFNAR	B16/F10	Decreased anti-tumor CD8 ⁺ T cell response in IFNAR ^{-/-} , IRF3 ^{-/-} and STING ^{-/-} mice. DC from IRF3 and STING ^{-/-} mice show significantly reduced type I IFN upon phagocytosis of dying tumor cells.
Diamond et al. (2011)	IRF3 ^{-/-} STING ^{-/-} IFNARfl-CD11c-CRE	B16-OVA H31m1, d38m2 MCA sarcoma	Induction of anti-tumor CD8 ⁺ T cell responses depends on type I IFN sensing by DC.
Woo et al. (2014)	IFNAR Ab STING ^{-/-}	B16-S1Y	STING and IRF3 are required for spontaneous anti-tumor CD8 ⁺ T cell responses and control of tumor growth. Type I IFN production to tumor DNA in DC requires STING, cGAS and IRF3.
Deng et al. (2014a)	IRF3 ^{-/-} IFNAR ^{-/-}	MC38 + radiation	Type I IFN sensing and STING are required for the anti-tumor effect or radiation. IRF3, STING and cGAS are required for optimal cross-priming in vitro. Intratumoral administration of cGAMP promotes anti-tumor responses.
Chandra et al. (2014)	IFNAR Ab STING ^{-/-} IRF3 ^{-/-} cGAS ^{-/-} r-cGAMP	4T1	Treatment with a listeria based vaccine in combination with c-di-GMP significantly reduced the number of metastases.
Curran et al. (2016)	listeria based vaccine + c-di-GMP DMXAA	C1498	Systemic administration of DMXAA improves outcomes in AML models and increased anti-leukemic T cells.

Author	Pathway	Model	Outcome
Ohkuri et al. (2014,2015)	STING ^{-/-}	C1498-SIY	STING ^{-/-} mice succumb faster in a model of de novo glioma and have increased MDSC and Treg and reduced IFN- γ and CD8 ⁺ T cells. C-di-GMP enhanced vaccine effect against an implanted glioma line.
	IFNAR ^{-/-}		
Baird et al. (2016)	STING ^{-/-}	Genetic model of de novo induction of glioma	Intratumoral administration increases radiation therapy efficacy, prevents recurrences and promotes abscopal effect.
	c-di-GMP + peptide vaccination	GL261-OVA	
Wang et al. (2017)	STING ^{-/-}	Panc02, 3LL	STING and cGAS promote tumor-specific CD8 T cells. STING and cGAMP are essential for the therapeutic effect of PDL-1 blockade. Intratumoral cGAMP administration enhances anti-tumor effects by PDL-1.
	CDG	SCCVII, Genetic pancreatic adenocarcinoma model	
	STING ^{-/-}	B16/F10	
Liu et al. (2015)	cGAS ^{-/-}	B16-OVA	CD47 blockade promotes T cell mediated tumor killing which is dependent on type I IFN sensing in DC and STING.
	PDL-1 blockade		
	STING ^{-/-}	MC38	
Li et al. (2016)	CD11c-Cre IFNAR1 IFNAR Ab		Intratumoral administration of cGAMP reduces tumor growth rate in a STING-dependent manner. Combinations of cGAMP with 5-FU improves anti-tumor activity and reduces 5-FU toxicity.
	CD47 Ab	CT26	
	STING ^{-/-}		
	cGAMP		
	5-FU		