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New perspectives on type I IFNs in cancer

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Abstract

Although type I IFNs were initially described based on their anti-viral properties, it was quickly realized that these cytokines had anti-proliferative and anti-cancer activities. These observations ultimately led to the clinical development and utility of IFN- α 2b for the treatment of patients with melanoma, renal cell carcinoma, and chronic myelogenous leukemia, among others. However, the mechanism of action of type I IFNs in vivo was never fully elucidated, and the pleiotropic effects of IFNs on multiple cell types had made it challenging to decipher. Advancement of genetically engineered mouse models has provided new tools for interrogating these mechanisms. Recent evidence has indicated that spontaneous innate immune sensing of cancers that leads to adaptive immune responses is dependent on host type I IFN production and signaling. The major innate immune receptor pathway that leads to type I IFN production in response to a growing tumor appears to be the STING pathway of cytosolic DNA sensing. STING agonists drive type I IFN production and are impressively therapeutic in mouse tumor models. Targeting low doses of type I IFNs to the tumor microenvironment also promotes anti-tumor activity via host adaptive immunity that is T cell-dependent. However, high doses of intratumoral type I IFNs largely function via an anti-angiogenic effect. Understanding these mechanistic details should enable improved clinical manipulation of the type I IFN system in cancer.

1. Endogenous innate immune sensing of cancer involves host type I IFN signaling

A major subset of human cancer patients shows evidence for a spontaneous T cell response against their tumor as evidenced by a T cell-inflamed tumor microenvironment gene expression signature and the presence of CD8⁺ T cells by immunohistochemistry (1–3). Tumor antigen-specific T cells have been identified among this infiltrate, arguing that at least a component of this T cell population is directly tumor-reactive (4–6). The positive prognostic import of this phenotype (7, 8) suggests that this smoldering immune response is attempting to control the tumor, but without the ultimate success of tumor elimination. In

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fact, recent evidence suggests that this subset of tumors is dominated by immune inhibitory pathways that restrain T cell function and ultimately allow tumor outgrowth (9, 10). Targeting these immune inhibitory pathways has led to a new class of cancer immunotherapies, including anti-CTLA-4 and anti-PD-1/PD-L1 mAbs (11–13). As such, understanding the underlying molecular mechanisms that control the presence or absence of this spontaneous T cell-inflamed tumor microenvironment phenotype has evolved into an active area of investigation.

Productive T cell activation and differentiation into effector cells is thought to depend upon proper innate immune signaling upstream, particularly at the level of dendritic cells (DCs). However, how a sterile tumor could potentially lead to T cell priming in vivo in the absence of exogenous pathogen-associated molecular patterns (PAMPs) had been elusive. Interrogation of melanoma gene expression profiles for evidence of innate immune activation pathways that might be associated with the presence of T cell transcripts revealed evidence for a positive correlation with a type I IFN gene signature (14, 15). Based on this observation, preclinical mouse model experiments were performed and indeed revealed that type I IFN signaling was required upstream for spontaneous T cell priming against tumor-associated antigens in vivo (14, 15). Similarly, host type I IFN signaling was required for spontaneous regression of immunogenic tumors. IFN- β was found to be rapidly induced upon tumor implantation in vivo, largely by CD11c⁺ DCs. Detailed mapping using mixed bone marrow chimeras and conditional type I IFNR^{-/-} mice demonstrated that type I IFN signaling had to occur within the Batf3-lineage CD8 α ⁺ subset of DCs (16). Thus, like for most viral infections, host type I IFN signaling is crucial for an adaptive immune response against tumors in vivo. Knowledge of this requirement for natural immunity against tumors has provided new insights to guide therapeutic considerations for type I IFNs in the cancer context.

2. A major mechanism of innate immune sensing that leads to type I IFN production is through the STING pathway

The observation that type I IFN production was induced in response to a growing tumor in vivo raised the next level question of which innate immune pathway might be “sensing” the presence of tumor and thereby promoting induction of type I IFN gene expression. From the infectious disease context, several distinct receptor and signaling systems have been identified that could ultimately lead to type I IFN transcription. These are the TLR pathways that signal via MyD88 and/or TRIF (15), the cytosolic RNA sensing pathways that signal via MAVS, and the cytosolic DNA sensing pathway that signals through STING (17). Gene-targeted mice lacking these individual pathways were employed in order to evaluate whether each of these might be required for induction of type I IFN production and spontaneous T cell responses against tumor-associated antigens in vivo. Interestingly, no defect was seen in mice deficient in MyD88, TRIF, or MAVS, nor was any defect observed in specific TLR^{-/-} mice. However, a major defect in both type I IFN induction and T cell priming was observed in STING^{-/-} mice, as well is in mice lacking the downstream transcription factor IRF3. In vitro, tumor-derived DNA was capable of inducing STING pathway activation and type I IFN production when introduced into the cytosol of DCs in vitro. Moreover, labeled tumor-

associated DNA was identified within the cytosol of host DCs within the tumor microenvironment in vivo. Together, these data argue that a major mechanism for innate immune sensing of tumors occurs via cytosolic DNA sensing through the STING pathway within tumor-infiltrating DCs (Woo, Gajewski et al., Immunity, In Press 2014).

Recent data have indicated that radiation therapy for cancer also involves induction of type I IFNs by host DCs and facilitation of an adaptive immune response that contributes to tumor control in vivo (18). Thus, it was of interest to determine whether the STING pathway was also important for the therapeutic effect of radiation. Indeed, the efficacy of radiation was largely ablated in STING^{-/-} hosts, whereas no defect was observed using mice lacking specific TLR signaling pathways (Deng et al, Immunity, In Press 2014). Thus, it is tempting to speculate that radiation facilitates the proper acquisition of tumor-derived DNA by host DCs in the tumor microenvironment as a major mechanism of immune priming in response to tumor irradiation.

3. Therapeutic strategies to promote endogenous type I IFN production

Based on the discovered importance of the STING pathway and host type I IFN induction as a bridge to adaptive immune responses against tumors in vivo, strategies to mimic or activate host innate immune sensing pathways as a cancer therapeutic are being considered. 5,6-Dimethylxanthenone-4-acetic acid (DMXAA) is a drug that has anti-tumor activity in mouse models but which failed to show benefit in advanced stage clinical trials in cancer patients (19). However, the molecular target of DMXAA had not been defined at the time of those studies. Recently, it was reported that DMXAA directly binds to murine STING, and a crystal structure of the interaction has been solved (20). Importantly, this compound fails to bind human STING, which probably explains the failure of this agent clinically (21). Using mouse transplantable tumor models, we have observed that intratumoral administration of DMXAA exerts profound anti-tumor activity that is completely dependent on host STING. Treatment is associated with a marked increase in systemic anti-tumor T cell responses, which is capable of regressing non-injected distant tumors. Novel cyclic dinucleotides have been synthesized that do indeed bind human STING and induce type I IFNs from human dendritic cells in vitro (Corrales, Gajewski et al., manuscript submitted). Thus, it is hoped that clinical grade drug will be developed for future translation into human phase I trials.

4. Therapeutic strategies to target type I IFNs to the tumor microenvironment

As an alternative to eliciting type I IFN production from host DCs in vivo, targeting of type I IFNs directly into the tumor site also is being explored. Interestingly, the mechanism of anti-tumor activity seems to vary depending on the specific strategy utilized, which likely is related to the dose and duration of type I IFN presence within the tumor microenvironment. We found that transfection of tumor cells to express high levels of IFN- β led to tumor regression that was largely independent of host adaptive immunity. Rather, this approach led to an elimination of the tumor vasculature, consistent with an anti-angiogenic effect. Most of the therapeutic effect was preserved in RAG^{-/-} and NK cell-depleted mice. Surprisingly, bone marrow chimera experiments revealed a major role for type I IFN signaling on non-

hematopoietic cells. Moreover, conditional type I IFNR^{-/-} mice lacking type I IFN signaling exclusively on Tie2⁺ cells lost the therapeutic effect of high-dose IFN- β , arguing for a direct effect on the tumor vasculature (22).

In contrast, an alternative approach to introduce IFN- β into the tumor microenvironment is by the use of tumor-targeting monoclonal antibodies (mAbs) coupled to IFN- β protein as a payload. Indeed, coupling of IFN- β either to anti-Her2 or anti-EGFR mAbs led to a synergistic therapeutic effect in the appropriate models, that was completely T cell-dependent. In this case, type I IFN signaling was required on bone marrow-derived cells. Conditional type I IFNR^{-/-} mice lacking type I IFN signaling specifically on CD11c⁺ cells lost the therapeutic effect with this strategy (23). Thus, transient expression of lower doses of IFN- β within the tumor microenvironment may facilitate adaptive immunity to tumors. The distinction between these in vivo therapeutic effects is summarized in Figure 1.

5. Implications and future directions

The identification of the host STING pathway as a critical mechanism of innate immune sensing of cancers, that drives type I IFN production and facilitates anti-tumor immunity, raises the question of whether non-T cell-inflamed tumors lack activation of the STING pathway in vivo. It will thus be critical to phenotype DC subsets within human tumors, and understand if Batf3-lineage DCs are present and activated within the non-T cell-inflamed tumors. Understanding the level of block in tumors of this phenotype should instruct on how to intervene therapeutically to overcome the putative immune defect involved. The development of human STING agonists for clinical translation should move forward, to test whether direct STING activation within the tumor microenvironment might be therapeutic in patients. Such studies should involve careful pharmacodynamic biomarker evaluation, for example to determine whether post-treatment biopsies show upregulation of type I IFNs. The pursuit of IFN- β /mAb fusions is also ripe for clinical translation, for example with anti-Her2 mAb. The recent success of TDM1 as a mAb-chemotherapeutic toxin fusion has increased enthusiasm for such payload approaches. Ultimately, refining strategies for delivering type I IFNs directly within the tumor microenvironment should improve the therapeutic window for these cytokines and increase effectiveness against cancer.

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Figure 1. Therapeutic mechanisms of intratumoral type I IFNs appears to vary with dose
 Low doses of endogenous IFN-β produced in response to innate immune sensing of cancer is upstream from T cell priming and drives an adaptive immune response. Targeting of low doses of IFN-β into tumor sites with mAb conjugation also facilitates induction of T cell responses against tumor antigens. However, high sustained doses of IFN-β, which also has a therapeutic effect, largely works via a T cell-independent mechanism. Instead, a direct anti-angiogenic effect is observed, which is mediated through the type I IFN receptor (IFNAR) expressed by endothelial cells.