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Minireview

Combination Cancer Therapies with Immune Checkpoint Blockade: Convergence on Interferon Signaling

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Improving efficacy of immune checkpoint blockade for cancer can be facilitated by combining these agents with each other and/or with other conventional or targeted therapies. Interferon and innate immune signaling pathways in immune and tumor cells have emerged as intriguing determinants of response and resistance, often in complex and seemingly paradoxical ways.

Checkpoint Blockade and Combination Therapy

Harnessing the immune system against cancer is becoming an increasingly effective therapy option that can result in dramatic and durable responses in several cancer types. One approach to achieve the reactivation of endogenous anti-tumor T cells is by blocking inhibitory receptors, or immune checkpoints, expressed on T cells and other leukocytes. These inhibitory receptors are transiently upregulated on activated T cells after priming but are sustained on chronically stimulated T cells, such as those in tumors, to negatively regulate the function of these ''exhausted'' T cells (Pauken and Wherry, 2015). Exhausted T cells have diminished proliferative capacity and have poor cytokine production and effector function compared to non-exhausted effector or memory counterparts. As a result, these cells fail to eradicate tumors and provide poor protective immunity. Blocking inhibitory receptors can at least partially reverse T cell exhaustion and improve anti-tumor T cell responses. Seminal examples of this immunobiology include the demonstration that a blocking antibody to CTLA4, an immune checkpoint receptor on T cells, results in long-term survival in ~20% of patients with metastatic melanoma, and blockade of the T cell immune checkpoint receptor PD-1 or its ligand PD-L1 achieves responses as high as 30%–40% in several solid tumors (Topalian et al., 2015). Despite these impressive clinical results, the majority of patients are either resistant or relapse after therapy, highlighting the need to understand mechanisms that drive resistance.

The determinants of response and resistance to immune checkpoint blockade (ICB) are clearly multi-faceted (Chen and Mellman, 2013). As such, the use of combination therapy that non-redundantly targets the steps that stall the generation of an effective anti-tumor immune response can improve therapeutic efficacy. Interestingly, many combination approaches that are being explored engage innate immune signaling pathways that converge onto interferon (IFN) signaling in an effort to boost innate immune activation (Figure 1). However, IFN-related signaling can have opposing effects on anti-tumor responses, a phenomenon consistent with the complex biology unraveled by studies on host-virus interactions. Thus, the success of ICB combination therapies employing innate immune activation via IFN pathways may rely on a detailed understanding of immune-suppressive and stimulatory IFN signaling.

Engaging IFN Pathways in Immune Cells

Most of the antigen-presenting cells such as dendritic cells (DCs) and tumor-associated macrophages in the tumor microenvironment are thought to be dysfunctional (Broz et al., 2014), which can lead to ineffective activation of T cells despite the availability of tumor antigens. Recent evidence highlights how DC activation through the cGAS/STING pathway can promote tumor rejection after ICB and after conventional cancer therapies such as radiation therapy (RT) (Deng et al., 2014; Woo et al., 2014). cGAS/STING is a pattern recognition receptor (PRR) that normally recognizes pathogenic cytosolic DNA. STING in DCs can be activated by DNA originating from dying cancer cells and is critical for high levels of type 1 IFN (IFN-I) generated during an anti-tumor immune response and for optimal cross-priming of T cells. Thus, activating the STING pathway in DCs represents one approach to enhance ICB when immune infiltrate and tumor antigen are present, but additional signals for DC activation are required.

Several therapeutic approaches have been used to activate STING in DCs during ICB. Direct STING agonists such as synthetic cyclic dinucleotides (CDN) mimic cyclic GMP-AMP normally produced by cGAS after recognizing cytosolic DNA. CDNs can significantly improve responses to a GM-CSF-secreting tumor vaccine (GM-vaccine) combined with anti-PD-1 in mouse models, leading to tumor eradication (Fu et al., 2015). The effect of CDNs on DCs is particularly dependent on $CD8^+CD103^+$ DCs that possess superior T cell cross-priming ability (Corrales et al., 2015). Another approach to

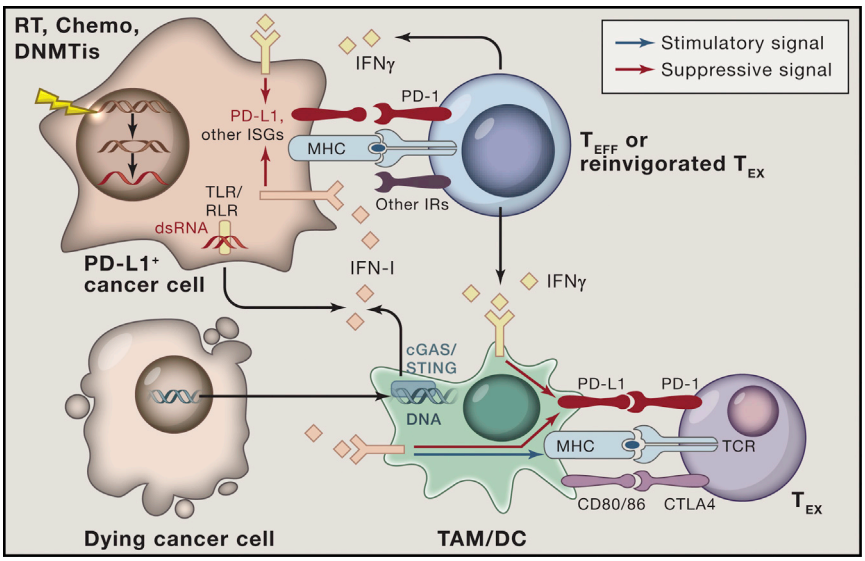

Figure 1. Combination Therapy with ICB and Opposing Roles of IFN Signaling in Tumor and Immune Cells

Therapeutically reinstating T-cell activation can result in upregulation of PD-L1 on tumor cells, DCs, and/or tumor-associated macrophages (TAMs) through T-cell-derived IFNγ or IFN-I, necessitating blockade of PD-1/PD-L1 to reinvigorate exhausted T cells (T_{EX}) or allow for development of effector T cells (T_{EFF}). Other suppressive ISGs can be induced, and blockade of other T cell inhibitory receptors (IR) may be necessary. Cytotoxic agents can increase IFN-I through the de-repression of endogenous RNA DAMPs that activate TLRs/RLRs or through the DNA from dying cells stimulating STING in DCs. Suppressive effects of IFN-I may occur, particularly with IFNβ. ISG expression in cancer cells can also directly impact resistance to cytotoxic cancer therapies independent of the adaptive immune system.

activate STING and other PRR pathways in DCs is using cytotoxic/genotoxic agents such as RT and chemotherapy. In addition to tumor-intrinsic DNA damage, RT and chemotherapy can have immunogenic effects that elicit a T-cell-dependent anti-tumor response (Kroemer et al., 2013). RT can activate STING in DCs to augment IFN-I production and contribute to immune-mediated regression of irradiated tumors (Deng et al., 2014). Although mechanistic details are unclear, DNA from dying tumor cells appears to be a ligand for cGAS. Interestingly, promoting phagocytosis with anti-CD47 antibodies can also elicit anti-tumor T-cell responses in a manner dependent on STING in DCs (Liu et al., 2015), further suggesting that tumor material can act as damage-associated molecular patterns (DAMPs). Like with direct STING agonists, RT can enhance the efficacy of ICB or ICB combinations in several mouse models (Demaria et al., 2015; Twyman-Saint Victor et al., 2015). Similar to RT, chemotherapy can improve responses to ICB in pre-clinical models (Pfirschke et al., 2016), and both can activate TLR4 in DCs by engaging HMGB1, another DAMP released by dying tumor cells (Kroemer et al., 2013). Whether the ability of RT, chemotherapy, or other cytotoxic agents to enhance ICB is solely dependent on PRR signaling in DCs is unclear, as additional routes to activate IFN and innate immune signaling in the tumor microenvironment exist, as discussed below.

Engaging IFN Pathways in Tumor Cells

In addition to stimulating STING and IFN pathways in DCs, genotoxic anti-cancer agents also can activate PRRs and IFN signaling in cancer cells. Treatment with DNA methyltransferase inhibitors (DNMTis), RT, and chemotherapy can all lead to enhanced expression of interferon-stimulated genes (ISGs) (Chiappinelli et al., 2015; Roulois et al., 2015; Sistigu et al., 2014; Weichselbaum et al., 2008). The expression of these ISGs can correlate with tumor response and is driven by PRRs in cancer cells, including Toll-like receptors (TLRs) and RIG-like receptors (RLRs) that normally recognize viral dsRNA. In the case of DNMTis, DNA demethylation increases expression of human endogenous retroviruses (ERVs). De-repressed ERVs appear to function as tumor-intrinsic DAMPs that activate cancer cell TLRs/RLRs and induce IFN and ISGs. Chemotherapy and RT can also de-repress retroelements (Rudin and Thompson, 2001), which may also activate PRRs.

What is the significance of IFN and ISG induction in cancer cells with disrupted genomic integrity? Unabated retrotransposition can cause host genome mutation. In this regard, the IFN/ISG response may restrict retroelement activity (Yu et al., 2015). For some cancer cells that remain sensitive to the anti-apoptotic effects of IFN, cell death is a possible outcome (Leonova et al., 2013). However, as is the case after viral infection, another important property of anti-viral signaling is instigation of additional innate and adaptive immune responses. Retroelement transcripts may serve as DAMPs to marshal the adaptive immune system to eliminate compromised cells with aberrant retroelement activity. Thus, the activation of tumor-intrinsic innate immune signaling pathways by retroelement-mediated viral mimicry may contribute to the ability of DNMTis, RT, or chemotherapy to enhance ICB in cancer. Because genotoxic agents alone often fail in settings where combining these agents with ICB can succeed, ICB may facilitate an adaptive immune response initiated by these other interventions. Nonetheless, how these observations are mechanistically linked to elicitation of a PRR-mediated IFN response by retroelements remains incompletely defined.

Immune-Suppressive Effects of IFN Signaling

Although IFNs are generally immune stimulatory and these functions may explain the efficacy of certain combination ICB regimens, both type 1 and type 2 IFNs can have suppressive immunoregulatory effects. Examples of this dual role for IFN-I come from studies on chronic viral-host interactions (Teijaro et al., 2013; Wilson et al., 2013). Intriguingly, in the setting of chronic viral infection, IFN-I signaling persists, and rather than helping to contain the virus, this persistent IFN-I signaling

switches from immune stimulatory to immune suppressive. In particular, IFNβ, rather than IFNα, appears to tip the balance toward immune suppression (Ng et al., 2015). Persistent IFNβ promotes expression of suppressive factors such as PD-L1, IL-10, and indoleamine 2,3 dioxygenase (IDO) by DCs and other myeloid cells; may have direct suppressive effects on CD4 and CD8 T cells; and disrupts lymphoid architecture. Blockade of sustained IFN-I receptor signaling or of IFNβ improves viral clearance during chronic infection. In total, these data indicate that IFN-I can have dichotomous immune potentiating or immunoregulatory functions.

The opposing properties of IFN signaling may also contribute to the immune-suppressive tumor microenvironment. Although more focused on IFNγ, studies from patients and mice indicate that immune cell IFN production can lead to the inducible expression of PD-L1 within the tumor microenvironment, a phenomenon called "adaptive resistance" (Topalian et al., 2015; Tumeh et al., 2014). Here, the production of IFNγ is believed to either be a consequence of a pre-existing non-productive immune response against the tumor or coerced by therapeutic intervention. Regardless, IFNγ drives high levels of PD-L1 that engage PD-1 on T cells and drive T cell dysfunction. This elevated expression of PD-L1 can be found on both cancer cells and immune cells. Notably, IFN-I can also upregulate tumor PD-L1, consistent with what is observed in chronic viral infection (Minn, 2015). Thus, both type 1 and 2 IFNs are capable of contributing to immune suppression in cancer.

A Cornerstone for Combination Therapy

Considering that IFN signaling serves essential stimulatory roles in T-cell activation, differentiation, and effector function, the regulation of PD-L1 by IFN may inextricably link the suppressive action of the PD-1 pathway with robust T-cell responses. Such a model would suggest that adaptive resistance might be a common immune-suppressive response and PD-L1/PD-1 blockade may have a cornerstone role in combination immunotherapy (Figure 1). In support of the potential prevalence of IFN-driven adaptive resistance, widespread expression of ISGs is observed in primary human tumors (Weichselbaum et al., 2008). Furthermore, the ability of anti-PD-1/PD-L1 to evoke response across multiple cancer types may reflect at least a partial reversal of adaptive resistance in some high-ISG tumors with pre-existing tumor-infiltrating T cells. Similarly, when T-cell infiltration and activation is therapeutically enhanced, ensuing IFN secretion and the onset of adaptive resistance may make PD-L1/PD-1 blockade an important adjuvant for sustained anti-tumor activity. Indeed, high levels of PD-L1 are often seen when resistance to immunotherapies emerges. For example, PD-L1 and/or ISG expression was noted to increase in treated or relapsed tumors after the combination of RT and anti-CTLA4 (Twyman-Saint Victor et al., 2015), RT combined with TGF-β inhibition (Vanpouille-Box et al., 2015), and concurrent therapy with STING agonists and tumor vaccine (Fu et al., 2015). Accordingly, addition of anti-PD-1/PD-L1 improved anti-tumor responses in these cases. Thus, the PD-1/PD-L1 inhibitory axis in the tumor microenvironment may be inextricably linked to essential stimulatory functions of IFNs, which would make PD-L1/PD-1 blockade a necessary component of effective combination immunotherapy for many cancers.

Despite the importance of PD-1/PD-L1 blockade, it is likely that, in many tumors, additional suppressive pathways need to be concurrently disabled. Approximately 50% of melanoma and other solid tumors deemed to be PD-L1(+) fail to derive a clinical benefit or do not respond after anti-PD-1/PD-L1 monotherapy (Herbst et al., 2014; Taube et al., 2014). One possibility is that IFN drives the expression of additional suppressive ISGs besides PD-L1. USP15 knockout mice that have excessive IFNγ production by T cells unexpectedly show increased tumor formation in the MCA-induced fibrosarcoma model (Zou et al., 2015). Although part of this effect was attributed to elevated PD-L1, PD-L1-independent effects were also suggested. As an example, IFNγ can enhance expression of IDO1, and inhibiting IDO can improve anti-tumor activity (Spranger et al., 2013). Thus, in some situations, although PD-1/PD-L1 blockade may be a necessary part of combination therapy to block adaptive resistance, the existence of IFN-driven PD-L1-independent suppressive mechanisms argues that combination therapy may require targeting additional suppressive ISGs.

Immune-Independent Effects of IFN

Whether and how cancer cells die influences their immunogenic properties through multiple mechanisms (Kroemer et al., 2013), suggesting that cancer cell resistance to the direct cytotoxic action of cancer therapies might impact their ability to enhance ICB. Indeed, in addition to immunomodulatory actions, elevated expression of ISGs can promote immune-independent cancer cell resistance to RT and chemotherapy. In a variety of cancers, the transcription factor STAT1 drives the expression of a subset of ISGs and both influences cell-intrinsic sensitivity to genotoxic agents and regulates cell-extrinsic resistance mechanisms by non-immune cells such as stromal fibroblasts (Boelens et al., 2014; Weichselbaum et al., 2008). In the latter context, stromal cell exosomes, which are enriched in non-coding RNA and repeat/transposable elements, can be transferred to cancer cells to activate STAT1 through RIG-I. This "viral mimicry" leads to RT and chemotherapy resistance through the cooperation of STAT1 with NOTCH3, an effect that is independent of the adaptive immune system. Thus, IFN-related signaling pathways can influence both cell-intrinsic and non-immune cell-extrinsic determinants of response to cytotoxic agents. Given that dying cancer cells liberate DAMPs, inhibiting the ability of conventional cancer therapies to kill tumor cells may interfere with the ability of these agents to enhance ICB.

Dual Functions of IFN Signaling

What are the mechanisms that account for the complex and opposing functions of IFNs on the immune system and its non-immune effects on cancer cell resistance? Qualitative and quantitative differences in signaling properties of IFNs are likely important. Structural differences with how IFNβ interfaces with the type 1 IFN receptor contribute to a higher binding affinity compared to IFNα, and these differences may contribute to the bias in immune suppression observed with IFNβ (Ng et al., 2016). Besides the potential impact of signaling

magnitude, the timing and duration may also be critical parameters. In a simian immunodeficiency virus model, blockade of the IFN-I receptor decreased viral control, while initial IFNα treatment conversely improved disease parameters. However, with prolonged IFNα therapy, viral control unexpectedly worsened (Sandler et al., 2014). Such differences in the phenotypic outcome of IFN signaling may result from how direct antiviral effects are integrated with immunoregulatory effects on APCs and T cells. Also, negative regulators or post-translational modifications of STAT1 may sustain a subset of ISGs associated with chronic viral infection. Interestingly, these ISGs also overlap with ISGs that regulate DNA damage resistance in cancer (Minn, 2015). Thus, proximal and distal signaling differences dictate how IFN signaling impacts tumor and immune cells.

Conclusion

Enhancing the effectiveness of ICB can be facilitated through combination therapies that utilize multiple ICB antibodies, conventional cytotoxic cancer therapies, and other targeted agents. The PD-1/PD-L1 pathway represents a cornerstone for combination checkpoint blockade regimens, but optimal combinations will likely require antagonizing additional inhibitory signals. IFNs and innate immune signaling pathways have emerged as complex regulators of resistance and response to both ICB and the cytotoxic agents that can be effectively combined with ICB. Complexity arises from their immune stimulatory and suppressive effects and from their activation by endogenous DAMPs that may not only account for immunogenic effects, but also influence resistance to cytotoxic agents independently of the immune system. An inextricable link between IFNs with the reinstatement of antitumor T cell responses may make deconvoluting the complexities of IFN signaling difficult to avoid on the path to improving combination immunotherapies.

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