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Leptin-producing oncolytic virus makes tumor-infiltrating T cells fit, not fat

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Abbreviations: ICIs: immune checkpoint inhibitors; ACT: adoptive cell transfer, TIL: tumor infiltrating lymphocytes, TME: tumor microenvironment; TAA: tumor associated antigens; I-O: immuno-oncology; OV: oncolytic virus;

Abstract

Effective anticancer immunosurveillance after oncolytic viral infection is often hindered by the defective metabolic function of tumor infiltrating lymphocytes (TILs). A recent paper by Rivadeneira et al. demonstrates that intratumoral delivery of leptin by a recombinant oncolytic vaccinia virus can metabolically enhance TIL effector and memory functions through improved mitochondrial oxidative phosphorylation, thereby enhancing therapeutic efficacy.

Main text

Immuno-oncology (I-O) therapies such as immune checkpoint inhibitors (ICIs) or adoptive T cell therapies (ACT) are improving cancer treatments, leading to regulatory approvals in a broad range of indications. However, only a minority of patients, especially those with immunologically “hot” tumors, i.e. cancers full of tumor-infiltrating lymphocytes (TILs), exhibit objective responses and survival benefits. Moreover, I-O therapies can be harmful at two levels. On the one hand, they can induce debilitating autoimmunity affecting any organ system. On the other hand, the excessive price tag attached to I-O treatments drains resources that might be invested more effectively in cancer prevention or low-cost therapies. In this issue of *Immunity*, Rivadeneira et al. (Rivadeneira et al., 2019) demonstrate that local delivery of the adipokine leptin by oncolytic viruses can metabolically reprogram newly generated antitumor T lymphocytes, protecting the host against aggressive melanoma and pancreatic cancers.

Intratumoral immunotherapy is a therapeutic strategy that aims at converting the tumor into its own vaccine, offering several advantages over systemic delivery. First, a high concentration of immuno-stimulatory products can be achieved *in situ*, while using small amounts of drugs. Second, local delivery of immunotherapies allows multiple combination therapies, while preventing significant systemic exposure and off-target toxicities. Third, local

I-O may elicit a broad spectrum of T cell receptor (TCR) specificities across neo-antigens or tumor-associated antigens (TAAs) in a personalized manner, while being agnostic with regard to the relevant epitopes (Kepp et al. 2019).

One local I-O approach consists in the intratumoral injection of oncolytic viruses (OVs). OVs have a tropism for malignant cells, thereby causing lysis of malignant cells with consequent local inflammation of the tumor microenvironment (TME). OV-mediated tumor cell killing may result in "immunogenic cell death", associated with effective cross-presentation of tumor antigens by dendritic cells to T lymphocytes, thus igniting a systemic "abscopal" immune response at distant sites (Fend L, et al. 2017). Preclinical studies have confirmed that OVs can cause systemic reactivation and/or proliferation of adoptively transferred T cells and mediate synergistic antitumor effects with other I-O modalities, especially when OVs have been engineered to express immunostimulatory genes. Thus far, the sole FDA-approved oncolytic virus, T-VEC (official name: Talimogene Laherparepvec), a genetically modified herpes simplex-1 (HSV) virus expressing granulocyte macrophage colony-stimulating factor (GM-CSF), is used for the local treatment of inoperable melanoma (Andtbacka et al. 2015). Clinical trials revealed that T-VEC can induce "abscopal" effects upon local injection into melanomas, thus reducing the volume of non-injected lesions, especially in the context of immune checkpoint blockade (Ribas et al. 2018). Nonetheless, durable remission rates remain exceptional with this new class of I-O therapy (Andtbacka et al., 2015), highlighting the need to further elucidate the mechanisms of action of OVs. In fact, the immune cell subtypes contributing to OV-induced immune response have not been fully defined, and the functional status of newly infiltrating lymphocytes has been poorly investigated.

Cancer cells and TILs, including effector CD8⁺ T cells, have similar metabolic requirements and compete for nutrient resources, including glucose and amino acids, within the TME. Furthermore, tumors create a challenging environment characterized by hypoxia, acidosis and high concentrations of immunosuppressive metabolites. Chronic protein kinase B signaling in T lymphocytes induces a defect in transcription factor PPAR γ coactivator 1 α (PGC-1 α)-driven mitochondrial biogenesis and oxidative phosphorylation (Scharping et al. 2016) associated with T cell dysfunction. Thus, new approaches that combine existing I-O with strategies that overcome the metabolically challenging TME and enhance infiltration by, and longevity of, antitumor effector memory responses are actively investigated.

Rivadeneira et al. sought to determine the immune cell subtypes contributing to OV-induced immune responses and to determine why OV responses are typically incomplete. When attempting to treat an oncogene-driven established melanoma line (CL24) derived from Pten^{f/f} Braf^{V600E} Tyr2^{Cre,ER} mice using Western Reserve laboratory strain Vaccinia OV, the authors obtained substantial tumor growth retardation but failed to observe complete tumor eradication. Single-cell sequencing of the OV-induced TILs led to the identification of a subset of CD8⁺ T cells harboring hallmarks of activation and exhaustion, such as high PD1 and TIM3 expression, coupled with reduced mitochondrial biogenesis.

Although leptin was first discovered as the satiety hormone, meaning that its (rare) genetic defect causes hyperphagy and obesity, this hormone produced by adipocytes, also known as an adipokine, is chronically elevated in the vast majority of obese patients. The rationale to use leptin to metabolically reprogram the TME came from pioneering work highlighting that this adipokine could reverse the immunosuppressive effects of acute starvation in mice. Administration of leptin reinstated T helper 1 (Th1) immune responses, acting on activated T cells to stimulate glucose uptake and metabolism (Lord GM, et al. 1998).

Rivadeneira et al. found higher expression levels of leptin receptors on PD1⁺Tim3⁺ CD8⁺ T cells residing in tumor beds compared with secondary lymphoid organs or compared with PD1⁻ T cells and then observed that, in activated T cells *in vitro*, leptin boosted the mitochondrial mass and induced the phosphorylation of p38 MAPK as well as the transcription factor ATF2. Encouraged by these findings, the authors then conducted several experiments to confirm the beneficial effects of leptin on TIL cell fitness in the hostile TME. First, genetic engineering of CL24 melanomas to enforce expression of leptin (CL24^{leptin}) led to CD8⁺ T cell-dependent tumor growth retardation associated with the influx of cytotoxic T lymphocytes (CTLs) and NK cells into the tumor bed. Importantly, despite high PD1 and Tim3 expression levels, CD8⁺ T cells homing into CL24^{leptin} tumor beds were specific for cancer antigens, remained proliferative and polyfunctional, exhibited phosphorylation of PKB, p38 MAPK and STAT3, and showed increased mitochondrial biogenesis. Moreover, the anticancer effects of leptin release into the TME were lost when recombinant CL24^{leptin} tumor cells were inoculated into hosts harboring a T cell-restricted heterozygous deletion of the leptin receptor (Rivadeneira et al. *Immunity in press*).

Next, Rivadeneira et al. equipped OV_s with a gene encoding leptin under the control of the Vaccinia p7.5 promoter. Local delivery of leptin by OV^{leptin} into 5mm CL24^{wt} tumors recapitulated the effects observed with CL24^{leptin} cells, leading to tumor infiltration by proliferative polyfunctional CD8⁺ T lymphocytes with amplified mitochondrial mass, as well as to complete tumor eradication in 27% of the cases. These beneficial effects of OV^{leptin} were corroborated in a second tumor model resistant to PD1 blockade, the mouse Panc02 pancreatic cancer model. Single-cell RNA sequencing of TILs in OV^{leptin} tumor beds revealed a change in the effector memory phenotype of CD8⁺ cells, towards a more polyclonal and precursor memory CD127⁺Tcf7⁺ profiling (Figure 1) as well as a type 1 IFN signature. Animals that

received intratumoral OV^{leptin} developed not only a memory T cell response against viral antigens, but more importantly, a protective immunity against tumor antigens (Rivadeneira et al. Immunity in press).

This study employed leptin therapeutically as a metabolic modulator of the immune response against cancer using oncolytic viruses that were poorly efficient in these tumor models resistant to anti-PD1 antibodies. This strategy may invigorate pre-existing immunity by stimulating PPAR γ coactivator 1 α dependent- mitochondrial oxidative phosphorylation, reinstating T cell fitness, similar to prior findings with agonistic CD137 antibodies (Menk, et al. 2018).

However, it is intriguing to note that some of the findings apparently conflict with clinical data reporting superior clinical benefit of anti-PD1 and/or anti-PDL-1 Abs in obese melanoma patients (McQuade, et al. 2018). Obesity is associated with inflammaging, hyperleptinemia and deregulated immune functions. An unfavorable role of leptin signaling in increasing PD-1 and other hallmarks of T cell exhaustion (Tim3, Lag3, Eomes and fatty acid oxidation) and culminating in accelerated tumor growth has been recently reported in obese tumor-bearing mice (Wang Z, et al. 2019). These discrepancies might stem from the differential effects of leptin on naive *versus* memory T cells, on the bioactivity of leptin on distinct cellular compartments of the TME (such as myeloid derived- suppressor cells or macrophages), or from the confounding factor of sex in these effects, PD1 blockade being more effective in obese males than obese females. It is of note that Rivadeneira et al. failed to obtain beneficial effects of systemic administration of recombinant leptin, suggesting that local delivery of this adipokine is key for the anticancer effects (Rivadeneira et al. Immunity in press). In addition, Rivadeneira et al. obtained most of the findings in lean but not obese tumor bearers, highlighting

the yet-to-be-explored possibility of an acquired T cell resistance to leptin in organisms chronically exposed to obesity-related systemic hyperleptinemia.

Regardless of these open issues, the possibility to modulate immunometabolism by OV represents a promising strategy for stimulating anticancer immunity in a way that synergies with current systemic I-O strategies.

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Figure 1. Metabolic reprogramming of TIL by oncolytic viruses engineered to express leptin. Following oncolytic virus (OV) infection, melanoma cells undergo immunogenic cell death. T cell incomers that are newly recruited into the inflamed tumor bed (along with NK cells and monocytes) exhibit inhibitory (PD1, Tim3) and functional leptin receptors. However, such tumor infiltrating lymphocytes (TILs) are poorly proliferative, contain low mitochondrial mass, and are defective in T helper 1 (Th1) cytokine secretion and memory responses. Following local delivery of leptin by OV-infected target cells, TILs become metabolically empowered, recover a polyfunctional phenotype (Ki67, IFN γ , TNF α) and acquire hallmarks of "memory resource" T cells (Tcf7, CD127, type 1 IFN signature). This metabolic switch is associated with complete tumor eradication, antigen spreading and long term protective immune responses against various tumor -associated antigens.

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Conflicts of interest: GK is a scientific co-founder of Samsara Therapeutics. GK and LZ are co-founders of everImmune. LZ is member of scientific advisory board of Transgene and co-inventor of a patent dealing with "Combination of oncolytic virus with immune checkpoint modulators".

