OncoImmunology 4:3, e989762; March 2015; Published with license by Taylor & Francis Group, LLC

In situ immunization via non-surgical ablation to prevent local and distant tumor recurrence

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Keywords: adenovirus, CpG, cryoablation, GM-CSF, Her2/neu, IL-12, immunization, mouse mammary tumor, suicide gene

Host immunities are induced during cryoablation or oncolytic adenovirus therapy when the entire repertoire of tumor associated antigens (TAA) is released. Local and systemic protection is enhanced by the combined treatment with toll-like receptor agonist or immune stimulating cytokines. Non-surgical tumor ablation is an effective platform for *in situ* immunization.

Introduction

As cancer immunotherapy becomes a mainstream treatment modality, development of novel strategies to fully exploit tumor associated antigens (TAA) is a priority. Since tumor tissues harbor the entire repertoire of TAA, it would be opportune to immunize with these TAA *in situ* via non-surgical ablation to achieve long-term protection. Several barriers to such *in situ* immunization are recognized, such as immune tolerance to TAA, immune suppression by the tumor itself or insufficient co-stimulation during massive release of TAA. To enable the manifestation of innate or adaptive immunity, we tested the feasibility of modifying the tumor microenvironment to favor immune activation. Intratumoral electroporation of mouse mammary tumors with plasmid DNAs encoding strong foreign antigens such as tetanus toxoid fragment C and cytokines such as interleukin (IL)-12 resulted in T-cell response to endogenous TAA with heavy inflammatory infiltration, partial tumor regression and protection from repeated tumor challenge.[1] Therefore, the tumor microenvironment can be modified with immune modulating molecules to enable *in situ* immune activation.

In the presence of an established tumor, it is critical to ensure complete tumor ablation while attempting *in situ* immunization. Non-surgical ablation such as cryoablation[2] or oncolytic adenoviral therapy[3] with radiation,[4] can destroy the tumor tissue while releasing the full content of TAA for immunization. The FDA approved cryoablation procedure induces tissue necrosis by damaging cell membranes and organelles via the formation of ice crystals, and indirectly through osmotic stress and microvasculature thrombosis. The oncolytic adenovirus (Adv/CD-TK) containing two suicide genes:[5] the cytosine deaminase (CD) and herpes simplex virus thymidine kinase (TK) has been used in our phase I/II trials for non-metastatic prostate cancer.[4] Long-term prostate-specific antigen (PSA) kinetics suggested the induction of anti-tumor immunity in some treated patients.[6] While debulking the tumor, the necrotic tissue from cryoablation or Adv/CD-TK therapy becomes a rich reservoir of TAA that are cleared by antigen presenting cells, creating a unique environment to prime or boost antitumor immune responses (**Fig. 1**). Induction of innate and adaptive immunity was further investigated in animal models.

Treatment of mouse mammary tumors with Adv/CD-TK induced modest adaptive immunity whether pro-drugs are included in the regimen, showing immune activation by Adv which exhibits significant danger signals by itself.[3] Treatment with Adv/GM that expressed mouse granulocyte macrophage colony-stimulating factor (GM-CSF) enhanced tumor immunity to confer protection against secondary tumors. Adv/IL-12 similarly enhanced tumor immunity.[7] These findings support the feasibility of immune activation by oncolytic Adv therapy, but additional intervention such as radiation will be required to achieve complete tumor ablation.

A comprehensive evaluation of innate immunity and α-Her2/neu adaptive immunity following cryoablation was conducted in wild type (WT) BALB/c, neu-tolerant BALB NeuT, and SCID mice. In BALB/c mice, cryoablation alone resulted in a systemic immune priming and partial protection against secondary tumors, but the same treatment had little to no impact in neu-tolerant or SCID mice, suggesting that TAA released by cryoablation induced varying levels of innate and adaptive immunity in different host environments. Peritumoral injection of toll-like receptor 9 (TLR9) agonist CpG following

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Submitted: 11/12/2014; Accepted: 11/13/2014
http://dx.doi.org/10.4161/2162402X.2014.989762
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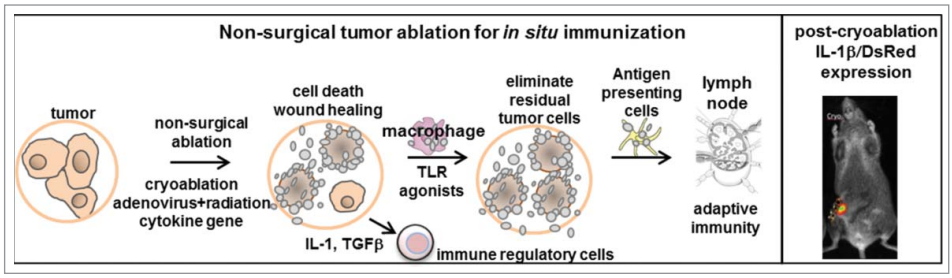

Figure 1. Non-surgical tumor ablation *in situ* immunization.

cryoablation, significantly improved treatment outcome in all three mouse strains. CpG-induced innate immunity significantly contributes to the control of local recurrences as evidenced by the delay in tumor recurrence seen in SCID mice. However, long-term recurrence-free survival is dependent on adaptive immunity, which is demonstrated by the incremental increase in reoccurrences seen in WT, tolerant NeuT, and SCID mice, respectively. For high-risk tumors and oligometastatic disease, residual tumor microfoci and subsequent recurrence following cryoablation is a significant clinical consideration. The added protection provided by CpG may expand the utility of cryoablation to successfully treat more patients, such as for nerve sparing prostate cryoablation. Cryoablation is a strong platform for combination with immune modulation to achieve non-surgical tumor destruction and systemic protection.

IL-1β and transforming growth factor beta (TGF-β) are released in the tumor microenvironment during necrotic tumor resolution. In IL-1 DsRed mice, DsRed fluorescence driven by IL-1β promoter is detected for over two weeks after cryoablation to illustrate sustained inflammation (**Fig. 1**). TGF-β is produced by platelets and macrophages during wound healing process with the potential to downregulate tumor immunity. A transient immune refractory period is observed during this phase that dampens existing immune effector function[2] and likely impedes immune priming. To exploit TAA released during tumor ablation, strategies to overcome immune suppression may be particularly beneficial. There lies an opportunity for immune intervention. In murine studies, depletion of $CD25^+$ regulatory T cells significantly enhanced immune response to TAA[8] or DNA vaccination.[9] We showed the role of TGF-β inducible early gene 1 (*TIEG1*) in the conversion of naïve $CD4^+$ T cells to $Foxp3^+$ regulatory T cells.[10] It is possible that TGF-β released during necrotic tumor resolution enhances Treg activity via *TIEG1* activation. Control of TGF-β/TIEG1 pathway and other immune regulatory mechanisms may improve *in situ* immunization. Activation of additional co-stimulatory molecules or blockade of immune checkpoints could provide further benefit in this setting. Overall, TAA harbored in the tumor tissue are potential cancer vaccines *in situ*. Several strategies have demonstrated promising results in ablating tumors while activating innate and adaptive immunity. To achieve therapeutic success, existing and novel reagents should be fully investigated to overcome immune suppression and boost immune priming after non-surgical ablation.

Disclosure of Potential Conflicts of Interest

The authors disclose no potential conflicts of interest.

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