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RESEARCH HIGHLIGHT

Highlights on FOXO3 and tumor-associated dendritic cells in prostate cancer

Kwong Y Tsang and James L Gulley

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Cancer immunotherapy sometimes fails to provoke effective immune responses because of immunosuppressive mechanisms present in the tumor-bearing host. Dendritic cells (DCs) are the most potent antigen-presenting cells. After internalizing tumor-associated antigens (TAAs) at the tumor site, $CCR7^+$ DCs traffic to the tumor-draining lymph nodes, where they influence the maturation of T cells. DCs may also be present at the tumor site. These tumor-associated DCs (TADCs) cross-present TAAs to recruited $CD8^+$ T cells, which develop into TAA-specific effector cells. When activated, TADCs can mediate the sensitization of naive T cells that have been recruited into the tumor site. For this reason, interaction between tumor-infiltrating T cells and TADCs is essential for activating and maintaining specific antitumor immune responses.

DCs can also induce tolerance, rather than immune activation, to the TAAs which they are presenting, depending on how DCs integrate information collected from the innate and adaptive immune systems. DCs may respond actively, in part, to tolerogenic signals such as IL-10 and TGF-beta that exist in the immunosuppressive local milieu. Watkins *et al.*[1] have identified a gene, *FOXO3*, in TADCs that can induce immune suppression in human and murine prostate cancer. They discovered that TADCs in prostate tumors in both humans and transgenic adenocarcinoma of mouse prostate (TRAMP) mice expressed high levels of *FOXO3* compared to DC from non-tumor prostate tissues and no longer activated T cells. Instead, these TADCs suppressed T-cell responses and promoted tolerance to TAAs, causing T cells to lose their ability to destroy tumor cells and even to be converted to T regulatory cells (Tregs).

Watkins *et al.*[1] analyzed gene expression patterns of TADCs isolated from TRAMP mice and compared them to gene expression patterns of DCs in normal tissue and found that genes overexpressed in the TADCs included *Cxcl10*, *Cxcl9*, *Ccl5*, *Il-6*, *Tgfb1*, *Vegfa*, *IL1b*, *FasL*, *Ido1*, *Arg1*, *Nos2*, *Cd274*, *Stst3* and *Foxo3*. *FOXO3* is known to regulate DC function and to modulate immune responses. To confirm that overexpression of *Foxo3* in TADCs correlated with their suppressive function, Watkins *et al.*[1] demonstrated that silencing *Foxo3* with siRNA abrogated the ability of both mouse and human TADCs to induce tolerance and suppressive activity in T cells. They also showed that silencing *Foxo3* in TRAMP TADCs correlated with increased expression of costimulatory molecules such as CD80 (but not CD86) and proinflammatory cytokines such as IL-6, and diminished expression of tolerogenic factors such as TGF-beta, arginase and indoleamine-2,3-dioxygenase. More importantly, they found that transferring tumor-specific CD4 T cells into TRAMP mice can destroy the tolerogenicity of TADCs, a phenomenon associated with reduced *FOXO3* expression.

To determine whether *FOXO3* induces tolerogenicity in TADCs only in prostate cancer, Watkins *et al.*[1] evaluated the expression of *FOXO3* and the function of TADCs isolated from other tumor models, including B16 melanoma and orthotopic renal tumors. They found that TADCs in these tumor models had elevated levels of *FOXO3* comparable to those found in TRAMP TADCs, and that these TADCs also induced T-cell tolerance. Watkins *et al.*[1] thus demonstrated that TADCs are key regulators of immune outcomes, capable of suppressing T-cell responses in the tumor microenvironment.

Prostate cancer is the most common malignancy in men in the United States and a leading cause of cancer death in North America.[2] Treatment for localized disease includes radical prostatectomy or radiation therapy, but approximately 30% of patients will subsequently develop rising prostate-specific antigen. While androgen-deprivation therapy can temporarily control the disease, the majority of patients will eventually develop metastatic castration-resistant disease. Docetaxel-based chemotherapy has been shown to improve survival by approximately 2–3 months in this setting.[3] Immunotherapeutic approaches using peptide, DC-based, or poxviral-based ('TRICOM') vaccines have also shown evidence of clinical benefit.[4] A recently reported phase III trial of sipuleucel-T (Provenge) vaccine in patients with metastatic castration-resistant prostate cancer was the first trial to demonstrate a statistically significant improvement in overall survival (the primary end point) with a cell-based therapeutic vaccine.[5]

Tregs ($CD4^+CD25^{high}FoxP3^+$) play an important role in immune homeostasis through their ability to suppress activated T cells. An increase in number or functionality of Tregs could favor tumor development. Increased levels of $CD4^+CD25^{high}FoxP3^+$ Tregs have been detected in the peripheral blood mononuclear cells, tumor microenvironment and draining lymph nodes of patients with prostate cancer.[6,7] Clinical studies have demonstrated that Tregs can inhibit both antigen-specific and nonspecific T-cell responses and reduce the efficacy of cancer immunotherapy, while depletion of Tregs can augment vaccine-mediated antitumor immune response.[8] This study by Watkins *et al.*[1] suggests that it may be possible to enhance T-cell responses to prostate cancer and decrease T-cell suppression by directly targeting both *FOXO3* and Tregs with small

Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA
Correspondence: Dr KY Tsang (tsangkwo@mail.nih.gov)

molecules, in combination with therapeutic cancer vaccines such as prostate-specific antigen-TRICOM[9,10] and Provenge. It can also be inferred from these findings that enhancing expression of *FOXO3* could be a strategy for treating autoimmune diseases.

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