A master switch for regulating tolerance and immunity in dendritic cells

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Recent findings demonstrate that dendritic cells in prostate tumors induce immune tolerance in tumor antigen-specific CD8+ T cells. We propose that DC tolerogenicity can be regulated by expression of Foxo3, silencing Foxo3 expression enhances anti-tumor immune responses and renders FOXO3 a potential target for immunotherapy.

FOXO3

Dendritic cells (DC) are among the most versatile cells of the immune system. DC are essential to the initiation of a productive immune response by presenting Ag to T cells and secreting pro-inflammatory cytokines, but are also responsible for controlling over-active immunity by regulating immune tolerance. While tolerance is an essential component of preventing autoimmune disease, it is also a complication and major hurdle to overcome in the quest to improve immune-based therapies for cancer.

Tumors create a highly complex micro-environment rich in cytokines, chemokines and other factors that attract various populations of immune cells including DC and T cells. Although the infiltration of immune cells is seemingly beneficial, the suppressor factors has proven to be insufficient for sustaining anti-tumor immunity. Therefore, we sought to gain a better understanding of the mechanisms that regulate tumor-associated DC (TADC) induced immune tolerance.

We initially confirmed that tolerogenic mediators present in the tumor microenvironment (IDO, Arginase, TGFβ) are responsible for the protracted transferred CD8+ T cells. However, blocking these TADC-derived tolerogenic mediators individually, or in combination, only transiently enhanced T cell effector function. In contrast, depleting TADC restored anti-tumor effector functions of tumor-infiltrating cells. Subsequent in vitro studies demonstrated that TADC were not only poor stimulators of T cells, but they actively induced T cell tolerance and were capable of inducing suppressive activity. Building on our previous observations, we used an adoptive transfer model (Fig. 1A). Importantly, our results observed in the TRAinguezi Renin-angiotensin of the Mouse Prostate (TRAMP) model were consistent with studies using TADC isolated from human prostate tumor tissues suggesting that our results may have significant clinical relevance.

Analysis of gene expression patterns in TRAMP TADC revealed increased expression of chemokines (Ccl5, Ccl2 and Ccl3), growth factors (Vegfa and Tgfβ), and other genes associated with tolerance (Foxo3, Arg1, Ccl5, Ccl2). Additionally, increased expression of the gene Foxo3 was observed in TADC compared with non-tumor associated prostate DC (Fig. 1A). Foxo3 is a member of the Forkhead box transcription factor class-O family and was previously reported to play a role in the regulation of DC function through a “reverse signaling” process mediated by CTLA-4 interaction with B7 molecules, leading to increased TGFβ levels. Therefore, to determine the role of Foxo3 expression in TADC tolerogenicity, we silenced Foxo3 expression using siRNA and observed reduced DC tolerogenicity, decreased expression of suppressive factors Tgfβ, Arg1 and Idol and a concomitant increase in expression of the co-stimulatory molecule CD80 and the pro-inflammatory cytokines, IL-6.

Interestingly, we previously reported that TRAMP TADC could be activated or “Reversed” to favor tumor antigen specific CD4+ T cells, resulting in more stimulation DC. We now observed that the activation induced by CD4+ helper cells also resulted in reduced TADC immunosuppressive activity with downregulation of Foxo3 expression. In general, our findings suggest that the downregulation of Foxo3 allows for the conversion of
Tolerogenic DC to immune stimulatory, including increased expression of B7, MHC and CD40 molecules as well as increase in pro-inflammatory cytokines IL-12, IL-15 and IL-6 (Fig. 1B). These data suggest that TADC can be a useful target in immune based therapies and furthermore, that FOXO3 may be part of a regulatory mechanism that programs the inflammatory or tolerogenic potential of dendritic cells.

In summary, we conclude that TADC are critical in determining the effectiveness of anti-tumor immune responses, especially with respect to maintaining activation of Ag-specific CD8+ T cells. We reported that TADC tolerogenicity is regulated by FOXO3 in both murine and human prostate cancer as well as murine models of melanoma and renal cell carcinoma. However, the mechanisms responsible for increased Foxo3 expression in TADC as well as the mechanism(s) by which FOXO3 mediates tolerogenicity are unclear and are currently under investigation. Foxo3 expression may be induced in response to stimuli such as reactive oxygen species in the TME or in response to interactions with other inflammatory cells (e.g., macrophages or mast cells) and their products within the TME. FOXO3 may induce expression of genes associated with tolerance, such as Ido, Tgfβ, and/or Il-10. A second possible hypothesis is that FOXO3 may play a less direct or active role in inducing expression of genes associated with tolerance but rather, may mediate the inactivation of pro-inflammatory cytokines such as Il-12, Il-15 and Il-6 through downregulation of Foxo3 expression to dendritic cells, thus preventing their differentiation to immunosuppressive cells.

**Figure 1.** Increased FOXO3 expression is associated with TADC induced tolerance. (A) TADC produce tolerogenic mediators (DC costimulate TEs) and express increased PDL1 and FOXO3 interaction between TADC and CTLs induced T cell tolerance. (B) Inhibiting Foxo3 or providing a potent pro-inflammatory stimulus converts TADC to immune stimulating and promotes CTL effector functions and anti-tumor immunity.

**References**

1. Anderson MJ, Shafer-Weaver K, Greenberg NM, Hurwitz AA. Tolerization of tumor-specific T cells in autoimmunity and tumor immunity. J Immunol 2009; 183:4848-52.

2. Pinzon-Chanu M, Adachi T, Z vitamins and cancer: A review. Integr Cancer Ther 2005; 4:314-36; PMID:16174093.

3. Shafer-Weaver KA., Hurwitz AA. Cutting Edge: Increased FOXO3 expression is associated with TADC induced tolerance. (A) TADC produce tolerogenic mediators (DC costimulate TEs) and express increased PDL1 and FOXO3 interaction between TADC and CTLs induced T cell tolerance. (B) Inhibiting Foxo3 or providing a potent pro-inflammatory stimulus converts TADC to immune stimulating and promotes CTL effector functions and anti-tumor immunity.

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6. Shafer-Weaver KA, Watkins SK, Anderson MK, Draper LJ, Marygeline A, Aroyo WG, et al. Immunity to murine prostatic tumors: continuous provision of T-cell help prevents CD8 T-cell tolerance and activates tumor-infiltrating dendritic cells. Cancer Res 2009; 69:6256-64; PMID:19622771; http://dx.doi.org/10.1158/0008-5472.CAN-08-4516

7. Nakamura T, Sakamoto K. Forkhead transcription factor FOXO subfamily is essential for reactive oxygen species-induced apoptosis. Mol Cell Endocrinol 2008; 281:47-55; PMID:18035477; http://dx.doi.org/10.1016/j.mce.2007.10.007

8. Lin L, Hron JD, Peng SL. Regulation of NF-κB, Th activation, and autoinflammation by the forkhead transcription factor Foxo3a. Immunity 2004; 21:203-13; PMID:15308101; http://dx.doi.org/10.1016/j.immuni.2004.06.016

9. Komarova EA, Krivokrysenko V, Wang K, Nekrassova N, Chernov MV, Komarov PG, et al. p53 is a suppressor of inflammatory response in mice. FASEB J 2005; 19:1030-2; PMID:15811878

10. Dijkers PF, Medema RH, Lammers JW, Koenderman L, Coffer PJ. Expression of the pro-apoptotic Bel-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1. Curr Biol 2000; 10:1201-4; PMID:11050388; http://dx.doi.org/10.1016/S0960-9822(00)00728-4