FOXO3
A master switch for regulating tolerance and immunity in dendritic cells

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Keywords: tumor, dendritic cells, tolerance, Foxo3, immunotherapy

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.

Dendritic cells (DC) are among the most versatile cells of the immune system. DCs 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 microenvironment 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 suppressive tumor microenvironment (TME) quickly neutralizes anti-tumor responses and cells such as antigen-specific cytotoxic T lymphocytes (CTL) with the potential to control tumor burden lose their function. Several therapies have aimed to target and neutralize DC-produced immunosuppressive factors such as Interleukin (IL)-10, indoleamine-2,3-dioxygenase (IDO), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) in an attempt to enhance T cell responses to tumors. However, inhibition of select suppressive 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) dampened responsiveness of adoptively 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 T 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, confirming our previous observations using an adoptive transfer model (Fig. 1A). Importantly, our results observed in the TRAngenic Adenocarcinoma of the Mouse Prostate (TRAMP) model were consistent with studies using TADC isolated from human prostate tumor tissue sections suggesting that our results may have significant clinical relevance.

Analysis of gene expression patterns in TRAMP TADC revealed increased expression of chemokines (Ccl5, Cxcl9 and Cxcl10), growth factors (Vegfa and Tgfβ), and other genes associated with tolerance (Ido1, Arg1, Cd274). 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 in IDO levels. Therefore, to determine the role of Foxo3 expression in controlling TADC tolerogenicity, we silenced Foxo3 expression using siRNA and observed reduced DC tolerogenicity, decreased expression of suppressive factors Tgfβ, Arg1 and Ido1 and a concomitant increase in expression of the co-stimulatory molecule CD80 and the pro-inflammatory cytokine, IL-6.

Interestingly, we previously reported that TRAMP TADC could be activated or “licensed” in situ by tumor-antigen specific CD4+ T cells, resulting in more stimulatory DC. We now observed that the activation induced by CD4+ helper cells also resulted in reduced TADC tolerogenicity concomitant with down-regulation of Foxo3 expression. In general, our findings suggest that the down-regulation of Foxo3 allows for the conversion of
tolerogenic DC to immune stimulatory, including increased expression of B7, MHC and CD40 molecules as well as an 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 vs. 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 signals such as inhibition of NFkB or STATs. Lin et al. previously reported that FOXO3 regulates helper T cell activation to control autoimmunity by inhibiting NFkB. This is an interesting perspective as similar mechanisms have been described involving transcription factors overexpressed in inflammatory cells that infiltrate tumors, such as p53. Elucidation of these mechanisms may be critical to unveiling the balance between inflammation and tolerance in DC.

In terms of therapy, given that FOXO3 is also important for cell cycle regulation by activating genes that induce apoptosis, it may be most beneficial to utilize an approach that specifically targets silencing expression to dendritic cells, thus preventing downregulation of Foxo3 in tumor cells. One such approach currently under investigation is to administer small molecules and peptide-based inhibitors to FOXO3. Ideally, these inhibitors could be linked to molecules that would be taken up by DC-specific receptors, such as DEC-205. A second strategy may be to inhibit DNA binding by administering decoy DNA containing the motif recognized by Foxo3 thus preventing the induction of Foxo3 regulated genes associated with tolerance. These discoveries may have great impact for not only efficiently targeting and improving DC function in cancer immunotherapy, but may also have significant implications for designing therapeutics to utilize DC to dampen inflammation in autoimmune disease.

Acknowledgments

The authors would like to acknowledge the critical review of Dr. Scott Durum. Some research described in this manuscript was supported by the Intramural Research Program of the NIH, NCI.

References


