

Tumors induce immune tolerance through activation of β -catenin/TCF4 signaling in dendritic cells: A novel therapeutic target for cancer immunotherapy

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Tumors promote immune suppression and dendritic cells (DCs) play a key role in this. However, signaling networks that program DCs to induce immune suppression are unknown. In our recent study, we showed that tumors activate β -catenin/TCF4 in DCs programming them to a regulatory state, which promotes T regulatory responses while suppresses effector T cell responses. Thus, targeting DCs- β -catenin pathway represents a promising target for anticancer immunotherapy.

Tumor-Induced Immune Tolerance via. β -catenin/Retinoic Acid Pathway

Tumor-induced immune tolerance is a central impediment for effective anticancer immunotherapy. Tumors use multi-layered regulatory mechanisms to evade antitumor immunity. One such immune suppression mechanism involves induction of regulatory DCs that limits antitumor immunity.¹ However, the factors or signaling pathways that programs DCs to a regulatory state are not known. Our previous studies on mouse models of gut tolerance and neuroinflammation have shown that Wnt/ β -catenin/TCF pathway in DCs plays a critical role in balancing immunity and tolerance.^{2,3} Interestingly, tumor microenvironment (TME) contains high-levels Wnts and aberrant β -catenin signaling occurs in many tumors.⁴ However, it is possible that tumors can induce regulatory DCs by activating β -catenin/TCF pathway and promote immune suppression. In our recent study,⁵ we have put this idea to the test and showed

tumors exploit β -catenin/TCF pathway as a potential immune evasion mechanism.

Previous studies have shown that β -catenin pathway is active in intestinal DCs but not in the peripheral DCs. Interestingly, recent studies have shown that tumors activate β -catenin in DCs in the periphery. Consistent with these studies, our data showed that this pathway is active in DCs isolated from tumor draining lymph node (TDLN) and tumor.⁵ To further examine the role of this pathway in DC-mediated immune suppression, we have generated mice that selectively lacked β -catenin in DCs (referred to as β -cat ^{Δ DC} mice). DC-specific deletion of β -catenin resulted in delayed melanoma (MO4) and thymoma tumor growth compared with control mice. Further examination of the effector phenotype of tumor-infiltrating lymphocytes (TILs) isolated from β -cat ^{Δ DC} mice showed a significant increase in effector T cell (IFN γ ⁺CD4⁺ and IFN γ ⁺CD8⁺ T cell) responses with concomitant decrease in regulatory cell (Foxp3⁺CD4⁺ Tregs, IL-10⁺CD4⁺ Tr1 cells, IL-10⁺CD8⁺

T cell) responses, indicating that β -catenin activation in DCs induces regulatory T cell response and limits effector T cell response to tumors.

To identify the possible mechanisms by which β -catenin pathway in DCs drive T regulatory responses, we looked at the expression of immune regulatory genes (which are involved in Treg cell induction) such as ALDH1 (enzyme involved in retinoic acid (RA) synthesis) and IL-10. Tumor draining lymph node DCs (TDLN DCs) isolated from tumor bearing β -cat ^{Δ DC} mice expressed lower *Aldh1a1* and *Aldh1a2* isoforms (key rate-limiting enzymes in RA synthesis) than control TDLN DCs. Further, our data showed that *Aldh1a1* and *Aldh1a2* are β -catenin target genes and their expression is dependent on TCF4 (downstream mediator of β -catenin signaling). RA is an active metabolite of vitamin A and plays a key role in the induction of regulatory T cell and immune tolerance.⁶ Interestingly, TME DCs also produce large quantities of RA suggesting possible role in tumor tolerance.⁷ However, how DCs in TME

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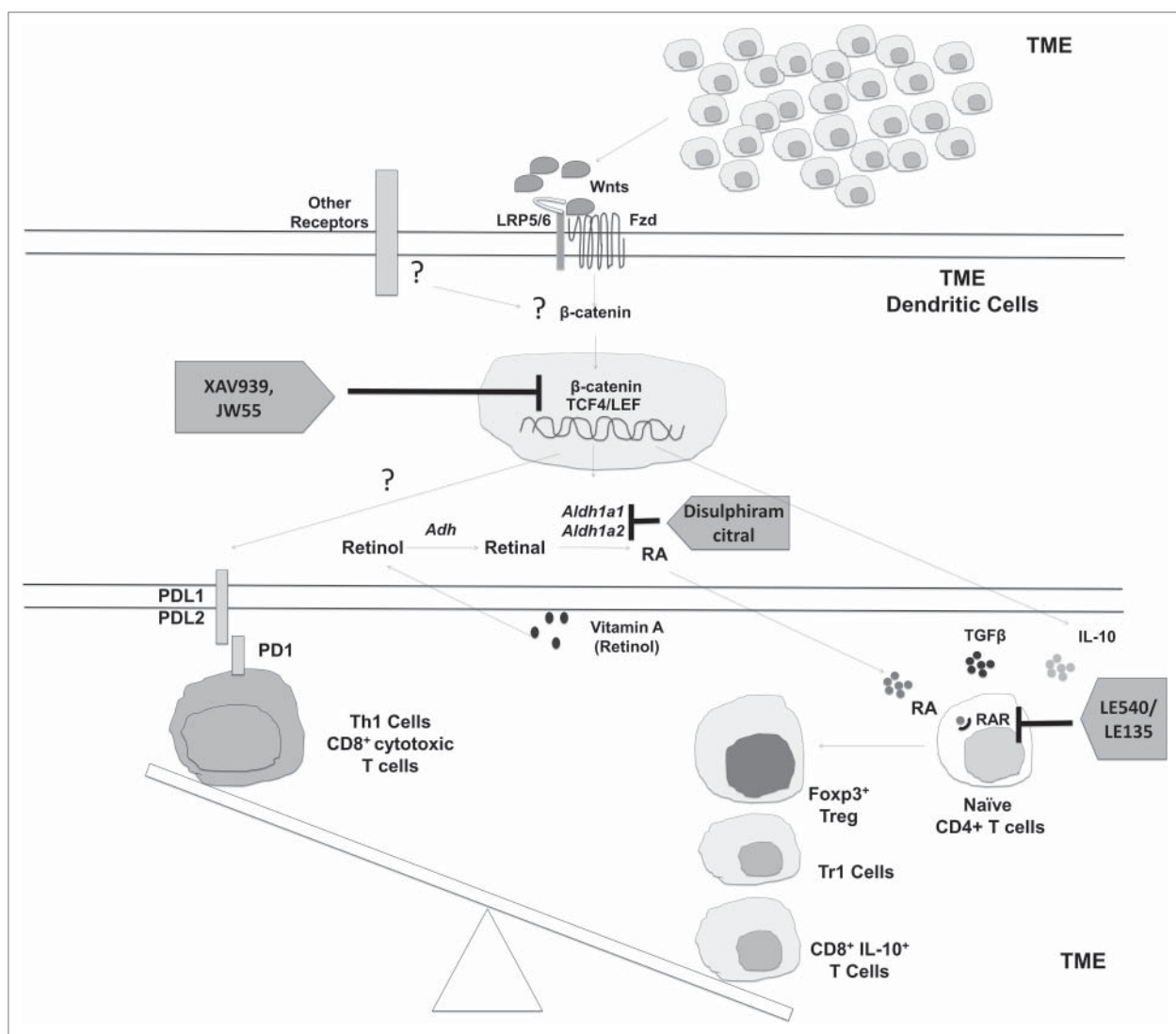


Figure 1. Activation of β -catenin/TCF4 signaling in tumor DCs induces RA-mediated immune tolerance. Wnt ligands secreted by various cells in TME bind frizzled and LRP5/6 co-receptors of DCs, leading to activation and accumulation of β -catenin in the cytoplasm and translocation into the nucleus. In the nucleus, β -catenin binds to TCF4/LEF complex initiating transcription of several target genes including *Aldh1a1* and *Aldh1a2*, key enzymes involved in RA synthesis. In addition, activation of β -catenin signaling in DCs increases expression of IL-10 and co-inhibitory molecules such as PDL1 and PDL2. Thus, β -catenin mediated reprogramming of DCs induces tumor tolerance through increased differentiation of naive T cells to Tregs, Tr1 and IL-10⁺ CD8⁺ T cells. This shifts the balance from effector T cells (IFN γ producing CD4⁺ and CD8⁺ T cells) to regulatory T cells leading to suppression of antitumor immunity. Accordingly, blocking activation of β -catenin using small molecule inhibitors (XAV939, JW55) and/or inhibition of RA synthesis using disulfiram, citral and LE540/LE135 represents an attractive therapeutic target for effective cancer immunotherapy.^{5,9}

acquire ability to metabolize vitamin A to RA, and its role in induction of Tregs and tumor-induced immune tolerance remain poorly understood. Thus, using RA inhibitors, we showed that TDLN DCs promote naive T cell differentiation to Treg through its ability to produce RA. Accordingly, addition of disulfiram (Aldh1 inhibitor) and LE540/LE135 (pan-retinoic acid receptor/RAR-inhibitor) significantly reduced the ability of TDLNs to induce Treg. Further, adoptive transfer of naive

OT-II cells in MO4 tumor bearing mice showed increased conversion to Treg in TDLN and tumor. Interestingly, treatment of these mice with disulfiram resulted in significant reduction in Treg generation suggesting that tumors actively use this pathway to induce immune tolerance through RA-induced Treg generation.

Moreover, phenotypic characterization of TDLN DCs from β -cat^{ΔDC} mice showed increased expression of activation

markers (CD80 and CD86) with reduced expression of co-inhibitory molecules (PDL1 and PDL2), indicating that activation of β -catenin also induces immune tolerance through regulation co-inhibitory and co-stimulatory molecules. Similarly, a recent study has shown that activation of β -catenin in DCs also affect the DCs ability to cross-prime CD8⁺ T cell responses, indicating that activation of β -catenin in DCs promote tumor tolerance through multiple mechanisms.⁸

These data prompted us to investigate the therapeutic benefits of modulating this pathway in a clinically relevant model. Accordingly, pharmacological inhibition of β -catenin/TCF4 pathway using XAV939⁹ in WT tumor bearing mice significantly diminished tumor growth. Consistent with the previous data, we noted a significant reduction in RA-synthesizing enzymes in TDLN DCs from XAV939 treated mice compared to control treated-mice. Although our data suggest that TME DCs promote immune tolerance through RA, additional studies are needed to understand molecular mechanisms that induce β -catenin in DCs and role of this

pathway in induction of other immunosuppressive mechanisms (Fig. 1). In this regard, our unpublished data suggests that expression of co-receptors LRP5 and six on DCs is critical for activation of β -catenin and inducing tumor tolerance.

In summary, our results suggest for the first time that TME induces immune tolerance through activation of β -catenin/TCF4 pathway, which promotes RA synthesis and Treg responses in TME and TDLNs (Fig. 1). Our finding suggest that blocking β -catenin/TCF4 pathway during tumor progression represent a promising novel therapeutic target for achieving effective antitumor immunotherapy. In

this regard, numerous small molecule activators or inhibitors of this pathway already exist with several more in active preclinical development opening new avenues to target tumor tolerance.⁹

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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