TARGET THE ACTIVIN RECEPTOR 1C ON CD4+ T CELLS TO ACHIEVE ANTI-TUMOR THERAPEUTIC EFFECTS

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Background Activins, members of the transforming growth factor- β (TGF- β) superfamily, were isolated and identified in endocrine system, and have been widely studied in endocrine-related cancers,^{1, 2} but not substantially in the context of immune system and endocrine-unrelated cancers.^{3–5} It has been reported that upon binding to the receptors, activins cause the intracellular recruitment and phosphorylation of smad proteins, which mediate the expression of Foxp3.^{6–9} Therefore, we hypothesized that the blockade of the interaction of activins and their receptors will inhibit the activin-mediated Foxp3 induction in CD4+ T cells, thus modify the immune suppressive tumor microenvironment and achieve the goal of cancer immunotherapy.

Methods ELISA (enzyme-linked immunosorbent assay) has been performed to determine the plasma level of Activin A in tumor-bearing mice and cancer patients. In vitro iTreg (induced regulatory T cells) differentiation has been done to naïve CD4+ cells isolated from wild type mice in the presence or absence of Activin A, and the percentage of Foxp3+ cells was demonstrated by flow cytometric analysis. qRT-PCR analysis has been conducted to determine the mRNA level of activin receptor isoforms in the immune subpopulations sorted from Foxp3-YFP mice. In the end, in vivo subcutaneous transplanted tumor studies have been done to evaluate the anti-tumor therapeutic effects of activin-receptor 1c blockade.

Results We show here that tumor-bearing mice had elevated Activin A levels, which correlated directly with tumor burden. Likewise, cancer patients had elevated plasma Activin A compared to healthy controls. Importantly, our in vitro studies suggested that Activin A promoted differentiation of conventional CD4+ cells into Foxp3-expressing induced Tregs, especially when TGF- β was limited. Database and qRT-PCR analysis of sorted major immune cell subsets in mice revealed that activin receptor 1C (Acvr1c) was uniquely expressed on Tregs and was highly upregulated during iTreg differentiation. Mice deficient in Acvr1c were more resistant to cancer progression compared to wild type mice. This phenotype correlated with reduced expression of the FoxP3 transcription factor in CD4+ cells. Similar phenomena were observed when we treated the mice with anti-Acvr1c antibody after tumor inoculation. This anti-tumor therapeutic effect was more significant when anti-Acvr1c antibody was administered in combination with anti-PD-1 antibody.

Conclusions Blocking Activin A signaling through its receptor 1c is a promising and disease-specific strategy for preventing the accumulation of immunosuppressive iTregs in cancer. Hence it represents a potential candidate for cancer immunotherapy.

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Ethics Approval All animal experiments were performed under protocols approved by the Johns Hopkins University Institutional Animal Care and Use Committee (IACUC).

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