Evidence for a Role of GPR35 in IDO1-mediated Tumor Immune Escape by Regulating Hippo-YAP Pathway

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IDO1 is an immune checkpoint regulator and mediator of tumor immune evasion [(1)]. The cellular and molecular mechanisms of IDO1-mediated immune suppression are not fully understood. We describe here that GPR35, the putative receptor for kynurenic acid (KYNA), a metabolite of IDO1-mediated tryptophan catabolism, couples to Hippo-YAP pathway. GPR35 activation, either by overexpression or by treatment with tryptophan metabolites resulted in Hippo inactivation and YAP-directed transcription via TEAD family of transcription factors. Recently, YAP is found to be essential for tumor immune escape. Yap1 deficiency in T-lineage-specific knockout mice, or by dosing a YAP inhibitor, impairs T_{reg} formation and function, leading to tumor growth inhibition [(2)]. YAP also plays an inhibitory role in CD8 T cell function, especially in activated CTL usually found in the TME [3,4]. Through medicinal chemistry design and a structure-vs-activity relationship study effort, we discovered a GPR35-selective inhibitor, here termed TMER1i. TMER1i potently blocks GPR35 in Hippo suppression and YAP activation. We showed that GPR35 is expressed in the T-cell lineage of the immune cells including naïve CD4 T cells, Treg and CD8 T cells. Treatment of purified human naïve CD4 T cells with anti-CD3/anti-CD28 under an $IDO1^+$ TME-like culture condition *in vitro* induced a robust T cell activation and differentiation toward Treg phenotype, as shown previously using mouse naïve CD4 T cells by others [(5)]. Importantly, TMER1i efficiently blocked the Treg differentiation under these conditions. When dosed in human-PBMC reconstituted NCG mice bearing HT29 tumors, TMER1i dose-dependently increased the infiltration of human CD8 T cells within tumors in a statistically significant manner, and a trending lower Treg cell infiltration, accompanied by a tumor growth inhibition. qPCR analysis of the HT29 tumors grown in the PBMC-NCG mice indicated that IDO1 was highly expressed in the tumors. These results indicated that GPR35 selective inhibitor TMER1i enhanced an anti-tumor immune activity in an $IDO1^+$ tumor microenvironment. Taken together, these studies provided evidence for a role of GPR35 in mediating IDO1 immune suppression by regulating Hippo-YAP pathway in Treg and CTL cells in the immune system. Thus, GPR35 inhibitors have a potential to be novel immuno-therapeutic agents effective in treating patients with IDO1-positive tumors.

Reference

1. Munn, DH, and Mellor, AL. (2007) Indoleamine 2,3-dioxygenase and tumor-induced tolerance. ***J. Clin. Invest.*** 117:1147–1154
2. Ni, X. *et al*. Yap is essential for Treg-mediated suppression of antitumor Immunity. ***Cancer Discovery*** 2018; 8:1026-1043
3. Lebid, A. *et al*. (2020) YAP attenuates CD8 T cell-mediated anti-tumor response. ***Frontiers in Immunology*** 11:1-8
4. Stampouloglou, E. *et al.* (2020) YAP suppresses T-cell function and infiltration in the tumor microenvironment. ***PLoS Biol*** 18(1): e3000591
5. Fallarino, F. *et al.* (2006) The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor ζ-chain and induce a regulatory phenotype in naïve T cells**.** ***J Immunol*** 176:6752-6761