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"Cblb-Notch1 regulation as a new target for cancer immunotherapy"

Notch1 (N1) is a highly conserved transmembrane receptor that is essential for many developmental functions and processes in the body. Upon binding of its ligands, N1 undergoes proteolytic cleavages that lead to the release of its intracellular domain, which enters the nucleus and activates the transcription of target genes. In CD8+ T-cells, N1 is responsible for the regulation of proliferation, cytokine production and cytotoxic activity. One of the major functions of CD8+ T-cells is the elimination of pathogens and cancer cells within the body. However, cancer cells have several strategies to evade T-cells responses. For example, the tumor microenvironment produces elevated levels of adenosine, an immunosuppressive ATP metabolite that dampens immune responses by signaling through adenosine receptors on immune cells. Specifically, we recently described that activation of Adenosine A2A Receptor (A2AR) in CD8+ T-cells decreases N1 and T-cells activation, thus allowing tumors to evade T-cells responses. Modulation of adenosine receptors on T-cells is the object of intense research activity as blocking A2AR receptor or its downstream signaling has the potential to boost anti-cancer T-cells responses. In this study, we investigated signaling through A2AR in CD8+ T-cells, its effect on N1 and T-cell activation, with the ultimate goal to find strategies to target this new pathway for therapeutic purposes.

Primary CD8+ T-cells were isolated from the spleens and lymph nodes of mice and used as a model to investigate signaling pathways through the A2AR. These cells were activated and cultured in the presence of a selective A2AR agonist and/or an antagonist, small molecule compounds which activate and block A2AR, respectively. Stimulation of the A2AR with an A2AR agonist resulted in decreased cell proliferation, cytokine production and a reduction in the levels of N1. Blocking A2AR signaling with the A2AR antagonist reversed the suppressive effects of the agonist. Additionally, we observed that ubiquination and degradation of N1 increased in cells treated with the A2AR agonist, suggesting A2AR may control N1 degradation. This observation led us to investigate an ubiquitin ligase that may regulate the degradation of N1: Casitas B-Lineage Proto-oncogene B (CBLB). An experimental drug inhibitor of CBLB was used in combination with the A2AR agonist in CD8+ T-cells to test the effect of CBLB inhibition on N1 levels and T-cells functions. Treatment with the CBLB inhibitor rescued N1 from the A2AR agonist-induced degradation and restored proliferation and cytokines production. Importantly, treatment of tumor-derived breast cancer organoids with the CBLB inhibitor resulted in increased cancer cell death. These results suggest that A2AR may control CBLB-mediated N1 degradation and blocking A2AR or CBLB could rescue N1 and T-cells functions, thus boosting anti-tumor T-cells responses.

Our data indicate that modulation of signaling through the A2AR is capable of manipulating N1 levels through CBLB and, in turn, T-cell functions in CD8+ T-cells. Importantly, CD8+ T-cells treated with drugs that increase N1 activation showed enhanced T-cell functions and anti-tumor responses, suggesting that this pathway is a promising new target for cancer immunotherapy.