Cblb-Notch1 regulation as a new target for cancer immunotherapy

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Background

- Notch1 (N1) is a transmembrane receptor that regulates proliferation, cytokine production and cytotoxic activity of CD8+ T-cells.
- One of the major functions of CD8+ T-cells is the elimination of pathogens and cancer cells within the body. However, cancer cells can evade T-cell responses, for example, by producing adenosine, an immunosuppressive metabolite.
- Adenosine blocks T-cells immune responses in part through activation of the Adenosine A2A Receptor (A2AR). Activation of this receptor by adenosine or an A2AR agonist (CGS) decreases Notch1 in CD8+ T-cells and, in turn, T-cell functions.
- It is not known how A2AR regulates N1, however, a recent study from our group identified the ubiquitin liga Cblb as a potential negative regulator of N1.

Objectives: We investigated signaling through A2AR, its effect on N1 and activation in CD8+ T-cells. The final goal of this work is to find strategies to target this new pathway for therapeutic purposes.

Methods

- Primary CD8+ T-cells were isolated from the spleens and lymph nodes of mice, treated with anti-CD3/CD28, and cultured for 72 hours with an A2AR agonist (CGS), antagonist (ZM) and a Cblb inhibitor.
- T-cells were lysed and lysates were analyzed by Immunoprecipitation and Western Blot to detect N1 protein levels
- ELISA was conducted on T-cell culture supernatants to quantify the production of Interferon-Gamma (INF-gamma)
- T-cells were stained with carboxyfluorescent diacetate succinimidyl ester (CFSE) to measure proliferation by flow cytometry
- Tumor 3D-cell cultures (organoids) were derived from a mouse model of breast cancer and were treated with a Cblb inhibitor to test its anti-cancer activity

Results

1. A2AR stimulation increases Notch1 degradation

![Western Blot showing N1FL, and Transmembrane Notch1 (N1TM) protein levels in activated CD8+ T-cells untreated (control) or treated with CGS (left image). Immunoprecipitation of Notch1 and detection of Ubiquitinated Notch1 (central image). Western blot showing cleaved Notch1 (CN1) and N1TM (right image) in CD8+ T-cells treated with a protein synthase inhibitor for 0 or 2 hours.](image)

2. A2AR stimulation decreases T-cells cytotoxic activity

![Graphs showing CD8+ T-cells proliferation and Interferon-Gamma production.](image)

3. CBLB inhibition rescues Notch1 from A2AR-dependent suppression

![Graphs showing inhibition of CBLB with an experimental pharmacologic inhibitor rescues N1 protein level from CGS-induced suppression.](image)

4. CBLB inhibition restores cytotoxic activity

![Graphs showing the amount of Interferon Gamma production from CD8+ T-cells treated with Cblb inhibitor, CGS and their combination.](image)

5. CBLB inhibition shows anti-tumor activity

![Graph showing the inhibition of CBLB in organoids derived from a triple-negative breast cancer (TNBC) mouse model.](image)

Conclusions

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.

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