Non- Canonical Notch signaling also regulates the metabolism and proliferation of CD8 T-cells

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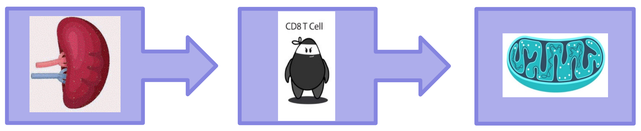
Introduction

Triple-negative breast cancer (TBNC) is one of the harder forms of breast cancer to treat due to it lacking three receptors that are essential to the targeted therapy used to treat other forms of breast cancer. The Notch pathway is a protein pathway that has been proven essential in both healthy cells, specifically CD8 T-cells, and cancer cell growth and is generally overexpressed in cancer cells, including TBNC. Its prevalence in TBNC subsequently makes it a focus in finding targeted therapies for TBNC. $CD8^+$ T-cells are immune cells that eliminate harmful substances, including tumors. The Notch pathway is also known to play a role in T-cell activation, which is necessary for the anti-tumor response in the body. With the non-canonical Notch pathway regulating both mitochondrial metabolism in TBNC cells and T- cell activation in CD8 T-cells, attempting to target this pathway in TBNC cells while not affecting $CD8^+$ T-cells is a daunting task.

Objective

The aim of this project is to better understand the role of mitochondrial Notch (non-canonical Notch) in $CD8^+$ T-cell metabolism, cell proliferation, and cytotoxic activity.

Methods



1. Extract $CD8^+$ T-cells from the spleen of C57BL/6 mice, mitochondria from isolated cells, and immunoprecipitate Notch-1 from mitochondrial fraction.

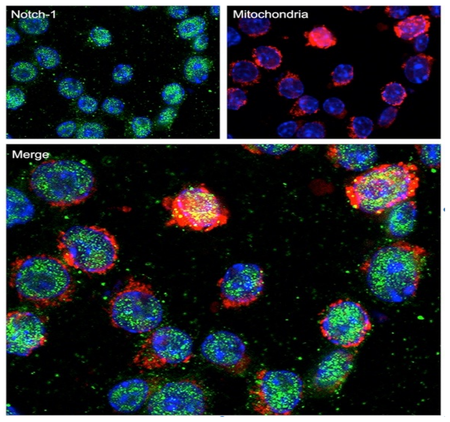
Immunofluorescence Assay → **Western Blot**

2. Confirm the isolation of mitochondrial Notch-1.

Seahorse Assay → **T-cell proliferation assay** → **ELISA Assay**

3. Analyze the role of Notch-1 in metabolic processes, cell proliferation, and cytotoxic activity.

Results



Blue: Nucleus
Green: Notch-1
Red: Mitochondria

Figure 1. Confocal imaging showing the co-localization of Notch-1 and the mitochondria of stimulated (a-CD3/CD28 for 72 hrs) mouse $CD8^+$ T-cells.

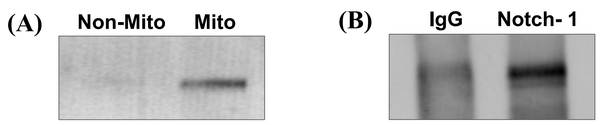


Figure 2. Western blot analysis to confirm the presence of Notch-1 in mitochondria of $CD8^+$ T-cells. $CD8^+$ T cells were isolated from C57BL/6 mouse spleen using EasySep $CD8^+$ T cell isolation kit (STEMCELL Technologies) and then stimulated with plate bound a-CD3/CD28 for 72 hours. (A) Mitochondrial fractions were purified using a mitochondrial isolation kit (Abcam). Western blot shows the presence of Notch-1 in the mitochondrial fractions. (B) Immunoprecipitation (IP) of mitochondrial fractions with Notch-1 and IgG antibody, followed by Western blot analysis with Notch-1 antibody.

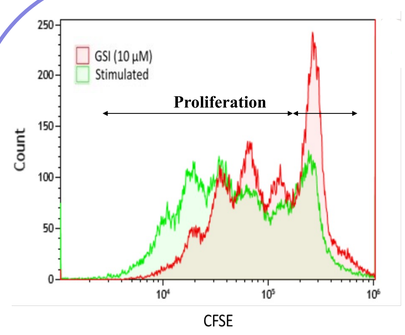


Figure 3A. T-cell proliferation assay demonstrating proliferation levels of CD8 T-cells. Gamma-secretase inhibitor (GSI) (PF-3084014) (10 µM) reduced $CD8^+$ T-cells proliferation as measured with carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution by Flow Cytometer.

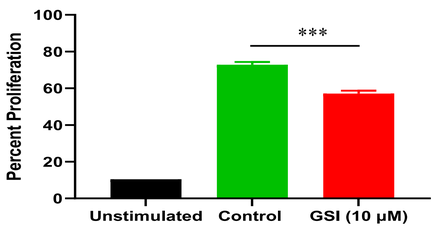


Figure 3B. T-cell proliferation assay results quantify the percent of proliferation. GSI (PF-3084014) (10 µM) significantly reduced $CD8^+$ T-cells proliferation.

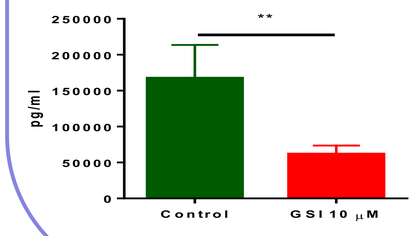


Figure 3C. Granzyme B ELISA assay. GSI (PF-3084014) (10 µM) treated $CD8^+$ T-cells show reduced cytotoxic activity.

Results

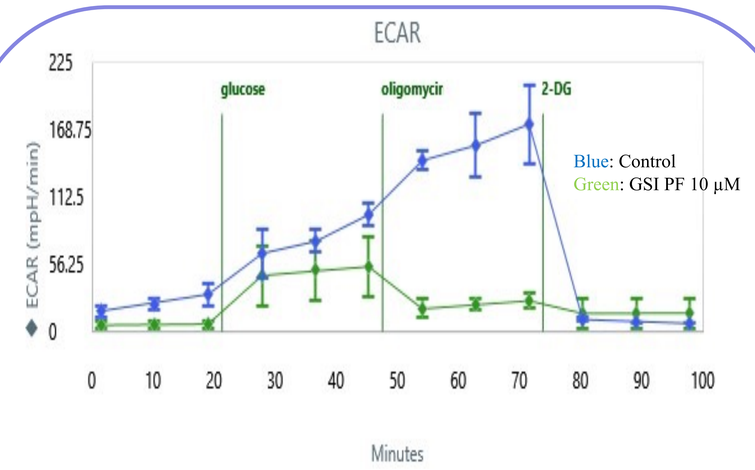


Figure 4A. Glycolytic flux of $CD8^+$ T cells as measured by extracellular acidification rate (ECAR) using Seahorse analyzer (Agilent Technologies). Metabolism of activated $CD8^+$ T cells is characterized by elevated level of glycolytic flux. GSI (PF-3084014) (10 µM) reduced both the basal and reserve ECAR capacity of $CD8^+$ T cells

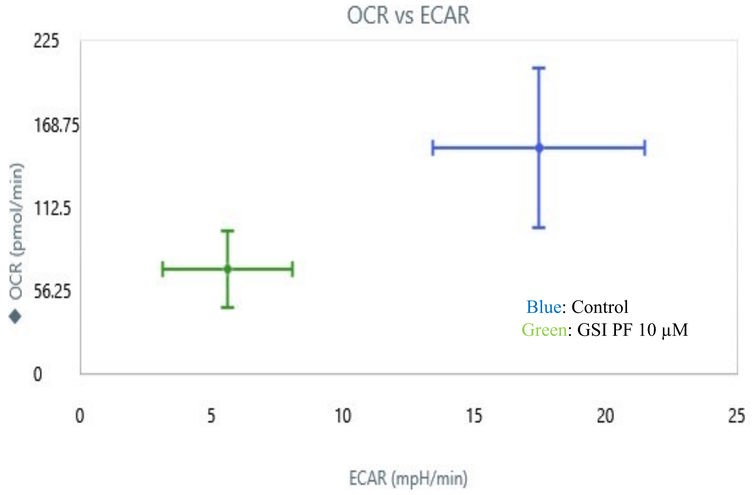


Figure 4B. Seahorse assay showing cellular energetics as measured by oxygen consumption rate (OCR) vs. ECAR. GSI (PF-3084014) (10 µM) treated $CD8^+$ T-cells show reduced metabolic activity as evidenced by the reduction of both OCR and ECAR.

Conclusion

- ❑ Notch-1 is present on the mitochondria of $CD8^+$ T-cells.
- ❑ Our results suggest that in addition to the canonical pathway, non-canonical Notch does play a role in metabolic processes, cell proliferation processes, and cytotoxic activity of $CD8^+$ T-cells.
- ❑ From these findings, future research will be geared toward determining the specific role Notch-1 plays in these necessary biological processes.

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