Kaitlyn Hawkins
Undergraduate
Xavier University of Louisiana, LA

Mentor: Fokhrul Hossain Ph. D
Louisiana Cancer Research Center

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

Triple-negative breast cancer (TBNC) is aggressive cancer that is very difficult to treat due to it lacking the three receptors, estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 (HER2), that are used in formulated targeted therapies for other forms of breast cancer. The Notch pathway is a signal transduction pathway that has been proven essential in both healthy and cancer cell proliferation and is generally overexpressed in cancer cells, including TBNC. Immune cells, specifically CD8 T-cells, are cells that are important in fighting cancer in the body. Posing a treatment that could target the pathway that is known to play a pivotal role in the proliferation of TBNC is complex due to the treatment possibly targeting immune cells as well as cancer cells, which would limit the already weakened immune systems of TBNC cancer patients. The Notch pathway is known to play a role in T-cell activation, which is necessary for the anti-tumor response in the body. Previously, we also reported that the non-canonical Notch pathway regulated mitochondrial metabolism in TBNC cells. In this study, the role of mitochondrial Notch (non-canonical Notch) in CD8 T-cells activity, specifically cell metabolism, and proliferation, was explored to attempt to gain insight into the significance of the non-canonical Notch pathway in cytotoxic T-cell antitumor activity.

CD8 T- cells were extracted from the spleen of mice and the mitochondria were isolated. An immunoprecipitation assay was subsequently used to isolate Notch1 from the mitochondria of the CD8 T-cells, and Western blot analysis was performed to confirm the presence of the mitochondrial Notch-1. Immunofluorescence and AMNIS were performed as a secondary measure to confirm the presence of Notch-1 in the CD8 T-cell mitochondria. The remaining isolated T- cells were treated with 5uM and 10uM of gamma-secretase inhibitors (PF-3084014), a class of drugs determined to inhibit the Notch pathway by preventing the cleavage of gamma-secretase. The treated cells underwent a T-cell proliferation assay as well as a Seahorse assay to determine the extent of the role of Notch in T-cell proliferation and metabolic processes respectively in CD8 T-cells.

From the experiments carried out, it was confirmed that Notch-1 is present in the mitochondria of CD8 T-cells. The results from the assays conveyed that in addition to the canonical pathway, non-canonical Notch does play a role in both metabolic processes as well as proliferation processes of CD8 T-cells. From these findings, future research will be geared toward determining the specific role Notch-1 plays in both of these necessary biological processes.