STRONG CHILDREN'S RESEARCH CENTER

Summer 2019 Research Scholar

Name: **Jacob Perkins**

School: **University at Buffalo**

Mentor: **Dr. George Porter**

ABSTRACT

Title: Impact of Cyclophillin D Mutations on Mitochondrial Transition Pore Opening

Background:
The mitochondrial permeability transition pore (mPTP) is hypothesized to be derived from ATP synthase, an inner mitochondrial membrane protein that produces ATP. Opening of the mPTP, dissipates the mitochondrial membrane potential, resulting in a decreased production of ATP and increased production of reactive oxidative species. Cyclosporine A (CsA) is an inhibitor of cyclophillin D (CyPD) and delays opening of the mPTP. In addition, ADP prevents mPTP opening, while mitochondrial calcium overload opens the mPTP.

Objectives:
- To investigate the function and mechanisms of the mPTP using a 143B cell line.
- To determine the effect of the CyPD mutations K166Q and K166R on mPTP opening in response to calcium.
- To determine whether mutated CyPD (K166Q, K166R and R96G) binds to the ATP synthase.

Results:
The cancer cell line, 143B was transfected with mutated CyPD: acetylation mimic (K166Q), deacetylation mimic (K166R), and enzymatic inactive (R96G). Calcium retention capacity (CRC) was used to analyze the onset of mPTP opening in untransfected 143B cells (WT), K166Q and K166R transfected cells. CRC was measured with no treatment (Ctrl), in the presence of CsA and ADP. The CRC increased significantly ($p \leq 0.05$) in ADP treated samples compared to Ctrl. There was no significant CRC increase when samples were treated with CsA.

Next, the transfected CyPD was immunoprecipitated using the DDK-tag attached to the mutation. Results indicate that ATP5A precipitated with the DDK-tag in K166Q, K166R, and R96G transfected cells. Immunoprecipitation of the ATP synthase indicated that mutated CyPD precipitated with the ATP synthase in K166R and R96G transfected cells. Using 2D electrophoresis, a signal for WT CyPD was detected in protein complexes with the molecular weight of ATP Synthase monomers and dimers. The signal for K166Q mutated CyPD occurred in a protein complex with molecular weight lower than the ATP synthase monomer.

Conclusion:
In 143B cell cultures ADP is an effective inhibitor of mPTP and thereby delays pore opening across WT, K116Q, and K166R conditions. CsA is not an effective inhibitor of CyPD in 143B cells. Mutated CyPD K166R and R96G most likely bind to ATP synthase and K166Q may not.