STRONG CHILDREN'S RESEARCH CENTER

Summer 2013 Research Scholar

Name: **Katie Hockensmith**

School: **Drury University**

Mentor: **Dr. George Porter, Jr., M.D., Ph.D.**

ABSTRACT

Title: Immunoprecipitation of ATP synthase using c subunit antibodies

Background: ATP synthase is the fifth complex in the oxidative phosphorylation pathway. This multisubunit protein complex is more than 600 kDa, consisting of two distinct portions, F_O and F_1. The hydrophobic F_O portion, that performs proton translocation, is embedded into the inner mitochondrial membrane and consists of subunits a, b, c, d, e, f, g, F6, and A6L. The hydrophilic F_1 portion, that performs ATP synthesis, protrudes from the membrane and consists of subunits α, ß, γ, δ, ε, and OSCP. The c subunit of the F_O portion of this complex is hypothesized to be the location of the mitochondrial permeability transition pore (mPTP), the opening of which increases mitochondrial inner membrane permeability, energetic failure, and cell death.

Objective: The Porter lab has been working to determine whether the mPTP is derived from ATP synthase. The objective of this particular project is to identify if antibodies specific for the c subunit (ATP5G) will immunoprecipitate ATP synthase.

Methods: Although the c subunit of ATP synthase is difficult to identify due to its hydrophobicity, we obtained an antibody raised against the sequence common to the three c subunit isoforms (ATP5G 1/2/3), and we used this antibody to immunoprecipitate proteins from homogenates of mitochondria from the adult mouse heart. Precipitated (sediment) and unprecipitated (supernatant) proteins were separated by electrophoresis in polyacrylamide gels based on their molecular weights and blotted to nitrocellulose membranes for immunodetection using antibodies to various ATP synthase subunits.

Results: We found that the immuneprecipitation using the ATP5G 1/2/3 antibody was successful. First, this antibody precipitated the 15 kDa c subunit proteins using a number of anti-c subunit antibodies. It was previously thought that the mature c-subunit protein was 7.6 kDa, based on work in bacteria, but we have demonstrated that the mitochondrial targeting sequence of the mammalian c subunit is not cleaved. The results from this project support that the working c subunit is 15 kDa in mammalian cell. Then, the following proteins were identified in the sediment, indicating that they were specifically precipitated as a complex with the c subunit: OSCP, α, g, and A6L.

Conclusion: The identification of ATP5G 1/2/3 as an immunoprecipitant has allowed for study of the relationship between ATP synthase and the mPTP. This anti-c subunit antibody successfully immunoprecipitates the entire ATP synthase complex and also helps identify the composition of the working c subunit within that complex. Further work from the lab demonstrates that octameric rings of the 15 kDa c subunit create the pore of the mPTP, which is an important advancement in the field of mitochondrial biology. These findings may lead to therapies targeting the c subunit to treat diseases in which the mPTP plays a role, such as congenital heart disease, cardiomyopathies, and myocardial infarction.