STRONG CHILDREN'S RESEARCH CENTER

Summer 2019 Research Scholar

Name: **Erin Ritzer**

School: **Lake Erie College of Osteopathic Medicine – Bradenton**

Mentors: **Ethan David Cohen PhD and Michael O'Reilly PhD**

ABSTRACT

Title: *Role of AMPK in Neonatal Hyperoxia-induced Cardiovascular Disease*

Background: Premature infants needing the most supplemental oxygen have higher risk of cardiovascular disease due to poorly understood mechanisms. A mouse model was established in which exposure to hyperoxia from birth to postnatal day (PND) 0 to 4 causes pulmonary hypertension and heart failure. These effects are preceded by the failure to expand cardiomyocytes (CMs) lining the pulmonary vein (PV) and left atrium (LA). Gene expression profiling shows that fatty acid synthesis is suppressed in the atria of mice exposed to neonatal hyperoxia on PND4. Enzymes needed for fatty acid synthesis such as Fatty acid synthase (FASN) and Stearoyl-CoA desaturase (SCD1) are permanently reduced in the atria but not ventricles of hyperoxia-exposed mice. FASN and SCD1 inhibition also reduces the proliferation of the HL-1 atrial cardiomyocyte cell line while FASN and SCD1 overexpression increases HL-1 cell proliferation in hyperoxia. KEGG pathway analysis of our microarray data suggests that AMPK signaling may be responsible for the hyperoxia-induced suppression of fatty acid synthesis.

Objective: To determine if AMPK is responsible for the hyperoxia-induced loss of fatty acid synthesis genes and CM proliferation in the PV and LA.

Methods: *Cell Proliferation Analysis* HL-1 cells plated at equal density in 96 well plates were also treated with increasing doses of the AMPK activators Metformin (Dosages: 0, 0.75, 1.5, 3, 6 and 12mM) and AICAR (Dosages: 0, 0.5, 1, 2mM) and grown for 12, 24, 36 and 48 hours before being fixed and stained with DAPI so that the numbers of cells in each well could be counted using a Nexcelon Celigo Imaging Cytometer. *In Vitro and In Vivo P-AMPK Activation* Western blotting with antibodies for the activation state specific phosphorylation of AMPK on threonine 172 (p-AMPK), total AMPK and Actin was performed on HL-1 cells grown in room air or hyperoxia for 36 hours as well as the left and right atria of PND56 mice that were exposed to room air or hyperoxia from PND 0-4. Membranes were exposed using BioRad ChemiDoc imagery and densitometry was done on ImageJ/Fiji.

Results: *Cell Proliferation Analysis* Metformin and AICAR similarly reduced the growth of HL-1 cells in room air similar to the effects of hyperoxia or the inhibition of fatty acid synthesis in these cells. *In Vitro P-AMPK Activation* The levels of p-AMPK were approximately 2 fold higher in HL-1 cells grown in hyperoxia than in those grown in room air. *In Vivo P-AMPK Activation* P-AMPK levels were approximately 1.75 fold higher in the left atria of PND 56 mice exposed to hyperoxia than in those of room air treated control mice. P-AMPK levels showed no significant change in the right atria.

Conclusion: The AMPK activators Metformin and AICAR also reduce the proliferation of HL-1 cells in vitro, similar to the effects of hyperoxia or the inhibition of fatty acid synthesis. Hyperoxia activates AMPK in HL-1 atrial CMs and the left atria of PND56 mice exposed to neonatal hyperoxia relative to those of room air treated controls. Together, these data suggest that hyperoxia may suppress fatty acid synthesis and reduce proliferation through the persistent activation of AMPK signaling in atrial and pulmonary vein CMs.