STRONG CHILDREN'S RESEARCH CENTER

Summer 2016 Research Scholar

Name: **Hannah Latta**

School: **University of Kentucky**

Mentor: **Dr. Laurie Steiner**

ABSTRACT

Title: *Determining the Role of gamma-H2AX in Erythropoiesis*

Background: Erythropoiesis is the multi-faceted process by which erythrocytes, or red blood cells, are formed. Several proteins are implicated in this developmental story including histone variant H2AX and its phosphorylated form, gamma-H2AX. Histones are utilized to package DNA into chromatin and gamma-H2AX is also implicated in the DNA double-strand break (DSB) repair process. ATM, ATR, and DNA-PK are three kinases that phosphorylate H2AX to gamma-H2AX. Interestingly, the levels of gamma-H2AX are elevated in certain erythroid precursors in the absence of any evidence of DNA damage.

Objective: To elucidate the functions of gamma-H2AX in erythroblasts, several experiments were performed using extensively self-renewing erythroblasts (ESREs) in vitro. ATM, ATR, and DNA-PK expression was knocked down using siRNA transfection. Transfected cultures were induced to mature for an additional 48 hours to ascertain the effect of treatment on viability and maturation.

Results: A reduction in expression of ATM, ATR, and DNA-PK expression was confirmed using qPCR. Knockdown was also verified by a decrease in gamma-H2AX signal observed with immunostaining. Cell concentration was greater in matured treatment wells when compared to a scramble control which suggests that selective kinase knockdown positively influences proliferation. This preliminary data is consistent with literature reports of similar phenomena occurring in cancer cell models. Since ATM, ATR, and DNA-PK are implicated in DNA repair, knocking down these genes can be implicated in the bypassing of critical cell cycle checkpoints.

Conclusion: Future direction for this project will include conducting ChIP assays to verify the non-canonical role of gamma-H2AX in erythropoiesis. Additional siRNA transfection experiments and qPCR will be used to assess relative expression of other kinase-encoding genes in cells that display a knockdown phenotype for one kinase. These experiments will also be used to investigate the relationship between cell cycle inhibitor abundance and the absence of ATM, ATR, and DNA-PK kinase molecules.