

STRONG CHILDREN'S RESEARCH CENTER

Summer 2018 Research Scholar

Name: Lisa Yoder
School: Allegheny College
Mentor: Dr. Laurie Steiner, MD

ABSTRACT

Title: *Elucidating Intrinsic and Extrinsic Factors of Myelodysplastic Syndrome (MDS) in Human Erythropoiesis*

Background: Erythropoiesis is the differentiation and maturation of hemopoietic stem cells into red blood cells (RBCs) in the bone marrow of adult humans. Red blood cells transport oxygen through the body via hemoglobin. In order to have room for ample hemoglobin storage in the cell, erythroid precursor cells (erythroblasts) must prepare their nucleus for expulsion by compacting DNA. DNA is wrapped tightly around histone proteins and packaged into chromatin through activation and silencing of chromatin modifiers. Over two million red blood cells are made each second in healthy humans in order to transport enough oxygen throughout the body. Thus, the changes that need to occur for compaction of DNA and the ejection of the nucleus must occur rapidly. Disruption of chromatin modifiers can lead to diseases where erythroblasts cannot mature properly.

Myelodysplastic syndrome (MDS) is a cluster of diseases where blood cells can not differentiate and mature properly. Patients with MDS have an average survival rate of five years with little treatment options. Specifically, erythropoiesis is affected in patients with MDS resulting in lower levels of healthy red blood cells produced by the bone marrow and leading to anemia. Previous studies have identified DNA mutations in erythroblasts of patients with MDS. Additionally, microenvironments of the bone marrow in patients with MDS is altered which may have implications in erythropoiesis.

Objective: The goal of this research project is to form a better understanding of how DNA mutations and the microenvironment affects the development of red blood cells in patients with MDS.

Methods: An immortalized human cell line, human umbilical cord blood derived erythroid progenitor cells (HUDEP-2), were used as a model for erythroblasts in the laboratory to elucidate intrinsic and extrinsic factors of MDS in human erythropoiesis. Cultures of HUDEP-2 cells were grown in expansion and matured in maturation media. Cluster, regularly interspaced, short palindromic repeat (CRISPr), was used to mimic specific DNA mutations in HUDEP-2 cells that are found in erythroblasts of patients with MDS. Additionally, protein signaling molecules, cytokines, were used to treat HUPEP-2 cells to mimic potential altered microenvironments found in patients with MDS which may impact red blood cell development.

Results: Intrinsically, CRISPr/Cas9 was used to successfully make MDS mutations in exon 13 of the ASXL1 gene in HUDEP-2 cells. Extrinsically, we hypothesized that treatment with cytokines will slow the maturation of HUDEP-2 cells as compared to controls. Treatment of HUDEP-2 cells with the cytokine interleukin-1 β (IL-1 β) and its antagonist anakinra did not result in a significant difference in cell maturation or development compared to the vehicle treatment. Treatment of HUDEP-2 cells with the cytokine interferon- γ (IFN- γ) showed a dose-dependent response with a decrease in erythropoietic proliferation. Imaging flow cytometry also showed that cells treated with IFN- γ were less mature as compared to the vehicle supported by larger cell area and nuclear area.

Conclusion: Cytokine treatment has demonstrated that the microenvironment of progenitor cells is important for erythropoiesis. The effects of other cytokines on erythropoiesis can be studied. In the future, the new HUDEP-2 cell lines created to mimic MDS mutations can be used as a model for understanding how proper erythropoiesis is prevented. Forming a better understanding of what intrinsic mutations and the extrinsic microenvironments contribute to MDS will help in identifying possible mechanisms that are responsible for erroneous erythropoiesis and potential pharmacological treatments which could improve survival rates.