STRONG CHILDREN'S RESEARCH CENTER

Summer 2013 Research Scholar

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ABSTRACT

Title: **Inhibition of Stat3 Signaling in Primitive and Definitive Erythropoiesis**

Background:

During development, distinct primitive and definitive erythroid lineages sequentially generate the red blood cells necessary for embryonic growth and our postnatal well-being. Erythropoietin (EPO) is the primary cytokine regulator of erythropoiesis, and signals primarily through Jak2-Stat5 in definitive erythroid cells. Gene expression studies revealed the differential expression of Stat3 in primitive and definitive erythroid cells. Here the function of Stat3 in primitive and definitive erythroid cells was tested. Primary tissue from E8.5 mouse embryos was used to model primitive erythropoiesis using a "two step" in vitro maturation system. To model definitive erythropoiesis in vitro, mouse fetal livers (E13.5-E14.5) were used to isolate erythroid progenitors by FACS. As the progenitors matured to precursors in vitro, the role of Stat3 signaling was explored by adding a Stat3 inhibitor (S3I201), in the presence or absence of the growth factor erythropoietin (Epo). The role of Stat3 in the context of Epo was assayed by monitoring cell division (cell counts), viability (trypan negativity), and hemoglobin accumulation (benzidine positivity). Morphology was also assessed (cytospins stained by giemsa and benzidine). Gene expression (Bcl-xl, Bid, Bax) was determined using qPCR.

Objective:

The goals of this project were to better understand maturation of erythroid cells both in their primitive stage and in their adult definitive stage, as well as see how Stat3 signaling affected the erythroid cell maturation process by inhibition of its function.

Results:

Addition of the Stat3 inhibitor caused a decrease in hemoglobin accumulation and viability in primitive erythroid cells compared to cells without the inhibitor. This is in contrast to definitive erythropoiesis in which inhibition of Stat3 showed no significant effect on cell maturation both in terms of viability and hemoglobin accrual. Bid, Bax, and Bcl-xl genes all showed a decrease in expression in primitive erythropoiesis relative to the presence of EPO without the inhibitor while both Bid and Bax showed an increase in expression in definitive erythropoiesis.

Conclusion:

We conclude that Stat3 signaling plays a differential role in primitive, but not definitive, erythroblast maturation and proliferation.