

STRONG CHILDREN'S RESEARCH CENTER

Summer 2013 Research Scholar

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ABSTRACT

Title: **Elucidating the mechanism underlying the protective effects of erythropoietin in the developing brain.**

Background: Hypoxic Ischemic Encephalopathy (HIE), a brain injury caused by a combination of inadequate blood flow and oxygen to the brain, is a common occurrence in newborns leading to death in 15-20% of cases and severe neurological damage in another 25%. While therapeutic hypothermia can have some benefit on the prognosis of an infant suffering from hypoxic insult, this treatment can only be utilized in term or near term infants and its availability is limited. Recent research has shown that administering exogenous erythropoietin (EPO) may have neuroprotective effects on infants suffering from hypoxic insult and further that the EPO pathway plays an important role in normal neural development, however, little is known regarding the underlying mechanisms.

Objective: The goal of our study is to elucidate the molecular mechanisms underlying the neuroprotective effects of EPO in the developing brain.

Hypothesis: We hypothesize that in the developing brain, EPO signaling promotes specific patterns of epigenetic modifications, transcription factor binding, and mRNA expression.

Methods and Results: To study the role of EPO in neurodevelopment, primary cells were dissected from 15.5-day mouse embryos obtained from a timed pregnancy. Both bright field and immunofluorescence images were used to confirm that our cells were neural. mRNA expression analyses demonstrated that EPO and EpoR, as well as their downstream effectors, are developmentally regulated, with higher levels of expression early in development and decreasing levels as maturation occurs. To study the role of EPO in the response to hypoxia, we cultured and differentiated NTERA-2 cells for 7 days using retinoic acid (RA). We found that when supplemented with RA, NTERA-2 cells will differentiate down a neuronal pathway as illustrated by mRNA expression analyses through an increase in the expression of neural gene markers. Once differentiated, we incubated the NTERA-2 cells +/- EPO in a hypoxic (5% O₂) environment for 24 hours. Finally, using both mRNA gene expression and chromatin immunoprecipitation, we were able to show that EPO plays a role in the response to hypoxia with the increase in expression of downstream EPO effectors subsequent to incubation in a hypoxic environment.

Conclusion: Together, our findings suggest that not only is EPO developmentally regulated in the embryonic nervous system, but further that EPO plays an important role in the response for hypoxia, making elucidation of the underlying mechanism of EPO in the developing brain an important goal for future studies.

