

**Neonatal hypoxia/ischemia expands the numbers of neural stem cells in the subventricular zone by enhancing EGFR and IGF-1R responsiveness.** Amber Ziegler, Dhivyaa Alagappan, William Tyler, Claudine Bitel, Matthew V. Covey, Steven W. Levison and Teresa L. Wood. *Department of Neurology and Neurosciences, UH Cancer Center, UMDNJ-New Jersey Medical School, Newark, NJ, USA*

**Objectives:** The brain was long regarded as a non-regenerating organ; however, studies performed over the last decade have demonstrated that there is limited CNS regeneration after injury. Our previous studies demonstrated an expansion in the numbers of neural stem/progenitors (NSPs) in the subventricular zone (SVZ) of the neonatal brain in response to hypoxic/ischemic (H/I) injury. Towards understanding which extrinsic factors regulate NSP expansion after injury, we have analyzed changes in the expression of several growth factors and their receptors in the SVZ following H/I and have begun to determine the function of these growth factors on NSPs.

**Methods:** Levels of insulin-like growth factor-1 (IGF-1), the IGF type 1 receptor (IGF-1R) and the epidermal growth factor receptor (EGFR) were analyzed by gene array and verified by q-PCR. RNA was isolated from 7 day and 14 day brains of neonatal rats following unilateral carotid artery ligation and exposure to 8% oxygen for 90 min. To evaluate the importance of growth factor signaling for NSP proliferation we analyzed the proliferation, self-renewal and survival of NSPs in vitro using neurosphere assays. To analyze the role of the IGF-1R in neurosphere growth, two culture conditions were tested where insulin levels varied: 1) normal growth medium containing EGF (2 ng/ml) and micromolar insulin (25  $\mu$ g/ml) that will stimulate both the insulin receptor and the IGF-1R, and 2) low insulin growth medium containing EGF (2 ng/ml) and nanomolar insulin (25 ng/ml) that will activate the insulin receptor but not the IGF-1R.

**Results:** Expression of IGF-1 mRNA was increased 25-fold within the SVZ with peak IGF-1 expression occurring between 7 to 14 days post-H/I, whereupon it returned to baseline. The IGF-1R also increased within the SVZ after H/I. EGFR mRNA expression also increased 4-fold by 7 days post-H/I; this was correlated with increased EGFR phosphorylation. Pharmacologically inhibiting EGF receptor signaling, reduced the expansion of the NSPs after H/I. Placing NSPs into cell culture medium with physiological concentrations of insulin (i.e. in the absence of IGF-1R stimulation) compromised the formation and size of neurospheres compared to neurospheres grown in normal medium containing superphysiological levels of insulin and EGF. The reduced growth capacity cannot be attributed simply to failure to survive, as NSPs maintained in low insulin and EGF for 7 days retained the capacity to form spheres when transferred to high insulin containing medium and EGF. Moreover, the expansion of NSPs observed after perinatal H/I was curtailed when NSPs post-insult were grown in physiological vs supraphysiological insulin. Lowering the concentration of insulin in the medium abrogated the expansion in the number of spheres formed post injury and reduced the size of the spheres. This result was observed despite increased sensitivity to EGF. To establish whether the IGF-1R was required in the neurospheres for high insulin-mediated growth, IGF-1R expression was decreased using an shRNA to the IGF-1R. NSPs transfected with the IGF-1R shRNA generated spheres of reduced size (14% of control), but the number of spheres produced was not different.

**Conclusions:** Based on these data we conclude that the IGF-1R is necessary for the normal proliferation of NSPs as well as for their reactive expansion after injury and that coordinate EGFR and IGF-1R signaling are required for optimal proliferation. Supported by MH 59950 awarded to SWL.