Directing Neural Stem Cell Recruitment: Crosstalk Signaling Between ECM and SDF-1α

C.P. Addington, C. Pauken, M.R. Caplan, S.E. Stabenfeldt
School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ

Statement of Purpose: Traumatic brain injury inititiates numerous injury-induced signaling events in effort to maintain homeostatsis. Pro-regenerative signaling occurs in conjunction with these injury sequelae resulting in the recruitment a variety of stem cell types (e.g. neural, hematopoietic, mesenchymal). [1,2] The mechanisms underlying endogenous neural progenitor/stem cell (NPSC) recruitment have not fully been elicudated, but inflammatory and immune related cytokines such as stromal cell derived factor 1α (SDF-1α) have been implicated as potential chemotractant signaling molecules. The adult neural stem cell niche is comprised of a microenvironment highly enriched with extracellular matrix (ECM) proteins derived from neighboring blood vessels. Subsequently, we are interested in the potential role of crosstalk between the injury-induced cytokine, SDF-1α, and the ECM in directing endogenous NPSC fate after injury. Therefore, the objective of this study was to investigate the influence of ECM-SDF-1α crosstalk on NPSC behavior. Results from this study will facilitate the development of the next generation biomaterials that exploit endogenous NSPC regenerative signaling cascades.

Methods: NPSCs were harvested according to an approved protocol from the Arizona State University Institutional Animal Care and Use Committee. Briefly, NPSCs were obtained from the developing germinal eminence of E14.5 C57BL/6 mice and were cultured in neurospheres as previously described by Reynolds et al. [3] NPSCs were plated in ECM coated 24-well plates (groups: poly-L-lysine, fibronectin, vitronectin, laminin, Matrigel; 6 μg/cm^2) with or without SDF-1α (1μg/mL). Cell migration was tracked via phase contrast imaging 1hr after seeding and every 24hrs for 7days. Cell differentiation was assessed through immunocytochemistry for proteins indicative of specific cell types: neural stem cells (nestin); oligodendrocytes (O4); astrocytes (GFAP); and neurons (beta III tubulin). Proliferation was also assessed at 24hr and 4days after plating with Click-it EdU Proliferation Assay (Life Technologies). Statistical significance was analyzed using a two-way ANOVA test and Tukey's post-hoc pairwise test.

Results: In the radial migration assay, the presence/absence of SDF-1α significantly influenced migration rates of NPSCs on laminin and Matrigel (Figure 1); however, no significant differences in migration were observed on the other substrates (data not shown). After 7 days in culture, immunohistochemistry demonstrated increased neuronal phenotype on Matrigel and laminin over PLL, fibronectin, and vitronectin in basal media. However, in the presence of SDF-1α, all substrates (PLL, laminin, Matrigel, fibronectin, vitronectin) displayed enhanced neuronal phenotype. All substrates supported astrocytic and oligodendrocytic phenotypes regardless of SDF-1α supplementation. Proliferation results demonstrated a significantly increase in the number of proliferating cells as measured by the incorporation of EdU stain when plated on the ECM-derived substrates (Matrigel, laminin, vitronectin, and fibronectin) only when in the presence of SDF-1α.

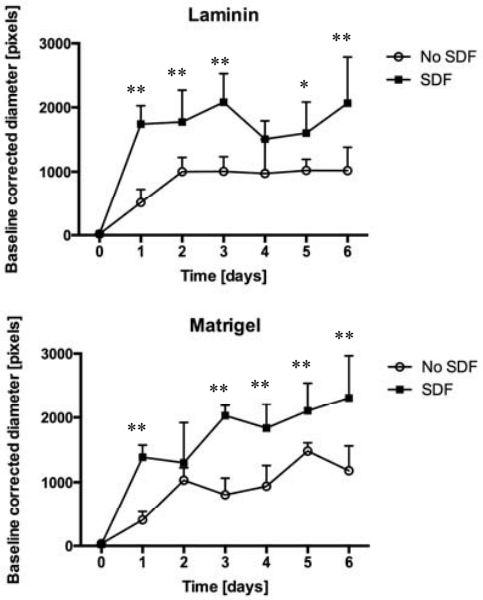

Figure 1: Radial migration of NPSC on laminin (top) and Matrigel (bottom) in absence or presence of SDF-1α. Asterisks indicate significance with respect to the appropriate non-SDF-1α supplemented samples. (*p<0.01,**P<0.0001)

Conclusions: Our findings suggest that SDF-1α acts synergistically with certain ECM proteins (particularly laminin) to enhance NPSC migration. We also observed an enhancement of neuronal differentiation on all substrates in the presence of SDF-1 α with the greatest increase on laminin and Matrigel. Additionally, our findings suggest that SDF-1α significantly influences NSPC proliferation on ECM substrates. Thus, crosstalk signaling between SDF-1α and ECM substrates may play a large role in neural stem cell recruitment following a traumatic brain injury. Modulating NPSC response after injury will be a valuable tool in developing the next generation of regenerative strategies for neural repair.

References:

1. Hill, WD et al., J Neuropathol Exp Neurol (2004) 63(1):84-96.
2. Kucia, M. et al., J Mol Histol (2004) 35 : 233-245.
3. Reynolds et al., J Neurosci (1992) 12(11): 4565-4574.