Characterization of the Neural Stem Cell Response to Extracellular Matrix Produced by Dynamically-Stimulated Endothelial Cells

by

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ABSTRACT

Neural stem cells are multi-potential precursors that can be used as an autologous cell source for cell-mediated therapies to treat neurodegenerative diseases and injury to the nervous system. *In vivo,* neural stem cell proliferation and differentiation is regulated by a complex interaction of cues, soluble factors (growth factors and cytokines), insoluble factors (extracellular matrix), and cell-cell contacts, in a specialized microenvironment known as the stem cell niche. This niche is located near blood vessels suggesting an important interaction between neural stem cells and the endothelial cells that comprise the lining of the microvasculature [1-3]. *In vitro* studies have demonstrated that soluble factors released by endothelial cells cultured under normal static conditions stimulate neural stem cell self-renewal and inhibit differentiation, while direct contact between the statically-cultured endothelial cells and neural stem cells promoted neuronal differentiation [4,5]. In vascular biology, it is well-known that the endothelial phenotype is sensitive to the local hemodynamic environment eliciting an altered production of soluble factors and insoluble extracellular matrix proteins [6]. However, *in vitro* models of neural stem cell – endothelial cell interactions have not considered these phenotypic changes to the endothelial phenotype which likely impact neural stem cell fate. I hypothesize that changes to the endothelial phenotype and the altered production of soluble factors and extracellular matrix proteins will impact neural stem cell fate. While there are many ways the endothelial cells can influence neural stem cell function (direct cell contact, release of soluble factors and the production of extracellular matrix), the focus of my doctoral thesis is to isolate one component of this niche, the extracellular matrix, from dynamically-stimulated endothelial cells and examine the response of neural stem cells to this naturally-derived biomaterial. To accomplish this objective, my thesis is comprised of three specific aims. The first aim focuses on the isolation and processing of the extracellular matrix to form a biomaterial wherein the native structure is preserved. In the second aim, the extracellular matrix will be used to evaluate changes in the neural stem cell response (morphology, proliferation and differentiation). The third aim examines the importance of signaling from the laminin integrin α6β1, an integrin chosen due to the importance of laminin in the nervous system. The results identified qualitative and quantitative increases in the production of laminin, fibronectin,

and type IV collagen due to the dynamic stimulation of endothelial cells (aim 1). This upregulation in protein production by dynamically-stimulated endothelial cells was found to impact neural stem cell spreading, proliferation, and metabolism relative to extracellular matrix produced in static conditions (aim 2). Furthermore, α6β1 integrin signaling was found to significantly contribute to these changes in neural stem cell behavior as examined with antibody blocking assays (aim 3). Determining the factors that regulate neural stem cell fate is critical for improving the strategies to expand and control stem cell differentiation for cell-based therapies. This study has extended previous work by demonstrating the importance of endothelial factors on neural stem cell fate and has specifically demonstrated that dynamic stimulation of endothelial cells can impact neural stem cell fate through altered protein production. Furthermore, this was the first study to isolate and utilize natural cell-derived matrix as a culture substrate for neural stem cells studies. This work may ultimately lead to a better understanding of what components of the complex niche control neural stem cell fate and may also inspire the development of an engineered biomaterial to promote neural stem cell proliferation for expanding rare patient-specific cells for cell-mediated therapies.