An Immunohistochemical Evaluation of Integrin and Cadherin Expression within Mesenchymal Stem Cell Spheroids

Original Title: A Spatial Investigation of Integrin and Cadherin Expression in Mesenchymal Stem Cell Spheroids Using a Laminate Microfluidics System

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Hypothesis

Mesenchymal Stem Cells (MSCs) formed into three-dimensional spheroids have increased therapeutic potential compared to dissociated MSCs, as they have increased cell survival and trophic factor secretion. MSCs exhibit changes associated with different regions in the spheroid, but our lab has demonstrated that these changes are not due to hypoxic gradients, as was previously thought. This study aims to characterize the spatial differences in integrin and cadherin expression within MSC spheroids to better understand the differentiation potential, and thereby optimize spheroid synthesis. We hypothesize that MSC spheroids will exhibit increased cadherin expression towards the periphery of the spheroid and increased integrin expression towards the center.

Research Accomplished

The original experimental design of this study is largely based on prior investigations of tumor cell spheroids in which serial trypsinization was used to systematically evaluate protein expression within different spheroid regions that could represent tumor microenvironments. I adapted this model in multiple ways: (1) I collaborated with another lab and developed a high throughput microfluidics system to more efficiently, systematically, and consistently trypsinize MSC spheroids. (2) I learned a new spheroid synthesis method to eliminate sample contamination from trophic factor secretion via shared media. (3) I planned to evaluate mRNA integrin and cadherin expression via single-cell PCR. It has been shown that MSC osteogenic differentiation is heavily regulated by both integrin and cadherin signals, specifically N-cadherin, E-cadherin, Cadherin-11, and integrin subunits $\alpha 2$, $\alpha 5$, the vitronectin receptor of the α subunit (αv), and $\beta 1$. Therefore, the expression levels of each of these were to be evaluated from the PCR results.

Since summer of 2017, I have been designing and prototyping the microfluidics system using Inventor, a CAD software. I had also been conducting preliminary experiments ensuring cell viability post-trypsinization and proving the consistent removal of layers with sectioning (via a microtome) and hematoxylin and eosin (H&E) staining. The first half of the summer was dedicated to measuring spheroid diameter after removal of each layer as a basis for the experimental controls and prepping samples for the first round of PCR. However, during the sixth week of the program, the single-cell PCR was unexpectedly no longer feasible. Therefore, a new plan was implemented: immunohistochemistry for all the integrins and cadherins. I then designed the IHC layout (primary and secondary antibody selection). The STAR project ended shortly after the arrival of the appropriate antibodies, and, as a year-round researcher, I plan to continue this project, which will involve optimizing at least 5 different IHC protocols (as we have none for integrins and cadherins, specifically). I hope to have preliminary results by the end of 2018.