

STRONG CHILDREN'S RESEARCH CENTER

Summer 2015 Research Scholar

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ABSTRACT

Title: Mesenchymal stromal cells isolated from pediatric lungs: analysis of viability, differentiation potential, and protein expression.

Background: The Molecular Atlas of Lung Development Program (Lung MAP) aims to unravel the secrets of the developing lung. The University of Rochester is serving as the Human Tissue Core (HTC). The function of the HTC is to process lung tissue received from organ procurement agencies and to provide varied lung and cell samples to four collaborating research centers. The dissociated lung tissue samples will be shipped off to research centers in a variety of ways, from heterogeneous cell mixtures to isolated cell type samples.

Hypothesis: Mesenchymal stromal cell (MSC) phenotype will vary between donors based on the finding that percentage of MSCs in the total lung population varied between donors.

Objective: The goal of this project is to determine if fibroblasts can be cultured from a double magnetic column separation (CD105-/EPCAM-), to determine if CD105+ lung mesenchymal stromal cell (MSC) expansion, long term viability, and protein expression varies from based on donor profile, provide firm evidence that lung MSCs can be differentiated into adipocytes, osteoblasts, and chondrocytes, and take publication quality photos of cells throughout these experiments to determine if cell morphology is impacted by donor profile.

Results: It was determined that fibroblasts can be grown out via magnetic column sorting, though purity and phenotype of these cells has not yet been demonstrated. Lung MSC cultures can be passaged out to at least passage 9 (where cells are at the writing of this abstract). Fold expansion varied substantially between passages of individual donors and did not appear to show any pattern in regards to donor profile. MSCs will continue to be passaged and analyzed via flow cytometry. So far there has been no substantial difference in protein expression between donors or passages. The Miltenyi Differentiation Kit and methods for detecting differentiated mesenchymal stem cells work well and with numerous lung MSC donors, though it should be noted that chondrocyte staining has not yet been completed. Many publication quality photos have been taken of numerous cell types. No substantial difference in cell morphologies was noticed between donors and different passages.

Conclusion: Our hypothesis was not supported. Fold expansion does not appear to be correlated to donor profile and even varied when looking at individual donors between passages. The ability to easily phenotype lung MSCs means that we should be able to attempt to draw conclusions about protein expression as passages go on and compare them to the individual donor profiles. So far, it appears that MSC protein expression is not affected by donor profile. Miltenyi differentiation kits, however, were successful. We will continue to passage out lung MSCs in hopes of determining if there are differences in MSC phenotype and viability at later passages.