STRONG CHILDREN'S RESEARCH CENTER

Summer 2016 Research Scholar

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ABSTRACT

Title:
A Comparison of Lung Derived Mesenchymal Stromal Cells and Fibroblasts Using qRT-PCR

Background:

In recent years, stem cells have shown great promise for the repair and regeneration of tissues and organs through stem cell therapy. Access to developing human lung tissue is a major barrier to advancing medical treatments for neonatal and pediatric lung disease. Many recent studies suggest mesenchymal stromal cells could be the start to an answer for treating these chronic developmental barriers faced by a premature infant. The LungMAP was established by NHLBI to provide an atlas of normal human lung development to the scientific community for improved understanding of lung disease. The data presented is a continuation of a study that showed the diversity of samples isolated from 6 donor pilots. We have continued to characterize these 6 donor pilots but now in comparison to 7 fibroblast donors. MSCs are fibroblast-like shaped cells residing in mesodermal tissues of bone marrow, adipose tissue, lung, placenta, and the umbilical cord stroma[1]. MSCs have identifying features including their ability to adhere to plastic surfaces in cell culture laboratory practices, their expression of CD105, CD73, and CD90, and their potential to differentiate into osteogenic, adipogenic, and chondrogenic cell lineages in vitro. The preliminary data includes flow cytometry showing MSCs present in each donor and overtime, CD105+ decreases and cell recovery (cell counts and viability) decreases over time (P0 to P18). Through qRT-PCR, the 6 MSCs and 7 fibroblasts gene expression will be compared to further characterize the cells over time.

Objective:

Based on cellular differentiation, mesenchymal stromal cells and fibroblasts will inherently express different genes in all passages. The MSCs will begin to lose their stemness characteristics as well as CD105, CD73, and CD90 in later passages as shown in the preliminary flow cytometry data. In later passages, it is possible that the MSCs will resemble fibroblasts to a greater degree, but it is unknown at what point in their differentiation these changes are detectable. **Objective 1:** To test the hypothesis that as time increases, the MSCs will lose their inherent MSC markers, CD 105, CD73, and CD90. **Objective 2**: To compare the expression of the selected genes between the MSCs and fibroblasts at early and late passages.

Results:

The qRT-PCR data from both the early passage and late passage comparing the fibroblasts and mesenchymal stromal cells show few significant differences in the expression of genes selected. The data was run in duplicate, leading us to believe these two cell types are much more similar than initially predicted.

Conclusion:

MSCs are quintipotential cells at the top of their differentiation hierarchy and have the ability to become osteocytes, adipocytes, chondrocytes. Fibroblasts are terminally differentiated cells at the bottom of the MSC differentiation hierarchy. RNA from MSCs and fibroblasts from 6 and 7 respective donors was collected at P4/5 and P10/11 for analysis by qRT-PCR. Primers were selected from published literature to test the expression of various markers within the cells to characterize at the early and late passages. After analysis, it was found that the MSCs and fibroblasts from P4/5 and P10/11 were not as different as predicted. Future directions for the project include continuing to expand the cells to P15 and test the same primers with RT-PCR to see if there is a significant difference after more time. DO42 adult lung MSCs and fibroblasts as well as 5 cord donor cells are currently being grown; once at P15, another project could be to test these cells alongside the MSCs at P5, P10, and P15. Lastly, it would be beneficial to review new published literature to look for genes that may show larger differences, including an exploration of the difference in contact inhibition between the MSCs and fibroblasts.