

# STRONG CHILDREN'S RESEARCH CENTER

## Summer 2017 Research Scholar

**Name:** Benjamin C. Reeves

**School:** Yale University

**Mentor:** Gloria Pryhuber (M.D) & Ravi Misra (PhD)

---

### ABSTRACT

**Title:** Comparative Characterization of Matched Human Lung Mesenchymal Stromal Cells and Fibroblasts by qRT-PCR and Antioxidant Activity

**Background:** The LungMAP was established by the NHLBI and is comprised of physicians and scientists spread throughout one human tissue core (HTC), one data coordinating center, and four research centers. The organization aims to further advance the treatment of pediatric patients suffering from chronic respiratory diseases by better understanding the process of lung development. The HTC, located at the University of Rochester Medical Center, receives transplant quality lungs from deceased patients across the country and a portion of each lung is dissociated into specific cell types. Those of particular interest are mesenchymal stromal cells (MSCs) and fibroblasts. MSCs have a high regenerative power and an ability to differentiate into adipogenic, chondrogenic, and osteogenic lineages making them a candidate for stem cell-like therapies. These properties suggest that MSCs have the therapeutic potential for treating chronic diseases associated with lung development that primarily affect premature infants such as Bronchopulmonary Dysplasia (BPD). Fibroblasts are terminally differentiated cells that play a critical role in the production and maintenance of the extracellular matrix. While such properties make fibroblasts vital to the wound healing process, excessive accumulation can produce high concentrations of extracellular proteins leading to the potentially fatal condition of fibrosis. While MSCs share fibroblast morphology, MSCs are distinguished in the lab by their expression of CD105, CD90, and CD73. To further understand the differences between these cell types, we compared gene expression of 25 genes, both at passage 6 and passage 10, using qRT-PCR. RNA was isolated from the cell lysate of six lungs procured from donors ranging from a newborn to 8 years of age. The selected genes were chosen from secreted proteomics data collected on MSCs and fibroblasts from six donors. Additionally, we completed superoxide dismutase (SOD) activity assays on both cell lysate and cell supernatant from both cell types.

**Objective 1:** To further analyze gene expression in both MSCs and Fibroblasts at both early and late passages. **Objective 2:** To quantify relative activity of superoxide dismutase, an antioxidant that catalyzes the partitioning of superoxide to molecular oxygen or hydrogen peroxide, in both the cell lysates and supernatants of MSCs and fibroblasts.

**Results:** The qRT-PCR data showed several significant differences in the gene expression between MSCs and fibroblasts at both early and late passages. There were also significant differences in gene expression between passage 6 and passage 10 of the same cell types. The data also suggests that some changes in gene expression are correlated with the cell's ability to expand. Of those genes, we noted a 56-fold increase in expression of insulin like growth factor-binding protein 5, a member of a family of proteins believed to be related to BPD, in cells with reduced expansion ability. Interestingly, SOD and thioredoxin, both antioxidants, increased significantly from passage 6 to passage 10 in the fibroblasts indicating that their antioxidant capacity increases with passage number. There were no significant changes in expression of these genes from passage 6 to passage 10 in the MSCs. To explore this finding further, a total antioxidant capacity assay on both passage 6 and passage 10 cells could be done. Additionally, the MSCs displayed an increasing trend from passage 6 to passage 10 in the anti-inflammatory proteins transforming growth factor beta induced protein ig-h3 and transforming growth factor beta binding protein 2. There were no significant differences in the fibroblasts from passage 6 to 10. The lysates of the MSCs and the fibroblasts have significantly higher SOD activity than either supernatant. The SOD activity in the MSC supernatant was significantly higher than the supernatant of the fibroblasts.

**Conclusion:** The data supports the findings of the preliminary studies that showed MSCs have a longer life span in culture than fibroblasts. Many of the studied genes saw a decrease in their expression in fibroblasts from passage 6 to passage 10. This was not the case for the majority of the MSCs which leads us to believe that the fibroblasts are becoming quiescent at earlier time points than the MSCs.