

HCT Gene Expression by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

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INTRODUCTION

Lung diseases, especially asthma and Chronic Obstructive Pulmonary Disease (COPD), are responsible for the death of approximately 4 million people yearly worldwide. Air pollution, smoking, inflammation, and genetic mutations contribute to lung diseases by deteriorating lung capacity and impeding ventilation and the supply of adequate oxygen to the body. The symptoms of lung disease may include dyspnea, wheezing, cough, and chest pain with the underlying pathogenic mechanisms for most lung disease being inflammation and infection. Severe inflammation and the inability to resolve it ultimately leads to build up of infectious pathogens, such as *Pseudomonas aeruginosa*, and can generate severe lung damage, resulting in pulmonary failure. Human Umbilical Cord Blood (HUCB) has been previously utilized as a source of hematopoietic stem cells. Here HUCB stem cells are multipotent and have been shown to have regenerative, anti-inflammatory, and bioactive properties. hMSCs derived from cord blood and Bone Marrow (BM) are bioactive and can be used to ameliorate inflammation and to augment bactericidal capabilities in asthma and CF. In the majority of clinical trials, hMSCs have been derived from BM aspirates of healthy volunteers, which is both an invasive and a costly procedure. HCT has been investigated as a potential, rich source of hMSCs, as it is often discarded as human medical waste. Further, with the availability and abundance of a source material, the non-invasive collection procedure, and the lack of ethical concerns that are associated with other sources, HCT are an attractive option for therapeutic hMSC sourcing. In these studies, we profiled HCT hMSCs ability to produce and secrete anti-inflammatory products, ultimately pursuing the identification of the mechanistic response of HCT hMSCs through phenotypic properties in inflammatory scenarios, such as bacterial analog exposure. We further determined the functional capacity of HCT

hMSCs by examining effects on cellular activity with airway

Epithelial cells. Finally, we present options for HCT growth optimization towards specific anti-inflammatory properties, and define donor variability as a factor to be considered when utilizing HCT hMSCs as a therapeutic source. The innovative nature of the results in this manuscript, as well as their alignment with our findings in BM derived hMSCs demonstrates the unique role hMSCs can play in terms of their therapeutic potential for chronic inflammatory diseases. Thus, HCT hMSCs may be considered as an economical, advantageous, and potent resource of stem cells for therapeutic development. As approved by Institutional Review Board Committees, HCT hMSCs in frozen (-80°C) vials were obtained from Cord Blood Registry as repaired above, and were identified by donor number. The hMSCs were isolated and grown following the standard operating procedures using Mesen Cult (Mes) Medium (STEMCELL Technologies Inc. Cambridge MA) and Low Glucose (LG) Dulbecco's Modified Eagle's medium and High Glucose (HG) methods for which clinical trial based hMSCs are maintained. hMSCs were cultured in the following environments: no stimulant and Streptomycin to prevent bacterial contamination of the epithelial cells and were utilized at passage 8 or 9. Lung diseases, especially asthma and Chronic Obstructive Pulmonary Disease (COPD), are responsible for the death of approximately 4 million people yearly worldwide. Air pollution, smoking, inflammation, and genetic mutations contribute to lung diseases by deteriorating lung capacity and impeding ventilation and the supply of adequate oxygen to the body. Cells were grown in 2 mL of A549 media. One day prior to harvest, 1 mL of cord tissue media was added in place of 1 mL of A549 media. Here hours later, they were cultured with cord tissue media, followed by stimulation with or without LPS. Cells were harvested 24 hours post stimulation and cell pellets were saved for gene expression.

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