INFORMED TARGET DISCOVERY FOR GENE AND STEM CELL THERAPY IN ACUTE LUNG INJURY

Zhou, Dun1; Amatullah, Hajera1; Shan, Yuexin1; Gali, Patricia1; Hu, Pingzhao2; dos Santos, Claudia1

1St Michael's Hospital, Critical Care, Toronto, Canada; 2Sick Children's Hospital, The Centre for Applied Genomics, Toronto, Canada

Introduction: Sepsis-induced acute respiratory distress syndrome (ARDS) accounts for 9% of all patient deaths in the ICU. In spite of a growing and urgent need for treatment, the main clinical strategy is still low VT supportive mechanical ventilation which improves survival, but there are no specific pharmacological treatments for sepsis-induced ALI. Mesenchymal stem cells (MSC) have been shown to have reparative potential in both sepsis and ALI.

Objectives: To identify novel molecular targets for ARDS treatment.

Methods: Microarray, a high-throughput technology, was used to identify genes that showed differential expression in sepsis that could have potential therapeutic value in ARDS/ALI treatment. In the murine model of cecal ligation perforation (CLP)-induced ARDS, changes in global gene expression in the lung following sepsis and MSC treatment were profiled using microarray. In parallel, microRNA expression was also determined via microarray. LIMMA analysis was used to identify genes that showed significant transcriptional changes between sham, CLP-operated, and CLP+MSC treated mice; as well, LIMMA selected microRNAs that exhibited significant fold changes across the three treatment groups.

Results: Putative targets of microRNAs of differential expression between CLP and MSC groups were joined with LIMMA-selected mRNAs to generate a list of target mRNAs of significant changes in their expression. To confirm our in silico data, we used Human Pulmonary Microvascular Endothelial Cells (HPMEC) as an in vitro model. Two junctional proteins, occludin and claudin-2 – both are classical markers in sepsis-induced ARDS and indicative of endothelial leakage, were putative targets of our microRNA of interest. The expression patterns of occludin and claudin-2 in vitro matched closely to the in silico, as well as in vivo data. In the mRNA profile, occludin was down-regulated after CLP-operation compared with sham, and this was confirmed by western blots (in vivo and in vitro), and qRT-PCR results also showed a 50% reduction. Similarly, the expression pattern of claudin-2 from the in silico data matched the protein level analysis by western blot and qRT-PCR (in vivo and in vitro). Subsequently, following transfection of the inhibitor of the microRNA of interest, occludin’s mRNA level was significantly up-regulated. In the permeability assay we used to assess the change in phenotype, the use of microRNA inhibitor significantly reversed the increase in permeability induced by TNFa. The ability of the microRNA inhibitor to rescue of the integrity of the endothelium was also confirmed by trans-endothelial electrical resistance and dextran fluorescence.

References: Mei et al. AJRCCM, 2010.