ROLE OF MIR-27A MEDIATED REGULATION OF VAV3 IN SEPSIS-INDUCED ARDS

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Introduction: Despite recent advances in medicine, sepsis remains a disease with high mortality (30-50%). Of the many complications of sepsis, acute respiratory distress syndrome (ARDS) remains one of the most common and life-threatening illnesses. To date, there is no specific treatment for ARDS, and therapy is mainly supportive. Recent research show that mesenchymal stem cells (MSCs) have immunomodulatory and reparative potential in sepsis and ARDS (Mei et al. 2010), although the mechanism through which MSCs confer their beneficial effects is yet undetermined. To study the protective mechanisms of MSCs, we took interest in the growing field of study of microRNAs (miRs). MiRs are emerging as important post-transcriptional gene regulators in various diseases, and have potential of being novel therapeutic targets. We have identified miRs that are differentially expressed in septic lungs from MSC-treated vs non-treated mice, and generated a list of predicted targets for each miR. Particularly, miR-27a and its putative target gene VAV3 have been of interest. VAV3 is a guanine nucleotide exchange factor (GEF) for Rho family GTPases. It functions in a signaling pathway that alters actin structures, and thus has roles in cell migration.

Objectives: Our objectives are to 1) verify the regulation of VAV3 by miR-27a in our models of sepsis, 2) determine an observable phenotype through regulation of VAV3, and 3) establish the role of miR-27a mediated gene regulation in disease states of ARDS.

Methods: For our in vitro model of sepsis, we use Human Pulmonary Microvascular Endothelial Cells (HPMECs) treated with 10ng/mL of TNF-α. Through qRT-PCR and western blot, we examine changes in levels of miR-27a and differential expression of the target gene, VAV3, at RNA and protein levels. To verify regulation of VAV3 by miR-27a, we treat HPMECs with miR-27a specific inhibitor and mimic from QIAGEN, and examine subsequent changes in VAV3 expression. To observe VAV3 in a migration phenotype, we use a scratch migration assay on HPMECs treated with miR-27a specific inhibitor and mimic, and stimulated with TNF-α. Preliminary in vivo studies involve using C57BL/6 mice to perform intratracheal lipopolysaccharide (LPS) instillation for 8 hours. Lungs are collected, homogenized, and measured for RNA and protein levels of miR-27a and VAV3.

Results: We observe that VAV3 is down-regulated in both in vitro and in vivo models of sepsis, concomitant to increased levels of miR-27a. Administering the miR-27a mimic to HPMECs also demonstrates increased levels of miR-27a and down-regulation of VAV3, and vice-versa upon administering the miR-27a inhibitor. In scratch migration assays with HPMECs, both TNF-α and the miR-27a mimic are observed to decrease cellular migration, while the miR-27a inhibitor attenuates the decrease resulting from TNF-α stimulation.

Conclusion: MiR-27a functions as a post-transcriptional regulator of VAV3 in our models of sepsis. A decrease in VAV3 results in decreased cell migration, which may play important roles in wound healing and inflammation during sepsis-induced ARDS.