The impact of aging on pulmonary microvascular endothelial cell barrier dysfunction

Elderly individuals have substantially elevated morbidity and mortality during conditions of lung injury, though the precise underlying mechanisms are ill-defined. Lung injury is associated with damage to pulmonary microvascular endothelial cells (PMVEC), leading to a compromised vascular barrier, fluid and protein leakage within the tissue, and subsequent respiratory dysfunction. Cell-cell junctions, including adherens and tight junctions, are crucial for maintaining the endothelial barrier and have been shown to be dysregulated in various disease models. Pilot data from our lab showed increased pulmonary vascular leak in aged mice with lung injury compared to young mice. We hypothesized that aging contributes to PMVEC barrier dysfunction due to impaired cell-cell junction integrity. To address this, PMVEC isolated from young and aged mice were cultured *in vitro*, until a confluent monolayer was formed. Endothelial barrier integrity was assessed by Evans blue-labelled albumin flux across the monolayers, as well as immunofluorescence staining of the adherens junction protein, VE-cadherin, and tight junction protein, claudin-5. Localization of leak in relation to junctional proteins was performed using the fluorescently labelled macromolecule, NeutrAvidin. Proteomics analysis was conducted to identify differentially enriched pathways in young and aged PMVEC. Compared to the young, aged PMVEC monolayers exhibited a significant increase in albumin leak associated with augmented VE-cadherin and claudin-5 disruption. NeutrAvidin staining localized to paracellular regions with VE-cadherin discontinuity was increased in aged PMVEC, suggesting the junctional disruption was directly associated with leak. Proteomics analysis revealed pathways known to be implicated in endothelial barrier dysfunction, including regulation of oxidoreduction and protein processing and trafficking, that were altered in aged PMVEC. We will next target protein pathways dysregulated in aged PMVEC to potentially rescue age-induced barrier dysfunction. These findings may highlight molecular pathways involved in predisposing aged individuals to worsened outcomes during lung injury, which can help in the development of therapeutics.