DEFINING THE MOLECULAR SIGNATURE OF THE PULMONARY ENDOTHELIUM IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Introduction COPD is thought to result largely from neutrophilic inflammation. Relatively little focus has been given to the endothelium, through which neutrophils transmigrate to reach the lung, despite observation of abnormal amounts of endothelial tissue in COPD which behaves in a dysfunctional manner. We sought to determine if coding (mRNA) and non-coding (miRNA) alterations in pulmonary endothelium exist in COPD, and if so what their effect is on endothelial function.

Methods Patients with and without COPD undergoing thoracic surgery were recruited (n=52); power calculations indicated that 10 in each group were required to detect differences in miRNA between health and COPD. Human Pulmonary Endothelial Cells (HPECs) were isolated from lung tissue by positive selection using Ulex europaeus lectin-coated magnetic beads and extracted RNA used for miRNA and mRNA microarrays, as well as confirmatory qPCR. Significance Analysis of Microarrays (SAM) was used to perform differential miRNA or mRNA analysis; ingenuity pathway analysis (IPA) was also carried out to guide functional work. The miRNA which differed most between health and COPD was transfected into endothelial cells and a range of cellular functions were assessed, including matrigel and spheroid assays.

Results 2071 genes and 43 miRNAs were significantly upregulated in COPD. 6 mRNAs and 8 miRNAs were appropriate for further validation. 4 targets were validated by qPCR, of which mir-181b-3p exhibited the most significant differential expression and was chosen for functional validation. IPA suggested that cell growth/proliferation, eicosanoid signalling and TGFB (transforming growth factor beta) driven pathways were the most relevant areas for further study. There was significantly reduced tube formation (figure 1) and endothelial sprouting in Human umbilical vein endothelial cells (HUVECs) transfected with mir-181b-3p, consistent with an effect on angiogenesis.

Conclusions Upregulation of miR-181b-3p reduces tube formation and sprouting by endothelial cells. This might be significant in the development of emphysema as lung vasculature is important in the structural maintenance of alveoli. Correcting miR-181b-3p expression levels could be a route for treating emphysema by promoting support of alveolar structure and regeneration.

REFERENCES