Mechanistic insights into COPD

S113 DEFINING THE MOLECULAR SIGNATURE OF THE PULMONARY ENDOTHELIUM IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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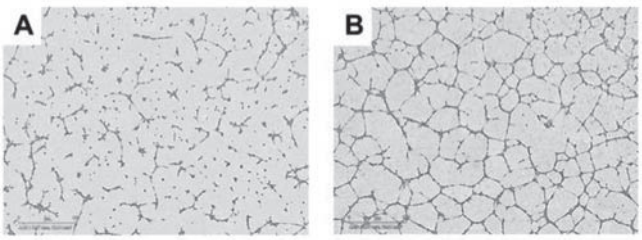
Introduction COPD is thought to result largely from neutrophilic inflammation. Relatively little focus has been given to the endothelium, through which neutrophils transmigrate to reach the lung, despite observation of abnormal amounts of endothelial tissue in COPD which behaves in a dysfunctional manner.[1] 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.



Abstract S113 Figure 1 An example of image from a matrigel experiment. A: HUVECs transfected with miR-181b-3p mimic. B: HUVECs transfected with negative siRNA. There is reduced tube formation seen in HUVECs transfected with the mimic.

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.[2] Correcting miR-181b-3p expression levels could be a route for treating emphysema by promoting support of alveolar structure and regeneration.

REFERENCES

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S114 HYPOXIA DRIVES NEUTROPHIL-MEDIATED ENDOTHELIAL DAMAGE IN COPD

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Introduction COPD is a progressive neutrophilic lung disease associated with increased risk of cardiovascular complications. Neutrophil elastase (NE) is implicated in COPD pathogenesis but the precise mechanisms of neutrophil-mediated tissue damage are unknown, particularly with respect to systemic manifestations. Inflamed COPD airways are profoundly hypoxic. We therefore hypothesised that hypoxia synergises with inflammatory cytokines to promote a destructive neutrophil phenotype with enhanced capacity for tissue damage, both locally and systemically.

Methods Neutrophils isolated from exacerbating COPD patients and age/sex-matched healthy volunteers were incubated under normoxia (21% O_2) or hypoxia (0.8% O_2) for 4 hours, before treatment with priming (PAF) and stimulating (fMLP) agents, with/without PI3Kinase inhibitors. NE activity was measured by Enzchek assay. Neutrophil supernatants were incubated with primary human pulmonary artery endothelial cells (HPAEC); cell damage was assessed by confocal microscopy. Normoxic/hypoxic neutrophil supernatants underwent tandem mass tag-labelled mass spectrometry (TMT-MS), and identified protein abundance was quantified. Neutrophil-derived microparticles (NDMPs) were isolated by ultra-centrifugation and quantified by NanoSight nanoparticle tracking technology.

Results Hypoxia increased NE release in a PI3K-dependent manner, with significantly more NE secreted by hypoxic neutrophils from exacerbating COPD patients *versus* healthy controls ($p<0.0001$). Supernatants generated from hypoxic, but not normoxic, stimulated neutrophils induced extensive HPAEC damage. Comparing the secretomes of supernatants derived from normoxic/hypoxic stimulated neutrophils, TMT-MS identified several additional proteins with potential to cause tissue damage as upregulated in hypoxia, including resistin and NGAL (neutrophil gelatinase-associated lipocalin). Notably, several of these proteins were not granule-associated, and some granule proteins were downregulated in hypoxia, indicating additional/alternative release mechanisms. Preliminary data show an increase in NDMP release under hypoxia,