

Mechanistic insights into COPD

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

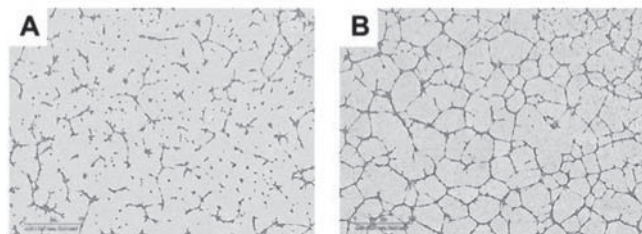
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Introduction COPD is thought to result largely from neutrophilic inflammation. Relatively little focus has been given to the endothelium, through which neutrophils transmigrate to reach the lung, despite observation of abnormal amounts of endothelial 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% O₂) or hypoxia (0.8% O₂) 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,

potentially contributing to the observed differential protein release.

Conclusions Hypoxia augments NE release in a PI3K-dependent manner, further increased during COPD exacerbations, and hypoxic neutrophil supernatants injure endothelial cells *in vitro*. Unbiased characterisation of hypoxic neutrophil secretomes identified several upregulated proteins which may contribute to cellular/tissue damage. In addition to degranulation, NDMP release may underpin differential protein secretion under hypoxia. Hypoxia engenders a neutrophil phenotype with potential to cause local and distant tissue damage in COPD; novel targets in the hypoxic neutrophil secretome may identify new therapeutic opportunities.

S115 MECHANISMS TO REVERSE IMPAIRED MACROPHAGE EFFEROCYTOSIS IN COPD

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COPD patients have defective innate immunity, characterised in part by macrophage dysfunction. In established disease, both monocyte derived macrophages (MDM) and alveolar macrophages (AM), have impaired phagocytosis of bacteria and apoptotic cells (efferocytosis). Failure to adequately efferocytose dying cells leads to further release of inflammatory mediators and ongoing recruitment of immune cells, culminating in inflammation that is both damaging and ineffective. The transcription factor, Nrf2 is the master regulator of the antioxidant-response-element. Previous work using the non specific Nrf2 agonist, Sulforaphane, has partially restored defective bacterial phagocytosis in COPD AMs, however the specificity and mechanism of action in this context remains unknown. The role of Nrf2 activation in restoring macrophage efferocytosis in COPD has yet to be examined. We questioned if impaired macrophage phagocytosis and efferocytosis in COPD share a common mechanism and consequently if the defect in macrophage efferocytosis can be therapeutically

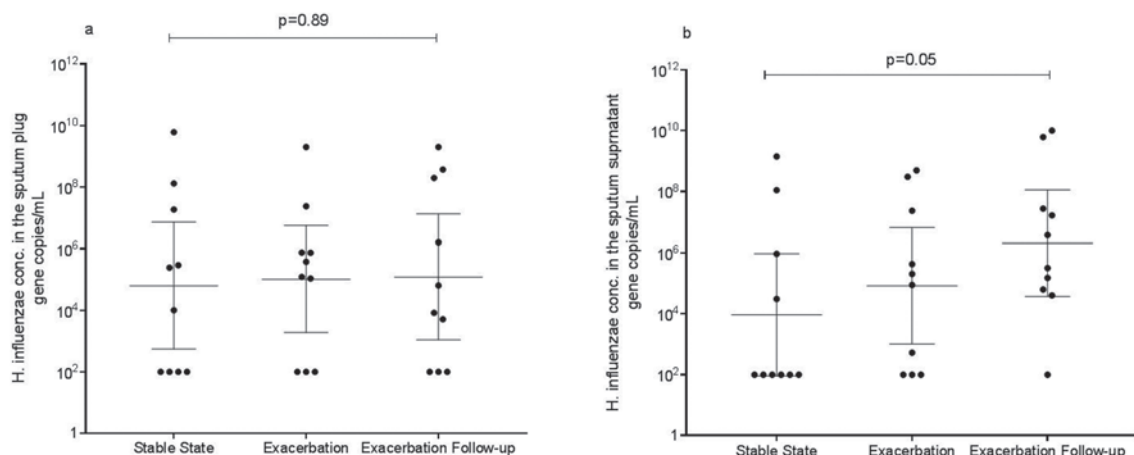
manipulated using highly selective Nrf2 agonists. AMs and MDMs were isolated from patients with established COPD (GOLD stage 1–3). Macrophage efferocytosis of apoptotic neutrophils from each donor was correlated with bacterial internalisation of *Streptococcus pneumoniae*. Efferocytosis assays were carried out in the presence or absence of Sulforaphane and Compound 7, a highly specific Nrf2 agonist (supplied by GSK). Both COPD MDMs and AMs have significantly impaired phagocytosis of bacteria and apoptotic cells compared to Healthy Controls (p values all<0.05). Moreover, there was a correlation between donor macrophage phagocytosis of apoptotic cells and of bacteria (r=0.71). *In vitro* studies using Sulforaphane enhanced efferocytosis of apoptotic cells in both COPD MDMs and AMs (p<0.01). This effect was replicated using Compound 7 in both MDMs (p<0.05) and AMs. This was more pronounced in current smokers. In summary, we observe a correlation between impaired macrophage phagocytosis and efferocytosis in COPD, suggesting a common mechanism. Furthermore, we describe partial rescue of defective COPD MDM and AM efferocytosis by Sulforaphane and via specific activation of the Nrf2 pathway using Compound 7. Together, these data highlight the importance of the Nrf2 pathway in reversing macrophage dysfunction in COPD patients and provide key mechanistic insights into the underlying defect.

S116 CELL-DISSOCIATED HAEMOPHILUS INFLUENZAE AND BACTERIA-ASSOCIATED INFLAMMATORY MEDIATORS IN THE AIRWAYS OF PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Background Patients with COPD have a susceptibility to respiratory tract infections associated with increased pulmonary inflammation. Bacteria can reside within the host as cell-associated (attached to host cells via adhesins, pili or biofilm formation) or cell-dissociated bacteria. It is unclear how bacteria-to-cell interactions affect pulmonary inflammation and whether



Abstract S116 Figure 1 Levels of *H. influenzae* in the sputum plug (A) and in the sputum supernatant (B) during an exacerbation time course in 10 paired subjects with COPD.