found that pulmonary endothelial specific deletion of HIF2 α , achieved using murine cre-loxp technologies (L1 or alk1-cre), offers protection against hypoxia-induced PAH. The rise in pulmonary artery pressure (PAP) normally observed following chronic hypoxic challenge was absent in mice with pulmonary endothelial HIF2 α deletion. The right ventricular systolic pressure of L1cre- HIF2 α mice post hypoxic challenge (26.17 ± 1.67 mmHg, $n=7$) was not significantly different from untreated WT mice (22.48 ± 1.19 mmHg, $n=9$) and much lower than the hypertensive values seen in WT littermate controls (41.91 ± 1.88 mmHg, $n=12$, $p<0.0001$) and L1cre-HIF1 α mice (36.25 ± 2.37 mmHg, $n=7$, $p<0.005$). Only minimal remodelling was observed in lung sections from L1cre-HIF2 α mice reflecting the normal physiological PAPs following chronic hypoxia. We next questioned whether deletion of lung endothelial HIF2 α would be sufficient to reduce downstream arginase-1 and -2 gene expression and in turn influence plasma nitrite/nitrate (NO_(X)) concentrations, which would be indicative of changes in nitric oxide homeostasis. The expression of both arginase-1 and -2 were significantly reduced in hypoxia-conditioned whole lung samples from L1cre-HIF2 α mice relative to WT littermate controls. Plasma NO_(X) concentrations were also significantly elevated in the HIF2 α mutant mice when compared to plasma from WT control mice. These observations fit a model whereby reduced arginase-1/2 expression leads to increased availability of L-arginine, and in turn increased NO synthesis via NO synthases. These data offer new insights into the role of pulmonary endothelial HIF2 α in causing PAH, and offer new therapeutic opportunities for the treatment of this condition.

S111 ALTERED NEUTROPHIL PHENOTYPES IN PULMONARY ARTERIAL HYPERTENSION

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Introduction Evidence has implicated neutrophil elastase (NE), a proteolytic enzyme, as a key driver of the pulmonary vascular remodelling that underlies pulmonary arterial hypertension (PAH). Moreover, studies using animal models and explanted human lung tissue have demonstrated that inhibition of NE attenuates pulmonary hypertension. However, there has been little investigation into neutrophil function in PAH patients even though their azurophilic granules are the main physiological reservoir of NE. We investigated neutrophil phenotypes in patients with PAH versus healthy controls, with a focus on neutrophil degranulation.

Methods Neutrophils were isolated from venous blood of PAH patients and healthy controls (HC) and treated with LPS; viability was assessed at 20 hours by morphology. Cell surface receptor expression was determined by flow cytometry. To evaluate degranulation, neutrophils were treated with priming agents, platelet activating factor (PAF, 1 μ M) or tumour necrosis factor- α (TNF, 20 ng/ml), and subsequently stimulated with N-formylmethionyl-leucyl-phenylalanine (fMLP; 100 nM). NE release was measured by ELISA and released NE activity and myeloperoxidase (MPO) activity were determined by

fluorogenic (Enzchek) and colorimetric (o-dianisidine oxidation) assays respectively.

Results Neutrophil apoptosis 20 hours following stimulation with LPS was significantly lower in PAH ($25.4\% \pm 2.2$, $n=12$) versus HC samples ($44.9\% \pm 4.7$, $n=9$), $p<0.001$. There were no differences in TLR2 or TLR4 expression between PAH and HC neutrophils. PAH neutrophils released greater amounts of NE following stimulation (e.g., TNF- α priming: PAH 675.2 ng/ml ± 77 vs. HC 277 ng/ml ± 18.4 , $p<0.0001$) but there was no increase in NE activity in the same supernatants, nor any difference in released MPO activity compared to healthy controls.

Conclusions Our Results indicate that neutrophil phenotype is altered in PAH, with a prolonged lifespan in response to a pro-inflammatory stimulus and increased release of NE. However, we did not detect a corresponding increase in NE activity, suggesting a concomitant increase in NE inhibitor release from PAH neutrophils. The potential role of this altered neutrophil phenotype in vascular remodelling requires further investigation.

S112 CONDITIONED MEDIA FROM HUMAN PULMONARY ARTERIAL ENDOTHELIAL CELLS TREATED WITH HEPCIDIN OR HAEMOGLOBIN CAUSE PROLIFERATION AND MIGRATION OF HUMAN PULMONARY ARTERY SMOOTH MUSCLE CELLS

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Introduction Pulmonary arterial hypertension (PAH) is characterised by vascular remodelling of pulmonary arterioles. Disrupted iron homeostasis including subclinical haemolysis are implicated in PAH, although exact mechanisms remain unknown. Interactions linked to altered iron handling consequent to release of mediators by human pulmonary artery endothelial cells (hPAECs) and/or human pulmonary artery smooth muscle cells (hPASMCs) may be important in this regard.

Objectives This study explored proliferative and migratory responses in hPASMCs linked to the primary exposure of hPAECs to indices of dysregulated iron homeostasis i.e., hepcidin or haemoglobin.

Methods hPAECs were challenged with haemoglobin (10 μ M) or hepcidin (100–1000 ng/mL). Proliferation and migration of hPASMCs were analysed using xCELLigence RTCA instrument by measuring the changes in cell index/impedance.

Results Novel findings demonstrate that media conditioned with hepcidin for 48 hour from hPAECs increased proliferation of hPASMCs by 24% (95% CI 0.256–47.9, $p<0.05$) after 48 hour of treatment. Additionally, 24 hour conditioned media from both hepcidin and haemoglobin treated hPAECs caused 3.27 and 2.28 fold increases in migration respectively in hPASMCs.

Conclusion These findings highlight a direct role for variant iron homeostasis in hPAECs which is linked to subsequent functional responses in hPASMCs of importance to vascular remodelling. These studies may provide novel insights regarding mechanisms for haemoglobin and hepcidin driven proliferative and migratory responses of relevance to PAH.

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.

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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,