whereas TXAS and COX-2 expression was markedly induced by CSE and hypoxia, either individually or in combination in both hPASMCs and hPAECs. ET-1 expression was increased by CSE and hypoxia in hPAECs. Interestingly, ET-1 was up-regulated by hypoxia, but reduced by CSE, with a net increase when both were combined in hPASMCs.

Conclusion These findings support our hypothesis that CS and hypoxia can cause an imbalance between excessive vasoconstrictors and deficient vasodilators in hPASMC and hPAECs. This imbalance may eventually lead to aberrant PASM proliferation and vascular remodelling in COPD-associated PH. Further experiments are being conducted to confirm this by analysing vasoactive gene expression and mediator release in both hPASMCs and hPAECs. Our findings also pave the way for further studies on cellular functions and intervention drug effects.

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HYPERCAPNIA IMPAIRS THE ABILITY OF MESENCHYMAL STEM CELLS TO PROMOTE DISTAL LUNG EPITHELIAL WOUND REPAIR IN ARDS

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Background Alveolar epithelial cell death and denudation of the basement membrane are hallmarks in the pathophysiology of Acute Respiratory Distress Syndrome (ARDS). Successful recovery requires basement membrane re-epithelialisation. While no pharmacological therapy exists for ARDS to date, Mesenchymal Stem Cells (MSCs) demonstrate promising therapeutic potential and are being tested in early-phase clinical trials. Heterogeneity of patients with ARDS is an obstacle for further development of an MSC-based therapy. While 20% of patients with ARDS develop hypercapnia (high CO₂) as a result of lung protective ventilation, the efficacy of MSCs has never been studied in this setting. We have previously found that transfer of functional mitochondria to surrounding cells is an important mechanism of the MSC therapeutic effect. The *aims* of this study therefore were to investigate the effect of MSCs on repair of the distal lung epithelium in normocapnia and hypercapnia in an *in vitro* model of ARDS, and to assess the role of mitochondrial transfer in mediating the MSC effect.

Methods Primary, human small airway epithelial cell (SAEC) monolayers were wounded in an *in vitro* scratch assay, stimulated with cytomix (IFN-gamma, I L-1 beta, TNF-alpha), and co-cultured with MSCs in normocapnia (5% CO₂) or hypercapnia (15% CO₂). Percentage wound closure was measured at 24 hour. SAEC proliferation was assessed by Ki67 staining. Mitochondrial transfer from MSCs to SAECs was assessed by flow cytometry using MitoTracker Green dye. MSC mitochondrial membrane potential was analysed by flow cytometry using JC-1. ATP production was measured by luminescent assay.

Results Epithelial wound closure was impaired by hypercapnia. MSCs promoted epithelial wound closure in the inflammatory setting in normocapnia via enhanced migration. This reparative capacity was lost in hypercapnia. Mitochondrial transfer from

MSCs to SAECs was observed to a similar extent in normocapnia and hypercapnia. However, hypercapnia attenuated mitochondrial membrane potential and ATP production in MSCs.

Conclusion While they promote epithelial wound repair in an inflammatory environment in normocapnia, MSCs lose this ability in hypercapnia. This suggests that their therapeutic efficacy may be lost in such an environment. An inhibitory effect of hypercapnia on MSC mitochondrial function may be at least partially responsible for this effect.

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THE EFFECTS OF TGF-B AND IL-33 ON THE PRO-FIBROTIC ACTIVITY OF PRIMARY HUMAN LUNG FIBROBLASTS DURING THE DEVELOPMENT OF IPF

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Introduction Idiopathic pulmonary fibrosis (IPF) is a debilitating interstitial lung disease with a poor prognosis and limited treatment options. It is a chronic, progressive, condition characterised by the excessive deposition of extracellular matrix by fibroblasts. Transforming growth factor-β (TGF-β) is central to this process and is regarded as a key pro-fibrotic mediator in IPF. Recently, emerging evidence suggests that the cytokine interleukin-33 (IL-33) may also be important in the development of IPF. However, the cellular and molecular mechanisms by which IL-33 promotes fibrosis are unknown. In particular, whether TGF-β and IL-33 have independent pro-fibrotic effects on fibroblasts remains unclear.

Methods Primary human lung fibroblasts (HLFs) from non-IPF and IPF patients were treated with either 2 ng/ml TGF-β or 10 ng/ml IL-33. The levels of *IL6* and *IL8* mRNA were used as readouts of IL-33-mediated NF-κβ activation whilst *ACTA2* and *COL1A1* mRNA were used as readouts of fibrosis. mRNA levels were measured by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR). Finally, the levels of IL-33, and its receptor ST2, were measured by qRT-PCR to assess mRNA expression and western blotting to measure protein.

Results Stimulation with TGF-β for 8 hours induced statistically significant increases in *IL33* gene expression by both non-IPF and IPF HLFs. Moreover, TGF-β stimulation of HLFs from patients with and without IPF induced IL-33 protein as assessed by immunoblotting. To assess the functional consequence of TGF-β induced IL-33 from fibroblasts, the effect of recombinant IL-33 on HLFs was determined. Increasing concentrations of IL-33 failed to stimulate *IL6*, *IL8*, *ACTA2* and *COL1A1* gene expression by either IPF or non-IPF fibroblasts. To understand the lack of IL-33 responsiveness in HLFs, ST2 levels were measured and neither ST2 mRNA or protein were detectable in IPF, or non-IPF, HLFs.

Conclusion TGF-β increases IL-33 production by HLFs, however fibroblast-derived IL-33 does not act directly on fibrotic fibroblasts as they fail to express the receptor required for IL-33 responsiveness. Therefore, the pro-fibrotic effects of IL-33 are likely mediated via alternative cell types.