processing before being secreted as the mature form, but several pathogenic *SFTPC* mutations associated with familial interstitial lung disease impede this process. Mutations in the C-terminal BRICHOS domain of SFTPC (ΔEx4 and L188Q) lead to retention of the protein within the endoplasmic reticulum (ER), while other mutations (e.g. I73T) cause SFTPC mis-trafficking.

Methods To study these mutants *in vivo* in a genetically tractable organism, we generated lines of *Drosophila melanogaster* expressing wild type or mutant human SFTPC. The transgenic proteins could be tagged with green fluorescent protein (GFP) to facilitate *in vivo* visualisation. These fusion proteins were expressed under the control of tissue-specific drivers. Components of the ER associated degradation (ERAD) machinery or of the autophagy pathway were depleted in those tissues by RNA interference. Lines expressing an ER stress reporter or autophagy reporter were used as readouts for these phenomena.

Results Expression of the BRICHOS mutants ΔEx4 and L188Q led to the progressive deposition of protein aggregates when expressed in the fly eye. In contrast, the I73T mutant accumulated in a more diffuse distribution. When expressed in the larval salivary gland, the BRICHOS mutants where retained within the cell, in contrast to the wild type protein that was trafficked to the cell surface. The I73T mutant showed low-level cell surface and weak intracellular fluorescence. Depletion of the ERAD E3 ubiquitin ligase Hrd1 or its associated E2 ligases failed to affect mutant protein levels arguing against an important role of ERAD in the degradation of SFTPC in this model. In contrast, inhibition of autophagy by depletion of Atg8 enhanced the accumulation of L188Q SFTPC. Accordingly, robust activation of autophagy was detected in L188Q SFTPC-expressing tissue. Interestingly, ER stress was not detected.

Conclusion In a *Drosophila* model of hSFTPC trafficking, autophagy was the major degradation pathway for L188Q mutant SFTPC.

S63 HUMAN MESENCHYMAL STROMAL CELL (HMSC) REGULATION OF HUMAN MACROPHAGES IN *IN VITRO* MODELS OF THE ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

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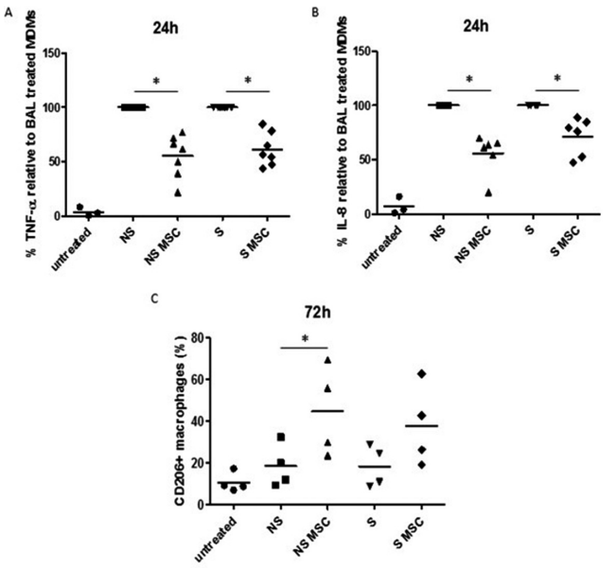
Background Currently there is no effective therapy which targets the mechanisms underlying the development of ARDS. MSCs present a promising candidate therapy and are being tested in clinical trials for ARDS however their mechanisms of effect in ARDS are not fully understood. Since the alveolar macrophage is key to orchestrating the alveolar inflammatory response, it was hypothesised that hMSCs induce an anti-inflammatory M2-like phenotype in human macrophages. The aim of this study therefore was to determine the effect of MSCs on macrophage phenotype and function and to elucidate the mechanisms of these effects.

Methods Using an *in vitro* non-contact co-culture system, human MSCs and human monocyte-derived-macrophages (MDMs) were stimulated with *E.coli* lipopolysaccharide (LPS). Cytokine and marker expression profiles were examined using ELISAs, multiplex and flow cytometry. Phagocytic capacity of MDMs was measured using fluorescent *E.coli* bioparticles by flow cytometry. For additional clinical relevance, the ARDS microenvironment was mimicked by using bronchoalveolar lavage fluid (BALF) obtained from patients with ARDS to examine the effect of MSCs.

Results MSCs suppress the production of both pro-inflammatory and anti-inflammatory cytokines by MDMs stimulated with LPS. MSCs increase expression of M2 markers CD163 and CD206 and have no effect on M1 markers CD80 and ICAM-1. Importantly, in spite of the immunosuppressive effect on macrophages, MSCs increase their phagocytic capacity. MSC effects on cytokine secretion and marker expression were maintained in the presence of BALF from patients with ARDS (Figure 1).



hMSCs suppress the pro-inflammatory response of human MDMs stimulated with BALF from patients of ARDS and induce an M2-like macrophage phenotype

Abstract S63 Figure 1 MSCs decrease secretion of pro-inflammatory cytokines TNF-α (A) and IL-8 (B) and increase expression of M2 macrophage marker CD206 (C) by MDMs stimulated with BALF from non-septic (NS) or septic (S) patients of ARDS. (A + B, n = 3–7, Kruskal Wallis *p < 0.05) (C, n = 4, ANOVA *p < 0.05)

Conclusions Human bone marrow-derived MSCs induce an M2-like phenotype and suppress cytokine secretion in primary human MDMs stimulated with LPS or ARDS patient BALF. Importantly, these effects are coupled with augmentation of macrophage phagocytosis which may be important in the clearance of bacteria and apoptotic cells. Uncovering the paracrine mechanisms responsible for the MSC effects on human macrophages remain the focus of ongoing work.

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S64 ALVEOLAR EPITHELIAL TYPE II CELL EXPRESSION OF VEGF-AXXXA IS CRITICAL FOR DEVELOPMENT OF IDIOPATHIC PULMONARY FIBROSIS (IPF): AN ANTI-FIBROTIC ROLE FOR VEGF-AXXXB ANTI-ANGIOGENIC ISOFORMS?

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Introduction VEGF has been implicated in the development of IPF. Alternative splicing of the VEGF-A gene generates numerous isoforms. The differential effects of these isoforms, in particular the VEGF-A_{xxx}b family, thought to have several opposing functions to the conventional family of isoforms (VEGF-A_{xxx}a), have not been considered.

Hypothesis

- The balance of VEGF-A_{xxx}a:VEGF-A_{xxx}b isoform expression is important in the pathogenesis of IPF.
- VEGF-A_{xxx}b isoforms may be protective against the formation of pulmonary fibrosis (PF).

Methods Normal and IPF lung lysates (n = 5) were analysed by western blotting (WB), and ELISA using an antibodies specific for PanVEGF-A and VEGF-A_{xxx}b isoforms.

The Bleomycin (BLM)-induced model of PF was used in conjunction with two transgenic (TG) mouse models, developed to explore the role of ATII-derived VEGF in the development of PF: 1) a conditionally inducible, ATII-specific, VEGF knock-out mouse (STCLL mice) and 2) a TG mouse over-expressing VEGF-A_{xxx}b in ATII cells (MMTV-$VEGF_{165}$b).

To explore the therapeutic potential of VEGF-A_{xxx}b in PF, wild-type mice were administered intraperitoneal (IP) injections of VEGF-A_{165}b, commencing 10 days after BLM challenge.

In all experiments fibrosis was assessed histologically using Masson's Trichrome, with blinded scoring of tissue sections.

Results By WB (n = 3) and ELISA (n = 5) there was no significant difference in PanVEGF-A expression between normal and IPF lung homogenates (*t*-test, $p > 0.05$). In contrast, VEGF-A_{xxx}b expression was significantly increased in these same IPF samples compared to control, by ELISA (*t*-test, $^{****}p < 0.0001$) and WB (Densitometry: *t*-test, $^{*}p < 0.05$).

Specific deletion of VEGF-A from ATII cells of mice ameliorated the development of BLM-induced pulmonary fibrosis (n = 5, Lung fibrosis score: ANOVA with Holm's Sidak $^{**}p < 0.01$). Over-expression of VEGF-A_{xxx}b in ATII cells also ameliorated the development of pulmonary fibrosis (n = 6, Lung fibrosis score: ANOVA with Holm's Sidak $^{***}p < 0.001$). Furthermore, delivery of VEGF-A_{165}b, specifically during the fibrotic phase of the BLM model, also attenuated lung fibrosis development (n = 6, Lung fibrosis score: ANOVA with Holm's Sidak $^{*}p < 0.05$).

Conclusion Changes in the bioavailability of ATII cell-derived VEGF-A, namely the ratio of VEGF-A_{xxx}a:VEGF-A_{xxx}b, appear critical to the development of pulmonary fibrosis. This data suggests that more a targeted approach to anti-VEGF-A therapy in IPF should be explored.

S65 THE ROLE OF SRC KINASE IN INSPIRATORY RESISTIVE BREATHING-INDUCED PULMONARY INFLAMMATION

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Introduction and objectives Inspiratory resistive breathing (IRB), a hallmark of obstructive pulmonary diseases, is characterised by large negative intrathoracic pressures. IRB is shown to induce pulmonary inflammation in previously healthy rats. Src is a multifunctional kinase that is activated by phosphorylation upon mechanical stress and plays a significant role in inflammatory processes. The aim of our study was to investigate the role of Src in IRB-induced pulmonary inflammation.

Methods Anaesthetised, tracheostomised rats were breathing spontaneously through a 2-way non rebreathing valve. The inspiratory port was connected to a resistance, setting peak tidal tracheal pressure at 50% of maximum (IRB). Quietly breathing animals served as controls. After 6 h of IRB, the mechanics of the respiratory system were assessed with the forced oscillation technique. Bronchoalveolar lavage (BAL) was performed to measure total and differential cell count and total protein levels. Phosphorylation of Src and ERK was detected in lung tissue samples by Western blot analysis at 30 min, 3 and 6 h of IRB. The Src inhibitor PP2 was administered intraperitoneally (1 mg/kg), 30 min prior to IRB, in a subgroup of animals.

Results After 6 h of IRB, increased tissue elasticity was measured, compared to control. Increased BAL cellularity was also found (2-fold increase to control), due to raised numbers of both macrophages and neutrophils. Total protein levels were elevated in BAL fluid. Src activation was detected at 30 min of IRB (3-fold increase to control), while ERK was phosphorylated at 3 and 6 h. Inhibition of Src kinase attenuated the increase in tissue elasticity after 6 h of IRB. Following inhibition of Src kinase, the total cell number after 6 h of IRB was not increased compared to control. Neither macrophage nor neutrophil count was elevated after 6 h of IRB, following Src inhibition. Total protein levels were not altered by Src inhibition. Src inhibition attenuated the activation of ERK only at 3 h of IRB.

Conclusion Src kinase activation partly mediates IRB-induced pulmonary inflammation.

S66 CAFFEINE INHIBITS TGFβ ACTIVATION BY EPITHELIAL CELLS, INTERRUPTS FIBROBLAST RESPONSES TO TGFβ, AND REDUCES PULMONARY FIBROSIS IN *EX VIVO* PRECISION-CUT LUNG SLICES

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Caffeine (1, 3, 7-tri-methylxanthine) is a common food additive found naturally in many products. It is a non-selective competitive antagonist of G-protein coupled adenosine receptors and can inhibit phosphodiesterases. Caffeine has anti-fibrotic effects in the liver and increased caffeine consumption has been associated with reduced liver fibrosis in patients with chronic hepatitis C infection. The effect of caffeine on pulmonary fibrosis has not been investigated, however, it has been shown to inhibit TGFβ-induced Smad signalling in epithelial cells. This study aimed to investigate the anti-fibrotic effects of caffeine in the lung using lung epithelial cells, fibroblasts and an *ex vivo* precision-cut lung slice (PCLS) model of fibrosis.

Immortalised human bronchial epithelial cells (iHBECs) and primary human lung fibroblasts from were used. TGFβ activation was assessed using an *in vitro* TGFβ reporter cell assay and assessment of phosphorylated Smad2. Expression of pro-fibrotic genes was assessed by quantitative polymerase chain reaction. Proliferation of fibroblasts was assessed by brdU incorporation assay. Finally, the effect of caffeine on established lung fibrosis was investigated *ex vivo* using PCLS. Mice were instilled with saline or 60 IU bleomycin and PCLS obtained after 28 days. PCLS were treated with increasing concentrations of caffeine for five days prior to measurement of collagen by high-performance liquid chromatography. Viability of the PCLS following caffeine treatment was assessed by MTT assay.