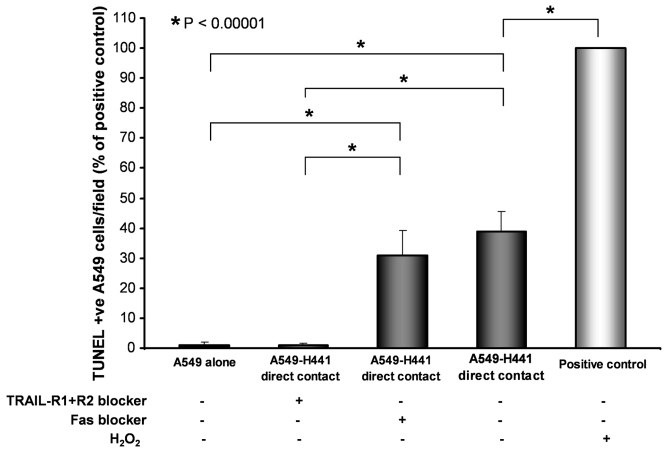

Abstract S138 Figure 1 H441 direct contact induced apoptosis in A549 cells through TRAIL-R1+R2 (10 µg/ml each) blockade. Fas blocker (10 µg/ml) failed to block direct cell contact- induced apoptosis. In positive control, apoptosis was induced with 200 µM H2O2. Negative control was represented by A549 cells cultured alone in monolayer.

Methods Using an in vitro wound repair model we explored the interaction of human Clara cells (H441 cell line) and type II AEC (A549 cell line). A transwell co-culture system was developed to determine the direct contact effect of densely populated Clara cells on wounded AEC monolayers.

Results In serum-free media, lone H441 cell wound repair was higher than equivalent A549 cells, despite the fourfold slower doubling time of H441 cells. Serum-free conditioned media obtained from un-wounded and wounded H441 monolayers did not show any significant influence on A549 wound repair. However, in a direct contact co-culture A549-H441 cell model significant inhibition of A549 wound repair ($p<0.005$) was observed. Interestingly, H441 migration into the injured A549 layer was seen after 24 h; with a significant proportion of migrated H441 cells found at the wound margins. Coupled to this migration we observed a 50% reduction in A549 cell number at the wound margins. TUNEL assay detected about 40% A549 apoptosis in juxta-wound monolayers in A549-H441 direct contact ($p<0.00001$). This direct contact-induced apoptosis was significantly blocked by TRAIL-R1 and R2 combined receptor blockers ($p<0.00001$); whereas, Fas blocker failed to block this apoptosis.

Conclusion In summary, direct contact of H441 cells induces apoptosis in the A549 monolayers through a TRAIL-dependent mechanism which disrupts wound margin integrity, inhibiting wound repair. This novel observation warrants further exploration of the role of Clara cell-alveolar epithelial cell interaction within the context of aberrant wound repair associated with chronic fibrotic lung disorders.

S139 THE K^+ CHANNEL KCa3.1 IS A NOVEL TARGET FOR THE TREATMENT OF IDIOPATHIC PULMONARY FIBROSIS

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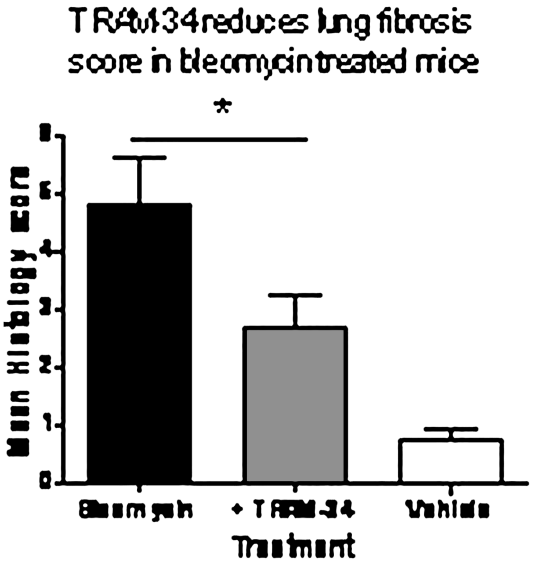
Introduction and objectives Idiopathic pulmonary fibrosis (IPF) is common, largely unresponsive to treatment with a median survival of 3 years. New therapies are urgently required. IPF is characterised by proliferation of pulmonary mesenchymal cells through epithelial mesenchymal transition, resident fibroblast proliferation and circulating fibrocyte recruitment. We have previously demonstrated that the potassium channel $K_{Ca}3.1$ regulates lung mesenchymal cell proliferation, is up-regulated by TGFβ, an important driver of IPF, and is present in fibrocytes in peripheral blood. We tested the hypotheses that $K_{Ca}3.1$ is up-regulated in IPF using the bleomycin-induced pulmonary fibrosis murine model and that $K_{Ca}3.1$ inhibition reduces pulmonary fibrosis.

Methods Prophylactic (Day -3) and daily thereafter, sub-cutaneous TRAM-34, a specific $K_{Ca}3.1$ inhibitor, was administered to C57BL/6 mice later exposed to nasal bleomycin (Day 0) and culled on day +21. Mice exposed to PBS or bleomycin acted as negative and positive controls. The primary endpoint was histological fibrosis score. Inflammation was assessed by bronchoalveolar lavage. Collagen deposition and $K_{Ca}3.1$ expression were assessed by Masson's trichrome staining and qPCR.

Results Bleomycin-induced pulmonary fibrosis characterised by thickened alveolar septae, architectural destruction and collagen deposition. Co-administration of TRAM-34 significantly reduced pulmonary fibrosis (Modified Ashcroft's score +/− SEM: 4.8+/−0.8 bleomycin group vs 2.6+/−0.6 TRAM-34 group: $p=0.02$). Bleomycin increased lung $K_{Ca}3.1$ (55-fold versus PBS control) and collagen Iα mRNA (fourfold) expression ($n=3$ in each case). Mice receiving bleomycin lost more weight (2.39 vs 0 g) and had greater mortality than those co-administered TRAM-34. BAL cellularity did not differ between the groups. Collagen staining was reduced in the TRAM-34 group.

Conclusions $K_{Ca}3.1$ expression is increased in a model of pulmonary fibrosis and inhibition with TRAM-34 significantly improves pathological outcome. The mechanism is likely to involve the modulation of cells involved in the fibrotic process. Previous clinical studies have shown KCa3.1 inhibition to be safe in humans and our study provides a rationale for a clinical trial of $K_{Ca}3.1$ inhibitors in human IPF.



Abstract S139 Figure 1

S140 THE ROLE OF TRANSFORMING GROWTH FACTOR-β ACTIVATED KINASE-1 (TAK-1) IN THE DEVELOPMENT OF AIRWAY FIBROSIS

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Introduction and aims Fibrotic disorders of the lung are characterised by an increase in fibroblast numbers and excessive deposition of extra

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