at surgery for carcinoma. Myofibroblasts grown in vitro were characterised by Western blot, immunofluorescence and RT-PCR to determine $K_{Ca}3.1$ channel expression. Patch clamp electrophysiology was used to demonstrate functional $K_{Ca}3.1$ channels. Wound healing and proliferation assays were performed using two specific $K_{Ca}3.1$ blockers (TRAM-34, ICA-17043 [Senicapoc]). Both NFC and IPF myofibroblasts expressed $K_{Ca}3.1$ channel mRNA and protein. Using the $K_{Ca}3.1$ channel opener 1-EBIO, $K_{Ca}3.1$ ion currents were elicited in 59% of NFC and 77% of IPF myofibroblasts tested (p=0.0411). These currents were blocked by TRAM-34 (200 nM). The 1-EBIO-induced currents were significantly larger in IPF cells compared to NFC cells (p=0.0124). Basic fibroblast growth factor (bFGF) (10 ng/ml) significantly increased the frequency of $K_{Ca}3.1$ currents across groups (p=0.0046). Similarly bFGF stimulation significantly increased myofibroblast wound healing (p=0.002). Following $K_{Ca}3.1$ blockade bFGF-stimulated wound healing was attenuated dose-dependently. Thus at the 48 h time-point wound healing was reduced by 22.2±11.1% and 27.3±9.5% for TRAM-34, 20 nM and 200 nM respectively (p=0.0467 across groups), and reduced by 16.9±8.1% and 24.4±6.6% for ICA-17043, 10 nM and 100 nM respectively (p=0.0076 across groups). $K_{Ca}3.1$ blockade had no effect on myofibroblast proliferation. We show for the first time that human lung myofibroblasts express the $K_{Ca}3.1$ K^+ channel. $K_{Ca}3.1$ currents are larger and more frequently present in cells from patients with IPF, and functional channel expression is increased by pro-fibrotic growth factors. $K_{Ca}3.1$ inhibition attenuates bFGF stimulated myofibroblast wound healing. These findings raise the possibility that blocking the $K_{Ca}3.1$ channel will inhibit pathological myofibroblast function in IPF, and thus offer a novel approach to IPF therapy.

T6 TRAIL IS A POTENTIAL NOVEL THERAPEUTIC TARGET IN PULMONARY ARTERIAL HYPERTENSION

doi:10.1136/thoraxjnl-2011-201054a.6

[1]A G Hameed, [1]N D Arnold, [1]J Pickworth, [1]J C Chamberlain, [1]C M H Newman, [2]D C Crossman, [1]S E Francis, [1]A Lawrie. *[1]University of Sheffield, Sheffield, UK; [2]University of East Anglia Medical School, Norwich, UK*

Introduction and Objectives Pulmonary Arterial Hypertension (PAH) is a life threatening disease characterised by the progressive narrowing and occlusion of small pulmonary arteries, driven by the dysregulated growth of vascular cells. Current therapies are unable to reverse PAH and so identifying key pathways in disease pathogenesis should permit the development of more targeted therapeutics. The cytokine, Tumour Necrosis Factor (TNF)-Related Apoptosis-Inducing Ligand (TRAIL) induces EC apoptosis and SMC proliferation in the systemic circulation, but hitherto has not been studied in PAH. We recently determined that TRAIL is a mitogen for human PA-SMCs in-vitro and was associated with pulmonary vascular lesions in humans and rodent models. We thus hypothesised that TRAIL is an important mediator in the pathogenesis of PAH and now describe the potential therapeutic benefits of targeting TRAIL in-vivo using two animal models.

Methods To test whether blocking TRAIL could prevent the development of PAH, an anti-TRAIL antibody was delivered via osmotic mini-pump coincident with the induction of PAH in the MCT rat model. We also tested whether genetic deletion of TRAIL (ApoE−/−/TRAIL−/− mice) would confer protection to diet-induced PAH. To test whether inhibiting TRAIL could reverse established disease we again treated both models with an anti-TRAIL antibody starting from day 21 in the rat and 8 weeks in the ApoE−/− mouse. Phenotyping included echocardiography, closed chest cardiac catheterisation followed by immuno-histological and biochemical analyses of the lung.

Results Antibody blockade (MCT) and genetic deletion (ApoE−/−) of TRAIL prevented the development of PAH in both models. Interestingly a PAH disease phenotype was restored in ApoE−/−/TRAIL−/− mice by the administration of recombinant TRAIL. In rodents with established PAH, an anti-TRAIL antibody, significantly increased survival and reduced pulmonary vascular remodelling in the fatal rat MCT model ($p<0.05$ cf control). In the murine model, an anti-TRAIL antibody treatment reversed both haemodynamics (RVSP 27 mm Hg vs 88 mm Hg, $p<0.001$) and pulmonary vascular remodelling.

Conclusions Our preclinical investigations are the first to determine the importance of TRAIL to disease pathogenesis and highlight its potential as a novel and rational target to direct future translational therapies for PAH.