Emerging evidence suggests a role for PI3K/mTOR signalling in the pathology of organ fibrosis. The aim of this study was to delineate PI3K/mTOR signalling in response to TGF-β1 stimulation of primary human lung fibroblasts (HLFs), and to investigate the role of this pathway in TGF-β1 mediated myofibroblast differentiation and collagen synthesis.

A time-course of SMAD 2/3 and Akt (Ser473) phosphorylation, the major downstream effectors of the PI3K/mTOR pathway, was constructed to assess TGF-β1 induced signalling kinetics in HLFs. TGF-β1 (1 ng/ml) induced rapid phosphorylation of SMAD2/3, peaking at 1 h, followed by Akt phosphorylation which peaked 12 h after initial stimulation. Maximal expression of ACTA2 and COL1A1 was observed 36 h after TGF-β1 stimulation, correlating with the delayed time-course of Akt phosphorylation.

To investigate the role of the PI3K/mTOR pathway in TGF-β1 induced myofibroblast differentiation and collagen gene expression, HLFs were treated with pharmacological titrations of potent pathway inhibitors. Maximal Akt signalling and expression of ACTA2 and COL1A1 were significantly inhibited by a dual PI3K/mTOR inhibitor, while SMAD phosphorylation was unaffected. Treatment with an ATP competitive mTOR inhibitor also resulted in significantly reduced Akt phosphorylation and expression of ACTA2 and COL1A1, in response to TGF-β1. In contrast, treatment of HLFs with either an allosteric or ATP competitive Akt inhibitor showed no inhibitory effect on TGF-β1 induced gene expression.

These data suggest PI3 kinase/mTOR signalling is an important component in TGF-β1 induced αSMA and collagen gene expression and that an mTOR dependent, Akt independent pathway mediates this functional response in primary HLFs.

**Abstract S137 Figure 1** The effect of VEGF165b on the development of murine BLM-induced pulmonary fibrosis.

### Methods

Human lung sections and BALF were used to quantify isoform expression in the IPF lung and were compared to controls (ELISA and IHC). Exemplified ‘normal’ (NF) and ‘fibrotic’ (FF) fibroblasts were grown in culture with subsequent total RNA and cell lysate extraction (qPCR and WB). Wild-type mice were administered bleomycin (BLM) then received bi-weekly therapeutic intraperitoneal (IP) injections of rhVEGF165b (from day 10). Fibrosis was assessed histologically (Mason’s Trichrome and Lung fibrosis score).

### Results

In the IPF lung, the alveolar epithelium was the most prominent site for total VEGF (PanVEGF isoforms) but also for VEGF165b (n = 10). Additional staining was noted in fibroblasts and lung inflammatory cells. Alveolar and fibroblastic cells in the least fibrotic areas of the IPF lung expressed significantly less VEGF165b than severely fibrotic areas (p < 0.001, n = 10). Examination of IPF BALF by ELISA revealed that total VEGF expression was significantly lower compared to control (IPF: 18.04 pg/ml +/- 6.13 n = 15, CTRL 85.72 pg/ml +/- 17.08 n = 13), whilst VEGF165b could not be detected in identical samples.

Explanted NF and FF express comparable quantities of VEGF165 and VEGF165b isoforms at the mRNA and protein level. Rh VEGF165 increases the mRNA expression of fibronectin (p < 0.001, n = 4) an effect not seen following the administration of rhVEGF165b.

Administration of rhVEGF165b to mice attenuated the development of BLM-induced pulmonary fibrosis (Mason’s Trichrome (Figure 1) and lung fibrosis score (mean score: BLM alone 41.20 vs VEGF165b 30.67, p < 0.001, n = 6 per group)).

### Conclusion

Differential expression of VEGF165 and VEGF165b isoforms occurs in the IPF lung. In vitro, recombinant proteins appear to have differential effects on ECM synthesis and in vivo attenuate the formation of pulmonary fibrosis. A mouse overexpressing VEGF165b in the lung has been developed to study this concept in greater detail.

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**NANODIAMOND DELIVERY OF VASCULAR ENDOTHELIAL GROWTH FACTOR PROMOTES FETAL LUNG DEVELOPMENT IN A RAT MODEL OF CONGENITAL DIAPHRAGMATIC HERNIA**

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New insights in skeletal muscle wasting and weakness

A PARADOXICAL RISE IN RECTUS FEMORIS MYOSTATIN (GDF-8) AND GDF-15 IN RESPONSE TO NEUROMUSCULAR ELECTRICAL STIMULATION IN CRITICAL CARE

Introduction Neuromuscular electrical stimulation (NMES) is widely used in rehabilitation and muscle disease. Recently there is increasing interest in its use as a prevention and treatment for intensive care unit acquired weakness (ICUAW). ICUAW is a common and often devastating disease resulting as a consequence of critical illness. The molecular mechanisms are not understood, however early mobilisation and rehabilitation are to date the most effective treatments. NMES has been shown to help prevent muscle wasting in some clinical studies in the ICU setting, however the evidence is inconclusive. We hypothesised that the NMES of a single leg in critical care patients would be associated with reduced muscle wasting and down regulation of molecular pathways involved in muscle breakdown. Specifically myostatin (GDF-8), a potent negative regulator of muscle mass, and GDF-15, a potential novel driver of muscle atrophy.

Methods We conducted a single-blinded, single leg, contralateral controlled trial of NMES in patients admitted to a specialist cardiothoracic ICU. Patients were recruited prior to elective high-risk cardiac surgery or during ICU admission. Baseline bilateral rectus femoris cross sectional area (RFcsa) was measured by ultrasound and rectus femoris biopsies were taken. 2 × 1 hour sessions of NMES were then conducted for 1 week and ultrasound and biopsies were repeated. Biopsy specimens were examined for mRNA expression of genes of interest and results analysed in paired analysis relative to baseline. (NCT013221320).

Results 12 patients completed the study protocol. Myostatin and GDF-15 mRNA expression were both significantly elevated in NMES legs compared to baseline (p = 0.03 and p = 0.04 respectively), but remained unchanged in control legs. There was no significant change in RFcsa.

Discussion It is believed that NMES will have beneficial effects in the ICU setting in terms of preservation of muscle function. However it is recognised to also have potential to cause muscle damage. In the setting of sedated patients who cannot report pain or those in whom the nutritional and metabolic status of the muscle may be expected to be poor, researchers should be aware that NMES may promote muscle breakdown.