S136 MTOR SIGNALLING IS AN ESSENTIAL PATHWAY FOR TGF-β1 INDUCED αSMA AND COLLAGEN GENE EXPRESSION

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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-β_1 stimulation of primary human lung fibroblasts (HLFs), and to investigate the role of this pathway in TGF-β_1 mediated myofibroblast differentiation and collagen synthesis.

A time-course of SMAD 2/3 and Akt (Ser473) phosphorylation, the major downstream effector of the PI3K/mTOR pathway, was constructed to assess TGF-β_1 induced signalling kinetics in HLFs. TGF-β_1 (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-β_1 stimulation, correlating with the delayed time-course of Akt phosphorylation.

To investigate the role of the PI3K/mTOR pathway in TGF-β_1 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-β_1. In contrast, treatment of HLFs with either an allosteric or ATP competitive Akt inhibitor showed no inhibitory effect on TGF-β_1 induced gene expression.

These data suggest PI3 kinase/mTOR signalling is an important component in TGF-β_1 induced αSMA and collagen gene expression and that an mTOR dependent, Akt independent pathway mediates this functional response in primary HLFs.

S137 VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION IN THE IPF LUNG – A ROLE FOR ANTI-ANGIOGENIC ISOFORMS?

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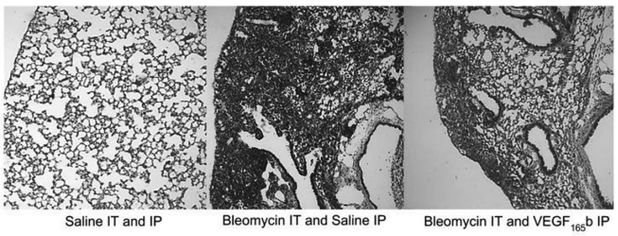
Introduction VEGF has been implicated in the pathogenesis of IPF. Differential splicing of the VEGF gene produces an alternative family of isoforms ($VEGF_{xxx}b$) that have anti-angiogenic properties, in contrast to conventional isoforms ($VEGF_{xxx}$). Currently available literature on the role of VEGF in IPF has not differentiated between these families of isoforms and thus a degree of literature re-appraisal is required.

Hypotheses

- The balance of $VEGF_{xxx}$:$VEGF_{xxx}b$ isoforms may be important in IPF pathogenesis
- $VEGF_{xxx}b$ isoforms may abrogate the development of IPF



Abstract S137 Figure 1 The effect of $VEGF_{165}b$ on the development of murine BLM-induced pulmonary fibrosis
Bleomycin was given to WT type mice (n = 6 per group) by oro-pharyngeal aspiration (Day 0). $rhVEGF_{165}b$ was administered by IP injection (1μg per mouse, bi-weekly) from days 10 to 21. Fibrosis was scored and examined histologically by Masson's trichrome staining. The development of BLM-induced fibrosis was attenuated in mice receiving $rhVEGF_{165}b$

Methods Human lung sections and BALF were used to quantify isoform expression in the IPF lung and were compared to controls (ELISA and IHC). Explanted '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 $rhVEGF_{165}b$ (from day 10). Fibrosis was assessed histologically (Masson'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 $VEGF_{165}b$ (n = 10). Addiitonal staining was noted in fibroblasts and lung inflammatory cells. Alveolar and fibrotic cells in the least fibrotic areas of the IPF lung expressed significantly less $VEGF_{165}b$ 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 $VEGF_{165}b$ could not be detected in identical samples.

Explanted NF and FF express comparable quantities of $VEGF_{xxx}$ and $VEGF_{xxx}b$ isoforms at the mRNA and protein level. Rh $VEGF_{165}$ increases the mRNA expression of fibronectin ($p < 0.001$, n = 4) an effect not seen following the administration of $rhVEGF_{165}b$.

Administration of $rhVEGF_{165}b$ to mice attenuated the development of BLM-induced pulmonary fibrosis (Masson's Trichrome (Figure 1) and lung fibrosis score (mean score: BLM alone 41.20 vs $VEGF_{165}b$ 30.67, $p < 0.01$, n = 6 per group)).

Conclusion Differential expression of $VEGF_{xxx}$ and $VEGF_{xxx}b$ 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 $VEGF_{165}b$ in the lung has been developed to study this concept in greater detail.

S138 NANODIAMOND DELIVERY OF VASCULAR ENDOTHELIAL GROWTH FACTOR PROMOTES FETAL LUNG DEVELOPMENT IN A RAT MODEL OF CONGENITAL DIAPHRAGMATIC HERNIA

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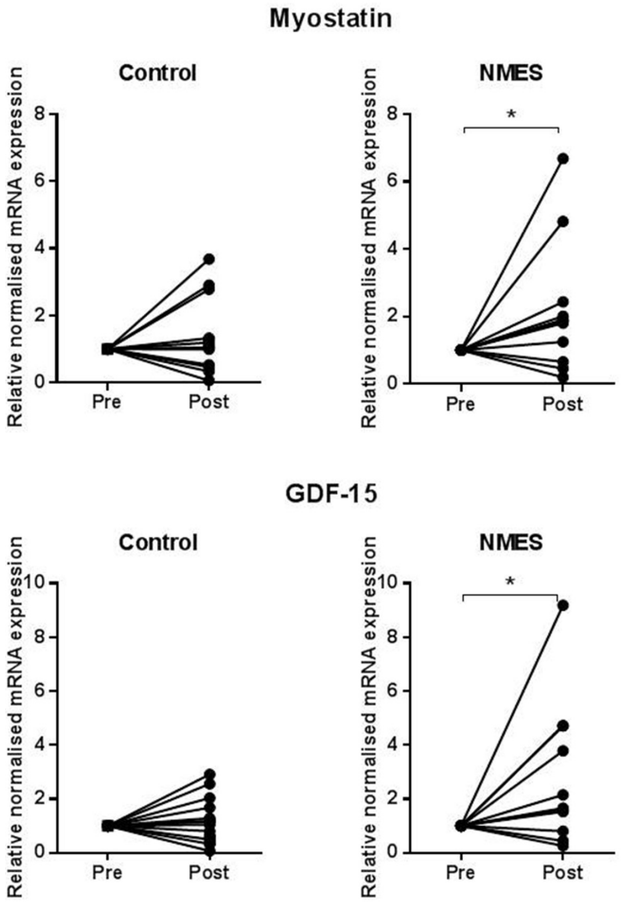
Congenital diaphragmatic hernia (CDH) is a developmental diaphragmatic anomaly resulting in pulmonary hypoplasia and consequent pulmonary hypertension and respiratory failure sequelae. Despite advances in treatment, CDH remains associated with high morbidity and mortality rates. Reduced levels of vascular endothelial growth factor (VEGF) have been implicated in CDH pathogenesis. Animal studies have shown that intrauterine VEGF replacement enhances pulmonary vascularisation and lung epithelial cell proliferation. This study aimed to deliver VEGF through the engineering of a biocompatible and slow releasing nanodiamond (ND) platform, in a rat model of CDH.

NDs were either fluorescently labelled (ND-FL) or conjugated to recombinant VEGF164 (ND-VEGF; 2 μg/mL VEGF164). Nitrofen was administered to pregnant Wistar rats at E9 (term=E22) to induce fetal CDH. At E19, maternal hysterotomy was performed, and NDs (75 μg/mL in 50 μL vehicle/saline) were administered intratracheally followed by fetal tracheal occlusion (TO). Blinded assessment of lung-to-body weight ratio (LBWR) and lung morphometric parameters was performed at E21.5 in CDH offspring.

Prenatal ND administration did not have overt adverse effects. ND-FL biodistribution indicated that NDs localised in type II pneumocytes. ND-VEGF+TO was associated with improved lung growth (LBWR: 5.9 ± 0.2%), which was greater than that observed in VEGF+TO (3.5 ± 0.4%; $p < 0.01$), vehicle+TO (3.9 ± 0.1%; $p < 0.01$), and sham surgery (2.0 ± 0.2%; $p < 0.001$) groups. Moreover, ND-VEGF+TO resulted in thinner alveolar septa (mean transection length/airspace: 18.9 ± 0.5) and increased alveolar size (mean airspace chord length: 31.4 ± 0.6) compared to other treatment groups (p).

This is the first study to show that nanoparticle-mediated prenatal delivery of VEGF induces significant lung growth in CDH and suggests that sustained cargo release is pivotal in mimicking the temporal expression of VEGF in normal lung development.



Abstract S139 Figure 1 Rectus Femoris mRNA expression of Myostatin or GDF-15 in ICU patients (n = 12) relative to baseline (pre) and following 1 week of neuromusclular electrical stimulation (NMES) or control. *$p < 0.05$ Wilcoxon paired analysis of post study comparison relative to baseline

New insights in skeletal muscle wasting and weakness

S139 A PARADOXICAL RISE IN RECTUS FEMORIS MYOSTATIN (GDF-8) AND GDF-15 IN RESPONSE TO NEUROMUSCULAR ELECTRICAL STIMULATION IN CRITICAL CARE

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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 atropy.

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 (RF_{csa}) 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. (NCT01321320).

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 RF_{csa}.

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.