S67 WNT11 INHIBITS TGFB-INDUCED EPITHELIAL-MESENCHYMAL TRANSITION IN A 3-DIMENSIONAL HUMAN LUNG TISSUE MODEL

doi:10.1136/thoraxjnl-2012-202678.073

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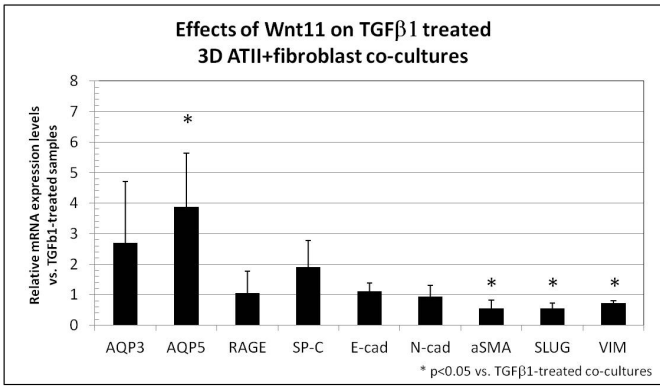
TGFβ1 has been implicated as a key effector in mediating Epithelial-Mesenchymal Transition (EMT), my ofibroblastic transdifferentiation and fibroproliferation in the fibrotic diseases of the lung. ATII cells show high differentiational plasticity: either transdifferentiation into ATI or a myofibroblastic phenotype might occur during the disease course. Factors regulating alveolar epithelial differentiation and EMT have high importance in lung regeneration therapy. The support of mesenchymal cells likely contributes to the homeostasis and regeneration of pulmonary epithelium. To study these mesenchymal effects, we constructed a 3-dimensional (3D) human tissue model of primary human ATIIs and pulmonary fibroblasts to mimic epithelial-mesenchymal interactions in the lung.

ATII cells were obtained from lung cancer resection specimens from patients with normal lung function (n=10). ATII cells in the 3D model retain more differentiated epithelial phenotype compared to conventionally cultured cells (n=4). This is indicated by the higher expression levels of Aquaporin (AQP)3 (p=0.04) and AQP5 (p=0.19), Surfactant Protein (SP)-C (p=0.11) and E-cadherin. (p= 0.37) Cells cultured in 2D showed elevated markers of EMT over time whereas 3D cultured cells had significantly lower expression of mesenchymal markers N-cadherin (p<0.01), α-Smooth Muscle Actin (αSMA, p<0.01) and the transcription factor SLUG (p<0.01). This suggests that our co-culture model is more relevant in modelling lung diseases in vitro than monolayer epithelial cultures.

We found that externally added recombinant Wnt11 (administered at 1μg/ml) inhibits phenotypic changes induced by TGFβ1 in 3D ATII+fibroblast co-cultures (n=6). Lung epithelial differentiation markers AQP3 (p=0.19), AQP5 (p=0.04), and SP-C (p=0.12) showed elevated expression levels upon the addition of Wnt11 to TGFβ1-treated cell cultures. Moreover, 1μg/ml Wnt11 blocked the EMT-inducing effects of 10ng/ml TGFβ1 in terms of the expression levels of SLUG (p=0.01), Vimentin (p<0.01) and αSMA (p=0.04).

Our findings indicate that Wnt11 – which is naturally secreted by pulmonary cells - might contribute to the homeostasis and repair of epithelial cells. Our finding that the effects of TGFβ1 can be antagonised by Wnt11 may mark Wnt11 as a potential therapeutic target in fibrotic diseases of lung.



Abstract S67 Figure 1

S68 BIOAVAILABILITY OF VEGF IN IDIOPATHIC PULMONARY FIBROSIS

doi:10.1136/thoraxjnl-2012-202678.074

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Introduction Vascular endothelial growth factor (VEGF) is both a growth and permeability factor implicated in the pathogenesis of idiopathic pulmonary fibrosis (IPF). VEGF exerts its biological effects through receptors: VEGFR-1, VEGFR-2, Neuropilin-1(NRP-1) and Neuropilin-2 (NRP-2). The presence of hypoxia and its treatment by high flow oxygen have been proposed to contribute to lung injury. VEGF gene transcription is tightly regulated by a hypoxia response element (HRE).

We hypothesised that:

1. VEGF and its receptors would be differentially expressed between normal (NF) and fibrotic fibroblasts (FF).
2. Hypoxia and hyperoxia may alter fibroblast VEGF expression.

Methods NF(n=5) and FF(n=5) (from patients with proven usual interstitial pneumonia) were extracted from lung biopsies using the explant method. VEGF receptors levels were analysed at the mRNA and protein level (qPCR and western blotting (WB)). Pan VEGF isoforms were detected at the protein level using WB and ELISA. Fibroblasts were grown in culture for 24 hours in normoxic, hyperoxic (90% O_2) and hypoxic conditions (21% O_2 with $CoCl_2$).

Results Both NF and FF expressed VEGFR1, VEGFR2, NRP-1 and NRP-2. No significant difference was detected in receptor expression at mRNA or protein level, but a trend towards reduced protein expression of VEGFR1 and NRP-2 in FF vs NF was observed. FF expressed significantly more total VEGF than NF by ELISA (Figure 1): (NF 180.5 pg/ml vs FF 332.0 pg/ml, p=0.01) and by WB (normalised densitometry value: NF 116 vs 162, p=0.046). Moreover, a significant increase in VEGF expression was observed in both normal and fibrotic fibroblasts in response to hypoxic growth conditions (NF 205.7 pg/ml vs 1382.0 pg/ml, p=0.05, FF 394.6 pg/ml vs 1113 pg/ml.0, p=0.01). A trend towards increased VEGF expression was also seen in FFvsNF exposed to hyperoxic conditions.

Conclusions Differential expression of VEGF between NF and FF suggests a potential role in the development of IPF. The observation that hypoxia and possibly hyperoxia may alter VEGF bioavailability has implications in the use of oxygen therapy in the management of this disease.

S69 TRANSCRIPTIONAL MECHANISMS REGULATING EXPRESSION OF THE AVB6 INTEGRIN IN IPF

doi:10.1136/thoraxjnl-2012-202678.075

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TGF-β is fibrogenic cytokine implicated in the pathogenesis of idiopathic pulmonary fibrosis (IPF). The integrin αVβ6 can activate TGF-β, and dysregulation of this pathway is thought to play a role in the pathogenesis of pulmonary fibrosis. TGF-β induces αVβ6 integrin expression and aVb6 integrins are upregulated in fibrotic regions of lungs from patients with IPF. This raises the possibility that dysregulation of a self-regulating feedback loop may promote IPF. This study aims to investigate the mechanisms involved TGF-β-induced β6 expression and how this may be dysregulated in IPF.

Using QPCR and flow cytometry we assessed expression of the integrin β6 (ITGB6) in lung epithelial cells. An ITGB6 promoter-luciferase construct and truncated mutants were used to identify

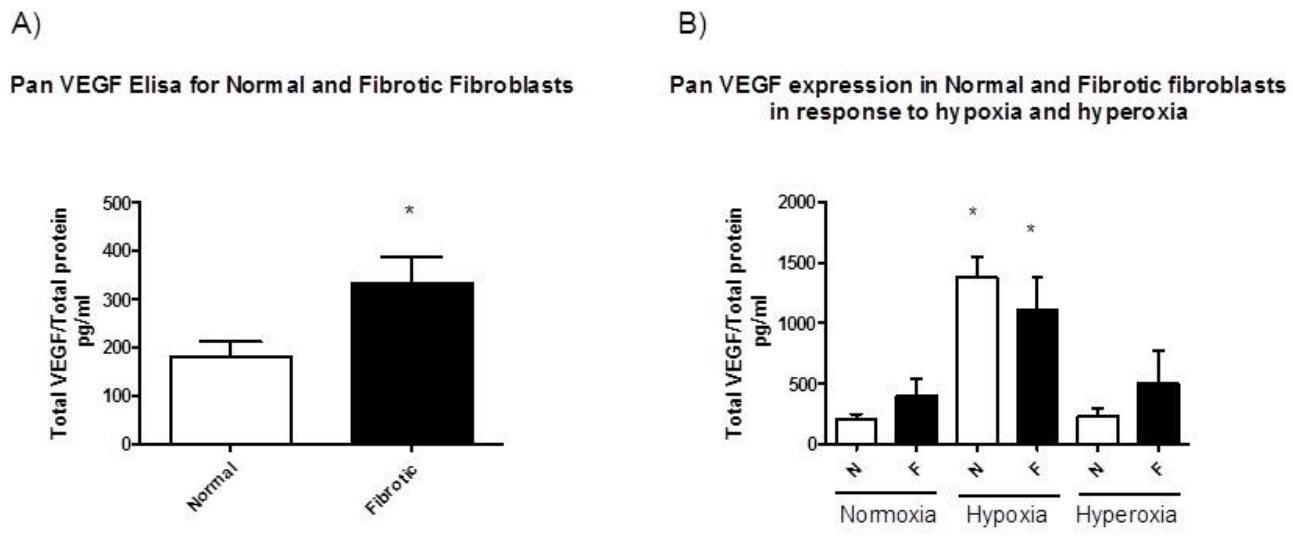

Figure 1.
A) Pan VEGF expression in Normal and Fibrotic fibroblasts (p = 0.01)
B) Pan VEGF expression in Normal and Fibrotic fibroblasts in response to normoxic, hypoxic and hyperoxic growth conditions (NF normoxia vs hypoxia: p = 0.05, FF normoxia vs hypoxia: p = 0.01)

Abstract S68 Figure 1

the regulatory region of the promoter. Dominant negative (dn) constructs were used to assess the role of Smad proteins. Binding of transcription factors to the promoter was assessed by chromatin immunoprecipitation (ChIP).

TGF-β caused concentration- and time-dependent increases in αVβ6 and ITGB6 mRNA, and increased activity of the ITGB6 promoter. Truncated mutants of the promoter showed that loss of 2 Smad binding sites resulted in loss of promoter activity. Co-transfection of dnSmad3 with the ITGB6 promoter reporter inhibited basal and TGF-β-induced promoter activity whereas dnSmad2 had little effect. dnSmad3 reduced TGF-β-induced αvβ6 cell surface expression. ChIP demonstrated binding of Smad3 and Smad4 to the promoter in response to TGF-β. Binding of Smad3 to the ITGB6 promoter was higher in lung tissue derived from IPF patients compared with controls. Finally, we identified a region of the ITGB6 promoter responsible for repressing transcription of the gene and demonstrate that siRNA targeting the transcription factor ELK1 increases αvβ6 expression.

In conclusion, TGF-β increases expression of αVβ6 by transcriptional regulation involving Smad3. Furthermore, enhanced binding of Smad3 to the ITGB6 promoter in patients with IPF suggests dysregulated synthesis of αvβ6 integrins may promote IPF. Finally, we identified ELK1 as a potentially important negative regulator of αvβ6 expression. This highlights a positive feedback loop which could be dysregulated through hyperstimulation and impaired repression leading to amplification of αvβ6 mediated TGF-β activation that could be fundamental to IPF pathogenesis.

S70 MESENCHYMAL STEM CELL CONDITIONED MEDIA (MSC-CM) SUPPRESS WNT-3A AND TGF-β1-INDUCED MYOFIBROBLASTIC DIFFERENTIATION

doi:10.1136/thoraxjnl-2012-202678.076

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Hypothesis Idiopathic pulmonary fibrosis (IPF) remains an incurable fibrotic lung disease. A mesenchymal stem cell (MSC)-mediated regenerative approach has been proposed; MSC-mediated antifibrotic effects have been demonstrated in animal lung-fibrosis models. However the mechanism of action and effect on myofibroblastic differentiation are unknown. The Wnt family member, Wnt-3a, has been implicated as an inducer of myofibroblastic differentiation in fibroblast cell models. This study explores the influence of MSC secreted factors on Wnt-3a and TGF-β1-induced lung myofibroblastic differentiation.

Method Human normal lung (CCD-8Lu) and IPF (LL29) fibroblasts were differentiated with Wnt-3a for 72-hours and TGF-β1 for 24-hours. MSC-mediated differentiation inhibition was assessed by co-incubation of fibroblasts with MSC-CM and either Wnt-3a for 72-hours or TGF-β1 for 24-hours. TGF-β1-induced myofibroblastic differentiation reversal was explored with MSC-CM incubation for 24, 48 and 72-hours. Myofibroblast differentiation was assessed by immunocytochemical detection of α-smooth muscle actin expression.

Results Myofibroblastic differentiation following TGF-β1 treatment was achieved in 86.27±2.57% CCD-8Lu cells and 86.69±2.51% LL29 cells respectively. Similar, though reduced, levels of myofibroblastic differentiation were achieved in 52.9±0.2% CCD-8Lu and 55.6±5.9% LL29 cells respectively following Wnt-3a treatment.

In contrast, a percentage reduction in myofibroblastic differentiation was achieved in CCD-8Lu 31.40+1.44% and LL29 35.69+7.47% cells following exposure to TGF-β1 in the presence of MSC-CM versus TGF-β1 alone ($p<0.001$). Similarly, we observed a striking percentage reduction in myofibroblastic differentiation following co-incubation with Wnt-3a and MSC-CM versus Wnt-3a alone ($p<0.001$); 80.76±3.64% of CCD-8Lu and 79.67±3.94% of LL29 cells.

A reversal of TGF-β1-induced myofibroblastic differentiation was observed following 72-hours administration of MSC-CM compared to serum-free culture media ($p<0.001$). Interestingly, we observed a MSC-CM exposure duration effect on the total myofibroblast percentage present in both CCD8-Lu and LL29 cells; 81.7+0.43% and 73.26+0.70% respectively at 24-hours, 72.15+0.81% and 60.57+4.27% at 48-hours, 57.63+4.54% and 60.65+4.9% at 72-hours.