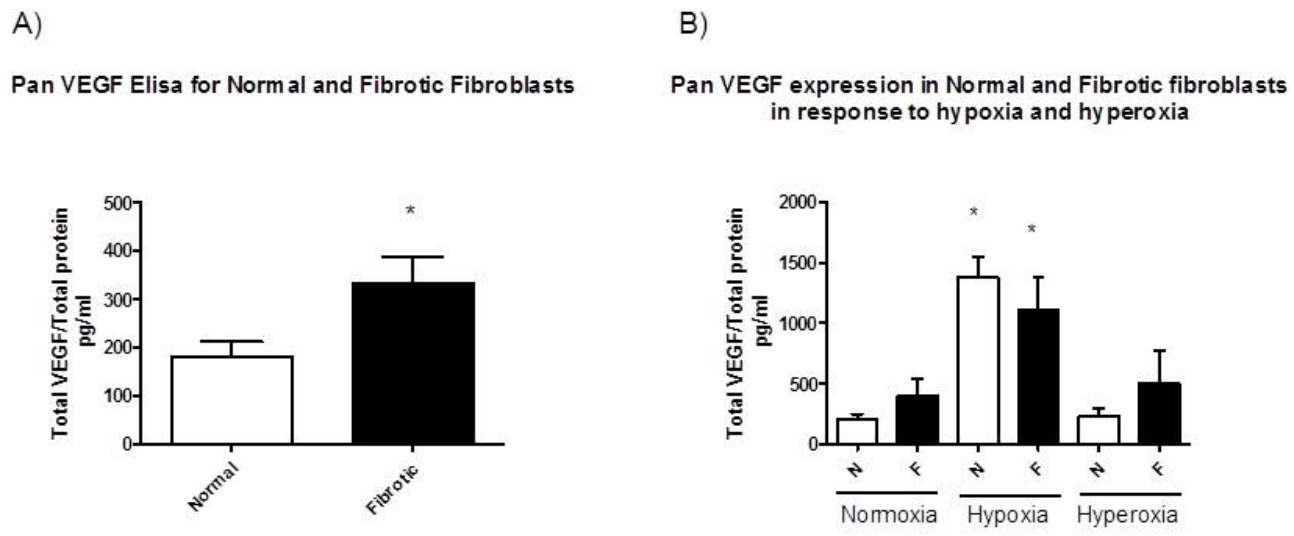

Figure 1.
A) Pan VEGF expression in Normal and Fibrotic fibroblasts (p = 0.01)
B) Pan VEGF expression in Normal and Fibrotic fibroblasts in response to normoxic, hypoxic and hyperoxic growth conditions (NF normoxia vs hypoxia: p = 0.05, FF normoxia vs hypoxia: p = 0.01)

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

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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.

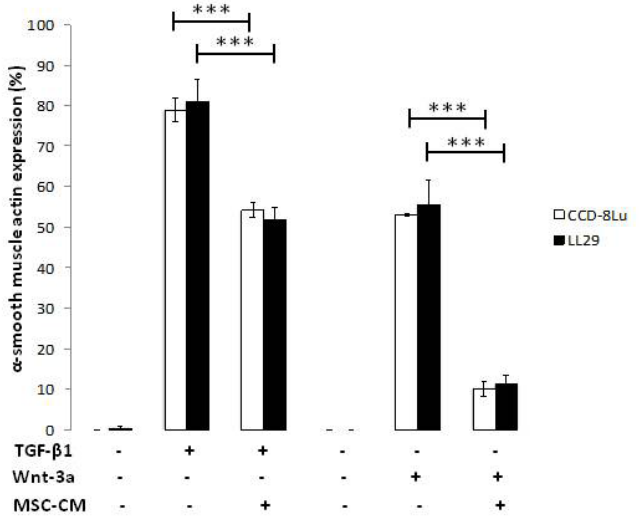
Method Human normal lung (CCD-8Lu) and IPF (LL29) fibroblasts were differentiated with Wnt-3a for 72-hours and TGF-β1 for 24-hours. MSC-mediated differentiation inhibition was assessed by co-incubation of fibroblasts with MSC-CM and either Wnt-3a for 72-hours or TGF-β1 for 24-hours. TGF-β1-induced myofibroblastic 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.

Conclusion MSC-CM appears to inhibit fibroblast to myofibroblast differentiation, over-riding the pro-differentiation effects of Wnt-3a and TGF-β1. Whilst both TGF-β1 and Wnt-3a have emerged as key players in IPF pathogenesis, we are the first to demonstrate that MSC-CM may be crucial in modulating their pro-fibrogenic effects. These actions of MSC-CM demonstrate exploitative potential for future anti-IPF therapeutic strategies.



Percentage of cells (CCD-8Lu and LL29) expressing α-SMA after 72 hours incubation with Wnt-3a and Wnt-3a+MSC-CM and 24 hours incubation with TGF-β1 and TGF-β1+MSC-CM. N= 3 per group
***P<0.001.

Abstract S70 Figure 1

S71 MECHANISMS OF LUNG REPAIR POST INJURY: THE ROLE FOR NON-CANONICAL WNT SIGNALLING AND PLANAR CELL POLARITY

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Introduction Fibrosis is a constituent of various respiratory diseases including asthma, COPD, idiopathic pulmonary fibrosis (IPF) and pulmonary hypertension. Research shows that Wnt genes, critical for lung development, are reactivated post cellular injury and that the canonical Wnt/β-catenin pathway is mis-expressed in these diseases. The non-canonical Wnt/PCP pathway can inhibit canonical signalling and also cause co-ordinated, directional cell migration. Moreover components of this pathway are suppressed in many respiratory fibro-proliferative disorders. We investigated the role of non-canonical Wnts in a tractable model of lung repair, by comparing the rate of wound healing using either canonical Wnt4 or non-canonical Wnt5a ligands. We hypothesised that Wnt5a would exhibit a higher rate of wound healing than Wnt4, coupled with increased cytoskeleton dynamics, favouring co-ordinated and directional cell migration.
Methods Confluent A549 cells were scratched with a 10μl pipette tip to induce a 'wound' and treated with either recombinant canonical Wnt4 or non-canonical Wnt5a, or cultured in control serum free medium. Wound area was measured at t=0, t-6 and t=24h post scratch to determine the rate of wound healing using time-lapse imaging. Phalloidin labelling and imaging was used to determine levels and distribution of F-actin at those times.
Results The addition of Wnt5a healed 4 times the area either Wnt4 or Control did using concentrations that induce maximal activity at t=6 (5.49, 1.23 and 1.01×105μm2 respectively) and t=24 (8.62, 2.42 and 2.39×105μm2 respectively) hours post scratch ($p<1x10–7$). At 0.5μg/ml Wnt5a healed 2 times the area Wnt4 did at it's ED50 dose with Wnt4 at 5xED50, at t=6 (1.99 and 1.01 x105μm2 respectively) and t=24 (4.29 and 1.80x105μm2 respectively) hours post scratch ($p<0.01$). Wnt5a treatment increased the frequency of focal F-actin enrichment towards the wound edge versus Wnt4 or control.
Conclusion Wnt5a is more efficient at wound healing in A549 cells than Wnt4. Wnt5a associated increase in focal F-actin enrichment amplified wound healing is associated with increased cytoskeletal dynamics and directional movement. In the future, novel therapies based around Wnt5a have the potential to be used to enhance repair in fibrotic respiratory diseases.

Translational studies in critical care

S72 RAGE ACTIVATION AND ENDOTHELIAL CELL INJURY ASSOCIATED WITH CARDIOPULMONARY BYPASS

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Introduction and objectives: The systemic inflammatory response syndrome (SIRS) which complicates most cases of surgery necessitating cardiopulmonary bypass (snCPB) is associated with endothelial cell injury and activation of the receptor for advanced glycation end products (RAGE). We hypothesised that RAGE activation leads to endothelial cell damage and that plasma levels of RAGE ligands, S100A8/A9 and S100A12, increased following snCPB will be positively associated with raised plasma syndecan-1 shed from damaged endothelial cells. We also hypothesised that S100 proteins and for comparison TNFα directly modulate syndecan-1 expression in cultured endothelial cells.
Methods Enzyme-linked immunosorbent assay measurements of syndecan-1, S100A8/A9 and S100A12 in plasma samples collected from patients pre- and post-snCPB (n=12); real-time-PCR determination of syndecan-1 expression and the house-keeping gene, GAPDH, in human umbilical vein endothelial cells (HUVEC) following incubation with TNFα (20ng/ml), S100A8/A9 (2μg/ml) and S100A12 (2μg/ml) for 3 to 24h.
Results Plasma levels of syndecan-1, S100A12 and S100A8/A9 levels increased following snCPB. Post-snCPB levels of syndecan-1 (86.1±16.2ng/ml) correlated, positively ($r^2=0.437$, $p=0.019$; and $r^2=0.729$, $p=0.0004$, respectively) with post-snCPB levels of S100A8/A9 (4.5±0.6ng/ml) and S100A12 (92.9±22.8ng/ml). In cultured HUVEC, TNFα significantly ($p<0.01$, n=3) decreased syndecan-1 mRNA expression by 75% at 6h and expression remained suppressed at 24h. By contrast, neither S100A8/A9 nor S100A12, under the conditions investigated in this study, significantly altered syndecan-1 mRNA expression.
Conclusion A positive association between post-operative plasma levels of RAGE ligands, S100A8/A9 and S100A12, and syndecan-1 is suggestive of a link between RAGE activation and endothelial injury, key feature of SIRS following snCPB. However, in cultured endothelial cells only TNFα and not S100A8/A9 or S100A12 decreased syndecan-1 mRNA expression; where decreased expression is indicative of reduced endothelial protective function. Possible explanations for the differences with S100A8/A9 and S100A12 in vivo and in vitro are that effects on syndecan-1 shedding in patients undergoing snCPB are indirect; and/or that in vitro, pre-activation of endothelial cells is required to up-regulate RAGE expression in order for S100 proteins to modulate