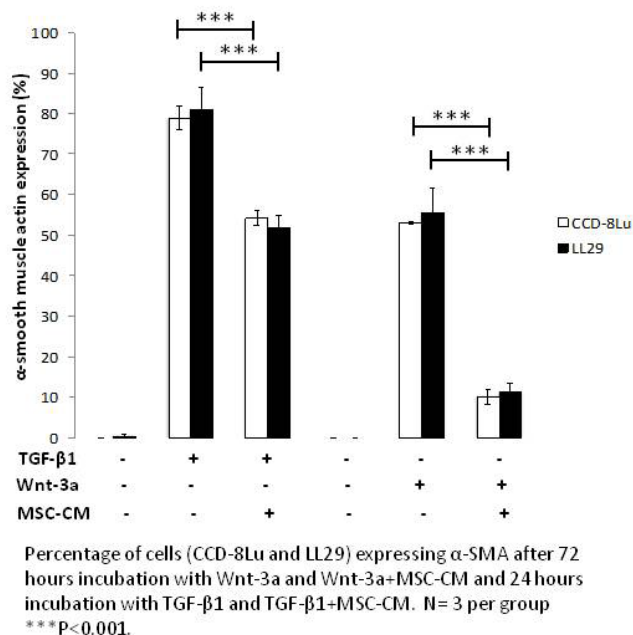


Conclusion MSC-CM appears to inhibit fibroblast to myofibroblast differentiation, over-riding the pro-differentiation effects of Wnt-3a and TGF- β 1. Whilst both TGF- β 1 and Wnt-3a have emerged as key players in IPF pathogenesis, we are the first to demonstrate that MSC-CM may be crucial in modulating their pro-fibrogenic effects. These actions of MSC-CM demonstrate exploitative potential for future anti-IPF therapeutic strategies.



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 10 μ l 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 \times 10⁵ μ m² respectively) and t=24 (8.62, 2.42 and 2.39 \times 10⁵ μ m² respectively) hours post scratch (p<1 \times 10⁻⁷). At 0.5 μ g/ml Wnt5a healed 2 times the area Wnt4 did at it's ED50 dose with Wnt4 at 5 \times ED50, at t=6 (1.99 and 1.01 \times 10⁵ μ m² respectively) and t=24 (4.29 and 1.80 \times 10⁵ μ m² 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 TNF α 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 TNF α (20ng/ml), S100A8/A9 (2 μ g/ml) and S100A12 (2 μ g/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 \pm 16.2ng/ml) correlated, positively (r²=0.437, p=0.019; and r²=0.729, p=0.0004, respectively) with post-snCPB levels of S100A8/A9 (4.5 \pm 0.6ng/ml) and S100A12 (92.9 \pm 22.8ng/ml). In cultured HUVEC, TNF α 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 TNF α 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