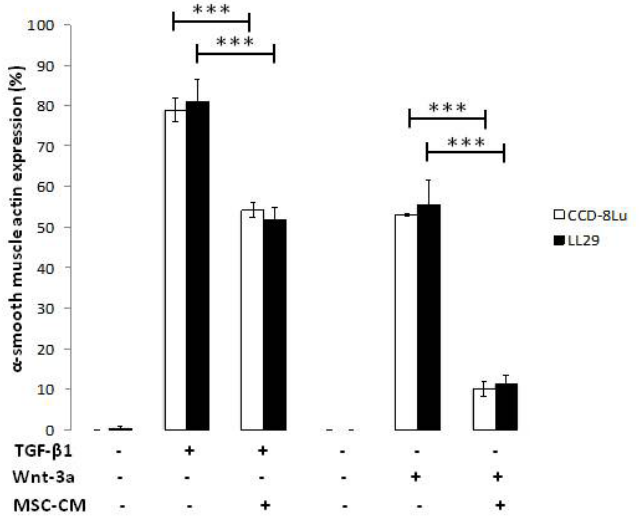
Conclusion MSC-CM appears to inhibit fibroblast to myofibroblast differentiation, over-riding the pro-differentiation effects of Wnt-3a and TGF-β1. Whilst both TGF-β1 and Wnt-3a have emerged as key players in IPF pathogenesis, we are the first to demonstrate that MSC-CM may be crucial in modulating their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

doi:10.1136/thoraxjnl-2012-202678.077

[1]NA Hasan, [2]M Hind, [3]CH Dean. *[1]Medical Student, Imperial College London, London, UK; [2]NIHR, Respiratory Biomedical Research Unit, Royal Brompton and Harefield NHS foundation trust, NHLI, London, UK; [3]Leukocyte Cell biology, NHLI, Imperial College London, London, UK*

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

doi:10.1136/thoraxjnl-2012-202678.078

M Patel, T Svermova, L Ramakrishnan, BC Creagh-Brown, M Griffiths, A Burke-Gaffney . *Imperial College London, London, UK*

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

syndecan-1 expression. These questions are the subject of ongoing studies into the role of RAGE activation in endothelial dysfunction in SIRS.

S73 CIRCULATING REGULATORS OF ACUTE MUSCLE WASTING IN THE CRITICALLY ILL: GDF-15 A POTENTIAL NOVEL DRIVER OF ACUTE MUSCLE WASTING

doi:10.1136/thoraxjnl-2012-202678.079

[1]SAA Bloch, [1]JY Lee, [2]SJ Wort, [2]MI Polkey, [1]PR Kemp, [2]MJD Griffiths. *[1]Imperial College, London, UK; [2]Department of Respiratory and Critical Care, Royal Brompton Hospital, London, UK*

Introduction Acute muscle wasting in the critically ill manifesting as intensive care unit acquired paresis (ICUAP) is common, and causes significant morbidity and mortality. The pathological mechanisms underlying ICUAP are poorly understood and, other than rehabilitation, no therapeutic interventions exist. It was hypothesised that known or potential circulating modulators of muscle mass: insulin-like growth factor-1 (IGF-1), myostatin and growth and differentiation factor-15 (GDF-15) would have distinct dynamic profiles in patients who develop acute muscle wasting in a novel human model of ICUAP.

Methods In a prospective observational study of 42 patients undergoing elective high-risk cardiothoracic surgery (at the Royal Brompton Hospital, London, UK) those who developed muscle wasting were identified by ultrasound. Circulating IGF-1, myostatin and GDF-15 were assayed pre-operatively and over the first week post-operatively. The ability of GDF-15 to cause muscle wasting in vitro was determined in cultured C2C12 myotubes.

Main results 23 of 42 patients (55%) developed quadriceps atrophy. Plasma concentrations of IGF-1 and unexpectedly myostatin, known mediators of muscle hypertrophy and atrophy respectively, both decreased from baseline in the first post operative days. By contrast, plasma GDF-15 concentrations increased in all patients. This increase in GDF-15 was higher (535%±308% versus 495%±247%) and sustained at day 7 in those who developed muscle wasting (day 7 compared with baseline, $p<0.01$), but recovered in the non-wasting group ($p>0.05$). Furthermore IGF-1 also did not recover in those who developed muscle wasting (day 7 compared with baseline, $p<0.01$) but did in the non-wasting group ($p>0.05$). Myostatin recovered to baseline in both groups. Finally, we demonstrated that GDF-15 caused atrophy of myotubes in vitro (average control myotube diameter was 26.3±9.0 μm versus 22.3±7.5 μm for GDF-15 treated myotubes, equating to 15% myotube atrophy, $p= 0.011$).

Conclusion This data supports the hypothesis that acute muscle loss occurs as a result of an imbalance between drivers of muscle atrophy and hypertrophy. GDF-15 is a potential novel mediator of muscle atrophy in ICUAP, which may become a therapeutic target in patients with ICUAP and other forms of acute muscle wasting.

S74 INCIDENCE AND ANALYSIS OF RISK FACTORS FOR THROMBOCYTOPENIA IN CRITICALLY ILL PATIENTS

doi:10.1136/thoraxjnl-2012-202678.080

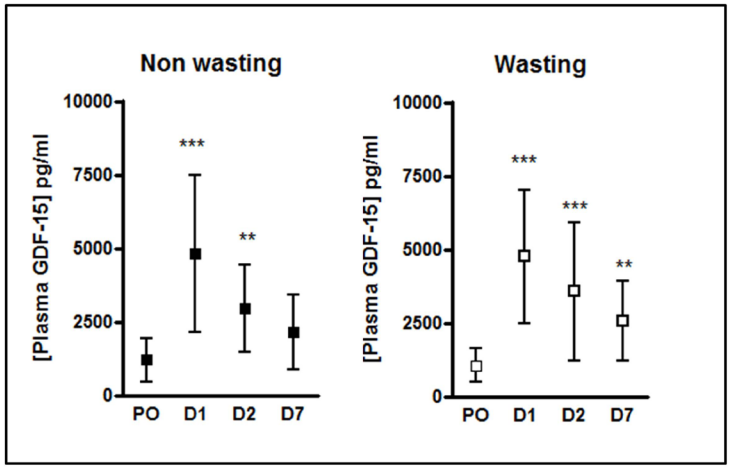
[1]LK Gupta, [2]B James. *[1]Geisinger Medical Center, Danville, PA, USA; [2]Regional Medical Center of San Jose, San Jose, CA, USA*

Introduction Thrombocytopenia occurs in 15% to 58% of intensive care unit patients. Up to 25% of acutely ill patients develop drug-induced thrombocytopenia. Establishing the etiology of secondary thrombocytopenia is challenging, since many medications and co morbidities have been implicated.

Objective To define the incidence and severity of thrombocytopenia in critically ill patients and to examine risk factors that may be related to the development of thrombocytopenia.

Method This is a retrospective chart review of 328 critically ill patients with thrombocytopenia requiring at least 72 hours of critical care. 30 suspected risk factors, including patient characteristics, drug effects, and associated diseases were identified from the literature and further evaluated using a multiple logistic regression. Mild Thrombocytopenia defined as platelet count less than 200 x 10(9)/L was observed frequently, but only 9 percent had severe thrombocytopenia defined as less than 20 x 10(9)/L.

Results Demographic characteristics of patients predisposed to thrombocytopenia are males, older than 55, and Hispanic population. Hemodynamic instability and/or heparin exposure appear to be the strongest identifiable correlates with thrombocytopenia. Sepsis, acute kidney injury and respiratory failure were strongly correlated ($p<0.0001$) with thrombocytopenia. Patients with severe thrombocytopenia were found to have concurrent alterations in



Plasma GDF-15 concentration (pg/ml) in non-wasting (n=19) and wasting patients (those with >9.24% muscle loss: n=23) pre-operatively (PO), on day 1 (D1), day 2 (D2) and on day 7 (D7). Data presented as mean ± SD.** p<0.01, *** p<0.001, repeated measures ANOVA with Bonferroni correction for comparison with pre-operative baseline.

Abstract S73 Figure 1