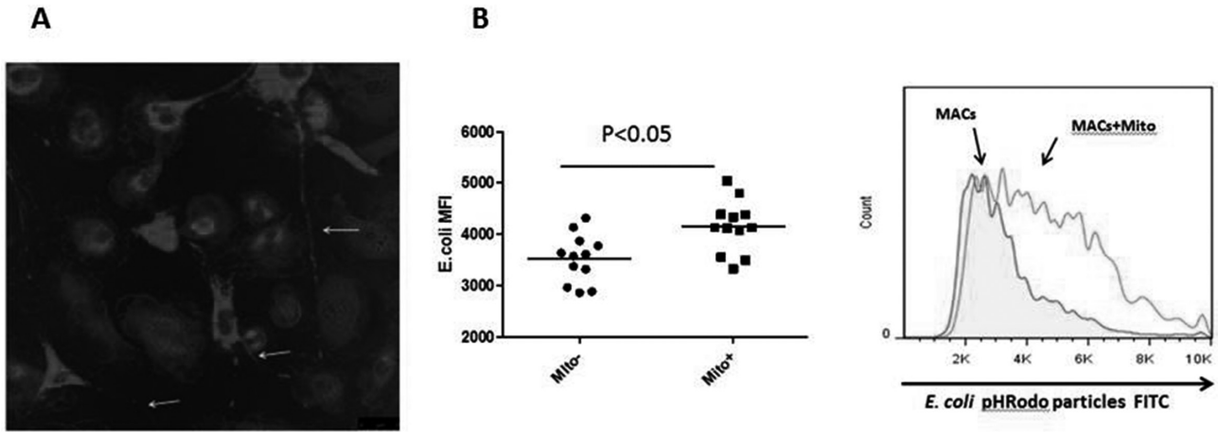

Abstract T3 Figure 1 Mitochondrial transfer from MSC to macrophages can enhance macrophage phagocytic activity *in vivo*. (A) MSC use tunnelling nano tubules (TNT) structures to transfer mitochondria (arrows). MSC were pre-stained with MitoTracker Red before co-culture with macrophages, 6 hr later sides were fixed and stained for(blue) to visualise macrophages. Almost allpositive cells demonstrate acquisition of red mitochondria from MSC. (B) In the *in vivo* model, MSC (MitoTracker)-treated mice BALF was taken and alveolar macrophages assessed for phagocytic activity using fluorescent E.coli bioparticles by flow cytometry. Macrophages that had acquired MSC mitochondria showed a higher phagocytic index in comparison to those without. This was assessed by an increase in Mean fluorescence Intensity (MFI). Some of the materials employed in this work were provided by the Texaz A&M Health Science Centre College of Medicine Institute for Regenerative Medicine at Scott and White through a grant from NCRR of the NIH, Grant #P40RR017447

Background ARDS remains a major cause of respiratory failure in critically ill patients with no specific therapy. MSC based cell therapy is a promising candidate and is being used in clinical trials for ARDS. However the mechanisms of MSC effect in lung injury are not very well understood. Islam *et al*., 2012 showed mitochondrial transfer from MSC to alveolar epithelial cells was protective in the mouse model of LPS induced pneumonia. Pathophysiology of ARDS is underpinned by dysregulated inflammation and pulmonary macrophages are key cellular mediators of the lung immune response. This study was undertaken to test if MSC could transfer their mitochondria to macrophages and to investigate the effects of MSC mitochondria transfer on macrophage function in the *in vivo* and *in vitro* models of ARDS.

Methods *In vivo* studies were performed using a mouse model of *E.coli* pneumonia induced ARDS. C56BL/6 mice were infected with *E.coli*, human bone marrow-derived MSC or PBS instilled intra-nasally 4 h after infection. For *in vitro* studies primary human monocyte-derived macrophages (MDM) were infected with *E.coli* and co-cultured with MSC in contact. MSC mitochondria were pre-stained with MitoTracker Red and MDM stained for CD45 expression. Double positive cells were visualised with confocal microscopy and quantified using flow cytometry. Phagocytosis was assessed using fluorescent *E.coli* bioparticles by flow cytometry.

Results When co-cultured with MSC >90% of MDMs acquired MitoRed fluorescence, indicating mitochondrial transfer from BM-MSC. Confocal imaging revealed presence of Mito-Red positive tunnelling nanotubules (TNTs) formed by MSC. *In vivo* >78% of $CD11c^{hi}$/F4–80+ alveolar macrophages retained MSC mitochondria at 24 hr post infection. Alveolar macrophages that had acquired MSC mitochondria had a significantly higher phagocytic index compared to those without suggesting enhancement of phagocytic capacity. Inhibition of TNT formation in MSC resulted in decreased transfer to macrophages by 60%, coupled with significant abrogation of MSC effect on macrophage phagocytosis *in vitro* and anti-microbial effect seen with MSC *in vivo*.

Conclusions Our findings suggest that anti-microbial activity of macrophages is enhanced at least partially by transfer of BM-MSC mitochondria through TNTs, representing an important mechanism of MSC effect in ARDS.

Supported by: MRC MR/L017229/1.

REFERENCE

1 Islam MN, Das SR, Emin MT, *et al*. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. *Nat Med*. 2012;**18**:759–65

T4 OPTICALLY DETECTABLE ANTIMICROBIAL PEPTIDES ENABLE THE IMMEDIATE DETECTION OF BACTERIA AND FUNGI IN THE LUNG

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10.1136/thoraxjnl-2015-207770.4

Introduction The immediate detection of pathogens in the lungs of patients with unexplained pulmonary opacities in the intensive care unit would represent a significant advance in their management. An optical imaging strategy, including the endobronchial administration of bacterial specific Smartprobes, would confer a number of advantages over conventional techniques such as bronchoalveolar lavage, principally real-time detection to immediately inform antimicrobial therapy. The aims of this study were to fluorescently label and iteratively develop anti-microbial peptides to image bacteria *in situ* in the lung using fibered confocal fluorescence microscopy (FCFM).

Methods Antimicrobial peptides (AMP) have been synthesised on a dendrimeric scaffold (AMP-1) and conjugated to an environmentally sensitive fluorophore called NBD, following the continuous development a linear counterpart. A further construct consists of an AMP with gram-selectivity conjugated to the NBD fluorophore (AMP-2). These are combined with FCFM to

allow distal alveolar imaging at micron resolution in an *ex vivo* ovine model of bacterial infection.

Results AMP-1 demonstrates bacterial binding affinity in a concentration dependent manner and labels a diverse panel of bacteria, including a panel consisting of >70% of ventilator-associated pneumonia causing organisms and the pathogenic fungi *Aspergillus fumigatus*. AMP-1 demonstrates significantly higher fluorescence over isomolar linear equivalents for *E. coli, K. pneumoniae, P. aeruginosa*, MSSA, *A. baumannii* and *S. pneumoniae* (all $p < 0.01$), is selective for bacteria over mammalian cells and has improved chemical stability over the linear equivalent when incubated with bronchoaoveolar lavage from patients with acute respiratory distress syndrome. Furthermore, AMP-1 can label *E. coli, K. pneumoniae, P. aeruginosa* and MSSA *in situ* in an *ex vivo* ovine model when instilled endobronchially and imaged with FCFM (pin vitro and remains selective for gram-negative bacteria over mammalian cells. In the *ex-vivo* model AMP-2 selectively labels the gram-negative bacterial segments (*P. aeruginosa, K. pneumonia* and *E. coli*) over the gram-positive (MSSA, MRSA and *S. pneumoniae*) or control pulmonary segments (all $p < 0.05$).

Conclusions A Smartprobe/FCFM strategy to immediately detect bacteria with gram selectivity in size relevant pre-clinical models is described, and are undergoing first-in-man translation.

T5 MICRORNA-140–5P REGULATES DISEASE PHENOTYPE IN EXPERIMENTAL PULMONARY ARTERIAL HYPERTENSION VIA SMURF1

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10.1136/thoraxjnl-2015-207770.5

Introduction and objectives Clinical therapies for the treatment of pulmonary arterial hypertension (PAH) target vasoconstriction. However, the proliferative pulmonary vascular remodelling that drives disease persists contributing to significant patient morbidity and mortality. MicroRNA (miR) are short non-coding RNA that mediate post-transcriptional regulation of mRNA targets. We hypothesise that dysregulation of miR leads to de-repression of cellular targets central to disease pathogenesis. We sought to identify dysregulated circulating miR in patients with PAH, determine their phenotypic effect using *in vitro* and *in vivo* models and identify key mechanistic regulators that may represent novel therapeutic targets.

Methods Two patient cohorts were used to identify and validate differential expression of miR in whole blood by microarray and single assay qPCR. Binding site and network analysis was used to identify key miR targets. Effect of miR on identified targets and disease phenotype was determined in pulmonary artery smooth muscle cells (PASMC) and in the monocrotaline (MCT) and Sugen5416 plus Hypoxia (SuHx) models of PAH.

Results Expression of miR-140–5p was reduced in whole blood samples from patients with PAH and experimental models of PAH. Network and pathway analysis identified key regulators of TGFß and PDGF signalling as miR-140–5p targets. Transfection with miR-140–5p inhibitor resulted in increased proliferation and migration of PASMC and de-repression of key targets. Nebulised delivery of miR-140–5p mimic prevented the development of PAH in the MCT rat model and attenuated progression of established PAH in MCT and SuHx rat models. In experimental models levels of SMURF1 protein correlated inversely with miR-140–5p. Direct regulation of SMURF1 by miR-140–5p was demonstrated *in vitro* by 3'UTR luciferase activity. Both miR-140–5p mimic and SMURF1 siRNA increased BMP response element activity identifying SMURF1 as a key negative regulator of BMP signalling in PASMC. Genetic ablation of SMURF1 in C57BL6 mice conferred allele dependent protection from SuHx induced PAH. Finally, whole blood mRNA and pulmonary vascular immunoreactivity of SMURF1 was increased in patients with PAH.

Conclusions These studies suggest that miR-140–5p and SMURF1 are key regulators of BMP signalling and disease pathology in PAH and highlight SMURF1 as a potential novel therapeutic target.

T6 MET TARGETED THERAPY IN LUNG ADENOCARCINOMA: DOES 'RESISTANT' EGFR MAKE A MET-RESPONSIVE DIMER?

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10.1136/thoraxjnl-2015-207770.6

Introduction Lung cancer has extremely poor survival with few effective treatments. Activating EGFR mutations (e.g. L858R), select for impressive EGFR tyrosine kinase inhibitor (TKI) responses but most develop resistance e.g. T790M mutations or MET amplification, which is thought to mediate EGFR-HER3 kinase switching. Preclinical studies suggest EGFR-MET TKI synergy and whilst phase III trials have failed, optimism remains for biomarker driven therapy.

Hypothesis EGFR-MET dimerisation determines MET TKI response.

Objectives

1. Explore MET TKI responsiveness in EGFR mutant lung adenocarcinoma.
2. Develop an EGFR-MET FLIM assay indicative of MET TKI responsiveness.

Methods An *in vitro* and murine xenograft model was derived from EGFR TKI resistant NCI-H1975 lung adenocarcinoma cells (L858R T790M). After shEGFR knockdown we re-expressed EGFR L858R or WT to represent three clinical scenarios: 1. H1975: 'EGFR-TKI Resistant' 2. H1975 L858R: 'EGFR Responders' or 3. H1975 WT (wildtype EGFR).

Cells and xenografts (gavage) were challenged with exquisitely selective MET TKI, SGX523. Response was assessed by BrdU proliferation assays *in vitro* alongside random migration assays mimicking tumour cell motility. Xenograft tumours (FFPE) were stained for Ki67/phosphohistoneH3 (proliferation) and Masson's trichrome/anti-sma (Collagen/stroma).

EGFR-MET interaction was assessed by co-immunoprecipitation and Forster resonance energy transfer (FRET) FLIM to quantify EGFR-MET dimers after SGX523 treatment.

Results SGX523 treatment significantly reduced H1975 xenograft tumour growth/weight. Proliferation was suppressed *in vitro* (BrdU %) and *in vivo* (phosphohistone-H3). Conversely in H1975 L858R, SGX523 reduced stroma (Masson's trichrome) and migration. EGFR-MET dimers were more common in H1975 than H1975 L858R/WT. This was associated with a