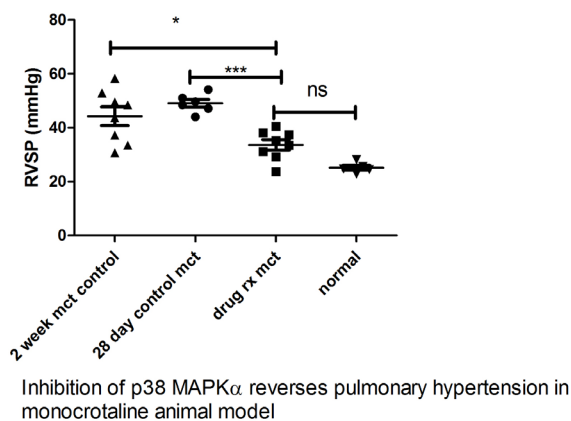


in a prevention strategy, or after 14 days in a treatment strategy. Both prevention and treatment methods were further employed in a second monocrotaline animal model. Haemodynamic measurements (right ventricular systolic pressure RVSP, right ventricular hypertrophy RVH) were performed, and lungs were removed for immunohistochemistry (IHC) and biochemical analysis. Multiplex ELISA was used to analyse cytokine profile in rat serum. Primary PAF were isolated and siRNA techniques employed to knockdown p38MAPK α . IHC for p38 MAPK α was performed on human tissue from patients with idiopathic pulmonary arterial hypertension.

Results siRNA to p38MAPK α inhibited the hypoxic induced proliferation of PAFs. Increased levels of total p38 MAPK activity and increased expression of the alpha isoform was found in the lungs of both chronic hypoxic and MCT animals compared to normal. Using the p38 MAPK inhibitor in the chronic hypoxic and monocrotaline *in vivo* prevention study resulted in lower RVSP and RVH in the drug treated animals ($p < 0.005$). In the reversal study of both animal models the inhibitor reversed established pulmonary hypertension as determined by RVSP and RVH ($p < 0.001$). Both serum and whole lung levels of IL-6 were lower in the drug treated animals compared to normal. Increased expression of p38 MAPK was observed in lungs from IPAH patients compared to control.

Conclusions Our study suggests p38 MAPK alpha is important in pulmonary hypertension. Inhibition of this pathway can prevent the development of PH and perhaps more clinically relevant, can reverse established disease *in vivo*. Reduction in IL-6 may be a mechanism underlying this process.



Abstract S36 Figure 1

S37 CAN THE LUNG REVERSE REMODEL? GENE THERAPY FOR CARDIAC FAILURE ALTERS PULMONARY GENE EXPRESSION

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Introduction Irreversible alveolar capillary membrane (ACM) remodelling accompanies chronic heart failure (CHF), contributing to dyspnoea, the predominant symptom that limits quality of life in CHF. Gene therapy is aimed at improving myocardial function in CHF. Restoration of Sarco-endoplasmic reticulum calcium ATPase (SERCA2a) gene expression in animal models of CHF restores haemodynamic parameters towards normal.

The lungs are the direct upstream target of raised left atrial pressure and hence pulmonary venous hypertension. We hypothesised that mechanical strain at the pulmonary micro vasculature associated with PVH up-regulates mediators leading to pulmonary inflammation and ACM remodelling. We have previously shown that gene expression of monocyte chemoattractant protein (MCP)-1,

interleukin(IL)-6, endothelin (ET)-1, endothelin receptors (ETR) A and B, and endothelial converting enzyme (ECE) are altered in the lungs of Sprague-Dawley rats at 16 weeks after left coronary artery ligation. We now sought to determine the effect of SERCA2a gene therapy on gene expression of these mediators in the lung.

Methods Gene expression of components of the ET-1 pathway, MCP-1 and IL-6 were investigated in whole lungs of rats at 16 weeks after LCA, at 16 weeks post LCA with tail vein injection of adeno-associated viral (AAV) gene transfer of SERCA2a at 12 weeks post LCA, or sham procedure ($n=5$ in each group). Lungs were snap frozen in liquid nitrogen, RNA extracted using a modified Trizol and RN easy protocol and gene expression determined in reverse transcribed cDNA by qPCR.

Results Expression of ET-1, ETAR, MCP-1 and IL-6 genes were elevated in heart failure animals and reduced to or towards normal in SERCA2a treated animals. In heart failure animals there was a trend towards reduced ETRB expression which was significantly improved by SERCA2a gene therapy (figure 1). ECE gene expression was not altered by LCA or gene therapy.

Conclusion SERCA2a gene therapy directed at the myocardium in heart failure also affects gene expression in the lungs of CHF animals. This may provide therapeutic benefit to the lungs in addition, reducing inflammation and stimuli associated with structural and vascular remodelling.

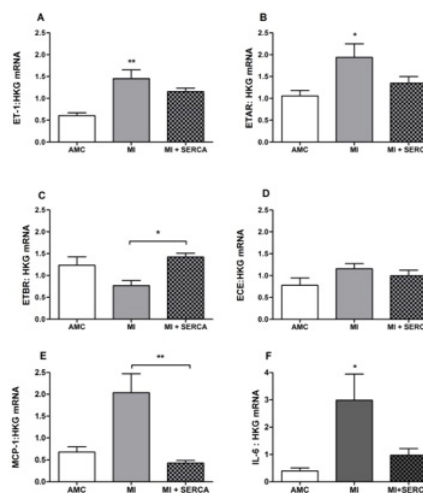


Figure 1 Effect of treatment with SERCA gene therapy on endothelin-1 (ET-1), endothelin receptor (ETR) a & B, endothelin converting enzyme (ECE), Monocyte chemoattractant protein (MCP)-1 and Interleukin (IL)-6 mRNA expression in chronic heart failure in the rat.

Abstract S37 Figure 1

S38 TGF-BETA1 NEGATIVELY REGULATES BMP4 SIGNALLING IN HUMAN PULMONARY ARTERY SMOOTH MUSCLE CELLS VIA A SMAD3-DEPENDENT MECHANISM

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Introduction BMP4 signals via the Smad pathway to induce the expression of the ID dominant-negative basic helix-loop-helix transcription factors (ID1-4) that regulate cell differentiation. We have shown that ID induction is blunted in human pulmonary artery smooth muscle cells (HPASMCs) from pulmonary arterial hypertension (PAH) patients with mutations in the bone morphogenetic type-II receptor (BMPRII). TGF β 1 is implicated in the pathogenesis of PAH. We therefore examined whether TGF β 1 and BMP4 signalling directly interact in HPASMCs.

Methods Explant-derived HPASMCs from unaffected donors or PAH patients with identified BMPR-II mutations were studied. The transcriptional responses of ID1, ID2, PAI1 and CTGF to BMP4, TGFβ1 or co-treatment were examined by qPCR. In some experiments, cells were pre-incubated with cycloheximide or pharmacological inhibitors of ALK5 (SD208), MAP kinase (U0126), TAK1 or p38 MAP kinase (SB203580). The roles of Smad2, Smad3, Smad6 and Smad7 were investigated using siRNAs. Smad-dependent transcription was examined using the BMP-responsive luciferase reporter (BRE-luc) and the TGF-responsive luciferase reporter, CAGA12-luc. Protein lysates collected at 1 and 4hrs were immunoblotted for phosphorylated and total Smads and candidate kinases. In some experiments, nuclear and cytoplasmic fractions were prepared and immunoblotted.

Results BMP4 induced ID1 and ID2 expression at 1, 4 and 24h whereas TGFβ1 induced ID1/2 at 1h and repressed them at 4h and 24h. TGFβ1, but not BMP4, induced PAI1 and CTGF expression. BMP4 did not alter TGFβ1-mediated transcriptional responses. In contrast, TGFβ1 attenuated BMP4-mediated ID1/2 induction and the BRE-luc response in donor HPASMCs. Moreover, TGFβ1 abolished BMP4 responses in PAH PSMCs with BMPR-II mutations. This was reversed by the ALK5 inhibitor, SD208, but not by cycloheximide or a TAK1 inhibitor. BMP-Smad phosphorylation and nuclear translocation did not differ between co-treatment and treatment with individual ligands. Smad3 siRNA, but not Smad2, Smad6 or Smad7, reversed the inhibitory effect of TGFβ1.

Conclusions TGFβ1 negatively regulates BMP4-mediated ID genes transcription by interfering with BMP-Smad signalling via Smad3, abolishing the BMP signals in cells with BMPR-II mutations. These data reinforce the notion that TGFβ1 plays a pathogenic role in PAH, via direct inhibition of the attenuated BMP response caused by BMPR-II insufficiency.

S39

WHOLE EXOME SEQUENCING IN CHRONIC THROMBOEMBOLIC PULMONARY HYPERTENSION REVEALS BIOLOGICALLY PLAUSIBLE NOVEL GENETIC VARIATION

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Chronic thromboembolic pulmonary hypertension (CTEPH) is a disease with no known heritability, the cumulative incidence of which has been placed at between 0.5 and 9.1% following acute pulmonary embolism [1,2]. A majority consensus on its development has

emerged predicated on the theory of disordered thrombus resolution following venous thromboembolism. This in part stems from epidemiological data but is also based on studies implicating established clinical and laboratory factors, a central theorem being that individuals respond differently to acute thrombotic insult.

Given traditional prothrombotic factors explain less than 10% of reported cases of CTEPH, we hypothesised a significant burden of disease is accounted for by unidentified genetic factors. Using an unbiased approach of exome capture, we selected 20, deeply phenotyped, young individuals (11 female) who had suffered large PE that subsequently developed haemodynamically confirmed CTEPH despite anticoagulation. Individuals with known prothrombotic tendency and significant comorbidity were excluded. Selected patients were White Caucasian origin.

Following ethical approval, DNA was extracted from 20 whole blood samples and libraries prepared. Whole exome sequencing was undertaken using Agilent's Sure Select Exome capture kit on the HiSeq 2000 platform prior to bioinformatic analysis. 75 bp paired-end reads were aligned to the H19 Reference genome and the pipeline annotated for SNPs and indels. Intergenic, intronic and UTR variants were removed from the dataset with minimum mapping quality score and 20x read-depth coverage stipulated for variant calls.

Consecutive filters were applied, firstly retaining those predicted to be pathogenic flagged as splice-site, essential splice-site, missense, frameshift-coding, non-synonymous coding, NMD-transcript, within-mature-miRNA, STOP-gained and STOP-lost. Variants also present in twenty locally sourced normal controls were discarded. Additional filters were subsequently applied to exclude variants already listed in dbSNP/1000 genomes data with remaining variants split into heterozygous and homozygous groups (Table 1). Our final candidate list contains novel DNA variants lying within pathways known to mediate both inflammation and endothelial dysfunction in the pulmonary vasculature. Detailed downstream biological analysis of our variant effects following validation measures may lead to the uncovering of novel mechanisms in this difficult disease.

References

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Abstract S39 Table 1

N=20 (>5samples)	Heterozygous SNP	Gene	Homozygous SNP	Gene
Pathogenic	33260 (13767)	11009 (6267)	1251 (423)	587 (112)
Novel (dbSNP1000 genomes)	6662 (332)	5868 (268)	447 (70)	312 (65)
Not in Normal Controls	5002 (43)	3741 (57)	241 (10)	189 (10)

Table 1 shows the number of SNPs and genes containing those SNPs for both heterozygous and homozygous variants following each filtering step. Bracketed values show the number of SNPs/genes with those SNPs present in 5 or more samples.