S154 IS THERE A ROLE FOR IL-33 IN THE PATHOGENESIS OF PULMONARY ARTERIAL HYPERTENSION?

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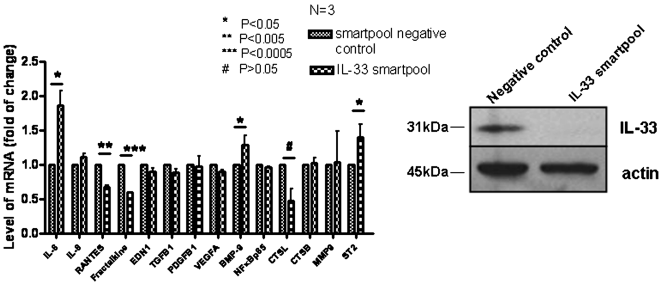
Introduction and objectives IL-33 is a 31KDa cytokine which is a member of the IL-1 family. It resides in the nucleus of endothelial and epithelial cells as a chromatin-associated factor in vivo. IL-33 is thought to be released from stressed or necrotic, but not apoptotic, endothelial or epithelial cells in response to cell injury or infection, acting as an endogenous 'alarmin', to alert the immune system of cell and tissue damage. IL-33 is then able to bind to its receptor (ST2) on immune cells, thereby stimulating immunoregulatory activity through induction of NF-κB and mitogen-activated protein kinases, as well as enhancing the production of the Th2 cytokines IL-5 and IL-13. There is increasing evidence that IL-33 may have a protective role in terms of endothelial integrity and function. As the pathogenesis of pulmonary arterial hypertension (PAH) is thought to involve endothelial cell dysfunction, we were interested to see whether there may be a role for IL-33 in this condition.

Methods RT-PCR for IL-33 was performed on human pulmonary arterial cells (HPAECs) derived from normal healthy controls and patients with idiopathic PAH. siRNA IL-33 knockdown was performed using smartpool duplexes on normal HPAECs. RT-PCR was then performed using QuantiTec primer assays.

Results IL-33 mRNA expression was decreased 2.1-fold in PAH patient samples (0.369±0.02, n=10) compared to controls (0.761±0.06, n=14) $p<0.005$. In normal human lung tissue, intense IL-33 staining was shown in the ECs nuclear in blood vessels. It is also worth to note that there was little or no IL-33 staining in non-ECs. siRNA knockdown IL-33 in HPAECs resulted in a 1.8±0.22, 1.3±0.13 and 1.4±0.20 (n=3) fold increase of mRNA IL-6, BMP-9 and ST2 mRNA, respectively; whereas RANTES, fractalkine and cathepsin-L mRNA was decreased by 1.5±0.03, 1.7±0.01 and 2.1±0.17 fold respectively (n=3) (Abstract S154 Figure 1).

Conclusion IL-33mayplay an important role in the pathogenesis of PAH through regulating the expression ofcytokines and chemokines known to be involved in vascular remodelling. In particular, it is of interest that Il-33 may regulate IL-6 production and ST2 which acts as an endogenous IL-33 inhibitor.



Abstract S154 Figure 1 Effect of IL-33 on gene expression.

S155 THE ROLE OF ST2 IN A MODEL OF PULMONARY HYPERTENSION

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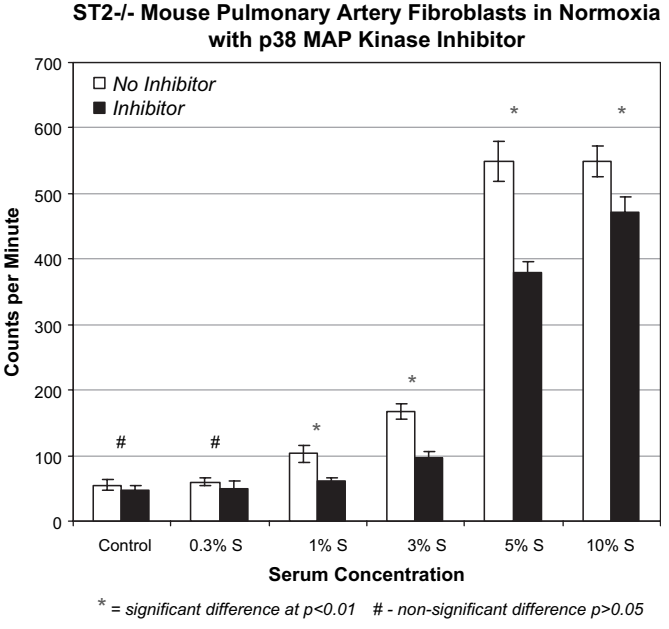
Background Pulmonary arterial hypertension (PAH) is a fatal condition involving remodelling of the pulmonary vessel wall leading to elevated pulmonary arterial pressure and eventually right heart failure. ST2 is a transmembrane receptor with ligand interleukin-33 (IL-33). p38 MAP kinase is an intracellular signalling molecule shown to be involved in the vascular remodelling associated with PAH; and is also involved in ST2/IL-33 signalling. ST2/IL-33 signalling has been shown to reduce fibrosis in the heart following pressure overload in animal models.

Aims To determine whether ST2/IL-33 signalling was involved in the proliferation of mouse pulmonary artery fibroblasts and if p38 MAP kinase was involved.

Methods Two cell types were used—wild type (WT) and ST2 knockout (ST2−/−) mouse pulmonary artery fibroblasts. Proliferation was assessed by [^{3}H] Thymidine incorporation. Expression of p38 MAP kinase was detected by Western blotting. Cells were cultured at various serum concentrations in normoxia and hypoxia. A p38 MAP kinase inhibitor (SB203580) was used to assess its role in cell proliferation. The effect of IL-33 on cell proliferation and expression of p38 MAP kinase was studied.

Results WT and ST2−/− cells proliferated in response to increasing serum concentration. WT cells hyperproliferated in response to hypoxia. ST2−/− cells hyperproliferated in normoxia and hypoxia compared to WT cells. The hyperproliferation of ST2−/− cells in normoxia and hypoxia could be reduced by p38 MAP kinase inhibition (p38) (see Abstract S155 Figure 1). Phosphorylated p38 MAP kinase was detected in all ST2−/− cells and in WT cells in hypoxia with 10% serum, demonstrating p38 MAP kinase is involved in the



Abstract S155 Figure 1 Proliferation in Response to pp38 MAP Kinase Inhibition in Normoxia ST2-/- cells were cultured in normoxia at serum concentrations 0, 0.3, 1, 3, 5 and 10%, with and without the p38 MAP kinase inhibitor SB203580. Proliferation was reduced in the presence of the inhibitor with statistical significance at serum concentrations 1% serum suggesting that p38 MAP kinase is involved in the hyperproliferation seen in ST2-/- cells.

hyperproliferation observed. Hyperproliferation in response to hypoxia in WT cells could be blocked by addition of IL-33. IL-33 stimulation also decreased the phosphorylation of p38 MAP kinase. There was no effect of IL-33 on ST2−/− cells.

Conclusions Mouse pulmonary artery fibroblasts hyperproliferate in response to hypoxia and in the absence of the ST2 receptor. This hyperproliferation involves phosphorylation of p38 MAP kinase. This phosphorylation and excessive cell proliferation can be blocked by ST2/IL-33 signalling. ST2 may be a potentially novel therapeutic target in the PAH.

S156 CHLORIDE INTRACELLULAR CHANNEL PROTEIN 4 (CLIC4) IN THE REGULATION OF HUMAN PULMONARY ENDOTHELIAL RESPONSES TO HYPOXIA

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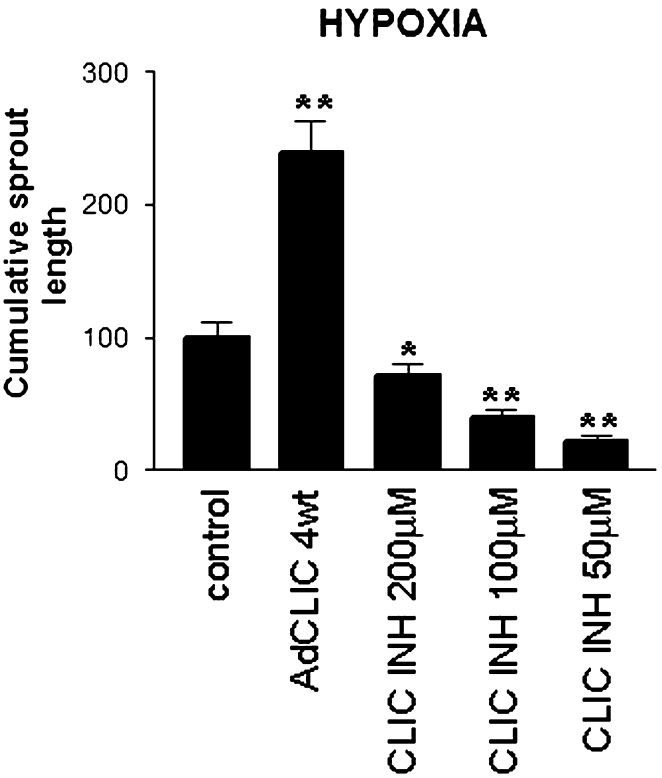
Introduction and Objectives CLIC-4 belongs to the family of chloride intracellular channels, proteins structurally homologous to glutathione transferases. CLIC4 has been implicated in tumour angiogenesis and signalling pathways important in the pathogenesis of pulmonary arterial hypertension (PAH). We have previously demonstrated increased CLIC4 protein expression in whole lung samples from patients with PAH and animals with chronic hypoxia- and monocrotaline-induced PH. CLIC4 is particularly abundant in pulmonary endothelium of patients with PAH. In this project we aimed to establish a role of CLIC4 plays in the regulation of pulmonary endothelial cell responses to hypoxia.



Abstract S156 Figure 1 Endothelial spheroids were left untreated, were infected with adenoviruses to overexpress wtCLIC4, or were treated with NPPB, as indicated. The spheroids were then left in normoxia (not shown) or placed in hypoxia (2% O_2, 5% CO_2, 93% N_2) for 24 h. The cumulative sprout length was studied with image analysis software (Image J). *P<0.05; **P<0.01; n=4.

Methods Human pulmonary artery endothelial cells (HPAECs) were cultured in normoxic conditions or were exposed to hypoxia (2% O_2, 5% CO_2, 93% N_2) for 1–24 h. CLIC4 expression and localisation was studied by Western blotting, immunofluorescence and confocal microscopy. The wildtype and nuclear-targetted CLIC4 were overexpressed via adenoviral gene transfer while chloride channel inhibitor NPPB (5-Nitro-2-(3-phenylpropylamino)benzoic acid) was used to inhibit CLIC4 activity. We studied the effects of CLIC4 on endothelial permeability, angiogenesis and proliferation.

Results Overexpression of the wildtype CLIC4 in HPAECs increased pulmonary endothelial proliferation, compromised barrier function and increased angiogenic responses to chronic hypoxia in vitro (Abstract S156 Figure 1). These effects were prevented by chloride channel inhibitor NPPB. In normoxic HPAECs CLIC4 was localised predominantly to the cell nucleus and cytoplasm. Upon stimulation with hypoxia, CLIC-4 translocated to the cell periphery, localising in particular to membrane protrusions (filopodia and lamellipodia), the effect mimicked by overexpression of CLIC4.

Conclusions Hypoxia induces translocation of CLIC-4 to the membrane of HPAECs. This behaviour has been linked to increased motility and malignant phenotype in other cell types. CLIC-4 overexpression in HPAECs also increases endothelial cell responses to hypoxia in vitro. These findings suggest increased CLIC-4 expression in PAECs in PAH may play a role in pathogenesis of PAH and provide novel insights in to disease pathogenesis and treatment strategies.

Occupational asthma

S157 PREVALENCE OF ASTHMA RELATED TO EMPLOYMENT IN THE UK

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It is widely held that 10–15% of adult asthma is causally related to occupation. It is likely that this fraction varies importantly depending on historical and international variations in employment. Further uncertainties arise from misclassification in the diagnosis of asthma and in exposure assessment, particularly if based on self-report. We carried out a postal survey of adults listed as asthmatic through general practices across the UK. Cases, who were defined as those who had experienced onset of asthma or worsening of pre-existing childhood asthma within 2 years of starting a new job, were compared to controls who declared an equivalent onset or deterioration more than 2 years from the start of a job. Of 8535 individuals targeted, 3115 (37%) returned a completed questionnaire. Almost 40% of these (n=1198) experienced a deterioration of pre-existing childhood asthma or onset of adult asthma whilst working; 441 were cases and 757 controls. A priori analysis of risk was performed using an asthma-specific JEM, the ECRHS asthma 'high risk occupations' and data from the UK SWORD surveillance scheme. Odds ratios (adjusted for sex, smoking and era of onset and stratified by onset type)—displayed in Abstract S157 Table 1—did not suggest an increased risk of asthma within 2 years of starting a high-risk job. A posteriori analysis of all occupations demonstrated an increased risk of asthma within 2 years of starting a new job in sales and elementary occupations. The calculated population attributable risk (PAR) for these occupations was 15.9% and was higher in women than men. The results from this study suggest that a priori assessment of risk does not identify occupation as a significant cause of asthma in this contemporary adult workforce in the UK. Jobs which do appear to increase risk of new asthma are not those typically associated with an excess risk of the disease. These findings highlight the disparity between epidemiological and clinical assessments of asthma related to occupation, and the need to consider novel occupations as a cause of asthma.