sleep in both stable and exacerbating asthma. Cough frequency increases during the morning and late afternoon.

Mechanistic insights into interstitial lung disease

S74 ENDOPLASMIC STRESS IS ASSOCIATED WITH FIBROSIS IN INTERSTITIAL LUNG DISEASE

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Interstitial lung diseases (ILD) are a heterogeneous group characterised by variable amounts of inflammation and fibrosis. However, the development of pulmonary fibrosis is associated with a poorer prognosis. Although distinct histological features differentiate between the ILDs, it is unknown if there are shared pathogenic mechanisms involved in the development of fibrosis. Endoplasmic reticulum (ER) stress has been implicated in the pathogenesis of familial and sporadic idiopathic pulmonary fibrosis (IPF). In response to ER stress, cells trigger the unfolded protein response (UPR) and upregulate chaperones, such as BiP, and the phosphatase GADD34, which can regulate epithelial to mesenchyme transition, cell proliferation, apoptosis and cell survival.

Aims We hypothesise that ER stress may be involved in the pathogenesis of fibrosis in all interstitial lung diseases.

Methods Paraffin embedded sections, obtained from video assisted thoracoscopic diagnostic lung biopsies, from 8 patients with familial pulmonary fibrosis, 11 sporadic idiopathic pulmonary fibrosis (IPF), 12 non-specific interstitial pneumonia (NSIP) and 10 hypersensitivity pneumonitis (HP) were evaluated for BiP and GADD34 by immunohistochemistry. Using light microscopy, 6 high power fields were scored for fibrosis, inflammation, BiP and GADD34 using semi-quantitative analysis by 2 blinded, independent investigators. Data were analysed by linear regression using Prism software.

Results Of the 41 biopsy samples analysed, 20 (49%) were non-smokers and 18 (44%) were male. BiP and GADD34 were localised to reactive type II pneumocytes and columnar epithelium within areas of fibrosis. GADD34 was also evident in the endothelium. No staining was detected within fibroblasts or fibroblastic foci. Epithelial GADD34 correlated with extent of fibrosis in familial pulmonary fibrosis ($r^2=0.72$ $p<0.001$), IPF ($r^2=0.51$ $p<0.0001$) and NSIP ($r^2=0.46$ $p<0.0001$). In contrast, BiP was associated with fibrosis in IPF ($r^2=0.49$ $p<0.0001$) and HP ($r^2=0.59$ $p<0.0001$). There was no association with inflammation.

Conclusion These data show that ER stress and the UPR are associated with fibrotic ILDs. Hence targeting ER stress may be a novel therapeutic option for pulmonary fibrosis. Work is on going to identify a peripheral biomarker signature for ER stress.

S75 CYCLICAL STRETCH INDUCES $G_{\alpha Q/11}$ MEDIATED TGF β ACTIVATION IN LUNG FIBROBLASTS

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Introduction Idiopathic pulmonary fibrosis (IPF) is an incurable and progressive fibrotic condition of the lung with a poor prognosis. Activation of the profibrotic cytokine transforming growth factor- β (TGF β) is central to the pathogenesis of IPF. Mechanical signals are emerging as key mediators of TGF β activation, and cyclical stretch is a ubiquitous stimulus in the lungs. Stretch of fibrotic lung induces TGF β activation, and alveolar epithelial $G_{\alpha Q/11}$ signalling is important in stretch-mediated TGF β activation. However, the role of $G_{\alpha Q/11}$ signalling in stretch-mediated TGF β activation by lung fibroblasts is unclear.

Methods Murine embryonic fibroblasts (MEFs) and human lung fibroblasts (HLFs) were subject to cyclical stretch using the Flexcell system, and stretch regimens designed to mimic tidal breathing in the relevant organism (15% elongation unless otherwise stated, frequency: 1 Hz in MEFs, 0.3 Hz in HLFs). Phosphorylated Smad2 (pSmad2), total Smad2, and alpha smooth muscle actin (α SMA) protein expression were assessed by western blot. TGF β activation was determined using the ratio of pSmad2 to total Smad2 on densitometry. Comparisons of stretch-mediated TGF β activation were made between wild-type (WT), $G_{\alpha 12/13}^{-/-}$, and $G_{\alpha Q/11}$ -deficient MEFs, and between HLFs from patients with and without IPF.

Results Cyclical stretch induced a significant increase in pSmad2 expression after 48 hours in both MEFs and HLFs ($p<0.05$). However, stretch did not affect total α SMA protein expression in either cell type. There was no 'dose-response' relationship in stretch-induced pSmad2 expression in WT MEFs (5%, 10%, 15% and 20% elongation). MEFs deficient in $G_{\alpha Q/11}$ signalling, but not $G_{\alpha 12/13}$ signalling, activated significantly less TGF β than WT MEFs in response to cyclical stretch ($p<0.05$). HLFs from both IPF and non-IPF donors activated TGF β after 48 hours of cyclical stretch, but IPF fibroblasts activated significantly more TGF β than fibroblasts from patients without IPF (6 IPF and 6 non-IPF cell lines; $p<0.05$).

Conclusion Cyclical stretch is a physiologically relevant stimulus that drives $G_{\alpha Q/11}$ -mediated TGF β activation in lung fibroblasts. IPF fibroblasts have enhanced stretch-mediated TGF β activation, indicating that dysregulation of breathing-related stretch signalling is a potential mechanism of IPF progression. A greater understanding of these pathways may identify targets for new therapies that could halt the progression of IPF.