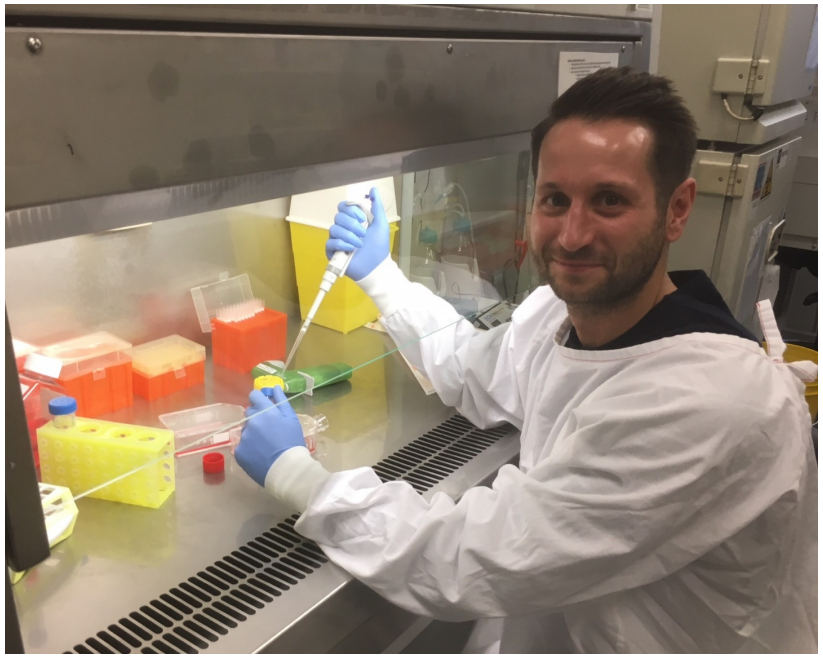
ATS 2018 Highlights

Respiratory Structure and Function Early Career Professionals

Get to know members of the RSF Assembly



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Is your research clinical, basic science or translational?

Basic Science

Tell us about your research?

In the lung, senescent cells are strongly implicated in the pathogenesis of IPF. Senescent epithelial cells are thought to be primarily pro-fibrotic but there is little understanding of the contribution of senescent fibroblasts to the fibrosis observed in IPF. My research aims to develop and characterise a model of induced senescence, using primary human lung fibroblasts, and to ultimately determine whether senescent fibroblasts are pro- or anti-fibrotic. I also aim to identify whether senescent fibroblasts are capable of driving non-senescent fibroblasts into senescence, and determine if senescent fibroblasts promote fibroblast-to-myofibroblast differentiation of neighbouring non-senescent fibroblasts.

Where do you see yourself in 5 years?

Upon completion of my PhD in Australia, I plan on moving back to Europe to join a team involved in IPF research. In the shorter term, my goal is to contribute to the field through hypothesis generating and performing basic science in the lab. In 5 years I aim to be driving the research of more than a one-researcher band and to begin to see my science translated to clinical applications.

What do you find is the major benefit of RSF Assembly Membership?

As an early career researcher the networking opportunities that the RSF assembly provides are invaluable, they have allowed me to interact with senior researchers in the field which is always inspiring.

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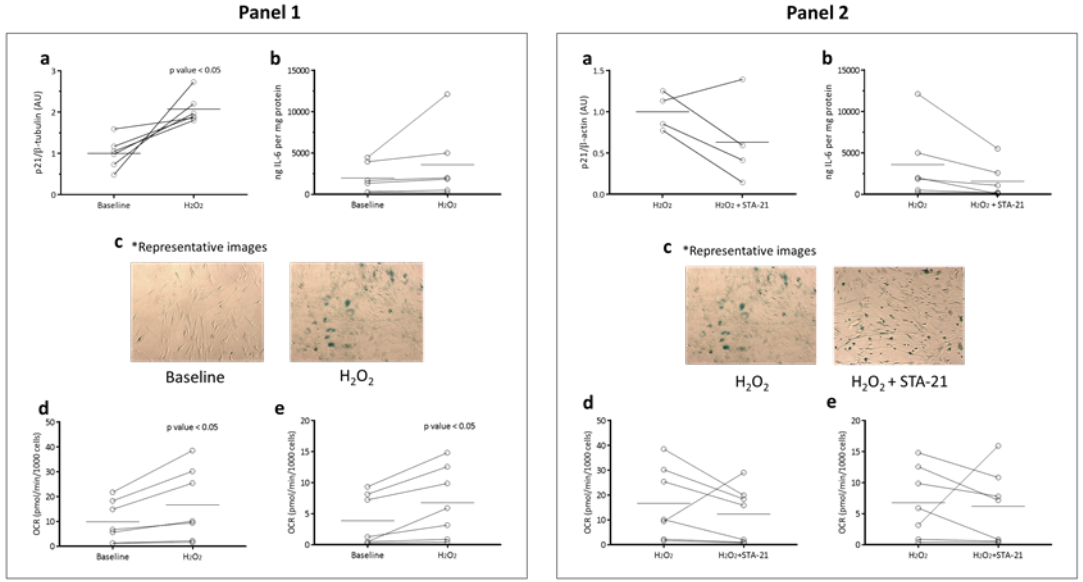

Panel 1: A shift in phenotype after H_2O_2 treatment evidenced by a) graphed densitometry of a p21 immunoblot, expression at baseline and a significant increase in p21 expression after H_2O_2 treatment. b) IL-6 production as measured by ELISA, the increased IL-6 expression of treated LFs indicates a shift in excretory profile. c) β-Galactosidase content of fibroblasts at baseline and after H_2O_2 treatment visualised through a cytochemical assay in which β-Galactosidase stains blue. Mitochondrial analysis revealed increased basal respiration (d) and increased proton leak (e). **Panel 2**: STAT3 inhibition reduced levels of p21 (2a) suggesting fewer cells in cell cycle arrest in culture. IL-6 production (2b) is reduced, as is β-Galactosidase content (2c). Mitochondrial respiration decreased through a reduction in basal respiration (d) and decreased proton leak (e).

STAT3 activation reinforces senescence in human lung fibroblasts

Idiopathic pulmonary fibrosis (IPF) is a chronic fibrosing interstitial pneumonia of unknown cause and has a median survival of only 3 years after diagnosis. We have shown that fibroblasts derived from IPF-lungs clearly display characteristics of senescent cells. The question of whether the senescent phenotype can be modulated to restore normal fibroblast function remains unanswered. Dysregulated activation of the transcription factor STAT3 has previously been shown to correlate with IPF progression and has the potential to drive the phenotypic divergence observed in lung fibroblasts (LFs) from IPF patients. We hypothesise that inhibiting STAT3 activation after induced senescence will attenuate characteristics of the senescent phenotype. **Aim**: To determine the effect of inhibiting STAT3 activation on the development of fibroblast senescence.

Methods: Primary cultures of LFs were established from macroscopically normal lung tissue of thoracotomy patients. Senescence was induced by exposing cells to H_20_2 (150 µM) for 2hr and confirmed by measuring senescence-associated-β-galactosidase (SA-β-Gal), IL-6 production, and cell-cycle arrest protein p21 by cytochemistry, ELISA, and immunoblotting respectively. STAT3 activation was inhibited using the pharmacological inhibitor STA-21 (10 µM), cellular characteristics were subsequently assessed via Immunoblot, fluorescence, and the Seahorse Mito Stress test.

Results:. The induction of senescence resulted in increased SA-β-Gal activity, p21 levels, and IL-6 production. Mitochondrial analysis revealed evidence of increased mitochondrial respiration in senescent fibroblasts characterized by increased basal respiration, proton leak and an associated increase in superoxide production. Targeting STAT3 activity after senescence induction attenuated IL-6 production and prevented senescence-associated increases in mitochondrial-respiration.

Conclusion: The results of this study suggest that induced senescence involves the activation of STAT3. **Future perspectives:** To determine whether senescent LFs are pro- or anti-fibrotic and to assess the impact of senescent LFs on non-senescent LFs.

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