**A high-throughput cell culture model of Idiopathic Pulmonary Fibrosis (IPF) for gene therapy applications**

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Idiopathic Pulmonary Fibrosis (IPF) is a chronic, progressive, and lethal disease of the lower respiratory tract. Emerging evidence suggests that repeated injury to the alveolar region could result in irreversible Epithelial to Mesenchymal Transition (EMT), activation of pulmonary fibroblasts and pathological deposition of extracellular matrix (ECM). This accumulation of ECM and fibronectin leads to stiffness in the lungs making it increasingly difficult to facilitate gas exchange, leading to respiratory failure.

We would like to develop a gene therapy for IPF but the lack of suitable models to recapitulate this complex disease is limiting and the underlying cellular/molecular mechanisms remain elusive. Use of the bleomycin-induced mouse model has identified some potential therapeutic compounds but few have shown beneficial effects in clinical trials. The standard TGFβ1-induced cell model makes only a limited contribution to the fibroblast activation signature and fails to upregulate some hallmarks of the disease including Actin Alpha 2 (*ACTA2*), relevant Mucin genes such as Mucin1 and Mucin16 (*MUC1, MUC16*) and Matrix Metallopeptidase 7 (*MMP7*), thought to be key effectors of collagen deposition and biomarkers of disease progression.

Our goal is to generate an *in vitro* model that can better represent key features of IPF and can be used for investigating new therapeutic modalities. We previously developed an air-liquid interface (ALI) cell culture model based on human lung adenocarcinoma cell line (H441) that recapitulates human lung parenchyma markers (Munis et al 2021 PMC7782204). Here, we adapt this model to study pathophysiological mechanisms of IPF. Human H441 cells grown in transwells with basal culture media lacking dexamethasone were air-lifted to differentiate at the ALI to allow the cells to acquire a phenotype similar to alveolar Type II (ATII) cells in the lung parenchyma. An IPF phenotyope was induced via exposure to cytokines relevant to tissue repair mechanisms (IL-25, IL-33, TSLP) as well as TGFβ1. RNASeq studies were performed (1E+05 cells per group, n=6 replicates) and libraries prepared and sequenced (NextSeq, Oxford Genomics Centre) using paired-end 150 bp reads. Differential expression

analysis was then performed to compare the induced samples (IL-25, IL-33, TSLP ± TGFβ1) against the naïve control.

As expected, RNAseq data revealed that addition of TGFβ1 can upregulate some key pathways involved in the progression of IPF such as EMT, Wnt and Notch. Importantly, the addition of type 2 cytokines (IL-25, IL-33, TSLP) upregulated many hallmarks of the condition including: *ACTA2, MUC1, MUC16, PCOLCE* and *FOXA1* genes (Log2 fold change 0.42, 0.72, 1.56, 0.46, and 0.29, with adjusted p-values of 4.50E-02, 6.12E-15, 5.91E-17, 2.67E-04, and 1.05E-04 respectively) compared with the naïve control, which TGFβ1 alone failed to upregulate. Interestingly, with addition of type 2 cytokines there was also a trend for upregulation of MMP7 (log2 fold change 0.35), which is a profibrotic mediator and important predictive biomarker for IPF disease progression. Key changes in RNA levels are currently being validated by qPCR assay through the $2^{-\Delta\Delta CT}$ method and protein levels such as fibronectin will be assessed by western blot.

In summary, a well characterised and patho-physiologically relevant model is needed for understanding IPF. Our human ALI culture model of lung parenchyma provides a new platform for modelling aspects of the formation and progression of IPF at the molecular (RNA and protein) level. It may also provide an opportunity to develop opportunities for screening of novel antifibrotic agents and validating gene therapy interventions.