Single-cell RNA sequencing identifies G-protein coupled receptor 87 as a basal cell marker expressed in distal honeycomb cysts in idiopathic pulmonary fibrosis

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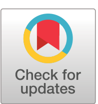

Shareable abstract (@ERSpublications)
Bronchiolisation and honeycombing are features of IPF. ScRNA sequencing identified GPR87 as a novel marker of basal cells in IPF, enriched in honeycomb cysts. GPR87 overexpression resulted in aberrant airway cell differentiation. https://bit.ly/3i4dXeT

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To the Editor:

Idiopathic pulmonary fibrosis (IPF) is a devastating and life-threatening lung disease characterised by epithelial reprogramming and increased extracellular matrix deposition leading to loss of lung function. Prominent histopathological structures in the distal IPF lung include honeycomb cysts in the alveolar space [1]. These are heterogeneous bronchiolised areas that feature clusters of simple epithelium with keratin (KRT)5^{+} basal-like cells interspersed with pseudostratified epithelium containing differentiated, hyperplastic epithelial cells, as well as aberrant ciliated cells [2–5]. Recent single-cell RNA sequencing studies of whole lungs from IPF and donor tissue revealed cellular subtypes unique to IPF, including basaloid KRT5^{-}/KRT17^{+} cells present in the distal lung [6–10]. However, IPF distal bronchiole KRT5^{+} basal cell subtypes still remain poorly characterised and their disease contribution remains under-investigated. Here, we report *G-protein coupled receptor (GPR) 87* as a marker of distal bronchioles and KRT5^{+} basal-like cells in IPF. We generated single cell transcriptomes from EpCAM^{+} cells isolated from parenchymal lung tissue from three IPF patients and three age-matched healthy donors. In short, fresh non-fixed human lung tissue from de-identified healthy donors and explants from IPF patients with end-stage disease was received from National Jewish Hospital/UC Health University of Colorado Hospital (Denver, CO, USA) (COMIRB 11–1664). Right lower or middle lobes of healthy donor (n=3, two males both aged 66 years, and a 68-year-old female) and IPF patient tissue (n=3, two males aged 45 and 65 years, and a 68-year-old female), respectively, were used. All tissues were obtained from non-smokers. Human lung tissue was homogenised and tissue was digested by dispase/collagenase (collagenase: 0.1 U·mL^{-1}; dispase: 0.8 U·mL^{-1}; Roche). Samples were successively filtered through nylon filters

(100 µm and 20 µm) followed by a percoll gradient and CD45 MACS sorting (Miltenyi Biotec). After FACS, $EpCAM^+/DAPI^-$ live single epithelial cell suspensions were used for single-cell RNA sequencing (scRNAseq). Detailed single cell methodology and data processing and analysis is reported in the GitHub repository (https://github.com/KonigshoffLab/GPR87_IPF_2022). The raw data have been deposited in NCBI's Gene Expression Omnibus with accession number GSE190889. Using the 10x Genomics platform, we generated a dataset of 46 199 cells and found nine distinct cell clusters, including main progenitor cell types of the alveolar region and distal airways as well as rare cell types, such as suprabasal cells, recently reported in the healthy lung (figure 1a) [11]. Cells from both conditions were found in all clusters with differentially distributed clusters between healthy and IPF (figure 1b). In line with previous single cell data [6–8], ciliated cells were predominantly found in IPF while ATII cells were largely present in non-diseased lungs, further suggesting a loss of ATII cells and distal bronchiolisation in IPF. Honeycomb cysts are an important histopathological criteria for the diagnosis of IPF; however, mechanistic insight in the process of bronchiolisation and remodelling of the terminal bronchiole in IPF remains scarce. To shed light into cell populations potentially contributing to honeycomb cysts, we analysed differentially expressed genes in all epithelial clusters and found cytokeratins such as *KRT6A*, *KRT5*, *KRT17*, and *KRT15* among the most upregulated genes in IPF (figure 1c). KRT5 is a well-characterised marker of basal and suprabasal cells, and $KRT5^+$ cells strongly accumulate in distal IPF lung tissues, mostly in areas of honeycombing [3, 4, 12]. To further identify cellular surface markers and potential pharmacological targets that might be expressed in $KRT5^+$ cells, we analysed transmembrane signalling receptors (GO:0004888) in all epithelial cells and found *GPR87*, a G-protein coupled receptor with unknown function in IPF, to be one of the highest regulated transcripts (figure 1c). Importantly, when we analysed transmembrane signalling receptors specifically in the (supra) basal cell population across individual tissue samples, we observed a strong and robust increase of *GPR87* (figure 1d). A limitation of our scRNASeq dataset is the small sample size used for scRNASeq (n=3 each); thus, we further confirmed upregulation of *GPR87* in (supra) basal cells in comparison to other epithelial cells not only in our own (figure 1e) but in two additional independently published datasets (figure 1f) [6, 8]. Notably, GPR87 showed further enrichment in basaloid $KRT5^-/KRT17^+$ cells, a cell type which we did not detect in our dataset (figure 1f).