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

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 basoloid 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 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...
a) Ciliated
Deuterosomal
Suprabasal
Basal
AT1
Cycling
Club
Goblet
AT2

b) AT1
AT2
Ciliated
Deuterosomal
Basal
Suprabasal
Cycling
Club
Goblet

Control–1
Control–2
Control–3
IPF–1
IPF–2
IPF–3

KRT5
KRT17
GPR87

Fraction of cells
in group (%)

Mean expression
in group

0.0
0.5
1.0

KRT17
GPR87

Fraction of cells
in group (%)

Mean expression
in group

0.0
0.5
1.0

GPR87

Fraction of cells
in group (%)

Mean expression
in group

0.0
0.5
1.0

KRT5
KRT17
GPR87

Fraction of cells
in group (%)

Mean expression
in group

0.0
0.5
1.0

i) ii) iii) iv)

Transforming growth factor beta production
Keratinisation
Interleukin-1 production
Extracellular matrix organisation
Extracellular structure organisation
Epithelial cell differentiation
Epithelial cell proliferation
Cytokine production
Tissue development
Epithelium development
Cell adhesion
Regulation of cell differentiation
Cell surface receptor signalling pathway
Signal transduction

Transduction Ctrl/GPR87 vector
Submerged
Air-liquid interface
Day –7 to –1
Day 0 to Day 20
Day 21 to Day 28
(–/+ TGF-beta (K))

10
30
70
170

Gene overlap

Log10(p-value)

–3
–4
–5
–6

Gene ratio

0.05 0.10 0.15

Fold change (relative to control)

Low
High

p=0.0571
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 dataset (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).

We focused on GPR87 for our subsequent studies for several reasons: First, it belongs to the class of G-protein coupled receptors, which are intensively studied drug targets with attractive pharmacological accessibility. Second, although classified as an orphan receptor, profibrotic ligands have been discussed, such as lysophosphatidic acid [13]. Third, GPR87 has been linked to aberrant cell cycle control [14, 15], which is a feature of epithelial reprogramming and bronchiolisation/honeycomb cyst development in IPF [1]. Thus, we aimed to investigate GPR87 expression within the distal IPF lung and its potential contribution to airway cell differentiation and bronchiolisation in IPF.

We confirmed GPR87 epithelial cell expression and distribution within the IPF lung in situ using fluorescent immunolabelling and RNASeq of human tissue section as previously described [4, 16]. RNAscope detected GPR87 RNA in KRT5+ cells in areas of bronchiolisation and honeycomb cysts in distal IPF tissue sections, respectively (figure 1g: arrowheads). The GPR87 RNA was also found in KRT5+/KRT17+ cells (figure 1g; open triangles). GPR87 function was further investigated in an air–liquid interface (ALI) cell culture model of primary human bronchial epithelial cells (HBECs), mimicking in vivo-like differentiation of basal cells to
more mature cell types, including ciliated and secretory cells (figure 1i) [4, 16]. GPR87 was expressed in KRT5+ basal cells of our human ALI culture (figure 1j). Transforming growth factor (TGF)-β treatment, inducing fibrotic epithelial reprogramming, led to increased GPR87 expression in mature ALI cultures (figure 1k). This was consistent with the functional annotation enrichment analysis of our scRNAseq data, which revealed tissue development, keratinocyte differentiation and extracellular matrix remodelling, as well as TGF-β production; all indicative of altered epithelial airway differentiation and integrity, to be correlated with GPR87 (figure 1j). Moreover, GPR87 overexpressing HBEcS cultured at ALI displayed impaired differentiation of KRT5+ cells into mature airway cells evidenced by altered epithelial structure and a decrease in cilia coverage (mean±SD: 27.65±6.21% for the control, compared to 12.90±4.47% for the GPR87 overexpression) (figure 1m).

Our data suggest that overexpression of GPR87 leads to impaired airway cell differentiation of KRT5+ basal cells, and thus support the hypothesis that GPR87 might contribute to bronchiolisation and honeycomb cyst formation. It will be important to further study the functional consequences of GPR87 expression in basal cells in vivo and to analyse whether inhibition of GPR87 would be able to revert impaired airway cell differentiation and prevent TGF-β induced fibrotic reprogramming, thus serving as a potential therapeutic target.

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