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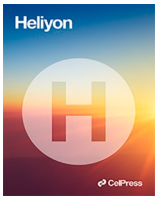

Research article

Disease-specific transcriptional programs govern airway goblet cell metaplasia

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ABSTRACT

Hypersecretion of airway mucus caused by goblet cell metaplasia is a characteristic of chronic pulmonary inflammatory diseases including asthma, cystic fibrosis (CF), and chronic obstructive pulmonary disease (COPD). Goblet cells originate from airway progenitor club cells. However, the molecular mechanisms and features of goblet cell metaplasia in lung disease are poorly understood. Herein, public single-cell RNA sequencing datasets of human lungs were reanalyzed to explore the transitional phase as club cells differentiate into goblet cells in asthma, CF, and COPD. We found that changes in club and goblet cells during pathogenesis and cellular transition were associated with signalling pathways related to immune response, oxidative stress, and apoptosis. Moreover, other key drivers of goblet cell specification appeared to be pathologically specific, with interleukin (IL)-13 and hypoxia inducible factor 1 (HIF-1)-induced genetic changes in asthma, cystic fibrosis transmembrane conductance regulator (CFTR) mutation being present in CF, and interactions with $CD8^+$ T cells, mitophagy, and mitochondria-induced apoptosis in COPD. In conclusion, this study revealed the similarities and differences in goblet cell metaplasia in asthma, CF, and COPD at the transcriptome level, thereby providing insights into possible novel therapeutic approaches for these diseases.

1. Introduction

Mucus is a gel composed of water, mucins, lipids, and cell debris. Mucus traps bacteria, allergens, and other harmful substances and removes them from the lungs through ciliary activity [1,2]. Airway obstruction caused by mucus hypersecretion and plugging contributes significantly to the high morbidity and mortality of chronic lung diseases including asthma, cystic fibrosis (CF), and chronic obstructive pulmonary disease (COPD), which share a common phenotype of goblet cell metaplasia [3–5].

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Goblet cells originate from airway progenitor club cells that express secretoglobin family 1A member 1 (SCGB1A1) [6]. Several transcription factors (TFs) are pivotal in regulating goblet cell transition, such as SAM-pointed domain-containing ETs-like factor (SPDEF), forkhead box A3 (FOXA3), FOXA2, and NK2 homeobox 1 [7]. However, it remains unclear whether control of goblet cell fate is identical to asthma, CF, and COPD. In addition, it is difficult to precisely capture and study the transitional phase of the club-to-goblet cell transition.

Single-cell RNA sequencing (scRNA-seq) is a powerful and rapid tool for high-throughput sequencing analysis of transcriptomes at the single-cell level. It can identify genetic differences among thousands of cells in multiple samples. The advent of scRNA-seq has enabled us to better understand dynamic gene expression within and heterogeneity between related cell types in diseased human lungs [8]. Herein, we integrated five public scRNA-seq datasets from human lungs to analyse the transcriptional similarities and differences of goblet cell metaplasia in asthma, CF, and COPD. Our results clarify the mechanism of airway mucus production and expression of signaling pathway genes, and suggest possible therapeutic targets for these diseases.

2. Materials and methods

2.1. Asthma, CF, and COPD datasets

scRNA-seq data from bronchoscopies of six healthy controls and six patients with asthma from the proximal airways (third to sixth generations of the right lower and middle lobe) were kindly provided by FA Vieira Braga [9]. scRNA-seq data of proximal airway epithelia (GSE150674) from 20 healthy controls and 22 patients with CF were downloaded from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo) [10]. scRNA-seq data from lung distal parenchymal samples (GSE136831, GSE173896, and GSE168191) from 39 healthy controls and 28 patients with COPD were downloaded from the GEO database [11–13]. The demographics and clinical data of the patients are shown in Supplementary Table 1.

2.2. Data processing

The expression matrices and patient metadata of the five datasets were downloaded and analysed using R software (version 4.2.1). Doublets of raw data (GSE168191 and GSE173896) were removed using the DoubletFinder package (version 2.0.3) before analysis. Doublets in other datasets were previously removed and were not considered in this study. The Seurat package (version 4.1.1) was used for subsequent analysis [14]. Briefly, batch effects between the datasets and samples were removed using the harmony package (version 0.1.0) [15]. Cells with $<$200 and $>$6000 genes or those composed of $>$20 % mitochondrial genes were considered low-quality cells and were excluded from the asthma and COPD datasets. For the CF dataset, quality control had already been performed on the downloaded data, therefore cell filtering was not conducted. Then, epithelial cells ($EPCAM^+$ and $CDH1^+$) in the five datasets were then integrated and analysed using the standard workflow of the Seurat package (normalisation, dimension reduction, and clustering) with default parameters (R codes are provided in the Supplementary Materials). Canonical marker genes were used to annotate the cell types in the dataset [16–18]. Club and goblet cells from the three diseases were extracted from the integrated data for downstream analysis.

2.3. Cell trajectory analysis

Cell trajectory analysis measures the transcriptional differences in individual cells during cell transition and ranks each unit according to its progress on the trajectory. Herein, the monocle package (version 2.20.0) was used for cell trajectory analysis of club-to-goblet cell transition in the three diseases [19]. The genes along the cell differentiation trajectory (CTG) were determined based on a q-value $<$0.01. Club cells were defined as the root of the cell differentiation trajectory in each dataset.

2.4. Pathway enrichment analysis

The club and goblet cells were reclassified according to the cell order from the cell trajectory analysis. To study goblet cell metaplasia in the three diseases, club and goblet cells were analysed for differentially expressed genes (DEGs). The DEGs in airway club and goblet cells from the disease groups compared with cells from the control groups were identified using the run_de function of Libra (version 1.1.0) [20]. |Fold change (FC)| $>$1.5 and an adjusted p-value $<$0.05 were used to screen DEGs. Gene Ontology biological process (GO BP), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of the DEGs were performed using the Database for Annotation, Visualisation and Integrated Discovery (DAVID, 2021 Update) website with default parameters. The top five upregulated and downregulated DEGs of club and goblet cells for each disease were visualised using volcano diagrams.

2.5. Transcriptional regulatory network analysis

To determine the crucial TFs among the CTGs of the disease and control groups, club and goblet cells were used to perform standard single-cell regulatory network inference and clustering (SCENIC version 1.3.1) analysis [21]. Regulons (TFs and their target genes) were identified using the GENIE3 package (version 1.18.0). The AUCell package (version 1.18.0) was used to score the regulatory activity of TFs in each disease. Regulons with scores beyond the calculated thresholds were considered active regulons and were retained in the regulatory network. Eventually, active TFs and target genes that met the screening criteria (|FC| $>$1.2 and adjusted

p-value <0.05) were visualised using Cytoscape software (version 3.9.1) [22].

2.6. Transitional state analysis of the CTG

To further understand the CTGs in the three diseases, the genes obtained from cell trajectory analysis were divided into three states (start, middle, and end) using the monocle package. The CTGs in the middle state, which represent the differentiating state were pulled out for pathway enrichment analysis by DAVID. GO BP terms and KEGG pathways with Kappa scores of >0.4 were visualised, and a two-sided hypergeometric test was used to determine whether these terms should be retained, based on an adjusted p-value of 0.05.

2.7. Gene module score and gene set variation analysis (GSVA)

The following 10 pathway gene sets related to club-to-goblet cell transition reported in previous studies were downloaded from the KEGG pathway database (https://www.kegg.jp): GABAergic synapse (hsa04727), WNT signalling pathway (hsa04310), Hedgehog signalling pathway (hsa04340), JAK-STAT signalling pathway (hsa04630), MAPK signalling pathway (hsa04010), NF-kappa B signalling pathway (hsa04064), NOTCH signalling pathway (hsa04330), PI3K-AKT signalling pathway (hsa04151), TGF-beta signalling pathway (hsa04350), and VEGF signalling pathway (hsa04370). To determine the functional activities of these pathways in our scRNA-seq data, the average score of each airway epithelial cell type was calculated using the corresponding gene set via the AddModuleScore function of the Seurat package and visualised using heatmaps. The normalised expression matrix of club and goblet cells was used for GSVA (version 1.44.1) [23], and the results were visualised using divergent bar plots.

2.8. Statistical analysis

All statistical analyses were calculated using R software. For the DEG analysis, we used the edgeR algorithm [24] based on the run_de function of the Libra package. To compare the cell proportion and regulon activities in different groups, we used the two-sided Student's t-tests via the t.test function of the stats package if the data were normally distributed, and the two-sided Wilcoxon rank-sum test via the wilcox.test function of the stats package if the data were not normally distributed. Statistical significance was set at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

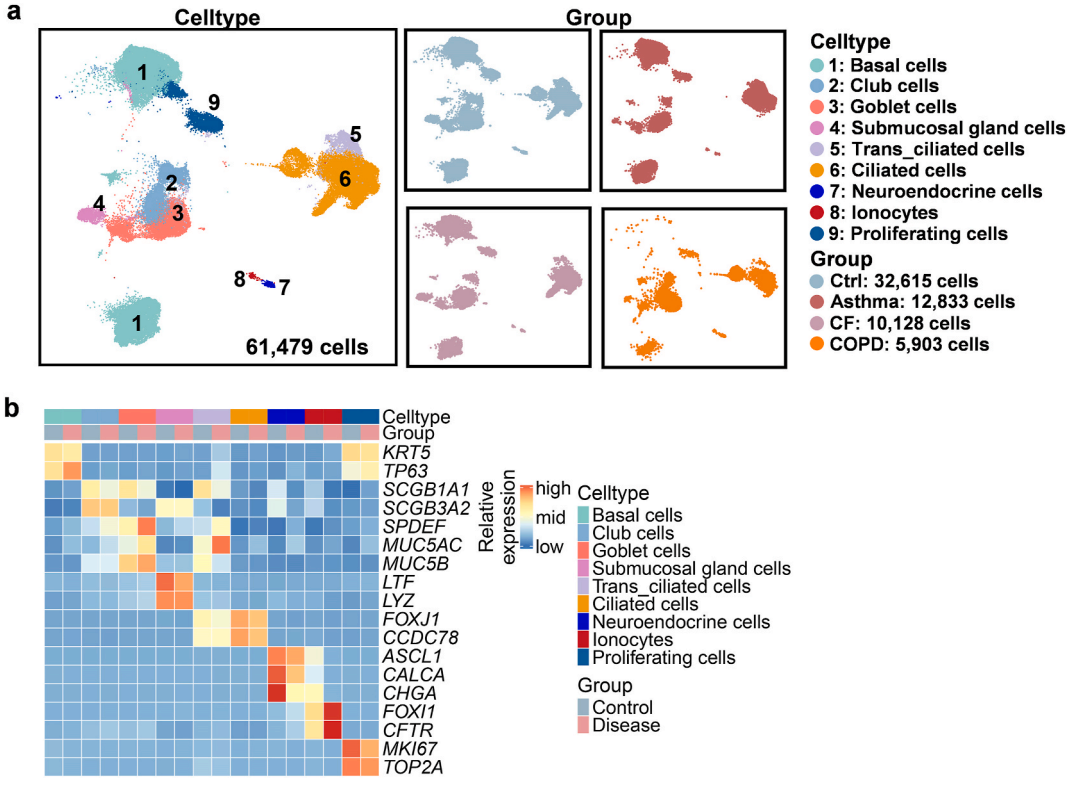

Fig. 1. Single-cell RNA-sequencing analysis of airway epithelial cells in asthma, cystic fibrosis (CF), and chronic obstructive pulmonary disease (COPD). a Uniform manifold approximation and projection (UMAP) graphical representation of 61,479 epithelial cells from control and diseases. b Heatmap of canonical marker gene expression levels in nine airway epithelial cell types.

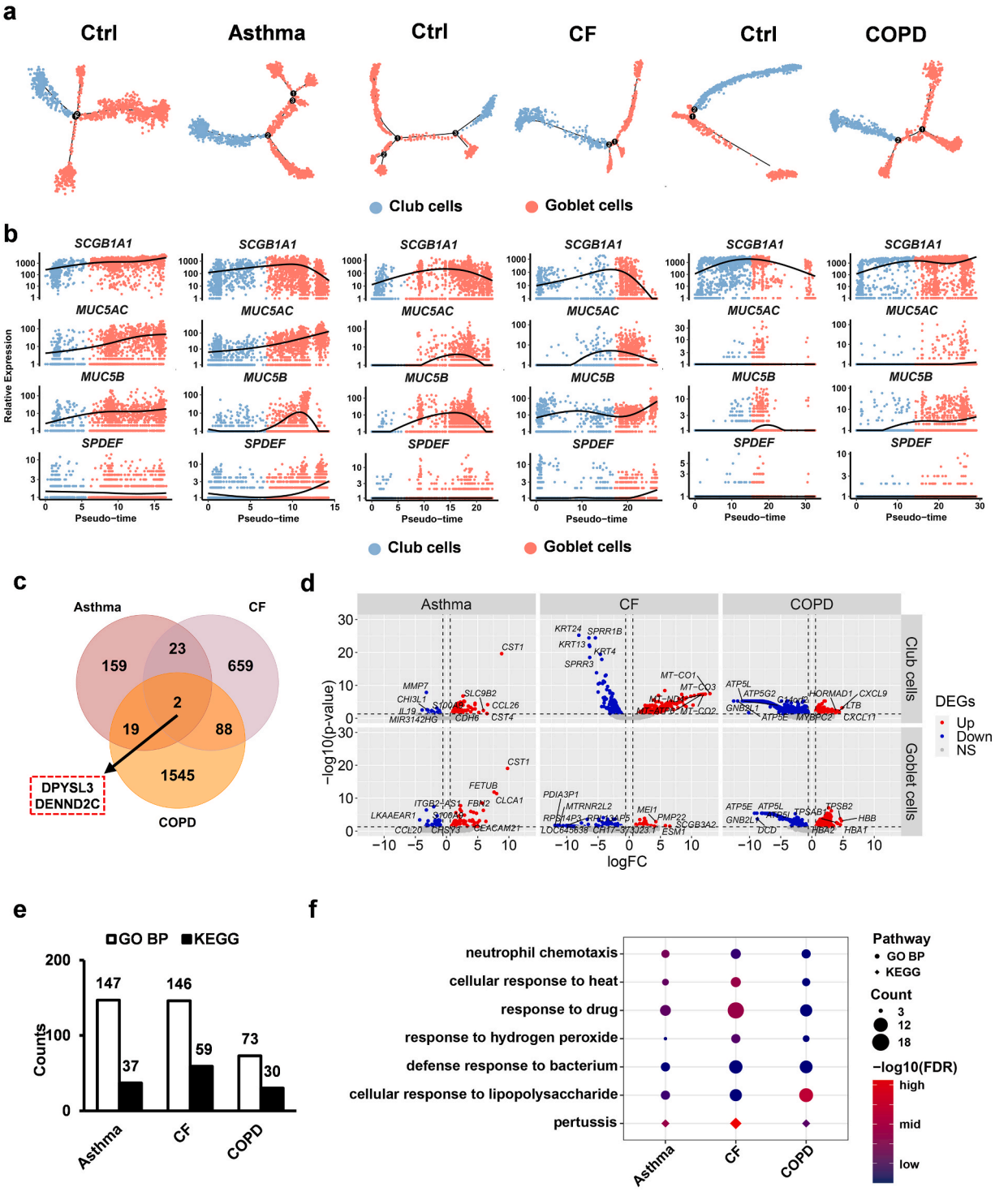

Fig. 2. Transcriptomic changes in club and goblet cells in asthma, cystic fibrosis (CF), and chronic obstructive pulmonary disease (COPD). a The cell trajectory from club to goblet cells in the control and disease group. Each point represents a cell. b The relative expression of SCGB1A1, MUC5AC, MUC5B and SPDEF during club-to-goblet cell transition. c Venn diagram showing the intersections of differentially expressed genes (DEGs) in asthma, CF, and COPD. d Volcano diagrams showing the top five upregulated and downregulated DEGs of club and goblet cells in each disease. e The number of Gene Ontology biological process (GO BP) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched for DEGs from the three diseases. f The common GO BP and KEGG pathways among the three diseases.

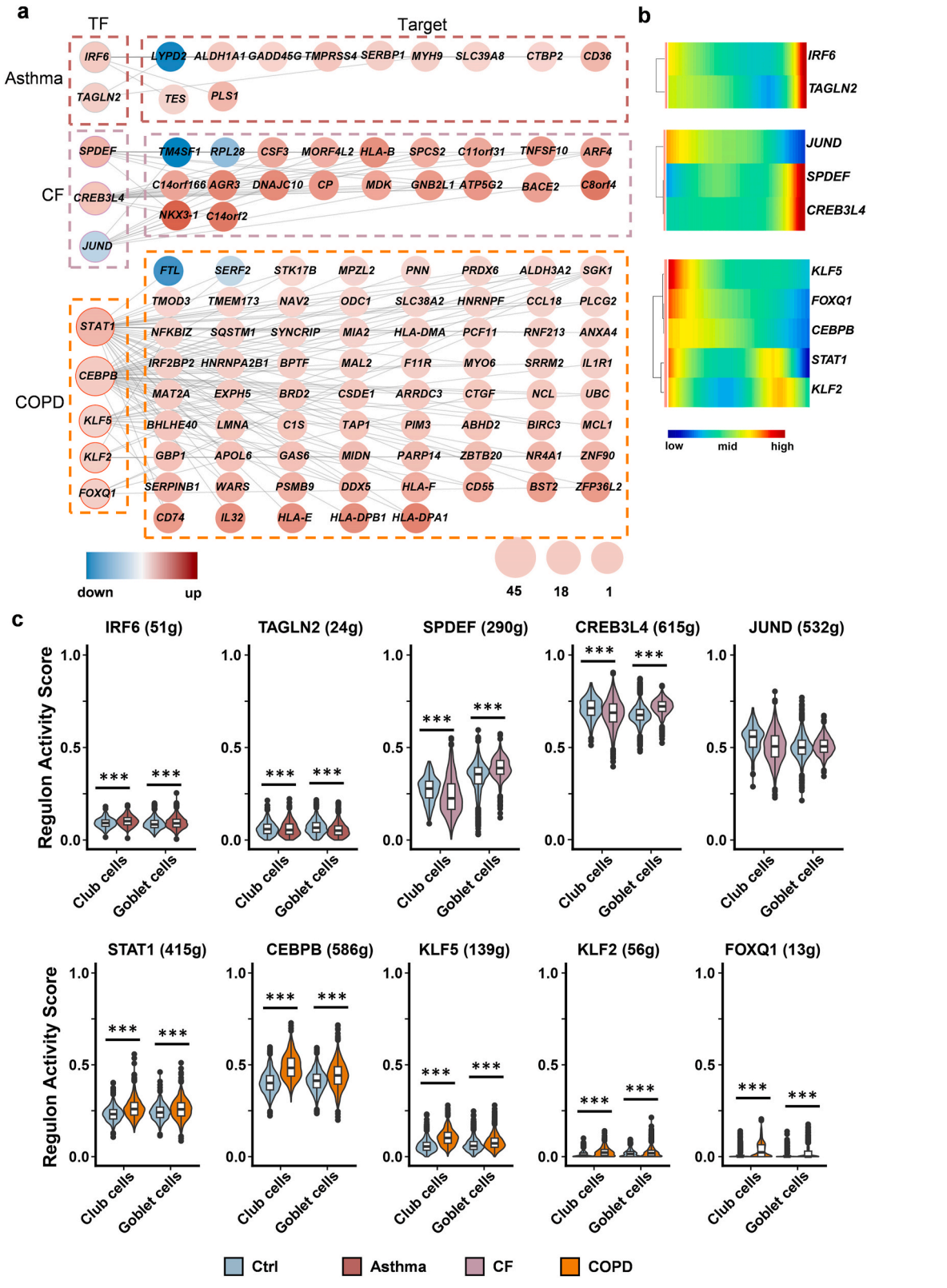

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Fig. 3. Distinct transcription factors (TFs) govern club-to-goblet cell transition in asthma, cystic fibrosis (CF), and chronic obstructive pulmonary disease (COPD). a The network of TFs (left) and their target genes (right) for three diseases. The colours of the nodes represent the fold change in upregulation (red) and downregulation (blue) of genes in the disease group compared with the control group. The size of the TF nodes indicates for the number of their target genes. b Heatmaps of TF expression along the cell differentiation trajectory in each disease. c Violin plots showing the activities of the TF regulatory units in club and goblet cells of the three diseases. ***$p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3. Results

3.1. scRNA-seq analysis of airway goblet cell metaplasia

Goblet cell metaplasia is commonly found in chronic airway inflammatory diseases such as asthma, CF, and COPD. To further understand the similarities and differences in the molecular mechanisms that regulate goblet cell metaplasia, we retrieved and analysed scRNA-seq data collected from the lungs of patients with these three diseases. After removing low-quality cells and batch effects, the standard workflow of the Seurat package was used for downstream analysis. The epithelial cells ($EPCAM^+$ and $CDH1^+$) of five datasets were first integrated and clustered, and airway epithelial cells from each disease were selected for further analysis. Airway epithelial cells from the three diseases totalled 61,479 cells (control: 32,615 cells, asthma: 12,833 cells, CF: 10,128 cells, COPD: 5,903 cells) and were divided into nine cell types according to canonical marker genes (Fig. 1a and b), including seven normal clusters comprising basal cells ($KRT5^+$ and $TP63^+$), club cells ($SCGB1A1^+$ and $SCGB3A2^+$), goblet cells ($MUC5AC^+$, $MUC5B^+$, and $SPDEF^+$), submucosal gland cells (LYZ^+ and LTF^+), ciliated cells ($FOXJ1^+$ and $CCDC78^+$), neuroendocrine cells ($CALCA^+$, $CHGA^+$, and $ASCL1^+$), and ionocytes ($CFTR^+$ and $FOXI1^+$); one transitional cluster comprising *trans*-ciliated cells ($SCGB1A1^+$, $MUC5AC^+$, and $FOXJ1^+$); and one proliferating cluster comprising proliferating cells ($MKI67^+$ and $TOP2A^+$). Furthermore, the proportion of each type of lung epithelial cell was analysed in patients with asthma, CF, and COPD (Supplementary Fig. 1).

3.2. Disease-specificity of the club-to-goblet cell transition trajectory

To avoid the clinical-group batch effect and determine the real differences in club-to-goblet cell transition between the disease and control groups, cell trajectory analysis was performed separately in different clinical groups using the monocle package. We annotated club and goblet cells in our data according to the cell order calculated by monocle (Fig. 2a and b). We then used the pseudo bulks method [20] for DEG analysis of club and goblet cells in each disease individually. The DEGs of the three diseases were combined, and the results showed 203 DEGs for asthma, 772 for CF, and 1654 for COPD (Supplementary Table 2). DPYSL3 and DENND2C were the common DEGs in all three datasets (Fig. 2c). The top five upregulated and downregulated DEGs of each disease were visualised using volcano plots (Fig. 2d). In asthma, CST1, CST4, and CCL26 levels were increased in club cells, and CST1, FETUB, and CLCA1 levels were upregulated in goblet cells. In CF, club cells showed high expression levels of mitochondrial genes. In COPD, CXCL9 and CXCL11 levels were increased in club cells, while the levels of ATP5E, ATP5I, ATP5L and other genes encoding adenosine triphosphate (ATP) synthase were decreased in club and goblet cells.

We then conducted enrichment analysis using the DEGs to identify possible biological processes and signalling pathways associated with each disease. The number of enriched terms was higher in asthma and CF than in COPD (Fig. 2e), indicating that the alteration of club and goblet cells in COPD was more complicated and might be associated with multiple physiological activities. Thereafter, we performed intersection analysis using the enrichment results of the three diseases and extracted the top 10 pathways of DEG enrichment for each disease (Supplementary Tables 3 and 4). We found that the mutually enriched terms in the three diseases were mainly involved in neutrophil chemotaxis, response to lipopolysaccharide, defence response to bacterium, and response to hydrogen peroxide (Fig. 2f).

3.3. Distinct TFs regulate the goblet cell transition in different lung diseases

To study the TFs that control the goblet cell transition in the three lung diseases, we performed the single-cell regulatory network inference and clustering (SCENIC) workflow with disease-specific CTGs and set the selection criteria as: $|FC| > 1.2$ and and adjusted p-value < 0.05. We identified two active TFs in asthma, three in CF, and five in COPD (Fig. 3a). Along the asthmatic cell differentiation trajectory, IRF6 and TAGLN2 were highly expressed in the end state. In CF, JUND was initially highly expressed, whereas SPDEF and CREB3L4 were mainly concentrated in the end state. Additionally, in COPD, the KLF5, FOXQ1, and CEBPB played roles in the initial transition state, while STAT1 and KLF2 were expressed at the initiation and intermediate stages (Fig. 3b). The activity scores of the TF regulatory units suggested that most regulons may be more active in the goblet cells of the disease groups compared with the control group (Fig. 3c); therefore, these regulators may be important targets for biological therapy. To map the TFs of club and goblet cells in the steady state, we extracted CTGs related to the transition of club cells to goblet cells in normal samples of the three datasets. TFs that may be responsible for goblet cell transition at the steady-state included CREB3L1, FOSB, GTF2B, MAFB, and CHD2 (Supplementary Fig. 2A, Supplementary Table 5).

3.4. Key pathways govern the transition of the goblet cell transition trajectory

The dynamic expression patterns of CTGs along the pseudotime trajectory from club cells (start) to club-goblet transitional cells (middle) and goblet cells (end) are presented as pseudotemporal expression heatmaps (Fig. 4a). We then focused on the CTGs in the middle state, which might be vital in the transitional cell state during the club-to-goblet cell transition, particularly in the disease groups [25] (Fig. 4a). Subsequently, many complex pathways were identified using DAVID. We summarised the results with similar functions and compared the summarised pathways of the enrichment results based on the 'GeneRatio' (Supplementary Table 6). Several pathways were observed in at least two datasets, and 15 major pathways associated with apoptosis, cellular energy metabolism, immune defence response, phagocytosis, metal ion-related signalling, response to stimulus, response to hormone, response to hypoxia, oxidative stress and retina homeostasis appeared in all three diseases (Fig. 4b and c). In addition, the top major pathways unique to each disease are shown in Fig. 4d, and the results showed that the CTGs upregulated in the asthmatic middle state were primarily involved in signal transduction, digestive system processes, and protein kinase B and HIF-1 signalling pathways. In CF, they were mainly associated with the cell cycle; NF-kappa B transcription factor activity; cytokine-cytokine receptor interaction; cell migration; protein kinase A; tumor necrosis factor (TNF); IL-17; and transforming growth factor (TGF)-beta signalling pathways. In

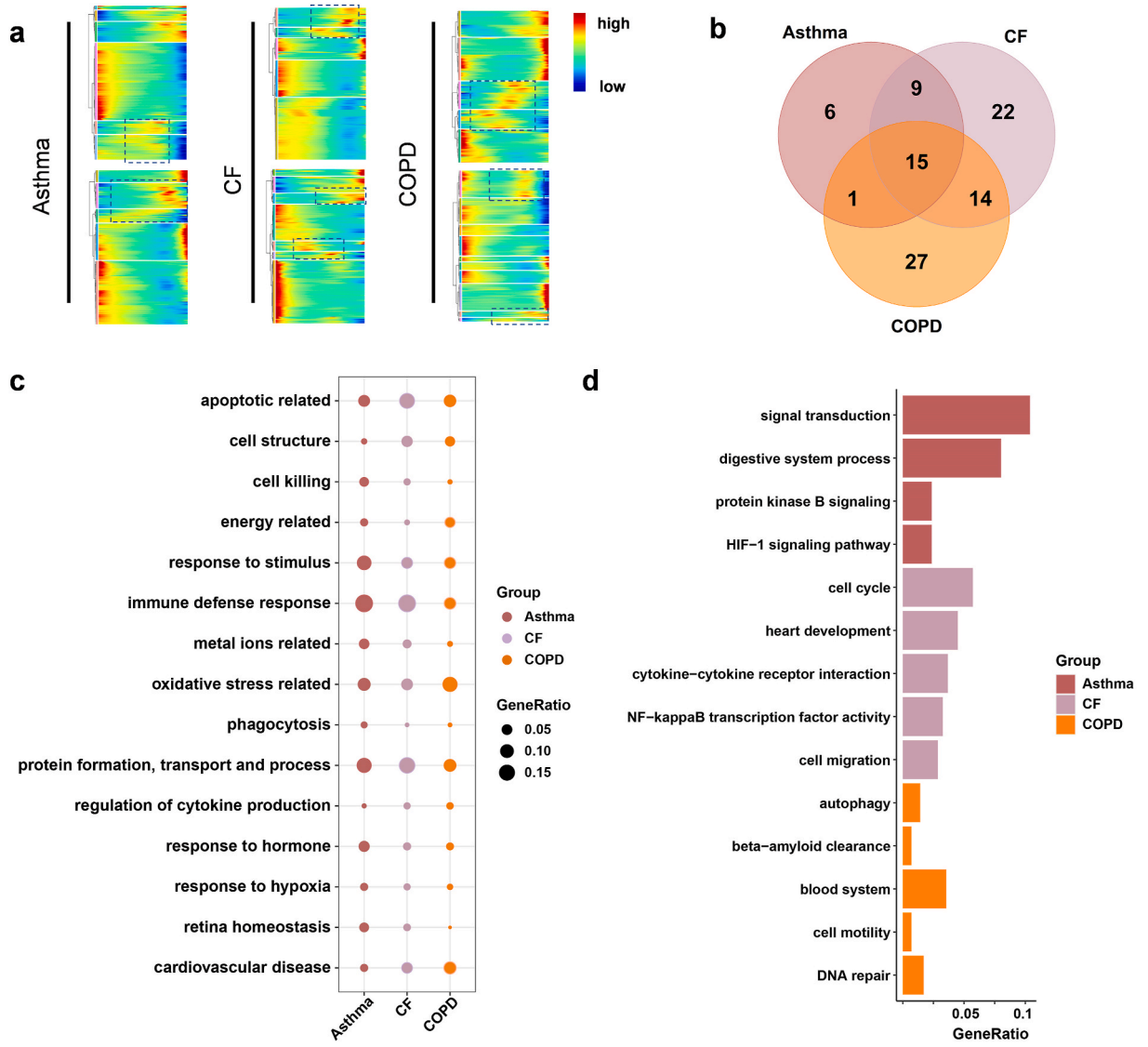

Fig. 4. Enrichment analysis of the genes highly expressed in the club-to-goblet transitional state in asthma, cystic fibrosis (CF), and chronic obstructive pulmonary disease (COPD). a Heatmaps of CTGs in the control (up) and disease (down) groups of the three datasets along the cell differentiation trajectory. The dashed box marks the middle state of the trajectory. b The number of summarised GO BP and KEGG pathways enriched by the DAVID website using the middle-state CTGs in three diseases. c The common summarised pathways among the three diseases. d Top summarised pathways according to 'GeneRatio' of the enrichment results for each disease.

COPD, they were mainly associated with metabolic pathways, interaction with T cells, autophagy, mitophagy, and mitochondria-induced apoptosis (Supplementary Table 7). In addition, signalling pathways related to the transition of club cells to goblet cells in normal lungs are shown in Supplementary Table 8 and Supplementary Fig. 2B. These data suggested that the goblet cell transition is regulated by distinct programs, in addition to common oxidative stress pathways in asthma, CF, and COPD.

3.5. Module analysis and GSVA of the pathways involved in goblet cell transition

Goblet cell metaplasia in the lung airway epithelium has been reported in many studies, but its molecular mechanism is poorly understood. We summarised 10 signalling pathways associated with goblet cell transition from previous studies and conducted gene module analysis and GSVA to determine their performance in club and goblet cells in our datasets (Fig. 5). Except for goblet and club

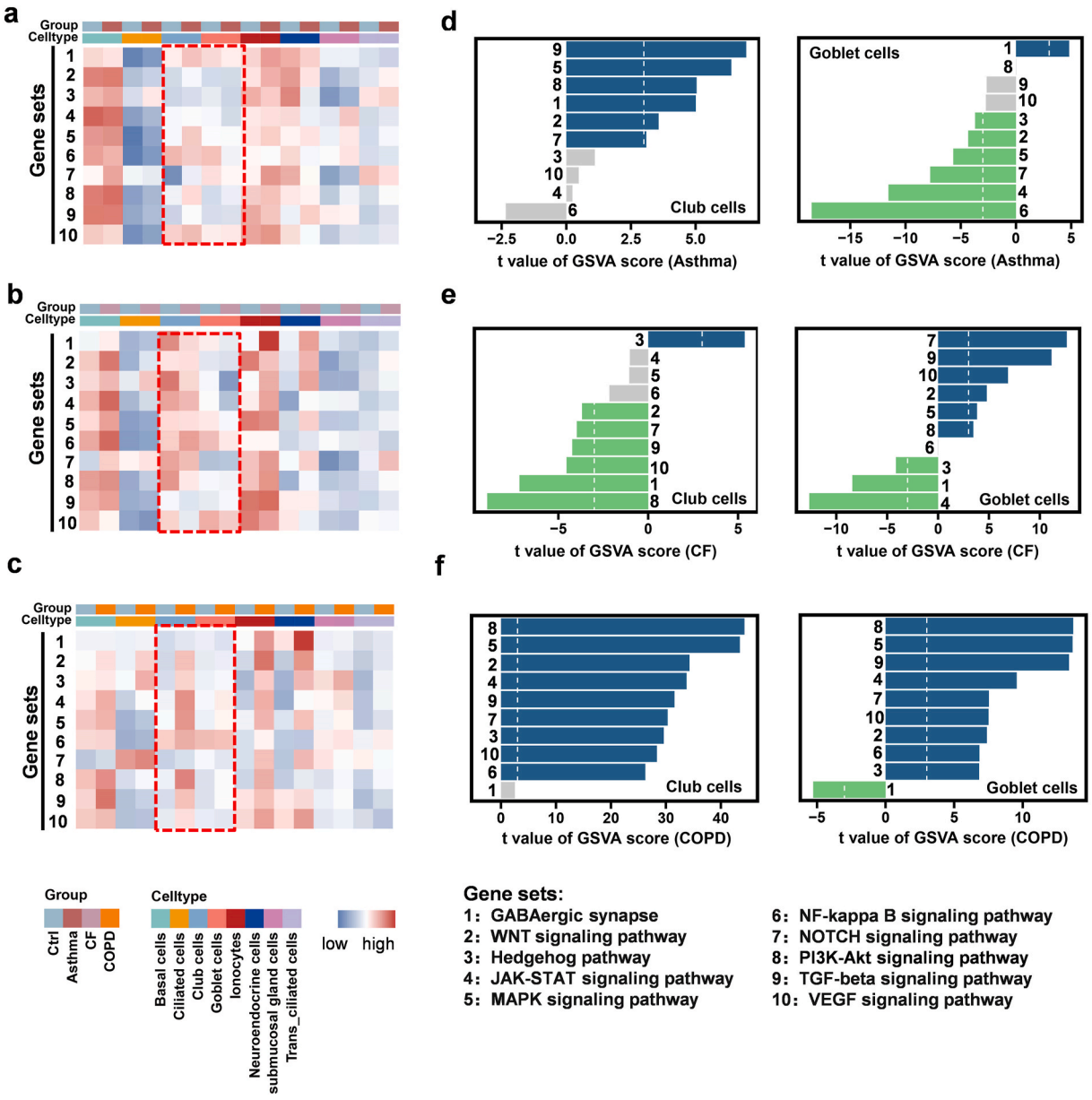

Fig. 5. Behaviour of known signalling pathways associated with club-to-goblet cell transition in our modules. a–c Heatmaps of the average module scores on the airway epithelial cells of the three diseases. The red dashed box marks the club and goblet cells. d–f The GSVA scores of the 10 pathway gene sets in club and goblet cells in asthma, CF, and COPD, respectively. The coloured bars (blue for upregulated; green for downregulated) represent gene set scores that were statistically different between the disease and control groups at a significance level of 0.001. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

cells in asthma and CF, respectively, in all three lung diseases, the TGF-beta signalling pathway was activated, which is consistent with a previous study showing that TGF-beta 1 signalling, especially the SMAD-dependent pathway, promotes goblet cell hyperplasia [26]. The expression level of members of NOTCH signalling pathway was consistent with the expression level of members of the TGF-beta signalling pathway. A study showed that NOTCH signalling promotes goblet cell metaplasia [27], however another study showed the opposite effect [28]. In addition, the results showed that the Hedgehog signalling pathway, which promotes goblet cell metaplasia [29], was significantly activated in club cells in CF, and may play an important role in goblet metaplasia in CF. The upregulated JAK-STAT signalling pathway in club and goblet cells of COPD also promotes goblet metaplasia [30]. Activation of the PI3K-AKT and MAPK pathways is known to be beneficial for goblet cell metaplasia [31,32]. Our results showed that the PI3K-AKT signalling pathway was significantly inhibited in club cells in CF, and the MAPK signalling pathway was inhibited in goblet and club cells in asthma and CF, respectively. GABAergic signalling was inhibited in the CF cells and goblet cells of COPD. Previous studies have demonstrated that blocking the GABA/Gabrp pathway inhibits club cell differentiation, and the GABAergic system is upregulated in the airway epithelium of patients with asthma, which might affect mucus production [33]. The WNT signalling pathway was only inhibited in goblet and club cells in asthmatic and CF, respectively. Activation of the classic WNT/β-catenin pathway could promote goblet cell proliferation by inhibiting FOXA2 [34]. In addition, the NF-Kappa B and vascular endothelial growth factor (VEGF) signalling pathways were significantly activated in club and goblet cells in COPD and may participate in goblet cell transition [35,36]. These results indicate that airway goblet cell metaplasia is directed by different signalling pathways in asthma, CF, and COPD.

In addition, we observed that the activity of the JAK-STAT, MAPK, NF-kappa B, PI3K, TGF-beta, and VEGF pathways were stronger in basal cells than in ciliated cells in all of three diseases (Fig. 5a–c). However, the activity of the NOTCH pathway was greater in ciliated cells than in basal cells in COPD (Fig. 5c). The WNT and JAK-STAT pathways were activated in basal cells in CF and COPD, here as NOTCH activation was observed in basal cells in patients with asthma compared with control patients.

Overall, these results showed the similarities and differences between the three diseases regarding the molecular mechanisms that the control club-to-goblet cell transition (Fig. 6). We found that asthma, CF, and COPD shared features related to immune defence response, response to stimulus, response to hypoxia, response to hormone, cellular energy metabolism, metal ions, oxidative stress, apoptosis, phagocytosis, and retina homeostasis. The TFs IRF6 and TAGLN2 were involved in the club-to-goblet cell transition in asthma, and transition-related signalling pathways included protein kinase B and HIF-1 signalling pathways. In CF, the TFs SPDEF, JUND, CREB3L4, and NF-kappa B, and the protein kinase A, TNF, IL-17, and TGF-beta signalling pathways participated in the club-to-

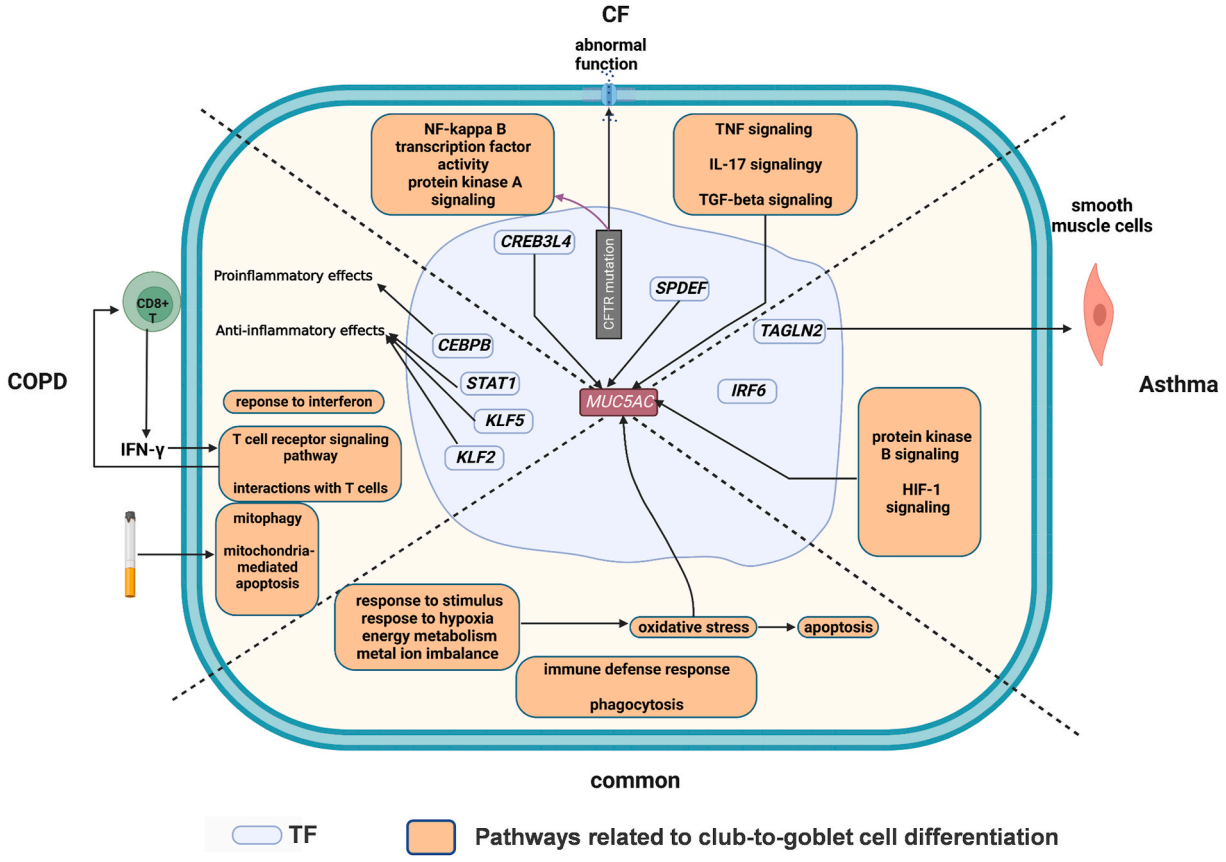

Fig. 6. Schematic illustration of distinct programs causing goblet cell metaplasia in asthma, cystic fibrosis (CF), and chronic obstructive pulmonary disease (COPD). The cell represents the transitional phase as club cells differentiate into goblet cells. Created with BioRender.com.

goblet cell transition. In COPD, the TFs STAT1, CEBPB, KLF5, KLF2, and FOXQ1 were activated, and interaction with T cells, autophagy, mitophagy, and mitochondria-induced apoptosis may be associated with club-to-goblet cell transition.

4. Discussion

Mucus represents the first line of defence against external microorganisms and pathogens, followed by the airway epithelium which is a physical barrier that regulates innate and adaptive immunity [37]. Alterations of airway epithelial cells and metaplasia of goblet cells, for instance, produce excessive mucus, which is an important feature of many respiratory diseases, including asthma, CF, and COPD [38]. In this study, we reanalyzed publicly available scRNA-seq data for these three diseases and observed distinct transcriptional programs, notwithstanding the feature of goblet cell metaplasia, which is shared by all three of these lung diseases.

Simulating an accurate club to goblet cell transition state using conventional methods is unfeasible, however the advent of scRNA-seq technology provides an opportunity [19]. We used well-developed scRNA-seq analysis methods and developed a thorough workflow to study the transition states of the cell types of interest. This approach aided us in locating the phase from club cells transiting to goblet cells, and to analyse thousands of genes associated with the transition process, clarifying the similarities and differences among the three diseases in the human lung.

With the aforementioned results, we found some common features between club and goblet cells among the three diseases in pathogenesis and cellular transition. In addition, enrichment analysis of the DEGs showed that the transcriptional alterations of club and goblet cells in the three diseases were enriched in neutrophil chemotaxis, response to lipopolysaccharide, defence response to bacterium, and response to hydrogen peroxide, which suggested that the transcriptional alterations in club and goblet cells were associated with focal inflammation reactions and an antioxidant imbalance [39–44]. In addition, we found that the club-to-goblet cell transitions in the three diseases were associated with response to stimulus, hypoxia, hormone, metal ion-related, apoptosis, phagocytosis, and retina homeostasis processes. Several studies have shown that these processes induce oxidative stress and reactive oxygen species (ROS) production, eventually leading to apoptosis [45,46]. However, oxidative stress has been reported to stimulate goblet cell metaplasia [47]. Therefore, we hypothesised that oxidative stress promotes club cells differentiation into goblet cells and reverses apoptosis. Thus, inhibiting oxidative stress during disease development might be an effective therapeutic approach, but this needs further investigation. ClueGO software suggested that antigen processing and presentation and antioxidant activity played a crucial role in club-to-goblet cell transition in the three diseases. Taken together, these common features highlight the similarities in the downstream molecular network of the goblet cell metaplasia phenotype.

We also determined the disease-specific features of club and goblet cells in asthma, CF, and COPD. Asthma is a common disease caused by chronic inflammation of the lower respiratory tract and is characterised by airway remodelling and hyper-responsiveness. Most patients with asthma experience type 2 inflammation [39]. We found that the upregulation of CST1, CST4, CLCA1, and CCL26 in asthma may be mediated by IL-13, a type 2 inflammatory factor that induces MUC5AC expression, causing goblet cell metaplasia [48,49]. The DEGs identified in the club and goblet cells of patients with asthma were also mainly enriched in inflammatory response and goblet cell hyperplasia pathways, such the ErbB, PI3K-AKT, and JAK-STAT signalling pathways and positive regulation of nitric oxide biosynthetic process [50]. IRF6 and TAGLN2 are asthma-specific TFs involved in multiple lung diseases [51] and airway resistance [52], implying that the club-to-goblet cell transition might interact with the airway's smooth muscle and cooperate to mediate the airway lumen size during transition. Moreover, the HIF-1 signalling pathway was enriched in the club-to-goblet cell transition in asthma, indicating that hypoxia might promote goblet cell transition to some extent during asthma pathogenesis.

Autosomal recessive inheritance of CFTR mutations is the basis of multiorgan CF and CF is characterised by chronic bacterial airway infection and goblet cell metaplasia [40]. The upregulated genes of patients with CF club cells were mainly mitochondrial genes, indicating that club cells may compensate for ionocyte ion transport defects and, thus, increase mitochondrial gene expression levels to generate more energy and secrete more ions. Enrichment analysis showed that disease-related pathways were mainly associated with the loss of CFTR function in CF, such as hydrogen ion transmembrane transport and glutathione metabolism [53], indicating that CFTR was involved in ion transport and pH regulation. CREB3L4, as an active TF, may also participate in induction of MUC5AC expression in human airway epithelial cells [54]. Furthermore, according to previous studies, the TNF, TGF-beta, and IL-17 signalling pathways are associated with the club-to-goblet cell transition and airway homeostasis maintenance in CF [55,56].

COPD is a systemic chronic inflammatory disease that affects the parenchyma and distal airways and causes progressive airflow limitation in the lungs [41]. In our study, the upregulated DEGs were associated with T cell recruitment, activation and proliferation [57]. TFs involved in regulating inflammation, including STAT1, KLF5, KLF2, and CEBPB, were activated in club-to-goblet cells transition, implying that club and goblet cells also participated in the immune response during transition [58,59]. Moreover, the enrichment results of DEGs and CTGs indicated that club and goblet cells may interact with T cells during COPD pathogenesis. Numerous $CD8^+$ T cells are present in the airways of patients with COPD and they directly bind to antigens through MHC I, producing interferon-γ and granzyme. $CD8^+$ T cells act as central regulators of inflammatory networks in cigarette-smoke-induced emphysema [60,61]. Mitophagy and mitochondria-mediated apoptosis induced by smoke stimulation are also important pathological processes of COPD [62]. The enrichment of these pathways suggested that $CD8^+$ T cells crosstalk and mitochondrial damage and dysfunction play special roles in club-to-goblet cell transition in COPD.

In summary, we focused on the similarities and differences among shared goblet cell metaplasia phenotypes in asthma, CF, and COPD. Our investigation revealed that several genes and biological processes are associated with goblet cell metaplasia in these three diseases and suggested that goblet cell metaplasia may be relieved by inhibiting oxidative stress in club cells. In addition, the biological functions of IL-13- and HIF-1-related pathways in asthma and interactions with T cells when managing COPD should be a focus for this research subject, whereas restoring the normal functions of CFTR with gene modification or exogenous modulators might be the most

promising strategy for CF treatment. In addition to disease-associated factors affecting goblet cell metaplasia, the differential microenvironment of the proximal and distal airways may also play a regulatory role. This study has several limitations. The sample source and characteristics may have biased our analysis. Among the 12 patients with asthma analysed in this study, only one female patient was included in the control and asthma groups. Moreover, tissues were sampled distally in patients with COPD versus proximally in patients with asthma and CF. Future large-scale studies are needed to verify these finding.

Data availability statement and materials

The asthma dataset was kindly provided by FA Vieira Braga (Vieira Braga, Kar, Berg, Carpaij, Polanski, Simon, Brouwer, Gomes, Hesse, Jiang, Fasouli, Efremova, Vento-Tormo, Talavera-Lopez, Jonker, Affleck, Palit, Strzelecka, Firth, Mahbubani, Cvejic, Meyer, Saeb-Parsy, Luinge, Brandsma, Timens, Angelidis, Strunz, Koppelman, van Oosterhout, Schiller, Theis, van den Berge, Nawijn and Teichmann 2019). Publicly available datasets were analysed in this study. These data can be found here: NCBI GEO accession GSE150674, GSE136831, GSE173896, and GSE168191. The code for our analysis was attached in Supplementary Materials.

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CRediT authorship contribution statement

Kuan Li: Writing – original draft, Software, Methodology. **Zhaoyu Song:** Writing – original draft. **Qing Yue:** Writing – original draft. **Qi Wang:** Software. **Yu Li:** Visualization. **Yu Zhu:** Writing – review & editing. **Huaiyong Chen:** Writing – review & editing.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34105.

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