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Charting a New Path: A Single-Cell Atlas of Porcine Cystic Fibrosis Airways at Birth

The development of the porcine model of cystic fibrosis (CF) disease marked a leap forward in our ability to understand CF pathogenesis while keeping a high degree of fidelity with human disease (1, 2). Similarly, the emergence of single-cell RNA sequencing (scRNAseq) as a tool for gene expression analysis rapidly expanded our CF-specific transcriptome knowledge with unprecedented resolution (3, 4). Early studies of scRNAseq in airway epithelial and immune cells provided data on dysfunctional transcriptional programs controlling ion transport, host defense, and immunity within CF airways and even enabled the discovery of novel airway epithelial cell types (4–6).

In this issue of the *Journal*, Thurman and colleagues (pp. 612–622) integrate these groundbreaking developments to chart the transcriptional topography of the porcine bronchial tree at birth, providing for the first time a detailed landscape of gene expression and epithelial cell–type distribution in newborn CFTR (CF transmembrane conductance regulator)–deficient ($CFTR^{-/-}$) lungs (7). Through this work, the authors inch ever closer to answering a long-standing question: does CF disease originate from intrinsic epithelial dysfunction, or is epithelial dysfunction a result of cyclical infection and inflammation in the postnatal airway environment?

The authors microdissected large and small airways from newborn $CFTR^{-/-}$ pigs for scRNAseq analysis. Despite CFTR deficiency, the transcriptome of CF and non-CF porcine airways exhibited minimal transcriptomic differences at birth, suggesting that CFTR deficiency itself did not cause extensive epithelial gene expression dysregulation. This remarkable finding brings forth the idea that CF lung disease may be a modifiable, largely postnatal process rather than a predetermined outcome due to intrinsic epithelial dysfunction and inflammation already evolving *in utero*.

Although minimal transcriptional differences were observed between CF and non-CF airways, the authors show vastly different transcriptomic and protein marker profiles between small and large airways in $CFTR^{-/-}$ and wild-type porcine lungs. Apart from CFTR, a considerable number of differentially expressed genes distinguished basal, secretory, and ciliated cells from large and small airways, with remarkably fewer differentially expressed genes in "deeper" cells (e.g., muscle cells, endothelial cells) than in those closer to the apical surface (e.g., ciliated cells, secretory cells).

Although others have characterized epithelial and immune cell subgroups in human CF and experimental models using scRNAseq, this work offers a detailed map of the airway epithelium to understand cell subset distributions and transcriptional topography in the earliest stages of CF. This work also provides a point of reference to investigate CF pathogenesis, from relatively unaffected airways to the chronic infection and inflammation in our closest animal model of CF lung disease to date. This may be particularly relevant to the role of small airways, an incompletely characterized, yet often proposed site of early CF lung disease development (8–10).

This study confirms the presence of common airway epithelial cells in small and large airways, including ciliated, secretory, and club cells. It also identifies rare epithelial cells such as the recently described ionocytes in a nearly exclusive large airway distribution (4). The paucity of ionocytes in small airways concurred with increased expression of non-CFTR ion channels in small airway epithelia, highlighting how much remains to be understood about the function of these cells and their transcriptional and functional equivalents in smaller airways.

Ion transport is critically disrupted in CF, leading to abnormal solute concentration in respiratory secretions, mucus desiccation, and impaired mucociliary clearance (8, 11, 12). Expression of CFTR, a major driver of epithelial chloride, bicarbonate, and fluid transport, was overall low in wild-type airways. It was measured at the highest concentration in ionocytes, followed by secretory, ciliated, and basal cells. Regionally, CFTR expression was highest in ionocytes of large airways and in secretory and ciliated cells of small airways. Small airways expressed more epithelial sodium channel subunits, less of its negative regulator, BPIFA1 (bactericidal/permeability-increasing fold containing family A, member 1) (13), and more aquaporins than large airways, suggesting a program that enhances sodium movement and osmotic water transport in smaller airways. Functionally, large and small airways had similar paracellular conductance, but transcriptional differences suggest that small airways were more permeable to cations than anions, whereas large ones had similar permeability to both. These findings will require further functional validation.

Mucins are crucial contributors to the clinical manifestations of CF, and their expression is influenced by inflammation and epithelial dysfunction (14, 15). The authors found that MUC5AC (mucin 5AC, oligomeric mucus/gel-forming) and MUC5B (mucin 5B, oligomeric mucus/gel-forming) expression was site specific, with a transition from MUC5AC-rich secretory cells to MUC5AC-low club cells distally in small airways. These observations suggested a site-specific transcriptional program that promotes higher mucin content in large airways. These initial observations provide a reference to investigate epithelial cell and mucin production by lower airways that may foment chronic inflammation and promote mucoid impaction.

The secretion of host defense proteins and peptides (AMPs) is a key component of epithelial immunity (16, 17). In neonatal CF porcine lungs, secretory cells expressed the most AMPs, again with remarkable regional differences: large airways expressed higher concentrations of lysozyme, lactoferrin, calprotectin, and BPIFA1 (a surfactant AMP with ion transport and immunomodulatory properties) (18). In contrast, the small airway epithelium differentially

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expressed higher concentrations of surfactant proteins (SFTPB [surfactant protein B], SFTPA1 [surfactant protein A1]). In addition, the authors observed differential expression of other host-defense genes, including pathogen recognition receptors, IFNs, and IFN-stimulated genes critical for bacterial and antiviral defense.

The work presented by Thurman and colleagues has some limitations. First, submucosal glands, immune cells, and the transitional region from large to small airways were not included in the analysis. Although this is a reasonable design choice, future work could clarify the role of these compartments and help us better understand the transcriptional progression from neonatal airways to a CF phenotype. Second, although $CFTR^{-/-}$ pigs are a clinically relevant model of CF, CFTR deletions are not the most prevalent CF-causing mutation. Therefore, a future focus on F508del mutations that cause CFTR misfolding, early degradation, and disruption of cellular proteostasis may uncover a different transcriptional profile from the one observed here. As F508del mutations are present in at least one allele of more than 85% of patients with CF, findings on such a model would yield more broadly generalizable information. Finally, work remains to be done in understanding how regional differences in inflammation, pH, metabolite content, and microbiome influence cell distributions and the transcriptome in early CF development.

The findings of this study have impact within and beyond the CF field. The differential expression of host defense, mucins, and solute transport genes between large and small airways instructs us to consider these differences when modeling and interpreting pulmonary pathophysiology. We are also reminded to consider the contributions of small airways in novel models of disease pathogenesis. In the CF field, the absence of intrinsic transcriptional changes between CF and wild-type airways suggests that lung disease may not be a predetermined outcome, reinforcing that early interventions to correct CFTR dysfunction and prevent cycles of inflammation could delay its emergence. Furthermore, the versatility of the porcine model, in which multiple CF-causing mutations can be studied, creates an opportunity to follow transcriptional effects of inflammation and infection in those with rare CF-causing mutations for whom CFTR modulators are not yet available. This map of neonatal CF airways lays the groundwork for a new path to answer a crucial question: could early CFTR modulation maintain this neonatal airway phenotype and prevent CF lung disease altogether? The answer may be transcribed along the way. ■

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