8. Katira BH, Osada K, Engelberts D, Bastia L, Damiani LF, Li X, *et al.* Positive end-expiratory pressure, pleural pressure, and regional compliance during pronation: an experimental study. *Am J Respir Crit Care Med* 2021;203:1266–1274.

9. Keenan JC, Cortes-Puentes GA, Zhang L, Adams AB, Dries DJ, Marini JJ. PEEP titration: the effect of prone position and abdominal pressure in an ARDS model. *Intensive Care Med Exp* 2018;6:3.

10. Gattinoni L, Vagginelli F, Carlesso E, Taccone P, Conte V, Chiumello D, *et al.*; Prone-Supine Study Group. Decrease in PaCO2 with prone position is predictive of improved outcome in acute respiratory distress syndrome. *Crit Care Med* 2003;31:2727–2733.

11. Guérin C, Reignier J, Richard JC, Beuret P, Gacouin A, Boulain T, *et al.*; PROSEVA Study Group. Prone positioning in severe acute respiratory distress syndrome. *N Engl J Med* 2013;368:2159–2168.

12. Gerbino AJ, McKinney S, Glenny RW. Correlation between ventilation and perfusion determines VA/Q heterogeneity in endotoxemia. *J Appl Physiol (1985)* 2000;88:1933–1942.

13. Tomashefski JF Jr, Davies P, Boggis C, Greene R, Zapol WM, Reid LM. The pulmonary vascular lesions of the adult respiratory distress syndrome. *Am J Pathol* 1983;112:112–126.

14. Richter T, Bellani G, Scott Harris R, Vidal Melo MF, Winkler T, Venegas JG, *et al.* Effect of prone position on regional shunt, aeration, and perfusion in experimental acute lung injury. *Am J Respir Crit Care Med* 2005;172:480–487.

15. Prisk GK, Yamada K, Henderson AC, Arai TJ, Levin DL, Buxton RB, *et al.* Pulmonary perfusion in the prone and supine postures in the normal human lung. *J Appl Physiol (1985)* 2007;103:883–894.

Check for updates

Where Is the Cystic Fibrosis Transmembrane Conductance Regulator?

Cystic fibrosis (CF) is a mucoobstructive pathology associated with chronic inflammation and chronic bacterial infection of the lungs. Mutations in the CF gene lead to dysfunction of the CFTR (CF transmembrane conductance regulator) and development of clinical symptoms. Two central characteristics of CF lung disease are an inadequate hydration and a defective transport of the mucous layer that covers and protects the airway surface. Despite the huge progress made in CF care, the exact relationships between primary defects and different manifestations of the disease are lacking. In that context, it is important to establish a precise map of CFTR expression along the airways. Such studies, begun immediately after the discovery of the CF gene, gradually concluded that CFTR was detected in airway surface epithelial cells, including in airway multiciliated cells, as well as in rare "CFTR hot" cells near or within airway submucosal gland acini or gland duct cells (1, 2).

In 2018, Montoro and colleagues (3) and Plasschaert and colleagues (4) applied single-cell RNA sequencing (scRNAseq), a technology that allows unbiased transcriptional profiling of tens of thousands of individual cells. They generated a catalog of the cells expressed in the lung, and, more particularly, they revealed a population of rare cells that they entitled pulmonary ionocytes. This name reflected a population of cells found in fish gills and frog skin, which contribute to ion homeostasis and hydration. Interestingly, pulmonary ionocytes were not only established by Montoro and colleagues and Plasschaert and colleagues as the sites of highest CFTR expression in airway cells but

were also characterized by their high expression of other ion-transport genes, including subunits of the amiloride sensitive Na^+ channel, and components of H^+-ATPases. This peculiar gene expression program, also showing similarities with renal intercalated cells, suggests that ionocytes could be directly involved in active absorption of fluids (5) and/or regulation of acid-base homeostasis (6). In the murine tracheal epithelium, the majority of CFTR was present in pulmonary ionocytes but basal and secretory cells also expressed CFTR (3). Remarkably, little to no CFTR expression was detected in multiciliated cells, which were thought to be the main harbor of CFTR expression.

In this issue of the *Journal*, Okuda and colleagues (pp. 1275–1289) are now providing a comprehensive description of CFTR-expressing cell types in normal human conducting airways (7). To do so, they have combined scRNAseq technologies, single-cell quantitative RT-PCR, and RNA *in situ* hybridization methods, validating some of their results by electrophysiological approaches. Their measurements also provide information about variations of gene expression between large and small airway epithelia. This work confirms that CFTR is strongly expressed in ionocytes but also underlines the rarity of these cells in human small airway epithelium. The authors' conclusion is that ionocytes represent a fraction of the total CFTR signal. Instead, more abundant cell types that express lower individual levels of CFTR represent a much larger fraction of the total signal. Secretory cells are thus the dominant cell type that expresses CFTR in the surface epithelium of large and small airways. CFTR is also significantly expressed in basal cells, suprabasal cells, and, to a lesser extent, multiciliated cells. Finally, the authors directly measured CFTR-mediated Cl^- secretory function, demonstrating a better correlation between this signal and the presence of secretory cell types than with ionocytes. Secretory cells from CF airway epithelia, but not multiciliated cells, were capable of CFTR-mediated Cl^- secretion after transduction with wild-type CFTR.

The results of Okuda and colleagues fit well with independent data sets that were recently published on human lung and airway (Table 1). Deprez and colleagues provided an scRNAseq atlas of 77,969 cells from 35 healthy human airway samples derived from 10 subjects, in which they defined 28 distinct cell types/states (8). They confirmed the high

---

Table 1. Characteristics of the Five Different Data Sets Discussed Herein

Authors	Data Set	CFTR Expression	Reference
Okuda *et al.*	16,643 cells, 7 donors	9 clusters (10x): secretory cells (43.6%) > suprabasal cells (23.5%) > cycling/deuterosomal cells (15.9%) > basal cells (9.2%) > ionocyte + neuroendocrine cells (4.8%) > multiciliated cells (3%)	7
	26,319 cells, 7 donors	11 clusters (Drop-Seq scRNAseq): secretory cells (43.6%) > suprabasal cells (20.7%) > multiciliated cells (5.6%) > ionocytes + neuroendocrine cells (3.6%) > cycling/deuterosomal cells (3.2%) > basal cells (2.9%) https://cells.ucsc.edu/?bp=lung&ds=lung-airway	
Deprez *et al.*	77,969 cells, 35 human airway samples, 10 healthy volunteers	28 distinct cell types, including ionocytes (10x): secretory cells (65.5%) > suprabasal cells (23.3%) > basal cells (5.1%) > ionocytes (2.5%) > multiciliated (1.5%) https://www.genomique.eu/cellbrowser/HCA	8
Habermann *et al.*	114,000 cells, 20 pulmonary fibrosis and 10 control lungs	31 distinct cell types: alveolar type 2 cells (48.1%) > secretory cells (34.6%) > multiciliated cells (8.4%) > basal cells (4.9%) https://cells.ucsc.edu/?ds=lung-pf-control	9
Miller *et al.*	8,443 EPCAM$^+$ cells, 8 human fetal lung samples (11.5–21 wk of development)	12 distinct cell types: secretory progenitors (39.1%) > bud tip adjacent cells (17.8%) https://cells.ucsc.edu/?ds=covid19atlas+c19a-miller20	10
Goldfarbmuren *et al.*	36,248 epithelial cells, 15 donors (including 6 never-smokers and 6 heavy smokers, i.e., ≥15 pack-years)	10 epithelial cell clusters: KRT8-high intermediate cells (46.3%) > basal cells (19%) > mucus secretory cells (17.9%) > ionocytes (11.2%) > multiciliated cells, submucosal gland cells https://cells.ucsc.edu/?ds=lung-smoking-effect	11

Definition of abbreviations: CFTR = cystic fibrosis transmembrane conductance regulator; Drop-Seq = droplet-based scRNAseq; EPCAM = epithelial cell adhesion molecule; scRNAseq = single-cell RNA sequencing.

expression of CFTR in ionocytes (2.5% of total CFTR signal in 0.15% of total cells), but they also detected it in secretory (65.5% of signal), suprabasal (23.3% of signal), basal (5.1% of signal), and multiciliated cells (1.5% of signal) at four different levels of the airways. Habermann and colleagues analyzed 114,000 cells from 20 pulmonary fibrosis and 10 control lungs, including cells from the parenchyma (9). After defining 31 distinct cell types, they detected CFTR in alveolar type 2 cells (48.1% of total signal), secretory cells (34.6% of total signal), multiciliated cells (8.4% of total signal), and basal cells (4.9% of total signal). This data set includes a few ionocytes, which were not formally typed but which express *bona fide* ionocyte markers, including CFTR. Miller and colleagues analyzed 8,443 human fetal lung cells (11.5–21 wk of development), defining 12 distinct epithelial cell types. In this data set, the major sites of expression for CFTR correspond to secretory progenitors (totaling 39.1% of the CFTR signal) and bud tip adjacent cells (17.8% of the CFTR signal) (10). Finally, Goldfarbmuren and colleagues defined 10 epithelial cell clusters in 36,248 epithelial cells isolated from 15 donors (either smokers or never-smokers), in which they detected 46.3% in KRT8-high intermediate cells, 19% of total CFTR signal in basal cells, 17.9% in mucus secretory cells, and 11.2% in ionocytes (11). Collectively, these results draw a very consistent picture with secretory cells as major sites of expression for CFTR in human airways. It illustrates the interest of integrating well-standardized data sets from different origins in powerful atlases (12). Sharing of common ontologies, proper definitions of gene markers, and continuous improvement of the existing resources are key to ensure their overall quality (13). As nicely illustrated by Okuda and colleagues, maintaining a constant dialogue between production of single-cell data and independent biological results is also important.

By establishing a cellular context that link CFTR with MUC5AC and MUC5B, that is, the two main synthetic and secreted respiratory mucins, the work by Okuda and colleagues defines possible scenarios to finely control mucus hydration. This may also fit well with a recent observation of some very early posttranslational modifications that can lead to hypersialylation of mucin O-sugars in a CF pig model (14).

The airway surface liquid is made of two distinct components: a mucus layer principally made of MUC5AC and MUC5B and a periciliary layer (gel) formed by tethered macromolecules, including MUC1, MUC4, and MUC16 (15). Small changes in mucus concentrations alter the osmotic pressures in the two phases, resulting in a severe impairment of the mucociliary transport rate. A direct control of ion fluxes by CFTR in cells that do secrete MUC5B and MUC5AC makes a lot of sense and should now be tested quantitatively.

A final point is that the importance of secretory cells does not preclude additional roles of CFTR in other cells. The function of ionocytes and ionocyte-localized CFTR remains unknown. It is also likely that CFTR located in different cell types subtends different functions in different parts of the respiratory tree (5). ∎

Pascal Barbry, Ph.D.
Brice Marcet, Ph.D.
Institut de Pharmacologie Moléculaire et Cellulaire
Université Côte d'Azur–CNRS
Sophia Antipolis, France

Ignacio Caballero, Ph.D.
UMR-1282 Infectiologie et Santé Publique
INRAE
Nouzilly, France

ORCID IDs: 0000-0001-9632-6483 (P.B.); 0000-0001-5015-2297 (B.M.); 0000-0002-0628-4134 (I.C.).

References

1. Kreda SM, Mall M, Mengos A, Rochelle L, Yankaskas J, Riordan JR, et al. Characterization of wild-type and deltaF508 cystic fibrosis transmembrane regulator in human respiratory epithelia. *Mol Biol Cell* 2005;16:2154–2167.
2. Engelhardt JF, Zepeda M, Cohn JA, Yankaskas JR, Wilson JM. Expression of the cystic fibrosis gene in adult human lung. *J Clin Invest* 1994;93: 737–749.
3. Montoro DT, Haber AL, Biton M, Vinarsky V, Lin B, Birket SE, et al. A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. *Nature* 2018;560:319–324.
4. Plasschaert LW, Žilionis R, Choo-Wing R, Savova V, Knehr J, Roma G, et al. A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. *Nature* 2018;560:377–381.
5. Fang X, Fukuda N, Barbry P, Sartori C, Verkman AS, Matthay MA. Novel role for CFTR in fluid absorption from the distal airspaces of the lung. *J Gen Physiol* 2002;119:199–207.
6. Eladari D, Chambrey R, Peti-Peterdi J. A new look at electrolyte transport in the distal tubule. *Annu Rev Physiol* 2012;74:325–349.
7. Okuda K, Dang H, Kobayashi Y, Carraro G, Nakano S, Chen G, et al. Secretory cells dominate airway CFTR expression and function in human airway superficial epithelia. *Am J Respir Crit Care Med* 2021;203:1275–1289.
8. Deprez M, Zaragosi L-E, Truchi M, Becavin C, Ruiz García S, Arguel M-J, et al. A single-cell atlas of the human healthy airways. *Am J Respir Crit Care Med* 2020;202:1636–1645.
9. Habermann AC, Gutierrez AJ, Bui LT, Yahn SL, Winters NI, Calvi CL, et al. Single-cell RNA sequencing reveals profibrotic roles of distinct epithelial and mesenchymal lineages in pulmonary fibrosis. *Sci Adv* 2020;6:eaba1972.
10. Miller AJ, Yu Q, Czerwinski M, Tsai YH, Conway RF, Wu A, et al. In vitro and in vivo development of the human airway at single-cell resolution. *Dev Cell* 2020;53:117–128, e6. [Published erratum appears in *Dev Cell* 54:818.]
11. Goldfarbmuren KC, Jackson ND, Sajuthi SP, Dyjack N, Li KS, Rios CL, et al. Dissecting the cellular specificity of smoking effects and reconstructing lineages in the human airway epithelium. *Nat Commun* 2020;11:2485.
12. Schiller HB, Montoro DT, Simon LM, Rawlins EL, Meyer KB, Strunz M, et al. The human lung cell atlas: a high-resolution reference map of the human lung in health and disease. *Am J Respir Cell Mol Biol* 2019;61:31–41.
13. Weber GM, Ju Y, Börner K. Considerations for using the vasculature as a coordinate system to map all the cells in the human body. *Front Cardiovasc Med* 2020;7:29.
14. Caballero I, Ringot-Destrez B, Si-Tahar M, Barbry P, Guillon A, Lantier I, et al. Evidence of early increased sialylation of airway mucins and defective mucociliary clearance in CFTR-deficient piglets. *J Cyst Fibros* [online ahead of print] 23 Sep 2020; DOI: 10.1016/j.jcf.2020.09.009.
15. Boucher RC. Muco-obstructive lung diseases. *N Engl J Med* 2019;380:1941–1953.

Check for updates

🔓 Knocking Out Smoking and Pulmonary Hypertension with a K$^+$

Although many patients with chronic obstructive pulmonary disease (COPD) develop mild pulmonary arterial hypertension (PAH), believed to result from hypoxia-induced pulmonary vasoconstriction, a small but significant fraction of patients with COPD develop more severe PAH, often without clinical evidence of hypoxemia or out of proportion to their degree of emphysema (1, 2). The mechanism for the development of pulmonary hypertension in these patients is not entirely clear, but pathologically, the pulmonary arterioles of these patients demonstrate endothelial cell dysfunction (3), smooth muscle cell hyperplasia, and arterial intimal fibrosis (4), features commonly observed in other forms of primary or group I PAH. As a consequence, these patients suffer significant morbidity and mortality, often independent of the severity of their obstructive airway disease (5). Why some patients with COPD develop significant PAH and the mechanisms that drive this process are not completely understood.

The harmful effects of cigarette smoke are well known to not only directly affect cells of the respiratory epithelium but also other cell types in the lung, including mesenchymal cells and vascular endothelial cells (6). Indeed, the ability of toxins from cigarette smoking to traverse the epithelial barrier and effect pulmonary and systemic vasculature is an oft-cited mechanism for how smoking contributes to cardiovascular disease, stroke, and other systemic diseases (7). Not surprisingly, tobacco smoking is also a risk factor for PAH (8).

Dynamic vasoconstriction and vasodilation are mediated by the contraction and relaxation of smooth muscle and mesenchymal cells of the vasculature, and like most muscle cells, they are mediated by the opening and closing of various ion channels. From the initial discovery of action potentials described by Hodgkin and Huxley in 1952, ion channel behavior is one of the oldest and most fundamental processes that has been studied in cell and molecular physiology. Patch clamp recordings, invented by Neher and Sakmann, provided a technique to study electrophysiology at the level of individual ion channels and cells. Today, we know the human genome codes for more than 300 different ion channels, whose function are not only limited to electrochemical homeostasis or neuronal communication but also to diverse functions including cell proliferation, differentiation, mitochondrial function, cellular metabolism, DNA repair, and cell–cell communication. Beyond muscle contraction, ion channels play a role in organ development, repair and regeneration, aging, and cellular senescence.

Potassium (K$^+$) channels themselves have been in eukaryotic, bacterial, and archaeal existence since before the evolution of neuronal signaling (9). They are found in nearly all organisms and cell types (10). In humans, they are often classified by their structure (for example, inward-rectifying K$^+$ channels have two transmembrane domains, whereas others have six) and gating mechanisms, where they may either remain constitutively open to help maintain resting membrane potential or open only in response to changes in voltage (often designated K$_v$) or calcium. Abnormalities