Toward a Cell Atlas of the Human Airway

The cellular composition of the human airway is of significant interest because of the role of the airway in normal lung development, gas delivery to the alveoli, and mucociliary clearance and its involvement in two of the most common human diseases, asthma and chronic obstructive pulmonary disease, as well as many rarer conditions. Single-cell RNA sequencing (scRNAseq), a technology that allows unbiased transcriptional profiling of tens of thousands of individual cells, facilitates the generation of detailed catalogs or atlases of all cells in an organ or anatomic compartment (1). Several initiatives have been formed to profile the entirety of cellular diversity in the human body, including the "Human Cell Atlas" (HCA) and NIH's "Human BioMolecular Atlas." The potential of these collaborative efforts was exemplified by the recent integrated analysis of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) entry mediators *ACE2*, *TMPRSS2*, and *CTSL* in over 1 million cells from over 150 subjects (2). Within the HCA community, the lung has been identified as a priority organ because of its cellular and anatomical complexity, and the rationales, objectives, and approaches of the explicitly modular and collaborative HCA lung network have been presented (1).

In this issue of the *Journal*, Deprez and colleagues (pp. 1636–1645) provide an scRNAseq atlas of the healthy human airway by profiling 77,969 cells from 10 subjects (3). By diligently sampling multiple locations along the respiratory tract, from the nasal epithelium to the distal tracheobronchial tree, they survey the cellular diversity of the airways and transcriptional profiles of constituent cell types. They discover all major epithelial cell types and contribute specifically to the transcriptional characterization of rare airway cell types, including ionocytes, the first novel cell type previously discovered by scRNAseq (4, 5); pulmonary neuroendocrine cells; and serous and mucous cells from submucosal glands (6–8). As expected, the method of sampling influences the composition of recovered cells: a higher proportion of cells originating from deeper anatomical structures such as submucosal glands was observed in samples taken with forceps biopsies compared with bronchial brushings. Comparison of gene expression signatures of cells from the nasal and the tracheobronchial epithelium revealed a preservation of patterns that recapitulate embryonic development and confirm similar bulk RNA sequencing results in children (9). *PAX7* and *SIX3* were expressed in major nasal epithelial cell types, both of which are associated with neural crest–derived development in general and the olfactory epithelium specifically. In contrast, major tracheobronchial epithelial cell types express *NKX2-1* (TTF-1), master regulator of lung and thyroid development, and *IRX2* (3). Secretory cells from nasal and tracheobronchial epithelium were found to be similar at a global transcriptomic perspective. However, there are important differences. In line with Vieira Braga and colleagues (6), the authors observed that *SCGB1A1* and *SCGB3A1* are only sparsely expressed in nasal secretory cells and therefore not sufficient to identify them. Vice versa, nasal secretory cells can be identified by *PI3* but not bronchial ones (6). Of notice, goblet and club cells were not found to form separate populations on the transcriptional level but rather are archetypes within secretory cells (3).

Two additional novel observations deserve a separate discussion. The first novel finding is a population of *NREP*-positive "undefined rare" cells, potentially reflecting a differentiation state between basal and brush cells or ionocytes. The differentiation of pulmonary neuroendocrine cells and ionocytes remains at the center of some controversy, with Goldfarbmuren and colleagues suggesting that tuft-like cells are the precursors of both (7). However, the gene expression profile of these tuft-like cells overlaps only loosely with the $NREP^{+}$ undefined rare or with brush cells. The presence of this $NREP^{+}$ undefined rare population is not validated by histology or another data set. The second novel finding is the $KRT13^{+}$ "hillock" cells, previously only found in mouse airways (4). These cells express regulators of cellular adhesion and squamous epithelial differentiation, immunomodulation, and asthma and are characterized by expression of genes associated with rapid cellular turnover and squamous barrier function. Deprez and colleagues transcriptionally and histologically identify $KRT13^{+}$ hillock cells in human nasal epithelium but only in a very limited number of subjects ($n = 3$). Both findings are exciting and may represent an important complement to the full atlas of airway cells but require substantial validations in larger populations and research on their functional roles.

Although this study excels regarding sample collection and data analysis, it is not without limitations; probably the most important are the limited size and diversity of the population. This is essential, as age, sex, race, and ethnicity may have substantial effects on the cellular content of the human airway, a compartment constantly in contact with environmental exposures. It would be regrettable if single-cell profiling, a transformative cutting-edge technology, repeats errors of past eras and sets its reference of cells based on one homogenous and limited population. Of course, as more scRNAseq data sets of the lung and respiratory tract become available, the integration of these data sets into an airway cell atlas that is truly representative of human diversity becomes possible. For this to happen, several conditions are required. First, the data has to be publicly available and deposited on a public database with equal access to all to allow integration, synergy, and validation of results by others in the community. Second, a convention is needed about what constitutes a cell, the transcriptional and quantitative attributes and experimental validations required to define an scRNAseq finding as a "real cell," and the nomenclature used to name it.

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Third, we as a community must move beyond convenience cohorts to carefully planned cohorts, taking into account the diversity parameters mentioned above. Finally, a minimum set of metadata must be defined for human samples. It is possible that integrating this data set with currently available normal human lung and airway data sets (3, 7, 10, 11) as well the recent very large human lung disease data sets (6, 8, 12, 13) will allow immediate validation and augmentation of the exciting results in this manuscript. In summary, the study by Deprez and colleagues is a major milestone toward the generation of a comprehensive cell atlas of the human airway. Providing such a comprehensive and accurate atlas will improve our understanding of the airway and its function in development, health, and disease. Combining this effort with similar efforts from diseased tissues will eventually lead to better approaches in the diagnosis and management of airway diseases. ■

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Jonas Christian Schupp, M.D.
Section of Pulmonary, Critical Care and Sleep Medicine
Yale University School of Medicine
New Haven, Connecticut

Xiting Yan, Ph.D.
Section of Pulmonary, Critical Care and Sleep Medicine
Yale University School of Medicine
New Haven, Connecticut
and
Department of Biostatistics
Yale School of Public Health
New Haven, Connecticut

Naftali Kaminski, M.D.
Section of Pulmonary, Critical Care and Sleep Medicine
Yale University School of Medicine
New Haven, Connecticut

ORCID ID: 0000-0002-7714-8076 (J.C.S.).

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Birth Cohort Studies: Their Next Coming of Age

The fetus was traditionally considered to have a privileged place *in utero*, where it was protected from harmful environmental exposures. The paradigm that we are all born in a state of good health was challenged by research in the 1980s and 1990s that demonstrated links between reduced birth weight and increased risk for noncommunicable diseases (NCDs) in later life. The paradigm that antenatal exposures can have lifelong implications for health and well-being is now well accepted (1).

Inspired by the concept of "fetal origins of disease" and later concepts such as developmental plasticity and predictive adaptive responses, recruitment began in the 1980s to birth cohort studies designed to give insight into the premorbid physiological mechanisms linking the antenatal environment to later NCDs. To

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