GENOMICS

Profile of an unknown airway cell

RNA sequencing of single cells in the mammalian trachea reveals a previously unknown airway cell that expresses genes involved in fluid and solute balance, and that might play a part in cystic fibrosis. SEE ARTICLE P.319 & LETTER P.377

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Over the past two centuries, light and electron microscopy have enabled scientists to identify six or more cell types that line the mammalian airways, transporting oxygen into the body and protecting us from microbes and particles in the air we breathe. In the past two decades, the identification of molecular markers for most of these cell types has provided insight into their functions, and given researchers and physicians a way to locate and characterize the cells in clinical specimens. Montoro *et al.*[1] (page 319) and Plasschaert *et al.*[2] (page 377) now describe the full gene-expression profiles of cell types that line the mammalian trachea. In doing so, they have discovered a previously unknown cell type that could hold the key to understanding the disease cystic fibrosis.

The genomics revolution was spurred by the development of techniques to measure messenger RNA levels for every gene[3,4] and thus to profile gene expression across the genome. However, these early approaches required millions of cells to generate enough mRNA for analysis. As such, they were limited to producing a profile of average gene expression among all the cells in a population analysed.

It is only in the past decade that more-sensitive methods have become available to profile individual cells[5]. In the past couple of years, improvements in cell isolation and in mRNA capture, amplification and sequencing have allowed single-cell mRNA sequencing (scRNAseq) to be broadly applied across biology[6]. This technique has begun to reveal the diversity of cells in developing, mature and diseased tissues, without any need for prior knowledge of the cells or the ability to purify individual cell types[7]. Many groups are now reporting gene-expression profiles for thousands or tens of thousands of individual cells. These profiles can be used to reveal the gene-expression programs that govern dynamic biological processes such as cell differentiation[7], and to create molecular cell atlases of entire organs and even whole organisms[8–10].

In the current studies, both groups used scRNAseq to analyse tens of thousands of cells from the lining (epithelium) of the trachea of mice, and Plasschaert *et al.* also analysed human airway cells that had proliferated and differentiated in culture. Their analyses confirmed gene-expression profiles for the two most common cell types: club cells, which secrete components of the mucus that lines the airways, including antimicrobial and immune modulatory proteins; and ciliated cells, which carry protruding structures called cilia that swirl to clear mucus and debris.

In addition, the large number of cells analysed revealed expression profiles for some rare and less-well-characterized cell types: goblet cells, which produce mucus proteins; tuft cells, which are thought to act as immune sensors[11]; and neuroendocrine cells, which sense oxygen levels, irritants and stretch in the airways, and signal to other lung cells and the central nervous system. The analyses also uncovered molecularly distinct subpopulations of club, goblet and tuft cells.

The two studies then established the gene-expression profile of basal cells, which are located below the other cells in the epithelium (Fig. 1) and function as stem and progenitor cells. Montoro *et al.* combined scRNAseq with a technique to track basal cells, showing that basal cells can directly give rise not only to club cells, as previously shown[12], but also to all the minor cell types analysed except goblet cells. Goblet cells, like ciliated cells[12], seem to arise from club cells. The groups identified previously unknown molecular markers for each cell type, along with type-specific combinations of transcription factors, which are presumably needed to select and maintain each cell type's distinct properties during airway development and renewal.

The groups' most surprising and important finding was the discovery of a previously unknown cell type. The authors dubbed these rare cells pulmonary ionocytes, because the cells' gene-expression profile overlaps with that of ionocytes found in fish gills. In fish, these cells maintain normal solute concentrations by regulating the exchange of sodium, chloride and calcium ions between the animals' tissues and the surrounding water[13]. It is not yet clear whether pulmonary ionocytes serve a similar function in mammalian airways, although the cells do express multiple ion-transport genes.

In addition, both the fish and the mammalian cells produce a transcription factor of the Foxi family. In fish, the Foxi protein is required for fish cells to adopt the characteristics of ionocytes. Likewise, Montoro *et al.* found that the *Foxi1* gene is necessary for the expression of ionocyte markers in the mouse trachea, and Plasschaert *et al.* showed that the FOXI1 protein governs ionocyte identity in cultured human airway cells. Notably, both groups report that Foxi1 controls the expression of the gene *cystic fibrosis transmembrane conductance regulator* (*CFTR*) in pulmonary ionocytes.

The CFTR protein transports chloride ions

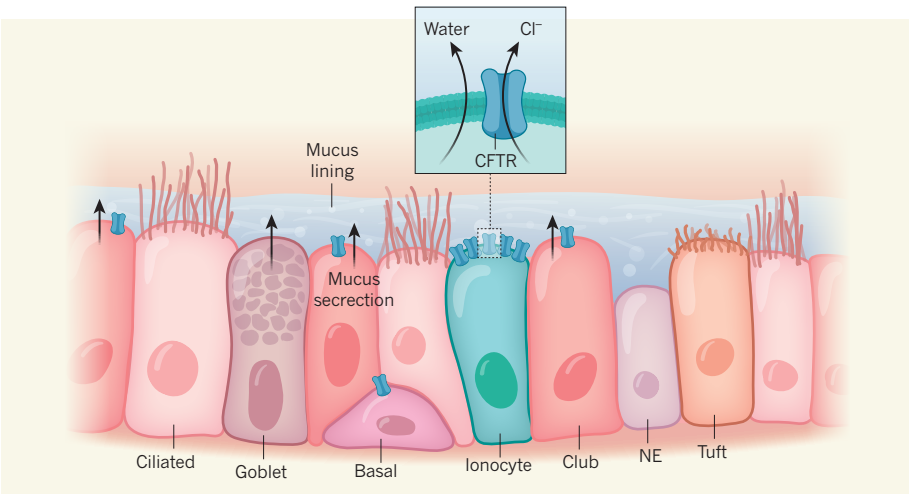

Figure 1 | Cells lining the trachea in mice. The inner surface of the trachea harbours multiple cell types. A protective lining of mucus is secreted by abundant club cells, rare goblet cells and submucosal glands (not shown). Ciliated cells bearing protrusions called cilia slowly propel mucus out of the lung. The surface also contains rare sensory neuroendocrine (NE) cells, tuft cells and basal progenitor cells. Two studies[1,2] have now identified another rare cell type on the airway surface: the ionocyte. Ionocytes highly express the gene *Cftr*, which encodes the CFTR ion channel, through which chloride ions (Cl^-) pass from the cell into the mucus, followed by water passage through a different channel (not shown). Basal cells and club cells also express *Cftr*, but at much lower levels than ionocytes. In people with the disease cystic fibrosis, CFTR is missing or defective, leading to thickening of the mucus, clogging of the airway, and repeated infections.

out of airway cells, causing water to flow out and so thinning the airway's lining of mucus (Fig. 1). When CFTR is absent or inactive, as in people who have cystic fibrosis, the mucus thickens and accumulates, causing airway obstruction and repeated infections and inflammation[14]. Determining which lung cells express *CFTR* and are directly affected in people with cystic fibrosis has been difficult because expression of this gene seems to be complex and variable along the airways[15]. The current papers demonstrate that the gene's expression is not as random as it had seemed: the bulk of the *CFTR* mRNA detected was from the rare pulmonary ionocytes, each of which highly express the gene. People with cystic fibrosis can also experience gastrointestinal symptoms and infertility. Perhaps *CFTR*-expressing ionocytes will be discovered in organs involved in these problems, too.

Mice harbouring mutations in *Cftr* do not develop cystic fibrosis — a curious fact that has long hampered research into the disease. Montoro *et al.* found that cultured airway epithelial cells generated from *Foxi1*-mutant mice have low *Cftr* expression but, paradoxically, higher than normal Cftr activity. Differences in pulmonary ionocytes between mice and humans, such as compensatory expression of another chloride channel when *Cftr* expression is lost in mice, might explain both this paradox and why *Cftr*-mutant mice do not model the disease.

Although these results suggest that ionocytes have a key role in airway biology and cystic fibrosis, much work is still needed to define their physiological functions, the role of CFTR in these functions, and how loss of CFTR causes or contributes to disease symptoms. Developing methods to genetically and pharmacologically manipulate ionocytes or replace them in model systems, and ultimately in patients, is another priority.

These papers provide excellent examples of how scRNAseq can transform long-established views of a tissue and a human disease. As scRNAseq tools improve and costs continue to drop, we will probably soon witness something similar for many human organs and diseases. ■

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ORGANIC CHEMISTRY

A practical route to 3D molecular diversity

Cycloaddition reactions are powerful tools for synthesizing three-dimensional molecules, but their scope has been limited. A creative solution to this problem opens up opportunities for drug discovery. SEE LETTER P.350

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Reactions known as cycloadditions are unparalleled in their ability to construct ring-containing molecules in a way that precisely controls the geometric arrangement of groups attached to the carbon atoms in the molecules — that is, the reactions offer great stereoselectivity. The power of these reactions has been demonstrated in numerous syntheses of complex natural products[1,2]. However, the scope of cycloadditions is limited to certain combinations of starting materials, which has restricted their use for making libraries of compounds in drug-discovery programs[3]. On page 350, Chen *et al.*[4] report a strategy that combines cycloadditions with another type of reaction, known as carbon–carbon cross coupling, to enable the modular and programmable preparation of cycloaddition-derived molecules.

Carbon–carbon (C–C) cross-coupling reactions are often used to form bonds between carbon atoms that are already part of a carbon–carbon double bond; such carbon atoms are said to have sp^2 orbital hybridization. Cross couplings between sp^2 carbons lend themselves to the modular synthetic routes