Single cell analysis of human lung development: knowing what mesenchymal cells are and what they may be

Jennifer M.S. Sucre[1,2] and James Hagood[3,4]

Affiliations: [1]Mildred Stahlman Division of Neonatology, Dept of Pediatrics, Vanderbilt University, Nashville, TN, USA. [2]Dept of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA. [3]Division of Pulmonology, Dept of Pediatrics, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. [4]Program for Rare and Interstitial Lung Disease, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

Correspondence: Jennifer M.S. Sucre, Vanderbilt University, 2215B Garland Ave, 1125 Light Hall, Nashville, TN 37232, USA. E-mail: jennifer.sucre@vanderbilt.edu

@ERSpublications
Single cell sequencing has identified new mesenchymal lineage markers in early human development, allowing for the more precise identification and characterisation of mesenchymal subpopulations in lung development and disease http://bit.ly/2NpX6UY

Cite this article as: Sucre JMS, Hagood J. Single cell analysis of human lung development: knowing what mesenchymal cells are and what they may be. *Eur Respir J* 2020; 55: 1902327 [https://doi.org/10.1183/13993003.02327-2019].

Examination of the transcriptomic activity of an organ at single cell resolution is one of the major breakthroughs in modern biology. Single cell RNA sequencing (scRNA-seq) is built on the paradigm-shifting work of Brady and Iscove [1], who described a method for generating microgrammes of cDNA from samples as small as a single cell almost 30 years ago. This discovery was followed closely by single cell transcriptomic analysis in neurons, accomplished by microinjecting primer, nucleotides and reverse transcriptase enzyme into dissociated cells [2]. Next generation sequencing technology afforded the opportunity to sequence thousands of genes from a single cell, with the first reported whole transcriptome analysis from a single mouse blastomere in 2009 [3]. Additional technical breakthroughs have refined library preparation and sequencing, which, combined with microfluidics-based single cell separation with reactions occurring in nanolitre scale droplets, have greatly increased the throughput and depth of analysis [4]. The potential for scRNA-seq to answer fundamental questions of cellular identity and heterogeneity, and to generate new hypotheses in lung developmental biology and disease pathogenesis, has driven the rapid adoption of this approach, with more than 40 papers published in the past 5 years that apply scRNA-seq techniques in the lung. From these data, there is an emerging appreciation of the contribution of rare subpopulations of cells to lung development and disease. With this new knowledge has come the challenge of defining cell types and subpopulations of cells by function and transcriptomic features. Clearly defining cellular identity is even more complex during lung development, when cells are in a state of rapid transition, a conundrum aptly described by Shakespeare's Ophelia in *Hamlet* when she said, "Lord we know what we are, but not what we may be."

In the recent past, our understanding of the heterogeneity of the pulmonary mesenchyme and appreciation for the pivotal role that mesenchymal cells play in directing lung organogenesis has grown tremendously [5, 6]. Defining cellular identity, and delineating which specific markers characterise specific subpopulations, have been significant hurdles in understanding the role of mesenchymal cells in lung

Received: 04 Dec 2019 | Accepted after revision: 06 Jan 2020

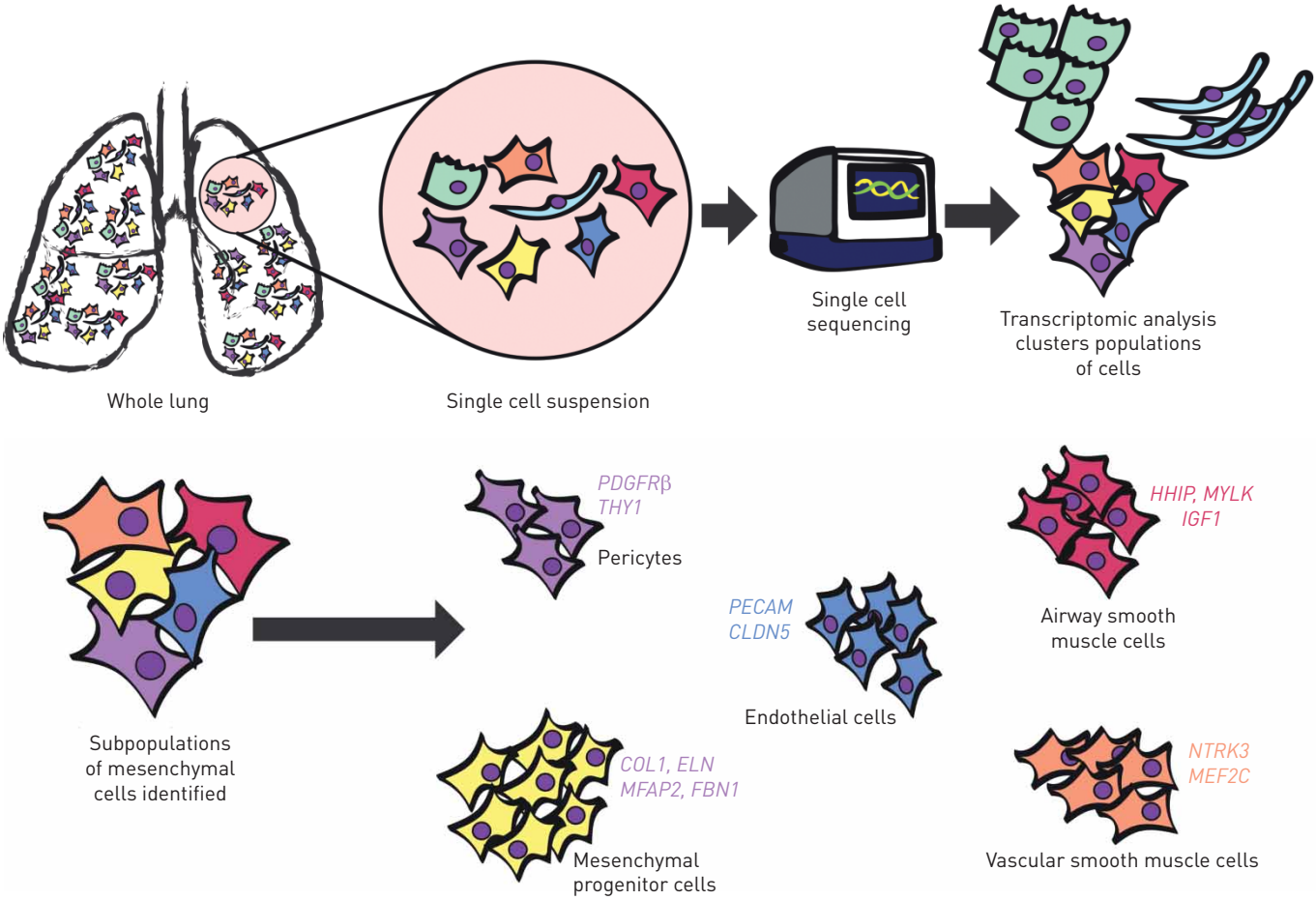

FIGURE 1 Schematic overview of single cell RNA sequencing and of the human lung. Single cell suspensions were made from human lungs at 11.5 and 18.5 weeks of gestation, with a total of >3000 cells analysed with a detection rate of 2000–3000 genes per cell. Analysis of the transcriptomics allows for clustering of cells, identifying 10 clusters with corresponding cluster marker genes. Within the mesenchymal cell population, several subpopulations were identified, including pericytes, endothelial cells, a large population of non-specific human lung progenitors and two distinct populations of *ACTA2*-positive cells, airway smooth muscle cells and vascular smooth muscle cells.

development and disease [7]. Heretofore, the identification and nomenclature of subpopulations of mesenchymal cells has been based largely on the phenotype or location of these cells (*e.g.* lipofibroblasts, myofibroblasts). Furthermore, much of this work has been based on rodent models [8]. Specific and fundamental differences between human and mouse development (*e.g.* the unique presence in humans of multipotent epithelial progenitor tip cells and diverging roles of FGF ligands in humans and mice [9–11]) underscore the need for a greater understanding of mesenchymal cells during human lung development. Achieving such understanding requires a reliable way of identifying the different populations of human mesenchymal cells as a basis for interrogating their function during development. Addressing this important knowledge gap, Danopoulos *et al.* [12] report in this issue of the *European Respiratory Journal* that scRNA-seq of the developing human lung identified subpopulations of ACTA2-expressing cells early in development, allowing identification of unique cell markers for airway smooth muscle cells (HHIP, MYLK, IGF1) *versus* vascular smooth muscle cells (NTRK3, MEF2C) (figure 1). The group reliably identified these distinct subpopulations of airway and vascular smooth muscle cells in human lung sections *via in situ* hybridisation using probes for the aforementioned cell markers, validating their utility. Whereas prior work has focused on specific gene products of these cells, such as ACTA2, the new markers provide the basis for future work to identify the fates of these highly specialised cells in disease states of the airways and vasculature, and to develop novel insights into their developmental roles. Indeed, these data strongly suggest that using subpopulation-specific mesenchymal markers will improve the accuracy and rigor of research on the lung microenvironment compared to currently used markers. For example, *COL1A1* expression was found to be present across a diversity of sub-populations including matrix fibroblasts, pericytes, and myoepithelial cells, indicating *COL1A1* expression alone is insufficient to determine cellular identity without the presence of additional markers.

An additional striking finding in this study is that whereas many cells were committed to particular lineages at 11 weeks, a substantial portion of cells remained as non-specific progenitor cells [12]. This raises questions of the precise timing and pathways of differentiation of these uncommitted cells in later stages of lung development, and of how long they may persist. A deeper understanding of mesenchymal plasticity in the adult lung creates the potential for novel therapeutic strategies, whereby differentiated mesenchymal cells could be driven towards an uncommitted progenitor state in order to repair the lung after injury. In addition, these findings add further context for multiple recent publications and preprints featuring scRNA-seq data in humans with various chronic pulmonary diseases, such as idiopathic pulmonary fibrosis (IPF) [13–16]. Because mesenchymal cell phenotypes and epithelial-mesenchymal interactions are believed to be key drivers of the pathogenesis of fibrosis, harmonising the scRNAseq data from this manuscript with the recently published data from adults with and without IPF may yield insights into developmental origins of mesenchymal subpopulations present in this disease.

Additional data from this study indicate early differentiation of inflammatory cell types in the human lung, as well as the presence of myoepithelial cells. Although these were not validated using *in situ* techniques, these data are available to other investigators for further *in silico* analyses. The study has some important acknowledged limitations, including low sample numbers for these limited early human specimens, and use of dissociation techniques which favour mesenchymal cells at the expense of epithelial cells. Nevertheless, these data address an important knowledge gap in human lung development, and provide an important resource for the field.

Comprised of diverse cell subpopulations which heretofore have lacked clearly distinguishing features, the pulmonary mesenchyme has been challenging to parse into specific groups with distinct markers and functions. Use of scRNA-seq allows not only for the analysis of the developing lung at single cell resolution, but also within the context of transcriptomic differences between groups of cells [17]. Ongoing studies to characterise the regulatory landscape of cellular identity using epigenomic approaches, such as single cell assay for transposase-accessible chromatin using sequencing (scATAC-seq), will provide an additional dimension to understanding the lineages and roles of specific mesenchymal cell subpopulations in human lung development and disease.

Conflict of interest: None declared.

Support statement: This work was supported by NIH K08 HL143051 (J.M.S. Sucre) and the Parker B. Francis Fellowship (J.M.S. Sucre), as well as by the NIH Common Fund, through the Office of Strategic Coordination/Office of the NIH Director under NIH U54 HL145608 (J. Hagood). Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 Brady G, Barbara M, Iscove NN. Representative *in vitro* cDNA amplification from individual hemopoietic cells and colonies. *Methods Mol Cell Biol* 1990; 2: 17–25.
2 Eberwine J, Yeh H, Miyashiro K, *et al.* Analysis of gene expression in single live neurons. *Proc Natl Acad Sci USA* 1992; 89: 3010–3014.
3 Tang F, Barbacioru C, Wang Y, *et al.* mRNA-Seq whole-transcriptome analysis of a single cell. *Nat Methods* 2009; 6: 377–382.
4 Klein AM, Mazutis L, Akartuna I, *et al.* Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. *Cell* 2015; 161: 1187–1201.
5 Warburton D. Overview of lung development in the newborn human. *Neonatology* 2017; 111: 398–401.
6 McCulley D, Wienhold M, Sun X. The pulmonary mesenchyme directs lung development. *Curr Opin Genet Dev* 2015; 32: 98–105.
7 Endale M, Ahlfeld S, Bao E, *et al.* Temporal, spatial, and phenotypical changes of PDGFRalpha expressing fibroblasts during late lung development. *Dev Biol* 2017; 425: 161–175.
8 Metzger RJ, Klein OD, Martin GR, *et al.* The branching programme of mouse lung development. *Nature* 2008; 453: 745–750.
9 Danopoulos S, Alonso I, Thornton ME, *et al.* Human lung branching morphogenesis is orchestrated by the spatiotemporal distribution of ACTA2, SOX2, and SOX9. *Am J Physiol Lung Cell Mol Physiol* 2018; 314: L144–L149.
10 Danopoulos S, Thornton ME, Grubbs BH, *et al.* Discordant roles for FGF ligands in lung branching morphogenesis between human and mouse. *J Pathol* 2019; 247: 254–265.
11 Nikolic MZ, Caritg O, Jeng Q, *et al.* Human embryonic lung epithelial tips are multipotent progenitors that can be expanded *in vitro* as long-term self-renewing organoids. *eLife* 2017; 6: e26575.
12 Danopoulos S, Bhattacharya S, Mariani TJ, *et al.* Transcriptional characterisation of human lung cells identifies novel mesenchymal lineage markers. *Eur Respir J* 2020; 55: 1900746.
13 Xu Y, Mizuno T, Sridharan A, *et al.* Single-cell RNA sequencing identifies diverse roles of epithelial cells in idiopathic pulmonary fibrosis. *JCI Insight* 2016; 1: e90558.
14 Reyfman PA, Walter JM, Joshi N, *et al.* Single-cell transcriptomic analysis of human lung provides insights into the pathobiology of pulmonary fibrosis. *Am J Respir Crit Care Med* 2019; 199: 1517–1536.

15 Habermann AC, Gutierrez AJ, Bui LT, *et al.* Single-cell RNA-sequencing reveals profibrotic roles of distinct epithelial and mesenchymal lineages in pulmonary fibrosis. *bioRxiv* 2019; preprint [https://doi.org/10.1101/753806].
16 Adams TS, Schupp JC, Poli S, *et al.* Single Cell RNA-seq reveals ectopic and aberrant lung resident cell populations in idiopathic pulmonary fibrosis. *bioRxiv* 2019; preprint [https://doi.org/10.1101/759902].
17 Klein AM, Macosko E. InDrops and Drop-seq technologies for single-cell sequencing. *Lab Chip* 2017; 17: 2540–2541.