24. De Rossi G, Evans AR, Kay E, Woodfin A, McKay TR, Nourshargh S, *et al.* Shed syndecan-2 inhibits angiogenesis. *J Cell Sci* 2014;127:4788–4799.
25. Whiteford JR, Behrends V, Kirby H, Kusche-Gullberg M, Muramatsu T, Couchman JR. Syndecans promote integrin-mediated adhesion of mesenchymal cells in two distinct pathways. *Exp Cell Res* 2007;313: 3902–3913.
26. Couchman JR. Syndecans: proteoglycan regulators of cell-surface microdomains? *Nat Rev Mol Cell Biol* 2003;4:926–937.
27. Adams TS, Schupp JC, Poli S, Ayaub EA, Neumark N, Ahangari F, *et al.* Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. *Sci Adv* 2020;6.
28. 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.
29. Redente EF, Black BP, Backos DS, Bahadur AN, Humphries SM, Lynch DA, *et al.* Persistent, progressive pulmonary fibrosis and epithelial remodeling in mice. *Am J Respir Cell Mol Biol* 2021;64:669–676.

All Roads Lead to Rome? Resident or Interspecies Chimera-derived Pulmonary Endothelial Progenitors for Cell-based Therapy

Aberrant development or extensive damage to the lung endothelium are involved in diseases such as bronchopulmonary dysplasia (1), pulmonary arterial hypertension (2), or even coronavirus disease (COVID-19) (3). Coordinated regenerative responses of the lung endothelium and epithelium are necessary to restore lung function and architecture after various types of injury (4). Cell-based therapeutic approaches for pulmonary vascular diseases are currently at various stages of development, including clinical trials (5, 6). These approaches are propelled by basic studies that aim to elucidate endothelial cell heterogeneity and mechanisms of epithelial–endothelial cross-talk in lung development and disease (7, 8).

Endothelial cell heterogeneity across tissues has recently been described and analyzed in depth with the introduction of single-cell RNA-sequencing (9). The lung vascular field has also benefited from this tool that has allowed for the identification of capillary subpopulations in the mouse lung. The main source of endothelial heterogeneity arises from capillaries (7–10), an observation that is also conserved across organs (9) and that has been depicted in the human lung (11).

Pulmonary endothelial cells, specifically, include two distinct capillary types: general capillaries labeled by *cKit* (cKit tyrosine kinase, 85% of the endothelium) and Car4 endothelial cells (15%), named after their marker gene carbonic anhydrase 4 (7, 10). The morphology, location, and direct association of the Car4 cell to the alveolar type 1 cell (7) indicate a possible role in gas exchange, which has led to the name "aerocytes" (10); still, there is no functional data to support that role thus far. Car4 endothelial cells first appear at Embryonic Day 19, specified by VEGFA (vascular endothelial growth factor A) secreted by alveolar type 1 cells. However, earlier in lung development at Embryonic Day 17, there is only one type of capillary cell type that can be identified by the expression of *cKit*, a progenitor population that gives rise to the mature *cKit*$^+$ general capillaries and to the Car4 population (7).

In this issue of the *Journal*, Wang and colleagues (pp. 326–338) have identified additional heterogeneity within this cKIT$^+$ progenitor population consisting of *Foxf1* (forkhead box F1)-positive or -negative cells by means of single-cell RNA-sequencing (12). FOXF1$^+$cKIT$^+$ cells, also present in the human lung, decrease in the adult and are enriched in genes related to angiogenesis and endothelial cell proliferation. Underlining their progenitor potential, these cells were able to rescue neonatal angiogenesis in a mouse model of alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV), a rare disease related to mutations in the *Foxf1* gene. Moreover, their presence improved alveologenesis, highlighting the developmental association and coordinated growth of the endothelium and epithelium.

In a similar vein to their previous work on lung epithelial reconstitution via intraspecies (mouse–mouse) blastocyst complementation (13), the authors then produced mouse endothelial cells via interspecies (mouse–rat) complementation (i.e., mouse embryonic stem cell injection into rat blastocysts). Mouse-derived cells were found incorporated in most tissues, including mouse endothelial cells in the rat pulmonary vasculature. Flow-cytometry–purified mouse FOXF1$^+$cKIT$^+$ lung endothelial progenitors from chimeric animals were able to improve neonatal angiogenesis and alveolarization in their ACDMPV mouse model after adoptive transfer. These cells were transcriptionally similar to endogenous FOXF1$^+$cKIT$^+$ progenitors, overall indicating that interspecies chimeras are, in principle, a viable way of producing functional endothelial progenitors via *in vivo* differentiation of pluripotent stem cells.

The presence of "transitional cells" in the vasculature—cells that coexpress markers of distinct phenotypes simultaneously—raises interesting questions about identity and potential (9). In the lung, we can find this phenomenon as part of the anatomical transition along the vascular tree, exemplified by cells that express *Esm1* (endothelial cell specific molecule 1), as well as macrovasculature and capillary genes, which are located in the transition zone from arteries to capillaries (7). Nevertheless, this transition could also be the result of cell fate change. Mainly, these cKIT$^+$ progenitors, which then subdivide into FOXF1-expressing cells to finally give rise to mature capillaries, point to the dynamic nature of the lung during development and how little is known about the mechanisms for endothelial heterogeneity and its role in regeneration (7). Whether FOXF1$^+$cKIT$^+$ progenitors play a part in endothelial repair during disease, what cell types they are able to give rise

Originally Published in Press as DOI: 10.1164/rccm.202103-0786ED on April 26, 2021

to, and why and how only a subset of cKIT$^+$ cells are capable of undergoing this process remain unknown.

Overall, this present study, together with work done previously by the same group (14), offers insight into the future of cell-based therapy for diseases such as ACDMPV or bronchopulmonary dysplasia. Importantly, it also expands the repertoire of lung cell types that have been successfully derived via blastocyst complementation. This technique not only will be important for disease management but possibly for evolutionary studies of the respiratory system, as it also allows interspecies experiments that can further advance our knowledge of which genes and signals are relevant for lung development and regeneration. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Lisandra Vila Ellis, M.D.
Department of Pulmonary Medicine
The University of Texas M.D. Anderson Cancer Center
Houston, Texas

Laertis Ikonomou, Ph.D.
Department of Oral Biology
University at Buffalo
Buffalo, New York

ORCID IDs: 0000-0002-2000-5592 (L.V.E.); 0000-0003-0993-6713 (L.I.).

References

1. Bhatt AJ, Pryhuber GS, Huyck H, Watkins RH, Metlay LA, Maniscalco WM. Disrupted pulmonary vasculature and decreased vascular endothelial growth factor, Flt-1, and TIE-2 in human infants dying with bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;164:1971–1980.
2. Budhiraja R, Tuder RM, Hassoun PM. Endothelial dysfunction in pulmonary hypertension. *Circulation* 2004;109:159–165.
3. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, *et al.* Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* 2020;383:120–128.
4. Ding BS, Nolan DJ, Guo P, Babazadeh AO, Cao Z, Rosenwaks Z, *et al.* Endothelial-derived angiocrine signals induce and sustain regenerative lung alveolarization. *Cell* 2011;147:539–553.
5. Granton J, Langleben D, Kutryk MB, Camack N, Galipeau J, Courtman DW, *et al.* Endothelial NO-synthase gene-enhanced progenitor cell therapy for pulmonary arterial hypertension: the PHACeT trial. *Circ Res* 2015;117:645–654.
6. Ikonomou L, Wagner DE, Turner L, Weiss DJ. Translating basic research into safe and effective cell-based treatments for respiratory diseases. *Ann Am Thorac Soc* 2019;16:657–668.
7. Vila Ellis L, Cain MP, Hutchison V, Flodby P, Crandall ED, Borok Z, *et al.* Epithelial Vegfa specifies a distinct endothelial population in the mouse lung. *Dev Cell* 2020;52:617–630.e6.
8. Niethamer TK, Stabler CT, Leach JP, Zepp JA, Morley MP, Babu A, *et al.* Defining the role of pulmonary endothelial cell heterogeneity in the response to acute lung injury. *eLife* 2020;9:28.
9. Kalucka J, de Rooij LPMH, Goveia J, Rohlenova K, Dumas SJ, Meta E, *et al.* Single-cell transcriptome atlas of murine endothelial cells. *Cell* 2020;180:764–779.e20.
10. Gillich A, Zhang F, Farmer CG, Travaglini KJ, Tan SY, Gu M, *et al.* Capillary cell-type specialization in the alveolus. *Nature* 2020;586:785–789.
11. Schupp JC, Adams TS, Cosme C, Raredon MSB, Omote N, De Frias SP, *et al.* Integrated single cell atlas of endothelial cells of the human lung. *bioRxiv* 2020: 2020.2010.2021.347914.
12. Wang G, Wen B, Ren X, Li E, Zhang Y, Guo M, *et al.* Generation of pulmonary endothelial progenitor cells for cell-based therapy using interspecies mouse–rat chimeras. *Am J Respir Crit Care Med* 2021;204:326–338.
13. Wen B, Li E, Ustiyan V, Wang G, Guo M, Na CL, *et al. In vivo* generation of lung and thyroid tissues from embryonic stem cells using blastocyst complementation. *Am J Respir Crit Care Med* 2021;203:471–483.
14. Ren X, Ustiyan V, Guo M, Wang G, Bolte C, Zhang Y, *et al.* Postnatal alveologenesis depends on FOXF1 signaling in c-KIT$^+$ endothelial progenitor cells. *Am J Respir Crit Care Med* 2019;200:1164–1176.

The Legacy of Racial and Ethnic Segregation on Health: The Story of Continuous Positive Airway Pressure Use

Health inequities—unjust and avoidable differences in health status—arise from unequal distribution of finances, power, and resources (1, 2). Historically disadvantaged populations, Black, Indigenous, and people of color (BIPOC), suffer a greater burden of sleep health disparities. Shorter duration of sleep, more fragmented sleep, delayed sleep onset, and poorer quality sleep are more common in non-white populations (3). Disparities in obstructive sleep apnea (OSA) recognition, diagnosis, and treatment have also been identified by race, ethnicity, and socioeconomic status (SES) (4). Results from observational cohort studies and clinical trials suggest that adherence to positive airway pressure (PAP), the most effective treatment for OSA, differs by race and ethnicity (5, 6). However, little "real world" data exists. Failure to meet the Centers for Medicare and Medicaid Services (CMS) criteria for adherence (≥4 hours of use per night on ≥70% of nights) often leads to loss of PAP coverage, so reduced PAP use in BIPOC may further exacerbate sleep health disparities.

In this issue of the *Journal*, Borker and colleagues (pp. 339–346) analyzed data from a nationwide sample of adults with OSA who used their PAP for at least 30 seconds, exploring differences in use by neighborhood racial and ethnic composition (7). Nearly 800,000 individuals were included together with data regarding their PAP use. Each person's 5-digit ZIP code was mapped to ZIP code tabulation areas

Originally Published in Press as DOI: 10.1164/rccm.202103-0649ED on April 12, 2021