in scleroderma lung disease treated with oral cyclophosphamide. *Eur Radiol* 2011;21:2455–2465.
13. ClinicalTrials.gov. A trial to evaluate the efficacy of PRM-151 in subjects with idiopathic pulmonary fibrosis (IPF); 2015 [updated 2016 Oct 26; accessed 2016 Nov 1]. Available from: https://clinicaltrials.gov/ct2/show/NCT02550873
14. ClinicalTrials.gov. Safety, tolerability, and efficacy study of idiopathic pulmonary fibrosis (FGCL-3019-049); 2010 [updated 2014 Jan 15; accessed 2016 Nov 1]. Available from: https://clinicaltrials.gov/ct2/show/NCT01262001
15. Steyerberg EW, Vergouwe Y. Towards better clinical prediction models: seven steps for development and an ABCD for validation. *Eur Heart J* 2014;35:1925–1931.

Induced Pluripotent Stem Cells in Pulmonary Arterial Hypertension

Pioneering work by Yamanaka's group showed that overexpression of four transcription factors, octamer-binding protein 4 (*OCT4*), Krüppel-like factor 4 (*KLF4*), sex determining region Y-box 2 (*SOX2*), and c-myc avian myelocytomatosis viral oncogene homolog (*c-MYC*), could reprogram somatic cells into induced pluripotent stem cells (iPSCs), which could then be differentiated into all cell types (1). Since that finding, it has become clear that iPSCs hold great promise as models for diseases, drug discovery, and testing of cell-based therapeutic strategies.

Pulmonary diseases are one leading cause of morbidity and mortality worldwide. Currently available treatments can only alleviate symptoms or delay disease progression within a limited time range for patients with end-stage pulmonary diseases. Pulmonary arterial hypertension (PAH) is a complex disorder of pulmonary microvasculature, circulating cells, and right heart, with poor prognosis (2, 3). Animal models of PAH have existed for some time and have provided key insights into disease pathogenesis. Attempts to translate these findings into treatment for patients, however, have been imperfect at best, because at a molecular level animal lungs and lung tissues are different from human lungs and tissues, and thus it has been difficult to recapitulate the disease process *in vitro*. Use of human lung cells could address some of the deficiencies. However, it is technically difficult to isolate and characterize human cells in enough number to be useful in laboratory studies. iPSCs provide one potential solution to this problem. iPSCs have been used to derive respiratory epithelial cells, vascular endothelial cells, and vascular smooth muscle cells (4–6) and have also been used to study lung development and vascular modeling (4, 7–9). However, whether they can serve as tools to investigate the potential for new therapeutic agents in PAH is unknown.

In this issue of the *Journal*, Sa and colleagues (pp. 930–941) present data from proof-of-principle studies that address an important question: whether iPSCs derived from patients with PAH have potential as tools for drug discovery and testing (10). They compared the pulmonary artery endothelial cells isolated from patients with idiopathic or heritable PAH to endothelial cells (ECs) derived from fibroblast-derived iPSCs from the same patients. The authors show that there are many similarities between the iPSC-ECs and native pulmonary artery endothelial cells, including morphology, functional deficits, reduction of bone morphogenetic protein receptor (BMPR)-II signaling, and, importantly, response to bone morphogenetic protein 9 stimulation and drug treatment, as well as some differences. Using RNA-seq analyses, they further identified molecular signatures responsible for the observed functional and drug response differences between native ECs and iPSC-ECs. In summary, their data show that iPSC-derived ECs can serve as surrogates for native ECs in both functional and drug discovery studies.

Their work suggests that the iPSC-ECs model has the potential to serve as a precision/personalized medicine tool—to determine which one of the many drugs would be effective in a particular patient—because the cells are derived from the same patient who would, in the end, receive the drug. Although this concept maybe exciting, their data also show that the use of these cells is not straightforward, and additional data are needed before broadly generalizable conclusions can be made. For example, they found that only one of two IPSC-ECs derived from patients with heritable PAH (with the same *BMPR2* mutation) and only two of six iPSC-ECs derived from patients with idiopathic PAH showed a functional response to Elafin or FK506. Why the treatments did not have a universal effect is currently unknown. One potential mechanism could be preserved fibroblast-specific epigenetic signatures; it is well known that iPSCs can retain some of the epigenetic signatures of the parent cell. Another could be that patient-specific molecular modifiers, such as *BMPR2* expression and alternative splicing and estrogen metabolism (11–13), retain their effect regardless of programming. One way to address this question would be to compare iPSC-ECs derived from different originating tissues from the same patient and with each and with other types of endothelial cells, such as circulating ECs, also from the same patient. Another possibility could be that because both Elafin and FK506 specifically affect BMPR-II signaling (14, 15), they might not be effective if BMPR-II signaling is not the primary disease driver in a particular cell line. It may thus be useful to determine if iPSC-ECs and native ECs show similar responses to a wider variety of potential therapeutic agents.

It is important to note that this work involved a small number of cell lines derived from a limited number of patients from a particular pulmonary hypertension (PH) category (Group 1). A larger number of iPSCs from a larger cohort of patients with PH, including patients from the other PH groups and subgroups (Groups 2–5), will need to be analyzed to more conclusively determine the usefulness of this approach to the PH field as a whole.

Given the paucity of treatment options in PAH, there is an urgent need for improved understanding of the molecular

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mechanisms of PAH in human tissues—a vital prerequisite for developing disease models and eventually therapies for PAH. Directed differentiation of iPSCs and generation of pulmonary vascular cell lines and tissues could be an important tool for such studies. This promising work from Sa and colleagues addresses many of these needs and has the potential to provide unparalleled insight into PAH development and pathogenesis and, importantly, significantly speed up new drug discovery. ■

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References

1. Yamanaka S, Takahashi K. Induction of pluripotent stem cells from mouse fibroblast cultures [in Japanese]. *Tanpakushitsu Kakusan Koso* 2006;51:2346–2351.
2. Farber HW, Loscalzo J. Pulmonary arterial hypertension. *N Engl J Med* 2004;351:1655–1665.
3. Leopold JA, Maron BA. Molecular mechanisms of pulmonary vascular remodeling in pulmonary arterial hypertension. *Int J Mol Sci* 2016;17: 761.
4. Huang SX, Islam MN, O'Neill J, Hu Z, Yang YG, Chen YW, Mumau M, Green MD, Vunjak-Novakovic G, Bhattacharya J, *et al*. Efficient generation of lung and airway epithelial cells from human pluripotent stem cells. *Nat Biotechnol* 2014;32:84–91.
5. Ghaedi M, Calle EA, Mendez JJ, Gard AL, Balestrini J, Booth A, Bove PF, Gui L, White ES, Niklason LE. Human iPS cell-derived alveolar epithelium repopulates lung extracellular matrix. *J Clin Invest* 2013; 123:4950–4962.
6. Nakayama KH, Joshi PA, Lai ES, Gujar P, Joubert LM, Chen B, Huang NF. Bilayered vascular graft derived from human induced pluripotent stem cells with biomimetic structure and function. *Regen Med* 2015;10:745–755.
7. Snoeck HW. Modeling human lung development and disease using pluripotent stem cells. *Development* 2015;142:13–16.
8. Hawkins F, Kotton DN. Embryonic and induced pluripotent stem cells for lung regeneration. *Ann Am Thorac Soc* 2015;12:S50–S53.
9. Belair DG, Whisler JA, Valdez J, Velazquez J, Molenda JA, Vickerman V, Lewis R, Daigh C, Hansen TD, Mann DA, *et al*. Human vascular tissue models formed from human induced pluripotent stem cell derived endothelial cells. *Stem Cell Rev* 2015;11:511–525.
10. Sa S, Gu M, Chappell J, Shao N-Y, Ameen M, Elliott KAT, Li D, Grubert F, Li CG, Taylor S, *et al*. Induced pluripotent stem cell model of pulmonary arterial hypertension reveals novel gene expression and patient specificity. *Am J Respir Crit Care Med* 2017;195:930–941.
11. Hamid R, Cogan JD, Hedges LK, Austin E, Phillips JA III, Newman JH, Loyd JE. Penetrance of pulmonary arterial hypertension is modulated by the expression of normal BMPR2 allele. *Hum Mutat* 2009;30: 649–654.
12. Cogan J, Austin E, Hedges L, Womack B, West J, Loyd J, Hamid R. Role of BMPR2 alternative splicing in heritable pulmonary arterial hypertension penetrance. *Circulation* 2012;126:1907–1916.
13. Chen X, Talati M, Fessel JP, Hemnes AR, Gladson S, French J, Shay S, Trammell A, Phillips JA, Hamid R, *et al*. Estrogen metabolite 16α-hydroxyestrone exacerbates bone morphogenetic protein receptor type II-associated pulmonary arterial hypertension through microRNA-29-mediated modulation of cellular metabolism. *Circulation* 2016;133:82–97.
14. Nickel NP, Spiekerkoetter E, Gu M, Li CG, Li H, Kaschwich M, Diebold I, Hennigs JK, Kim KY, Miyagawa K, *et al*. Elafin reverses pulmonary hypertension via caveolin-1-dependent bone morphogenetic protein signaling. *Am J Respir Crit Care Med* 2015;191:1273–1286.
15. Spiekerkoetter E, Tian X, Cai J, Hopper RK, Sudheendra D, Li CG, El-Bizri N, Sawada H, Haghighat R, Chan R, *et al*. FK506 activates BMPR2, rescues endothelial dysfunction, and reverses pulmonary hypertension. *J Clin Invest* 2013;123:3600–3613.