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Precision Modeling of Pulmonary Hypertension Pathology with Induced Pluripotent Stem Cell-derived Cells

Pulmonary arterial hypertension (PAH) is a progressive, ultimately fatal disease characterized by vascular remodeling via endothelial cell (EC) dysfunction and apoptosis, smooth muscle cell (SMC) proliferation and contraction, and formation of plexiform lesions. These pathologic processes all contribute to the increased vascular resistance that defines PAH. Heritable PAH is most often associated with mutation in BMPR2 (bone morphogenetic protein receptor 2), and PAH from other causes is usually correlated with alterations in BMPR2 signaling. However, despite its strong linkage to PAH, only about 30% of people who carry a pathogenic BMPR2 mutation go on to develop the disease. This is due to yet unknown combinations of environmental modifiers and variants of other genes. These disease modifiers not only affect penetrance but ultimately lead to substantially different responses to treatment in patients with PAH. Although targeted precision medicine may ultimately prove to be the most effective overall strategy for therapy (1), the challenge remains to recapitulate and tease apart various environmental and genetic modifiers *in vitro* to determine the role of modifiers in disease penetrance.

A number of cell lines exist for this purpose. Pulmonary arterial ECs (PAECs) and SMCs (PASMCs) can be derived from explanted tissue for *in vitro* study, and recapitulate the apoptotic, proliferative, and mitochondrial dysfunction seen in PAH (2, 3).

More recently, patient-specific induced pluripotent stem cells differentiated into ECs (iPSC-ECs) have been characterized (3, 4). Although there was a brief report of successful SMC differentiation from PAH iPSCs (5), their phenotype is unknown, and no iPSC-SMC line has been characterized as a model of PAH. Derivation of ECs or SMCs from iPSCs not only removes the requirement for patient lung samples but also may enable researchers to evaluate the probable success of patient-tailored therapies. However, because cells from each patient contain unique genetic modifiers, the effects of genetic differences or varied gene expression among cell lines confound basic research, and multiple patient-derived iPSC-EC lines must be evaluated to pool results. Moreover, although endothelial BMPR2-associated mitochondrial dysfunction is an important contributor to the metabolic defects and pathogenesis of PAH (6, 7), patient-derived iPSC-ECs were unable to replicate this mitochondrial pathology (3). As reported in this issue of the *Journal*, Kiskin and colleagues (pp. 271–275) addressed these issues by deriving and characterizing wild-type iPSC-SMC and iPSC-EC lines containing introduced BMPR2 mutations (8).

The study by Kiskin and colleagues has several points of significance. First, this study's careful attention to the details of the differentiation protocol is a model of how to handle the derivation of a particular cell type from iPSCs. Three SMC differentiation protocols led to quite different outcomes, as assessed by a principal component analysis on expression array data. The iPSC-SMCs derived via a lateral plate mesoderm lineage had intact

contractile function, gene expression similar to both proximal and distal adult-derived PSMCs in a principal component analysis, and responded to BMP4 treatment similarly to peripheral PSMCs.

A second strong point of interest is the authors' demonstration that the details of the cell culture conditions have a huge impact on the mutation effect. iPSC-SMCs with an introduced BMPR2 mutation have decreased apoptosis and increased proliferation at a basal level compared with wild-type, and the addition of serum hyperpolarizes the inner mitochondrial membrane (IMM) in these cells, similar to what is observed with patient-derived PSMCs. BMPR2 mutant iPSC-ECs are similar to the wild-type in serum-free conditions, but in the presence of serum they display increased apoptosis and proliferation and IMM hyperpolarization like patient-derived PAECs. This strong dependence of phenotype on available substrates is a good match to the body of literature that has been developed relating to metabolic alterations in PAH (9, 10), but is often neglected in cell culture studies in this disease. It also suggests that there may be some lab-to-lab variations in the resulting phenotype when this approach is used, due to possible batch differences in the serum used.

The authors' central claim is that many of the phenotypes associated with disease can be reproduced by an introduced BMPR2 point mutation in iPSCs, without directly requiring additional mutations or environmental effects. Although this sort of work has been done before, it required larger numbers of cell lines to overcome the variation caused by the genetic background. Of course, environmental and other genetic factors may still contribute to penetrance, and Kiskin and colleagues demonstrate that TNF- α (tumor necrosis factor- α) exposure hyperpolarized the IMM in iPSC-SMCs and ECs with a BMPR2 mutation to a greater extent than did serum, and concomitant BMP9 addition reversed the hyperpolarization in iPSC-ECs. This strengthens the argument that exposure to an immune trigger may help drive the disease phenotype, as well as the potential of these iPSC-derived cells to screen for treatment efficacy. These results provide an excellent model system in which to evaluate the contribution of other environmental effects related to PAH, such as mechanical stretch, changes in glucose levels, and the cytokine contribution to PAH cellular phenotypes. Similarly, other gene variants could be introduced.

There are still drawbacks to this approach, of course, and chief among them is the fact that we still do not have differentiation protocols that can produce distinctly pulmonary (as opposed to systemic) smooth muscle and endothelium. Behaviors unique to the pulmonary vasculature thus cannot be directly tested in this model, although, as the authors suggest, the cells can be combined with pulmonary-specific cells in organoid or artery-on-chip setups for more targeted evaluations. Nevertheless, this report describes a

model system—and a model approach—that is likely to be of tremendous utility for future research in PAH. ■

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