Investigating Pulmonary Arterial Hypertension from ‘Stem’ to Stern

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For decades, primary cell culture *in vitro* and animal models *in vivo* have remained the cornerstones of functional modeling and drug discovery in the context of human disease. While both have proved to be invaluable tools, they are inherently limited in their ability to replicate human pathology. Primary cells are often difficult to maintain for long periods in culture, and animal models often provide little insight into species-specific facets of disease. Induced pluripotent stem cells (iPSCs) have earned tremendous attention in the last decade as a novel model system for the study of human pathobiology. In 2006, seminal work by Sinya Yamanaka and collaborators revealed that the retroviral gene transfer of four transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) was sufficient to induce a pluripotent state in fibroblasts taken from both embryonic and adult mice (8). These findings were later replicated using human fibroblasts and blood lymphocytes (2). The resulting cells can be grown in culture and are morphologically indistinguishable from embryonic stem (ES) cells, with nearly equivalent differentiation potential (8).

An important feature of iPSCs is the fact that they typically retain genetic information from their donors, including any genetic abnormalities that may have contributed to disease. This lends them a wide range of potential applications, from regenerative medicine to personalized drug screening (2). Notably, they provide an invaluable opportunity for the study of genetic diseases where the accuracy of more traditional models has remained a persistent problem. Pulmonary arterial hypertension (PAH) is a complex panvasculopathy, characterized by a program of endothelial and epithelial cell dysfunction throughout the pulmonary vasculature, with contributions from the surrounding mesenchymal stroma (1). Both heritable and idiopathic forms of the disease have been linked to dysregulation of the BMPR2 signaling pathway, yet the role of BMPR2 mutation in the development of PAH is not well-understood, due in part to the deficiency of available PAH disease models. *In vitro* knockdown of BMPR2 in culture
does not fully suppress wild-type signaling (7), while human PAH tissue can be scarce, and is typically available only following transplant or autopsy, when the disease has inevitably reached a very late stage. Animal models are available, but tend to display differences in pulmonary vascular remodeling relative to what is observed in patients, calling into question their biological fidelity (7).

Pioneering the use of iPS cells in the investigation of PAH, a new study by West and colleagues has readdressed the role of BMPR2 mutation in the pulmonary vasculature (9). Working with skin fibroblasts isolated from PAH patients, their team produced a line of PAH-specific iPS cells from which they derived endothelial cell (EC) and mesenchymal stromal cell (MSC) lineages. Importantly, cells produced in this manner recapitulated the genetic background of disease in these patients, but they lacked the acquired changes in phenotype that might be seen in primary cells taken directly from the pulmonary vasculature. In doing so, this platform allowed for any genetic abnormalities to be studied in isolation, without concern for the confounding factors present in late-stage disease (9). Through expression array profiling of these iPS-derived ECs and MSCs, West and colleagues were able to detect dysregulation of several members of the canonical Wnt signaling pathway. The upregulation of noncanonical Wnt signaling has been previously linked to vasoconstriction and vascular remodeling in PAH (4), and the BMPR2 and Wnt pathways are known to interact closely in the regulation of body axis patterning in early-stage development. However, their specific relationships in the adult cardiovascular system have not been fully defined, especially in the context of heritable PAH (9).

This finding represents a novel contribution to our understanding of the genetic factors that contribute to PAH, but more importantly, it showcases the power of PAH-specific
modeling with this exciting new technology (Fig. 1). The field of iPSC biology is growing rapidly in concert with technologies in human genomics and genetics, and soon, patient-specific iPS cells could prove invaluable in unraveling the growing list of genetic abnormalities associated with PAH (5). As iPS cell techniques mature, it may even be possible that compilation of these cells from a broad cohort of individuals will become a platform by which additional genetic factors may be identified in this disease, independent of classical human disease phenotyping. As technology for iPS cell generation become further streamlined, generating high volumes of these cells from broad cohorts of PAH patients may become feasible, as many current protocols can be pursued without the need for retroviral gene transfer and further genomic alteration (10). In addition, the utility of iPSCs is quickly progressing towards targeted drug and personalized toxicology screening (2). Finally, by coupling an iPSC platform with genome editing technology, as has been attempted in other cardiovascular contexts (3), it is conceivable that causal disease mutations in PAH could be corrected in pulmonary vascular cell populations for transplantation into human patients.

However, numerous caveats still exist that may temper expectations of iPSC technology in this disease. Because PAH spans multiple vascular cell types, it is not well modeled by any single cell population in culture (7) - a limitation which may muddle interpretations of the functional importance of mutations in iPSC-derived PAH models in cell culture. Particularly for the study of PAH, further improvement of the technology is also crucial to ensure that iPSC-derived vascular cell types accurately recapitulate activity of pulmonary vascular cells, as most differentiation protocols to generate vascular endothelium, smooth muscle cells or fibroblasts are not specific for the pulmonary system (6). Finally, the safety of iPSCs for transplantation into PAH patients has not been fully vetted, and given the potential for tumorigenicity, immunogenicity, and
other unexpected genetic and epigenetic abnormalities, there are important safety
considerations for genomic editing in iPSCs prior to human transplantation (2).

Even with these caveats, however, the advent of iPSC technology in the study of PAH is
a significant first step forward. It now remains to be seen if the grand potential of iPSCs
can be fully realized in truly altering the way in which we model, treat, and prevent
human PAH.

**Figure Legends:**

**Figure 1:** PAH-specific iPS cells promise myriad functional applications, both in the
realm of basic science and in translational research for the treatment of patients afflicted
with this disease.
References:


**Basic Science**
- Discovery of novel genetic factors
- Advanced disease modeling
- Drug discovery and screening

**Difficulties**
- Unable to model multiple cell types in one system
- Must reflect cell types found specifically in the pulmonary vascular bed

**PAH Patient**

**Difficulties**
- Safety considerations (tumorigenicity, genetic abnormalities)

**Tissue collection (e.g., fibroblast, peripheral blood mononuclear cells)**

**Patient-derived Primary Cells**
- Reprogramming of somatic cells (OCT4, KLF4, MYC, SOX2)

**iPS Cell Colonies**
- Directed differentiation (endothelial, epithelial, fibroblast, mesenchymal stromal)

**Differentiated Cells**

**Translational Research**
- Personalized toxicology screening
- Genome editing and transplantation of repaired vascular cells
- Combination of PAH specific cell banks