Programmatic change: lung disease research in the era of induced pluripotency

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Human lung research has made remarkable progress over the last century largely through the use of animal models of disease. The challenge for the future is to translate these findings into human disease and bring about meaningful disease modification or even cure. The ability to generate transformative therapies in the future will require human tissue, currently scarce under the best of circumstances. Unfortunately, patient-derived somatic cells are often poorly characterized and have a limited life span in culture. Moreover, these cells are frequently obtained from patients with end-stage disease exposed to multiple drug therapies, leaving researchers with questions about whether their findings recapitulate disease-initiating processes or are simply the result of pharmacological intervention or subsequent host responses.

The goal of studying early disease in multiple cell and tissue types has driven interest in the use of induced pluripotent stem cells (iPSCs) to model lung disease. These cells provide an alternative model for relevant lung research and hold promise in particular for studying the initiation of disease processes in genetic conditions such as heritable pulmonary arterial hypertension as well as other lung diseases. In this Perspective, we focus on potential iPSC use in pulmonary vascular disease research as a model for iPSC use in many types of advanced lung disease.

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perspective.

The feasibility of using human iPSCs for drug development was also demonstrated in the case of the long QT syndrome, an autosomal dominant disease characterized by delayed repolarization of cardiomyocytes that results in potentially lethal tachycardia. This syndrome is due to mutations of genes encoding potassium channel subunits such as KCNQ1 and KCNH2. Two independent groups demonstrated that iPSC-derived cardiomyocytes from long QT syndrome patients show longer action potentials (characteristic signature of the disease) compared with control cardiomyocytes (21, 35). Furthermore, aberrant ion flux in these cells in vitro was responsive to drugs, such as beta blockers that ameliorate the disease phenotype in vivo (21).

Nevertheless, most human diseases, even if genetic in origin, manifest their phenotype as a result of a complex interplay of genetic and environmental factors; thus their correct in vitro modeling using iPSCs presents formidable challenges (48). For instance, final manifestation of cystic fibrosis phenotype is not just a function of CFTR mutation but also includes the lung microbiome, anatomy, drug therapy, and other factors (6). Such challenges will be discussed in the context of iPSC modeling of pulmonary arterial hypertension (PAH), a highly morbid cardiopulmonary disease resulting from aberrant remodeling of the pulmonary vasculature. We have previously derived more than 100 iPSC lines from patients with diseases affecting the epithelial, endothelial, or interstitial compartments of the lung (48). Cells from these compartments are not readily accessible or amenable to culture; thus deriving these differentiated cell types de novo in culture from iPSCs may provide important in vitro correlates to their naturally occurring in vivo counterparts. We believe the same approach can be successfully applied to PAH, since it involves several cell types.

Use of iPSC Technology To Model Lung Diseases, Exemplified by PAH

PAH is characterized by elevated pulmonary artery pressures and widespread obstruction and obliteration of small pulmonary arteries (Fig. 2) (3, 47). Arterial changes occur in all the layers of the vascular wall. This includes occlusion, or near occlusion, of the lumen due to neointimal formation characterized by the expansion of endothelial cells, cells with
markers of vascular smooth muscle cells, and inflammatory cells, as well as obstructive microthrombi. A fibroproliferative response characterized by an increase in extracellular matrix components may be seen, and vessels may display extensive development of endothelial channels (plexiform lesions) characteristic of PAH. The surrounding adventitia is typically thickened, as well (14, 43). Progressive vascular remodeling results ultimately in right ventricular (RV) failure. Whereas PAH is associated with a wide array of comorbid conditions, it also occurs as a primary pulmonary vascular disease known as either idiopathic (IPAH) (no known family history or genetic abnormality) or heritable (HPAH) PAH (50) (positive family history and/or genetic abnormality).

The majority of HPAH cases are due to mutations in bone morphogenetic protein receptor type 2 (BMPR2) (31). BMPR2 expression is typically reduced in the lungs of not only HPAH patients with BMPR2 gene mutations, but also among those with IPAH (1). Therefore, understanding the exact mechanisms by which dysfunctional bone morphogenetic protein (BMP) signaling results in PAH should be important for the development of effective therapies more broadly than just for those with HPAH. However, ~20% of HPAH cases and at least 60% of IPAH cases are associated with non-BMPR2 variants, the majority of which are not described (31). iPSC should facilitate the study of PAH regardless of its genetic underpinnings, and human iPSC offer a unique platform for mechanistic studies of and drug development for PAH. For example, direct comparison of specific patient genotype drug responses, in variable cell types with the same genotype, may lead to novel information not only for genotype and cell-specific drug discovery, but also for drug toxicities in PAH. The technology also holds the potential for direct cell transplant to treat PAH in patients. Such cell therapies could be directed toward the pulmonary microvasculature and the failing right heart (57). iPSC could be used to generate mesenchymal stem and endothelial cells, which have been shown to be effective in animal models of pulmonary hypertension directly (2, 22, 63) or with further molecular modification of expression of key genes like eNOS to improve microvasculature structure and function (5, 23, 64).

For example, the PHACeT (Pulmonary Hypertension: Assessment of Cell Therapy) Trial (clinicaltrials.gov, identifier NCT00469027) is a phase I trial of autologous progenitor cell-based gene therapy currently underway; in theory, autologous iPSCs derived from skin or circulating cells could be differentiated and manipulated to effectively deliver cell-based therapies in a similar manner.

Although dysfunction of the BMP signaling pathway in HPAH results in severe vascular remodeling and occlusion of small peripheral pulmonary microvasculature, the precise mechanisms responsible for disease pathogenesis are elusive.
With the advent of iPSCs, new venues of research are now available to elucidate the underlying mechanisms in several ways. For example, since multiple cell types are likely to contribute to HPAH pathogenesis, the ability to differentiate iPSCs into multiple lineages will allow the analysis of multiple cell types derived from a single patient, including vascular endothelial cells (ECs), smooth muscle cells and cardiomycocytes (25). In addition, multiple developmental pathways of lineage differentiation may be evaluated. For example, one may evaluate the commitment of iPSCs into mesenchymal cells (4) or iPSCs into ECs via hemangioblast (7) stages of commitment.

Once purified differentiated cells are available, in vitro and ex vivo tissue engineering approaches may be used to reconstruct arteries and study the development of the disease in a physiologically relevant system (48). For example, we and others previously described a “decellularization-recellularization” approach to create bioartificial lungs for autologous lung transplantation (39, 42). This platform may also be relevant for ex vivo studies of HPAH using iPSCs. ECs, smooth muscle cells, and lung epithelial cells could conceivably be derived in vitro and used to seed decellularized lungs. Liquid or air ventilation in combination with continuous media perfusion in the endothelial compartment would create an experimental setup that approximates the function of the native lung. This model may also be used to evaluate potential disease modifiers, such as suspected environmental insults previously associated with PAH [e.g., fenfluramine derivatives (18) and serotonergic agents (30)].

In addition to pulmonary vascular remodeling, PAH is associated with RV dysfunction (15). On average, patients with HPAH die at a younger age than those with IPAH (53). Because RV function is the primary determinant of mortality in PAH, RV dysfunction exacerbated by BMPR2 mutation or reduced BMPR2 expression may underlie this finding. Consistent with this possibility, BMP signaling appears critical to proper cardiac development (58), and mutations in BMPR2 have been linked to congenital heart disease-associated PAH (45, 46).

Given the critical role of BMP signaling in cardiac differentiation (58), it is conceivable that iPSCs derived from HPAH patients will manifest differences in cardiogenic differentiation compared with iPSCs from healthy wild-type subjects, and this may yield new mechanistic insights. In vitro studies show that the differentiation of iPSCs into cardiovascular progenitors, and ultimately to mature ECs and cardiomycocytes, is enhanced by proper exposure to BMP morphogens at specific time points of the differentiation process (25, 60). When directing the differentiation of normal ESCs or iPSCs, antagonists of BMP signaling, such as Noggin and PRDC, or chemical compounds such as dorsomorphin, influence both the yield and physiological properties of stem cell-derived cardiomycocytes (16). Thus iPSCs derived from HPAH patients carrying BMPR2 mutations may manifest differences in cardiomycocyte differentiation compared with iPSCs from healthy individuals without BMPR2 mutations; this would provide a useful model for comparative studies. In addition to disease modeling of HPAH, employing iPSC lines with known mutations in receptors that activate BMP signaling will help to advance our understanding of one of the key signaling pathways of cardiovascular development, embryonic patterning, and musculoskeletal formation. On this basis, the novel tool of iPSCs with known BMPR2 mutations may present a unique opportunity to address the cellular and molecular basis of cardiovascular diseases more broadly.

It has long been known that only ~20% of BMPR2 mutation carriers develop PAH. Differences in epigenetic modification(s) among carriers have been proposed as one hypothesis to explain this observation. If epigenetic modifications are important for the manifestation of BMPR2 mutation defects in vitro, the “epigenetic memory” of the original somatic cells from which iPSCs are derived may be important; this is a potential deficiency of fibroblast-derived iPSCs. Epigenetic memory refers to the presence of epigenetic marks from the cell type of origin in the resulting iPSCs that continues to influence gene expression. However, this epigenetic memory appears to be transient and is lost at later passages following the onset of...
reprogramming (44). In the context of PAH, if environmental insults mediate reversible epigenetic modifications in tissues that exhibit the HPAPH phenotype, then early passage iPSCs should retain epigenetic modifications peculiar to their cell of origin (e.g., lung vascular cells) whereas late passage iPSCs will not. Because using the somatic cell from which the iPSC is derived may improve this memory, novel iPSC derivation techniques, such as those that derive iPSC directly from the particular diseased cell type (for example, a pulmonary microvascular endothelial cell) rather than skin fibroblasts, may provide a more effective technology from which to derive iPSC depending upon the goals of the study. In fact, derivation of a given cell type may be more efficient if the iPSC is derived from that somatic cell type both because of and also of irrespective of epigenetic memory (38). This may be a challenge in PAH research given the paucity of lung tissue for study.

Although HPAPH has the luxury of a known mutation in the majority of cases, we and others are identifying candidate genes that underlie susceptibility to development of a number of lung diseases including those involving the airways, such as chronic obstructive pulmonary disease, asthma, idiopathic pulmonary fibrosis, and familial interstitial pneumonia (9, 27). The same iPSC techniques discussed above could be used to study how these identified genes affect epithelial lineage differentiation, molecular signatures, cell phenotype, and function.

Thus iPSC technology offers the potential to investigate the full complement of disease spectrum, from early to advanced, and to dissect genetic and environmental influences on phenotype. Moreover, in the years ahead iPSCs should have enhanced applicability to human lung disease by 1) providing broad access to a patient-derived expandable pluripotent human cell population, 2) recapitulating disease pathophysiology in a cell-specific manner, and 3) giving rise in vitro to multiple cell lineage derivatives with the same genetic makeup. The opportunity for cell-based therapies exists, although a number of impediments exist, including the choice of what cell type to reprogram (may be different for different diseases and/or therapeutic goals), need for high-throughput reprogramming techniques with lower costs, as well as safety issues including but not limited to genetic stability of the derived cells (52). Nonetheless, iPSC should ultimately help us translate science from “bench to bedside” in the most meaningful form: healing human lung disease.

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DISCLOSURES

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