Modeling Pulmonary Arterial Hypertension Using Induced Pluripotent Stem Cells

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Abstract

Heritable pulmonary arterial hypertension belongs to a group of pulmonary vascular diseases for which there is currently no cure other than a heart and lung transplantation. Increased endothelial and smooth muscle cell proliferation leads to vascular remodeling, resulting in increased blood pressure in the lungs which ultimately causes right heart failure. Understanding the molecular mechanisms implicated in the establishment and progression of pulmonary arterial hypertension is hampered due to a lack of disease models, including a limited availability of patient-derived cells and the low penetrance of *BMPR2*, the major disease-associated gene. Induced pluripotent stem cells provide a series of advantages for disease modeling that could potentially allow these issues to be overcome. These include (1) a limitlessly self-renewing capacity that overcomes the restricted supply of cells from diseased patients, (2) an epigenome that has been wiped of pathogenic modifications which have accumulated during the disease process, (3) an amenability to gene editing which allows the introduction of specific pathogenic mutations in an otherwise isogenic background to facilitate the dissection of the precise contribution of disease-associated genetic mutations, and (4) the generation of isogenic cell, tissue, and organ-specific disease models.

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Keywords

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18.1 Heritable Pulmonary Arterial Hypertension

Heritable pulmonary arterial hypertension (HPAH) is a debilitating, often fatal disease caused by increased proliferation and apoptotic resistance of smooth muscle and endothelial cells which drive the vascular remodeling of small pulmonary arterioles and results in their narrowing and obliteration. This causes increased pulmonary vascular resistance and hence increased blood pressure in the pulmonary vessels, eventually leading to right-heart failure [1]. Although currently available therapies have improved life expectancy, they do not prevent disease progression.

18.1.1 Insights into the Pathobiology of PAH

Major insights into PAH pathobiology followed the identification of causal heterozygous germline mutations in the gene encoding the bone morphogenetic protein type II receptor (*BMPR2*) in over 80% of cases of families with PAH [2]. *BMPR2* is a receptor for bone morphogenetic proteins (BMPs), which are members of the transforming growth factor- β superfamily. Furthermore, around 20% of cases of sporadic or idiopathic PAH harbor mutations in *BMPR2* [2]. The *BMPR2* locus is marked by considerable allelic heterogeneity with over 660 germline variants identified to date. Approximately 70% of mutations lead to haploinsufficiency due to nonsense-mediated mRNA decay of the mutant transcript. Additional mutations include missense mutations that lead to substitutions in highly conserved amino acids in important functional domains of the receptor [2].

The central role of the *BMPR2* pathway in PAH is supported by the identification of causal mutations in other members of this pathway including *ACVRL1*, *ENG*, *GDF2* (BMP9), and *SMAD9* [3]. In addition, loss of *BMPR2* signaling appears to play a critical role in non-genetic forms of PAH in humans and in preclinical rodent models. Furthermore, treatment with the selective *BMPR2*/ALK1 ligand BMP9 has been shown to increase lung endothelial *BMPR2* expression and reverse PAH in rodent models [4].

18.1.2 Reduced Penetrance of *BMPR2* in PAH

An important observation in heritable PAH is that *BMPR2* mutations exhibit low penetrance (approximately 20–30% in families), suggesting a requirement for additional environmental or genetic triggers necessary for disease initiation and progression [5]. Interestingly, identical twins carrying mutations in *BMPR2*, where only

one sibling presents with PAH, have been identified [6]. Together, these argue that environmental factors must have a critical role in disease establishment, and in some contexts, these may be more important than the presence of particular genetic modifiers of *BMPR2*.

Insights into potential environmental factors that interact with *BMPR2* to cause PAH have come from animal models free of genetic mutations in *BMPR2*, including lipopoly-saccharide-, monocrotaline-, Sugen-5416/hypoxia-, and chronic hypoxia-induced mouse and rat models, as well as from mice with lung-specific overexpression of tumor necrosis factor (TNF α) [7]. However, a common and important feature of these non-genetic models of PAH is the general reduction in *BMPR2* at the protein level, which further supports a central role of *BMPR2* in the establishment of PAH. In recent work from our labs, we have dissected the mechanism of how inflammatory signaling in the form of TNF α can cause a reduction in *BMPR2* signaling [8]. This is achieved by via the shedding of *BMPR2* extracellular domain at the cell surface, which reduces intact receptor expression, as well as by the transcriptional repression of *BMPR2*, which reduces the total overall expression of *BMPR2* in cells. Taken together, these changes ultimately lead to hyper-proliferation of pulmonary artery smooth muscle cells.

18.2 Modeling Pulmonary Arterial Hypertension with Induced Pluripotent Stem Cells

The limited availability of human tissue from PAH patients with end-stage disease and only at the time of transplantation has hampered progress in understanding the initiation and progression of PAH. In addition, mouse knockout or transgenic models of *BMPR2* deficiency fail to fully recapitulate the pathology observed in humans [7]. Hence, there is a pressing need for alternative human models of PAH to accelerate the development of new treatments for this devastating disease.

Induced pluripotent stem cells (iPSCs) provide major opportunities for disease modeling because they can be derived from specific patients, possess a limitless ability to self-renew, differentiate into all somatic cell lineages, and are amenable to targeted genetic engineering [9]. Their epigenetic memory is also reset during the reprogramming process, wiping epigenetic remodeling marks accumulated during the disease process and therefore potentially providing a naïve non-diseased system which can be used to induce a disease state and thus elucidate hitherto unknown mechanisms of disease progression.

However, an important consideration is whether iPSC-derived cells resemble the relevant adult cell types closely enough. In the case of PAH, the goal is to mimic pulmonary arterial endothelial and distal smooth muscle cells. For example, fundamental differences in responses to hypoxia exist between the pulmonary vasculature and the systemic vasculature and also between the BMP responsiveness of proximal and peripheral (distal) portions of the pulmonary vascular branch.

While systemic arteries relax in response to tissue hypoxia in order to improve blood flow and oxygen supply in hypoxic tissues, pulmonary arteries respond by

contracting, thereby diverting blood flow from poorly ventilated regions toward the more oxygenated areas of the lungs [10]. Furthermore, previous work has demonstrated that in contrast to proximal cells, peripheral PASMCs are not growth-suppressed and exhibit reduced apoptosis when treated with BMP4 [11].

18.2.1 Embryological Origins of the Pulmonary Vasculature

In order to differentiate iPSCs toward pulmonary vascular lineages we must consider the embryological origins of the pulmonary vascular cells and the developmental pathways that have directed them toward their specific fate. For example, the differences in responsiveness to hypoxia and BMP signaling might be due to cells of the pulmonary vasculature having different embryological origins. Lineage tracing has shown that four major embryonic lineages contribute to the pulmonary vasculature: (1) Wnt1+ neural crest, which contributes to the SMCs at the root of the pulmonary artery, (2) cardiopulmonary progenitors (CPPs), which contribute ECs and SMCs to the proximal pulmonary artery and vein, (3) WT1+ mesothelium, which surrounds the lung and contributes widely to both ECs and SMCs throughout the vasculature, and (4) lateral plate mesoderm, which contributes to the PASMCs in peripheral pulmonary arteries [12]. The ability to recapitulate specific lineages and their vascular derivatives is likely to be critical for generating a high-fidelity model of PAH.

18.2.2 Current iPSC Models of PAH

Our group was the first to publish work on reprogramming patient-derived cells carrying *BMPR2* mutations [13]. Since then, other reports have used iPSC-derived cells to gain insights into PAH.

West et al. [14] used patient-derived iPSCs carrying mutations in *BMPR2* to generate mesenchymal and endothelial-like cells. The authors noted an increase in Wnt signaling in these cells and found this was also the case in adult *BMPR2* mutant fibroblasts and in human tissue. However, although the mesenchymal cells expressed some SMC markers, these cells were unable to model the characteristic hyper-proliferative phenotype observed in PASMCs. This highlights the important consideration that iPSC-derived cells must approximate closely enough the relevant adult cell types.

Sa et al. [15] performed RNA-seq on iPSC-derived endothelial cells (iPSC-ECs) and pulmonary artery endothelial cells (PAECs) with and without *BMPR2* mutations and observed that cell adhesion and migration were decreased in cells carrying *BMPR2* mutations compared to controls. This work also showed that *KISS1* was upregulated in *BMPR2* mutant cells compared to wild-type cells.

Gu et al. [16] reiterated the cell adhesion and cell migration defect in iPSC-ECs but went on to look at why not all *BMPR2* mutation carriers develop

PAH. Their approach was to take three different families, in which all individuals carried mutations in *BMPR2*, and then compare the phenotype and transcriptomes of iPSC-ECs of individuals within each family to see if there was a difference between those family members that present with PAH (FPAH) and those that did not (unaffected mutation carriers or UMC). Through this they found that apoptosis was higher in FPAH compared with UMC, while cell adhesion was reduced in both FPAH and UMC. This suggested that genetic modifiers might give UMCs a cell survival advantage. Through transcriptomic analysis, *BIRC3*, a gene implicated in cell survival, was found to be reduced in FPAH compared to UMC. Taken together, the authors concluded that genetic background can influence whether or not an individual will develop PAH. However, as discussed earlier, regardless of the initial instigator (genetic or non-genetic) of PAH, all patients ultimately show a reduction in *BMPR2* expression and signaling. Thus, the question of what *BMPR2* is doing in disease is fundamental and remained unanswered.

We recently addressed this issue using iPSCs to model arterial ECs and distal PASMCs where we were able to transition cells from a naïve state lacking disease-associated cellular phenotypes to a diseased state, showing that a *BMPR2* mutation *per se* is necessary and sufficient for the establishment of some but not all PAH-associated cellular phenotypes in iPSC-ECs and iPSC-SMCs [21]. Omics analysis of the changes associated with this transition will reveal potentially novel and/or druggable pathways to prevent or reverse PAH disease progression.

18.3 Future Direction and Clinical Implications

Primary future uses of iPSCs include drug screening and the generation of human 3D multi-tissue constructs of the pulmonary system for disease modeling. Protocols to generate endothelial cells (ECs), smooth muscle cells, and pericytes from iPSCs have been established [17, 18], and advanced protocols to generate cells more akin to pulmonary vascular cells are underway. Based on these protocols, functional evaluations in each cell type between normal and disease-carrying subjects can be performed to provide a powerful platform for cell type-specific drug discovery. Considering the importance of cell-cell communication during disease progression, modeling diseases will require using more than one cell type. Although it is possible to co-culture iPSC-derived ECs and perivascular cells [19], the highly specialized and complex nature of the pulmonary vascular bed poses challenges for constructing a 3D pulmonary-specific vasculature. In addition, investigating merely vascular cells might lead us to misinterpret pulmonary vascular disease mechanisms. Although animal models have been widely used to model diseases, they are expensive, time-consuming, and often do not fully recapitulate human pathology. iPSC-derived organ-like tissue, termed ‘organoids’, might eventually replace animal models [20]. Whilst liver and kidney organoids develop with vasculatures, current lung organoids lack vascular populations [20]. Developing better lung organoids or pulmonary artery-on-a-chip technologies would provide an advanced platform to model pulmonary vascular diseases.

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