Editorial

Stem Cell INVESTIGATION

Putting skin in the game: dermis-derived stem cells provide insight into familial pulmonary hypertension

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Pulmonary arterial hypertension (PAH) is a lethal disease characterized by pulmonary vascular remodeling due to smooth muscle hypertrophy, endothelial proliferation, and adventitial fibrosis. This remodeling progressively obliterates a large cross section of the lung vasculature, turning the normal low pressure pulmonary circulation into a hypertensive circuit and eventually leading to pump failure of the right ventricle. In the past 20 years, multiple effective therapies for PAH have been validated in clinical trials and these drugs have dramatically improved the quality of life of PAH patients. Yet drugs that halt or reverse vascular remodeling have remained elusive and many treated subjects still die (1). Like many seemingly sporadic diseases a subset of PAH is inherited (familial PAH, or FPAH) which enabled the discovery in 2000 via positional cloning that haplo-insufficiency of *BMPR2* is the genetic basis of FPAH (2). *BMPR2* is a receptor within the TGF-β superfamily. Subsequently, sporadic *BMPR2* mutations were also found in a minority of non-familial (idiopathic) PAH patients (3).

Further investigations of the dysregulated *BMPR2* pathway in FPAH may provide biologic insights that benefit the care of all PAH patients. Major limitations in the investigation of PAH and FPAH are that these subjects are typically identified late in the disease course, and that lung and heart tissue are rarely safe to obtain for scientific analysis. These tissues typically are available only at lung transplant or death—long after the onset of the cellular events that trigger disease progression. This has left investigators to study those cells safe to obtain such as peripheral blood lymphocytes and to rely on animal models such as *BMPR2* null or transgenic mice. Moreover, longitudinal observation of FPAH cohorts for early manifestations of disease is limited by the low penetrance of the *BMPR2* mutation—only 20% of carriers will develop PAH in their lifetime, and by the phenomenon of genetic anticipation where successive generations develop disease at earlier ages (4). Several studies have reported mutations or polymorphisms that affect TGF-β signaling as being either protective against or associated with the development of FPAH, but the events determining disease penetrance remain poorly understood for most families (4,5). Endothelial cell-line specific mutation of *BMPR2* in mice also leads to pulmonary hypertension, consistent with other data highlighting endothelial cells as a key factor in FPAH pathogenesis (6).

In the April 6, 2017 issue of *Cell Stem Cell*, Gu *et al*. utilized stem cells not as a therapy, but as a platform to assess the phenotypic differences between endothelial cells of *BMPR2* mutation carriers with PAH versus those of carriers without disease (7). Their approach was innovative and ambitious: use a cell line that can be safely obtained at any stage of disease from FPAH patients and unaffected relatives—skin fibroblasts, then perform *in vitro* reprograming to dedifferentiate cells to a stem cell lineage using retroviruses expressing Yamanaka factors, then differentiate these induced pluripotent stem cells (iPSC) into an endothelial lineage (iPSC-EC) using specific growth conditions. While the number of subjects they studied

was small (eleven, from three families), they generated credible endothelial phenotypes in sufficient amounts to assess the molecular differences between FPAH patients and unaffected relatives. They studied endothelial attributes relevant to FPAH (adhesion, migration, angiogenesis, integrin expression, and survival in stressed conditions) and evaluated perturbations of known FPAH-dysregulated members of the BMPR2 receptor signaling pathway (Id1, smads, *BMPR2*, GREM1, LRP1, caveolin, FKBP1A, and p38). They also pursued hypothesis-independent assessments of iPSC-EC (RNA expression by RNAseq, analysis for enrichment of transcription factor motifs in differentially regulated genes). Their goal was to identify protective modifiers that could explain the lack of disease penetrance in unaffected carriers—hoping to demonstrate that the development of individualized or "precision" treatments for FPAH families is feasible.

Interestingly, iPSC-EC from unaffected carriers typically had levels of adhesion, survival under stress, or other "normal" endothelial characteristics that approximated healthy control cell levels—or were intermediate between those observed in cells of FPAH-affected relatives and those of healthy controls. They also identified signaling pathways that were aberrant in FPAH iPSC-EC but preserved in the cells from unaffected carriers. Importantly, the unique mutations of each family had heterogeneous effects on perturbation of endothelial cell functions and signaling pathways, highlighting that the optimal molecular targets for treatment can be expected to vary between families. CRISPR/Cas9 genome editing of the *BMPR2* mutation in iPSC rescued many of the phenotypic defects of differentiated iPSC-EC. BIRC3, a protein important for endothelial survival, was expressed less in FPAH iPSC-EC compared to cells of unaffected mutation carriers. Of the genes differentially downregulated in FPAH iPSC-EC, 75% had promoters with putative smad2 binding motifs.

There are limitations to the approach used. The endothelial phenotype that was achieved seemed arterial, whereas pulmonary artery endothelial cells may have a more venous phenotype, for instance expressing higher levels of Von Willebrand factor (8). The numbers of subjects within groups for individual experiments were small, with no data given on the severity of PAH or on comorbidities that may have affected the condition of skin fibroblasts. It is unclear if they evaluated for abnormalities of wnt signaling in iPSC-EC that others have reported to exist in FPAH (9). A parallel study arm where iPSC are differentiated into vascular smooth muscle cells to address other key events of PAH pathogenesis would also have been of interest.

Gu and colleagues are to be credited with harnessing innovative stem cell techniques to improve our understanding of the pathogenesis of FPAH. The care of FPAH, and of all forms of PAH, may someday benefit from the serial analysis of skin-derived stem cells obtained before FPAH is manifest and later during its progression. Can we better understand genetic anticipation in these families, and detect which carriers are at risk of progressing to PAH by using scheduled analyses of their iPSC-EC? One can envision a predictable sequence of cellular events in such induced endothelial cells (and someday, smooth muscle cells) that can be modeled to predict disease onset in the pulmonary vasculature and used in parallel to screen drug candidates for identification of mutation specific therapies. We should expect continued insights into FPAH from stem cell techniques employed by these and other dedicated investigators as they continue to put more skin in the game.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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