Targeting BMPR2 Trafficking with Chaperones: An Important Step toward Precision Medicine in Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is characterized by an occlusive vasculopathy of the pulmonary arteries mediated by endothelial cell dysfunction, smooth muscle cell proliferation, and adventitial fibroblast activation. Novel therapies that target these deranged underlying cellular processes are highly sought after in order to halt or reverse disease progression and augment our current vasodilatory therapies (1).

BMPR2 (Bone Morphogenetic Protein Receptor 2) is recognized as a key signaling pathway receptor and potential master switch in PAH. Reduced BMPR2 expression and dysfunctional signaling recapitulate, *in vitro*, many of the above cellular derangements attributed to PAH pathogenesis (2). The BMPR2 pathway became the focus of the PAH research community 20 years ago when two independent groups described BMPR2 loss-of-function mutations as the disease-causing mutations in hereditary PAH (3, 4). Since then, more than 350 mutations have been described in the BMPR2 gene that target sequences encoding for the ligand-binding and kinase domain as well as the long cytoplasmic tail (5). Although BMPR2 mutations in general clearly confer a more severe clinical phenotype in hereditary versus idiopathic PAH (6), the kind of mutation (nonsense, missense, frameshift mutations, major gene rearrangements) as well as the affected domain of the BMPR2 receptor differentially determine the severity of disease (7). Patients with missense mutations are younger at time of diagnosis or death and have shorter survival from diagnosis to death or lung transplantation, potentially due to a dominant negative effect on downstream BMPR2 signaling caused by stable missense mRNA transcripts. Missense mutations in the cytoplasmic tail, however, appear to be less severe, having a later age of onset, lower pulmonary vascular resistance, and more vasoreactivity (8). Transcripts containing nonsense mutations or other premature translation stop signals undergo nonsense-mediated RNA decay resulting in haploinsufficiency, suggesting that there is a threshold of BMPR2 signaling ($<$50%) below which the disease is much more severe. Criticism regarding targeting BMPR2 in PAH stems from the fact that only $\sim$20% of patients with PAH harbor a BMPR2 mutation and that BMPR2 mutations show an incomplete penetrance (20–30%), implying that other genetic and environmental factors might equally contribute to the disease pathogenesis. However, it is apparent that patients with idiopathic or associated PAH without a BMPR2 mutation have reduced BMPR2 expression in the lung and blood cells, again stimulating a concerted effort to augment or rescue the BMPR2 pathway for therapy (2, 9). Although gene therapy was successful in animal models of PH, these approaches still face obstacles before they can be used in patients with PAH (10). Given the high attrition rates of PAH studies, substantial development costs, and the slow pace of new drug development, repositioning of "old" drugs is increasingly becoming an attractive path to identify novel treatment options, especially for a rare disease such as PAH (11). Repurposed drugs such as FK506 (tacrolimus) and enzastaurin (12, 13), recombinant BMP9 ligand, elafin, and a novel transforming growth factor-β ligand trap show promise in animal models, with some early encouraging results in selected patients and more clinical trials currently underway (2, 14, 15). Even established PAH therapies such as prostacyclin and sildenafil appear to potentiate the BMPR2 pathway somewhat, yet a direct comparison of their ability to increase BMP signaling has not yet been made (2).

Although broadly boosting the BMPR2 pathway with the above approaches might be beneficial even in hereditary PAH—by increasing BMPR2 signaling via their healthy BMPR2 allele/receptor—a targeted and more precise approach might be more effective in correcting the unique receptor dysfunction of specific BMPR2 mutations.

In this issue of the *Journal*, Dunmore and colleagues (pp. 160–171) focus on a specific BMPR2 mutation C118W that leads to cysteine substitutions within the extracellular ligand-binding domain of the BMPR2 receptor, resulting in the disruption of the three-dimensional folding of the protein and retention of the receptor in the endoplasmic reticulum (16). In a manner similar to CFTR-targeting therapies in cystic fibrosis, the authors use the chaperone 4-phenylbutyric acid (4PBA) to improve trafficking of the mutant BMPR2 receptor to the cell membrane, to restore normal BMPR2 localization and improve BMPR2 signaling as assessed by increased downstream targets such as phosphorylation of SMAD1/5 as well as expression of ID1–3. The authors confirmed dysfunctional BMPR2 signaling in dermal fibroblasts from patients harboring the C118W mutation, pulmonary artery smooth muscle cells (PASMCs) isolated from mice with a *Bmpr2* C118W knock-in mutation, and in *Bmpr2* C118W mice. Treatment with 4PBA improved downstream signaling in C118W dermal cells as well as in mouse PASMCs and decreased abnormal PASMC proliferation as well as the mild muscularization of distal pulmonary arteries observed in the *Bmpr2* C118W mice. Although the authors do provide proof of concept that 4PBA improves BMPR2 signaling in primary human C118W cells and in a genetic mouse harboring the same mutation, the effects of 4PBA are mild and do not lead to a full restoration of BMPR2 signaling. Furthermore, the *Bmpr2* C118W mouse model does not develop pulmonary hypertension or right ventricular hypertrophy over the time studied. Therefore, the model is not suited to evaluate

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treatment effects of 4PBA on experimental pulmonary hypertension in the setting of a *Bmpr2* C118W mutation. Despite this, the results are encouraging. Yet, as with other mouse models with reduced BMPR2 expression or signaling, additional injurious stimuli such as inflammation or hypoxia might be needed to tease out a pulmonary hypertension phenotype—a necessary requirement before attempting prevention or reversal strategies with 4PBA. Ultimately, given the incomplete penetrance of BMPR2 mutations, the question still remains regarding whether restoring the BMPR2 pathway will be sufficient to halt or even reverse disease, as likely other injurious stimuli or modifier genes are equally involved in the pathogenesis of PAH.

In summary, the development of 4PBA as a precision medicine for those patients with PAH with cysteine-substituted BMPR2 mutations affecting the ligand-binding domain is promising, yet because of the mild effect of 4PBA and only partial restoration of BMPR2 signaling as well as the subtle phenotype in *Bmpr2* C118W mice, further preclinical studies will most likely be needed before 4PBA could be tested as a repurposed precision drug for selected patients with PAH. ■

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