submit

Member Login:

submit

Forgot Password? | Register for an Account

- Courses
  - New Course Releases
  - Browse All Courses
- Advances in PH Journal
  - About the Journal
  - Journal Archives
  - Editorial Advisory Board
  - Instructions for Authors
- Resource Library
  - PHA Presentations
  - PHA Partner Presentations
  - Upcoming Webinars
  - Webinar Archives
  - Browse All Recordings
  - PH Learning Modules
  - History of PH

Advances in PH Journal

James West

James E. Loyd

Rizwan Hamid

//

Reviews

Sign in to add a review

0 comments
Leave a Comment

Vol, 11, No 4 (Sprint 2012)

- West, J., Loyd J.E., Hamid, R. Potential Interventions Against BMPR2 Related Pulmonary Hypertension. *Advances in Pulmonary Hypertension*. 2012; 11,1: 25-32.

Download This Issue
Download This Article

For more than 60 years, researchers have sought to understand the molecularbasis of idiopathic pulmonary arterial hypertension (PAH). Recognition of theheritable form of the disease led to the creation of patient registries in the1980s and 1990s, and discovery of BMPR2 as the cause of roughly 80% ofheritable PAH in 2000. With discovery of the disease gene came opportunity forintervention, with focus on 2 alternative approaches. First, it may be possibleto correct the effects of BMPR2 mutation directly through interventions targetedat correction of trafficking defects, increasing expression of the unmutatedallele, and correction

of splicing defects. Second, therapeutic interventionsare being targeted at the signaling consequences of BMPR2 mutation. Inparticular, therapies targeting cytoskeletal and metabolic defects caused byBMPR2 mutation are currently in trials, or will be ready for human trials in thenear future. Translation of these findings into therapies is the culmination ofdecades of research, and holds great promise for treatment of the underlyingmolecular bases of disease.

Prior to development of cardiac catheterization in the late 1940s, primary pulmonary hypertension (PPH) was rarely suspected or confirmed prior to autopsy. PPH first became a clinical entity when investigators were able to measure pulmonary artery pressure and thus make the diagnosis of pulmonary hypertension for the first time in living patients.[1,2] Cardiac catheterization also brought new understanding about the various causes of pulmonary hypertension and how these could be distinguished from one another. Physicians have long been fascinated by the many unique features of PPH, including its prevalence in healthy young women, its rarity, and its focal pathology within the pulmonary vascular bed, sparing the systemic circulation. Speculation by authorities about the origins of PPH favored abnormal vasoreactivity or microthrombosis, or both, until the past decade. Widespread interest for PPH among the general public and medical community was generated in the 1970s by the first large anorexigen epidemic related to aminorex/Menocil use in Europe,[3] and the subsequent pivotal WHO meeting in 1973, which developed, in part, as a consequence.[4]

Dr David Dresdale described the first PPH family in 1951, including a mother, her son, and her sister.[5] During the next 3 decades, several families (13) were reported in the US. In 1980, we met a young lady with PPH at Vanderbilt, and she described several young female family members with premature cardiorespiratory death, as young as age 23. That year, Dr John Newman joined the Vanderbilt pulmonary faculty and stimulated our investigation. We contacted the authors of the earlier reports, and they generously contacted the former families to join our developing study. We described 9 new cases, which occurred in 8 families during the interval after the original reports.[6] The transmission pattern was readily apparent even then, as vertical transmission (highly indicative of a single dominant gene) and father to son transmission (excluding X or Y linkage) were evident in multiple families. Incomplete penetrance, with multiple skip generations, was apparent; this phenomenon is still not understood and confounds any attempt at disease prediction or genetic counseling.

In the mid-1980s, Dr Newman served as Vanderbilt principal investigator in the National Institutes of Health (NIH) natural history study of PPH and enrolled 11 patients from Vanderbilt.[7] At semiannual NIH meetings for the study, the other investigators around the US learned of our growing interest in PPH families and encouraged PPH families at their centers to participate in our fledgling familial PPH registry. The NIH natural history study was the benchmark that defined its clinical features and identified a positive family history in 6% of the 187 patients enrolled.[7]

By the late 1980s, before any gene search, a major concern arose about the heterogeneity of the cohort. The pathologic literature in that era suggested that PPH was actually many different diseases, including plexogenic PPH, thomboembolic PPH, pulmonary veno-occlusive disease, isolated medial hypertrophy, and pulmonary capillary hemangiomatosis.[8] That set of pathologic observations and the related notion that PPH was not a single disease entity imposed serious limitations on a gene search: it would be illogical to search for one gene as a cause of different diseases. This conundrum was resolved by a study of the breadth of the pathologic findings in autopsies within the same family.[9] One family suffered 3 patients with

very different autopsy manifestations, and thus we concluded that the various pathologic findings are not different diseases, but have a single basis, because they occurred in a family with vertical transmission indicating a single gene. With that information we concluded that all the PPH families in our cohort could be joined into one group to conduct a gene search, which would be complete within the decade.

The familial PPH registry continued to grow, and collaboration by geneticist John Phillips after he moved to Vanderbilt in 1985 provided direction to envision a gene search. He urged us to bank patient and family specimens in the hope that a gene search would eventually become feasible. Gene discovery methods improved in the 1990s, and Bill Nichols at Michigan conducted a PPH microsatellite marker search. Using the patient and family samples from our familial PPH registry, he identified linkage on chromosome 2q32 in 1997,[10] and subsequently the underlying mutation responsible in most families was identified in bone morphogenic protein receptor 2 (*BMPR2*) in 2000.[11] Similar studies of linkage of PPH and discovery that *BMPR2* is the gene of interest were also accomplished independently in a nearly identical time frame at Columbia-Presbyterian by a team led by Drs Robyn Barst, Jane Morse, and Jim Knowles.[12] Mutation in *BMPR2* is now known to be the basis for the vast majority of families with PAH, and more than 120 *BMPR2* mutation families are known in the USA, with estimates of 500 families worldwide. Other genes related to TGF-? are less frequently responsible for the disease we now call hereditary pulmonary arterial hypertension (HPAH): *ACVRL1* (*ALK1*) and endoglin, which more frequently cause hereditary hemorrhagic telangiectasia.[13] More recently, an association between HPAH and SMAD 9 was reported.[14] In summary, germline *BMPR2* gene mutations cause HPAH in 80%-85% of families with a family history of PAH, while 5%-25% of patients diagnosed as having idiopathic pulmonary arterial hypertension (IPAH) actually have a detectable germline mutation in *BMPR2*, as well.[11,15–18] *BMPR2* mutations thus constitute the largest known risk for developing PAH.

THERAPEUTIC INTERVENTIONS AGAINST *BMPR2* EXPRESSION, SPLICING, AND TRAFFICKING

Currently there is no therapy known to prevent, delay, or reverse the pulmonary vasculopathy of pulmonary arterial hypertension (PAH). Perhaps the greatest barriers to developing effective treatments are 2 closely related knowledge gaps: what is the exact role of *BMPR2* in the pathologic signaling of PAH and what mechanisms cause the pulmonary vascular disease itself. Certain unique features of *BMPR2*-related PAH provide tantalizing clues into disease pathogenesis. For example, one of the most striking features of HPAH is its reduced penetrance; a mutation carrier has only a 20% chance of developing PAH. Thus nearly 80% of *BMPR2* mutation carriers have no clinical symptoms but can produce offspring that are affected. The first clue toward understanding reduced penetrance came from the recognition that *BMPR2* mutations can either be haploinsufficient (HI) or dominant negative. This distinction is important to understand, as it provides unique insights into disease pathogenesis and opens avenues toward better HPAH diagnosis and treatment. RNA studies have shown that some *BMPR2* mutations produce stable transcripts, while others contain premature termination codons (PTC) and are rapidly degraded through the nonsense mediated decay (NMD) pathway.[19] NMD is an mRNA surveillance system that degrades transcripts containing PTCs to prevent translation of unnecessary or harmful transcripts.[20,21] HPAH patients with *BMPR2* mutations that do not cause PTC and are therefore not subject to NMD (NMD-) have disease due to dominant negative effects of the mutated protein, while patients with mutations subject to NMD (NMD+) have disease due to functional HI of *BMPR2* (NMD degradation of the mutated allele's mRNA).[19,22] Thus, it appears that HI is a heterozygous state in which the

normal allele of *BMPR2* has insufficient expression to maintain normal cellular function and prevent disease (this “threshold effect” is illustrated in Figure 1). Mutations that cause HI of *BMPR2* are slightly more common (?55%-60% of HPAH).

Figure 1: *Cellular levels of mRNA are determined only by the WT allele if one inherits a mutated allele that degrades the product transcribed from the mutated allele. In this case if the expression from the nonmutated allele is enough to reach a presumed threshold for mRNA expression, that patient will not get PAH (hypothesis from Hamid et al. 2009).*

Thus, HI mutations teach us that *total* cellular *BMPR2* mRNA or protein levels are important for disease penetrance. This observation in families fits with the broader observation that decreased *BMPR2* expression is present in other human and experimental forms of pulmonary hypertension, though it remains unclear whether this is a precipitating event or a side effect.[23] For example, decreased *BMPR2* expression is present in pulmonary vascular tissue of patients with IPAH[23,24] and in multiple experimental animal models of PAH, including those induced by monocrotaline, chronic hypoxia, and chronic systemic-to-pulmonary shunting.[25–27] These studies thus clearly demonstrate that *BMPR2* expression is important in many forms of PAH.

In studying HI NMD+ *BMPR2* mutation carriers, we noticed that mutation carriers had variation in their total cellular *BMPR2* levels. Analysis of 4 HPAH kindreds showed that, indeed, the expression of wild-type *BMPR2* transcript was lower in afflicted patients compared to unaffected mutation carriers ($P < .0.005$).[28] This association of transcript levels with penetrance was not limited to a single type of NMD+ mutation since all 4 of the kindreds analyzed had different NMD+ mutations. These data strongly suggest that the level of expression of the wild-type (WT) (normal) *BMPR2* allele predicts the clinical development of HPAH in individuals who carry HI *BMPR2* mutations, and thus the expression of the WT *BMPR2* allele may be a primary modifier of HPAH penetrance.[28] These data also suggest that there is likely a cellular threshold for *BMPR2* expression; ie, once the cell loses one *BMPR2* allele secondary to an HI mutation, the cellular *BMPR2* levels are determined by the expression of the remaining (normal, wild-type) allele (Figure 1). Thus decreased expression of the WT allele in an HI background (caused by the heterozygous NMD+ mutation) lowers total *BMPR2* expression below a critical threshold needed for proper cellular function, thus causing disease. In contrast, higher expression of the WT *BMPR2* allele (higher than the presumed threshold) with the same heterozygous NMD+ mutation may prevent clinical disease in the mutation carrier.[28] Such modulation of disease penetrance by WT transcripts has until now been thought to be a rare phenomenon, being previously reported in only 3 genetic disorders: dominantly inherited erythropoietic protoporphyria, hereditary elliptocytosis, and autosomal dominant retinitis pigmentosa.[29–32] One explanation for how levels of WT transcripts might affect HPAH penetrance comes from the finding that *BMPR2* forms a heterotrimeric complex with *BMPR1A* and *BMPR1B*. The degree of deficiency of normal

BMPR2 could affect the receptor complex stoichiometry, leading to decreased signaling and disease.[28]

The molecular mechanisms behind this variability in *BMPR2* expression are not known. It is unlikely to be related to *BMPR2* promoter mutations (unpublished data) and more likely due to *cis* (function of proximal regulatory regions) or a *trans* (function of distal genes) effect. Our data showing that normal individuals have baseline variability in *BMPR2* expression (unpublished data) certainly support this hypothesis.

BMPR2 expression may also help explain another interesting aspect of this disease: that females are 1.9- to 4.1-fold more likely to develop disease than males.[33] It was recently shown that the *BMPR2* promoter contains an evolutionarily conserved estrogen receptor binding site that responds to estrogen by suppressing *BMPR2* expression.[34] This observation provides one clue as to why females are more likely to develop PAH than males and further highlights the importance of *BMPR2* expression levels in PAH pathogenesis.

Recent data, however, suggest that the eventual role of *BMPR2* expression in HPAH is likely to be even more complex then levels of total WT *BMPR2* expression. These data show that *BMPR2* alternative splicing may play a role in HPAH pathogenesis—it may not be a simple question of total *BMPR2* expression, but in fact the relative levels of alternatively spliced *BMPR2* transcripts. Alternative splicing is a mechanism by which a single gene can generate multiple transcripts with likely different functions through internal deletion (“skipping”) of exons in various combinations. This mRNA processing can have clinical consequences and has been shown to play a role in many human diseases.[35,36] In lung disease, alternative splicing has been shown to be important in chronic obstructive pulmonary disease (COPD), bronchopulmonary dysplasia (BPD), chronic interstitial lung disease, familial and sporadic interstitial lung disease,[35–39] and cystic fibrosis.[40,41] *BMPR2* has 13 exons and is alternatively spliced to produce 2 primary transcripts: isoform A, which is the full length gene product containing all 13 exons of the gene and isoform B, a much rarer transcript missing exon 12.[42–44] Several studies have hinted at the importance of isoform B in proper functioning of *BMPR2* and in the development of PAH. Deletion of exon 12 is a common *BMPR2* mutation found in HPAH patients, and previous studies have shown that it can disrupt *BMPR2* function in a dominant negative fashion.[45–47] Furthermore, studies in mice have shown that overexpression of a *BMPR2* transcript with an exon 12 deletion results in PAH.[48] Interestingly, our data (unpublished, in review) suggest that cells from patients are more likely to have higher levels of isoform B relative to levels of isoform A (B/A ratio) compared to carriers. Thus the relationship between *BMPR2* expression and PAH pathogenesis is complex and involves not only the expression of WT allele but also alternative splicing. The relative contributions of either of these mechanisms toward HPAH pathogenesis are currently not known; however, it is quite likely that there is some overlap at a molecular level.

These data then suggest several potentially novel approaches toward disease diagnosis and treatment. For example, could we use this information to design better diagnostic tools for HPAH patients? The fact that we cannot predict disease development in *BMPR2* carriers with extensive family history of PAH results in significant physical, emotional, and economic burden. Mutation carriers do not know whether or when they will develop clinical disease. Moreover, at the time of diagnosis, 75% of subjects with HPAH already have symptoms in New York Heart Association functional class III or IV, and this more advanced symptom complex predicts poor survival despite available treatment. Thus, any approach that will predict which mutation carriers are likely to develop disease would be tremendously helpful

both for those likely to develop disease and those likely to remain disease free. If ongoing studies prove that expression of the normal *BMPR2* allele predicts disease development, this finding could possibly be used as a diagnostic tool to reassure some patients and provide optimal surveillance for those at higher risk.

These data also raise intriguing treatment possibilities—what if cellular *BMPR2* expression levels could be increased over this critical threshold? Several approaches can now be used to identify drugs that will alter *BMPR2* cellular expression. For example, the Connectivity Map database[49] offers a novel way to identify and test drugs that may modulate *BMPR2* expression. Furthermore, since drugs within the Map are already FDA-approved, the time frame from bench to bedside is significantly shortened, with obvious benefits to patients.[50] Drugs that could upregulate *BMPR2* expression could be potential PAH treatments, while it might be wise to avoid those that downregulate *BMPR2* expression, which could increase an individual's risk of developing disease.

Modification of *BMPR2* alternative splicing may also offer an approach to change disease course. Several studies, including our own, have shown that splicing is a dynamic process and can be altered by the environment, including exposure to drugs.[51,52] This raises the intriguing possibility that we inadvertently make the disease worse by our selection of particular pharmacological agents. It is likely that *BMPR2* alternative splicing is dynamic both in its response to medication (patient's pharmacological milieu) and other environmental signals (such as hypoxia). A better understanding of the environmental determinants for alternative splicing could be highly relevant for clinicians if, in fact, the drugs we use favorably (or unfavorably) influence *BMPR2* expression and thus cellular function in HPAH patients.

THERAPEUTIC INTERVENTIONS AGAINST SIGNALING CONSEQUENCES OF *BMPR2* MUTATION

An alternative to targeting the expression and splicing of the *BMPR2* receptor itself is to target therapies at the downstream signaling consequences of *BMPR2* mutation. This will probably be necessary for most classes of NMD- (dominant negative) *BMPR2* mutation. Moreover, the molecular pathways affected in most IPAH patients are nearly identical to those in HPAH patients.[53] Since most IPAH patients lack identifiable defects in *BMPR2*, targeting downstream signaling will be required. In the 12 years since *BMPR2* was identified as the primary heritable PAH gene, substantial progress has been made in understanding the signaling consequences of *BMPR2* mutation in the pulmonary vasculature; some of these consequences are approaching readiness for therapeutic intervention in patients.

BMPR2 is a 1038 amino acid single pass transmembrane protein. Dimers of *BMPR2* in combination with dimers of type 1 BMP receptors, usually *BMPR1A* or *BMPR1B*, bind to BMP ligand[54] and signal directly through several different mechanisms (Figure 2). There may be alterations in signaling specificity based on whether the receptor complex is preformed or assembles in the presence of ligand,[55] and the specific type 1 receptors present in the complex may be important, but these details are currently poorly understood. The receptor itself consists of 4 domains: an extracellular ligand binding domain, a short transmembrane domain, a kinase domain, and a long cytoplasmic tail. *BMPR2* is highly homologous to other

type 2 TGF-? superfamily receptors, with the exception of its cytoplasmic tail domain, which is both unique and highly conserved across species.

Figure 2: *signals through multiple mechanisms. In addition to activation of SMAD transcription factors (green rectangles), signaling through the type 1 receptor regulates the TAB1/TAK1 complex, bridged by XIAP (green hexagons), and thence NF-kB and MAPK. In addition, the unique cytoplasmic tail binds and regulates SRC, LIMK1, and DYNLT1 (blue ovals), resulting in altered regulation of actin organization, caveoli, focal adhesions, and mitochondrial metabolism.*

The most well studied method by which *BMPR2* signals is through phosphorylation and activation of a type 1 receptor. The cytoplasmic tail domain is dispensable for this function; both tail domain mutations in *BMPR2* and naturally occurring *BMPR2* alternative splice isoform B, are capable of phosphorylating the type 1 receptor. On the other hand, the *BMPR2* cytoplasmic tail appears to be indispensable for binding and activation of at least 3 targets related to regulation of intracellular trafficking and the cytoskeleton, probably also requiring a functional *BMPR2* kinase domain. These targets are SRC, dynein light chain tctex-1 (DYNLT1), and LIM domain kinase 1 (LIMK1**,** Figure 2).

BMPR2 SIGNALING THROUGH THE *BMPR1A* OR *BMPR1B*

When phosphorylated by *BMPR2*, the type 1 receptor signals through 2 distinct pathways: it phosphorylates and activates SMAD transcription factors 1, 5, or 8, and under some circumstances also regulates the TGF-? activated kinase 1 and its binding protein (TAK1/TAB1) complex bridged by X-linked inactivator of apoptosis (XIAP).[56]

When phosphorylated, SMAD1, 5, and/or 8 bind to the co-SMAD, SMAD4, and enter the nucleus to drive transcription. The most well studied SMAD transcription targets regulate terminal differentiation of cells, a role for which BMP has been extensively studied in the developmental literature. The BMP pathway also suppresses inflammatory markers including interleukin-6 and STAT3 through SMAD-mediated mechanisms.[57,58] SMAD proteins also regulate micro RNA (miRNA) splicing through a nontranscriptional mechanism, not requiring binding to SMAD4, which promotes miRNA processing by DROSHA.[59] Micro RNAs are increasingly recognized as important signals in health and disease, including pulmonary hypertension. Thus, SMAD signaling regulates smooth muscle differentiation state[60,61] in 2 ways: by direct transcriptional regulation of genes involved in maintaining a fully differentiated, contractile state (in particular, KLF genes[62,63]) and by regulating maturation of miRNAs, which

further regulate differentiation state. In animal models, *BMPR2* mutations affecting SMAD signaling drive smooth muscle from a contractile to a synthetic state,[60] likely resulting in significant changes in cellular proliferation, extracellular matrix deposition, vascular inflammation, and mechanical stiffness.

In addition, decreased *BMPR2* signaling through the SMAD transcription factors may cause increased TGF-? signaling. Both pathways compete for use of the same co-SMAD, SMAD4, and so reduced use of SMAD4 by BMP may result in increased availability for TGF-? signal,[68] although there are hints that reciprocal regulation between the BMP and TGF-? signaling pathways is more complex than this. TGF-? -signaling in the lung is generally regarded as profibrotic and proinflammatory, and increased TGF-? signaling contributes to remodeling in some animal models.[69] Thus, decreased *BMPR2* mediated signaling may contribute to remodeling by allowing an increase in TGF-?-signaling.

There are potential interventions that could be targeted against defects caused by reduced *BMPR2* signaling through *BMPR1* receptors; however, such interventions are all currently at a very early stage of development. It would also be sensible to target the increased interleukin 6 and STAT3 signaling or to suppress increased TGF-? signaling. Inhaled miRNA targeting these or other *BMPR2* pathways would also be logical. However, these approaches would first need to be vetted for safety and efficacy in robust animal models, and such highly targeted therapies are likely more than a decade away.

BMPR2 SIGNALING THROUGH SRC, DYNLT1, AND LIMK1

In 2003, Ora Bernard's group in Australia found that the *BMPR2* cytoplasmic tail physically interacted with and regulated LIMK1,[70] and that when bound to *BMPR2*, LIMK1 function was reduced. *BMPR2* mutation led to decreased LIMK1 binding and increased activity. The primary phosphorylation target for LIMK1 is the actin binding and reorganization protein cofilin (CFL1); we have confirmed that *BMPR2* mutation leads to increased CFL1 phosphorylation in lungs from *BMPR2*^{R899X} mice, and this would have functional consequences to promote the pulmonary hypertension phenotype.

Also in 2003, Richard Trembath and Nick Morrell's group found that *BMPR2* colocalizes with, binds, and phosphorylates DYNLT1.[71] These functions were disrupted by PAH-causing mutations within the cytoplasmic tail of *BMPR2*. DYNLT1 also physically interacts with both the mitochondrial membrane permeability protein VDAC1[72] and the Rho/RAC guanine nucleotide exchange factor ARHGEF2.[73] We have found profound defects in energy metabolism in *BMPR2* mutant mice, cells, and patients,[74] and an increase in GTP-bound RAC1 in both whole mouse lung, vascular smooth muscle, and pulmonary microvascular endothelium with *BMPR2* mutations.[75] Especially because altered energy metabolism and Rho-kinase signaling are increasingly recognized as important to PAH pathogenesis in humans, our observations strongly implicate DYNLT1 as a functionally important downstream target of *BMPR2* mutations.

In summary, it seems that the *BMPR2* cytoplasmic tail regulates multiple critical cytoskeletal functions. Expression array experiments in mice and cells cultured from mice with *BMPR2*

mutation specific to the cytoplasmic tail show changes in metabolic, cytoskeletal, adhesion, and microtubule-related genes.[67,75,78] *BMPR2* regulation of the cytoskeleton may explain the defects in endothelial barrier function,[79] mitochondrial fission and fusion,[80] and motility[81] found in PAH.

Therapies targeted at the cytoskeletal or the metabolic defects are either currently in trials, or will be ready for human trials in the near future. Dichloroacetate is a direct inhibitor of pyruvate dehydrogenase kinase, a key enzyme regulating glycolysis, thus enhancing glucose oxidation, which has entered a Phase I trial for PAH at University of Alberta and Imperial College London.[82] Because this addresses only one of multiple metabolic defects found secondary to *BMPR2* mutation, it may not be sufficient in itself to restore normal metabolic function in PAH, but it is a solid first step. *BMPR2*-related PAH, both in patients and in animal models, is refractory to treatment: our group has tried multiple therapies on *BMPR2* mutant mice with limited success. The only class of treatment that reverses *BMPR2*-related PAH, done independently by 2 groups, is intervention targeted at restoring endothelial cell-cell junctions. Novartis has tested the Schering-Plough drug SCH 527123 against PAH in endothelial-specific *BMPR2* knockout mice, and found dramatic efficacy in improving cell-cell junctions, reducing leukocyte recruitment, and reversing PAH.[83] Our group used ACE2, which reversed the SRC and RAC1 defects, as well as many of the downstream metabolic consequences, and achieved reversal of elevated right ventricular systolic pressure in mice expressing the cytoplasmic tail domain mutant $BMPR2^{R899X}$.[75] Both of these drugs are currently in Phase I or II trials for non-PAH conditions,[84] and so are readily translatable to clinical trials for human PAH.

CONCLUSION

The search for the molecular etiology of PAH has stretched over decades, kicking into high gear in 2000 with the discovery that *BMPR2* was the most common heritable PAH gene. Now, therapies targeted at correcting the *BMPR2* mutation itself or at correcting the downstream consequences of *BMPR2* mutation are rapidly approaching human translation, with the promise of new, more effective treatments targeted specifically at the molecular defects that give rise to disease.

1. Cournand, A, Riley, RL, Breed, ES. Measurement of cardiac output in man using the technique of catheterization of the right auricle or ventricle. J Clin Invest. 1945;24(1):106–116.
2. Howarth, S, McMichael, J, Sharpey-Schafer, EP. Cardiac catheterization in cases of patent interauricular septum, primary pulmonary hypertension, Fallot's tetralogy, and pulmonary stenosis. Br Heart J. 1947;9(4):292–303.
3. Follath, F, Burkart, F, Schweizer, W. Drug-induced pulmonary hypertension? Br Med J. 1971;1(5743):265–266.
4. Hatano, E, Strasser, T, eds. Primary pulmonary hypertension: report on a WHO meeting. Geneva 15-17 October, 1973 Geneva: World Health Organization; 1975.
5. Dresdale, DT, Schultz, M, Michtom, RJ. Primary pulmonary hypertension. I. Clinical and

hemodynamic study. Am J Med. 1951;11(6):686–705.
6. Loyd, JE, Primm, RK, Newman, JH. Familial primary pulmonary hypertension: clinical patterns. Am Rev Respir Dis. 1984;129(1):194–197.
7. a. b. Rich, S, Dantzker, DR, Ayres, SM. Primary pulmonary hypertension. A national prospective study. Ann Intern Med. 1987;107(2):216–223.
8. Pietra, GG, Rüttner, JR. Specificity of pulmonary vascular lesions in primary pulmonary hypertension. A reappraisal. Respiration. 1987;52(2):81–85.
9. Loyd, JE, Atkinson, JB, Pietra, GG, Virmani, R, Newman, JH. Heterogeneity of pathologic lesions in familial primary pulmonary hypertension. Am Rev Respir Dis. 1988;138(4):952–957.
10. Nichols, WC, Koller, DL, Slovis, B. Localization of the gene for familial primary pulmonary hypertension to chromosome 2q31–32. Nat Genet. 1997;15(3):277–280.
11. a. b. International PPH Consortium, Lane, KB, Machado, RD, Pauciulo, MW. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. Nat Genet. 2000;26(1):81–84.
12. Deng, Z, Morse, JH, Slager, SL. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. Am J Hum Genet. 2000;67(3):737–744.
13. Trembath, RC, Thomson, JR, Machado, RD. Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. N Engl J Med. 2001;345(5):325–334.
14. Shintani, M, Yagi, H, Nakayama, T, Saji, T, Matsuoka, R. A new nonsense mutation of SMAD8 associated with pulmonary arterial hypertension. J Med Genet. 2009;46(5):331–337.
15. Thomson, JR, Machado, RD, Pauciulo, MW. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-beta family. J Med Genet. 2000;37(10):741–745.
16. Machado, RD , Koehler, R , Glissmeyer, E. Genetic association of the serotonin transporter in pulmonary arterial hypertension. Am J Respir Crit Care Med. 2006;173(7):793–797.
17. Fujiwara, M , Yagi, H , Matsuoka, R. Implications of mutations of activin receptor-like kinase 1 gene (ALK1) in addition to bone morphogenetic protein receptor II gene (BMPR2) in children with pulmonary arterial hypertension. Circ J. 2008;72(1):127–133.
18. Aldred, MA, Vijayakrishnan, J, James, V. BMPR2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. Hum Mutat. 2006;27(2):212–213.
19. a. b. Cogan, JD, Pauciulo, MW, Batchman, AP. High frequency of BMPR2 exonic deletions/duplications in familial pulmonary arterial hypertension. Am J Respir Crit Care Med. 2006;174(5):590–598.
20. González, CI, Bhattacharya, A, Wang, W, Peltz, SW. Nonsense-mediated mRNA decay in Saccharomyces cerevisiae. Gene. 2001;274(1–2):15–25.
21. Maquat, LE. Nonsense-mediated mRNA decay: splicing, translation and mRNP dynamics. Nat Rev Mol Cell Biol. 2004;5(2):89–99.
22. Cogan, JD, Vnencak-Jones, CL, Phillips, JA3rd. Gross BMPR2 gene rearrangements constitute a new cause for primary pulmonary hypertension. Genet Med. 2005;7(3):169–174.
23. a. b. Atkinson, C, Stewart, S, Upton, PD. Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of type II bone morphogenetic protein receptor. Circulation. 2002;105(14):1672–1678.
24. Dewachter, L, Adnot, S, Guignabert, C. Bone morphogenetic protein signalling in heritable versus idiopathic pulmonary hypertension. Eur Respir J. 2009;34(5):1100–1110.
25. Morty, RE, Nejman, B, Kwapiszewska, G. Dysregulated bone morphogenetic protein signaling in monocrotaline-induced pulmonary arterial hypertension. Arterioscler Thromb Vasc

Biol. 2007;27(5):1072–1078.
26. Rondelet, B , Kerbaul, F , Van Beneden, R. Signaling molecules in overcirculation-induced pulmonary hypertension in piglets: effects of sildenafil therapy. Circulation. 2004;110(15):2220–2225.
27. Takahashi, H, Goto, N, Kojima, Y. Downregulation of type II bone morphogenetic protein receptor in hypoxic pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol. 2006;290(3):L450–L458.
28. a. b. c. d. Hamid, R, Cogan, JD, Hedges, LK. Penetrance of pulmonary arterial hypertension is modulated by the expression of normal BMPR2 allele. Hum Mutat. 2009;30(4):649–654.
29. Gouya, L, Puy, H, Robreau, AM. The penetrance of dominant erythropoietic protoporphyria is modulated by expression of wildtype FECH. Nat Genet. 2002;30(1):27–28.
30. Gouya, L , Puy, H , Robreau, AM. Modulation of penetrance by the wild-type allele in dominantly inherited erythropoietic protoporphyria and acute hepatic porphyrias. Hum Genet. 2004;114(3):256–262.
31. Vithana, EN , Abu-Safieh, L , Pelosini, L. Expression of PRPF31 mRNA in patients with autosomal dominant retinitis pigmentosa: a molecular clue for incomplete penetrance? Invest Ophthalmol Vis Sci. 2003;44(10):4204–4209.
32. Wilmotte, R, Maréchal, J, Morlé, L. Low expression allele alpha LELY of red cell spectrin is associated with mutations in exon 40 (alpha V/41 polymorphism) and intron 45 and with partial skipping of exon 46. J Clin Invest. 1993;91(5):2091–2096.
33. Newman, JH, Wheeler, L, Lane, KB. Mutation in the gene for bone morphogenetic protein receptor II as a cause of primary pulmonary hypertension in a large kindred. New Engl J Med. 2001;345(5):319–324.
34. Austin, ED, Hamid, R, Hemnes, AR. BMPR2 expression is suppressed by signaling through the estrogen receptor. Biol Sex Differ. 2012;3(1):6.
35. a. b. Orengo, JP, Cooper, TA. Alternative splicing in disease. Adv Exp Med Biol. 2007;623:212–223.
36. Tazi, J, Bakkour, N, Stamm, S. Alternative splicing and disease. Biochim Biophys Acta. 2009;1792(1):14–26.
37. Dunbar, AJ , Goddard, C. Identification of an alternatively spliced mRNA transcript of human betacellulin lacking the C-loop of the EGF motif and the transmembrane domain. Growth Factors. 2000;18(3):169–175.
38. Hamvas, A , Nogee, LM , White, FV. Progressive lung disease and surfactant dysfunction with a deletion in surfactant protein C gene. Am J Respir Cell Mol Biol. 2004;30(6):771–776.
39. Lin, Z, Thomas, NJ, Wang, Y. Deletions within a CA-repeat-rich region of intron 4 of the human SP-B gene affect mRNA splicing. Biochem J. 2005;389(Pt 2):403–412.
40. Hinzpeter, A, Aissat, A, Sondo, E. Alternative splicing at a NAGNAG acceptor site as a novel phenotype modifier. PLoS Genet. 2010;6(10).
41. Ramalho, AS, Beck, S, Penque, D. Transcript analysis of the cystic fibrosis splicing mutation 1525-1GA shows use of multiple alternative splicing sites and suggests a putative role of exonic splicing enhancers. J Med Genet. 2003;40(7):e88.
42. Liu, F, Ventura, F, Doody, J, Massague?, J. Human type II receptor for bone morphogenic proteins (BMPs): extension of the two-kinase receptor model to the BMPs. Mol Cell Biol. 1995;15(7):3479–3486.
43. Rosenzweig, BL , Imamura, T , Okadome, T. Cloning and characterization of a human type II receptor for bone morphogenetic proteins. Proc Natl Acad Sci U S A. 1995;92(17):7632–7636.
44. Beppu, H, Minowa, O, Miyazono, K, Kawabata, M. cDNA cloning and genomic organization of the mouse BMP type II receptor. Biochem Biophys Res Commun.

1997;235(3):499–504.
45. Li, W, Dunmore, BJ, Morrell, NW. Bone morphogenetic protein type II receptor mutations causing protein misfolding in heritable pulmonary arterial hypertension. Proc Am Thorac Soc. 2010;7(6):395–398.
46. Rudarakanchana, N , Flanagan, JA , Chen, H. Functional analysis of bone morphogenetic protein type II receptor mutations underlying primary pulmonary hypertension. Hum Mol Genet. 2002;11(13):1517–1525.
47. Machado, RD, Aldred, MA, James, V. Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. Hum Mutat. 2006;27(2):121–132.
48. Yasuda, T, Tada, Y, Tanabe, N, Tatsumi, K, West, J. Rho-kinase inhibition alleviates pulmonary hypertension in transgenic mice expressing a dominant-negative type II bone morphogenetic protein receptor gene. Am J Physiol Lung Cell Mol Physiol. 2011;301(5):L667–L674.
49. Lamb, J, Crawford, ED, Peck, D. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. Science. 2006;313(5795):1929–1935.
50. Flynn, C, Zheng, S, Yan, L. Connectivity Map Analysis of NMD+ BMPR2 Related HPAH Provides Insights into Disease Penetrance. Am J Respir Cell Mol Biol. 2012 Feb 3. [Epub ahead of print]
51. Hamid, R, Phillips, JA3rd, Holladay, C. A molecular basis for variation in clinical severity of isolated growth hormone deficiency type II. J Clin Endocrinol Metab. 2009;94(12):4728–4734.
52. Poling, JS, Phillips, JA3rd, Cogan, JD, Hamid, R. Pharmacologic correction of dominant-negative GH1 deficiency causing mutations. Clin Transl Sci. 2011;4(3):175–179.
53. Austin, ED, Menon, S, Hemnes, AR. Idiopathic and heritable PAH perturb common molecular pathways, correlated with increased MSX1 expression. Pulm Circ. 2011;1(3):389–398.
54. Marom, B, Heining, E, Knaus, P, Henis, YI. Formation of stable homomeric and transient heteromeric bone morphogenetic protein (BMP) receptor complexes regulates Smad protein signaling. J Biol Chem. 2011;286(22):19287–19296.
55. Hassel, S, Schmitt, S, Hartung, A. Initiation of Smad-dependent and Smad-independent signaling via distinct BMP-receptor complexes. J Bone Joint Surg Am. 2003;85-A Suppl 3:44–51.
56. Yamaguchi, K, Nagai, S, Ninomiya-Tsuji, J. XIAP, a cellular member of the inhibitor of apoptosis protein family, links the receptors to TAB1-TAK1 in the BMP signaling pathway. EMBO J. 1999;18(1):179–187.
57. Hagen, M, Fagan, K, Steudel, W. Interaction of interleukin-6 and the BMP pathway in pulmonary smooth muscle. Am J Physiol Lung Cell Mol Physiol. 2007;292(6):L1473–L1479.
58. Ying, QL, Nichols, J, Chambers, I, Smith, A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell. 2003;115(3):281–292.
59. Davis, BN, Hilyard, AC, Lagna, G, Hata, A. SMAD proteins control DROSHA-mediated microRNA maturation. Nature. 2008;454(7200):56–61.
60. a. b. Tada, Y, Majka, S, Carr, M. Molecular effects of loss of BMPR2 signaling in smooth muscle in a transgenic mouse model of PAH. Am J Physiol Lung Cell Mol Physiol. 2007;292(6):L1556–L1563.
61. Young, KA, Ivester, C, West, J, Carr, M, Rodman, DM. BMP signaling controls PASMC KV channel expression in vitro and in vivo. Am J Physiol Lung Cell Mol Physiol. 2006;290(5):L841–L848.
62. King, KE, Iyemere, VP, Weissberg, PL, Shanahan, CM. Krup?pel-like factor 4

(KLF4/GKLF) is a target of bone morphogenetic proteins and transforming growth factor beta 1 in the regulation of vascular smooth muscle cell phenotype. J Biol Chem. 2003;278(13):11661–11669.
63. Helbing, T, Rothweiler, R, Ketterer, E. BMP activity controlled by BMPER regulates the proinflammatory phenotype of endothelium. Blood. 2011;118(18):5040–5049.
64. Matluk, N , Rochira, JA , Karaczyn, A , Adams, T , Verdi, JM. A role for NRAGE in NF-kappaB activation through the non-canonical BMP pathway. BMC Biol. 2010;8:7.
65. Lu, M , Lin, SC , Huang, Y. XIAP induces NF-kappaB activation via the BIR1/TAB1 interaction and BIR1 dimerization. Mol Cell. 2007;26(5):689–702.
66. Greenblatt, MB , Shim, JH , Glimcher, LH. TAK1 mediates BMP signaling in cartilage. Ann N Y Acad Sci. 2010;1192:385–390.
67. West, J, Harral, J, Lane, K. Mice expressing BMPR2R899X transgene in smooth muscle develop pulmonary vascular lesions. Am J Physiol Lung Cell Mol Physiol. 2008;295(5):L744–L755.
68. Phillips, JA3rd, Poling, JS, Phillips, CA. Synergistic heterozygosity for TGFbeta1 SNPs and BMPR2 mutations modulates the age at diagnosis and penetrance of familial pulmonary arterial hypertension. Genet Med. 2008;10(5):359–365.
69. Zaiman, AL, Podowski, M, Medicherla, S. Role of the TGF-beta/Alk5 signaling pathway in monocrotaline-induced pulmonary hypertension. Am J Respir Crit Care Med. 2008;177(8):896–905.
70. Foletta, VC, Lim, MA, Soosairajah, J. Direct signaling by the BMP type II receptor via the cytoskeletal regulator LIMK1. J Cell Biol. 2003;162(6):1089–1098.
71. Machado, RD, Rudarakanchana, N, Atkinson, C. Functional interaction between BMPR-II and Tctex-1, a light chain of Dynein, is isoform-specific and disrupted by mutations underlying primary pulmonary hypertension. Hum Mol Genet. 2003;12(24):3277–3286.
72. Fang, YD, Xu, X, Dang, YM. MAP4 mechanism that stabilizes mitochondrial permeability transition in hypoxia: microtubule enhancement and DYNLT1 interaction with VDAC1. PLoS One. 2011;6(12):e28052.
73. Meiri, D, Greeve, MA, Brunet, A. Modulation of Rho guanine exchange factor Lfc activity by protein kinase A-mediated phosphorylation. Mol Cell Biol. 2009;29(21):5963–5973.
74. Lane, KL, Talati, M, Austin, E. Oxidative injury is a common consequence of BMPR2 mutations. Pulm Circ. 2011;1(1):72–83.
75. a. b. c. Johnson, JA, Hemnes, AR, Perrien, DS. Cytoskeletal defects in Bmpr2-associated pulmonary arterial hypertension. Am J Physiol Lung Cell Mol Physiol. 2012;302(5):L474–L484.
76. Wong, WK , Knowles, JA , Morse, JH. Bone morphogenetic protein receptor type II C-terminus interacts with c-Src: implication for a role in pulmonary arterial hypertension. Am J Respir Cell Mol Biol. 2005;33(5):438–446.
77. Min, J , Reznichenko, M , Poythress, RH. Src modulates contractile vascular smooth muscle function via regulation of focal adhesions. J Cell Physiol. 2012 Jan 27. [Epub ahead of print]
78. Majka, S, Hagen, M, Blackwell, T. Physiologic and molecular consequences of endothelial Bmpr2 mutation. Respir Res. 2011;12:84.
79. Burton, VJ, Ciuclan, LI, Holmes, AM, Rodman, DM, Walker, C, Budd, DC. Bone morphogenetic protein receptor II regulates pulmonary artery endothelial cell barrier function. Blood. 2010;117(1):333–341.
80. Archer, SL, Gomberg-Maitland, M, Maitland, ML, Rich, S, Garcia, JG, Weir, EK. Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1alpha-Kv1.5 O2-sensing pathway at the intersection of pulmonary hypertension and cancer. Am J Physiol Heart Circ Physiol. 2008;294(2):H570–H578.

81. Spiekerkoetter, E, Guignabert, C, de Jesus Perez, V. S100A4 and bone morphogenetic protein-2 codependently induce vascular smooth muscle cell migration via phospho-extracellular signal-regulated kinase and chloride intracellular channel 4. Circ Res. 2009;105(7):639–647, 13 p following 647.
82. Dichloroacetate (DCA) for the Treatment of Pulmonary Arterial Hypertension. ClinicalTrials.gov Web site. http://clinicaltrials.gov/ct2/show/NCT01083524. Updated November 3, 2011. Accessed May 6, 2012.
83. Burton, VJ, Holmes, AM, Ciuclan, LI. Attenuation of leukocyte recruitment via CXCR1/2 inhibition stops the progression of PAH in mice with genetic ablation of endothelial BMPR-II. Blood. 2011;118(17):4750–4758.
84. Safety and Tolerability Study of APN01 (Recombinant Human Angiotensin Coverting Enzyme 2). ClinicalTrials.gov Web site. http://clinicaltrials.gov/ct2/show/NCT00886353. Updated December 30, 2009. Accessed May 6, 2012.

PREVIOUS * NEXT

Log In to Comment

Comments

Related Resources

- Repurposing FK506 to Increase BMPR2 Signaling and Improve Pulmonary Arterial Hypertension: A Fast Track From Cells to People
- Vasoreactivity Tests and TGF-B type II Receptor Gene Mutations
- BMPR2 Mutations Are Not Found In Portopulmonary Hypertension Patients
- Functional Genetic Variations in BMPR2 and Other Signaling Pathways Modify Clinical Expression of Familial Pulmonary Arterial Hypertension
- Rescuing the BMPR2 Pathway, Where Can We Intervene?

David Badesch, MD

PHA Scientific Leadership Council
University of Colorado Health Sciences Center
Aurora, Colo.

801 Roeder Road, Ste. 1000, Silver Spring, MD 20910
301-565-3004 | PHAOnlineUniv@PHAssociation.org