

Genetics of pulmonary arterial hypertension: do the molecular findings have translational value?

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Abstract

Pulmonary arterial hypertension (PAH) is usually a devastating condition with a poor prognosis. Nearly 10 years ago, the underlying molecular basis of heritable PAH was elucidated with the identification of mutations in the gene encoding the bone morphogenetic protein type II receptor (BMPR-II). This discovery is now beginning to suggest novel approaches to therapy in heritable PAH. Moreover, recent studies provide evidence that dysfunction of the BMPR-II pathway is a feature of non-familial forms of PAH, broadening the scope for intervention in this pathway.

Introduction and context

Idiopathic and familial forms of pulmonary arterial hypertension (PAH) continue to carry a poor prognosis despite significant improvements in the treatment of these and related conditions over the past 15 years. Existing therapies are based on the replacement of prostanoids, inhibition of the endothelin pathway, or enhancement of nitric oxide signaling. Although these therapies have improved symptoms and possibly survival of some patients, additional approaches founded on a more thorough understanding of the cell and molecular pathobiology of PAH are required. Nearly 10 years ago, heterozygous germline mutations in the gene encoding bone morphogenetic protein type II receptor (BMPR-II), on chromosome 2q33, were identified in families affected by PAH [1,2]. To date, mutations in BMPR-II have been identified in nearly 80% of affected families. In addition, 10-40% of apparently sporadic cases of idiopathic PAH have been found to harbour mutations in BMPR-II [3]. Mutations have been identified in almost all of the coding exons of the *BMPR2* gene. Approximately 30% of mutations are missense, causing substitution of highly conserved amino acids in important functional domains of the receptor (e.g., the ligand-binding or kinase domains) [3]. The remaining (approximately 70%)

comprise nonsense, frameshift, and splice-site defects, and gene rearrangements. These predict premature termination of the transcript with likely loss through the process of nonsense-mediated decay (NMD). Disease penetrance in mutation carriers varies between families but is usually less than 50%. This important observation suggests that, although heterozygous mutation in BMPR-II increases the risk for PAH more than 10^5 -fold, some additional environmental or genetic factor seems to be a requirement for disease manifestation [4]. Evidence for genotype-phenotype correlations is slowly emerging in that missense mutations have been associated with earlier age of onset and increased penetrance compared with other mutations [5]. In addition, certain low-penetrance alleles seem more likely to occur in idiopathic PAH or disease associated with other known triggers [3,6].

BMPR-II is a type II receptor member of the transforming growth factor-beta (TGF- β) superfamily. As with other TGF- β family members, BMPs signal via complexes comprising heterodimers of type I and type II receptors [7]. The type II receptor is a constitutively active serine-threonine kinase, which in the presence of ligand phosphorylates the type I receptor. The type I receptor then phosphorylates a family of proteins termed Smads,

which can bind to DNA either directly to alter gene transcription or in the presence of DNA-binding partners. BMPs typically activate Smads 1, 5, and 8, whereas the TGF- β receptors typically activate Smads 2 and 3. Smad 4 is a common partner Smad that lacks a DNA-binding domain but is necessary for entry of the receptor-activated Smads to the nucleus.

In lung tissue from patients with heritable PAH, BMPR-II protein expression and phospho-Smad1 expression are reduced [8,9]. Of note, expression of these key parts of the BMP signaling pathway is also reduced in PAH patients who have no identifiable mutation in BMPR-II [8]. In pulmonary artery smooth muscle cells (PASMCs) isolated from patients with BMPR-II mutations, phospho-Smad1/5 activation in response to BMPs is suppressed, as is the activation of key BMP target genes, including the inhibitors of differentiation (Id) genes [10]. The BMP/BMPR-II/Smad1/Id gene axis appears to be growth-suppressive in PASMCs and pro-apoptotic [9,11]. Overexpression of mutant BMPR-II in vascular smooth muscle cells of transgenic mice appears sufficient to induce the development of pulmonary hypertension in these animals [12], whereas heterozygous *BMPR2*-null mice have no clear phenotype [13,14]. These findings likely indicate the need for a critical reduction in the BMPR-II activity below a threshold for the manifestation of disease. It is possible that additional triggers necessary for disease manifestation may further disrupt expression of key components of this pathway or indeed BMPR-II itself. Immunohistochemistry and *in vitro* studies suggest that BMPR-II is most highly expressed on the vascular endothelium. In contrast to PASMCs, BMPs via BMPR-II/Smad1/5 and Id1 are thought to enhance proliferation and reduce apoptosis of endothelial cells [15]. Conditional knockout of endothelial BMPR-II is sufficient to cause pulmonary hypertension in a proportion of mice [16].

Major recent advances

In terms of the translational value of the molecular findings, two major questions arise. First, can the restoration of BMPR-II function or expression prevent, or preferably reverse, the development of pulmonary hypertension? Second, what are the consequences to the cell of reduced BMPR-II expression or function and can these be targeted? We now know that reduced BMPR-II expression is a key feature of the two most commonly used rat models of PAH: chronic hypoxia [17,18] and exposure to the plant alkaloid, monocrotaline [17,19]. Prevention of the reduction of BMPR-II expression by adenovirally transduced lung endothelial targeting of BMPR-II ameliorates the development of pulmonary hypertension in chronically hypoxic

rats [20]. The same targeted approach is successful in the monocrotaline model, although transduction via the airways failed to have an effect [21]. There are additional approaches that might be used to restore BMPR-II function. For example, many of the mutations result in NMD of the transcript. Agents that improve translational read-through of the transcript would therefore be useful for mutations in which the resulting protein would retain some function [22]. Many of the missense mutations occur in the ligand-binding domain of BMPR-II and involve the substitution of important cysteine residues that provide tertiary structure to the molecule. These cysteine-substituted mutants fail to traffic normally to the cell surface and are held up within the endoplasmic reticulum. Though mutated, these receptors retain kinase activity, can associate with type I receptors, and retain some ligand-binding capacity. It is possible to use chemical chaperones to enhance mutant BMPR-II trafficking to the cell surface and restore BMP signaling [23]. This approach may be useful in this particular form of mutation. In addition, it is possible that the endoplasmic reticulum stress response may be activated in the presence of these mutations, which could also be a target for intervention.

One example of a pathway that is altered as a consequence of reduced BMP signaling is the TGF- β pathway via the TGF- β type 1 receptor, activin receptor-like kinase 5 (ALK-5). Several studies have now confirmed that ALK-5 inhibition can prevent or reverse established pulmonary hypertension in rodent models [17,24,25]. Another consequence of reduced BMPR-II function appears to be the increased expression of pro-inflammatory cytokines and inflammation [26]. These findings are still emerging but again provide additional targets for intervention.

Future directions

Further advances are required to develop a robust mouse model of BMPR-II mutation that consistently leads to pulmonary hypertension and vascular remodeling. This will depend on the identification of a trigger or second hit that precipitates disease. The unique conditions faced by the lung circulation and the localization of disease to that vascular bed are likely clues to solving this enigma. Such models will allow the testing *in vivo* of novel therapies designed to target BMPR-II or the BMP signaling pathway. A further challenge is to more fully understand the consequences to the vascular cell of BMPR-II mutation. Evidence is emerging that non-canonical signaling or additional hitherto unknown functions of BMPR-II may provide further insight into pathobiology and ultimately therapy.

Abbreviations

ALK-5, activin receptor-like kinase 5; BMP, bone morphogenetic protein; BMPR-II, bone morphogenetic protein type II receptor; Id, inhibitors of differentiation; NMD, nonsense-mediated decay; PAH, pulmonary arterial hypertension; PASMC, pulmonary artery smooth muscle cell; TGF- β , transforming growth factor-beta.

Competing interests

The author declares that he has no competing interests.

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