The Mouse Through the Looking Glass
A New Door Into the Pathophysiology of Pulmonary Hypertension

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Idiopathic pulmonary artery hypertension (IPH) is a rare illness with a poor prognosis. Whereas chronic intravenous prostacyclin relieves some of the symptoms of progressive dyspnea and prolongs survival, most patients ultimately require a lung transplant.1

Newer therapies such as nonintravenously administered prostacyclin derivatives,2-3 endothelin receptor blockers,4-5 and, to some extent, phosphodiesterase inhibitors,7 hold some promise as alternatives for intravenous prostacyclin, but current expectation is that, like prostacyclin, they will, at best, retard disease progression, serving as a bridge to transplant rather than as an alternative. The pathological features of IPH are loss of small distal precapillary pulmonary arteries, obliterate changes (plexogenic lesions) in more proximal pulmonary arteries associated with migration and proliferation of smooth muscle cells, and increased extracellular matrix deposition. There is also dysregulation of endothelial cells associated with increased proliferation.8 The mechanism underlying the evolution of these changes is unknown, so there was great interest when 2 groups independently identified a mutation in bone morphogenetic protein receptor 11 (BMP-RII) in 60% of families with IPH.9,10 A BMP-RII mutation also occurs in 20% of sporadic cases of IPH,11 but the biological connection between the mutation and the pathobiology of IPH has been relatively elusive.

Recent studies using pulmonary artery smooth muscle cells from patients with IPH, including those with and without a BMP-RII mutation, showed similar abnormal proliferation in response to agents such as transforming growth factor-β (TGF-β) or BMP-2.12 In other studies, pulmonary artery smooth muscle cells were transfected with constructs encoding different mutant forms of BMP-RII expressing aberrant kinase or cytoplasmic domains, and impaired signaling was observed related to alterations in the induction of Smads and p38.13 Specifically, suppression of Smad1/5 and activation of p38 were related to smooth muscle cell proliferation. It is still unknown specifically how these abnormalities in signaling regulate genes that induce smooth muscle cell proliferation or the more complex features associated with obliterator vascular lesions and plexiform changes that are related to aberrant endothelial function, smooth muscle cell migration and abnormal matrix production,14 or sensitivity or resistance to apoptosis.15,16 One possibility it that there is altered function of a transcription factor that binds Smad proteins, such as AML117 (Figure). This is intriguing because we have related AML1 to the induction of an elastase enzyme18 that is pivotal to the progression of pulmonary hypertension in animal models.19 Quite recently, using a yeast 2-hybrid system, mutations in the cytoplasmic tail of BMP-RII have been linked to altered interaction with a microtubule-associated protein, but the significance of this abnormality remains to be determined.20

It would therefore be valuable to create a mouse in which the vascular pathology related to the mutation could be evaluated. Deletion of BMP-RII in the mouse is lethal in embryonic life,21 and the heterozygote has a relatively unremarkable phenotype. So it is with great interest that we read in this issue of Circulation Research a report from the laboratory of Dr David Rodman22 describing the development of pulmonary hypertension in a mouse induced by overexpressing the human BMP-RII mutation in vascular smooth muscle cells in the postnatal period. This is achieved by driving expression of the BMP-RII mutation with the SM22 vascular smooth muscle-specific promoter under the regulation of tetracycline. The mice described have pulmonary hypertension in room air and more severe pulmonary hypertension than control mice in Denver altitude or with hypoxia. This more severe pulmonary hypertension is associated with a relatively modest increase in the number of muscularized distal vessels and in the hypertrophy of the more proximal pulmonary arteries. Although these features do not recapitulate the full pathology found in IPH patients, they are common to all forms of pulmonary hypertension regardless of etiology. Thus, the authors of this report are able, for the first time, to link the mutation to the pathobiology of pulmonary hypertension in an intact animal. With the proviso that the mouse and human respond similarly, the report also suggests that there is a link between the early pulmonary arterial changes common to all patients with pulmonary hypertension and the later obliterator changes in IPH. The progression from increased muscularity of pulmonary arteries to obliterator neointimal formation has been observed in pulmonary hypertension associated with congenital heart defects but has not been shown in IPH, perhaps because the
changes in the pulmonary circulation are usually very advanced when the clinical diagnosis of IPH is made.

Whereas pulmonary artery endothelial and smooth muscle cells express BMP-RII, it is unknown whether the pathology of IPH is influenced by a mutation in BMP-RII affecting endothelial or smooth muscle cells or both. This article would implicate smooth muscle cells in the pathobiology of hypertension, but it could be argued that the full-blown pathology of IPH requires abnormal function of endothelial as well as smooth muscle cells. It is also possible that a fibroblast, a progenitor cell, or a nonvascular cell expressing BMP-RII plays a critical role in the pathology observed clinically. Thus, it would be interesting to study the phenotype resulting from loss of function of BMP-RII in all cells in the late embryonic or postnatal period when the lethality could be subverted. This could be achieved by tetracycline-regulated loss of gene expression downstream of a nontissue-specific promoter. Alternatively, it is possible that the full-blown phenotype requires expression of the mutation in smooth muscle cells in embryonic life. It is also possible that overexpression of the human mutation will not result in the same end point as replacement of the gene on 1 allele with the mutation. It will therefore be interesting to compare the mouse described in this study to one in which BMP-RII is deleted in all cells in late embryonic or postnatal period, and to one in which BMP-RII is deleted in endothelial or smooth muscle cells throughout fetal life or in the postnatal period. This would involve making a mouse in which flanking the BMP-RII gene with LoxP sites would enable deletion of the gene in a temporal or spatial specific pattern as a result of breeding with a mouse in which the enzyme Cre is regulated by a tissue-specific promoter or in an inducible manner. It will also be of interest to assess the response of the mouse described in this article to other factors known to present risks for the development of pulmonary hypertension, such as appetite suppressants and infection or inflammation.

Regardless of the model, there is the question of how the mutation leads to the vascular abnormality, in this case, the pulmonary hypertension and increased muscularity of the arteries, and why there appears to be a dissociation between the severity of the hypertension and the mild nature of the changes described. Harvesting smooth muscle cells would be of great value to enable more precise delineation of the interactions between the aberrant BMP-RII and the coreceptor BMP-RI and the pattern of downstream signaling events. Knowing which genes and which transcription factors are involved would be of interest in finding a common pathway that may be abnormal in patients that do not have the BMP-RII mutation. For example, because an increased frequency of polymorphisms causing heightened activity of the serotonin transporter has been implicated in IPH, it would be of interest to determine whether BMP-RII influences the activity of the serotonin transporter or whether breeding this BMP-RII mutant mouse with a mouse with serotonin transporter overexpression would result in more severe pulmonary hypertension and vascular disease. It would also be of interest to determine whether other gene products implicated in the pathobiology of pulmonary hypertension, such as elastase, tenascin, or Mts1, are upregulated when BMP-RII signaling is abnormal; whether there is K channel dysfunction or whether the induction of these gene products promotes the development of pulmonary vascular disease when BMP-RII is abnormal. These kinds of studies may also resolve the controversy in the literature as to whether under certain circumstances angiopoietin is protective or promotes the pathobiology of pulmonary artery hypertension.

That is, it has been previously shown that decreased K channel function is observed in cells from patients with IPH and is seen in response to pulmonary hypertension producing stimuli such as appetite suppressants and hypoxia. Cell culture studies indicate that BMP2 normally causes K channel dependent apoptosis of smooth muscle cells, which may not occur in cells with the BMP-RII mutation. Elastase activity is induced by plasma factors such as apoA1 and also by stimulation of serotonin receptors. Elastase activity can contribute to proliferation by releasing matrix-bound growth factors and by upregulating tenasin C, which facilitates phosphorylation of growth factor receptors. Elastase-mediated elastin peptide production can also promote migration of smooth muscle cells by increasing production of fibronectin. Angiopoietin I has been reported to protect against the loss of small vessels that occurs as a result of endothelial cell apoptosis. In contrast, it has also been suggested that angiopoietin induces serotonin release from endothelial cells, and, thus, is responsible for serotonin-mediated smooth muscle cell proliferation. How all these factors are linked to BMP-RII in a living animal can be tested in
this and other murine models. Thus, the article by West et al from the Rodman laboratory is the first major step toward linking a genetic defect seen in patients with the pathology of pulmonary hypertension in an intact animal and will be enormously useful in determining pathways that are potential targets to prevent and reverse pathology.

References


**KEY WORDS:** pulmonary hypertension ■ bone morphogenetic protein receptor ■ smooth muscle cells ■ transgenic mouse ■ hypoxia
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