Bone Morphogenetic Protein Receptor-2 and Pulmonary Arterial Hypertension
Unraveling a Riddle Inside an Enigma?

Duncan J. Stewart

Pulmonary arterial hypertension (PAH) is an intriguing condition that is characterized by dramatic changes in the structure and function of pulmonary microcirculation, particularly at the level of the distal arteriolar bed. In addition to exuberant hypertrophy of the medial smooth muscle layers, the pathological features of PAH include hypertrophy and fibrosis of the intima, and occasionally the appearance of plexiform lesions which often occur distal to regions of arterial occlusion.1 Plexiform lesions have attracted considerable attention even though these are not necessarily present in all patients with primary or idiopathic PAH, and they are also found in patients with PAH associated with known causes such as congenital heart disease, collagen vascular disease, and so forth. Hyperproliferative endothelial cells (EC) represent an important component of these complex glomeruloid structures, and have in some cases been shown to be of monoclonal origin.2 Interestingly, although increased smooth muscle cell (SMC) and EC growth may predominate in the advanced stages of disease, more recent evidence suggests that increased apoptosis and loss of pulmonary vascular endothelium may predominate at earlier stages. Indeed selection pressure created by profound EC loss may create the conditions necessary for the emergence of apoptosis-resistant and hyperproliferative endothelial cell “clones.”3

The discovery of heterozygous mutations of the BMPR2 gene, encoding for the bone morphogenetic protein receptor-II (BMPR-II), in a substantial proportion of patients with familial pulmonary arterial hypertension (FPAH), as well as many cases of sporadic or idiopathic PAH (IPAH),4–6 represents perhaps the single greatest advance toward an understanding of the molecular mechanisms that underpin this puzzling and often lethal vascular disease. Indeed, by definition, patients with IPAH (previously called primary pulmonary hypertension), have no evidence of vascular pathology in any other bed. Thus, the reason why mutations in a gene which is ubiquitously expressed in vascular as well as many other cell types would produce abnormal arterial remodeling only in the vasculature of the lungs is one of the most intriguing riddles that still need to be unraveled. Paramount among these is the unique predilection of this disease for the pulmonary bed. Indeed, by definition, patients with IPAH (previously called primary pulmonary hypertension), have no evidence of vascular pathology in any other bed. Thus, the reason why mutations in a gene which is ubiquitously expressed in vascular as well as many other cell types would produce abnormal arterial remodeling only in the vasculature of the lungs is one of the most intriguing riddles that still need to be unraveled.

Unfortunately, there are relatively few studies which have attempted to determine directly the functional impact of these mutations on relevant vascular cells. In this issue of Circulation Research, Yang et al8 examine the effect of bone morphogenetic protein-4 (BMP-4) on growth and survival of pulmonary arterial SMCs from normal subjects and patients with IPAH or FPAH, with and without known mutations in BMPR2. An earlier study from the same group showed that BMP-4 inhibited growth of pulmonary artery SMCs isolated from normal subjects but not those from patients with idiopathic PAH,9 whereas in another report BMP-2 and -7 were shown to induce apoptosis of normal SMC, an effect which was also reduced in cells derived from patients with IPAH.10 The present report extends these observations, by uncovering an intriguing discrepancy between the response of

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SMCs isolated from proximal pulmonary arterial segments, which exhibited the expected inhibition of proliferation in response to BMP-4, and those from more distal pulmonary arteries (ie, \( \sim 1 \) to 2 mm) which did not. This observation suggests that regional differences could explain to some extent the selectivity of the vascular phenotype for the pulmonary circulation.

However, the lack of a growth regulatory response to BMP in normal SMCs from the distal pulmonary arteries is somewhat counterintuitive because remodeling of the more distal pulmonary arteries and arterioles is thought to be of particular importance in the pathogenesis of PAH. In other words, based on these data it might be expected that loss-of-function mutations of BMPR2 would preferentially affect larger pulmonary arteries, rather than the smaller arteries and arterioles, which are generally thought to be more important in the initiation and progression of this disease. It could be argued that the so-called “distal” arteries, which still have diameters in excess of 1 mm, are still quite large from the perspective of the pathogenesis of PAH, whereas it is the remodeling of the much smaller intraductal or precapillary arterioles (ie, \(< 100\mu \) in diameter) which is truly of relevance for this disease. Unfortunately, it is not very practical to isolate SMCs of such small vessels, which normally show minimal or no muscularization, particularly in normal human lungs. Thus, the question remains as to whether these cells would behave more like the proximal or distal SMCs described in the present report.

Another powerful strategy to elucidate “genotype-phenotype” relationships is the use of transgenic mouse models. However, the role of BMPR2 mutations in predisposing to PAH mainly by an increase in pulmonary artery SMC growth has not been well supported by data from transgenic mouse models. Heterozygous BMPR2-deficient mice generally exhibit a very mild phenotype\(^{12}\) with slight increases in pulmonary pressures and if anything, evidence of reduced arterial remodeling after chronic exposure to hypoxia.\(^{12}\) The most successful strategy for inducing PAH so far has been the overexpression of a dominant negative BMPR2 mutation derived from a patient with FPAH.\(^{13}\) The expression of this mutant BMPR-II targeted to SMCs resulted in substantial increases in pulmonary arterial pressure but no significant increase in arteriolar muscularization.\(^{13}\) It may be that the lack of remodeling represents a limitation of mouse models which exhibit a lower tendency for arterial muscularization compared with more traditional rat models. Nonetheless, the overexpression of dominant negative BMPR2 still resulted in profound effects on pulmonary hemodynamics which cannot be attributed to enhanced SMC growth, and thus other mechanisms need to be considered such as enhanced vasoconstriction or paracrine effects on other cell types (ie, ECs).

This raises the important question of whether the effect of BMPs on ECs, particularly in the distal pulmonary vasculature, would be the same as for SMCs. Indeed, quite variable effects of BMPs have been described depending on cell type. Whereas BMPs have been reported to inhibit growth\(^{10}\) and increase apoptosis pulmonary artery SMCs,\(^{11}\) opposite influences have been described in other cell types. For example, BMPs enhanced survival in cardiomyocytes\(^{14}\) and in a mesenchymal cell line.\(^{15}\) In the human lung vasculature, BMPR2 has been shown to express predominantly in the endothelial layer, with less expression seen in medial SMCs.\(^{16}\) Moreover, recently it has been recognized that mutations in Alk-1, an endothelial-restricted member of the BMPR-I family, which previously have been implicated in hereditary hemorrhagic telangiectasia, have been linked to PAH.\(^{17}\) The observation that defects in a receptor that is expressed only in ECs can cause a disease indistinguishable from IPAH strongly points to the vascular endothelium as a critical target in this disease. Unfortunately, no reports as yet have directly assessed the effect of BMPs specifically in pulmonary ECs, which arguably may be as (or even more) relevant to the pathogenesis of IPAH. In a preliminary report from our group, BMPs inhibited apoptosis and promoted survival in normal human pulmonary artery ECs as well as endothelial progenitor cells derived from normal subjects.\(^{18}\) This raises the intriguing possibility of opposite consequences of BMPR2 mutations in the 2 principal vascular cell types, which may both contribute importantly to the development of PAH: ie, increased EC apoptosis leading to loss of distal arteriolar integrity may possibly be an initiating trigger with subsequently enhanced pulmonary arteriolar remodeling driven mainly by exaggerated SMC growth contributing to the full spectrum of PAH pathology.\(^{19}\)

As well, the present report by the Morrell group points to the activation of Smad-1 as a critical mechanism for the growth inhibitory effects of BMPs in pulmonary artery SMCs. It has previously been recognized that in addition to R-Smad signaling, BMPR-II induces the activation of mitogen-activated kinases (ie, ERK1,2, JNK, and p38MAPK)\(^{8}\) in many cell types.\(^{8}\) Previous work from the same group demonstrated that whereas most BMPR-II mutations showed loss of Smad-mediated signaling when expressed in mouse mammary gland epithelial cells, there was an exaggerated activation of p38MAPK\(^{20}\) leading to enhanced cell proliferation.\(^{20}\) Interestingly, Smad-1 phosphorylation was not different in normal human pulmonary arterial SMCs from proximal and distal arteries even though growth inhibition was seen only in the former, suggesting that the “utilization” of the Smad1 signaling is reduced or absent in distal SMCs for as yet undetermined reasons. In contrast, proximal pulmonary arterial SMCs from patients with known mutations in the kinase domain of BMPR2 showed both a defect in Smad1 activation and loss of growth inhibition in response to BMP-4, with no reduction in the action of p38. Moreover, reduced phosphorylated Smad-1 was also demonstrated by immunohistochemistry in the media and intimal layers of smaller pulmonary arterioles from patients with IPAH and FPAH compared with controls, consistent with the paradigm of altered balance between R-Smad and p38MAPK signaling in SMCs leading to exaggerated cell growth in this disease.

Thus, careful analysis of the functional consequences of known mutations in BMPR2 on the responses of pulmonary vascular cells to BMPs, such as that described by the Morrell group in this issue of Circulation Research,\(^{9}\) is beginning to provide answers to the challenging questions about how these mutations may predispose to the development of the FPAH, and possibly IPAH as well. Clearly there are many more
questions that still need to be addressed, particularly in relation to the effects of loss of BMPR-II signaling in other cell types, such as the pulmonary vascular endothelium. These questions will hopefully be answered in the near future, so that finally we can see through the mystery that surrounds pathogenesis of PAH, and resolve with clarity the enigmatic link between the genetic mutations and the molecular mechanisms underlying this disease.

References

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