BMPR-II heterozygous mice have mild pulmonary hypertension and an impaired pulmonary vascular remodeling response to prolonged hypoxia

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1Cardiovascular Research Center, Department of Medicine, and 2Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts 02129; 3Department of Anesthesia and Critical Care, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114; 4Department of Biochemistry, The Cancer Institute of the Japanese Foundation for Cancer Research, Tokyo 170-8455, Japan

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Beppu, Hideyuki, Fumito Ichinose, Noriko Kawai, Rosemary C. Jones, Paul B. Yu, Warren M. Zapol, Kohei Miyazono, En Li, and Kenneth D. Bloch. BMPR-II heterozygous mice have mild pulmonary hypertension and an impaired pulmonary vascular remodeling response to prolonged hypoxia. Am J Physiol Lung Cell Mol Physiol 287: L1241–L1247, 2004. First published July 30, 2004; doi:10.1152/ajplung.00239.2004.—Heterozygous mutations of the bone morphogenetic protein type II receptor (BMPR-II) gene have been identified in patients with primary pulmonary hypertension. The mechanisms by which these mutations contribute to the pathogenesis of primary pulmonary hypertension are not fully elucidated. To assess the impact of a heterozygous mutation of the BMPR-II gene on the pulmonary vasculature, we studied mice carrying a mutant BMPR-II allele lacking exons 4 and 5 (BMPR-II+/− mice). BMPR-II+/− mice had increased mean pulmonary arterial pressure and pulmonary vascular resistance compared with their wild-type littermates. Histological analyses revealed that the wall thickness of muscularized pulmonary arteries (<100 μm in diameter) and the number of alveolar-capillary units were greater in BMPR-II+/− than in wild-type mice. Breathing 11% oxygen for 3 wk increased mean pulmonary arterial pressure, pulmonary vascular resistance, and hemoglobin concentration to similar levels in BMPR-II+/− and wild-type mice, but the degree of muscularization of small pulmonary arteries and formation of alveolar-capillary units were reduced in BMPR-II+/− mice. Our results suggest that, in mice, mutation of one copy of the BMPR-II gene causes pulmonary hypertension but impairs the ability of the pulmonary vasculature to remodel in response to prolonged hypoxic breathing.

pulmonary vasculature; smooth muscle cells; bone morphogenetic protein; hypoxia

PRIMARY PULMONARY HYPERTENSION (PPH) is a disorder characterized by a marked and sustained elevation of pulmonary vascular resistance with consequent progressive right ventricular failure (23). Typical pathological features include vascular cell proliferation, muscularization, and obliteration of small pulmonary arteries and formation of plexiform lesions. Familial PPH is inherited as an autosomal dominant disorder with reduced penetrance. Germline mutations of the bone morphogenetic protein type II receptor (BMPR-II) gene have been identified in patients with familial and sporadic PPH (4, 14, 19, 29). However, the reduced penetrance of PPH in individuals with BMPR-II gene mutations suggests that additional genetic and/or environmental factors are required for the development of PPH.

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-β (TGF-β) superfamily and bind to two types of serine/threonine kinase receptors, type I and type II. Both type I and type II receptors are required for signal transduction (13, 16). After they bind to BMPs, type II receptors phosphorylate type I receptors, which in turn phosphorylate Smad1, Smad5, and Smad8, and thereby modulate gene transcription (17). Activation of BMP receptors can also modulate Smad-independent pathways, including signaling via mitogen-activated protein kinases (5).

Pulmonary arterial smooth muscle cells (PaSMCs) from patients with PPH have altered growth responses to TGF-β and BMPs (18) and are resistant to BMP-induced apoptosis (36). BMPR-II gene expression was reported to be markedly reduced in the peripheral lung tissue of PPH patients, especially in those harboring heterozygous mutations of the BMPR-II gene (1). A wide range of mutations, which are distributed throughout the coding region of the BMPR-II gene, have been identified in patients with PPH. Most of the mutations are predicted to lead to a premature termination codon (15), but some missense mutations have also been identified. BMPR-II mutations may cause PPH via haploinsufficiency or dominant-negative mechanisms (20, 22). The importance of BMP/TGF-β signaling in the pathogenesis of PPH is further supported by the finding that some patients with mutations of activin receptor-like kinase-1 (ALK-1), a type I receptor for TGF-β, develop PPH, as well as hereditary hemorrhagic telangiectasia (30). Moreover, expression of ALK-3/BMPR-IA, one of the BMP type I receptors, has been shown to be reduced in lungs of patients with nonfamilial pulmonary hypertension (7).

Mice carrying two copies of a mutant BMPR-II allele (lacking exons 4 and 5) fail to form mesodermal tissue and die during early development, whereas mice carrying only one mutant allele (BMPR-II+/−) can survive and reproduce normally (3). To examine the impact of a heterozygous BMPR-II mutation on the pulmonary vasculature, we compared pulmonary vascular hemodynamics and structure in BMPR-II+/− mice.

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mice and their wild-type littermates. Here, we report that, compared with wild-type mice, BMPR-II^{+/−} mice have an increased pulmonary vascular resistance and mean pulmonary arterial pressure (PAP), as well as increased wall thickness (WT) of the muscularized pulmonary arteries and an increased number of alveolar-capillary units. However, the pulmonary vascular remodeling response to prolonged hypoxic breathing was impaired in BMPR-II^{+/−} mice.

MATERIALS AND METHODS

Mice Heterozygous for a BMPR-II Mutation

Mice carrying a mutant BMPR-II allele were generated as reported previously (3). BMPR-II^{+/−} mice are maintained on a C57BL/6 background. We tested mice of both sexes (age range of 2–5 mo, weighing 18–30 g). All animal experiments were conducted under protocols reviewed and approved by the Subcommittee on Research Animal Care of the Massachusetts General Hospital.

Measurement of Hemodynamics in Mice

Surgical preparation of mice for hemodynamic study after thoracotomy was performed in anesthetized mice (intraperitoneal administration of 250 ng/g fentanyl and 50 µg/g ketamine), as described previously (11). Briefly, catheters (PE-10, Becton Dickinson, Sandy, UT) were inserted into the main pulmonary artery and into the right carotid artery to measure PAP and systemic arterial pressure (SAP), respectively. Cardiac output was estimated by measuring lower thoracic aortic flow (LTAF) with a small-vessel flow probe connected to a flowmeter (T106; Transonic Systems, Ithaca, NY). We recorded all hemodynamic data using a biocomputer amplifiers while mice were mechanically ventilated with room air. Total pulmonary vascular resistance (TPVR) and total systemic vascular resistance (TSVR) were calculated by dividing PAP and SAP, respectively, by LTAF. To further characterize the pulmonary vascular-flow relationship, the inferior vena cava was partially occluded with a circumferential 5-0 silk ligature to transiently reduce cardiac output, while PAP and LTAF were continuously recorded (11). We calculated the slope of the PAP/LTAF using linear-regression analysis and reported this as incremental pulmonary vascular resistance (PVRi).

Vessel Morphometry

The airway and pulmonary vessels of wild-type and BMPR-II^{+/−} mice were perfusion fixed with 3% paraformaldehyde-0.1% glutaraldehyde in phosphate-buffered saline at constant inflation pressures of 23 and 100 cmH2O, respectively. Lung tissue was embedded in Technovit 7100 resin (Heraeus Kulzer), and 2-μm-thick sections were stained with 0.01% toluidine blue. Alveoli and alveolar vessels were counted in 20 contiguous microscope fields. To assess vascular WT, the sections were stained with an antibody against α-smooth muscle actin (clone 1A4, Sigma, St. Louis, MO), as described previously (12). All of the α-smooth muscle actin-positive vessels in the sections, <100 μm in diameter, were assessed for their WT and external diameter and were landmarked by their location, i.e., position in relation to associated airway structures, such as terminal bronchiol (TB), respiratory bronchiol (RB), alveolar ducts (AD), or alveolar wall (AW). The endothelial component of the vessel wall was excluded from the measurement of WT. WT1 was measured at one point of the vessel wall and WT2 at the diametrically opposite point. The percentage of wall thickness (%WT) was calculated as (WT1 + WT2) × 100/external diameter.

RNA Blot Hybridization

We extracted total RNA from lungs using the guanidine isothiocyanate-cesium chloride method. RNA (15 µg) was fractionated in formaldehyde-agarose gels containing ethidium bromide and transferred to nylon membranes. Membranes were hybridized with a 32P-labeled 0.2-kb mouse BMPR-II cDNA probe corresponding to exons 4 and 5, washed at high stringency, and exposed to X-ray film.

Measurement of Smad1/5/8 Activation

Mouse PaSMCs were isolated as described previously (27) and maintained in RPMI 1640 (GIBCOB, Invitrogen, Grand Island, NY) with 10% fetal calf serum. After serum starvation in RPMI 1640 without fetal calf serum, cells were exposed to BMP-2 (100 ng/ml; R&D Systems, Minneapolis, MN) for varying durations. Cell lysates were fractionated by SDS-PAGE, transferred to PVDF membranes, and incubated with antibodies specific for phosphorylated Smad1/5/8 or with antibodies that recognize both phosphorylated and nonphosphorylated Smad1 (Cell Signaling Technology, Beverly, MA).

Effects of Prolonged Hypoxia on Pulmonary Circulation and Ventricular Hypertrophy

Wild-type and BMPR-II^{+/−} mice (2–5 mo old) were housed in specially constructed environmental chambers (11) wherein they breathed at an inspired O2 fraction (FIO2) of 0.11 (hypoxia) for 3 wk. At the end of the exposure period, the mice were removed from the chamber, and hemodynamic measurements and histological examination of pulmonary vessels were performed as described above. To assess the development of right ventricular hypertrophy in wild-type and BMPR-II^{+/−} mice, the ratio of the weight of the right ventricle (WRV) to the sum of the weights of the left ventricle and septum (WLV+s) was calculated (Fulton’s ratio: WRV/WLV+s). Hemoglobin levels were determined in whole blood after the hemodynamic measurements were performed.

Statistical Analysis

We determined differences between groups using Student’s t-test or a two-way ANOVA. A value of P < 0.05 indicated a significant difference. All data are expressed as means ± SE.

RESULTS

Hemodynamic Measurements in BMPR-II^{+/−} Mice at Baseline

To examine the impact of the heterozygous mutation of the BMPR-II gene on the pulmonary vasculature of the mice, we obtained hemodynamic measurements in BMPR-II^{+/−} mice (n = 12; 8 males, 4 females) and wild-type mice (n = 11; 7 males, 4 females) during ventilation with air. We found that, compared with their wild-type littermates, BMPR-II^{+/−} mice had a similar LTAF but had an increased mean PAP (21 ± 3 vs. 16 ± 2 mmHg, P < 0.05) and TPVR (77 ± 7 vs. 51 ± 3 mmHg·min·l−1·g, P < 0.05) (Table 1). PVRi was also greater in BMPR-II^{+/−} mice than in wild-type mice (44 ± 4 vs. 33 ± 5 mmHg·min·l−1·g, P < 0.05). In contrast, mean SAP and TSVR did not differ between the genotypes. No differences were observed in the hemodynamic measurements obtained in male and female mice of either genotype.

Lung Morphology of the BMPR-II^{+/−} Mice

The lung morphology of the BMPR-II^{+/−} mice (n = 3) and wild-type mice (n = 3) did not appear qualitatively different at baseline (data not shown). In the lungs of BMPR-II^{+/−} mice, there was no evidence of the pulmonary vascular abnormalities
Table 1. Hemodynamic measurements

<table>
<thead>
<tr>
<th></th>
<th>Wild-type</th>
<th>BMPR-II&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>Wild-type</th>
<th>BMPR-II&lt;sup&gt;−/−&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of mice</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Weight, g</td>
<td>25.3 ± 0.9</td>
<td>25.3 ± 0.7</td>
<td>22.7 ± 1.0</td>
<td>22.7 ± 0.8†</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>13.0 ± 0.4</td>
<td>12.6 ± 0.4</td>
<td>17.6 ± 0.9</td>
<td>17.3 ± 0.7‡</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>561 ± 18</td>
<td>510 ± 21</td>
<td>515 ± 16</td>
<td>481 ± 17</td>
</tr>
<tr>
<td>Mean PAP, mmHg</td>
<td>16 ± 1</td>
<td>21 ± 1*</td>
<td>26 ± 2†</td>
<td>26 ± 1†</td>
</tr>
<tr>
<td>Mean SAP, mmHg</td>
<td>65 ± 4</td>
<td>59 ± 5</td>
<td>49 ± 4†</td>
<td>47 ± 2†</td>
</tr>
<tr>
<td>LTAF, ml-min&lt;sup&gt;−1&lt;/sup&gt;·g&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>0.33 ± 0.02</td>
<td>0.28 ± 0.02</td>
<td>0.33 ± 0.03</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>TAPVR, mmHg·ml&lt;sup&gt;−1&lt;/sup&gt;·min&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>51 ± 3</td>
<td>77 ± 7*</td>
<td>85 ± 6‡</td>
<td>85.7</td>
</tr>
<tr>
<td>TSVR, mmHg·ml&lt;sup&gt;−1&lt;/sup&gt;·min&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>204 ± 18</td>
<td>216 ± 20</td>
<td>158 ± 11†</td>
<td>154 ± 12‡</td>
</tr>
<tr>
<td>PVRi, mmHg·ml&lt;sup&gt;−1&lt;/sup&gt;·min&lt;sup&gt;−1&lt;/sup&gt;</td>
<td>33 ± 3</td>
<td>44 ± 4*</td>
<td>46 ± 2†</td>
<td>46.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Hemodynamic measurements were made at baseline or after exposure to inspired O2 fraction (FIO2) of 0.11 for 3 wk in wild-type and BMPR-II<sup>−/−</sup> mice. Hb, hemoglobin; HR, heart rate; PAP, pulmonary arterial pressure; SAP, systemic arterial pressure; LTAF, lower thoracic aortic flow; TAPVR, total pulmonary vascular resistance; TSVR, total systemic vascular resistance, PVRi: incremental pulmonary vascular resistance. *P < 0.05 vs. wild-type mice at baseline; †P < 0.05 vs. BMPR-II<sup>−/−</sup> mice at baseline; ‡P < 0.05 vs. wild-type at baseline.†

Typically found in patients with PPH, such as neointima formation and plexiform lesions. To quantitatively analyze the pulmonary structure in the two genotypes, we counted pulmonary vessels and alveoli (Table 2). The number of pulmonary vessels (including both arterioles and venules) per millimeter squared was similar for both genotypes, but the number of alveoli (and associated capillaries together referred to as alveolar-capillary units) per millimeter squared was 40% greater in BMPR-II<sup>−/−</sup> mice than in their wild-type littermates (P < 0.01, see Table 2). When expressed as a ratio, the larger number of alveoli decreased the vessels-to-alveoli ratio in the BMPR-II<sup>−/−</sup> mice compared with the wild-type mice (P < 0.01).

At baseline, few muscularized vessels (α-smooth muscle actin positive) of a size <100 μm in diameter were found in the lungs of either genotype. Most of the muscularized vessels were found to be precapillary (TB), but some were intra-acinar (RB, AD, and AW). However, the average percentage of WT of the muscularized vessels (<100 μm in diameter) was greater in BMPR-II<sup>−/−</sup> mice than in wild-type mice (16 ± 2% vs. 11 ± 1%, P < 0.01; Fig. 1A).

BMP Signaling in the Lung and PaSMCs Isolated From BMPR-II<sup>−/−</sup> Mice

To assess the impact of carrying one mutant BMPR-II gene on pulmonary BMPR-II gene expression, we measured BMPR-II mRNA levels in the lungs of BMPR-II<sup>−/−</sup> and wild-type mice. BMPR-II mRNA levels were ~50% reduced in the lungs of BMPR-II<sup>−/−</sup> mice compared with the lungs of wild-type mice (Fig. 2A). Similarly, BMPR-II gene expression in PaSMCs from BMPR-II<sup>−/−</sup> mice was approximately one-half the level in PaSMCs from wild-type mice (data not shown). To evaluate the effect of reduced gene expression on BMPR-II signaling, we measured Smad1/5/8 phosphorylation in lung extracts prepared from BMPR-II<sup>−/−</sup> and wild-type mice. Phosphorylation of Smad1/5/8 did not differ in lungs of BMPR-II<sup>−/−</sup> and wild-type mice (data not shown). In serum-starved PaSMCs from BMPR-II<sup>−/−</sup> and wild-type mice, only very low levels of Smad1/5/8 phosphorylation were detected and did not differ between genotypes. Exposure of PaSMCs to BMP-2 markedly induced Smad1/5/8 phosphorylation in PaSMCs from both genotypes, but the magnitude of Smad1/5/8 phosphorylation was reduced in PaSMCs from BMPR-II<sup>−/−</sup> mice compared with those from wild-type mice (Fig. 2B). Three independent lines of PaSMCs were derived from each genotype and were examined with similar results. These findings indicate that PaSMCs from BMPR-II<sup>−/−</sup> mice have reduced ability to transduce BMP signals.

![Wall thickness of small pulmonary arteries at baseline and after 3 wk of exposure to inspired oxygen fraction (FIO2) of 0.11. BMPR-II<sup>−/−</sup> and wild-type mice were studied at baseline (n = 4 each) and after 3 wk of breathing FIO2 of 0.11 (n = 12 and 13, respectively). A: percentage of wall thickness of α-smooth muscle actin (αSMA)-positive vessels, including intra-acinar and precapillary vessels <100 μm in external diameter (total number of vessels examined was as follows: at baseline, 55 wild-type vessels and 37 BMPR-II<sup>−/−</sup> vessels; after hypoxia, 387 wild-type vessels and 391 BMPR-II<sup>−/−</sup> vessels). *P < 0.01, **P < 0.001 vs. corresponding wild-type. †P < 0.001 vs. wild-type at baseline. B: photomicrographs of alveolar wall vessels stained with αSMA antibody (red) from lungs of wild-type and BMPR-II<sup>−/−</sup> mice after 3 wk of hypoxia. The muscularized vascular wall in BMPR-II<sup>−/−</sup> mice is thinner than that in wild-type mice. Scale bars: 100 μm.](http://ajplung.physiology.org/)

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tension is associated with right ventricular hypertrophy, we from the lungs of wild-type and BMPR-II mice. A photograph of 28S ribosomal RNA in the ethidium bromide-stained gel is shown to confirm equal RNA loading. B: PaSMCs isolated from wild-type and BMPR-II mice were analyzed for the phosphorylation of Smad1/5/8 before and after exposure to bone morphogenetic protein 2 (BMP-2; 100 ng/ml) for up to 30 min. Total Smad1 expression is also shown. W, wild-type; H, BMPR-II/−.

**Impact of Prolonged Hypoxia on Marine Pulmonary Hemodynamics, Vessel Morphology, and the Right Ventricle**

**Hemodynamic measurements.** Prolonged breathing at low oxygen concentrations induces pulmonary hypertension with pulmonary vascular remodeling in mice. To test whether chronic hypoxia would cause more severe pulmonary hypertension in BMPR-II/+− mice than in wild-type mice, we obtained hemodynamic measurements in the mice that breathed at FIO2 of 0.11 for 3 wk (Table 1). After prolonged hypoxia, mean PAP, TPVR, and PVRi were increased in wild-type mice (n = 15; 7 males, 8 females), but mean SAP and TSVR were decreased. In BMPR-II/−− mice (n = 13; 8 males, 5 females), prolonged hypoxia caused an increase in PAP and a decrease in SAP and TSVR; however, TPVR and PVRi did not change significantly. After mice breathed at FIO2 of 0.11 for 3 wk, pulmonary artery and systemic hemodynamic measurements did not differ between genotypes. Moreover, no differences in hemodynamic measurements between male and female mice of either genotype were observed. Although the hemoglobin level increased similarly in both genotypes, the magnitude of the increase in mean PAP in response to exposure at FIO2 of 0.11 for 3 wk was less in BMPR-II/−− mice than in wild-type mice (P < 0.05).

**Ventricular hypertrophy.** Because chronic pulmonary hypertension is associated with right ventricular hypertrophy, we compared WRV and WLV+S in BMPR-II/−− and wild-type mice. Although at baseline BMPR-II/−− mice had an elevated mean PAP compared with their wild-type littermates, the WRV-to-WLV+S ratio did not differ between BMPR-II/−− and wild-type mice (Table 3). After the mice breathed at FIO2 of 0.11 for 3 wk, their body weights decreased by 10% in both genotypes. Although the WRV-to-WLV+S ratio was increased in both genotypes compared with at baseline, BMPR-II/−− mice had a greater WRV/WLV+S than did wild-type mice (P < 0.01) despite similar pulmonary hemodynamics. However, the WRV-to-body weight ratio did not differ between the genotypes under normoxic conditions or after prolonged hypoxia. Of note, at baseline, the WLV+S-to-body weight ratio did not differ between the genotypes, but the ratio was greater in wild-type mice after prolonged hypoxia than in BMPR-II/−− mice (P < 0.05).

**Alveolar-capillary units.** After mice breathed at FIO2 of 0.11 for 3 wk, the number of the vessels per millimeter squared decreased by approximately one-third (P < 0.01, Table 2) in both genotypes, consistent with vascular pruning. In wild-type mice, prolonged hypoxia was associated with a 42% increase in the number of alveoli (alveolar-capillary units) per millimeter squared, consistent with pulmonary capillary angiogenesis (P < 0.01) (10). In contrast, after 3 wk of hypoxia, BMPR-II/−− mice did not exhibit an increase in the number of alveolar-capillary units per millimeter squared.

**Vessel morphometry.** Prolonged hypoxia induced the extension of smooth muscle cells into peripheral pulmonary arteries of both genotypes. Neither obliteration of pulmonary vessels nor plexiform lesions were observed in either genotype. In wild-type mice, vascular WT doubled after exposure to FIO2 of 0.11 for 3 wk (P < 0.001). In contrast, in chronically hypoxic BMPR-II/−− mice, the %WT increased by only 20% (P = 0.07) and was 25% less than in wild-type mice (19 ± 1% vs. 25 ± 1%, P < 0.01, see Fig. 1). The impaired muscularization of small pulmonary arteries in BMPR-II/−− mice was evident both in the distal in-tract aerobic vessels (RB, AD, and AW vessels, %WT; 17 ± 1% vs. 24 ± 1%, P < 0.001, Fig. 1B) and in the proximal precapillaries (TB vessels, %WT; 25 ± 1% vs. 29 ± 1%, P < 0.01).

**DISCUSSION**

The present study demonstrates that mice carrying one copy of a mutant BMPR-II allele have pulmonary artery hypertension due to an elevated pulmonary vascular resistance. The pulmonary hemodynamic abnormalities detected in BMPR-II/−− mice were associated with morphological alterations, including increased WT of muscularized pulmonary arteries (<100 μm in diameter) and an increased number of alveolar-capillary units. However, in BMPR-II/−− mice, there was no evidence of pulmonary vascular obliteration or plexiform le-

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**Table 3. Comparison of ventricular weights of wild-type and BMPR-II/−− mice**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>3 wk at FIO2 of 0.11</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Wild-type</td>
</tr>
<tr>
<td>No. of mice</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>BW, g</td>
<td></td>
<td>25.3 ± 0.9</td>
</tr>
<tr>
<td>WRV/WLV+S</td>
<td>0.25 ± 0.01</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>WRV/BW</td>
<td>(×10−5)</td>
<td>0.83 ± 0.02</td>
</tr>
<tr>
<td>WLV+S/BW</td>
<td>(×10−5)</td>
<td>3.27 ± 0.10</td>
</tr>
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</table>

Values are means ± SE. The ratio of the weight of the right ventricle (WRV) to the sum of the weights of the left ventricle and septum (WLV+S) was calculated (WRV/WLV+S) to assess the right ventricular hypertrophy at baseline or after 3 wk at FIO2 of 0.11. The ratios of WRV and WLV+S to body weight (BW) are also shown. *P < 0.05 and #P < 0.01 vs. wild-type after 3 wk at FIO2 of 0.11. †P < 0.05, ‡P < 0.01, and §P < 0.0001 vs. same genotypes at baseline.
sions, the pulmonary vascular abnormalities typically found in patients with severe PPH.

In our mouse model, the presence of a mutant BMPR-II allele was associated with a 50% reduction in pulmonary BMPR-II gene expression. However, phosphorylation of Smad1/5/8 did not differ in lung extracts prepared from BMPR-II+/− or wild-type mice. Because the lung contains many kinds of cells, we considered the possibility that BMPR-II-dependent signaling might be altered in only certain cell types, particularly those likely to participate in the pathogenesis of PPH. To examine this possibility, we cultured smooth muscle cells from the pulmonary arteries of BMPR-II+/− and wild-type mice. We observed in PaSMCs derived from BMPR-II+/− mice that Smad1/5/8 phosphorylation was reduced in response to BMP-2 stimulation, whereas total Smad1 levels were unchanged. It is probable that reduced BMPR-II expression in PaSMCs from BMPR-II+/− mice was responsible for the decreased responsiveness of these cells to BMP-2. The attenuated activation of regulatory Smads and their downstream gene targets by BMP signals may have contributed to the alterations in pulmonary vascular structure and function that we found in BMPR-II+/− mice. However, it is also possible that the phenotype of BMPR-II+/− mice may be attributable to perturbations of Smad1/5/8-independent mechanisms (e.g., mitogen-activated protein kinases).

Two mechanisms have been proposed for the pathogenesis of PPH in patients carrying a mutant BMPR-II allele: haploinsufficiency and dominant-negative interference. The role of haploinsufficiency is supported by the observation that BMPR-II expression is markedly reduced in lungs of patients with PPH associated with BMPR-II mutations (1). In our murine model, the BMPR-II gene lacking exons 4 and 5 likely produces no functional BMPR-II protein: even if it were translated, the predicted truncated protein would contain only the extracellular domain (exons 1–3) without the transmembrane and kinase domains and would not be anticipated to bind BMP ligands and BMP type I receptors. These findings suggest that BMPR-II haploinsufficiency is responsible for the pulmonary vascular abnormalities found in our BMPR-II+/− mice. It is noteworthy that, in PPH patients, mutations in BMPR-II exons 3 and 4 have been reported (4, 15), which would be predicted to encode truncated proteins similar to those potentially encoded by the mouse BMPR-II mutant allele.

An important role for dominant-negative interference by mutant BMPR-II proteins in the pathogenesis of PPH is supported by in vitro studies demonstrating that transfected mutant BMPR-II alleles (found in patients with PPH) act in a dominant-negative manner, perhaps by sequestering type I receptors in the cytoplasm (20, 22). The importance of a dominant-negative mechanism is further supported by the recent report of West et al. (34), who studied transgenic mice in which a dominant-negative BMPR-II was expressed selectively in smooth muscle cells only after birth. They observed that the transgenic mice had pulmonary hypertension and an increased smooth muscle cell ratio after prolonged hypoxia: a finding that may suggest that cells other than smooth muscle cells are involved in the development of pulmonary hypertension.

We examined this possibility, we cultured smooth muscle cells from the pulmonary arteries of BMPR-II+/− and wild-type mice. After mice were exposed to FIO2 of 0.11 for 3 wk, the WLV-to-WTV ratio increased in BMPR-II+/− mice harboring the BMPR-II mutant in smooth muscle cells and mice heterozygous for a mutant BMPR-II allele. The transgenic approach directed expression of the BMPR-II mutant in only a subset of the cells (smooth muscle cells) that may contribute to the development of PPH, whereas heterozygosity of the BMPR-II gene occurs in all somatic cells. Moreover, the levels of the BMPR-II mutant encoded by the transgene likely exceeded those encoded by the endogenous BMPR-II gene and may interfere with other BMP type II receptors (i.e., activin type II and IIB receptors) by sequestering BMP type I receptors. Finally, the increased number of alveolar-capillary units observed in BMPR-II+/− mice but not in the transgenic mice suggests that cells other than smooth muscle cells are involved in the formation of alveolar-capillary units and/or that the production of alveolar-capillary units occurs during development before the transgene expression was induced. Nonetheless, the finding of pulmonary hypertension in transgenic mice expressing a dominant-negative BMPR-II and in mice with BMPR-II haploinsufficiency supports a role for BMPR-II in the regulation of pulmonary vascular structure and function.

One of the BMPs, BMP-4, has important, yet complex, roles in the mesenchymal-epithelial interactions involved in early lung development, including branching morphogenesis and formation of terminal sacculles (2, 25, 33). Alveolar-capillary units are formed in the latter stages of lung development through a process of terminal sacculle septation and microvascular growth (21). The finding of increased alveolar-capillary units in BMPR-II+/− mice at baseline suggests a new role for BMP signaling in the alveolar formation and/or vascularization.

The absence of the pulmonary vascular abnormalities characteristic of clinical PPH in BMPR-II+/− mice led us to examine whether environmental stimuli would elicit a more robust pulmonary vascular response in BMPR-II+/− mice than in wild-type mice. A number of studies have shown that prolonged exposure to hypoxia causes pulmonary vascular remodeling in mice and rats (11, 26, 28, 31). We therefore compared pulmonary hemodynamics and structure in wild-type and BMPR-II+/− mice exposed to FIO2 of 0.11 for 3 wk. Prolonged hypoxia increased mean PAP in both genotypes; however, despite pulmonary hypertension and an elevated pulmonary vascular resistance in BMPR-II+/− mice at baseline, mean PAP and pulmonary vascular resistance did not differ between BMPR-II+/− and wild-type mice after 3 wk at FIO2 of 0.11. This finding was not attributable to difference in the pulmonary vascular contractile response to acute hypoxia because the change in left lung pulmonary vascular resistance in response to left mainstem bronchus occlusion (11) did not differ in wild-type and BMPR-II+/− mice (data not shown).

After mice were exposed to FIO2 of 0.11 for 3 wk, the WRV-to-WLV+S ratio increased to a greater extent in BMPR-II+/− mice than in wild-type mice. However, the WRV-to-body weight ratio did not differ in the two genotypes after prolonged hypoxia. Instead, the difference in the WRV-to-WLV+S ratio between the two strains was attributable to a difference in the response of left ventricle to prolonged hypoxia: after mice were exposed to FIO2 of 0.11 for 3 wk, the WLV+S-to-body weight ratio increased in wild-type mice but not in BMPR-II+/− mice. It is known that BMP signaling plays a central role in the induction of cardiac myogenesis in the chick embryo (24). These findings suggest an unexpected role for BMPR-II in
modulating the changes in mass of the adult mouse left ventricle in response to prolonged hypoxia.

In wild-type mice after 3 wk of hypoxia, the number of alveolar-capillary units was increased. These findings are consistent with the recent report of Howell et al. (10) who proposed that lung angiogenesis may be an adaptive response to prolonged hypoxia. In contrast, in BMPR-II\textsuperscript{+/−} mice, exposure to FIO\textsubscript{2} of 0.11 for 3 wk did not increase the number of alveolar-capillary units, suggesting that the pulmonary capillary angiogenic response to prolonged hypoxia was impaired. We observed that, after breathing at FIO\textsubscript{2} of 0.11 for 3 wk, the number of pulmonary vessels (arterioles and venules) decreased in both wild-type and BMPR-II\textsuperscript{+/−} mice. In contrast, prolonged hypoxia increased pulmonary vessel WT in wild-type mice to a greater extent than in BMPR-II\textsuperscript{+/−} mice. It is known that BMP signaling stimulates endothelial migration and tube formation (32) and promotes smooth muscle cell migration and differentiation (6, 35). In BMPR-II\textsuperscript{+/−} mice, decreased endothelial cell migration and tube formation may have contributed to the inability to increase the number of alveolar-capillary units in response to prolonged hypoxia. Moreover, decreased smooth muscle cell migration and differentiation may have had a role in the attenuation of pulmonary vascular muscularization in chronically hypoxic BMPR-II\textsuperscript{+/−} mice. Together, these observations suggest that BMPR-II signaling is critically important for the pulmonary vascular remodeling response to prolonged hypoxia (i.e., angiogenesis and muscularization).

BMPR-II\textsuperscript{+/−} mice exposed to FIO\textsubscript{2} of 0.11 for 3 wk did not develop the obliteration of small pulmonary arteries and plexiform lesions typically found in the lungs of patients with severe PPH. A recent study demonstrating the presence of plexiform lesions in the pulmonary arteries of ~5% of transgenic mice overexpressing S100A4/Mts1 suggests that mice can develop the pathological abnormalities observed in PPH patients (8). We speculate that BMPR-II\textsuperscript{+/−} mice will be predisposed to the development of PPH-associated pulmonary abnormalities in response to environmental or genetic factors other than prolonged hypoxia.

Although most individuals who carry a BMPR-II gene mutation do not develop PPH, some asymptomatic carriers appear to have an abnormal pulmonary artery pressure response to exercise (9). Despite the limitations of extrapolating observations made in mice to human beings, our findings of moderate pulmonary hypertension in BMPR-II\textsuperscript{+/−} mice at baseline suggest that individuals carrying a BMPR-II gene mutation may have abnormal pulmonary vascular function before the development of severe PPH with symptoms. The identification of patients at risk of developing PPH using either genetic testing or physiological assessment may be critical to preventing the development of the disease.

In summary, we report reduced lung BMPR-II expression and pulmonary hypertension due to an elevated pulmonary vascular resistance in mice carrying a mutant BMPR-II gene. We also found that the pulmonary vascular remodeling response to prolonged hypoxic breathing, including pulmonary capillary angiogenesis and vascular muscularization, was attenuated in BMPR-II\textsuperscript{+/−} mice, confirming a complex role of BMP signaling in pulmonary vascular growth and differentiation.


