Genetic Ablation of the Bmpr2 Gene in Pulmonary Endothelium Is Sufficient to Predispose to Pulmonary Arterial Hypertension

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Background—Pulmonary arterial hypertension (PAH) is a rare but fatal lung disease of diverse origins. PAH is now further subclassified as idiopathic PAH, familial PAH, and associated PAH varieties. Heterozygous mutations in BMPR2 can be detected in 50% to 70% of patients with familial PAH and 10% to 40% of patients with idiopathic PAH. Although endothelial cells have been suspected as the cellular origin of PAH pathogenesis, no direct in vivo evidence has been clearly presented. The present study was designed to investigate whether endothelial Bmpr2 deletion can predispose to PAH.

Methods and Results—The Bmpr2 gene was deleted in pulmonary endothelial cells using Bmpr2 conditional knockout mice and a novel endothelial Cre transgenic mouse line. Wide ranges of right ventricular systolic pressure were observed in mice with heterozygous (21.7 to 44.1 mm Hg; median, 23.7 mm Hg) and homozygous (20.7 to 56.3 mm Hg; median, 27 mm Hg) conditional deletion of Bmpr2 in pulmonary endothelial cells compared with control mice (19.9 to 26.7 mm Hg; median, 23 mm Hg) at 2 to 7 months of age. A subset of mice with right ventricular systolic pressure >30 mm Hg exhibited right ventricular hypertrophy and an increase in the number and wall thickness of muscularized distal pulmonary arteries. In the lungs of these mice with high right ventricular systolic pressure, the expression of proteins involved in the pathogenesis of PAH such as serotonin transporter and tenascin-C was elevated in distal arteries and had a high incidence of perivascular leukocyte infiltration and in situ thrombosis.

Conclusions—Conditional heterozygous or homozygous Bmpr2 deletion in pulmonary endothelial cells predisposes mice to develop PAH. (Circulation. 2008;118:000-000.)

Key Words: endothelium ■ genetics ■ hypertension, pulmonary ■ receptors

Pulmonary hypertension (PH) is a lung disorder in which mean pulmonary arterial pressure rises above normal levels (25 mm Hg at rest and 30 mm Hg during exercise). PH is classified into arterial, venous, hypoxic, thromboembolic, and miscellaneous varieties. Of these varieties, pulmonary arterial hypertension (PAH) typically carries the worst prognosis. PAH is further subclassified as idiopathic (IPAH), familial (FPAH), and associated (APAH) subtypes. The previously used term primary PH comprises IPAH, FPAH, and the anorexigen-induced subset of APAH. Pathological features of PAH appear in small pulmonary arteries and include intimal fibrosis, distal localization and proliferation of vascular smooth muscle, and pulmonary arterial occlusion. Appearing in severe end-stage PAH are plexiform lesions that consist of multiple, irregular vascular lumens with highly proliferative endothelial cells (ECs). Increased pulmonary vascular resistance and tone associated with thickening of medial layer and occlusions of small arteries lead to right ventricular (RV) hypertrophy and eventually to right heart failure. The prognosis of PAH remains poor despite recent advances in therapeutic approaches that appear to prolong survival in some PAH patients.
Clinical Perspective p  ●●●

The pathogenesis of PAH is largely unknown, but there is ample evidence implicating the involvement of diverse vascular effectors. In general, PAH can be promoted by hormones, growth factors, neurotransmitters, and environmental stresses that induce pulmonary vascular constriction, cell proliferation, or remodeling. More specifically, a decrease in vasodilatory and/or antiproliferative effects such as prostacyclin, nitric oxide, and voltage-gated potassium channels or in an increase in vasoconstrictory and/or mitogenic effects such as endothelin-1 and serotonin have been implicated in PAH.

Genetic studies have shown that bone morphogenetic protein receptor type II (BMPR2) signaling plays a critical role in the pathogenesis of IPAH and FPAH. FPAH accounts for at least 6% of all cases of PAH and shows an autosomal-dominant manner of inheritance.5 Heterozygous mutations of the BMPR2 gene were found in ≈50% to 70% of FPAH cases. Furthermore, 11% to 40% of apparently sporadic IPAH cases also carry germline BMPR2 mutations.5 In addition to the BMPR2 gene, PAH develops in a subset of hereditary hemorrhagic telangiectasia patients harboring heterozygous mutations in the ALK1 (activin receptor-like kinase 1) or ENG (endoglin) gene.5,7 Because ALK1 and ENG are components of the BMP signaling pathway, these genetic data suggest that the deficiency of BMP signaling is a primary factor predisposing to FPAH.

Pedigree studies of FPAH families have shown that only ≈20% of people who are expected to harbor a heterozygous BMPR2 mutation exhibit PH.5 Genetic anticipation (more severe forms and earlier onset of clinical manifestations in successive generations) also was observed in FPAH families.5 The molecular mechanisms governing the low penetrance and genetic anticipation in FPAH cases are unknown. However, these genetic data suggest that the heterozygous BMPR2 mutations are by themselves insufficient to account for the clinical manifestation of IPAH and that multiple environmental or genetic “hits” may play a pivotal role in triggering the disease.

Several attempts have been made to investigate the impact of BMPR2 deficiency on the development of PAH in mice. Because the characteristics of BMPR2 mutations in human PAH patients indicate haploinsufficiency (reduced functional protein) as the molecular mechanism of disease,8 mice heterozygous for a Bmpr2-null allele (Bmpr2+/−) received much attention. Bmpr2+/− mice showed moderately elevated mean pulmonary arterial pressure and pulmonary vascular resistance.9 More recent studies using the same mouse strain, however, showed no significant difference in RV systolic pressure (RVSP) between Bmpr2+/− and control mice.10,11 Mice with smooth muscle–specific downregulation of BMPR2 signaling using a dominant-negative form of BMPR2, Tg(SM22α::dnBmpr2), showed elevated RVSP.12 Both Bmpr2+/− and Tg(SM22α::dnBMPR2) mice exhibited moderate increases in muscularization of small arteries, but these mice did not fully recapitulate the pathological features of severe PAH in patients such as intimal fibrosis, occlusion of arteries, or plexiform lesions.9,12 It is unknown why the lung histology of Bmpr2+/− mice containing a Bmpr2 mutation essentially the same as in humans does not recapitulate the histological characteristics seen in human PAH patients. Perhaps, compared with humans, mice have a lower threshold level of BMPR2 signaling required for the maintenance of pulmonary vascular homeostasis. Bmpr2-knockdown mice with an shRNA technology that showed up to a 90% reduction in Bmpr2 expression compared with normal had no significant elevation in pulmonary pressure.13 Unfortunately, no additional information regarding the role of BMPR2 deficiency in PAH could be obtained from mice with a further reduction in BMPR2 level such as Bmpr2 hypomorphs and Bmpr2−/− mice because of their embryonic lethal phenotypes.14,15 A report showed that BMPR2 expression or function may play a role in PAH pathogenesis.16,17

Here, using the Cre/loxP system, we show that a deficiency in BMPR2 signaling in pulmonary ECs (pECs) can elicit PAH. On the other hand, even homozygous Bmpr2 deletion in pECs is not sufficient to cause PAH in all mice by 7 months. Our data suggest that genetic and environmental factors beyond those that modulate expression or function of BMPR2 in pECs play an important role in the development of PAH. This novel genetic model represents a valuable resource with which to further our understanding of the cause and pathogenesis of PAH.

Methods

All animal procedures performed were reviewed and approved by the University of Florida Institutional Animal Care and Use Committee.

Homozgyous Deletion of Bmpr2 Gene in pECs

Generation of a conditional Bmpr2 allele (Bmpr2f) was described previously.16 Generation of Tg(ALK1-cre)-L1 (L1cre) and Tg(ALK1-cre)-B1 (B1cre) lines also was described recently.19 R26R mice were purchased from The Jackson Laboratory (Bar Harbor, Me). Bmpr2f mice were intercrossed with L1cre and R26R mice. The L1cre(-); Bmpr2f or L1cre(+)Bmpr2f mice were further intercrossed with Bmpr2fR26R females to produce L1cre(-);Bmpr2fR26R, L1cre(+); Bmpr2fR26R, and L1cre(+)Bmpr2fR26R. More than half of the experimental and control mice contained the R26R allele, which served to monitor the Cre activities. Polymerase chain reaction (PCR) primer sets detecting the conditional and null alleles of Bmpr2 were as previously described.18 Primer sets detecting either the Cre or lacZ gene were used to genotype L1cre or R26R, respectively.

Hemodynamic Analysis

Systemic blood pressure was recorded noninvasively with the tail-cuff method. A pneumatic pulse sensor was placed on the tail distal to an occlusion cuff controlled by a programmed electrophys- momanometer (PE-300, Narco Bio-Systems, Austin, Tex), which was connected to the Powerlab system (ADInstruments, Colorado Springs, Colo). To evaluate pulmonary artery pressure, RVSP was measured by right heart catheterization through the right jugular vein. Briefly, each mouse was anesthetized with ketamine (100 mg/kg) and xylazine (15 mg/kg) and placed with the supine position. A Mikro-Tip pressure transducer (SPR-835, Millar Instrument, Houston, Tex) was inserted into the right external jugular vein and advanced into the right ventricle. All electric outputs from the tail cuff, pulse sensor, and transducer were recorded and analyzed by the
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Figure 1. L1cre mice express the Cre recombinase in the pulmonary vascular ECs. A and B, Lungs of 5-month-old L1cre;R26R bigenic mice were stained with X-gal for β-galactosidase expression to visualize the cells in which Cre-mediated recombination has occurred. Sections of whole-mount X-gal–stained lungs were counterstained with nuclear fast red (NFR; A) or anti-α-SMA antibodies (B). Insets, Magnified views of the area indicated by arrows. Note that X-gal–positive cells reside in vascular ECs but not in airway epithelial cells or in SMCs. C, PCR detection of the Bmpr2-null allele (ie, Bmpr2loxP=deletion of exons 4 and 5 of Bmpr2 by Cre)18 from genomic DNA isolated from multiple organs/tissues of 2-month-old L1cre(−):Bmpr21loxP (left) and L1cre(−):Bmpr22loxP (right), demonstrating a lung-specific Cre activity. A primer set amplifying an Alk1 locus (~190 bp) was used as a control for PCR reaction. D, Representative Western blotting analysis with anti-BMPR2 antibody on whole-lung lysate shows reduced levels of BMPR2 protein (~120 kDa) in L1cre(+) Bmpr21loxP lungs vs L1cre(−):Bmpr22loxP lungs, ~30% reduction (P<0.09; n=3). GAPDH (~36 kDa) was used as a loading control.

Powerlab 8/30 data acquisition system and associated Chart software (ADInstruments).

Pulmonary Vessel Morphometry
After hemodynamic analysis, mice were euthanized, and the heart and lungs were isolated for measuring heart weight and for lung morphometry studies as described in the Methods section of the online Data Supplement.

Western Blotting and Immunohistochemistry
Detailed reagents and procedures used for immunoblotting and immunohistochemistry are described in the Methods section of the online Data Supplement.

Statistical Analysis
Data are presented as mean±SEM except for the RVSP data. One-way ANOVA was used to determine a statistical significance among groups, and multiple pairwise comparisons were made by posthoc tests (Tukey and Tukey-Kramer) with SigmaStat (SPSS, Inc) and Minitab (Minitab, Inc). For RVSP data (Figure 2A), nonparametric 1-way ANOVA (Dunn’s method) was used, followed by the Kruskal-Wallis test for pairwise comparisons. The Z test was used to evaluate the differences of proportion of PH mice. For comparison of proliferating cell nuclear antigen (PCNA)–positive vascular cells between 2 genotype groups, the t test was used.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results
Bmpr2 Deletion in pECs by a Novel Cre Transgenic Mouse Line
To investigate the role of BMPR2 signaling in pulmonary vascular endothelium, we used a Bmpr2 conditional knockout mouse strain18 and a novel Cre transgenic mouse line designated Tg(Alk1-cre)-L1 (or L1cre). The L1cre line was established during the screening of multiple transgenic founder lines in which the Cre recombinase is driven by a 9.2-kb Alk1 genomic fragment.20 Unlike other independent Tg(Alk1-cre) founder lines and previously reported endothelium-specific Cre lines, Cre-mediated gene excision (called Cre activity hereafter) was not detected in the endocardial cells and their progeny in L1cre mice.10 On the other hand, Cre activity was observed in pECs from the emergence of patent pulmonary vessel formation during embryogenesis until adulthood.18 We have monitored the Cre activity of neonatal and adult L1cre mice by crossing them with ROSA26-lacZ (R26R) mice in which constitutive and permanent lacZ expression is designed to be activated by Cre. As shown in Figure I of the online Data Supplement, strong and homogeneous lacZ activity was detected in the lungs of L1cre(+):R26R mice at a level comparable to an endothelial Cre-line B1cre(+):R26R newborn pup (supplementary Figure Ib and Ic). Unlike B1cre mice, however, the lacZ activity of L1cre in systemic vessels, except for brain (supplementary Figure Id and Ie), appeared to be mosaic or absent (supplementary Figure If through I). The Cre activity is persistent in adult lungs; it was widely detected in the lung of 5-month-old L1cre;R26R mice. Histological sections showed that pECs were positive for the Cre activity, whereas the bronchial epithelia and smooth muscle layers were negative (Figure 1A and 1B). We crossed L1cre mice with Bmpr21loxP females to generate L1cre(+); Bmpr21loxP male mice, which were subsequently crossed with Bmpr22loxP;R26R females.

The L1cre(+):Bmpr22loxP mice were viable over 8 months. Genomic PCR analysis on multiple organs/tissues of 2-month-old L1cre(+):Bmpr22loxP mice revealed that the Cre-
mediated Bmpr2 gene excision was detected only in the lung, indicating that Cre expression was lung specific in the L1cre line (Figure 1C). Western blot analysis on whole-lung extracts with anti-BMPR2 antibodies revealed ∼30% reduction in BMPR2 protein in L1cre(Bmpr2f/f) lungs compared with L1cre(Bmpr2f/f) controls (Figure 1D).

Bmpr2 Deletion in pECs Can Induce Elevation of RVSP and RV Hypertrophy

To assess the pulmonary artery pressure, RV pressure was measured by right heart catheterization through the right jugular vein in 2- to 7-month-old mice. The median RVSP of control [L1cre(-);Bmpr22f] mice was 23 mm Hg (n=18; Figure 2A). The median RVSP of L1cre(+);Bmpr22f mice (23.7 mm Hg; n=20) was similar to that of control mice, but the RVSP in L1cre(+);Bmpr22f mice (27 mm Hg; n=33) was significantly higher than that in control mice (Figure 2A). There was no difference in the systemic pressure among the different genotype groups (Figure 2B).

In a subset of L1cre(+);Bmpr22f (4 of 20) and L1cre(+);Bmpr22f (13 of 33) mice, RVSPs were >30 mm Hg (supplementary Tables I and II). These high-RVSP mice were designated the PH group, whereas those mice with RVSP <30 mm Hg were designated the non-PH (N-PH) group. The ratio of RV to left ventricle plus septum, an indicator of RV hypertrophy, was higher in L1cre(+);Bmpr22f mice compared with controls or the L1cre(+);Bmpr22f mice (Figure 2C). When the data were analyzed by PH and N-PH groups, the PH mice had greater ratios of RV to left ventricle plus septum than did N-PH mice, indicating the presence of sustained elevation of RV pressure in the PH mice (Figure 2D).

Increased Number and Medial Wall Thickness of α-Smooth Muscle Actin–Positive Distal Arteries in the Mutant Mice With Elevated RVSP

To determine whether the elevation of RVSP and RV hypertrophy were associated with pulmonary vascular remodeling, lung tissue sections were examined by hematoxylin and eosin staining and immunohistochemistry. General aspects of lung development and morphology were indistinguishable among genotypes and between the PH and N-PH groups. Immunostaining of the lung sections with anti-α-SMA antibodies revealed an increased number of and increased wall thickness of α-SMA-positive small arteries in the PH group compared with the N-PH group (Figure 3A and 3B). Quantitative morphometric analysis demonstrated that the percentages of αSMA-positive small arteries (30- to 70-μm outer diameter) in the PH lungs were greater than those in the N-PH lungs within each genotype group (Figure 3C). Likewise, the wall thickness of αSMA-positive small arteries in the PH group was greater than that in the N-PH group (Figure 3D). Both the percentage of
SMA-positive small arteries and wall thickness were greater in lungs of L1cre(+/H11001);Bmpr2f/f PH mice compared with L1cre(+/H11001);Bmpr2f/f PH mice, suggesting that loss of both alleles induced more marked pulmonary vascular remodeling than did loss of 1 allele.

Histopathological Features in the Mutant Mice With Elevated RVSP

A high incidence of focal leukocyte infiltrations surrounding pulmonary vessels was found in L1cre(+);Bmpr22f/2 (46%, 6 of 13) and L1cre(+);Bmpr22f/2 (50%, 12 of 24) mice but not in the control mice (0 of 11) (Figure 4A and 4B and supplementary Table III). Among L1cre(+);Bmpr22f/2 mice, the infiltration tended to be more frequent in the PH group (8 of 13) than in the N-PH group (4 of 11). Most of the infiltrated cells were CD68-positive monocyte/macrophages (Figure 4B inset). Conspicuous thickening of αSMA-positive cell layers was observed in small arteries of the PH lungs (Figure 4C and 4D), and some arteries appeared to be occluded (Figure 4E), resembling the concentric vascular lesion in human PAH lung samples.16 In situ thrombosis also was observed in the PH lungs. As shown in Figure 4F, the lumens of some affected vessels were partially or completely occluded by fibrin(ogen)-positive thrombi. The frequency of ≥1 thrombotic lesions was higher in PH lungs (53%, 9 of 17) than in N-PH lungs (14%, 3 of 22) of L1cre(+);Bmpr22f/2 and L1cre(+);Bmpr22f/2 mice (P<0.05) (supplementary Table III). In addition, we have examined the expression of 5-hydroxytryptamine transporter and tenascin-C in PH lungs because elevated serotonin signaling and tenascin-C expression have been implicated in the pathogenesis of PAH.21,22 As shown in supplementary Figure II, elevated 5-hydroxytryptamine transporter and tenascin-C expression was detected in the vascular smooth muscle cell (VSCM) layer of PH lungs but not in N-PH or control lungs.

Increased Proliferation Index in ECs and SMCs of L1cre(+);Bmpr22f/2 Mice

To investigate whether the histological alteration of pulmonary vessels was associated with an increase in proliferation and/or a decrease in (or resistance to) apoptosis of vascular cells, proliferation and apoptosis indexes were examined in 2- to 7-month-old mice. We found no significant differences between PH and N-PH lungs in the percentage of cells positive for a proliferation or apoptosis marker in distal arteries. Because muscularization of distal arteries in PH lungs was readily detectable in 2- to 7-month-old mice, the processes of proliferation or apoptosis might have already occurred in this age group. We then examined a younger set of mice (≤4 weeks of age). Because we could not perform cardiac catheterization in this young group, we randomly chose 7 mice each from the L1cre(+);Bmpr22f/2 and L1cre(−);Bmpr22f/2 groups and performed PCNA or terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay on their lung sections. We found only a very small number of TUNEL-positive vascular cells in the
lung sections regardless of their genotypes (data not shown), and no significant difference in TUNEL staining was seen between the 2 genotype groups. On the other hand, both PCNA-positive EC and SMC populations in distal arteries of the L1cre(+/H11001); Bmpr2<sup>f/f</sup> group appeared to be greater than those of controls (P<0.001) (Figure 5).

**Discussion**

We show here that some but not all mice with the genetic ablation of Bmpr2 in pECs exhibited an elevation of RVSP, RV hypertrophy, and histopathological features reminiscent of human PAH lungs, demonstrating for the first time in vivo that Bmpr2 mutation in endothelium is sufficient to predispose to PAH. We also show that homozygous Bmpr2 deletion is in itself not sufficient to cause PAH but can increase the susceptibility to PAH to a greater extent than that observed with heterozygous deletion.

PAH pathogenesis is associated with dysregulation of pulmonary arterial remodeling, which involves multiple vascular (endothelial, smooth muscle, and adventitial fibroblasts) and nonvascular cell types (leukocytes, platelets, and circulating endothelial progenitor cells).<sup>2</sup> BMPR2 is expressed in most of these cell types.<sup>16,23,24</sup> Predisposition to PAH is associated with mutations in the ALK1 or ENG genes.<sup>6,7</sup> which are expressed primarily in ECs, suggesting that pECs may be a primary cell type in which BMPR2 mutations elicit PAH.

To examine whether Bmpr2 deletion in pECs is sufficient to predispose mice to PAH, we exploited the Cre/loxP system. For the endothelium-specific Cre driver, we used the L1cre for 3 reasons. First, the Alk1 promoter could be the most relevant promoter to drive Cre expression because Alk1 is persistently expressed in pECs from embryonic to adult stages and ALK1 mutations are linked to IPAH. Second, unlike currently available transgenic mouse lines expressing Cre in endothelium by Tie1, Tie2, Flk, or Vecad regulatory sequences, L1cre mice do not express the Cre recombinase in the endocardial cells, the progenies of which constitute atrioventricular cushions during cardiogenesis stages.<sup>19</sup> Conditional deletion of several transforming growth factor-β/BMP receptors using these known EC-specific Cre lines causes cardiac malformations such as cardiac valve or ventricular septal defects.<sup>25,26</sup> When we deleted the Bmpr2 gene using Tg(Tie2-cre) mice, most Tg(Tie2-cre);Bmpr2<sup>f/f</sup> mice died before the weaning age as a result of cardiac malformations (H.B., unpublished data), which makes this model unsuitable for studying the pathogenesis of PAH. Third, the L1cre mice show strong Cre expression in pECs but mosaic or absent Cre expression in most systemic ECs (supplementary Figure I). The L1cre line is superior to the known endothelial Cre lines for evaluating the impact of a gene deletion.
preferentially in pECs without affecting cardiogenesis. The L1cre line also will be useful for assessing the role of other BMPR2 signaling partners (eg, BMPR1A and SMAD1) and of potential downstream mediators of BMPR2 in the pathogenesis of PAH.

L1cre(+);Bmpr2<sup>−/−</sup> mice at 2 to 7 months of age showed a wide range of RVSP (20.7 to 56.3 mm Hg; n = 33) compared with control mice (19.9 to 26.7 mm Hg; n = 18) (supplementary Tables I and II). Although there is no conventional definition of PH in mice, we designated the mice having RVSP > 30 mm Hg, beyond the range observed in the control mice, as the PH group (33.2 to 56.3 mm Hg; 41%, 13 of 33). The PH mice displayed higher RV weight, an increased number of muscularized distal arteries, and thicker smooth muscle layers compared with the N-PH or control mice (Figures 2d and 3). Similar to the L1cre(+);Bmpr2<sup>−/−</sup> group but to a lesser extent, 20% of the L1cre(+);Bmpr2<sup>−/−</sup> mice (4 of 20) also exhibited the PH phenotype. Interestingly, this frequency is similar to human studies showing that ≈20% of people harboring heterozygous BMPR2 mutations develop PAH. Although technical limitations in mice prohibit fully satisfying all clinical criteria for PAH, the strong correlations between RVSP and morphometric data imply that these PH mice may represent PAH.

It is unclear why some but not all mice with endothelial BMPR2 deficiency develop PAH. The frequency of PAH phenotypes in this study did not differ by gender of the mice. To examine the possibility that the phenotypic variation resulted from an inconsistency in the Bmpr2 deletion in pECs, we monitored the Cre activity by introducing the R26R allele. No differences in the intensity and pattern of X-gal staining were detected between the PH and N-PH groups regardless of their Bmpr2 genotype, indicating that the phenotypic variation should not be attributed to incomplete excision of Bmpr2 gene in pECs. Moreover, the PH phenotype was not more frequent in mice carrying the R26R allele than in those without the allele. When the data were analyzed by 2 age groups, 2 to 4 and 5 to 7 months of age, the older age group tended to have a higher frequency of the PH mice than the younger age group (67% [6 of 9] versus 30% [7 of 24]), indicating that the 2- to 7-month-old N-PH mice may eventually develop PAH when they get older. Studies on 12- and 18-month-old mice may address whether incomplete penetrance is the issue of onset. Because the mice used in this present study were on 129Sv, C57BL/6, and FVB mixed background, strain-specific genetic modifiers may have modulated the propensity to develop PAH in mice carrying mutant Bmpr2 alleles. Candidate modifiers may include genes involved in modulating the balance between TGF-β and BMP signal transduction pathways.

We have shown that both pECs and VSCMs in distal arteries of 4-week-old L1cre(+);Bmpr2<sup>−/−</sup> lungs had a higher number of proliferating cells compared with controls (Figure 5). Because the BMPR2 signaling is intact in VSCMs of L1cre(+);Bmpr2<sup>−/−</sup> mice, the higher proliferation index of VSCMs in the mutants is likely due to indirect effects of defects in ECs. It is conceivable that impaired BMPR2 signaling in pECs may result in dysregulated production of cytokines, which in turn promote VSCM growth. We observed a higher incidence of infiltration of CD68-positive mononuclear cells in the perivascular regions of L1cre(+);Bmpr2<sup>−/−</sup> mice (12 of 24) and L1cre(+);Bmpr2<sup>−/+</sup> mice (6 of 13) compared with L1cre(-);Bmpr2<sup>−/−</sup> controls (0 of 11) (supplementary Table III). Moreover, in situ thrombosis was observed more frequently in the PH group than in the N-PH or control group. These observations are consistent with pathological findings that human PAH lungs often contain inflammatory and/or thrombotic vascular lesions. Taken together, our data suggest that Bmpr2 deletion in pECs may increase the risk of pECs to damage that renders the pulmonary vessels more susceptible to inflammation or thrombosis. A recent report suggested that BMP signaling in ECs may...
protect them from apoptosis and thus BMPR2 deficiency may predispose the ECs to apoptosis. EC death can be a triggering point of endothelial dysfunction and PAH pathogenesis. In our system, however, no significant difference was found in the number of TUNEL-positive cells between normal and mutant lungs in the presymptomatic and symptomatic phases.

One of the major impediments for understanding the underlying pathogenetic mechanisms for PAH is limited access to biological samples. IPAH is a rare disease (2 to 3 per million per year), and pathological samples are available only from lung explants and autopsy specimens at the very late stage of the disease. Animal models that reproduce key features of PAH provide relevant pathological samples from early to late stages of PAH. The L1cre(+/H11001);Bmpr2f/f mouse lines presented here may serve as a useful genetic resource to further our knowledge about PAH. Incomplete penetrance of this genetic model is a limitation for studying PAH pathogenesis, but it can be a great opportunity to identify environmental or genetic factors that influence PAH pathogenesis in terms of frequency, onset, or severity. It was shown that Bmpr2f/f mice are susceptible to an inflammatory mediator or serotonin in the development of PH. Genetic crosses or pharmacological treatments that modulate inflammation, cell growth, or endothelial functions of L1cre(+/+);Bmpr2f/f mice may provide valuable insights regarding the source of “hits” for PAH pathogenesis. Because the readouts used in the present study, ie, right heart catheterization for RVSP, the Fulton index, and histopathology, are terminal procedures, it is difficult to monitor the progression of disease, which is a major limitation of this model for preclinical translational studies. Future work should include the use of noninvasive systems such as echocardiographic techniques for monitoring RV wall thickness and/or hemodynamics and identifying serum biomarkers for the PH group mice.

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Disclosures
None.

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**CLINICAL PERSPECTIVE**

Pulmonary hypertension (PH) is a lung disease of diverse origins. Pulmonary hypertension is classified into arterial, venous, hypoxic, thromboembolic, and miscellaneous varieties. Of these varieties, pulmonary arterial hypertension (PAH) typically carries the worst prognosis. PAH is promoted by the imbalance of hormones, growth factors, neurotransmitters, or environmental stresses, which leads to pulmonary vascular constriction, cell proliferation, or remodeling. Bone morphogenetic protein receptor type II (BMPR2) signaling plays a critical role in PAH pathogenesis because germline mutations of BMPR2 are associated with 25% to 30% of all PAH cases. PAH pathogenesis involves multiple vascular and nonvascular cell types. We show that mice with the genetic ablation of Bmpr2 in pulmonary endothelial cells exhibited an elevation of right ventricular systolic pressure, right ventricular hypertrophy, and histopathological features reminiscent of human PAH lungs, demonstrating for the first time in vivo that Bmpr2 mutation in endothelium is sufficient to predispose to PAH. Our data suggest that impaired BMPR2 signaling in pulmonary endothelial cells may increase the risk of pulmonary endothelial cells to damage that renders the pulmonary vessels more susceptible to dysregulated remodeling.

One of the major impediments for PAH studies is limited access to biological samples because pathological samples are available only from lung explants and autopsy specimens at the very late stage of the disease. Animal models that reproduce key features of PAH provide relevant pathological samples from early to late stages of PAH. If we are able to detect pulmonary hypertension from these mutant mice at an early phase with noninvasive monitoring systems, it will facilitate the usefulness of this animal model for various PAH studies.
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