Signaling Molecules in Nonfamilial Pulmonary Hypertension


BACKGROUND

Biochemical, genetic, and clinical evidence indicates that smooth-muscle proliferation around small pulmonary vessels is an essential part of the pathogenesis of pulmonary hypertension. Mutations in the bone morphogenetic protein receptor type 2 (BMPR2) have been linked to familial cases of pulmonary hypertension, but the molecular basis of the common nonfamilial forms is unknown.

METHODS

We evaluated the pattern of expression of angiopoietin-1, a protein involved in the recruitment of smooth-muscle cells around blood vessels; TIE2, the endothelial-specific receptor for angiopoietin-1; and bone morphogenetic protein receptor type 1A (BMPR1A) and BMPR2 in lung-biopsy specimens from patients with pulmonary hypertension and from normotensive control patients. The effect of angiopoietin-1 on the modulation of BMPR expression was also evaluated in subcultures of human pulmonary arteriolar endothelial cells.

RESULTS

The expression of angiopoietin-1 messenger RNA and the protein itself and the phosphorylation of TIE2 were strongly up-regulated in the lungs of patients with various forms of pulmonary hypertension, correlating directly with the severity of disease. A mechanistic link between familial and acquired pulmonary hypertension was demonstrated by the finding that angiopoietin-1 shuts off the expression of BMPR1A, a transmembrane protein required for BMPR2 signaling, in pulmonary arteriolar endothelial cells. Similarly, we found that the expression of BMPR1A was severely reduced in the lungs of patients with various forms of acquired as well as primary nonfamilial pulmonary hypertension.

CONCLUSIONS

These findings suggest that all forms of pulmonary hypertension are linked by defects in the signaling pathway involving angiopoietin-1, TIE2, BMPR1A, and BMPR2 and consequently identify specific molecular targets for therapeutic intervention.
The pulmonary vascular bed is a high-flow, low-pressure circuit that has the capacity to dilate and recruit unused vasculature in order to accommodate increases in blood flow. In pulmonary hypertension, this capacity is lost, resulting in elevated pulmonary arterial pressure at rest and further increases in pressure with exercise. At the cellular level, this pathologic process is characterized by the proliferation of vascular smooth-muscle cells and asymmetric neointimal hyperplasia in small pulmonary arteries and arterioles. The molecular mechanism underlying the vasculopathy responsible for pulmonary hypertension is unknown.

Recently, heterozygous mutations have been characterized in the gene for bone morphogenetic protein receptor type 2 (BMPR2) in families with inherited pulmonary hypertension. Mutations were found to occur at highly conserved sites, predicted to perturb heterodimerization with its sister receptor, bone morphogenetic protein receptor type 1A (BMPR1A), or to disrupt the ligand-binding function of this protein. In addition, missense, deletion, and nonsense mutations in the activin-receptor–like kinase 1 gene (ALK1) have been identified in patients with hereditary hemorrhagic telangiectasia in whom pulmonary hypertension develops. However, most cases of pulmonary hypertension are sporadic or are due to a variety of causes, including hypoxia, thromboembolism, left-sided heart failure, and drugs. One puzzling aspect of this disease is whether any common molecular pathway underlies the seemingly different causes of the same pulmonary vascular pathologic process.

Angiopoietin-1 is a 70-kD angiogenic factor essential for lung vascular development. Produced by smooth-muscle cells and precursor pericytes, angiopoietin-1 stabilizes the development of blood vessels by recruiting muscle cells, through migration and division, to endothelial tubes, creating mature arterial structures. Animals lacking angiopoietin-1 die in utero, with little arterial development in the lungs and other organs. The receptor for angiopoietin-1, TIE2, is present only on vascular endothelium. The ligand–receptor interaction between angiopoietin-1 secreted by smooth-muscle cells and endothelium-specific TIE2 during organ development induces the proliferation of muscle cells around the endothelial vascular network. After development is completed, angiopoietin-1 is expressed at a minimally detectable level in the human lung.

Since angiopoietin-1 has been linked to the proliferation of smooth-muscle cells during blood-vessel development, we postulated the following: first, that the excessive muscularization of pulmonary arterioles seen in most forms of pulmonary hypertension could be the result of aberrant overexpression of angiopoietin-1 in the adult lung and, second, that constitutive expression of angiopoietin-1 could be linked mechanistically to signaling by bone morphogenetic protein receptors (BMPRs) and the development of pulmonary hypertension through modulation of the expression or function of this receptor. To test our hypotheses, we studied the expression and localization of angiopoietin-1, TIE2, and BMPR1A gene products in the lung in various forms of acquired and nonfamilial primary pulmonary hypertension and investigated the ability of angiopoietin-1 to modulate the levels of BMPR in human pulmonary endothelial cells. Our goal was to establish a molecular fingerprint that unites different forms of this disease.

**METHODS**

**COLLECTION OF SPECIMENS**

Between July 2000 and January 2002, lung biopsy was performed in 22 consecutive patients undergoing pulmonary thromboendarterectomy for thromboembolic pulmonary hypertension, 8 patients undergoing lung transplantation (5 with primary pulmonary hypertension and 3 with pulmonary hypertension from scleroderma), 3 patients undergoing heart–lung transplantation as a result of Eisenmenger’s syndrome, 9 patients undergoing mitral-valve replacement because of mitral regurgitation and pulmonary hypertension, and 19 patients without pulmonary hypertension who were undergoing pulmonary resection for benign causes (i.e., noncancerous pulmonary nodules). Written informed consent was obtained from each patient. Patients who were undergoing thromboendarterectomy, transplantation, or mitral-valve surgery (the group with pulmonary hypertension) had a mean pulmonary vascular resistance of 929 dyn·sec·cm⁻⁵ (range, 402 to 2040) and a mean pulmonary-artery systolic pressure of 65 mm Hg (range, 50 to 110), whereas patients undergoing pulmonary resection for benign causes had a mean pulmonary vascular resistance of 174 dyn·sec·cm⁻⁵ (range, 145 to 210) and a mean pulmonary-artery systolic pressure of 18 mm Hg (range, 11 to 25). The group with pulmonary hypertension and the control group were...
similar (P > 0.08) with respect to age (52 ± 7.4 and 54 ± 9.3 years, respectively), proportion of men (48 percent and 53 percent), arterial oxygen tension at a fraction of inspired oxygen of 100 percent (291 ± 33.7 and 278 ± 60.2 mm Hg), pulmonary-capillary wedge pressure (9.5 ± 2.6 and 8.8 ± 2.7 mm Hg), carbon monoxide diffusing capacity (62 ± 6.6 and 69 ± 7.8 percent of the predicted value), forced expiratory volume in one second (67 ± 6.0 and 65 ± 11.5 percent of the predicted value), and hematocrit (38 ± 1.9 and 39 ± 3.4 percent). The study was approved by the institutional review board of the University of California, San Francisco.

For patients with thromboembolic pulmonary hypertension, tissue was obtained from the lobe determined to be most affected by occlusive disease on preoperative angiography. Patients with mitral-valve abnormalities underwent lung biopsy, patients who received a heart–lung transplant underwent biopsy of the upper and lower lobes of both lungs, and patients who received a single lung transplant and control patients underwent a biopsy of the upper and lower lobe on the affected side. Specimens were obtained before cardiopulmonary bypass was instituted or before lung resection was performed, during ventilation at a fraction of inspired oxygen of 100 percent.

SEQUENCE OF BMPR2 AND ALK1 IN PATIENTS WITH PRIMARY PULMONARY HYPERTENSION

The complementary DNA (cDNA) of BMPR2 and ALK1 was amplified from human-lung RNA by reverse transcriptase–polymerase chain reaction (RT-PCR), subcloned, and sequenced with use of an ABI-PRISM 3100 genetic analyzer (Applied Biosystems). Sequence data were compared with those in the National Center for Biotechnology Information Entrez Nucleotide data base (accession numbers, NM_001204 for BMPR2 and Z22533 for ALK1).

PURIFICATION OF ANGIOPOIETIN-1 AND TREATMENT OF ENDOTHELIAL CELLS

An epitope tag, a C-terminal polyhistidine, was added to murine angiopoietin-1 cDNA, the cDNA was subcloned into a plasmid, and recombinant angiopoietin-1 protein was produced in BL21 bacteria. The protein was purified with use of an affinity column (Ni-NTA, Stratagene). The size and the purity of recombinant angiopoietin-1 were verified by staining with Coomassie blue and immunoblotting, and the biologic activity of the protein was confirmed by the induction of TIE2-receptor phosphorylation in vitro.13

Primary pulmonary endothelial cells were isolated from arterioles that were 500 to 1500 μm in diameter from the lung tissue of normotensive subjects and grown to 60 percent confluence. Subcultures of these cells were incubated for two hours in serum-free medium and treated with recombinant angiopoietin-1 protein (50 ng per milliliter). Aliquots of cells were serially removed for the quantitation of TIE2 phosphorylation and the expression of BMPR1A.

IMMUNOPRECIPITATION AND WESTERN BLOTTING

Western blotting was performed as previously described12 with goat polyclonal anti-TIE2, mouse monoclonal anti-BMPR2 (R&D Systems), and goat polyclonal anti–angiopoietin-1, anti–angiopoietin-2, anti-BMPR1A, and anti-actin (Santa Cruz Biotechnology). TIE2 phosphorylation was assessed by first incubating protein extracts with anti-TIE2 antibody prebound to protein G–coated agarose beads (Roche). The immunoprecipitated proteins were then separated by sodium dodecyl sulfate–polyacrylamide-gel electrophoresis and transferred to nitrocellulose. Quantitative TIE2 phosphorylation immunoblotting was performed with anti-phosphotyrosine antibody (Cell Signaling Technology). The blots were washed and probed again with goat anti-TIE2 to verify equal levels of TIE2.

RT-PCR AND NORTHERN BLOTTING

RT-PCR was performed as previously described,14 with the use of the following primers: angiopoietin-1, 5’GGCAACTGTCTGAGATGAG3’ and 5’CATTAGATTGGAGGCGGCA3’; BMPR1A, 5’GGTAAAGGCCGATATGGAGAAG3’ and 5’TAGGCAGATGCTAGATGCA3’; BMPR2, 5’ATGAGCTTATTACTGAGACAG3’ and 5’GCCGACGCTTTAAGAGAATG3’; and glyceraldehyde-3-phosphate dehydrogenase, 5’CCTGCTTCACCCATCTTGTG3’ and 5’CATCATCTCTGCCCCTCTGTG3’. For Northern blotting, 20 μg of RNA was separated on 1.2 percent formaldehyde–agarose gels and then transferred to Nytran Supercharge membranes (Schleicher and Schuell). Membranes were hybridized with the use of probes labeled with phosphorus-32. Bands were visualized by autoradiography.

HISTOLOGIC AND IMMUNOHISTOCHEMICAL ANALYSES

Six lung sections from each patient were stained with hematoxylin and eosin and examined by means of digital photomicroscopy at various magnifica-
tions to determine the severity of pulmonary hypertension. A pulmonary pathologist who was unaware of the patients’ diagnoses reviewed the photomicrograph slides and determined, for vessels measuring 100 to 1000 µm in diameter, the percentage of vessels with medial hyperplasia or hypertrophy, the number of myocytes per vessel wall, the percentage of occluded vessels, and the percentage of vessels with plexiform lesions.

Immunohistochemical analyses were performed as previously described, with the same antibodies that were used in the immunoblotting experiments and with biotinylated anti-goat IgG as a secondary antibody (Vector). Each specimen had two negative controls: one section without primary antibody and one section with nonspecific goat IgG.

STATISTICAL ANALYSIS
The preoperative characteristics of patients with pulmonary hypertension and control patients were compared with use of Student’s independent-group t-test for normally distributed variables and the Wilcoxon rank-sum test for measures that were not normally distributed. The normality of distribution was evaluated with use of the Shapiro–Wilks test. Sex was compared with use of Pearson’s chi-square test. A P value of less than 0.05 was considered to indicate statistical significance, and all tests were two-sided. The relation between pulmonary vascular resistance and the level of TIE2 phosphorylation was assessed with linear-regression analysis. Copyright © 2003 Massachusetts Medical Society. All rights reserved.

**Figure 1. Angiopoietin-1 Expression in the Lung as a Molecular Marker for the Severity of Disease in Different Forms of Pulmonary Hypertension, as Assessed by Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR) and Western Blotting (Panel A), Northern and Western Blotting (Panel B), and Linear-Regression Analysis (Panel C).**

Panel A shows the results of RT-PCR and Western blotting analysis of the expression of angiopoietin-1 (Ang-1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and actin in lung samples from patients with pulmonary hypertension due to thromboembolic disease (lane 1), Eisenmenger’s syndrome (lane 2), idiopathic causes (i.e., primary pulmonary hypertension) (lane 3), scleroderma (lane 4), or mitral-valve regurgitation (lane 5) and two normotensive control patients (Controls 1 and 2 in lanes 6 and 7, respectively). Panel B shows the results of Northern blot analysis of angiopoietin-1 and actin messenger RNA, performed with human angiopoietin-1 and actin complementary DNA probes, and of Western blot analysis of angiopoietin-1 and actin proteins with the use of anti–angiopoietin-1 and anti-actin antibodies on lung tissue from patients and controls with various degrees of pulmonary vascular resistance. Control patients had pulmonary vascular resistance in the normal range of less than 220 dyn·sec·cm⁻⁵. Panel C shows the results of linear regression analysis of the correlation between the level of angiopoietin-1 protein in the lung and the degree of preoperative pulmonary vascular resistance in all 61 patients. For each sample, the densities of the angiopoietin-1 bands on Western blotting are normalized to the density of the corresponding actin band.
tion and angiopoietin-1 protein was characterized with the use of linear regression.

RESULTS

ANGIOPOIETIN-1 EXPRESSION IN DIFFERENT FORMS OF PULMONARY HYPERTENSION

The expression of angiopoietin-1 in lung tissue was measured by RT-PCR and immunoblot analysis (Fig. 1A). Before molecular analysis was performed, lung sections from each patient were examined under a light microscope to quantitate the pulmonary hypertensive changes according to the Heath–Edwards classification15 (Table 1). Specimens from patients with pulmonary hypertension demonstrated Heath–Edwards stage 2, 3, 4, or 5 disease, with diffuse medial arteriolar hypertrophy or hyperplasia and stenosis of at least one-third of all arterioles examined. There was a direct correlation between preoperative pulmonary arterial systolic pressure and pulmonary vascular resistance and the pathological grading of affected specimens.

Patients in the pulmonary hypertension group had higher steady-state levels of angiopoietin-1 mRNA and protein in their biopsy specimens (Fig. 1A) than did control patients. Angiopoietin-1 transcripts were not detected and angiopoietin-1 protein was barely detectable in biopsy specimens from control patients.

Correlation of angiopoietin-1 expression and tie2 phosphorylation with disease severity

Patients with pulmonary hypertension had greater amounts of steady-state angiopoietin-1 mRNA and protein in the lung than did control patients (Fig. 1B). The level of angiopoietin-1 correlated directly with the severity of pulmonary hypertension as measured by the preoperative pulmonary vascular resistance and pulmonary arterial systolic pressure (Fig. 1C). We found this correlation between gene expression and phenotype (elevated pulmonary vascular resistance) in all samples from the patients, irrespective of the cause of the disease. Angiopoietin-1 was not detected by immunoblotting in the serum or wall of the main pulmonary artery of patients with pulmonary hypertension, suggesting that this protein and its effect are confined to the lung.

We also examined the expression of angiopoietin-2, a competitive inhibitor of angiopoietin-1 in vasculogenesis,16 in lung tissue from patients with pulmonary hypertension and in lung tissue from those without it. In contrast to angiopoietin-1, the steady-state levels of angiopoietin-2 mRNA or protein did not differ significantly between lung tissue from normotensive patients and lung tissue from patients with pulmonary hypertension (data not shown).

Since angiopoietin-1 has been shown in other angiogenesis systems to bind and induce tyrosine phosphorylation of TIE2,17 we examined whether activation of TIE2 in the lung was a marker for the severity of pulmonary hypertension. Steady-state levels of TIE2 protein were similar in lung-biopsy specimens from patients with pulmonary hypertension and control patients (Fig. 2A). However, using quantitative phosphotyrosine immunoblotting, we found that the degree of TIE2 phosphorylation was directly proportional to the clinical severity of pulmonary hypertension (as measured by pulmonary arterial systolic pressure and pulmonary vascular resistance) in the group with pulmonary hypertension (Fig. 2B and 2C). In normotensive patients, minimal levels of phosphorylated TIE2 protein were detected.

BMPR2 AND ALK1 GENES IN PATIENTS WITH PRIMARY PULMONARY HYPERTENSION

We sequenced the BMPR2 and ALK1 genes in the five patients with primary pulmonary hypertension in this study to confirm the absence of familial disease. None of the patients had deletion, missense, or non-

Table 1. Semiquantitative Morphometric Analysis of Pulmonary Vascular Lesions.6

<table>
<thead>
<tr>
<th>Vessel Morphology</th>
<th>Control Subjects</th>
<th>Patients with Pulmonary Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PVR, 50–210 dyn·sec·cm⁻⁵ (N=19)</td>
<td>PVR, 400–1000 dyn·sec·cm⁻⁵ (N=22)</td>
</tr>
<tr>
<td>Vessels with medial hyperplasia (%)</td>
<td>1±1.4</td>
<td>53±18.7</td>
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<tr>
<td>Mean no. of myocytes/vessel wall</td>
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<td>Vessels occluded (%)</td>
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<td>22±12.7</td>
</tr>
<tr>
<td>Plexiform lesions (%)</td>
<td>0</td>
<td>6±4.2</td>
</tr>
<tr>
<td>Median Heath–Edwards stage†</td>
<td>0</td>
<td>3</td>
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* Plus–minus values are means ±SD. PVR denotes pulmonary vascular resistance.† The Heath–Edwards classification has six stages; stage 1 indicates normal pulmonary vascular morphology, and stage 6 end-stage pulmonary hypertension.
sense mutations in the BMPR2 or ALK1 genes. None had a polymorphism in the BMPR2 gene at position 354 in exon 3, as has previously been reported in such patients. These results illustrate that not all cases of primary pulmonary hypertension are defined by mutations in these two genes.

**DOWN-REGULATION OF BMPR1A IN THE LUNGS IN VARIOUS FORMS OF PULMONARY HYPERTENSION**

Using RT-PCR and immunoblot analysis, we found that BMPR1A and BMPR2 mRNA and protein were expressed in lung samples from normotensive patients. In contrast, despite the high sensitivity of our assays, almost no BMPR1A mRNA or protein was detected in lung tissue from patients with primary pulmonary hypertension and patients with four different acquired forms of the disease (Fig. 3). These results suggest that, although familial forms of pulmonary hypertension are defined by mutations in the BMPR2 gene, nonfamilial forms of pulmonary hypertension are characterized by severely diminished or absent steady-state levels of the BMPR2 coreceptor, BMPR1A, in the lung.

**DOWN-REGULATION OF BMPR1A IN PULMONARY ARTERIOLAR ENDOTHELIAL CELLS INCUBATED WITH ANGIOPOIETIN-1**

Our findings suggested a link between constitutive angiopoietin-1 levels in the lung, the transcriptional down-regulation of BMPR1A (either by decreased transcription or enhanced degradation) in pulmonary endothelium, and the genesis of pulmonary hypertension. On the basis of these results, we hypothesized that angiopoietin-1 signaling in pulmonary arteriolar endothelial cells down-regulates BMPR1A transcription. To test this hypothesis, we examined whether angiopoietin-1 could directly modulate the expression of BMPR1A. Purified recombinant angiopoietin-1 protein was added to human primary pulmonary endothelial cells in culture. On Northern blotting, subcultured human primary pulmonary endothelial cells exposed to recombinant angiopoietin-1 had undetectable steady-state levels of BMPR1A mRNA, whereas untreated endothelial cells strongly expressed BMPR1A (Fig. 4B). We found that in subcultured human primary pulmonary endothelial cells that were exposed to recombinant angiopoietin-1, levels of TIE2 tyrosine phosphorylation increased (Fig. 4A) and were followed by a rapid diminution in the steady-state levels of BMPR1A mRNA (Fig. 4C). These results suggest that, in vascular endothelial cells, angiopoietin-1–ligand binding results in both TIE2 phosphorylation and subsequent down-regulation of the BMPR1A gene product. The diminished BMPR1A
mRNA levels persisted for 36 hours in human primary pulmonary endothelial cells after exposure to recombinant angiopoietin-1.

**CELLULAR LOCALIZATION OF SIGNALING MOLECULES IN LUNG TISSUE**

Staining of sectioned specimens from patients with pulmonary hypertension with anti–angiopoietin-1 antibody revealed that angiopoietin-1 was confined to the cytoplasm of smooth-muscle cells in the media of pulmonary arterioles and small arteries measuring less than 800 µm in diameter (Fig. 5A). Angiopoietin-1 was not detected in the lungs of control patients with normal pulmonary vascular resistance (Fig. 5B). Angiopoietin-2 immunoreactivity was detected in the cytoplasm of vascular smooth-muscle cells in large and small vessels in the lung and was present in samples from patients with clinical pulmonary hypertension and from those without it (Fig. 5C and 5D).

TIE2 protein was limited to the cytoplasm and the surface of endothelial cells lining pulmonary vessels of all sizes from patients with pulmonary hypertension and normotensive patients (Fig. 5E and 5F), a finding consistent with previous reports that TIE2 is restricted to the endothelial-cell lineage. Angiopoietin-1 protein and the degree of phosphorylation of its receptor TIE2 are sensitive molecular markers of the severity of pulmonary hypertension in patients with nonfamilial forms of the disease. These results establish a link between the expression of angiopoietin-1, the
phosphorylation of TIE2, and the magnitude of pulmonary vascular resistance. Finally, we found that angiopoietin-1 down-regulates steady-state levels of BMPR1A mRNA and protein in subcultured human pulmonary arteriolar endothelial cells. These results unite nonfamilial primary pulmonary hypertension and multiple forms of secondary pulmonary hypertension by demonstrating that they have a similar pattern of aberrant gene expression and suggest that pulmonary hypertension may occur through a molecular cascade whereby angiopoietin-1 ultimately down-regulates steady-state levels of BMPR1A.

Our results link two seemingly unrelated observations in pulmonary hypertension research. First, experiments in our laboratory have shown that targeted overexpression of angiopoietin-1 in the lungs of rodents induces clinical and pathological pul-

Figure 4. Increased Phosphorylation of TIE2-Receptors and Down-Regulation of Bone Morphogenetic Protein Receptor Type 1A (BMPR1A) Protein in Pulmonary Endothelial Cells Treated with Angiopoietin-1 (Ang-1), as Assessed by Immunoprecipitation and Immunoblotting (Panel A), Northern Blotting (Panel B), and Serial Time-Point Analysis (Panel C).

In Panel A, immunoblot analysis shows that treatment of human pulmonary arteriolar endothelial cells with angiopoietin-1 results in the induction of TIE2-receptor phosphorylation but does not cause the modulation of TIE2 protein levels. In Panel B, Northern blot analysis of BMPR1A and actin in human pulmonary endothelial cells treated with angiopoietin-1 shows down-regulation of steady-state levels of BMPR1A messenger RNA (mRNA) by angiopoietin-1. Panel C shows the effect of exposure to angiopoietin-1 on levels of BMPR1A mRNA and TIE2-receptor tyrosine phosphorylation in human pulmonary arteriolar endothelial cells over time. BMPR1A mRNA levels were normalized to actin mRNA levels and are presented as the mean of four independent experiments at each time point. TIE2 phosphorylation is presented as the mean of four independent measurements at each time point.

Figure 5. Immunohistochemical Analysis of Representative Lung Samples from Patients with Pulmonary Hypertension (Panels A, C, E, and G) and Normotensive Control Patients (Panels B, D, F, and H) (×200).

Angiopoietin-1 (Ang-1) is present in vascular smooth-muscle cells in arterioles less than 800 µm in diameter in lung tissue from a patient with pulmonary hypertension (Panel A) but not lung tissue from a control patient (Panel B). Angiopoietin-2 (Ang-2) is present in vascular smooth-muscle cells in vessels of all sizes in lung tissue from a patient with pulmonary hypertension (Panel C) and a normotensive patient (Panel D). TIE2 is present in endothelial cells lining vessels of all sizes in a patient with pulmonary hypertension (Panel E) and a control patient (Panel F). Bone morphogenetic protein receptor type 1A (BMPR1A) is absent in endothelial cells in vessels less than 800 µm in diameter in lung tissue from a patient with pulmonary hypertension (Panel G) and present in lung tissue from a normotensive patient (Panel H). The scale bar represents 100 µm.
monary hypertension, specifically the hyperplasia of vascular smooth-muscle cells that is characteristic of the disease. This experiment confirmed that the expression of angiopoietin-1 in the adult lung is causal of pulmonary hypertension rather than secondary to it and parallels results in other laboratories that suggest that angiopoietin-1 induces muscularization of nascent arteries in utero. Second, familial pulmonary hypertension, a disease pathologically identical to many forms of secondary pulmonary hypertension, is characterized by haploid mutations in the BMPR2 gene, resulting in dose-dependent modulation of BMPR2 oligomerization with BMPR1A. Bone morphogenetic protein ligands exert their effects through the activation and heterodimerization of BMPR1 and BMPR2 on the cell surface, leading to Smad intracellular signaling. The effect of the activation of BMPR1A and BMPR2 depends on the type of cell and can result in either promotion or inhibition of transcription. Although BMPR2 mutations have been seen in patients with sporadic primary pulmonary hypertension, they have not been identified in most forms of the disease. Since we found that BMPR1A mRNA levels are controlled by angiopoietin-1 in pulmonary endothelium and that steady-state levels of BMPR1A are markedly diminished in the lung tissue of patients with pulmonary hypertension, we suspect that nonfamilial forms of pulmonary hypertension occur through an angiopoietin-1-BMPR1A pathway. Thus, dose-dependent inactivation of the BMPR complex either by a mutation (in BMPR2 in familial disease) or by regulation of steady-state levels of transcripts (BMPR1A, as we have shown here) is a hallmark of this disease.

In summary, we identified a partial molecular pathway for the generation of nonfamilial forms of pulmonary hypertension, representing a specific pattern of gene expression common to many forms of this disease. Further studies of the way in which angiopoietin-1–induced changes in the stoichiometry between BMPR1A and BMPR2 in vascular endothelium result in signals that stimulate the proliferation of vascular smooth-muscle cells should shed light on the fundamental mechanism of the patterns of endothelial and smooth-muscle cell growth in an organ in which these vessels are critical for gas exchange and survival.

Supported by the Charles B. Wang Foundation and by grants (1R01 HL70852-01, to Dr. Thistlethwaite, and M01 RR00827, to Dr. Deutsch) from the National Institutes of Health.

We are indebted to Feliz Garcia Bannach, Ph.D., Gordon Yung, M.D., and Huntington Potter, Ph.D., for helpful discussions regarding the manuscript.

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