Tenascin-C is induced by mutated BMP type II receptors in familial forms of pulmonary arterial hypertension

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Ihida-Stansbury, Kaori, David M. McKeain, Kirk B. Lane, James E. Loyd, Lisa A. Wheeler, Nicholas W. Morrell, and Peter Lloyd Jones. Tenascin-C is induced by mutated BMP type II receptors in familial forms of pulmonary arterial hypertension. Am J Physiol Lung Cell Mol Physiol 291: L694–L702, 2006. First published June 16, 2006; doi:10.1152/ajplung.00119.2006.—Familial forms of pulmonary arterial hypertension (PAH) have been linked to mutations in bone morphogenetic protein (BMP) type II receptors (BMPR2s), yet the downstream targets of these receptors remain obscure. Here we show that pulmonary vascular lesions from patients harboring BMPR2 mutations express high levels of tenascin-C (TN-C), an extracellular matrix glycoprotein that promotes pulmonary artery (PA) smooth muscle cell (SMC) proliferation. To begin to define how TN-C is regulated, PA SMCs were cultured from normal subjects and from those with PAH due to BMPR2 mutations. PAH SMCs expressed higher levels of TN-C than normal SMCs. Similarly, expression of Prx1, a factor that drives TN-C transcription, was elevated in PAH vascular lesions and SMCs derived thereof. Furthermore, Prx1 and TN-C promoter activities were significantly higher in PAH vs. normal SMCs. To delineate how BMPR2s control TN-C, we focused on receptor (R)-Smads, downstream effectors of wild-type BMPR2s. Nuclear localization and phosphorylation of R-Smads was greater in normal vs. PAH SMCs. As well, indirect blockade of R-Smad signaling with a kinase-deficient BMP receptor Ib upregulated TN-C in normal SMCs. Because ERK1/2 MAPKs inhibit the transcriptional activity of R-Smads, and because ERK1/2 promotes TN-C transcription, we determined whether ERK1/2 inhibits R-Smad signaling in PAH SMCs and whether this activity is required for TN-C transcription. Indeed, ERK1/2 activity was greater in PAH SMCs, and inhibition of ERK1/2 resulted in nuclear localization of R-Smads and inhibition of TN-C. These studies define a novel signaling network relevant to PAH underscored by BMPR2 mutations.

pulmonary hypertension; bone morphogenetic protein receptors; extracellular matrix

THE NORMAL ADULT PULMONARY circulation is a low-resistance, low-pressure, high-flow system, and the pulmonary arteries (PAs) are correspondingly thin walled and highly distensible. In contrast, pulmonary arterial hypertension (PAH) is characterized by sustained elevation of PA pressure, and this may be accompanied by pulmonary vascular wall remodeling and reduced vascular distensibility (14). Whether idiopathic (primary), familial, or secondary to some other disease, severe PAH is characterized in its more advanced stages by adventitial and medial hypertrophy, neo-intimal hyperplasia and fibrosis, and development of complex neo-intimal lesions that constrict and obliterate the lumen of medium and small PAs. The resultant increases in pulmonary vascular resistance and PA stiffness cause pressure and volume overloading of the right ventricle, which can lead to right heart failure and death.

In terms of etiology, PAH has been attributed to a variety of factors, including congenital heart defects, collagen vascular disease, portal hypertension, human immunodeficiency virus-1 infection, and the use of certain anorexic drugs. Recently, it was discovered that heterozygous germ line mutations in the bone morphogenetic protein (BMP) type II receptor (BMPR2) gene are associated with ~50% of familial and ~20% of idiopathic PAH cases (7, 26, 32, 37). As well, a subset of patients with secondary forms of PAH, associated with anorexic drug use or congenital heart defects, also carry BMPR2 mutations (1, 13, 36). Therefore, understanding the functions of wild-type and mutated BMPR2s may provide new clues concerning the pathobiology and treatment of multiple forms of PAH.

BMPs represent developmentally regulated ligands for BMP receptors, and they are TGF-β-related polypeptides that exert diverse cellular effects, depending on cell type, tissue context, and differentiation state (11, 42). Briefly, BMPs (including BMP-2, -4, -6, and -7) stimulate heterodimerization and activation of two types of BMP receptors (i.e., I and II), and initiate phosphorylation of the receptor (R)-Smad proteins (1, 5, and 8), which combine with the common mediator Smad-4. On translocation to the nucleus, this Smad complex binds target genes to activate or repress their transcription. BMPs are critical for development, because homozygous BMPR2-knockout mice die early in development because of gastulation defects (2) and ligand domain-deficient BMPR2-null mice undergo normal gastrulation but die at midgestation with cardiovascular and skeletal defects (6).

With respect to gene structure and function, exons 1–3 of wild-type BMPR2s encode an extracellular ligand-binding domain, exon 4, a transmembrane domain, exons 5–11, a serine/threonine kinase domain, and exons 11 and 13, a long intracellular, cytoplasmic tail domain. In PAH, a variety of heterozygous, inactivating missense, nonsense, deletion, and insertion mutations have been identified in all 13 coding exons.
of BMPR2 (41). Therefore, it is anticipated that either mutations in these exons would fail to generate a receptor or they generate a modified BMPR2 receptor that is deficient in ligand binding and/or dimerization (due to mutations in the extracellular domain), in kinase activity (due to mutations in cytoplasmic domains), or in its ability to bind downstream interacting effectors (such as LIMK-1, Tctex-1, MAPKK6, Forkhead box L1, and c-src) that may normally interact with the cytoplasmic tail (9, 12, 28, 43). Although not fully proven, a consensus view is that the resultant haploinsufficiency predisposes individuals to PAH, yet some mutants may have some dominant-negative effect. Regardless of their mode of action, BMPR2 mutations would be expected to subvert the normal ability of wild-type BMPR2s to promote endothelial cell (EC) regeneration and survival on the one hand while suppressing smooth muscle cell (SMC) proliferation on the other. Despite this understanding, the identity of gene targets that are activated or repressed by mutated BMPR2 in PAH remains to be discovered.

Tenascin-C (TN-C) is an extracellular matrix (ECM) glycoprotein induced in a number of experimental and clinical forms of PAH, where it promotes PA SMC proliferation (4, 5, 16, 19, 20, 24, 30). For example, adult rats treated with the alkaidox toxin monocrotaline develop a fatal and irreversible form of PAH, characterized in part by the appearance of TN-C in the pulmonary vascular wall (16, 24). Similarly, neonatal swine with PAH secondary to congenital heart defects also express high levels of TN-C within pulmonary vascular lesions (20). Functionally, higher-molecular-mass isoforms of TN-C generated by alternative splicing promote proliferation and survival of PA SMCs via their ability to cross-modulate the activity of tyrosine kinases, including EGF and FGF-2 receptors (21). The importance of these discoveries was further substantiated in studies showing that antisense ablation of TN-C expression ameliorates the severity of monocrotaline-induced pulmonary vascular lesions ex vivo (5). Similarly, inhibition of EGF receptor activity in monocrotaline-treated rats promotes PA SMC apoptosis and improves animal survival (30). The realization that TN-C directly contributes to the development and progression of PAH by promoting PA SMC proliferation and survival prompted us to identify factors that regulate TN-C. In this regard, both activation of ERK1/2 MAPKs and binding of the -paired-related homeobox gene transcription factor Prx1 to the TN-C gene promoter represent critical events in the transcriptional induction of TN-C in rodent ECs, vascular SMCs, and fibroblasts (15, 18, 29, 33), cell types that contribute to remodeling and/or occlusion of pulmonary vessels in PAH. Given the apparent prevalence and mitogenic function of TN-C in the pathobiology of PAH, we investigated the interrelationship between ERK1/2 MAPKs, R-Smads, Prx1, and TN-C in familial forms of PAH (FPAH) arising from mutations in BMPR2s.

**MATERIALS AND METHODS**

**Lung tissue preparation.** Lung sections from FPAH subjects were processed at Vanderbilt University in accordance with institutional and National Institutes of Health guidelines. Adult normal lung sections were obtained from ProSci. All protocols were reviewed and approved by the Vanderbilt University Institutional Review Board.

**Cell culture.** Normal medial human PA SMCs were from Cambrex. For FPAH SMCs, main PAs were dissected and the endothelial and adventitial surfaces were removed. Medial layer tissue pieces were then cultured to allow outgrowth of SMCs. Cells were maintained in SmBM-2 BulletKit medium (Cambrex). To examine how BMP2 ligand activation affects phosphorylation of Smad-1, -5, and -8 (i.e., R-Smads), normal and FPAH PA SMCs cells were incubated with SmBM-2 medium supplemented with 5% FBS, which contains BMPs.

**Immunohistochemistry and cytochemistry.** Cells were fixed in 3% paraformaldehyde in PBS. Prx1 and TN-C were detected on paraffin sections as described previously (15). To examine the expression and localization of phosphorylated R-Smads, TN-C, and Prx1, SMCs were incubated with primary antibodies for P-Smad-1, -5, and -8, TN-C, or Prx1. Mutated BMP receptor Ib (BMPRIB)-hemagglutinin (HA)-expressing SMCs were detected by using anti-HA antibody. Primary antibody application was followed by incubation with fluorophore-conjugated, species-specific secondary antibodies. Slides were mounted in Vectashield containing 4,6-diamidino-2-phenylindole.

**Semiquantitative RT-PCR.** Total RNA was extracted from cultured SMCs. The following primer pairs were used for RT-PCR: Prx1, 5'-TCCCCTTCTCAAATCCTAC-3' and 5'-ACTATATTCTTGGCGCTTCTC-3'; TN-C, 5'-CGGACAAAAACCCTAGTCG-3' and 5'-AAGCCGAGACCTAACCTCATAGC-3'. 18S rRNA was used as an internal control (Ambion). Products were separated on Tris-borate-EDTA agarose gels containing Vista Green; 28, 30, and 32 PCR cycles were used for each cDNA.

**Western immunoblotting.** Cells were lysed in radioimmunoprecipitation assay buffer. Equal amounts of protein were separated on SDS-polyacrylamide gels before being transferred to polyvinylidene difluoride membranes, which were blocked and then incubated overnight at 4°C with primary antibodies. Thereafter, membranes were incubated with peroxidase-conjugated secondary antibodies. Antigen detection was via enhanced chemiluminescence. -Actin and -β-actin were used as loading controls. In some instances, results were quantified by scanning densitometry.

**Cloning the Prx1 gene promoter.** An EcoR1/Taq1 4.5-kb fragment containing 900 bp of the 5' untranslated region, and 3.7 kb of the putative Prx1 mouse promoter, was subcloned into pBluescript from a 170-kb genomic DNA bacterial artificial chromosome (BAC) clone. Ultimately, exonuclease III-based deletion mutagenesis was used to generate a ~1.3-kb plasmid designated pPrx1promB5. This promoter construct was chosen because it contains putative BMP response elements.

**Transient transfection assays.** SMCs were transfected with pPrx1promB5, TN7-luciferase plasmid and pCMV-β-galactosidase, and pCMV-myc or pPrx1-myc as described previously (15). Extracts were prepared in lysis buffer. After normalizing for β-galactosidase activity, luciferase assays were performed. R-Smad activation was inhibited by overexpressing a kinase domain-mutated BMPRIB cDNA tagged with HA. Normal PA SMCs were transiently transfected with the Amaxa nucleoporation system or Lipofectamine. Mutated BMPRIB-HA expression was detected by immunocytochemistry using anti-HA antibody.

**ERK1/2 MAPK inhibition.** SMCs were incubated with DMSO with or without 50 μM PD-98059 for 1 h before being harvested or fixed. Cells were processed for detection of activated R-Smads by immunocytochemistry, whereas TN-C gene promoter activity was assessed with a luciferase assay.

**Statistics.** All experiments were performed at least in triplicate. Statistical assessments were made with an unpaired Student's t-test and PRISM software. P < 0.05 was considered statistically significant. Values are represented as means ± SE.

**RESULTS**

Prx1 and TN-C are expressed in FPAH. We used an immunohistochemical approach to assess expression of Prx1 and
TN-C in lung tissue sections derived from normal subjects and from 18 subjects with FPAH, 50% of whom have been confirmed to harbor BMPR2 mutations (Table 1). To reiterate, Prx1 and TN-C were chosen because Prx1 induces TN-C in rodent ECs, vascular SMCs, and fibroblasts and both molecules are upregulated in experimental forms of PAH, where they control SMC proliferation and/or survival (5, 18, 20, 21, 24). As reported (5, 20), PAs within normal lung tissue did not express Prx1 or TN-C (data not shown). In contrast, all 18 subjects with FPAH, including those with BMPR2 mutations, Table 1.

### Table 1. Summary of clinical and genetic data of 18 patients with familial forms of PAH

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<th>Patient</th>
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PAH, pulmonary arterial hypertension. Results from family 14 and family 28 patients are in bold.

Fig. 1. Colocalization of Prx1 and tenascin-C (TN-C) in occlusive lesions of subjects with familial pulmonary arterial hypertension (FPAH). A: representative photomicrographs showing expression of Prx1 (black nuclear stain, left) and TN-C (brown extracellular stain, right) in serial sections derived from lungs of 2 individuals from different families [family 14 (F14) and family 28 (F28)] bearing bone morphogenetic protein (BMP) type II receptor (BMPR2) mutations in exon 3 or 8 (see Table 1). Methyl green and hematoxylin were used as counterstains for Prx1 and TN-C, respectively. Scale bar = 50 μm. Tissue outlined with dashed box is represented at higher magnification in B.
expressed high levels of Prx1 and TN-C protein (Fig. 1A). Specifically, nuclear Prx1 and extracellular TN-C were observed both within occlusive vascular lesions and within the walls of remodeled PAs, including the tunica media (Fig. 1). Also of note, Prx1-expressing SMCs were often surrounded by a TN-C-rich ECM (Fig. 1B), reinforcing the idea that Prx1 controls TN-C expression (15, 18, 29). Thus Prx1 and TN-C are upregulated in remodeled PAs derived from subjects with FPAH, including those caused by BMPR2 mutations. Furthermore, Prx1 and TN-C were induced in individuals bearing different BMPR2 mutations. For example, family 14 (F14) members carry an exon 3 missense mutation in the BMP ligand-binding site of the BMPR2, whereas family 28 (F28) members possess an exon 8 nonsense mutation in the BMPR2 kinase domain (additional details of these mutations can be found in Table 1), yet both expressed high levels of Prx1 and TN-C (Fig. 1).

Prx1 and TN-C are expressed in isolated PA SMCs harboring BMPR2 mutations. To begin to define the BMPR2-related pathway(s) leading to induction of Prx1 and TN-C, we compared expression of these molecules in human PA SMCs derived from normal individuals and from F14 and F28 subjects who possess heterozygous germ line mutations in BMPR2s (Fig. 1 and Table 1). It should be noted that although SMCs derived from patients with nonfamilial forms of PAH might represent a preferential normal control, such cells were not available.

Semi-quantitative RT-PCR revealed that normal, F14, and F28 PA SMCs express two isoforms of Prx1, designated Prx1a and Prx1b, and a trend toward greater expression of Prx1a was noted in F14 and F28 SMCs vs. normal SMCs (Fig. 2, A and B). This is of interest because Prx1a stimulates TN-C transcription (33). More strikingly, immunofluorescence microscopy showed that Prx1 protein levels were greater in F14 and F28 PA SMC cytoplasm and nuclei (Fig. 2D; nuclear localization is denoted by white arrows) compared with normal PA SMCs (Fig. 2D). Similarly, semiquantitative RT-PCR, Western immunoblotting, and immunofluorescence studies showed that TN-C mRNA and protein expression levels were greater in F14 and F28 SMCs than normal SMCs, albeit at different levels (Fig. 2). Interestingly, F14 and F28 SMCs expressed a higher-molecular-mass (220 kDa) isoform of TN-C compared with
normal SMCs (Fig. 2C). This isoform has been shown to play a more active role in promoting cellular migration and proliferation compared with lower-molecular-mass TN-C isoforms (17, 22). Together, these results show that PA SMCs bearing BMPR2 mutations appear to maintain their ability to express higher levels of Prx1 and TN-C in culture compared with normal PA SMCs.

Next, we determined whether BMPR2s regulate Prx1 and TN-C expression at the transcriptional level and whether Prx1 (i.e., Prx1a) is able to trans-activate the TN-C gene promoter in normal PA SMCs. To accomplish this, 1.3 kb of the murine Prx1 gene promoter was isolated from a BAC clone and was placed upstream from a luciferase reporter gene in pGL3-Basic. This construct, designated pPrx1promB5, was chosen because it contains putative BMP signaling responsive elements and it is highly homologous to the human Prx1 promoter. For these and subsequent studies we only used F28 cells, because both F14 and F28 SMCs share common responses vis-à-vis Prx1 and TN-C production in culture compared with normal PA SMCs (Fig. 2).

Transient cotransfection of pPrx1promB5 and a β-galactosidase expression vector showed that the basal level of Prx1 gene promoter activity was significantly greater in F28 SMCs than normal SMCs (Fig. 3A). As well, cellular transfection assays involving a truncated TN-C gene promoter-luciferase reporter construct (which contains a Prx1-binding site) (29) and a β-galactosidase expression vector revealed that the activity of the TN-C promoter was also significantly greater in F28 SMCs (Fig. 3B). Also, because Prx1 is induced and coexpressed in FPAH SMCs, both in vivo and in tissue culture, we evaluated whether Prx1 is able to regulate TN-C gene transcription, as already demonstrated in rodent cells (29). Accordingly, normal PA SMCs were cotransfected with either pCMV-myc or pPrx1-myc expression vectors, together with a TN-C gene promoter-luciferase reporter construct and a β-galactosidase expression vector. Overexpression of Prx1 significantly increased TN-C gene promoter activity (Fig. 3C).

Suppression of BMP receptor signaling in normal PA SMCs induces TN-C. Having shown that Prx1 is able to trans-activate a truncated version of the TN-C gene promoter that contains a Prx1 binding site and that Prx1 and TN-C gene promoters are upregulated in FPAH SMCs with BMPR2 mutations, we focused our attention on the signal transduction pathway(s) regulating TN-C. Phosphorylation of R-Smads (i.e., Smad-1, -5, -8) occurs after stimulation of wild-type BMPR2s in normal SMCs, but this event fails to occur in SMCs bearing BMPR2 mutations (44). Because F28 SMCs harbor a kinase domain mutation in one copy of the BMPR2 allele, we used these cells to pursue the role of R-Smads in regulation of TN-C. Consistent with these findings, Western immunoblotting of F28 whole cell lysates showed that phosphorylation of R-Smads is significantly greater in normal vs. F28 SMCs under basal conditions in serum-containing media (Fig. 4, A and B). In contrast, total levels of Smad-1 were unchanged between normal and F28 SMCs (Fig. 4A). In this instance, α-actin was used as a loading control, and its presence also demonstrates that both normal and F28 cells express a vascular smooth muscle marker (Fig. 4A). Next, we immunostained SMCs for activated R-Smads and showed that nuclear accumulation of these proteins was greater in normal SMCs (Fig. 4C). Consistent with our Western immunoblot data (Fig. 4, A and B), immunocytochemistry studies showed that some R-Smad activity was apparent in F28 cells (Fig. 4C), which might be expected because these cells are derived from subjects that still express one copy of the wild-type BMPR2 allele. Next, to begin to determine whether BMP receptors play a causal role in TN-C regulation, we indirectly blocked R-Smad activity with a kinase domain-deficient BMPR Ib expression vector that was epitope tagged with HA. This receptor normally het-
erodimerizes with the BMPR2 receptor, and the kinase domain of BMPRIb is required for R-Smad activation. Thus we hypothesized and then showed that overexpression of mutated BMPRIb-HA in normal PA SMCs blocks phosphorylation and nuclear localization of R-Smads in normal SMCs (Fig. 4D, top) and that normal SMCs expressing mutated BMPRIb-HA produced higher levels of TN-C compared with their nontransfected counterparts (Fig. 4D, bottom). Thus inhibition of BMP receptor signaling in normal PA SMCs results in the induction of TN-C.

**ERK1/2 MAPKS antagonize BMPR signaling and induce TN-C.** Although activated R-Smads were detectable in F28 SMCs, the pattern of localization was abnormal (Fig. 4C). Specifically, activated R-Smads were mostly retained in the cytoplasm in F28 SMCs (Fig. 4C). In contrast, the majority of R-Smad activity in normal PA SMCs was found in the nucleus (Fig. 4C). This indicates that although F28 SMCs do contain some activated R-Smads (which is to be expected because they still carry 1 copy of the wild-type BMPR2 allele), one or more effectors that are present in F28 SMCs may act to limit their entry into the nucleus. Because activated ERK1/2 MAPK and some of its upstream effectors are known to phosphorylate and then antagonize R-Smad-dependent gene transcription during mammalian and amphibian development (25, 35, 38), and because ERK1/2 MAPK activity is required for induction of the TN-C gene promoter in rodent vascular SMCs (23), we hypothesized that 1) activated ERK1/2 would be greater in F28 SMCs, 2) activated ERK1/2 would limit nuclear localization of activated R-Smads, and 3) ERK1/2 inhibition would repress TN-C expression in F28 SMCs.

Western immunoblotting demonstrated that ERK1/2 activity was significantly greater in F28 vs. normal PA SMCs (Fig. 5, A and B). Total ERK1/2 MAPK levels remained unchanged (Fig. 5, A and B). Furthermore, blocking ERK1/2 activity with a MEK1 inhibitor, PD-98059, increased the appearance of activated R-Smads in F28 cell nuclei to levels similar to those observed in normal SMCs (Fig. 5C). Also consistent with our

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**Fig. 4. Inhibition of BMP receptor signaling-induced TN-C in normal PA SMCs.** A: Western immunoblotting for phosphorylated Smad-1, -5, and -8 [receptor (R)-Smad-P; top] and total Smad-1 (middle) using whole cell lysates derived from normal and F28 SMCs. α-Actin expression was used as a loading control (bottom). B: quantification of R-Smad-P protein levels relative to total Smad-1. C: representative photomicrographs showing localization of activated R-Smads (R-Smad-P) in normal and F28 SMCs. Scale bar = 10 μm. D: representative photomicrographs showing normal SMCs transiently transfected with a kinase domain-deficient BMPRIb-hemagglutinin (HA)-tagged vector (mBMPRIb-HA) after double immunocytochemical staining of HA (red) and activated R-Smads (green, R-Smad-P) (top) or HA (red) and TN-C (green) (bottom). Coexpression of HA and TN-C yields orange/yellow. Nuclei were detected with DAPI. Scale bar = 10 μm.
hypothesis, ERK1/2 blockade resulted in a significant suppression of TN-C gene promoter activity (Fig. 5D).

**DISCUSSION**

Heterozygous germ line mutations in BMPR2s predispose certain individuals to developing PAH (32, 37), yet the identity of gene networks that are activated or repressed in response to these mutations has remained elusive. In this study, we showed for the first time that Prx1 and TN-C are induced in response to mutated BMPR2s in hypertensive patients in vivo and that induction of TN-C may also rely on loss or reduction of BMP receptor signaling in cultured PA SMCs.

It is generally accepted that [depending on their location within the pulmonary vascular tree (44)] wild-type BMPR2s suppress postnatal pulmonary vascular cell proliferation, whereas haploinsufficiency or signaling deficiencies generated by heterozygous mutations leads to increased PA SMC proliferation. Indeed, BMP-2, -4, and -7 suppress normal main PA SMC proliferation, and this response is lost in cells isolated from idiopathic PAH patients (31). In keeping with this idea, we have shown that expression of the pro-proliferative ECM protein TN-C is suppressed in normal PA SMCs and is induced in FPAH SMCs. Although our previous studies showed that TN-C is necessary for PA SMC survival and proliferation, it is not sufficient for these functions (21). Rather, TN-C acts to physically and biochemically render PA SMCs receptive to additional factors, such as EGF and FGF-2 (21). It is therefore tenable to hypothesize that a dynamic and reciprocal relationship exists between BMPR2s, TN-C and 5-HT expression. How these factors interact in the setting of FPAH clearly warrants further study.

It has already been established that BMPR2 mutations in FPAH lead to reduced R-Smad signaling, unopposed p38 MAPK/ERK signaling, and abnormal SMC proliferation (44), yet the interrelationship between R-Smads, ERK1/2 activity, and changes in gene expression leading to the onset of PA SMC proliferation had not been determined. In the present study, we showed not only that ERK1/2 MAPK inhibits R-Smad activation but that this culminates in upregulation of TN-C. Furthermore, ERK1/2-dependent phosphorylation of Smad-1 in its linker region is known to inhibit Smad-1 transcriptional activity in nonvascular cell types (35), but the
ability of ERK1/2 MAPK to inhibit R-Smad signaling in PA SMCs had not been evaluated. Nevertheless, the question still remains as to how mutated BMPR2s activate ERK1/2 MAPKs. One possibility is via activation by c-src, an upstream activator of ERK1/2. In normal SMCs, c-src binds to the cytoplasmic tail of BMPR2s and this interaction suppresses c-src activity (43). In contrast, BMPR2 tail mutations release c-src, potentially allowing it to activate ERK1/2 MAPKs (43). Alternatively, changes in F-actin cytoarchitecture elicited by mutated BMPR2s could permit ERK1/2 activation, given that SMC morphology dictated by the F-actin cytoskeleton has been shown to be a critical mediator of ERK1/2 MAPK signaling (3).

Our studies established a link between BMPR2 mutations and the induction of Prx1-dependent TN-C gene transcription in FPAH SMCs, yet we do not yet know whether R-Smads or other effectors of BMPs directly control Prx1 transcription. It is of interest, however, that the portion of the Prx1 gene promoter that we isolated and analyzed contains a binding site for Vent-2, a homeodomain transcription factor family and immediate response gene of BMP-4 signaling (34). Future studies will identify upstream effectors and downstream cis- and trans-acting factors that are called forth to regulate Prx1 and TN-C in response to BMPR2 mutations in PAH.

Finally, a recent report has shown that the BMPR2 signaling is downregulated in hypoxia-induced PAH in rats (40). These results suggest that abrogation of BMP signaling may be a common event in the development of PAH. Because Prx1 and TN-C also appear to be ubiquitously expressed in experimental models, they may have relevance in other forms of PAH besides the familial.

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