Hypoxia induces downregulation of PPAR-γ in isolated pulmonary arterial smooth muscle cells and in rat lung via transforming growth factor-β signaling

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Gong K, Xing D, Li P, Aksut B, Ambalavanan N, Yang Q, Nozell SE, Oparil S, Chen Y-F. Hypoxia induces downregulation of PPAR-γ in isolated pulmonary arterial smooth muscle cells and in rat lung via transforming growth factor-β signaling. Am J Physiol Lung Cell Mol Physiol 301: L899–L907, 2011. First published September 16, 2011; doi:10.1152/ajplung.00062.2011.—Chronic hypoxia activates transforming growth factor-β (TGF-β) signaling and leads to pulmonary vascular remodeling. Pharmacological activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) has been shown to prevent hypoxia-induced pulmonary hypertension and vascular remodeling in rodent models, suggesting a vasoprotective effect of PPAR-γ under chronic hypoxic stress. This study tested the hypothesis that there is a functional interaction between TGF-β/Smad signaling pathway and PPAR-γ in isolated pulmonary artery small muscle cells (PASMCs) under hypoxic stress. We observed that chronic hypoxia led to a dramatic decrease of PPAR-γ protein expression in whole lung homogenates (rat and mouse) and hypertrophied pulmonary arteries and isolated PASMCs. Using a transgenic model of mouse with inducible overexpression of a dominant-negative mutant of TGF-β receptor type II, we demonstrated that disruption of TGF-β pathway significantly attenuated chronic hypoxia-induced downregulation of PPAR-γ in lung. Similarly, in isolated rat PASMCs, antagonism of TGF-β signaling with either a neutralizing antibody to TGF-β or the selective TGF-β receptor type I inhibitor SB431542 effectively attenuated hypoxia-induced PPAR-γ downregulation. Furthermore, we have demonstrated that TGF-β1 treatment suppressed PPAR-γ expression in PASMCs under normoxia condition. Chromatin immunoprecipitation analysis showed that TGF-β1 treatment significantly increased binding of Smad2/3, Smad4, and the transcriptional corepressor histone deacetylase 1 to the PPAR-γ promoter in PASMCs. Conversely, treatment with the PPAR-γ agonist rosiglitazone attenuated TGF-β1-induced extracellular matrix molecule expression and growth factor in PASMCs. These data provide strong evidence that activation of TGF-β/Smad signaling, via transcriptional suppression of PPAR-γ expression, mediates chronic hypoxia-induced downregulation of PPAR-γ expression in lung.

peroxisome proliferator-activated receptor-γ; pulmonary vascular remodeling

CHRONIC HYPOXIC STRESS RESULTS in pulmonary artery constriction, hypertension, and remodeling in human subjects and animal models (3). Characteristic features of chronic hypoxic pulmonary hypertension (PH) include neomuscularization of distal pulmonary arterioles, adventitial thickening of larger pulmonary arteries, perivascular extracellular matrix deposition, and hyperplasia and reduced apoptosis of pulmonary artery smooth muscle cells (PASMCs) (14, 30).

Transforming growth factor-β (TGF-β) and its Smad signaling pathway play an important role in the pathogenesis of PH (17, 27). Elevated levels of TGF-β expression and Smad2/3 phosphorylation have been reported in rat models of experimental PH in response to hypoxia or monocrotaline treatment, as well as in patients with idiopathic PH (1, 20, 29). On a cellular level, TGF-β has been shown to promote the proliferation and migration of PASMCs from rats and patients with PH and to increase extracellular matrix and endothelin-1 expression in rat and human PASMCs (14, 29). Furthermore, emerging evidence has implicated enhanced TGF-β signaling attributable to a loss of function mutation of the bone morphogenetic protein receptor type II in the pathogenesis of idiopathic PH in humans (26). In addition, we have demonstrated that disruption of TGF-β signaling by inducible overexpression of a dominant-negative mutant of TGF-β receptor type II (DnTGFbRII) significantly attenuated hypoxia-induced pulmonary artery remodeling and right ventricular hypertrophy in mice (5), providing in vivo evidence for a functional role of TGF-β signaling in hypoxia adaptation.

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a nuclear receptor that functions as a transcription factor to regulate a variety of biological processes, including hypertrophic, fibrotic, and inflammatory responses to stress (10, 22). Accumulating evidence has shown that treatment with PPAR-γ agonists, including rosiglitazone and pioglitazone, elicits antiproliferative, proapoptotic, and vasodilatory effects, improves endothelial function, and prevents chronic hypoxia-induced pulmonary arterial remodeling in rodent models (1, 6, 13, 24). Furthermore, transgenic mice with targeted deletion of PPAR-γ in SMCs or endothelial cells spontaneously develop right ventricular hypertrophy and increased muscularization of the distal pulmonary arteries (11). Together, these provocative findings suggest that PPAR-γ may act as an endogenous vasoprotective antiproliferative/fibrogenic factor in the process of chronic hypoxic-induced PH and pulmonary artery remodeling (32).

It has been shown that chronic hypoxia-induced PH and pulmonary artery remodeling are related to an imbalance in the normal counterregulatory relationships between mitogenic/extracellular matrix forming and antiproliferative/proapoptotic signaling pathways in lung and PASMCs (5, 17, 18). The
current study extended these findings by linking TGF-β and PPAR-γ signaling in lung and PASMCs in the context of hypoxic stress. We demonstrate that hypoxia inhibits PPAR-γ protein expression in rat lung and isolated rat PASMCs via a TGF-β-dependent mechanism. We further observed that TGF-β mediates hypoxia-induced PPAR-γ downregulation by suppressing PPAR-γ expression at the transcriptional level. Conversely, pharmacological activation of PPAR-γ by the agonist rosiglitazone attenuated TGF-β-induced extracellular matrix expression in isolated PASMCs. These findings provide strong evidence for a counterregulatory relationship between TGF-β and PPAR-γ signaling and PPAR-γ expression in the pathogenesis of hypoxia-induced pulmonary vascular remodeling.

**MATERIALS AND METHODS**

**Animal preparation and surgical procedures.** To test the hypothesis that chronic hypoxia leads to downregulation of PPAR-γ expression in rat pulmonary artery, adult (age 8 wk) male Sprague-Dawley rats, obtained from Charles River Breeding Laboratories (Wilmington, MA), were exposed to hypoxia (10% O2, 1 atm) in an 800-l model 818GGB Plexiglas glove box (Plas Laboratories, Lansing, MI) for 2 wk as previously described (5). Rats were fed a standard diet (Harlan Teklad, Madison, WI) and were housed in rooms maintained at a constant humidity (60 ± 5%), temperature (24 ± 1°C), and light cycle (6:00 AM-6:00 PM). All protocols were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham and were consistent with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (DHHS publication No. 96–01, revised in 2002). To test the effect of TGF-β signaling on PPAR-γ expression in lung under chronic hypoxic condition, adult (8–10 wk old) male C57BL6/J wild-type and transgenic mice with inducible overexpression of DnTGFβRII were exposed to hypoxia (10% O2, 1 atm) (1, 5, 21). DnTGFβRII mice were given drinking water with 25 mM ZnSO4 to activate the transgene 1 wk before the hypoxic exposure.

**Immunohistochemical analysis.** To determine the level of PPAR-γ protein expression in pulmonary artery, lungs from 2 wk-hypoxia-exposed rats were transferred into an air-tight hypoxic chamber (model 18). PASMCs were subjected to serum starvation for 24 h. To test the effects of PPAR-γ agonist on the expression of TGF-β target genes, cells were pretreated with rosiglitazone (Rosi. 0.1 µM; Cayman Chemical, Ann Arbor, MI) for 24 h and then treated with TGF-β1 for an additional 12 h. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) and the purified RNA was reverse transcribed to cDNA using the SuperScript First-Strand Synthesis System (Invitrogen). cDNA was amplified by real-time quantitative PCR using the SYBR Green RT-PCR kit (Applied Biosystems, Foster City, CA) in a Bio-Rad iCycler with specific primers [PPAR-γ: 5′-GCT TGG CTT ACG GAC TGA-3′ and 5′=AGG CGG GGC AG-3′; plasminogen activator inhibitor-1 (PAI-1): 5′-GTG GGC CAG AAT GGC A-3′; and 5′-AGC ATG GTG CCT TCG CTG ATG C-3′; connnective tissue growth factor (CTGF): 5′-GGT GTG AGG AGT GGG GGT TGT-3′ and 5′-TTG GCT GCC ATC ATA GTT-3′; plasminogen activator inhibitor-1 (PAI-1): 5′-GCG CTA CCA CGG CGA AAC C-3′ and 5′-AGG ATG GGG AGG CGG AGC AGC AG-3′; perioxidin: 5′-CCA GTG CTC TGA GGC TAT-3′ and 5′-TAC CAG GTG CTC GGT AGT-3′; osteopontin (OPN): 5′-GCT TGG CTT ACG GAC TGA-3′ and 5′-TGT TTC...
CAC GCT TGG TTC-3; PDGF-BB: 5'-AAT CGC CGA GTG CAA GAC GCG-3' and 5'-CGG CCA CAC CAG GAA GTT GGC-3'; 18S rRNA: 5'-GAA ACG GCT ACC ACA TCC-3' and 5'-CAC CAG ACT TGC CCT CCA-3']. Relative RNA levels were calculated using the iCycler software (Bio-Rad, Hercules, CA) and normalized using 18S rRNA.

**PPAR-γ promoter activity analysis.** To test the hypothesis that TGF-β inhibits PPAR-γ promoter activity in PASMCs, quiescent PASMCs were transiently cotransfected with a pGL3-Basic plasmid containing a human PPAR-γ gene promoter (~2.8 kb of the 5'-flanking region) fused with a firefly luciferase reporter gene and a pRL-TK plasmid containing a Renilla luciferase gene (as a control for transfection efficiency) using the Lipofectamine Plus Transfection Reagent (Invitrogen). One day after transfection, PASMCs were treated with TGF-β1 (2 ng/ml) or vehicle for 24 h. PASMCs were harvested, and luminescence from transfected PASMCs was quantified by measuring firefly/Renilla luciferase activity using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI).

Chromatin immunoprecipitation analysis. Quiescent PASMCs (1.8 × 10^7 cells per 150-mm dish) were treated with TGF-β1 (2 ng/ml) or vehicle for 1 h, and chromatin immunoprecipitation (ChIP) assays were carried out with anti-Smad2/3 (sc-8332, Santa Cruz), anti-Smad4 (sc-7154, Santa Cruz), anti-histone deacetylase 1 (HDAC1) (sc-7872, Santa Cruz) antibodies and normal rabbit IgG (sc-2027, Santa Cruz) as described previously (25). A pair of primers (5'-CTG GTG GAC AGT CAG GGC ACC-3' and 5'-CGG CCA CAC CAG GAA GTT GGC-3') were used to amplify a 70-bp fragment (~226 to ~295 bp) of the promoter region of the rat PPAR-γ gene for detection of binding of Smad2/3, Smad4, and HDAC1. PCR was run at 29 to 31 cycles, and the PCR products were detected in agarose gel with ethidium bromide. Selective PCR product levels were semiquantitated using densitometry and normalized by respective input values.

**Pulmonary arterial muscularization assessment.** Pulmonary arterial muscularization was assessed using α-smooth muscle actin-immunostained lung sections as described previously (7). Arterial muscularization was defined according to the degree of muscularization: muscularized arteries (with two distinct elastic laminae and...
complete medial coat), partially muscularized arteries (with a continuous external elastic lamina and an incomplete medial coat), and nonmuscularized vessels (with only one single elastic lamina but no VSMC apparent) were distinguished by observation and counted. The percentage of muscularization of each pulmonary artery relative to its size (25–200 μm in diameter) was calculated as an index of pulmonary arterial muscularization.

Cell migration assay. To test the effect of Rosi and TGF-β on PASMC migration, serum-starved cells (0.5 × 10^5) were seeded in Falcon cell culture inserts (cat. 35–3097; Becton Dickinson Labware, Franklin Lakes, NJ) with 8.0-μm-pore size in 1% FBS-DMEM with vehicle or 0.1 μM Rosi; 0.5 ml 10% FBS-DMEM with vehicle or Rosi were added to the 24-well plates. After incubation for 3 h, cells were treated with vehicle or TGF-β1 (2 ng/ml) for 12 h; the cells in the upper chamber and on the polyester-track-etched membrane were mechanically removed with a cotton swab and washed with ice-cold PBS. Cells adherent to the outer surface of the lower side of the membrane were fixed with methanol for 15 min and stained with hematoxylin for 20 s. Fifteen fields were randomly selected in each group and cells were counted.

Statistical analysis. Results were expressed as means ± SE. Analyses were carried out using the SigmaStat statistical package (Jandel Scientific, San Rafeal, CA). Our primary statistical test was ANOVA; one-way ANOVA to evaluate the differences in mean values attributable to main effects (hypoxia, TGF-β1) and two-way ANOVA to test their interactions. If ANOVA results were significant, a post hoc comparison among groups was performed with the Newman-Keuls test. A P value < 0.05 was considered statistically significant.

RESULTS

Chronic hypoxia decreases PPAR-γ protein expression in rat lung. Two weeks of exposure to normobaric hypoxia led to a significant decrease (~77%) in PPAR-γ protein expression in rat whole lung tissue extracts, compared with air controls (Fig. 1, A and B). Immunohistochemical staining demonstrated that PPAR-γ was expressed ubiquitously in air control lungs, including in alveolar septal structures and adventitia and media in pulmonary artery (size from 250 μm to 750 μm) (Fig. 2). In contrast, PPAR-γ expression was greatly reduced in hypoxia-exposed lungs, particularly in the hypertrophied pulmonary arteries, as evidenced by decreased numbers of PPAR-γ-positive cells in medial PASMCSs (Fig. 2).

Blocking of TGF-β signaling significantly attenuates chronic hypoxia-induced downregulation of PPAR-γ expression in DnTGFβRII mice. We have recently demonstrated that disruption of TGF-β signaling significantly alleviates chronic hypoxia-induced pulmonary hypertension and pulmonary arterial remodeling in DnTGFβRII mice (20). In the current study, we demonstrated that 2 wk chronic hypoxic exposure decreased PPAR-γ protein levels in lung of wild-type mouse (Fig. 3A). In contrast, hypoxia-induced downregulation of PPAR-γ expression was significantly attenuated in DnTGFβRII mice when TGF-β signaling was blocked (Fig. 3B). Hypoxia-induced arteriolar muscularization in small pulmonary arteries (50–100 μm, an index of vascular remodeling) (Fig. 3C) and right ventricular hypertrophy (an index of pulmonary hypertension)
(Table 1) were less marked in DnTGFβRII than that in wild-type mice. These results suggest that TGF-β has a dominant role in hypoxia-induced downregulation of PPAR-γ expression, pulmonary hypertension, and vascular remodeling.

**TGF-β is required for hypoxia-induced downregulation of PPAR-γ in isolated PASMCs.** Because TGF-β1 signaling plays an important role in rodent models of pulmonary hypertension and PASMCs are known to be important targets of TGF-β signaling, we used isolated rat PASMCs to test the hypothesis that activation of TGF-β signaling mediates hypoxia-induced downregulation of PPAR-γ in this critical cell type. Consistent with the results of the in vivo study, hypoxic exposure led to significant downregulation of PPAR-γ protein expression compared with air controls. Furthermore, TGF-β1 (2 ng/ml) treatment also suppressed PPAR-γ protein in air controls but did not enhance the inhibitory effect of hypoxia on PPAR-γ expression (Fig. 4A). Pretreatment with a neutralizing antibody against TGF-β (50 μg/ml) effectively attenuated hypoxia-induced downregulation of PPAR-γ in PASMCs, whereas control IgG had no effect on basal PPAR-γ expression (Fig. 4B), confirming the functional role of TGF-β in mediating the hypoxia effect.

We have also demonstrated that TGF-β1 (2 ng/ml) treatment suppressed PPAR-γ protein expression in isolated rat aortic SMCs (Supplemental Fig. S1; supplemental material for this article is available online at the American Journal of Physiology Lung Cellular and Molecular Physiology website), indicating that the downregulation of PPAR-γ in response to TGF-β1 is not unique to PASMCs.

**TGF-β suppresses PPAR-γ expression in PASMCs via a TGFβRI kinase-dependent pathway.** The intrinsic kinase activity of TGF-β receptor type I (TGFβRI) has an essential role in initiating the classic TGF-β/Smad pathway (8). However, Sorrentino et al. (28) recently demonstrated that the kinase activity of TGFβRI is not required for TGF-β to induce apoptosis via a Smad-independent, TRAF6-TAK1-p38/JNK pathway. To test whether TGFβRI kinase activity is required for TGF-β-induced downregulation of PPAR-γ, isolated PASMCs were pretreated with the selective TGFβRI inhibitor (activin receptor-like kinase 5, ALK5) inhibitor SB431542 (0.5 μM) for 1 h, and then exposed to TGF-β1 (2 ng/ml) for 30 min or 24 h. We observed that inhibition of TGFβRI kinase activation significantly inhibited TGF-β-induced Smad3 phosphorylation (Fig. 5A) and fully abolished TGF-β1-induced downregulation of PPAR-γ protein expression (Fig. 5B), suggesting that TGF-β induces downregulation of PPAR-γ expression in a receptor kinase-dependent manner. Consistent with the results of the study using TGF-β-neutralizing antibody, pretreatment with SB43542 significantly attenuated hypoxia-induced downregulation of PPAR-γ protein expression in isolated PASMCs (Fig. 5C).

**TGF-β suppresses PPAR-γ expression in PASMCs at the transcriptional level.** To test the hypothesis that TGF-β1-induced downregulation of PPAR-γ expression in PASMCs occurs at the transcriptional level, quiescent PASMCs were exposed to TGF-β1 (2 ng/ml) for 24 h, and steady-state PPAR-γ mRNA was measured using real-time quantitative RT-PCR analysis. Consistent with its effect on protein expression, TGF-β1 treatment significantly decreased PPAR-γ mRNA expression (Fig. 6A). To determine the effect of TGF-β1 on the transcriptional activity of the PPAR-γ gene, PASMCs were transfected with a plasmid containing a human PPAR-γ promoter and incubated with TGF-β1 (2 ng/ml) for 24 h. Measurement of luciferase activities demonstrated that TGF-β1 treatment decreased PPAR-γ promoter activity significantly (by 31%) (Fig. 6B). Together, these results provide evidence that TGF-β inhibits PPAR-γ gene expression in PASMCs at the transcriptional level.

**Fig. 5.** Effect of a TGFβRI antagonist on TGF-β1- and hypoxia-induced downregulation of PPAR-γ. Quiescent rat PASMCs were pretreated with the ALK5 inhibitor SB431542 (SB, 0.5 μM) for 1 h, then challenged with TGF-β1 (2 ng/ml) for 30 min (A) or 24 h (B), Smad3 phosphorylation (30 min after TGF-β1 treatment) (A) and PPAR-γ protein expression (24 h after TGF-β1 treatment) (B) were detected by Western blot analysis and normalized using α-tubulin as an internal control; C: PASMCs were pre-treated with SB431542 (0.5 μM) for 1 h, then exposed to hypoxia (1% O2) for 24 h. Western blot of PPAR-γ protein was normalized using α-tubulin. Results are means ± SE; n = number of wells. *P < 0.05 compared with vehicle control; #P < 0.05 compared with TGF-β1 alone or hypoxia.
**DISCUSSION**

The current study provides the first demonstration that hypoxia inhibits PPAR-γ protein expression in rat lung and isolated PASMCs via a TGF-β-dependent mechanism. We further demonstrated that TGF-β inhibits PPAR-γ downregulation at the transcriptional level through increasing Smad2/3 and HDAC1 binding at the promoter of PPAR-γ in isolated PASMCs. With the use of these isolated PASMC as an in vitro model, these provocative findings define a novel molecular mechanism for counterregulatory effects of TGF-β and PPAR-γ signaling in mediating pulmonary vascular responses to hypoxic stress.
PPAR-γ plays a modulatory role in the pathogenesis of pulmonary hypertension and pulmonary vascular remodeling via multiple mechanisms (32). In vivo studies have shown that transgenic mouse models with either SMC- or endothelial cell-specific deletion of PPAR-γ develop mild pulmonary hypertension and pulmonary vascular remodeling under normoxic conditions (9, 11). Furthermore, administration of PPAR-γ agonists both prevents and reverses hypoxia- and monocrotaline-induced right ventricular hypertrophy and pulmonary artery neomuscularization in normal mice and rats, as well as in apoE knockout mice fed a high-fat diet (6, 12, 24).

In this study, we have shown that hypoxic exposure downregulated PPAR-γ expression in medial (250 μm in diameter) and large (750 μm) pulmonary arteries. In small pulmonary arteries (<200 μm), the PPAR-γ was too low to be detected because of thin SMC media or partially muscularized vessels (data not shown). These results support the finding of Crossno et al. (6) that PPAR-γ may be effective in attenuating the (larger) pulmonary arterial remodeling but not the elevated pulmonary arterial pressure (pulmonary hypertension).

In additional experiments, we have observed that TGF-β1 treatment also suppressed PPAR-γ protein expression in isolated rat aortic SMCs (Supplemental Fig. S1), indicating that the downregulation of PPAR-γ in response to TGF-β1 is not unique to PASMCs. These results suggest that, in addition to hypoxic stress, the interactions between TGF-β/Smad and PPAR-γ signaling may also play an important role in the remodeling of systemic vasculature under stressful conditions (e.g., vascular endoluminal injury that activates TGF-β1 expression).

In vitro studies have demonstrated that reduced expression of PPAR-γ is associated with increased proliferation and extracellular matrix molecule expression by SMCs. For example, it has been shown that PPAR-γ protein expression in aortic SMCs from spontaneously hypertensive rats, a strain characterized by enhanced proliferation of arterial SMCs, is significantly lower than in nomotensive control Wistar-Kyoto rats, which have lower SMC proliferation rates. Adenovirus-mediated PPAR-γ overexpression significantly inhibited the exaggerated proliferation of aortic SMCs from spontaneously hypertensive rats (31). Clinically, PPAR-γ expression is reduced in pulmonary artery of patients with severe PH (2). Collectively, these findings suggest that reduced PPAR-γ expression plays a mechanistic role in chronic hypoxia-induced PH and pulmonary arterial remodeling.

More recently, Kim et al. (15) showed that PPAR-γ expression is downregulated in rat lung under chronic hypoxia stress (15). We also observed that chronic hypoxia led to downregulation of PPAR-γ protein expression in both rat and mouse lung, especially in hypertrophied pulmonary arteries. Importantly, we demonstrated that disruption of TGF-β signaling in DnTGFβRII mice attenuated chronic hypoxia-induced downregulation of PPAR-γ expression. Furthermore, our in vitro studies showed that blocking TGF-β signaling with either a TGF-β neutralizing antibody or a TGFRII inhibitor significantly attenuated hypoxia-induced downregulation of PPAR-γ in isolated PASMCs. These results indicate that, under chronic hypoxia conditions, TGF-β activation mediates downregulation of PPAR-γ expression in lung.

TGF-β, via its receptors TGFβRI and TGFβRII, triggers cytoplasmic Smad2/3 activation that depends on the phosphorylation of TGFβRII induced by constitutively active TGFβRII in the receptor complex. TGFβRII has recently been shown to interact directly with ubiquitin ligase (E3) TRAF6 and induce
Lys 63-linked polyubiquitylation of TAK1, thus activating the TAK1-p38/JNK pathway in a receptor kinase- and Smad-independent manner (28). Although vascular SMCs express multiple isoforms of TGFβRI, i.e., ALK1–7, most of the effects of TGF-β on SMC function appear to be mediated via the ALK5 (4). To determine whether TGF-β-induced inhibition of PPAR-γ expression requires intrinsic kinase activation of TGFβRI, we pretreated isolated PASMCs with the selective ALK5 inhibitor SB431542 before TGF-β administration. Our results clearly demonstrated that inhibition of TGFβRI kinase activation effectively abolished TGF-β-induced Smad3 phosphorylation and significantly attenuated downregulation of PPAR-γ. These findings suggest that TGF-β-induced Smad3 activation by TGFβRI plays an important role in hypoxia-induced downregulation of PPAR-γ expression. Our observations are consistent with the previous demonstration that inhibition of ALK5 activation with the selective antagonist SB525334 attenuated TGF-β-induced proliferation-related gene expression and cellular proliferation of PASMCs from patients with idiopathic PH in vitro and significantly decreased pulmonary arterial pressure and inhibited right ventricular hypertrophy in monocrotaline-treated rats in vivo (29).

Our observation that TGF-β signaling suppresses PPAR-γ expression at the transcriptional level in PASMCs is consistent with previous studies. Fu et al. (7) have reported that exposure to TGF-β1 for periods >12 h suppressed PPAR-γ mRNA expression in human aortic SMCs (7). Conversely, in activated rat hepatic stellate cells, interruption of TGF-β signaling by curcumin has been shown to upregulate PPAR-γ gene expression (33). TGF-β-mediated transcriptional modulation requires Smad protein binding to the promoters of target genes. Our ChIP indicated that the PPAR-γ promoter is constitutively bound by Smad2/3 and Smad4 proteins and that binding of Smad2/3/4 proteins to the PPAR-γ promoter increases in response to TGF-β1 treatment, suggesting a role for Smad protein in this process. Future studies will be needed to map the precise regions of Smad binding at the PPAR-γ promoter that mediate inhibition of PPAR-γ gene expression by TGF-β1. The observation that TGF-β1-stimulated binding of Smad2/3/4 enhances the recruitment of HDAC1 at the PPAR-γ promoter provides additional evidence that TGF-β1 downregulates PPAR-γ gene expression through a transcriptional mechanism.

PPAR-γ agonists have been shown to suppress proliferation of PASMCs by stabilizing the cyclin-dependent kinase inhibitor p27kip1 and to induce apoptosis of proliferating SMCs by blocking the phosphatidylinositol-3 kinase survival pathway (13). The current study added to that body of knowledge by demonstrating that the PPAR-γ agonist Rosi inhibits TGF-β1-induced stimulation of extracellular matrix molecule expression by PASMCs, thus suggesting that the modulatory effect of these agents on PH and pulmonary vascular remodeling may be related to attenuation of TGF-β signaling.

In summary, this study provides the first evidence that TGF-β/Smad signaling plays a critical role in hypoxia-induced downregulation of PPAR-γ in lung. In isolated PASMCs, we also demonstrated that activation of TGF-β signaling suppresses PPAR-γ expression via a transcriptional regulatory mechanism. These findings provide important information of the mechanism(s) of hypoxia-induced pulmonary hypertension and vascular remodeling.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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