PPARγ and the Pathobiology of Pulmonary Arterial Hypertension

Marlene Rabinovitch

Abstract Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor that functions as a transcription factor to regulate adipogenesis and metabolism by binding to PPAR response elements (PPAREs) in the promoter region of various target genes. Activation of PPARγ suppresses smooth muscle cell proliferation and migration. This chapter discusses the potential protective role of PPARγ and its downstream signaling cascades in the development of pulmonary arterial hypertension. Furthermore, the chapter also provides an overview on the cellular and molecular mechanisms involved in PPARγ-mediated inhibitory effect on pulmonary vascular remodeling, a major contributor to the elevated pulmonary vascular resistance in patients with pulmonary arterial hypertension.

Keywords Peroxisome proliferator-activated receptor γ (PPARγ) • nuclear receptor • pulmonary vascular remodeling • cell proliferation and migration

1 Introduction

Peroxisome proliferator-activated receptor γ (PPARγ) is one of a family of three nuclear receptors (PPARγ, -α, and -δ) that can function as transcription factors to regulate adipogenesis and glucose metabolism.[1–3] On activation by mechanisms not well understood, PPARs heterodimerize with the retinoid X receptor (RXR) and bind to PPAR response elements (PPREs) in regulatory promoter regions of their target genes.[4] Many of these are implicated in suppressing smooth muscle cell (SMC) proliferation and migration.[4] For example, PPARγ activation blocks platelet-derived growth factor (PDGF) gene expression[5] and induces the expression of

M. Rabinovitch
The Wall Center for Pulmonary Vascular Diseases, Stanford University School of MedicineStanford, CA, USA,
e-mail: marlener@stansford.edu

J.X.-J. Yuan and J.P.T. Ward (eds.), *Membrane Receptors, Channels and Transporters in Pulmonary Circulation*, AEME, Vol. 661, DOI 10.1007/978-1-60761-500-2_29,

lipoprotein-like receptor protein 1 (LRP1),[6] the receptor necessary for apolipoprotein (Apo) E-mediated suppression of PDGF-BB signaling[7,8] as discussed in this chapter. Moreover, PPARγ activation can induce apoptosis of SMCs by phosphorylation of the retinoblastoma (RB) gene[9] and by increasing the proapoptotic protein Gadd 45.[10] PPARs can also interact with signaling molecules to regulate gene expression independent of DNA binding. For example, PPARγ impairs phosphorylation (i.e., activation) of extracellular-regulated kinase (ERK),[11,12] a mitogen-activated protein kinase (MAPK), downstream of PDGF-BB/PDGFR-β signaling. Moreover, activated PPARγ stabilizes the cyclin-dependent kinase inhibitor p27KIP1[9] and inhibits telomerase activity,[13] retinoblastoma protein phosphorylation,[9] and cell cycle progression associated with vascular SMC proliferation.[9] By blocking important pathways downstream of activated PDGFR-β (i.e., phosphoinositol-3-kinase (PI3K))[14] PPARγ agonists can also cause apoptosis of proliferating vascular cells.[4,15] In addition, it is known that PPARγ ligands impair production of matrix metalloproteinases[16] that can be activated by elastase.[17] Our group has shown that inhibition of this proteolytic cascade not only prevents but also reverses advanced fatal pulmonary artery hypertension (PAH) in rats.[18]

In endothelial cells (ECs), PPARγ activation reduces levels of endothelin 1 (ET-1)[19] and the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA),[20,21] factors that are implicated in both insulin resistance and in the pathobiology of PAH.[21] PPARγ has anti-inflammatory properties that include suppression of factors implicated in PAH, such as vascular cell adhesion molecule (VCAM), interleukin 6,[22,23] fractalkine,[24,25] and monocyte chemoattractant protein 1.[26] PPARγ also protects ECs against apoptosis[27,28] and may also promote EC proliferation and migration through production of endothelial nitric oxide synthase (eNOS)[29] as well as hemoxygenase (HO) 1.[30] In contrast, HO-1 represses SMC proliferation,[30] consistent with PPARγ-mediated repression of proliferation and migration of arterial SMCs in culture[31,32] and in animal models.[33]

Thus, extrapolating from data in systemic vascular disease[2] suggests that impaired activation of PPARγ transcriptional targets could lead to the pathology of PAH. This is reinforced by studies showing that the levels of PPARγ and its putative transcriptional target ApoE are reduced on complementary DNA (cDNA) microarrays from lung tissues and vessels in patients with PAH.[34,35] There is supporting evidence that links PPARγ with transcription of ApoE. A functional PPRE is present in the ApoE promoter,[36] conditional disruption of the PPARγ gene in mice results in decreased ApoE expression in macrophages,[37] and PPARγ activation leads to ApoE messenger RNA (mRNA) expression and protein secretion in an adipocyte cell line.[38]

2 Insulin Resistance, Pulmonary Hypertension, and PPARγ Agonist Treatment

Mice that are null for ApoE are made insulin resistant by being fed a high-fat diet. We showed that these mice have PAH in association with abnormal muscularization of distal arteries, and that the muscularization could be reversed following treat-

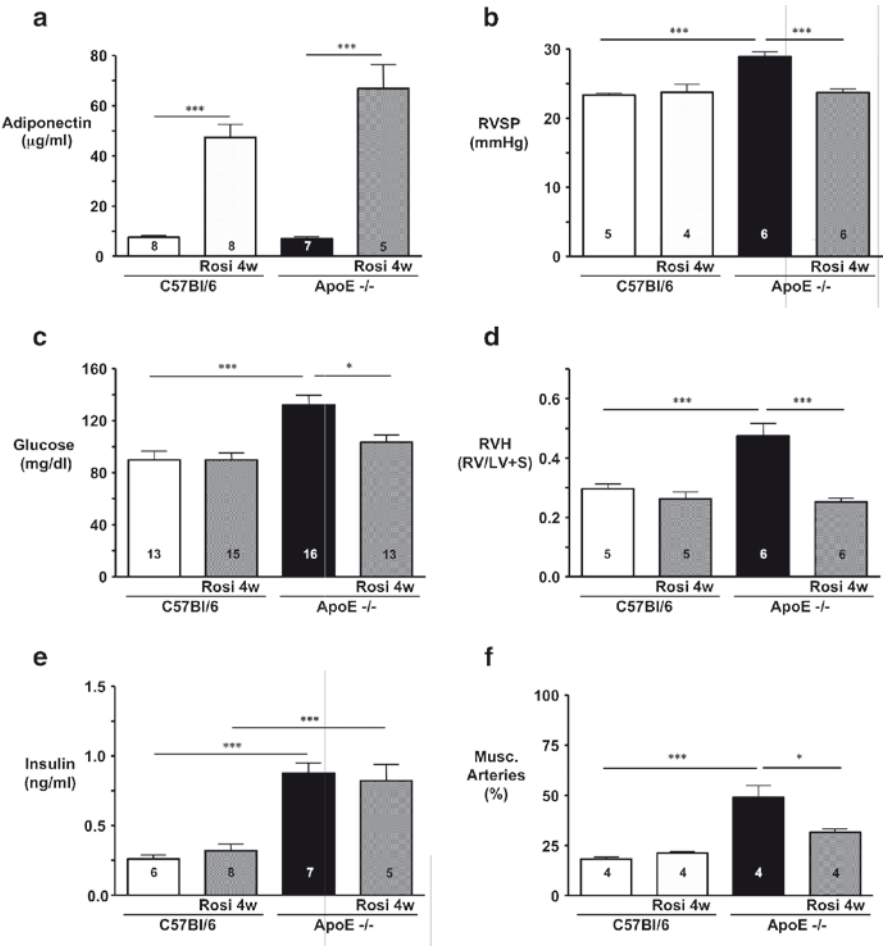

Fig. 29.1 Four-week treatment with the PPARγ agonist rosiglitazone reverses PAH, increases plasma adiponectin, and induces insulin sensitivity. Measurements of plasma adiponectin (**a**), right ventricular systolic pressure (RVSP) (**b**), blood glucose (**c**), right ventricular hypertrophy (RVH) (**d**), plasma insulin (**e**), and muscularization of alveolar wall arteries (**f**). Nineteen-week-old male C57Bl/6 and ApoE$^{-/-}$ mice, all on high fat (HF) diet for 15 weeks, were used. Bars represent mean ± standard error (SEM) (n = 4–16 as indicated in column graphs). *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$. Reproduced with permission[39]

ment with rosiglitazone, a PPARγ agonist[39] (Fig. 29.1). It was interesting that the female cohort of ApoE$^{-/-}$ mice had less-severe PAH as judged by lower levels of right ventricular systolic pressure, right ventricular hypertrophy, and muscularized distal arteries. We attributed this phenotype to the fact that the females had higher levels of adiponectin. The mechanism appears to be related to the fact that testosterone inhibits secretion of adiponectin,[40] and adiponectin can sequester PDGF.[41] We subsequently showed that adiponectin, a target of PPARγ-mediated transcriptional activity in adipocytes can repress pulmonary artery SMC (PASMC) proliferation in response to growth factors as does ApoE (Fig. 29.2).

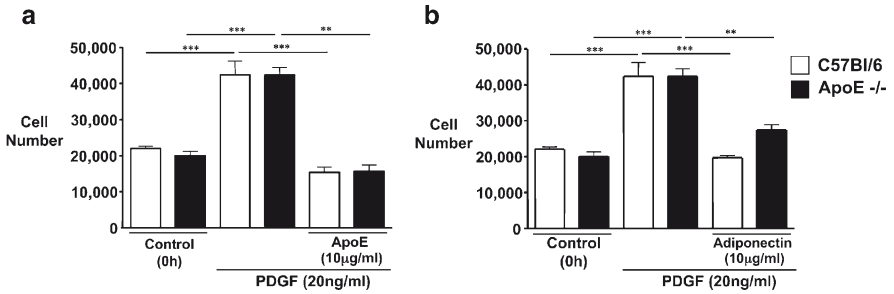

Fig. 29.2 Recombinant ApoE (**a**) and adiponectin (**b**) inhibit PDGF-BB-induced (20 ng/ml) proliferation of murine PASMCs harvested from both C57Bl/6 and ApoE$^{-/-}$ mice. Bars represent mean ± SEM ($n = 3$). *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$. Reproduced with permission[39]

In unpublished data from our group, we have found that older mice that are null for ApoE also develop PAH in the absence of a high-fat diet or insulin resistance. We speculate that this is related to the loss of the protective effect of ApoE in suppressing episodic PDGF-BB-mediated SMC proliferation over time. It has been shown that ApoE can bind to low-density LRP1, also a target of PPARγ-mediated gene transcription.[6] When this occurs, LRP1 targets the PDGFRβ for endocytosis,[42–44] repressing its function as an SMC mitogen. However, in contrast to its adverse effects as a smooth muscle mitogen, PDGF is also important in normal cell viability, in maintaining pericytes, and in the prevention of vascular endothelial growth factor (VEGF) overexpression and aberrant angiogenesis.[45] So, it could be proposed that PPARγ agonists may "fine-tune," allowing PDGF-mediated EC survival and pericyte recruitment while repressing aberrant angiogenesis and abnormal muscularization of distal vessels as well as proliferation of SMCs.

In light of these experimental studies and in keeping with clinical observations related to obesity in the population of patients with PAH, we investigated whether insulin resistance was prevalent in patients with PAH. Studies reported by Zamanian et al.[46] indicated a significantly higher proportion of female patients with PAH and insulin resistance when compared with the general population (45.7 vs. 21.5%), as judged by an abnormal elevation in the ratio of triglycerides to high-density lipoproteins.[46] It is of further interest that this high evidence of insulin resistance did not correlate with obesity as judged by the body mass index (BMI) or relate to the hemodynamic severity of the disease but was associated with a poorer 6-month event-free survival (58 vs. 79%) (Fig. 29.3).

3 PPARγ and the Bone Morphogenetic Protein Pathway

Mutations in bone morphogenetic protein receptor (BMPR) II that cause loss of function of the receptor are associated with familial and sporadic idiopathic PAH[47–49] and reduced expression of BMPR-II has been related to PAH regardless of etiology.[50] It was therefore of interest that in studies investigating transcription factors that are

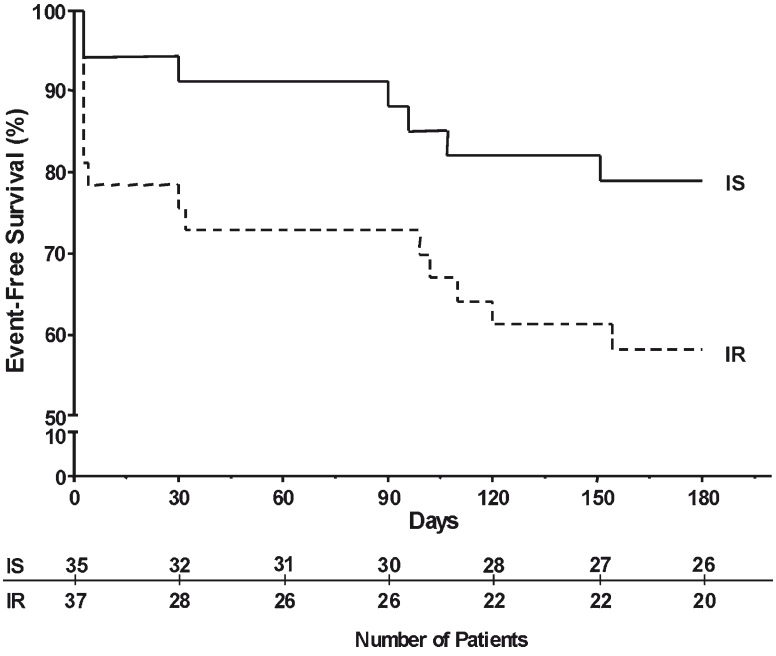

Fig. 29.3 Kaplan-Meier 6-month event-free survival curve in pulmonary arterial hypertension (PAH) females. Insulin-sensitive (*solid line*) PAH females had significantly better outcome compared with their insulin-resistant (*dashed line*) counterparts (79% vs. 58%; $P < 0.05$). Events were defined as death, transplantation, or acute hospitalization due to PAH exacerbation or right heart failure. Reproduced with permission[46]

regulated by signaling via BMPR-II, we identified PPARγ as a target.[51] We showed enhanced DNA binding of PPARγ following stimulation of PASMCs with bone morphogenetic protein (BMP) 2. We then showed that the ability of BMP2 to repress PDGF-BB-mediated PASMC proliferation depended on PPARγ (Fig. 29.4). Most interesting was the observation that loss of the function of BMPR-II to repress PDGF-BB-mediated proliferation could be rescued by a PPARγ agonist (Fig. 29.4). We then showed that BMP2-mediated inhibition of PDGF-BB-induced SMC proliferation requires not only activation of PPARγ but also that of its target of transcription, ApoE. We showed that both PPARγ and ApoE act downstream of BMP2/BMPR-II in human and murine PASMCs and prevent their proliferation in response to PDGF-BB. Bone morphogenetic protein-2 (BMP2)-mediated PPARγ activation occurs earlier than Smad1/5/8 phosphorylation and therefore appears to be independent of this established signaling axis downstream of BMPR-II. The BMPR-II ligand BMP2 induces a decrease in nuclear phospho-ERK, and rapid nuclear shuttling and DNA binding of PPARγ, whereas PDGF-BB has the opposite effects. Both BMP2 and the PPARγ agonist rosiglitazone stimulate production and secretion of ApoE in PASMCs (Fig. 29.5). Moreover, BMP2-mediated suppression of PDGF-BB-induced proliferation was absent in SMCs from a patient with a BMPR-II mutation and PAH, but this inhibition could be restored with a rosiglitazone (Fig. 29.6).

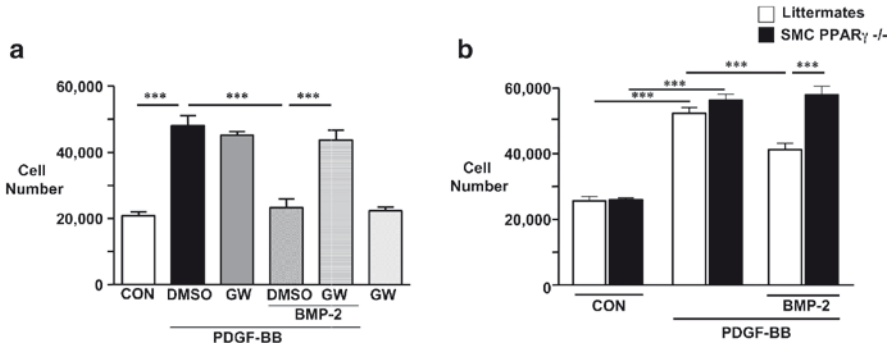

Fig. 29.4 (**a**) PASMCs were seeded at 2.5×10^4 cells per well of a 24-well plate in 500 μl of growth medium and allowed to adhere overnight. The cells were washed with phosphate-buffered saline (PBS) prior to the addition of starvation media (0.1% fetal bovine serum [FBS]) and incubated for 24 h (murine pulmonary artery smooth muscle cells (PASMCs)) or 48 h human PASMC (HPASMCs) and then stimulated with PDGF-BB (20 ng/ml) for 72 h. BMP-2 (10 ng/ml) was added to quiescent cells 30 min prior to PDGF-BB stimulation. The PPARγ antagonist GW9662 (GW; 1 μ*M*) was added 24 h prior to the addition of BMP2. Cells were finally washed twice with PBS, trypsinized, and counted in a hemocytometer (4 counts per well). Cell numbers in controls at time points 0 (CON) and 72 h were not significantly different. (**b**) Littermates, littermate control PASMCs; SMC PPAR$\gamma^{-/-}$, PASMCs isolated from *SM22α Cre PPARγflox/flox* mice. Bars represent mean ± SEM, $n = 4$ in (**a**) and 3 in (**b**). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ as indicated; analysis of variance (ANOVA) with Bonferroni's multiple-comparison test. Reproduced with permission[51]

Fig. 29.5 (continued) (**a**) BMP2 inhibits SMC proliferation via PPARγ and ApoE. ApoE impairs PDGF-BB/MAPK signaling by binding to low-density lipoprotein (LDL) receptor-related protein (LRP), thereby initiating endocytosis and degradation of the LRP/PDGFR-β/PDGF-B complex. PPARγ induces LRP and other growth-inhibitory/proapoptotic genes in SMCs and inhibits cell cycle and other growth-promoting genes, such as telomerase, cyclin D1, and retinoblastoma protein. Moreover, PPARγ induces phosphatases that can directly inactivate phospho-ERK. (**b**) BMPR-II dysfunction promotes SMC proliferation and survival in PAH. Heightened PDGF-BB signaling leading to SMC proliferation is a key clinical feature of PAH. Deficiency of both ApoE and LRP enhances mitogenic PDGF-BB/MAPK signaling. Loss-of-function mutations in the BMPR-II gene will decrease endogenous PPARγ activity, leading to unopposed MAPK signaling, SMC proliferation and survival, and ultimately development of PAH. *TF* transcription factor. (**c**) PPARγ agonists can rescue BMPR-II dysfunction and reverse PAH. PPARγ agonists such as rosiglitazone or pioglitazone might reverse SMC proliferation and vascular remodeling in PAH patients with or without BMPR-II dysfunction via induction of ApoE and other growth-inhibitory/proapoptotic genes (as indicated) and through repression of growth-promoting genes (not shown). Reproduced with permission[51]

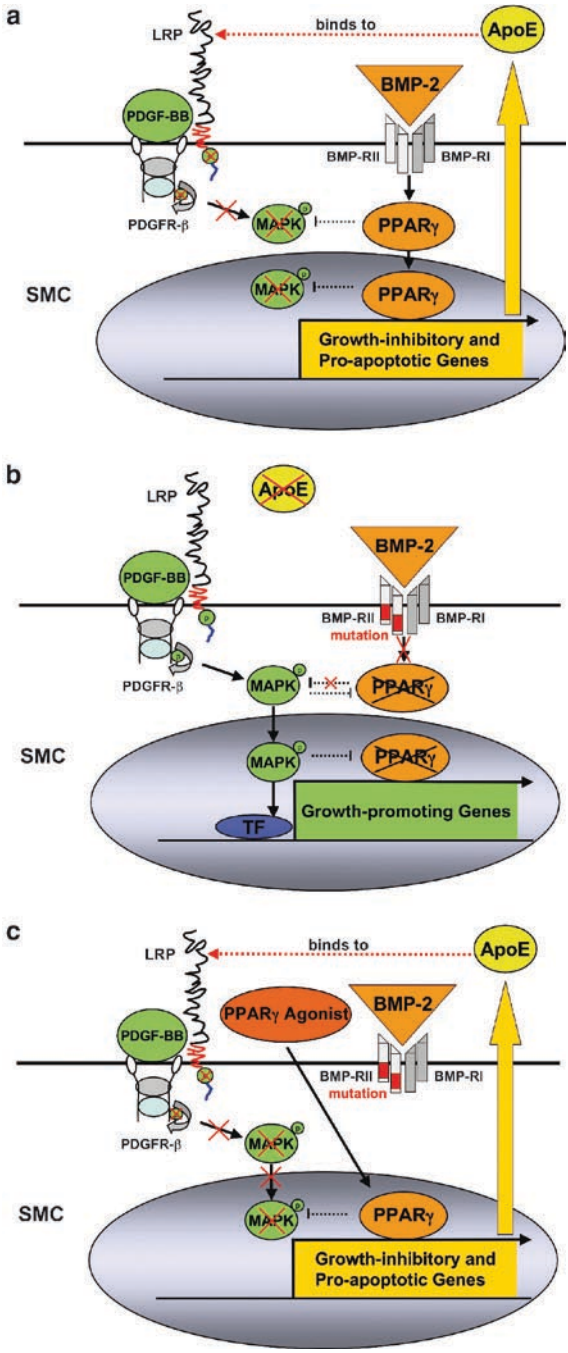

Fig. 29.5 Model: A novel antiproliferative BMP2/PPARγ/ApoE axis protects against PAH. This schema incorporates the findings described in our chapter and the literature to date as discussed.

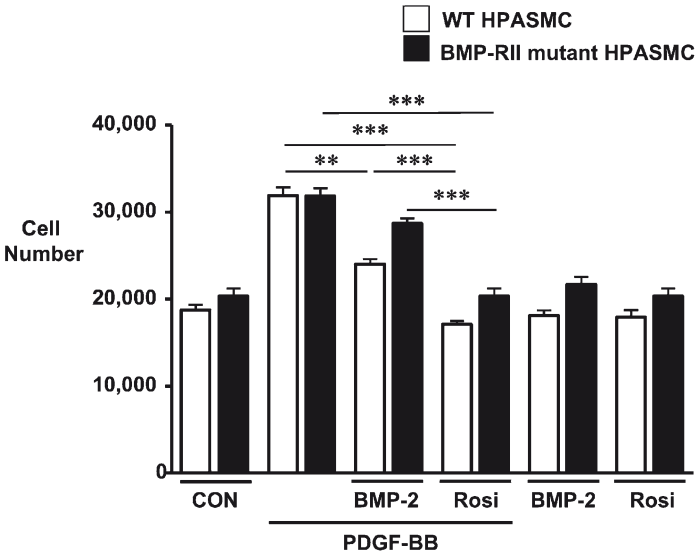

Fig. 29.6 Antiproliferative effects of BMP2 and the PPARγ agonist rosiglitazone on PDGF-BB-induced proliferation of human wild-type and BMPR-II mutant PASMCs. Control PASMCs were isolated from surgical resection specimens derived from patients undergoing lobectomy or pneumonectomy for suspected lung tumor. Additional peripheral pulmonary arteries (<1- to 2-mm external diameter) were obtained from a patient undergoing heart-lung transplantation for familial PAH (FPAH) and known to harbor a mutation (W9X) in BMPR-II. The nature of the BMPR-II mutation, cell isolation, culture techniques, and cell counts is the same as shown in Fig. 29.1. HPASMCs were incubated for 48 h in starvation media (0.1% FBS) and then stimulated with PDGF-BB (20 ng/ml) for 72 h. BMP2 (10 ng/ml) or rosiglitazone (1 μ*M*) were added to quiescent cells 30 min prior to PDGF-BB stimulation. Bars represent mean ± SEM ($n = 3$). $^{**}P < 0.01$; $^{***}P < 0.001$ as indicated; ANOVA with Bonferroni's multiple-comparison test. The number of PDGF-BB-stimulated cells was significantly higher than that of untreated control cells ($P < 0.001$). Reproduced with permission[51]

To determine whether, in addition to ApoE, other targets of PPARγ-mediated transcription in PASMCs were essential to inhibit the development of PAH, we made a mouse in which SM22-driven Cre was used to delete critical exons of a floxed PPARγ. This mouse had spontaneous PAH in the absence of a high-fat diet in association with muscularized distal arteries and right ventricular hypertrophy. Moreover, the pulmonary hypertensive response to chronic hypoxia is exaggerated in this mouse (Fig. 29.7).

We have bred the Tie2-expressing Cre mouse with the mouse in which PPAR is floxed. Our unpublished studies revealed that this mouse has a mild form of pulmonary hypertension under room air conditions but fails to show a heightened response to hypoxia. The mechanism does not appear to be related to ApoE but is associated with heightened signaling through PDGFRβ. While chronic hypoxia does not result in an exaggeration of PAH in these mice with EC deletion of PPARγ, reversal of PAH following exposure to chronic hypoxia is impaired.

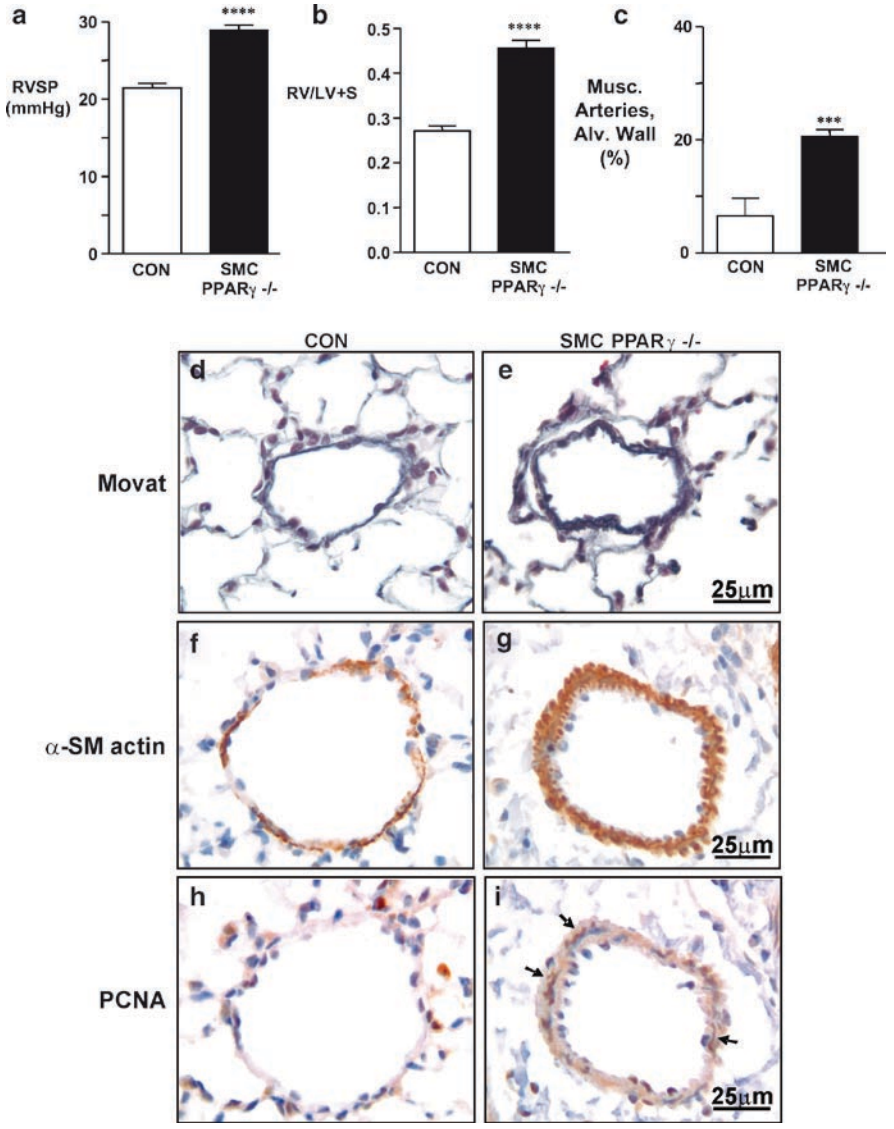

Fig. 29.7 PAH in mice with targeted deletion of PPARγ in SMCs. Thirteen- to 15-week-old mice underwent right ventricular (RV) catheterization, followed by organ harvest. (**a**) RVSP measurements. (**b**) Right ventricular hypertrophy (RVH), measured as ratio of the weight of the RV to that of the left ventricle (LV) plus septum (RV/LV + S). (**c**) Muscularization of alveolar wall arteries (Musc. Arteries Alv. Wall). (**d**) Representative photomicrographs of lung tissue (stained by Movat pentachrome) of 15-week-old mice showing a typical nonmuscular peripheral alveolar artery in a littermate control mouse. (**e**) A similar section in the *SM22α Cre PPARγflox/flox* (SMC PPARγ$^{-/-}$) mouse shows an alveolar wall artery surrounded by a rim of muscle. (**f–i**) Immunohistochemistry in serial lung tissue sections from littermate control (CON) and SMC PPARγ$^{-/-}$ mice stained for smooth muscle α-actin (α-SMA) (**f**, **g**) and proliferating cell nuclear antigen (PCNA; **h** and **i**). *Arrows* in (**i**) indicate enhanced PCNA staining in PASMCs. Bars represent mean ± SEM ($n = 5$). ***$P < 0.001$ vs. control; unpaired two-tailed t test. Reproduced with permission[51]

Studies have been carried out in which PPARγ agonists have been used to inhibit and reverse hypoxia-induced PAH. If is of interest that PPARγ agonists can completely reverse structural changes in response to chronic hypoxia but not the elevation in pulmonary arterial pressure.[52]

PPARγ agonists appear to act favorably to facilitate suppression of proliferation in PASMCs, but it will be important to determine whether they also support endothelial growth and stability because our studies in cultured cells suggest that certain agents could impede PPARγ-mediated endothelial gene regulation.

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