

## PULMONARY HYPERTENSION

PPAR $\gamma$  Activation: A Potential Treatment for Pulmonary HypertensionGeorg Hansmann<sup>1\*</sup> and Roham T. Zamanian<sup>2,3</sup>

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The pathobiology of pulmonary arterial hypertension (PAH) involves multiple molecular pathways and environmental modifiers and is characterized by progressive obliteration of pulmonary arterioles, leading to increased pulmonary vascular resistance (PVR), right heart failure, and death in  $\approx$ 40 to 60% of patients 5 years after diagnosis. There is emerging evidence that many key genes involved in PAH development are targets of the insulin-sensitizing transcription factor peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), and that pharmacological PPAR $\gamma$  activation would lead to their beneficial induction or repression and subsequent antiproliferative, anti-inflammatory, proapoptotic, and direct vasodilatory effects in the vasculature. PPAR $\gamma$  acts downstream of bone morphogenetic protein receptor II (BMP-RII), which is the cell surface receptor that is mutated or dysfunctional in many forms of PAH. Because our recent clinical observations indicate that insulin resistance may be an environmental risk factor or disease modifier ("second hit"), we suggest that PPAR $\gamma$ -activating agents might be beneficial in the future treatment of both insulin-resistant and insulin-sensitive PAH patients with or without BMP-RII mutations.

## INTRODUCTION

Pulmonary arterial hypertension (PAH) is characterized by progressive obliteration of small muscularized pulmonary arteries (arterioles), leading to increased pulmonary vascular resistance (PVR), right heart failure, and death. The pathobiology of PAH is complex and multifactorial: Pulmonary vascular remodeling includes structural and functional changes such as (i) (peri) vascular inflammation; (ii) endothelial cell (EC) damage and dysfunction; (iii) vasoconstriction; (iv) proliferation, migration, resistance to apoptosis, and survival of smooth muscle cells (SMCs), fibroblasts, and related cell types; (v) recruitment of progenitor cells; and (vi) crosstalks among vascular and circulating cells (1, 2). Therefore, it is unlikely that only one factor, pathway, or gene mutation (3) will explain all forms and cases. This underscores the need to explore several (frequently linked) signaling pathways and environmental modifiers of PAH. Because it is uncertain which pathway(s) are particularly active or inactive in an individual patient, tailored

PAH therapy is hardly possible. PAH biomarkers and validated imaging techniques indicating disease severity, progression, and prognosis of PAH and associated RV dysfunction would be extremely helpful in guiding established and more-experimental clinical therapies.

Currently, there are nine approved drugs for the treatment of PAH in adults in the United States, all of which are considered to be pulmonary vasodilators: endothelin-1 (ET-1) receptor antagonists, phosphodiesterase-5 (PDE-5) inhibitors, inhaled nitric oxide, prostanoids (PGI<sub>2</sub> analogs), and calcium channel blockers. However, none of the current medical therapies has been shown to be universally effective or able to reverse advanced pulmonary vascular disease. Promising drugs being explored for their potential to reverse PAH include platelet-derived growth factor receptor  $\beta$  (PDGFR- $\beta$ ) and epidermal growth factor receptor (EGFR) blockers; multi-kinase, elastase, and Rho-kinase (ROCK) inhibitors; mitochondrial metabolic modulators (dichloroacetate) (1, 2); and—as we suggest here—peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) agonists (4–6).

## GROWTH FACTOR SIGNALING IN SMCs AND ITS ROLE IN PAH

A hallmark of idiopathic (I) and heritable PAH (7) is enhanced signaling by growth

factors such as PDGF-BB, EGF, and transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) [reviewed in (1)]. PDGF-BB (through its receptor), PDGFR- $\beta$ , and EGF (via EGFR) activate mitogen-activated protein kinases (MAPKs) in vascular SMCs. Activated MAPKs such as extracellular-regulated kinase (ERK) induce cell cycle-promoting genes (encoding products such as cyclin D1/D2 and telomerase) and subsequently SMC proliferation, migration, resistance to apoptosis, and survival—all key features of pulmonary vascular remodeling (Figs. 1 and 2A). RhoA/ROCK pathways downstream of the aforementioned growth factors are also involved in the pathogenesis of PAH (2). ROCK is activated by angiotensin II and receptor tyrosine kinases (RTKs) such as PDGFR- $\beta$  and EGFR and participates in subsequent MAPK phosphorylation and SMC proliferation [reviewed in (8)].

Imatinib (STI571), a compound used to treat chronic myeloid leukemia, blocks the activity of the RTKs PDGFR- $\beta$ , c-Kit, and Bcr-Abl but hardly affects the EGFR (also an RTK). Hence, a drug target downstream of PDGFR- $\beta$  and EGFR that when activated inhibits MAPK activity and other growth-promoting pathways might be of additional and even greater benefit than RTK blockers, and be able to arrest or reverse advanced clinical PAH [Fig. 2B and supporting online material (SOM)].

## ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS IN VASCULAR BIOLOGY

PPARs ( $-\alpha$ ,  $-\beta$ ,  $-\gamma$ , and  $-\delta$ ) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. PPAR $\gamma$  is ubiquitously expressed and plays a major role in adipogenesis, glucose metabolism, and placental and cardiac development. PPAR $\gamma$  is an established vasoprotective transcription factor in systemic atherosclerotic diseases (9); however, its role in pulmonary vascular disease has not been explored in comparable depth. Upon ligand activation, PPAR $\gamma$  heterodimerizes with the retinoid X receptor and regulates multiple target genes, such as those encoding adiponectin, interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), ET-1, and the endogenous endothelial nitric oxide (NO) synthase (eNOS) inhibitor asymmetric dimethyl-arginine (ADMA), many of which are strongly implicated in the pathobiology of PAH (SOM). The antidiabetic drugs pioglitazone and rosiglitazone, both PPAR $\gamma$  li-

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gands of the thiazolidinedione (TZD) class, have anti-inflammatory properties through PPAR $\gamma$ -dependent and -independent effects in macrophages, adipocytes, and vascular cells (9). In addition, PPAR $\gamma$  ligands exert antiproliferative, proapoptotic, and direct vasodilatory effects (10) in the vasculature and improve endothelial dysfunction and systemic arterial hypertension in vivo (9). PPAR $\gamma$  agonists therefore have therapeutic potential beyond the treatment of insulin resistance (IR).

### MULTIPLE BENEFICIAL EFFECTS OF PPAR $\gamma$ ACTIVATION IN PULMONARY VASCULAR DISEASE

The unique potential of PPAR $\gamma$  agonists for the treatment of pulmonary vascular disease relates to the fact that many of its downstream targets are positively or negatively associated with the development of PAH.

**PPAR $\gamma$ : A drug target downstream of bone morphogenetic protein receptor II.** Heritable PAH includes nonfamilial PAH associated with germline mutations in the *bone morphogenetic protein receptor II (BMP-RII)* gene and familial cases with or without identified germline mutations. Loss-of-function mutations in the *BMP-RII* gene occur in up to 70% of PAH patients with a positive family history of PAH, in 10 to 40% of PAH patients without a family history of the disease (3, 7), and in 6 to 9% of patients with secondary forms of PAH associated with anorexic drug use or congenital heart defects. However, the inheritance pattern of *BMP-RII* is that of a dominant gene with low penetrance, in that only  $\approx$ 10 to 20% of affected family members develop the disease (3). This observation underscores the importance of environmental modifiers such as IR that could potentiate BMP-RII dysfunction, thereby triggering or worsening PAH (4, 6). Abnormalities in downstream effectors of BMP-RII probably contribute to PAH development.

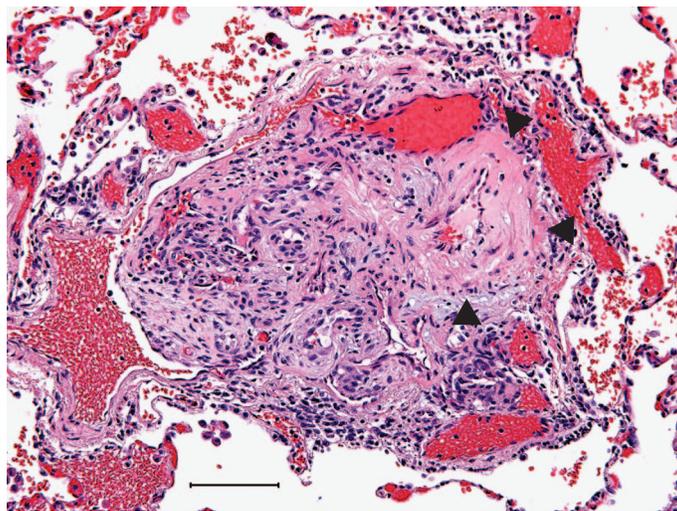
Recently, we demonstrated that BMP-2, an antiproliferative ligand of the crucial cell-surface receptor BMP-RII, induces nuclear shuttling and DNA binding of PPAR $\gamma$  in human pulmonary artery SMCs (PASMCs)

(5). Previous work by others showed that PAH patients have reduced pulmonary expression of *BMP-2* (11), *PPAR $\gamma$*  (12), and *apolipoprotein E (apoE)* (11) mRNAs. We found that all three factors are linked and demonstrated an antiproliferative BMP-2/PPAR $\gamma$ /apoE axis in human and murine PASMCs (5) (Fig. 2B): (i) Using small hairpin RNA interference and transgenic techniques, the antiproliferative effect of BMP-2 was shown to be BMP-RII-, PPAR $\gamma$ -, and apoE-dependent (4, 5); (ii) pharmacological PPAR $\gamma$  activation inhibited PDGF-BB-induced ERK phosphorylation and completely blocked PDGF-BB-induced proliferation of PASMCs isolated from a PAH patient with a frameshift mutation in the *BMP-RII* gene; and (iii) deletion of PPAR $\gamma$  in vascular SMCs caused PAH in vivo (5).

These findings suggest that BMP-RII dysfunction decreases endogenous PPAR $\gamma$  activity and enhances PDGF-BB/MAPK (e.g., ERK) pathways and associated vascular remodeling. Hence, a strategy aimed

at activating PPAR $\gamma$  could reverse the PAH phenotype (4). By blocking important survival pathways downstream of activated PDGFR- $\beta$  [such as those regulated by phosphatidylinositol 3-kinase (PI3K)], PPAR $\gamma$  agonists also induce apoptosis of proliferating SMCs (9) (Fig. 2B). In addition, fluid shear stress reduces PPAR $\gamma$  expression in ECV304 ECs. When transfected with a dominant negative version of PPAR $\gamma$  and injected into the tail vein of nude mice, ECV304 cells formed lumen-obliterating pulmonary vascular lesions (12). Hence, decreased PPAR $\gamma$  expression appears to characterize an abnormal, proliferating, apoptosis-resistant EC phenotype that may be reversed by pharmacological PPAR $\gamma$  activation.

**PPAR $\gamma$ , apoE, and adiponectin counterbalance growth factor-mediated vascular remodeling.** The PPAR $\gamma$  targets apoE and adiponectin inhibit PDGF-BB-induced PASMC proliferation, and deficiency of either target protein has been linked to PAH (4, 5, 15, 16). ApoE promotes the internalization of PDGFR- $\beta$  (17), and adiponectin sequesters the ligand PDGF-BB (18) (Fig. 2B). Both BMP-2 and a PPAR $\gamma$  agonist also induce apoE expression and secretion, thereby impairing PDGF-BB/MAPK signaling (i.e., ERK phosphorylation) (5) in human PASMCs. Activated PPAR $\gamma$  also blocks PDGF gene expression (19) and induces low-density lipoprotein receptor-related protein (LRP) (20), the receptor necessary for apoE-mediated suppression of PDGF-BB signaling (17) (Fig. 2B). Additional counteractive interactions between PPAR $\gamma$  and phosphoERK exist intracellularly; these include both the activation of phosphatases and the prevention of nuclear translocation in SMCs, and the phosphorylation of PPAR $\gamma$  at its N terminus that leads to its inactivation (Fig. 2; for details see the legend and SOM). Furthermore, transgenic mice with a targeted deletion of PPAR $\gamma$  in ECs and macrophages (Tie 2 Cre PPAR $\gamma^{\text{flox/flox}}$ ) have significantly higher pulmonary PDGFR- $\beta$  protein expression than littermate controls (21). Thus, decreased levels of PPAR $\gamma$ , apoE, and adiponectin are expected to enhance PDGF-BB-pERK signaling and pulmonary vascular remodeling (Fig. 2).

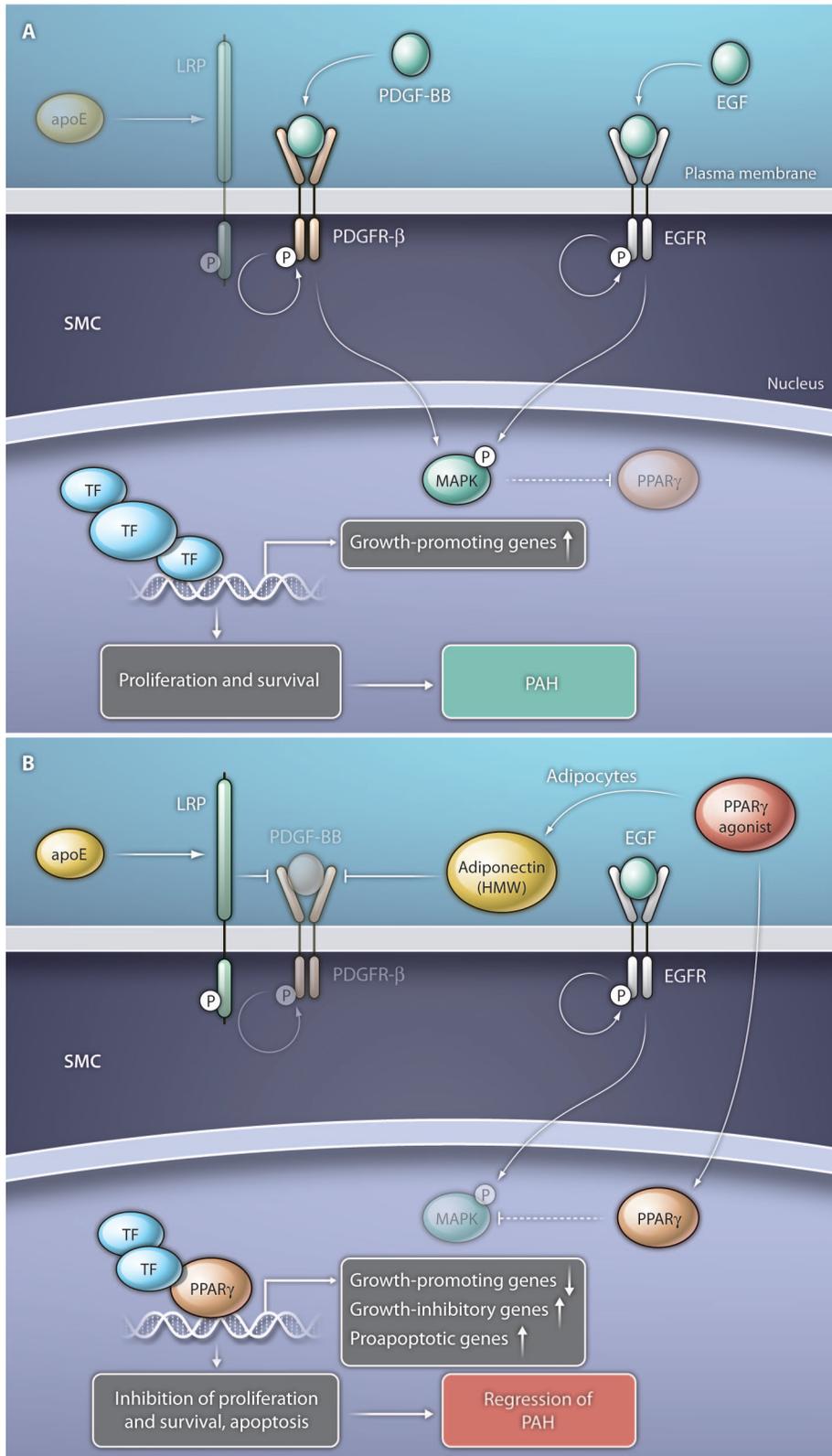


**Fig. 1.** Plexiform lesion in advanced pulmonary arterial hypertension. The pulmonary artery (arrowheads) is eccentrically expanded by cell proliferation surrounding slit-like and dilated vascular channels. The residual artery shows medial and intimal thickening (hematoxylin and eosin stain; scale bar, 50  $\mu$ m).

at activating PPAR $\gamma$  could reverse the PAH phenotype (4).

**PPAR $\gamma$ -mediated cell-cycle regulation and apoptosis.** PPAR $\gamma$  activation ultimately inhibits the G $_1$ →S-phase transition that is mandatory for cell cycle progression and vascular SMC proliferation, e.g., by stabilizing the cyclin-dependent kinase inhibi-

tor p27<sup>KIP1</sup> (13) or inhibiting telomerase (14). By blocking important survival pathways downstream of activated PDGFR- $\beta$  [such as those regulated by phosphatidylinositol 3-kinase (PI3K)], PPAR $\gamma$  agonists also induce apoptosis of proliferating SMCs (9) (Fig. 2B). In addition, fluid shear stress reduces PPAR $\gamma$  expression in ECV304 ECs. When transfected with a dominant negative version of PPAR $\gamma$  and injected into the tail vein of nude mice, ECV304 cells formed lumen-obliterating pulmonary vascular lesions (12). Hence, decreased PPAR $\gamma$  expression appears to characterize an abnormal, proliferating, apoptosis-resistant EC phenotype that may be reversed by pharmacological PPAR $\gamma$  activation.



**Fig. 2.** Model of how PPAR $\gamma$  activation might reverse pulmonary arterial hypertension (PAH). **(A)** Heightened PDGF-BB and EGF signaling leading to smooth muscle cell (SMC) proliferation and survival are key features of PAH. Deficiency of both apoE and LRP enhances mitogenic PDGF-BB–MAPK signaling that turns on the cell-cycle machinery, for example, expression of NOR1 and cyclin D1/D2, and induces other growth-promoting genes. PDGF-BB–/PDGFR- $\beta$ –mediated phosphorylation of MAPKs such as ERK has been shown to lead to N-terminal phosphorylation and thereby inactivation of PPAR $\gamma$ . **(B)** PPAR $\gamma$  activation induces growth-inhibitory and proapoptotic genes in SMCs and inhibits cell-cycle-promoting genes such as those encoding telomerase, cyclin D1, and retinoblastoma protein. Moreover, PPAR $\gamma$  induces phosphatases that can directly inactivate MAPKs (such as pERK) downstream of PDGFR- $\beta$  and EGFR. In addition, PPAR $\gamma$  activation can directly inhibit PDGF-BB–mediated pERK activity by blocking its nuclear translocation. Besides gene regulation in SMCs, PPAR $\gamma$  agonists induce the antimitogenic adipocytokine adiponectin, which [in its high molecular weight (HMW) form] sequesters the ligand PDGF-BB (“vasocrine signaling from fat cells”). Thus, decreased levels of PPAR $\gamma$ , apoE, and adiponectin are expected to enhance PDGF-BB/pERK signaling and pulmonary vascular remodeling. By blocking important survival pathways downstream of activated PDGFR- $\beta$  (such as PI3K), PPAR $\gamma$  agonists might also lead to SMC apoptosis. Therefore, PPAR $\gamma$  agonists have the potential to reverse SMC proliferation and vascular remodeling in PAH patients. TF, transcription factor.

op IR and severe PAH. A 4-week treatment with the PPAR $\gamma$  agonist rosiglitazone led to an eightfold induction of plasma adiponectin, improved insulin sensitivity, and complete regression of PAH (4) (Fig. 2B). Thus, PPAR $\gamma$  target genes other than *apoE* (e.g., the *adiponectin* gene) are also implicated in the antiremodeling and anti-inflammatory effects of PPAR $\gamma$  agonists (4, 5, 15, 16). Moreover, adiponectin is an endogenous antithrombotic factor (22). Hence, induction of adiponectin might be beneficial in pulmonary hypertension, even in the secondary forms associated with chronic thromboembolism, or parenchymal lung disease/chronic alveolar hypoxia (23).

**PPAR $\gamma$ , detrimental adipocytokines, endothelin-1, and the NO pathway.** Yudkin and colleagues (24) previously proposed that detrimental adipocytokines, such as tumor necrosis factor- $\alpha$  and IL-6, are secreted from perivascular fat cells and inhibit the eNOS pathway of insulin signaling, leaving unopposed vasoconstriction

FIGURE CONCEPT: G. HANSMANN, ILLUSTRATION: C. BICKEL/SCIENCE

Male apoE-deficient mice (*apoE*<sup>-/-</sup>), when fed a high-fat diet, do not up-regulate the insulin sensitizers adiponectin and leptin (in contrast to control mice), but devel-

mediated by endothelium-derived ET-1, a key player in PAH. PPAR $\gamma$  agonists would counteract these deleterious effects as they induce eNOS, and impair the expression of thrombin, ET-1, and the endogenous NO synthase inhibitor ADMA, thereby potentially reversing endothelial dysfunction. Increased pulmonary ET-1 expression and elevation of the ADMA plasma concentration were observed in PAH patients, and increased ADMA concentrations negatively correlated with cardiac index and human survival rates (25).

**Endothelial progenitor cells, PDE inhibitors, and PPAR $\gamma$  agonists.** Adult IPAH patients have reduced numbers of endothelial progenitor cells (EPCs) as compared with healthy controls; a reduced number of circulating EPCs correlated with poor hemodynamics and abnormal concentrations of inflammatory markers and ADMA. Treatment with sildenafil, a vasodilatory PDE-5 inhibitor commonly used for PAH therapy, led to a dose-dependent rise in EPC numbers that was greater than those found with other therapies (26). Intriguingly, rosiglitazone inhibited the negative effects of C-reactive protein (CRP) on EPC survival, differentiation, and function in a separate study (27), and transplantation of autologous EPCs improved mean pulmonary arterial pressure, PVR, cardiac output, and the distance walked in 6 min in patients with IPAH (28). Hence, sildenafil and PPAR $\gamma$  agonists might represent a pharmacological means of increasing circulating EPCs in PAH patients that probably can improve clinical outcome.

**PPAR $\gamma$  activation suppresses vascular and perivascular inflammation.** Circulating proinflammatory cytokines (e.g., IL-6 and MCP-1) that are mainly derived from monocytes and macrophages and regulated by nuclear factor kappa B (NF- $\kappa$ B), nuclear factor of activated T cells, and other transcription factors, substantially contribute to the development and/or progression of PAH (1, 2). TZDs reduce the activation and inflammation of ECs by suppressing the activity of NF- $\kappa$ B and activator protein-1 via PPAR $\gamma$ -dependent and -independent mechanisms. The overall result is an impaired production of chemokines, adhesion molecules, reactive oxygen species, and major histocompatibility complex class II proteins, which are crucial for T cell activation and initiation of an immune response (9). PPAR $\gamma$  agonists inhibit DNA binding of NF-

$\kappa$ B (29) and NF- $\kappa$ B-dependent matrix metalloproteinase (MMP)-9 activation in vascular SMCs (30), which are both implicated in PAH development (31). Thus, PPAR $\gamma$  agonists have the potential to counteract proinflammatory NF- $\kappa$ B pathways and associated MMP-9 hyperactivity in PAH patients.

**PPAR $\gamma$ -mediated inhibition of extracellular matrix proteinases.** PPAR $\gamma$  ligands impair MMPs that are activated by elastase. Elastase inhibitors not only prevent but also reverse advanced fatal PAH in rats (32). Rosiglitazone has been shown to activate glycogen synthase kinase-3 $\beta$  in vascular SMCs, which inhibits proliferation and migration by blocking NF- $\kappa$ B-dependent MMP-9 activation (30). Hence, along with elastase inhibitors such as elafin, PPAR $\gamma$  agonists could block the detrimental PAH-promoting effects of heightened elastase and MMP activity.

**Rho kinase inhibition by PPAR $\gamma$ .** Besides the induction of SMC proliferation (see above), RhoA/ROCK activation leads to endothelial dysfunction, induction of vasoactive cytokines via the NF- $\kappa$ B pathway, and chronic pulmonary remodeling that may involve up-regulation of TGF- $\beta$ 1 and down-regulation of the cell-cycle inhibitor p27<sup>KIP1</sup>. Pioglitazone improves endothelial dysfunction in the presence or absence of inflammation and inhibits ROCK pathways in vascular SMCs—the latter through up-regulation of the cytosolic protein tyrosine phosphatase SHP-2 (33). Thus, PPAR $\gamma$  agonists might provide antimitogenic ROCK inhibition, which has been effective in experimental and clinical PAH [reviewed in (8)].

#### THE LINK BETWEEN IR AND PAH

Low adiponectin and high CRP and IL-6 levels are associated with an increased risk for IR, metabolic syndrome, and systemic and possibly also pulmonary vascular disease in humans. ET-1 might be a key component of this pathobiology, because it inhibits adiponectin secretion and insulin sensitivity in healthy humans. We recently demonstrated that IR and dyslipidemia are more common in female PAH patients than in the general population (45.7% versus 21.5%;  $P < 0.001$ ) and may be environmental risk factors or disease modifiers that might increase the incidence of PAH in the upcoming years. The presence of IR was associated with poorer combined 6-month event-free survival from RV failure, transplantation, or death (58% versus 79%,

hazard ratio 2.57, 95% confidence interval 1.03-6.06;  $P < 0.05$ ) (6). Based on our preliminary experimental (4) and clinical data (6), we hypothesize that IR, dyslipidemia, and dysregulation of ET-1- and PPAR $\gamma$ -dependent factors such as adiponectin may increase the susceptibility to PAH that can be reversed by PPAR $\gamma$  activation. However, it is important to note that the beneficial effects of PPAR $\gamma$  agonists in the pulmonary (4, 23, 34) and systemic circulation (9) do not depend on the presence of IR. Recently, Walcher *et al.* demonstrated a very rapid effect of single-dose rosiglitazone treatment on endothelial function in nondiabetic healthy men, underscoring the fact that PPAR $\gamma$  agonists exhibit direct vascular (vasodilatory) effects (10).

#### PPAR $\gamma$ AGONISTS FOR THE TREATMENT OF PAH: TRANSLATION INTO CLINICAL PRACTICE

Prospective studies are needed to address whether IR, dyslipidemia, impaired PPAR $\gamma$  and apoE function, and low adiponectin levels are risk factors for the development or progression of PAH in humans. Most importantly, randomized controlled trials (RCTs) should investigate the potential of Food and Drug Administration-approved PPAR $\gamma$  agonists in the treatment of PAH in the presence or absence of IR. [For a discussion of the safety of PPAR $\gamma$  agonists (TZDs), see the SOM.] The molecular links and mechanisms that underlie IR, apoE, and adiponectin levels, PPAR $\gamma$  function, and known PAH pathways should also be explored. By applying advanced technologies such as proteomics and metabolomics, biomarkers might be found that will aid in (i) monitoring PAH progression and response to therapy, (ii) tailoring pharmacotherapy, and (iii) PAH screening, potentially, in certain at-risk populations. In addition, it will be important to study the prevalence and clinical relevance of genetic polymorphisms of PPARG 1/2 and PPAR $\gamma$  target genes in PAH patients.

#### INTERDISCIPLINARY INSIGHTS FROM STUDYING PPAR $\gamma$ FUNCTION IN THE PULMONARY CIRCULATION

Several RCTs currently investigate the therapeutic potential of pioglitazone in proliferative-inflammatory lung diseases other than PAH, such as non-small-cell lung cancer and asthma (35). The magnitude of these very recent research efforts underlines the potential of PPAR $\gamma$ -activating

drugs beyond diabetes therapy. The anti-proliferative BMP-2/PPAR $\gamma$ /apoE axis in SMCs (5) probably exists in other cell types and very likely has additional proapoptotic and anti-inflammatory properties, given that the key player PPAR $\gamma$  affects multiple genes besides *apoE* (9). Thus, the aforementioned translational studies on the protective role of PPAR $\gamma$  in PAH will have substantial impact on our understanding and future treatment of other emerging lung and collagen vascular diseases, as well as cancer, all of which are characterized by uncontrolled proliferation, inflammation, and resistance to apoptosis. Moreover, a connection between PPAR $\gamma$  and apoE has been made in patients with Alzheimer's disease, in that the improvement of cognitive function with rosiglitazone is not apparent in patients who carry the *APOE* epsilon 4 allele (36). We speculate that disruption of BMP-RII-PPAR $\gamma$  and PPAR $\gamma$ -apoE signaling might underlie many different pathologic processes.

## CONCLUSIONS

Taken together, emerging evidence indicates that multiple key genes involved in the pathobiology of PAH are targets of PPAR $\gamma$  and that pharmacological activation of PPAR $\gamma$  would lead to their beneficial induction and stabilization (genes encoding eNOS, p27<sup>KIP1</sup>, adiponectin, apoE, and LRP) or repression (genes encoding PDGF, MAPK, cyclin D1, telomerase, ET-1, ADMA, IL-6, NF- $\kappa$ B, elastase/MMP, and RhoA/ROCK). We suggest that PPAR $\gamma$  agonists might reverse pulmonary vascular remodeling in PAH patients with or without BMP-RII dysfunction and that IR might be a risk factor or disease modifier (so-called "second hit"). Hence, future treatment of both insulin-resistant and insulin-sensitive PAH patients might include PPAR $\gamma$  agonists or selective PPAR $\gamma$  modulators. RCTs on the use of these agents in pulmonary vascular disease are urgently needed.

## SUPPORTING ONLINE MATERIAL

www.sciencetranslationalmedicine.org/cgi/content/full/1/12/12ps14/DC1  
SOM Text  
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## Supplementary Materials for

### **PPAR $\gamma$ Activation: A Potential Treatment For Pulmonary Hypertension**

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#### **This PDF file includes:**

Supplementary Text  
References

## **SUPPLEMENTARY MATERIAL**

Imatinib (STI571), a compound used to treat chronic myeloid leukemia, blocks the activity of the receptor tyrosine kinases (RTK) PDGFR- $\beta$ , c-Kit and Bcr-Abl, but hardly affects EGFR (also an RTK) (S1). A recently completed phase II randomized controlled trial (RCT) on the RTK inhibitor imatinib as add-on therapy for severe PAH failed to show differences in exercise capacity but improved pulmonary vascular resistance (S2). Some PAH patients do not respond to PDGFR- $\beta$  blockade by imatinib (S3), probably due to ongoing signaling through EGF and other mitogens. Hence, a drug target downstream of PDGFR- $\beta$  and EGFR that--when activated--inhibits MAPK activity and other growth-promoting pathways, might be of additional and even greater benefit than RTK blockers, and able to arrest or reverse advanced clinical PAH (see Fig. 2, main manuscript).

### **Role of Peroxisome Proliferator-Activated Receptors in Vascular Biology**

(...) Upon ligand activation, PPAR $\gamma$  heterodimerizes with the retinoid X receptor and regulates multiple target genes, e.g., those encoding adiponectin (S4-8), interleukin 6 (IL-6) (S9-12), monocyte chemoattractant protein-1 (MCP-1, also known as CCL2) (S13-16), ET-1 (S17-19) and the endogenous endothelial NO synthase (eNOS) inhibitor asymmetric dimethyl-arginine (ADMA) (S20, S21), many of which are strongly implicated in the pathobiology of PAH. (...)

## **Multiple Beneficial Effects of PPAR $\gamma$ Activation In Pulmonary Vascular Disease**

*(...) PPAR $\gamma$ , Apolipoprotein E (ApoE), and Adiponectin (APN) Counterbalance Growth Factor-Mediated Vascular Remodeling*

(...) PPAR $\gamma$  has been shown to activate phosphatases (S22) and prevent ERK phosphorylation in vascular SMC (S23, S24). In addition, PPAR $\gamma$  activation can directly inhibit PDGF-BB-mediated pERK activity by blocking its nuclear translocation (S25). Conversely, PDGF-BB-/PDGFR- $\beta$ -mediated ERK phosphorylation leads to phosphorylation and thereby inactivation of PPAR $\gamma$  at its N-terminal (S26). Furthermore, transgenic mice with targeted deletion of PPAR $\gamma$  in ECs and macrophages (Tie 2 Cre PPAR $\gamma^{\text{flox/flox}}$ ) have significantly higher pulmonary PDGFR- $\beta$  protein expression than littermate controls (S27). Thus, decreased levels of PPAR $\gamma$ , apoE, and adiponectin are expected to enhance PDGF-BB-/pERK-signaling and pulmonary vascular remodelling (see Fig. 2, main manuscript).

## **Differential Safety Profile of PPAR $\gamma$ Agonists (TZDs)**

Currently, clinical data on the potential adverse effects of the TZDs rosiglitazone and pioglitazone are almost exclusively based on randomized controlled trials (RCTs) and meta-analyses investigating add-on therapy of either drug to oral glucose and lipid lowering medications in diabetic adults with cardiovascular co-morbidities. Particularly, the safety profile of rosiglitazone is the subject of an ongoing debate (S28-33). A systematic but limited meta-analysis of 42 trials reported a higher incidence of myocardial infarction (MI) and cardiovascular death in diabetic patients taking rosiglitazone (S28) (note: adverse event rate <1%; absolute

difference in MI rates <0.1%), whereas RECORD, a large RCT designed to study cardiovascular outcomes in diabetic patients, did not show such an association with rosiglitazone, but did report higher rates of non-fatal congestive heart failure (CHF), and limb fractures (mainly in women) (S32). A meta-analysis of 4 large RCTs with at least 12 months follow-up did find an association between rosiglitazone use and MI but not with cardiovascular mortality (29). However, there is no evidence that the other FDA-approved TZD, pioglitazone, is associated with higher MI rates as proposed for rosiglitazone (i.e., no TZD-class effect): In fact, a recent meta-analysis (n=16,390) shows that pioglitazone reduces macrovascular events including MI in patients with type 2 diabetes, and does not increase the incidence of death from CHF (34). In several but not all (S35) studies, pioglitazone was associated with mild to moderate, non-fatal CHF (meta-analysis: 200 vs. 138 of a total of 16,390 patients (S34))--an adverse event also observed with rosiglitazone (S29, S32). CHF in high risk (i.e., diabetic) patients given TZDs did not increase mortality and probably does not carry the risk of progressive left ventricular (LV) dysfunction (S36). At present, it is unclear whether direct renal (rather than cardiodepressant) effects of TZDs are the major cause of the fluid retention/peripheral edema seen in (diabetic) patients receiving either rosiglitazone or pioglitazone. The renal collecting duct appears to be the major site for increased fluid reabsorption in response to rosiglitazone or pioglitazone (S37), but it is controversial whether the underlying mechanisms involve altered activity of the epithelial sodium channel (ENaC) (S38).

It is also unknown whether the forementioned adverse effects of TZDs could be decreased in frequency or quantity by simply reducing the drug dose. Both fluid retention and heart failure observed with TZDs such as pioglitazone are reversible and do not appear to negate the beneficial effects of the drug on irreversible ischemic and fatal end points (S34). Nevertheless,

TZDs should be avoided in patients with significant preexisting LV dysfunction or chronic renal insufficiency.

Pioglitazone has more favourable effects on serum lipids than does rosiglitazone (S39, S40) and a large RCT on patients with existing macrovascular disease suggested that treatment with pioglitazone prevents cardiovascular events (S41). A recent population-based cohort study explored adverse cardiovascular events in 39,736 patients  $\geq$  66 years old during treatment with either pioglitazone or rosiglitazone (S33): During the six year study period, the primary composite outcome of death or hospital admission for either acute MI or CHF was reached in 895 (5.3%) of patients taking pioglitazone and 1563 (6.9%) of patients taking rosiglitazone. After extensive adjustment for demographic and clinical factors and drug doses, pioglitazone treated patients had a lower risk of developing the primary outcome than did patients treated with rosiglitazone (adjusted hazard ratio 0.83, 95% confidence interval 0.76 to 0.90). Juurlink *et al.* concluded that pioglitazone is associated with a significantly lower risk of heart failure and death than is rosiglitazone among older patients with diabetes (S33). A meta-analysis of 10 RCTs involving 13,715 participants and from 2 observational studies involving 31,679 participants (S42), demonstrate that both rosiglitazone and pioglitazone increase the risk of peripheral bone fractures in older diabetic patients -- mainly in women and increasingly with age (S42, S43).

Most importantly, the absolute cardiovascular risk attributed to PPAR $\gamma$  agonists of the TZD class is probably lower ( $\ll$ 1%) in PAH (vs. diabetic) patients who generally are less than 60 years old, have preserved LV function, normal blood pressure and blood glucose (S44). High dose pioglitazone used to treat non-diabetic children with autistic spectrum disorders had no significant cardiovascular adverse effects (weight gain and periorbital edema in 3-5%) (S45). In

line with this view, Hori et al. (2005) (S46) demonstrated that pioglitazone improves LV diastolic function in hypertensive patients without overt diabetes mellitus: the drug had no side effects except mild leg edema. Interestingly, the observed increase in plasma adiponectin concentrations after 6 months of treatment (+270%) correlated with echocardiographic improvement of LV diastolic function (S46). We speculate that pioglitazone/adiponectin-induced RV afterload-reduction and the drug's direct effects on the myocardium might improve RV function in future RCTs on PAH.

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