PPARγ as a potential therapeutic target in pulmonary hypertension

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Abstract: Pulmonary hypertension (PH) is a progressive disorder of the pulmonary circulation associated with significant morbidity and mortality. The pathobiology of PH involves a complex series of derangements causing endothelial dysfunction, vasoconstriction and abnormal proliferation of pulmonary vascular wall cells that lead to increases in pulmonary vascular resistance and pressure. Recent evidence indicates that the ligand-activated transcription factor, peroxisome proliferator-activated receptor gamma (PPARγ) can have a favorable impact on a variety of pathways involved in the pathogenesis of PH. This review summarizes PPARγ biology and the emerging evidence that therapies designed to activate this receptor may provide novel approaches to the treatment of PH. Mediators of PH that are regulated by PPARγ are reviewed to provide insights into potential mechanisms underlying therapeutic effects of PPARγ ligands in PH.

Keywords: PPARγ, pulmonary hypertension

Introduction
Pulmonary hypertension (PH) is a progressive disorder defined by an elevation in mean pulmonary artery (PA) pressure above 25 mmHg at rest that is not associated with elevation of the pulmonary capillary wedge pressure. In 2002, over 15,000 deaths and 260,000 hospital visits were attributed to PH [Hyduk et al. 2005]. Existing treatments for patients with PH include prostacyclin and its analogs, endothelin receptor antagonists, and phosphodiesterase type 5 inhibitors. Two recent meta-analyses examining the efficacy of these treatments arrived at different conclusions and determined that current therapies either fail [Macchia et al. 2007] or succeed [Galie et al. 2009] in reducing mortality in PH patients. Regardless of these conflicting results, the prognosis in PH remains poor, and there remains an urgent need for additional studies to enhance our understanding and treatment of this disorder. This review focuses on peroxisome proliferator-activated receptor gamma (PPARγ) as a novel target that regulates alterations in gene expression that contribute to increased vascular tone and vascular remodeling in PH.

Excellent reviews of the pathobiology of PH report that constriction of the pulmonary vasculature and abnormal proliferation of pulmonary vascular cells result in remodeling of small arterioles and increasing pulmonary vascular resistance [Hassoun et al. 2009; Morrell et al. 2009; Rabinovitch, 2008]. In the past 30 years, growing interest in the pathogenesis and management of PH has led to a more refined but still incomplete understanding of PH. For example, until recently, PH was classified into two main categories: primary and secondary PH. Primary PH was often referred to as familial or idiopathic pulmonary hypertension and considered sporadic in onset, whereas secondary PH was associated with other vascular disorders or chronic diseases [Humbert et al. 2004]. However, since 2003, the classification of PH has been refined in an attempt to create a more encompassing classification system that would permit investigators to more accurately group patients with similar pathogenesis and pathology for clinical trials [Simonneau et al. 2009] (for a review, see Hoeper [2009]). In the most recent categorization, category I includes pulmonary arterial hypertension (PAH) due to idiopathic or heritable disorders, drug and toxin-induced PAH, PAH associated with connective tissue diseases, HIV infection, portal hypertension, schistosomiasis, or chronic hemolytic anemias, persistent pulmonary hypertension of the newborn, and pulmonary veno-occlusive disease or pulmonary
capillary hemangiomatosis. Category II PH results from left-sided heart disease. Category III includes PH associated with hypoxemia or chronic lung disease. Category IV PH results from chronic thrombotic or embolic disease. Finally, category V includes PH associated with disorders such as sarcoidosis, histiocytosis X, lymphangiomatosis or diseases causing compression of the great vessels such as fibrosing mediastinitis. This classification scheme emphasizes not only the diversity of conditions that are associated with PH but also the challenges inherent in identifying pathogenic pathways or mediators common to all of these disorders and in successfully targeting these pathways and mediators for therapeutic advantage. This review focuses on one such potential therapeutic target, peroxisome proliferator-activated receptor gamma (PPARγ) and the mounting evidence for its role in pulmonary vascular biology.

**PPAR biology**

PPARs were originally described in 1990 as ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily that includes retinoid acid receptors, thyroid hormone receptors and steroid receptors [Laudet et al. 1992; Issmann and Green, 1990]. PPARs are ubiquitously expressed throughout the body and are composed of three distinct isotypes: α, β/δ and γ. These three subclasses possess similar structural and functional features but are distinguished by their tissue distribution, ligand specificity and regulation of unique target genes.

The human PPARγ gene is located at chromosome 3p25 and produces four different PPARγ mRNAs. PPARγ1, PPARγ3 and PPARγ4 encode the same protein and PPARγ2 has an additional 30 amino acids at the N terminus [Tontonoz et al. 1994]. PPARγ1 is expressed ubiquitously, PPARγ2 is predominantly expressed in adipose tissue, PPARγ3 is expressed in macrophages and white adipose tissue and tissue expression of PPARγ4 has not been determined [Braissant et al. 1996]. Like all other PAR subtypes, activation of PPARγ complexes is promoted by structurally diverse endogenous and exogenous ligands. Endogenous ligands include linoleic acid, 15d-PGJ2, and oxidized lipids such as 15-HETE, 9-HODE and 13-HODE [Kota et al. 2005; Forman et al. 1996]. Synthetic ligands for the PPARγ receptor include compounds in the thiazolidinedione (TZD) class of insulin sensitizing drugs such as troglitazone, rosiglitazone, ciglitazone, pioglitazone and englitazone. Troglitazone, rosiglitazone and pioglitazone have been employed clinically in the USA in the management of type 2 diabetes.

Despite promiscuity for activating ligands and broad tissue distribution, the specificity of PPARγ-mediated effects occurs through recruitment of ligand-specific populations of coactivators and corepressors [Camp et al. 2000; Kodera et al. 2000; Olefsky and Saltiel, 2000]. Ligands produce conformational changes in the PPARγ receptor. The ligand-induced conformational change allows PPARγ to heterodimerize with retinoid X receptor (RXR). The PPAR/RXR complex binds to DNA at PPAR response elements (PPREs) located in the promoter region of susceptible genes. The cellular PPAR response is heavily dependent on the recruitment or release of coactivators and corepressors [Zhu et al. 2000; Mizukami and Taniguchi, 1997]. Coactivator proteins either possess or recruit proteins with histone acetyltransferase activity. Acetylation of the histone proteins facilitates RNA polymerase binding by altering chromatin structure thereby facilitating transcription of the target gene [Chen et al. 2006]. PPARs can also repress gene expression through transrepression mechanisms. Potential mechanisms of transrepression that have been reported include: (1) PPARγ-stimulated nuclear export and inhibition of p65 and (2) ligand-dependent SUMOylation (small ubiquitin-like modifier) of the PPARγ binding domain which targets PPARγ to nuclear corepressors and prevents their proteosomal degradation and clearance from the promoters of regulated genes [Pascual et al. 2005; Kelly et al. 2004]. In general, transrepression of proinflammatory transcription factors, such as NF-κB and AP-1, is believed to be responsible for many of the anti-inflammatory effects of PPARγ. Thus, PPARγ activation can either stimulate or inhibit the expression of selected genes through several discreet mechanisms.

**Role of PPARγ in pulmonary hypertension**

PPARγ is normally expressed in many cell types in the lung including those of the pulmonary vascular wall such as endothelial and smooth muscle cells. Mounting evidence suggests that loss of PPARγ expression and function may be associated with PH while stimulating this receptor may attenuate PH. These studies are reviewed here followed by a consideration of the mechanisms by which PPARγ regulates PH. Any data
obtained from animal models are subject to a number of limitations that limit their translation to the clinical condition. These limitations of available animal models of PAH have been recently and comprehensively reviewed [Stenmark et al. 2009]. In general, common rodent models of PH caused by stimuli such as hypoxia or monocrotaline fail to induce the proliferative plexogenic arteriopathy seen in patients with severe World Health Organization (WHO) Category I PAH. Despite these limitations, animal models, such as hypoxia-induced PH in mice, have and will continue to provide new insights into the pathobiology and treatment of PH. On the other hand, hypoxia-induced PH in mice likely bear considerably more relevance to pulmonary hypertension in patients with WHO Category III pulmonary hypertension caused by hypoxia secondary to lung disease, sleep apnea, or living at altitude.

**Reduced PPARγ expression is associated with pulmonary hypertension**

PH is characterized by complex precapillary arteriolar plexiform lesions [Edwards and Edwards, 1977]. Ameshima and coworkers provided the first evidence that PPARγ expression was reduced in lung tissue from patients with severe PH. PPARγ was abundantly expressed in lung tissue from normal patients as well as in lung tissue from patients with chronic obstructive pulmonary disease (COPD), but its expression was reduced or absent in the angiogenic plexiform lesions in lung tissue from patients with PH [Ameshima et al. 2003]. Furthermore, these investigators demonstrated that PPARγ expression was reduced in pulmonary vascular lesions from a rat model of severe PH. Fluid shear stress also decreased PPARγ expression in ECV304 endothelial cells *in vitro*. Collectively, these findings suggested that increases in vascular shear stress in the lungs of subjects with PH could reduce PPARγ expression, leading to enhanced cellular proliferation and angiogenesis. The authors concluded that fluid shear stress decreased PPARγ expression in endothelial cells and suggested that reductions in PPARγ could contribute to an abnormal, proliferative, apoptosis-resistant endothelial cell phenotype.

Since this initial report linking reductions in PPARγ to PH, several additional studies have provided evidence to support this association. While PPARγ knockout mice present a seemingly straightforward approach to examine the role of loss of PPARγ function in PH, global deletion of PPARγ results in embryonic lethality [Barak et al. 1999]. This potential limitation has been overcome by employing experimental animals with tissue-targeted deletion of PPARγ. For example, Guignabert and coworkers examined mice with targeted deletion of PPARγ in vascular endothelium (ePPARγ−/−). ePPARγ−/− mice developed spontaneous PH with right ventricular hypertrophy and muscularization of small distal pulmonary arteries. When exposed to chronic hypoxia (10% O2) for 3 weeks, wild-type (WT) and ePPARγ−/− mice developed a similar degree of PH. However, when allowed to recover for 4 weeks in normoxia following exposure to chronic hypoxia, PH persisted in the ePPARγ−/− compared with WT mice suggesting that reduced endothelial PPARγ signaling is sufficient to cause mild PH and impair recovery from chronic hypoxic exposure [Guignabert et al. 2009]. Similarly, targeted depletion of PPARγ from smooth muscle cells using a Cre-loxP approach resulted in spontaneous PH in mice [Hansmann et al. 2008b]. Taken together, these reports suggest that reduced PPARγ function in pulmonary vascular wall cells is sufficient to cause PH.

**Stimulating PPARγ attenuates pulmonary hypertension**

In contrast to studies examining loss of PPARγ function in PH, mounting evidence suggests that stimulating this receptor attenuates PH in several experimental models. For example, Matsuda and coworkers demonstrated that monocrotaline-induced PH and vascular remodeling in the rat were attenuated by treatment with PPARγ ligands. In this study, PPARγ ligands also inhibited monocrotaline-induced vascular wall thickening and staining for proliferating cell nuclear antigen suggesting that PPARγ ligands suppressed cell proliferation and vascular remodeling in monocrotaline-induced PH in the rat [Matsuda et al. 2005]. Crossno and colleagues showed that PH and pulmonary vascular remodeling were increased in Wistar-Kyoto rats exposed to continuous hypobaric hypoxia for 3 weeks and that treatment with rosiglitazone attenuated a number of hypoxia-induced derangements including right ventricular hypertrophy, vascular smooth muscle cell (VSMC) proliferation, collagen and elastin deposition, appearance of c-Kit-positive cells in the adventitia, and matrix metalloproteinase-2 activity [Crossno et al. 2007]. Interestingly, despite therapeutic effects on hypoxic vascular remodeling, rosiglitazone failed to attenuate hypoxia-induced
increases in pulmonary artery pressure, an observation attributed to the inability of PPARγ ligands to modulate pathways critical in vasoconstriction such as Rho kinase signaling in this model [Crossno et al. 2007]. In another model of PH, Hansmann and colleagues reported that male ApoE−/− mice, fed high fat diets, developed significant increases in right ventricular systolic pressure, pulmonary vascular remodeling and right ventricular hypertrophy and that PPARγ ligands attenuated PH in this experimental model [Hansmann et al. 2008a, 2007].

Our group recently reported that male C57Bl/6 mice exposed to chronic hypoxia (10% O2) for 3 weeks developed PH that was attenuated by treatment with the PPARγ agonist, rosiglitazone (10 mg/kg/day by gavage), during the final 10 days of hypoxia exposure [Nisbet et al. 2009]. Rosiglitazone treatment also reduced hypoxia-mediated right ventricular hypertrophy and muscularization of small pulmonary arterioles. From a therapeutic perspective, this study also demonstrated that rosiglitazone could reverse established PH by introducing rosiglitazone treatment only after animals had developed PH [Nisbet et al. 2009]. The mechanisms of these therapeutic effects were attributed to PPARγ-mediated reductions in Nox4 expression, oxidative stress, and platelet-derived growth factor (PDGF) signaling in the lung. Collectively these reports indicate that PPARγ ligands attenuated pulmonary vascular remodeling and hypertension caused by a variety of stimuli in experimental models. To better understand the mechanism of action of these PPARγ ligand effects in PH requires an overview of the pathobiology of PH.

Mechanisms of pulmonary hypertension

There is general agreement that multiple mechanisms contribute to the development/progression of PH. These factors include but are not limited to vasoconstrictor/vasodilator imbalance, vascular remodeling and inflammation. The synergism between these factors results in functional and structural changes in the pulmonary vasculature leading to increased pulmonary vascular resistance and the development of PH. Comprehensive reviews of these mechanisms have been published recently [Hassoun et al. 2009; Morrell et al. 2009; Rabinovitch, 2008].

PH patients have reduced levels of vasodilators and increased levels of vasoconstrictors. This imbalance results in increased pulmonary vascular resistance. For example, circulating levels of the vasodilator prostacyclin are decreased in PH [Christman et al. 1992], and nitric oxide (NO)-mediated vasorelaxation is impaired [Giaid and Saleh, 1995]. Conversely, levels of vasoconstrictors such as endothelin-1 [Li et al. 1994] and thromboxane [Christman et al. 1992] are increased in PH patients. Current therapeutic modalities for PAH are generally directed at reducing this imbalance in vasoconstricting and vasodilating mediators. These therapies include agents that restore vasodilator levels (e.g. prostacyclin therapy), agents that enhance vasodilating signaling mechanisms (e.g. the phosphodiesterase 5 inhibitor, sildenafil), which prolongs NO-mediated increases in cGMP), and agents that block vasoconstrictor effects (e.g. endothelin receptor antagonists and calcium channel blockers). These agents may also modulate pulmonary vascular remodeling and the structural changes to the pulmonary vasculature that occur in response to vascular injury or secondarily in response to increased intraluminal pressure. The remodeling of the pulmonary vasculature can reduce the cross-sectional diameter of the pulmonary vasculature and increase pulmonary vascular resistance causing sustained PH. Studies examining the molecular mechanisms underlying pulmonary vascular remodeling have implicated growth factor pathways [Jeffery and Morrell, 2002] as well as matrix remodeling [Novotna and Herget, 2002] in the development and progression of PH.

In addition to vasoconstriction and vascular remodeling, inflammation plays a significant role in the development of PH [Hassoun et al. 2009]. Inflammatory markers are elevated in PH, and the pleuriform lesions that characterize severe PH are surrounded by inflammatory cells including macrophages, T and B lymphocytes, and dendritic cells [Tuder et al. 1994]. These cells may exacerbate PH by releasing growth factors, reactive oxygen species and additional cytokines [Tuder and Voelkel, 1998]. Chemokines such as CX3CL1, CCL5 and MCP-1, which recruit inflammatory cells, are elevated in PH patients [Sanchez et al. 2007; Balabanian et al. 2002; Dorfmuller et al. 2002]. Therefore, agents that target these inflammatory pathways may reduce inflammation and vascular dysfunction.

The participation of these complex mechanisms and diverse mediators in PH pathogenesis suggest that there are many potential therapeutic targets.
and that successful therapies in PH may need to target multiple pathways simultaneously. As reviewed in the following section and Table 1 and summarized in Figure 1, PPAR\(_{\gamma}\) may represent a novel target that is capable of favorably regulating a variety of pathways that mediate PH pathogenesis.

### Potential pulmonary hypertension mediators that may be regulated by PPAR\(_{\gamma}\)

#### Nitric oxide

NO represents a well-studied endothelium-derived mediator that plays a critical role in normal pulmonary vascular physiology. Impaired NO bioavailability contributes to PH [Steinhorn et al. 2001; Adnot et al. 1991]. Although downregulation of endothelial nitric oxide synthase (eNOS) has been described in PH in some studies [McQuillan et al. 1994], other reports have found reduced, unchanged, or increased levels of the enzyme [Tuder et al. 1999; Giaid and Saleh, 1995; Xue and Johns, 1995]. More consistent evidence demonstrates that endothelium-derived, NO-mediated vasodilation is impaired in models of PH [Mehta et al. 1995]. Collectively these studies suggest that post-translational alterations in eNOS regulation and/or enhanced NO degradation rather than reduced eNOS expression are important in PH. Supporting the important contribution of derangements in NO bioavailability in PH, genetic deletion of eNOS enhanced susceptibility to hypoxia-induced PH [Fagan et al. 1999], a defect reversed by adenoviral-mediated transfection of the pulmonary vasculature with eNOS [Champion et al. 2002]. Furthermore, overexpression of eNOS attenuated hypoxia-induced PH [Ozaki et al. 2001]. In addition, NO inhalation improves pulmonary hemodynamics and quality of life in a subset of patients with PH [Channick et al. 1996], and recent advances in cell-based eNOS gene transfer to the lung have demonstrated that eNOS can reverse established PH in animal models and facilitate restoration of the pulmonary microvasculature [Zhao et al. 2006].

NO is produced constitutively in vascular endothelial cells from the amino acid, L- arginine, by the Type III eNOS isoform. Enzyme activity is largely regulated by (1) the availability of intracellular Ca\(^{2+}\), (2) cofactor availability [Rosenkranz-Weiss et al. 1994], and (3) eNOS post-translational modifications [Fulton et al. 1999; Sessa et al. 1995; Busconi and Michel, 1993] including phosphorylation of specific serine, threonine, and tyrosine

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### Table 1. Pathways implicated in PH pathogenesis and the effects of PPAR\(_{\gamma}\) activation.

<table>
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<tr>
<th>Mechanism of PH</th>
<th>Effects of PPAR(_{\gamma}) activation</th>
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<tr>
<td>Vasoconstrictor/vasodilator imbalance</td>
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<tr>
<td>a) Decreased prostacyclin</td>
<td>Regulation of prostanoid production [Subbaramaiah et al. 2001]</td>
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<td>b) Decreased NO</td>
<td>Increase NO production [Calnek et al. 2003] and reduced superoxide generation [Hwang et al. 2005]</td>
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<td>c) Increased ET-1</td>
<td>Inhibit endothelial cell ET-1 secretion [Liu et al. 2006; Martin-Nizard et al. 2002; Fukunaga et al. 2001; Delerive et al. 1999]</td>
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<td>d) Increased thromboxane A(_2)</td>
<td>Suppression of thromboxane synthase [Ikeda et al. 2000] and thromboxane receptor expression [Coyle and Kinsella, 2006; Sugawara et al. 2002]</td>
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<td>Vascular remodeling</td>
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<td>a) Increased VSMC proliferation</td>
<td>c-kit staining [Crossno et al. 2007]</td>
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<td>b) Increased extracellular matrix proteins</td>
<td>Decreased collagen production and elastin deposition [Crossno et al. 2007]</td>
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<tr>
<td>c) Increased muscularization</td>
<td>Decreased alpha actin staining in PH models [Nisbet et al. 2009; Crossno et al. 2007; Hansmann et al. 2007]</td>
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<td>Inflammation</td>
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<td>a) Migration of inflammatory cells into vessel wall</td>
<td>Reduced inflammatory response [Szanto and Nagy, 2008]</td>
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<td>Endothelial progenitor cells</td>
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<td>a) Impaired migration of EPCs to vasculature</td>
<td>Facilitate differentiation and increase number and migratory activity [Gensch et al. 2007; Pistrosh et al. 2005]</td>
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PH, pulmonary hypertension; PPAR\(_{\gamma}\), peroxisome proliferator-activated receptor gamma; NO, nitric oxide; ET-1, endothelin-1; VSMC, vascular smooth muscle cell; EPC, endothelial progenitor cell.
residues [Dimmeler et al. 1999; Fulton et al. 1999] and eNOS interaction with other proteins including caveolin [Garcia-Cardena et al. 1998] and heat shock protein 90 (hsp90) [Pritchard et al. 2001]. eNOS phosphorylation [Murata et al. 2004] as well as interactions between eNOS and caveolin [Konduri et al. 2003; Murata et al. 2002] are altered in selected models of PH. Our work demonstrated that PPARγ activation modulates endothelial NO bioavailability [Polikandriotis et al. 2005; Calnek et al. 2003]. Rosiglitazone and the naturally occurring PPARγ ligand, 15d-PGF2α, increased endothelial NO release not by increasing eNOS protein levels, but through PPARγ-dependent mechanisms involving alterations in post-translational regulatory mechanisms that increase eNOS activity. PPARγ ligands increased endothelial NO release by reducing the inhibitory interaction between eNOS and caveolin, increasing interactions between eNOS and the molecular chaperone, hsp 90, and enhancing phosphorylation of eNOS on serine 1177, all post-translational modifications associated with enhanced eNOS activity.

Figure 1. Putative pathways by which PPARγ participates in pulmonary hypertension pathogenesis and therapy. As illustrated in the left panel, under normal circumstances, current evidence indicates that PPARγ stimulates (→) or inhibits (→) several pathways involved in pulmonary hypertension pathogenesis including inhibition of NADPH oxidase and stimulation of endothelial nitric oxide (NO), effects that collectively enhance NO bioavailability. PPARγ can also inhibit endothelin-1 (ET-1) and platelet-derived growth factor (PDGF) signaling, stimulate phosphatase and tensin homolog deleted on chromosome 10 (PTEN) expression, inhibit inflammation and Cox-2 expression as well as thromboxane (Tx) production, and stimulate endothelial progenitor cell (EPC) activity. As shown in the middle panel, reduced PPARγ expression (red text) can promote pulmonary hypertension through abnormal proliferation of endothelial and smooth muscle cells resulting in intimal and medial proliferation and thickening that reduces the vascular lumen and increases pulmonary vascular resistance. The mechanisms for abnormal pulmonary vascular cell proliferation may relate to the effects of impaired PPARγ signaling (dashed lines). As depicted in the right panel, PPARγ ligands (green) and activation of the PPARγ receptor can normalize PPARγ signaling to attenuate or reverse endothelial dysfunction, abnormal cell proliferation, and pulmonary vascular remodeling.
Once produced, endothelial-derived NO reduces vascular tone [Palmer et al. 1987], reduces platelet activation and aggregation [Azuma et al. 1986], decreases stimulated vascular smooth muscle proliferation [Garg and Hassid, 1989], and impairs leukocyte adherence [Bath et al. 1991]. To exert many of these biological effects, NO must diffuse into the vascular wall. Its ability to do so may be limited by local concentrations of superoxide. As a result, NO bioavailability can be regulated not only by the rate of NO formation, but also by the rate of NO degradation. Thus, increased superoxide generation constitutes an important mechanism of NO inactivation and endothelial dysfunction in the vascular wall. Superoxide combines at diffusion-limited rates with NO forming the potent oxidant, peroxynitrite, thereby reducing the vascular protective effects of NO and enhancing oxidative stress. Peroxynitrite also oxidizes the NOS cofactor, tetrahydrobiopterin [Kuzkaya et al. 2003]. Deficiency of tetrahydrobiopterin leads electron flow through eNOS to molecular oxygen rather than arginine, producing superoxide rather than NO, a condition referred to as eNOS uncoupling. Enhanced superoxide production in the vascular wall may, therefore, reduce NO bioavailability through multiple mechanisms.

**NADPH oxidases**

NADPH oxidases constitute a major source of superoxide production in the vasculature which has been implicated in PH and that contributes to endothelial dysfunction and vascular cell proliferation [Bedard and Krause, 2007; Griendling et al. 2000]. Originally described in phagocytic cells, the gp91phox-based oxidase is a multicomponent, membrane-associated, enzyme that catalyzes the one electron reduction of oxygen to superoxide using NADH or NADPH as the electron donor [Griendling et al. 2000]. The classical phagocytic NADPH oxidase is composed of several components or subunits including the membrane bound gp91phox (also known as Nox2) and p22phox subunits as well as the cytosolic p47phox and p67phox subunits which, when stimulated, combine with the small G-protein, rac, and translocate to the membrane to activate the enzyme complex. On the other hand, in nonphagocytic cells, the catalytic moiety of NADPH oxidases is composed of one or more gp91phox (Nox2) homologs, Nox1, 3, 4, 5, Duox1 or Duox2 [Lambeth, 2002]. These Nox homologs associate with the membrane-bound p22phox subunit and are differentially regulated and targeted to distinct subcellular loci suggesting that these oxidases serve unique roles in cell function. Nox1 and 3 are activated through interactions with rac and the p47phox and p67phox homologs, NOXA1 and NOXO1.

Current evidence indicates that Nox4 expression is increased in hypoxia-induced PH in the mouse and in the pulmonary vasculature of patients with PH [Nisbet et al. 2009; Mittal et al. 2007]. Previous reports have demonstrated that Nox4 is highly expressed in vascular wall cells including smooth muscle and endothelial cells [Sorescu et al. 2002] where it is constitutively active [Ambasta et al. 2004]. Furthermore, hypoxia increased Nox4 expression and pulmonary artery smooth muscle cell (PASMC) proliferation [Mittal et al. 2007], and Nox4 stimulated the proliferation of endothelial [Chen et al. 2008] and smooth muscle cells [Djordjevic et al. 2005]. We have found that hypoxia stimulates Nox4 expression and cell proliferation in the mouse lung in vivo [Nisbet et al. 2009] and in human pulmonary artery endothelial cell (HPAEC) and human pulmonary artery smooth muscle cell (HPASMC) in vitro. Treatment with rosiglitazone during the last 10 days of hypoxia exposure reduced Nox4 levels and ROS production and attenuated hypoxia-induced PH, right ventricular hypertrophy and vascular remodeling. Similarly, rosiglitazone attenuated hypoxia-induced Nox4 expression and proliferation in HPAEC and HPASMC in vitro. Taken together, these findings suggest that NADPH oxidases are important mediators of cell proliferation and vasoconstriction in PH that can be regulated by PPARγ.

While it is clear that increases in Nox4 mRNA levels increase Nox4 activity [Serrander et al. 2007], a detailed understanding of Nox4 transcriptional regulation remains to be established, and additional studies will be required to clarify how PPARγ regulates Nox4 expression. Nox4 induction has been reported in response to diverse stimuli including hypoxia in kidney and ischemia in brain [Bedard and Krause, 2007]. In smooth muscle cells, activators of Nox4 transcription include urokinase, plasminogen activator, angiotensin II, transforming growth factor beta 1 (TGF-β1), and tumor necrosis factor alpha (TNF-α) [Lambeth et al. 2007]. In contrast, in endothelial cells, oscillatory shear stress [Sorescu et al. 2004] and PPARγ activation [Hwang et al. 2005] suppress Nox4 mRNA levels. However, few studies have examined
regulatory elements in the Nox4 promoter. Only the E2F family of transcription factors has been described to activate the Nox4 promoter [Zhang et al. 2008]. In as yet unpublished studies, we have observed that hypoxia stimulates activation of the Nox4 promoter in part through NF-κB-mediated signaling and enhanced p65 binding to the Nox4 promoter which increased Nox4 expression and activity. Furthermore, treatment with rosiglitazone inhibited hypoxia-induced Nox4 expression and activity, proliferation, and p65-Nox4 promoter interaction (unpublished observations). Based on evidence that Nox4 stimulates smooth muscle [Mittal et al. 2007] and endothelial cell [Chen et al. 2008] proliferation, these findings suggest that activation of PPARγ can attenuate the proliferation of pulmonary vascular wall cells.

Prostacyclin

Another critical regulator of pulmonary vascular function, the endothelial-derived mediator, prostacyclin, is a potent vasodilator that inhibits platelet aggregation and exerts anti-inflammatory, antithrombotic, and antiproliferative vascular effects [Olschewski et al. 2004]. Overexpression of prostacyclin synthase protected mice from chronic hypoxia-induced PH [Geraci et al. 1999] whereas prostacyclin-receptor deficient mice were sensitized to hypoxia-induced PH [Hoshikawa et al. 2001]. Decreased prostacyclin synthase expression has been noted in the pulmonary arteries of patients with severe PH compared with normal subjects, and the vascular endothelium was found to be the major site of lung vascular prostacyclin synthase expression [Tuder et al. 1999]. In patients with PH, prostacyclin derivatives decreased urinary isoprostane metabolites, an index of oxidative stress, without altering thromboxane A2 [Robbins et al. 2005]. Currently, augmenting prostaglandin levels constitutes a therapeutic strategy in PH, but the precise cellular mechanisms responsible for prostacyclin-mediated benefits remain to be defined. The classical signaling pathway activated by prostacyclin involves ligation of the G-protein coupled cell surface prostacyclin receptor (IP) which when activated stimulates adenyl cyclase and increases cellular cAMP content. However, prostacyclin and its analogs can also activate PPAR receptors [Forman et al. 1997] including PPARδ [Gupta et al. 2000; Lim and Dey, 2002] and PPARγ [Nemenoff et al. 2008; Falcetti et al. 2007] to mediate biological effects. Several studies have suggested additional potential relationships between PPAR, prostaglandin metabolism, and vascular disease. For example, inducible cyclooxygenase-2 (COX-2) is expressed in vascular endothelial cells and promotes vascular dysfunction. The ability of PPARγ ligands to inhibit COX-2 induction [Subbaramaiah et al. 2001] suggests potential relationships between PPARγ and altered prostaglandin metabolism in vascular dysfunction. Pioglitazone reduced vascular production of the potent vasoconstrictor, thromboxane, in a fructose-induced rat model of metabolic syndrome suggesting that PPARγ activation can also modulate the relative production of vasodilating and vasoconstricting arachidonic acid metabolites in some models [Peredo et al. 2008]. Rosiglitazone attenuated microparticle-induced vascular dysfunction in mouse aorta by preventing microparticle-induced iNOS and Cox-2 upregulation and the overproduction of NO and prostacyclin, respectively [Tesse et al. 2008]. These reports suggest that PPARγ plays an important role in the production of prostanooids that regulate vascular function.

Endothelin-1

The potent vasoconstricting polypeptide, endothelin-1 (ET-1), has been implicated in PH pathogenesis. ET-1 receptors are upregulated in the lung in both animal models [Frasch et al. 1999; Li et al. 1994] and patients with PH [Giad and Saleh, 1995]. ET-1, as well as endothelium-derived reactive oxygen species, attenuated NO-dependent pulmonary vasodilation following exposure to chronic hypoxia in isolated rat lungs [Jernigan et al. 2004]. ET-1-induced pulmonary vasoconstriction was markedly reduced by administration of Cu/Zn superoxide dismutase and was completely attenuated in gp91phox deficient mice [Liu et al. 2006]. These findings suggest that NADPH oxidase and superoxide play an important role in pulmonary vascular effects of ET-1. Endothelin-1 receptor antagonists have been employed in patients with PH to improve functional status and other indices of PH related morbidity [Jernigan et al. 2004], further suggesting that ET-1 is an important mediator of pulmonary vascular dysregulation. Emerging evidence in several disease states indicates that PPARγ activation attenuates ET-1 signaling. PPAR ligands inhibited ET-1 secretion by vascular endothelial cells in vitro [Liu et al. 2006; Martin-Nizard et al. 2002; Fukunaga et al. 2001; Delerive et al. 1999].
Similarly in nondiabetic patients with metabolic syndrome, treatment with rosiglitazone reduced several markers of vascular inflammation including plasma levels of ET-1 and improved markers of metabolic control while lowering blood pressure and improving flow-mediated vasodilation [Wang et al. 2006].

PPARγ activation also reduced hypertrophy [Bao et al. 2008] and anti-apoptotic effects [Ehara et al. 2004] caused by ET-1 in cardiac myocytes in vitro through altered nuclear factor of activated T cells (NFAT) signaling. Treatment with PPARγ ligands in several rat models of hypertension reduced ET-1 expression in cardiac [Iglarz et al. 2003b; Sakai et al. 2002] and vascular [Iglarz et al. 2003a] tissues. Collectively, these findings suggest that PPARγ activation can attenuate expression of ET-1 in cardiovascular tissues in response to a variety of stimuli and can attenuate ET-1-mediated signaling in selected models.

**PDGF signaling**

PDGF participates in PH pathogenesis. Two genes (A and B) produce three biologically active forms of PDGF protein (AA, AB, and BB) [Fredriksson et al. 2004; Raines, 2004]. These proteins activate one or more PDGF receptors (αA, αB, or βB) to stimulate cell migration and survival. Ligand binding promotes PDGF receptor tyrosine autophosphorylation and subsequent activation of several downstream signaling pathways including Src, phosphatidylinositol 3 kinase (PI3K), phospholipase Cγ1, and Ras. These signaling pathways are largely activated by recruitment of these enzymes to PDGF-R SH-2 domains. The composition of the downstream signaling pathways activated by PDGF and their integration into specific cellular responses continue to be defined [Tallquist and Kazlauskas, 2004]. Although the expression of PDGF and its receptors is limited in the vascular wall at baseline, several pathological stimuli, including alterations in blood pressure and shear stress, induce peptide and receptor expression [Raines, 2004]. PDGF receptor expression was increased in the lungs of patients with PH, and the PDGF receptor antagonist, imatinib, reversed monocrotaline- or hypoxia-induced PH in rodents and improved pulmonary vascular resistance and exercise capacity in a patient with severe idiopathic PH [Ghofrani et al. 2005; Schermuly et al. 2005; Balasubramaniam et al. 2003]. PPARγ ligands attenuated hypoxia-induced PDGF activation in a mouse model of PH in vivo [Nisbet et al. 2009]. Coupled with reports that NO inhibits PDGF signaling [Failli et al. 2000; Sandirasagarane et al. 2000; Fang et al. 1997] and that PPARγ ligands inhibit PDGF-stimulated VSMC migration in vitro [Law et al. 2000; Goetze et al. 1999; Marx et al. 1998], these studies suggest that PPARγ can regulate important proliferative signaling pathways in experimental PH including those activated by PDGF.

PDGF receptor activity can also be regulated by phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a dual specificity phosphatase that dephosphorylates both lipid and protein substrates [Gericke et al. 2006]. PTEN catalyzes the removal of the phosphate moiety from the 3-position of the phosphatidylinositol ring, converting the second messenger, PI(3,4,5)P3, to PI(4,5)P2 [Maehama and Dixon, 1998]. PTEN and PI3K thereby have opposing actions on cellular levels of PI(3,4,5)P3. PTEN also dephosphorylates the PDGF receptor. Because PDGF receptor activation mediates cell proliferation and migration in part through stimulation of PI3K, PTEN can inhibit PDGF signaling both at the receptor and through lowering of PI(3,4,5)P3 levels thereby lowering the activity of PI3K-related downstream mediators such as the protein kinase B, Akt, which mediates survival, growth, and proliferative signals by inhibiting apoptosis.

PTEN activity is regulated at the transcriptional and post-translational level. At the transcriptional level, pathological stimuli such as ischemia have been shown to reduce PTEN expression and promote hypertrophy and remodeling [Schwartzbauer and Robbins, 2001; Tong et al. 2000]. Limited evidence suggests that NF-κB activation may lead to suppression of PTEN expression [Wang et al. 2007; Han and Roman, 2006]. On the other hand, the PTEN promoter contains two PPAR response elements, and several studies have demonstrated that PPARγ ligands stimulate PTEN expression [Farrow and Evers, 2003; Patel et al. 2001]. Furthermore, PTEN overexpression reduced VSMC proliferation and migration and inhibited injury-induced vascular remodeling in vivo [Huang et al. 2005; Huang and Kontos, 2002]. PTEN activity is inhibited by ROS which cause reversible oxidation of cysteine residues in the phosphatase active site [Leslie et al. 2003; Lee et al. 2002]. In fact, NADPH oxidase-derived ROS facilitate PDGF
signaling by inhibiting PTEN [Kwon et al. 2004]. Based on these reports, we postulated that chronic hypoxia caused sustained PI3K/Akt activation, in part, through the generation of NADPH oxidase-derived ROS that stimulate PDGF and inhibit PTEN signaling pathways. We demonstrated that chronic hypoxia increased PDGF receptor activation and reduced PTEN expression in the lung [Nisbet et al. 2009]. Furthermore, treatment with rosiglitazone attenuated hypoxia-induced PDGF receptor activation and restored PTEN levels in hypoxic mice to levels comparable to control animals. The ability of PPARγ ligands to simultaneously stimulate PTEN expression and lower oxidative stress-induced PTEN inactivation while attenuating PH provides a unique strategy to lower PDGF receptor phosphorylation and activation and reduce cellular PI(3,4,5)P3 levels. We postulate that these integrated effects contribute to the ability of PPARγ to attenuate pulmonary VSMC proliferation and hypertrophy as well as vascular remodeling caused by chronic hypoxia.

**Inflammation**

Numerous studies have suggested that inflammation plays an important role in idiopathic PAH and in PH associated with connective tissue disease or human immunodeficiency virus infection [Hassoun et al. 2009]. Inflammatory cells including T and B lymphocytes, macrophages, and dendritic cells have been observed in the vascular lesions of patients with PH. Patients with PH also have increased levels of circulating markers of inflammation [Bakouboula et al. 2008]. Common stimuli of PH such as hypoxia cause inflammatory responses in the pulmonary vasculature [Stenmark et al. 2006], and experimental models associated with inflammation, such as mice with deficiency of the anti-inflammatory adipocytokine, adiponectin, develop PH and vascular remodeling [Medoff et al. 2009; Summer et al. 2009]. These reports suggest that therapies preventing or attenuating inflammation in the pulmonary vasculature could have therapeutic potential in PH. PPARγ is expressed in T cells, macrophages, leukocytes, and dendritic cells, suggesting that its activation could modulate inflammation in the pulmonary vasculature that contributes to the development of PH. Numerous studies have demonstrated that PPARγ ligands attenuate inflammation in numerous models [Szanto and Nagy, 2008]. Evidence indicates that PPARγ activation reduces macrophage recruitment and inflammatory mediator production, impairs dendritic cell priming of T cells as well as T lymphocyte proliferation and viability. PPARγ ligands also induce regulatory T cells which downregulate immune responses [Hontecillas and Bassaganya-Riera, 2007; Wohlfert et al. 2007]. Current evidence indicates that these anti-inflammatory effects of PPARγ are mediated not through transactivation of specific target genes, but rather through physical binding to other proinflammatory transcription factors such as NF-κB, AP-1, and STAT leading to suppression of their activities. The precise mechanisms of these transrepression effects remain to be completely defined but may involve ligand-dependent SUMOylation of PPARγ which targets the receptor to corepressor complexes on the promoters of inflammatory genes preventing proteosomal degradation of the corepressors and thereby causing inhibition of inflammatory gene expression [Pascual et al. 2005].

**Progenitor cell recruitment**

Endothelial progenitor cells (EPCs) are bone-marrow-derived cells that are mobilized into the systemic circulation in response to ischemia or vascular injury [Asahara et al. 1997]. The role of EPC in vascular repair, vascular homeostasis, and formation of new blood vessels continues to be defined [Rosenzweig, 2005]. Clinical trials suggest that EPCs may serve as markers of vascular dysfunction and cardiovascular disease [Werner et al. 2005; Hill et al. 2003]. Because endothelial dysfunction participates in the pathogenesis of PH, EPCs may play a role in pulmonary vascular disease [Sata, 2006]. The endogenous erythropoietin system recruits EPCs to the lung in experimental PH in mice [Sato et al. 2006]. Furthermore, mesenchymal stem cells overexpressing eNOS [Kanki-Horimoto et al. 2006] or EPCs expressing adrenomedullin attenuated monocrotaline (MCT)-induced PH in rats [Nagaya et al. 2003]. These reports suggest that migration of EPCs to the pulmonary vasculature during experimental PH can exert beneficial effects. PPARγ agonists facilitate the differentiation of angiogenic progenitor cells into EPCs [Wang et al. 2004]. PPARγ agonists also increased the number and migratory activity of EPC in patients with type 2 diabetes and impaired endothelial function [Pistrosch et al. 2005] and increased EPC migratory activity and reduced EPC apoptosis in mice [Gensch et al. 2007]. Collectively, these reports suggest that PPARγ ligands might stimulate EPC to reduce pulmonary vascular dysfunction and reduce PH.
Pulmonary vascular remodeling

PH involves remodeling of pulmonary vessels with muscularization of nonmuscular distal arterioles, proliferation and migration of VSMC, and increased production of extracellular matrix proteins including fibronectin, collagen, and elastin [Stenmark et al. 2006; Cowan et al. 2000]. Alterations in matrix composition may be related to increased matrix degradation resulting from an imbalance in the matrix metalloproteinases (MMP)–tissue inhibitor of metalloproteinases (TIMP) system [Lepeit et al. 2005; Frisdal et al. 2001; Himelstein and Koch, 1998] with deposition of collagen, elastin, fibronectin, and tenascin-C [Cowan et al. 2000]. Inhibition of MMPs and elastase prevented the progression and actually induced regression of vascular remodeling in an experimental model of PH [Cowan et al. 2000]. In addition, the elastase inhibitor, elafin, protected mice from chronic hypoxia-induced PH [Zaidi et al. 2002]. Recent evidence indicates that the PPARγ ligand, rosiglitazone, attenuated and reversed vascular remodeling in rat and mouse models of chronic hypoxia-induced PH [Nisbet et al. 2009; Crossno et al. 2007; Hansmann et al. 2007]. Rosiglitazone decreased collagen production and elastin deposition and increased MMP-2 activity [Crossno et al. 2007]. Treatment with PPARγ ligands has also been associated with attenuation of matrix deposition in disorders other than PH [Hao et al. 2008; Makino et al. 2006; Peng et al. 2006]. Taken together, these reports suggest therapeutic mechanisms of PPARγ activation in PH could involve attenuation of matrix deposition and remodeling in the pulmonary vasculature.

Conclusions and future directions

Abundant evidence suggests that PPARγ is a potential therapeutic target for the treatment of PH. It is important to recognize, however, that many of these studies have been performed in animal models that may not serve as adequate models of human PAH [Stenmark et al. 2009]. In addition, the precise molecular and biochemical mechanisms that promote PH remain to be defined. The studies reviewed in this article suggest that therapeutic targets such as PPARγ, which have the capacity to regulate numerous pathways involved in PH pathogenesis simultaneously merit additional investigation. To fully recognize the therapeutic potential of strategies targeting PPARγ, future studies must not only determine the relevant molecular pathways that are altered by PPARγ, but also the cellular site of action of these ligands and their relative dependence on the PPARγ receptor.

The completion of preclinical studies will further inform the consideration of PPARγ as a therapeutic target in PH. The current availability of oral thiazolidinedione PPARγ ligands can facilitate clinical trials examining the efficacy of these drugs in patients with PH. However, evidence supporting successful therapy of PH with existing PPARγ ligands could also stimulate the development of novel pharmacological PPARγ ligands with enhanced therapeutic efficacy and/or reduced side effects. The evidence presented in this review indicates that approaches directed at novel targets in the lung, such as PPARγ, have significant potential to promote the development of more effective therapies for PH.

Acknowledgments

The authors acknowledge grant support from the Research Service of the Atlanta Veterans Affairs Medical Center (to CMH), the National Institutes of Health (R01 DK 074518, to CMH and RLS), and Takeda Pharmaceuticals.

Conflict of interest statement

None declared.

References


of PPARgamma antagonize protection of cardiac myocytes by endothelin-1. *Biochem Biophys Res Commun* 321: 345–349.


