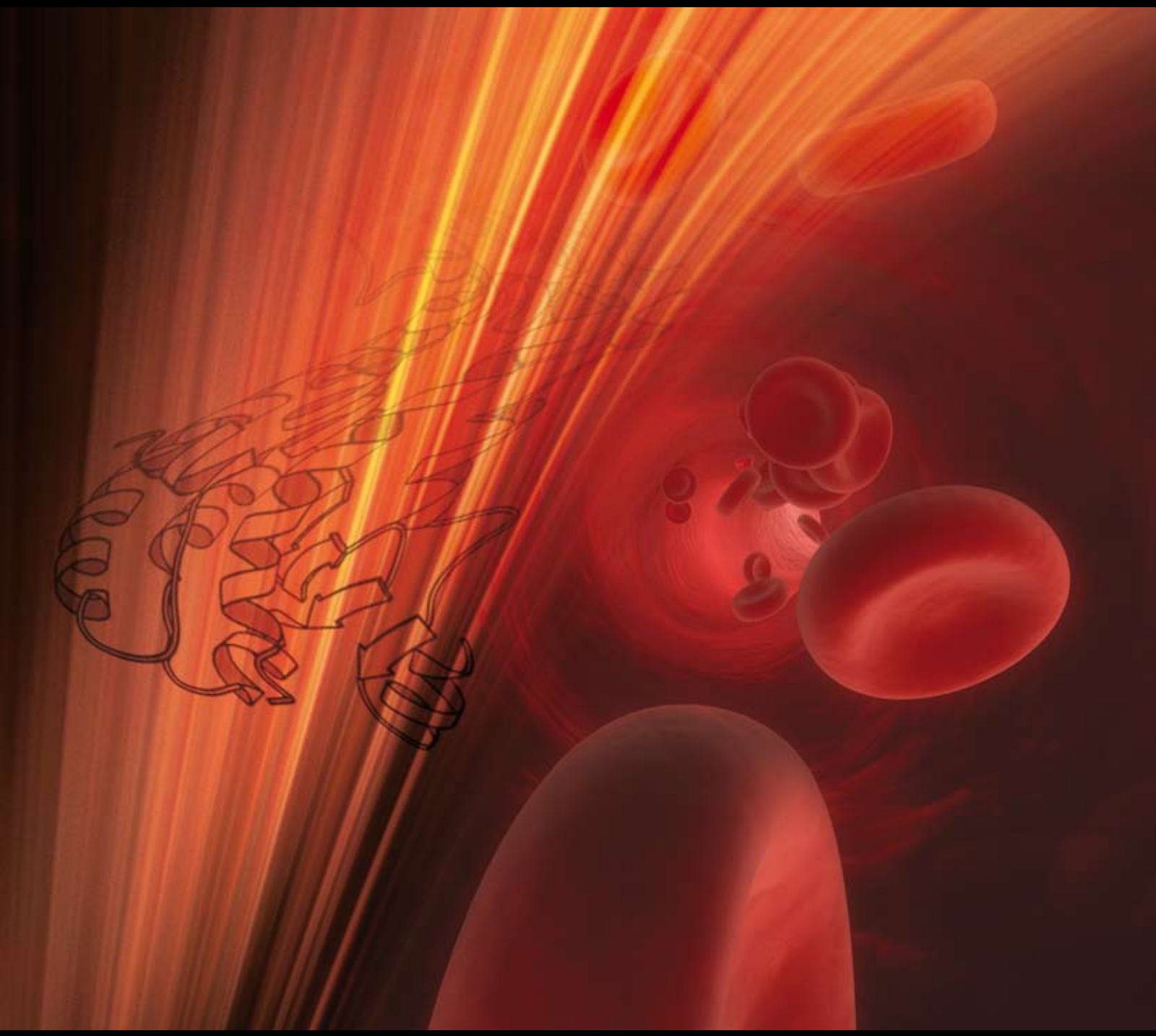
PPAR Research

PPARs in Lung Biology and Disease

Guest Editors: Theodore J. Standiford and Jesse Roman



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Contents

PPARs in Lung Biology and Disease, Theodore J. Standiford and Jesse Roman
Volume 2007, Article ID 28765, 2 pages

Role of PPARs and Retinoid X Receptors in the Regulation of Lung Maturation and Development, Dawn M. Simon and Thomas J. Mariani
Volume 2007, Article ID 91240, 8 pages

PPARs in Alveolar Macrophage Biology, Monica R. Smith, Theodore J. Standiford, and Raju C. Reddy
Volume 2007, Article ID 23812, 12 pages

Peroxisome Proliferator-Activated Receptors and Acute Lung Injury, Rosanna Di Paola and Salvatore Cuzzocrea
Volume 2007, Article ID 63745, 8 pages

Peroxisome Proliferator Activated Receptor Ligands as Regulators of Airway Inflammation and Remodelling in Chronic Lung Disease, Jane Elizabeth Ward and Xiahui Tan
Volume 2007, Article ID 14983, 12 pages

The Role of PPARs in Lung Fibrosis, Heather F. Lakatos, Thomas H. Thatcher, R. Matthew Kottmann, Tatiana M. Garcia, Richard P. Phipps, and Patricia J. Sime
Volume 2007, Article ID 71323, 10 pages

The Role of Peroxisome Proliferator-Activated Receptors in Pulmonary Vascular Disease, Rachel E. Nisbet, Roy L. Sutliff, and C. Michael Hart
Volume 2007, Article ID 18797, 10 pages

Peroxisome Proliferator-Activated Receptors in Lung Cancer, Venkateshwar G. Keshamouni, ShouWei Han, and Jesse Roman
Volume 2007, Article ID 90289, 10 pages

Stimulatory Effects of Peroxisome Proliferator-Activated Receptor-γ on Fcγ Receptor-Mediated Phagocytosis by Alveolar Macrophages, David M. Aronoff, Carlos H. Serezani, Jennifer K. Carstens, Teresa Marshall, Srinivasa R. Gangireddy, Marc Peters-Golden, and Raju C. Reddy
Volume 2007, Article ID 52546, 8 pages

Rosiglitazone, an Agonist of PPARγ, Inhibits Non-Small Cell Carcinoma Cell Proliferation In Part through Activation of Tumor Sclerosis Complex-2, ShouWei Han, Ying Zheng, and Jesse Roman
Volume 2007, Article ID 29632, 8 pages

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Volume 2007, Article ID 28765, 2 pages
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Editorial
PPARs in Lung Biology and Disease

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In this special issue of PPAR Research, we have assembled a comprehensive and complementary group of review articles and original investigations that illustrate the pivotal role of the PPAR family of nuclear hormone receptors in the regulation of fundamental cellular events in the lung. The lung performs the vital function of gas exchange required for the delivery of oxygen to tissues. In performing this function, the lung is continuously exposed to a diverse array of microbial pathogens, allergens, and toxic particulate matter. These insults require immune and reparative responses that are appropriate, yet tightly regulated in order to maintain the delicate alveolar structures required for efficient gas exchange. PPARs and their obligatory heterodimer partners retinoid X receptors (RXR) are well known to regulate the expression of genes involved in lipid and glucose metabolism. More recently, these transcription factors have been shown to modulate developmental, inflammatory, and reparative responses, including those that occur in the lung.

Articles included in this special issue highlight the importance of PPARs and RXR in lung biology and in the pathogenesis of lung disease. Both PPAR-γ and RXR participate in lung morphogenesis, including the processes of postnatal alveolar elastogenesis and maturation.The majority of studies reported to date support a role for PPAR-α and/or PPAR-γ in inhibiting the release of inflammatory mediators from lung immune and stromal/parenchymal cells in vitro, and dampening inflammation and damage in animal models of acute lung injury (ALI), ischemia-reperfusion injury, and allergic airways inflammation. Emerging evidence indicates that PPAR family members can also suppress proliferative and differentiation responses of lung epithelial cells, smooth muscle cells, and fibroblasts, which is of particular relevance to tissue remodeling and fibroproliferation that occur in chronic airways disease, ALI, pulmonary vascular disease, and pulmonary fibrosis. In contrast to these notable inhibitory effects, PPAR-γ can also activate key macrophage antimicrobial and reparative responses, in part by enhancing the expression of cell surface receptors required for ingestion of microbes and cellular debris present within the airspace. Finally, data is summarized to illuminate the central role of PPAR-γ in regulating critical aspects of lung tumor initiation, progression, and metastasis. Specific effects include promotion of tumor cell differentiation, induction of cell cycle arrest and apoptosis, suppression of angiogenesis, and modulation of immune/stromal cells within the tumor microenvironment.

The field of PPAR research continues to be hindered by the nonselective effects of synthetic and naturally occurring agonists, the limited availability of potent and specific inhibitors, and the absence of ideal genetic models of PPAR deficiency. The introduction of new molecular tools and the generation of conditionally targeted and site specific (including lung epithelial cell-specific) PPAR-γ deficient mice will allow better distinction between PPAR-dependent and PPAR-independent effects. Moreover, the search for relevant endogenous PPAR ligands continues. The recently described nitroalkene species are appealing candidates, although the presence of these molecules in lung tissues has not yet been characterized.

We thank the editors for the opportunity to share with the readership this important area of investigation. It is our hope that this special issue will serve as a catalyst for new initiatives in this exciting and rapidly evolving field. Observations made at the bench have already transitioned to the

bedside, as trials assessing effects of PPAR-γ agonists in the treatment of diverse lung diseases, including asthma and lung cancer, are ongoing.

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Review Article

Role of PPARs and Retinoid X Receptors in the Regulation of Lung Maturation and Development

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Understanding lung development has significant importance to public health because of the fact that interruptions in the normal developmental processes can have prominent effects on childhood and adult lung health. It is widely appreciated that the retinoic acid (RA) pathway plays an important role in lung development. Additionally, PPARs are believed to partner with receptors of this pathway and therefore could be considered extensions of retinoic acid function, including during lung development. This review will begin by introducing the relationship between the retinoic acid pathway and PPARs followed by an overview of lung development stages and regulation to conclude with details on PPARs and the retinoic acid pathway as they may relate to lung development.

1. THE RETINOIC ACID PATHWAY AND PPAR

The effects of retinoic acid are mediated by the retinoic acid receptors (RAR) and retinoid X receptors (or 9-cis retinoic acid receptor, RXR). RARs and RXRs each have 3 separate subtypes: α, β, and γ. RXR is specific for the 9-cis retinoic acid (9CRA) stereoisomer, while RAR binds both 9CRA and all-trans retinoic acid (ATRA). RARs form heterodimers with the three RXR subtypes and RXR's form heterodimers with members of the nuclear receptor family, including PPARγ. RXRs can also form homodimers, which among other effects, can activate PPAR target genes [1]. While as a group, the three PPAR isoforms (α, β/δ, and γ) function to regulate cellular lipid utilization and homeostasis, each isoform has discrete yet overlapping functions and ligand specificities. Upon activation by an appropriate ligand, PPARs form an obligate heterodimer with RXR to recruit nuclear receptor coactivators. Because they function as heterodimers with the RXR, PPARs could be considered an extension or modulator of the retinoic acid signaling pathway. The canonical pathway is that these ligand-activated PPAR-RXR heterodimers bind to peroxisome proliferator response elements (PPREs), and activate gene transcription, although PPARs can also serve as active transcriptional repressors [2]. Furthermore, nongenomic functions of PPARs upon gene regulation (e.g., regulatory effects independent of PPRE binding) have been reported [3–5]. For instance, PPARs are capable of trans-repression of other transcription factors, through direct interaction or through interaction with other coactivator/corepressors. Schupp and colleagues recently demonstrated that a RARα antagonist can directly affect PPARγ activity and therefore be considered both a PPARγ agonist and RARα antagonist [6]. Additionally, Szatmari and colleagues found that PPARγ regulates CD1d, a molecule involved in dendritic cell antigen presentation, by inducing retinoic acid synthesis through RARα [7]. These observations highlight the complex interplay between nuclear receptors. Given the numerous pathways through which PPARs could regulate gene expression either directly or indirectly, it is easy to envision that they may play a role in the complex regulatory mechanisms of lung development.

2. THE REGULATION OF MAMMALIAN LUNG DEVELOPMENT

Mammalian lung development follows a highly regulated, morphogenetic program beginning near mid-gestation and continuing through postnatal life [8, 9]. The mammalian

lung initiates as an out-pouching of the ventral foregut endoderm. Initially, during the "embryonic" stage of organ development, which occurs during 5th and 6th week of gestation in the human or embryonic days 9.5 (E9.5) and E10.5 in the mouse, the lung arises as a ventral diverticulum of the foregut endoderm, separating from the esophagus and elongating caudally. This bud branches to give rise to the main bronchi of the left and right lung. Significant recent advances have been made in the understanding of the genetic and molecular mechanisms governing many of the early processes of lung development [10, 11]. Lung bud initiation and outgrowth is controlled by both the Gli/Shh pathway [12–14] and FGF receptor signaling [15].

Beginning in the pseudoglandular stage (which occurs between 6 and 16 weeks of gestation in humans or E10.5–16.5 in mice) and continuing through the canalicular stage (which occurs between 16 and 26 weeks of gestation in humans or E16.5–17.5 in mice), this lung bud subsequently undergoes repeated rounds of dichotomous branching to produce the tree-like structure of the mature conducting airway. Numerous molecules are currently appreciated as playing a role in the branching process. Many, though certainly not all, of these molecules belong to the BMP and FGF signaling pathways [16–21]. BMP-4 and FGF-10 are believed to form signaling centers that specify branch initiation sites and outgrowth [22]. Locations of branching specificity are limited, in part, by molecules such as Sprouty and Noggin, which antagonize FGF and BMP signaling [23, 24]. Many other factors such as EGF, Shh, and Wnt also play a role in the regulation of branching morphogenesis. The involvement of these particular pathways also highlights the role of epithelial-mesenchymal interactions in lung development. It is well accepted that epithelial-mesenchymal interactions are essential for normal lung development, primarily during embryonic growth and differentiation [25, 26]. The specific role of epithelial-mesenchymal interactions in later stages of lung development, including postnatal lung maturation, is unclear. In addition to the continuation of branching morphogenesis, the canalicular stage is marked proximo-distal cell type specification and vascularization.

From 26 to 36 weeks of gestation (E17.5 through postnatal day 4 in mice), the "saccular" stage completes formation of the conducting airway tree and differentiation of distal epithelial cells. During this stage, the distal architecture of the lung dramatically changes due to further differentiation and flattening of distal airway epithelia. This process is coordinated by factors such as GATA-6, Nkx2.1, HNF3β, C/EBPα, glucococorticoid hormones, and FGFs [27]. At or near the end of the saccular stage, the lung becomes prepared for a transition to air breathing with the production of pulmonary surfactant. Recent studies support a role for the forkhead box transcription factor, Foxa2 as a master regulator of surfactant production [28], in coordination with the transcription factors, Ttf1 and C/EBPα [29]. The calcineurin/NFAT signaling pathway also appears to play a role in this process [30].

Finally, the gas exchange portions of the lung are formed during the alveolar stage of development. This occurs beginning in week 36 of human gestation and continues through early childhood. In mice, this stage occurs entirely during the postnatal period, beginning in the first week of life and continuing through the first month. Maturation of gas-exchange capacity involves airway wall secondary crest septation and elongation, a process referred to as alveogenesis. Elongation of secondary septae results in partitioning of saccules into alveolar ducts and alveoli with an increase in gas-exchange surface area. Lung maturation and alveogenesis continues after birth in both rodents and humans. Although the number of airway generations and branching pattern of the lung is established at birth, the morphology of the lung parenchyma is quite different between the newborn and the adult [31]. Alveoli continue to form for at least 2 years after birth in humans. A detailed understanding of the regulatory processes controlling alveogenesis is lacking. Retinoic acid (discussed further below), PDGF, and FGF signaling all contribute to the regulation of secondary crest elongation. PDGF-A is essential in alveolar formation as defined by failed alveogenesis in its deficiency state secondary to a lack of development of alveolar myofibroblasts [32]. FGF signaling is also critical to alveogenesis, again, as defined by combined deficiency in FGFR3 and FGFR4 [33]. Interestingly, the ligand(s) mediating this effect is unknown. These data can be integrated into a model predicting morphogenic gradients of RA and FGF signaling secondary crest elongation [34]. In recent years, the importance of coordinated development of the vasculature during alveolarization has gained appreciation. It is clear that the appropriate balance of VEGF activity, which is an important pathway for vascular development and maintenance, plays a critical role in alveogenesis [35–38]. VEGF also appears to play a critical role in promoting surfactant expression [39].

Boyden and Tompsett have described a mechanism for airspace formation distinct from the process of saccule subdivision by secondary septal elongation; the transformation of terminal or respiratory bronchioles into alveolar ducts [31, 40]. Massaro et al. corroborated this concept, finding that airspaces can develop through the nutritionally-dependent elongation of the conducting airway and de novo formation of alveoli (termed "retrograde alveolarization of bronchioles") [41]. Since the time of these seminal observations, only a few studies have clarified the regulation of alveolar duct formation and its contribution to airspace structure. Intact collagen and/or elastin fibers appear necessary for the development of alveolar ducts, as treatment of neonatal rats with the BAPN, an inhibitor of the collagen and elastin cross-linking enzyme lysyl oxidase results in increased volume density of alveolar ducts [42]. Indomethacin treatment of neonatal rats also results in increased alveolar duct formation, implicating endogenous prostaglandin levels as a regulatory component in this process [43].

3. RETINOIC ACID SIGNALING IS ESSENTIAL AT MANY POINTS IN LUNG DEVELOPMENT

The retinoic acid pathway can have effects on all stages of lung development (see Figure 1). The RARs and RXRs have distinct expression patterns, notably during mouse embryonic development [44–47]. Specifically, RXRs have been

shown to be expressed in the human lung during critical periods in development from 13 weeks gestation until term, then their expression becomes markedly reduced in the adult [44]. Interestingly, retinoic acid signaling is downregulated during lung epithelial tubule branching and differentiation, which ultimately allows formation of mature type I and II cells [46, 48].

To understand their functional role, gene-targeted mice have been generated for all 3 RARs and RXRs [49–51]. RAR single mutants are viable though they display a range of vitamin A deficiency syndromes, which increase when double null mutants are generated [49, 50]. RXRα loss results in fetal lethality at around E14.5 [52, 53]. Similar to PPARγ null mutants, these mice display severe myocardial hypoplasia. Because of the in utero lethality, mice with alleles for conditional gene targeting have been generated [51]. Based on these studies, RXRα has been found to be a crucial mediator of metabolism and skin development [54–57]. RXRβ mutant fetuses also have high mortality (50%) with infertility in viable male pups [58]. RXRγ mutant mice survive and are fertile though they have abnormal metabolism secondary to alterations in pituitary-thyroid axis [59, 60]. The development of these genetically altered mice has provided insight into the functional role of the RA signaling pathway as it relates to lung development. Targeted deletion of RARβ alters the regulation of lung septation [61]. RARγ deletion also results in reduced elastic tissue and alveolar number with increase in mean chord length [62]. The authors found similar results with RXRα deletion. Desai and colleagues demonstrated that balanced activation of RARα and β is critical for normal lung bud initiation and endodermal differentiation [63]. Mollard and colleagues determined that RA signaling through RARβ during the pseudoglandular stage promotes the formation of conducting airways [64]. Because single RAR mutants have few to no lung abnormalities [61, 64–68], double mutants have been developed because of the apparent redundancy in these receptors. For example, RARα/RXRα and RARα/β double mutants develop lung hypoplasia or agenesis [69–71]. Additionally, retinoids are capable of promoting the formation of alveoli in neonatal rats and in adult rats with elastase-induced emphysema [72, 73].

4. EPITHELIAL CELL PPARγ EXPRESSION CONTRIBUTES TO THE REGULATION OF LUNG MATURATION

Most of the literature regarding the role of PPARs in the lung has focused on understanding PPARγ. While PPARα shares the common characteristic of having potent anti-inflammatory properties with PPARγ, it has not been shown to have a role in regulating lung development. Similarly, there has been no description for a role of PPARβ/δ in modulating lung development though Matsuura and colleagues demonstrated upregulation of PPARβ/δ expression in induced human tracheobronchial epithelial (HBE) cells which suggests that PPARβ/δ may have a role in the squamous differentiation process of airway cells [74]. PPARγ is expressed as at least 2 different isoforms, γ1 and γ2. These isoforms differ only by the addition of 30 amino acids at the amino terminus of γ2, and appear to be functionally equivalent. While PPARγ2 is expressed primarily in adipose tissue, PPARγ1 is expressed in a broad range of tissues including the lung, heart, skeletal muscle, large and small intestine, kidney, pancreas, spleen, and breast [5, 75]. Within the lung, PPARγ expression has been reported in the airway epithelium [76, 77], bronchial smooth muscle [76, 78], endothelial cells [79], macrophages [80], eosinophils [81], and dendritic cells [82]. There is little data describing the expression of PPARγ in the developing lung. Barlier-Mur and colleagues found that PPARγ1 mRNA was detectable at 18 days gestation in fetal rat lungs, as well as the C/EBPs [83]. The expression of these factors increased during development, peaking just prior to delivery. While they and others [84] have reported PPARγ expression in type II alveolar cells, they did not see that this expression pattern was developmentally regulated, although it could be induced by exposure of cultured type II alveolar cells to dexamethasone, retinoic acid, EGF, and KGF. Interestingly, PPARγ protein concentrations were only induced by KGF, and not with EGF or dexamethasone. We observed a spatial and temporally restricted pattern of PPARγ expression, including prominent immunolocalization within the conducting airway epithelium of normal mouse lungs [85]. This pattern of staining was first detectable at birth and increased in intensity over the first few weeks of life in mice.

PPARγ can play a prominent role in regulating cellular differentiation. PPARγ is sufficient and necessary to promote the formation of adipocytes and the development of adipose tissue in vivo [75, 86]. This appears to be due, at least in part, to the ability of PPARγ to regulate numerous genes involved in lipid metabolism. Complete germ-line PPARγ deficiency in mice results in embryonic death at mid gestation, prior to lung development due to failed placental cytotrophoblast differentiation, which is necessary for placental vascularization [87]. Recently, Duan and colleagues generated a mouse model of complete PPARγ deficiency that spared the trophoblast, allowing delivery of viable pups that they used to study the role of PPARγ in the metabolic syndrome [88]. Unfortunately, there was no description of effect on lung development. A role for PPARγ in promoting cellular differentiation is also suggested by its antitumor effects in vivo and in vitro, which include suppressing cellular proliferation, promoting cell death, and inducing differentiation of malignant tumors cells from various organs including the lung [89], breast [90], colon [91], and adipose tissue [92]. In isolated lung epithelial cells, PPARγ can promote the expression of markers for terminal differentiation including the expression of surfactant associated protein genes [93–95].

In addition to its roles in cellular differentiation and organ/tissue development, PPARγ is widely appreciated as a regulator of tissue inflammation, which will be discussed in other sections of this review. In brief, PPARγ activation can modulate various immune cell functions. For example, PPARγ regulates monocyte/macrophage differentiation and promotes cellular activation as measured by increased production of metalloproteinases and reactive oxygen species [96]. Dendritic cells express PPARγ, which upon activation

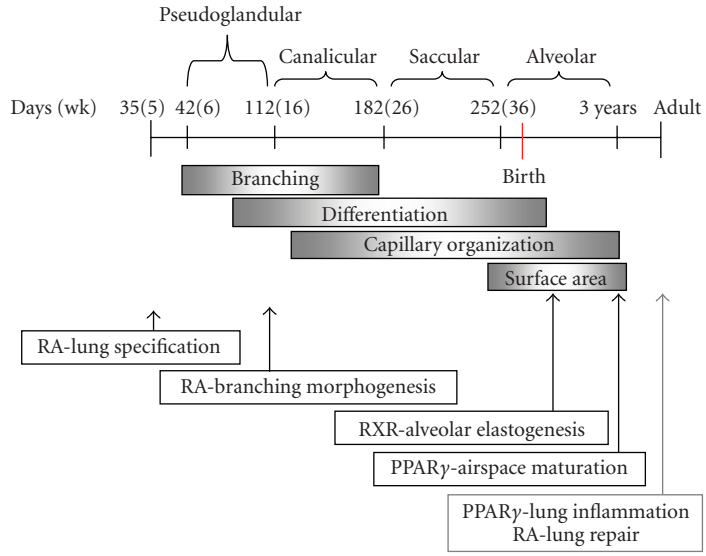

Figure 1: *Retinoic acid and PPARγ signaling are essential at many points during lung development.* Lung development occurs in multiple stages (top), each involving critical processes (middle) and multiple regulatory factors. This schematic highlights the timeline for human lung development, though murine lung development occurs in similar stages. It is widely appreciated that retinoic acid signaling has effects on all stages of lung development (bottom). Recently, PPARγ has also been found to be a critical modulator of postnatal lung development. (Adapted from Mariani, T.J. Developmental genetics of the pulmonary system. In: Moody, S.A., Editor, Principles of developmental genetics. Burlington, VT: Academic Press, 2007:932-945. With the permission of Elsevier Inc.)

can influence cell maturation and antigenic peptide presentation to T cells [82, 97]. PPARγ is expressed at low levels in resting T cells, but is increased following T cell activation where PPARγ can then inhibit T cell IL-2 and IFNγ production [98]. Additionally, PPARγ activation has an antiproliferative and cytotoxic effect on normal and malignant B cells [99]. While PPARγ expression has been reported in these various cell types, the target cells and mechanisms for the protective, anti-inflammatory activities of PPARγ ligands within the lung are unclear. Some of these inflammation-related functions of PPARγ appear to mediate, at least in part, the regulation of resident cell functions. PPARγ has been shown to be expressed in cultured human airway smooth muscle cells and its activation inhibits cell growth while inducing apoptosis and inhibits release of GM-CSF and G-CSF to a greater extent than dexamethasone, a medication frequently used in asthma [78]. Further, in cultured human airway epithelial cells, PPARγ activation can inhibit expression of proinflammatory mediators such as TNF-α, IL-8, iNOS, and MCP-1 [5, 77, 81].

Our laboratory sought to understand the physiological role of epithelial cell PPARγ and its potential contribution to lung development and homeostasis, considering the fact that PPARγ is capable of having a significant and complex influence upon cellular differentiation, organ development, and the control of tissue homeostasis. We hypothesized that epithelial cell PPARγ might be necessary for the establishment and maintenance of normal lung structure through regulation of epithelial cell differentiation and/or control of lung inflammation.

Using a conditional targeting strategy, we deleted the PPARγ gene specifically within conducting airway epithelial cells [85]. We started by generating a new line of Cre Recombinase-expressing targeting mice, termed CCtCre, where the rat CC10 promoter was used to drive Cre expression specifically within the lung conducting airway epithelium. Functional targeting specificity in these CCtCre mice was confirmed by crossing them to the ROSA26 reporter line. Crossing the CCtCre mice with mice engineered to have loxP sites (targets of Cre-mediated recombination) flanking exon 2 of the PPARγ gene led to targeted deletion within the airway epithelium (see Figure 2).

Lungs from PPARγ conditionally targeted, airway epithelial cell PPARγ deficient mice revealed structural and functional abnormalities at maturity, but not prior to maturity, including enlarged airspaces consistent with a deficiency in postnatal lung maturation (see Figure 1). Abnormal airspace structure persists throughout adulthood, but is not progressive and occurs in the absence of inflammation. While control animals show a reduction in mean airspace size between 2 and 8 weeks of age, conditionally targeted, airway epithelial cell PPARγ deficient animals do not. These data suggest that the phenotype results from an insufficiency in postnatal lung maturation. This does not appear to be the result of a defect in alveogenesis, as numerous normal-sized alveoli exist in conditionally targeted lungs. However, an abnormal distribution of airspaces, with increased numbers of alveolar ducts is observed (unpublished observations).

No qualitative or quantitative changes in the major classes of airway and airspace epithelial cells are evident, but

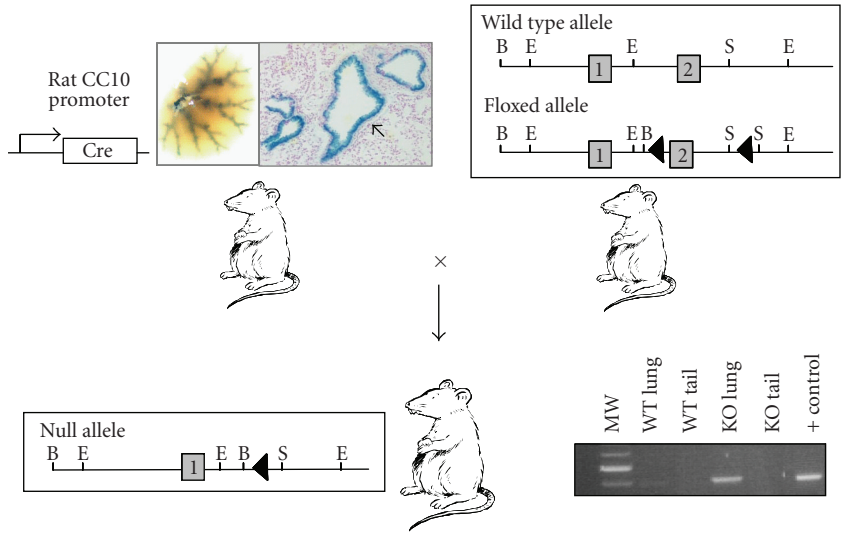

Figure 2: *The generation of conditionally targeted epithelial cell PPARγ deficient mice [85].* We developed a line of mice capable of targeting the airway epithelium by expressing Cre recombinase under the direction of the rat CC10 promoter (top, left). These mice, termed CCtCre, were crossed with the ROSA26 Cre reporter mouse to test the efficiency for recombining loxP sites in vivo which demonstrated β-galactosidase staining limited to the conducting airway epithelium (arrow within inset). We crossed the CCtCre mice with mice homozygous for a PPARγ allele with a pair of loxP sites flanking exon 2 of the gene (top, right) [100], creating mice with PPARγ deficiency limited to the conducting airway epithelium (bottom, left). The conditional targeted genotype was confirmed by identification of gene rearrangement specifically in the lung alone (bottom, right).

some characteristics of airway epithelial cell differentiation appear affected. We found, through genome wide expression analysis of targeted airway epithelial cells, changes consistent with alterations in PPARγ function (Lip1, Abca1, and Apoe) and cellular differentiation (Moesin, Ctsb, Klf13). We believe that altered epithelial-mesenchymal interactions, secondary to epithelial PPARγ deficiency, lead to changes in extracellular matrix gene expression and abnormal lung structure at maturity. Efforts to further define the mechanism(s) mediating this abnormality and to test the role of this transcription factor in regulating airway inflammation are the focus of current investigation.

In summary, it is well appreciated that the retinoic acid signaling pathway contributes to the regulation of lung development at many different stages, including during terminal maturation giving rise to the functional gas exchange units of the lung, the alveoli. Although retinoic acid activity during alveogenesis appears to be linked to elastin fiber formation, the cellular and molecular mechanisms for these effects are not well defined. It has recently become apparent that PPARγ has a role in contributing to these regulatory processes. Again, the mechanisms at work are yet to be defined. Potentially, they involve the regulation of epithelial cell differentiation, and may act in part through interaction with the RARs and RXRs. Tremendous current activities in the field of PPAR biology should rapidly lead to a better understanding of the role of these transcription factors in promoting lung maturation and their potential contribution to human lung disease.

REFERENCES

[1] A. Ijpenberg, N. S. Tan, L. Gelman, et al., "In vivo activation of PPAR target genes by RXR homodimers," *The EMBO Journal*, vol. 23, no. 10, pp. 2083–2091, 2004.

[2] C. Yu, K. Markan, K. A. Temple, D. Deplewski, M. J. Brady, and R. N. Cohen, "The nuclear receptor corepressors NCoR and SMRT decrease peroxisome proliferator-activated receptor γ transcriptional activity and repress 3T3-L1 adipogenesis," *Journal of Biological Chemistry*, vol. 280, no. 14, pp. 13600–13605, 2005.

[3] L. H. Wang, X. Y. Yang, X. Zhang, et al., "Transcriptional inactivation of STAT3 by PPARγ suppresses IL-6-responsive multiple myeloma cells," *Immunity*, vol. 20, no. 2, pp. 205–218, 2004.

[4] M. Gurnell, J. M. Wentworth, M. Agostini, et al., "A dominant-negative peroxisome proliferator-activated receptor γ (PPARγ) mutant is a constitutive repressor and inhibits PPARγ-mediated adipogenesis," *Journal of Biological Chemistry*, vol. 275, no. 8, pp. 5754–5759, 2000.

[5] R. A. Daynes and D. C. Jones, "Emerging roles of PPARs in inflammation and immunity," *Nature Reviews Immunology*, vol. 2, no. 10, pp. 748–759, 2002.

[6] M. Schupp, J. C. Curtin, R. J. Kim, A. N. Billin, and M. A. Lazar, "A widely used retinoic acid receptor antagonist induces peroxisome proliferator-activated receptor-γ activity," *Molecular Pharmacology*, vol. 71, pp. 1251–1257, 2007.

[7] I. Szatmari, A. Pap, R. Rühl, et al., "PPARγ controls CD1d expression by turning on retinoic acid synthesis in developing human dendritic cells," *Journal of Experimental Medicine*, vol. 203, no. 10, pp. 2351–2362, 2006.

[8] T. J. Mariani and N. Kaminski, "Gene expression studies in lung development and lung stem cell biology," *Current Topics in Developmental Biology*, vol. 64, pp. 57–71, 2004.

[9] T. J. Mariani, J. J. Reed, and S. D. Shapiro, "Expression profiling of the developing mouse lung: insights into the establishment of the extracellular matrix," *American Journal of Respiratory Cell and Molecular Biology*, vol. 26, no. 5, pp. 541–548, 2002.

[10] D. Warburton, J. Zhao, M. A. Berberich, and M. Bernfield, "Molecular embryology of the lung: then, now, and in the future," *American Journal of Physiology - Lung Cellular and Molecular Physiology*, vol. 276, no. 5, part 1, pp. L697–L704, 1999.

[11] D. Warburton, M. Schwarz, D. Tefft, G. Flores-Delgado, K. D. Anderson, and W. V. Cardoso, "The molecular basis of lung morphogenesis," *Mechanisms of Development*, vol. 92, no. 1, pp. 55–81, 2000.

[12] J. Motoyama, J. Liu, R. Mo, Q. Ding, M. Post, and C.-C. Hui, "Essential function of *Gli2* and *Gli3* in the formation of lung, trachea and oesophagus," *Nature Genetics*, vol. 20, no. 1, pp. 54–57, 1998.

[13] Y. Litingtung, L. Lei, H. Westphal, and C. Chiang, "Sonic hedgehog is essential to foregut development," *Nature Genetics*, vol. 20, no. 1, pp. 58–61, 1998.

[14] C. V. Pepicelli, P. M. Lewis, and A. P. McMahon, "Sonic hedgehog regulates branching morphogenesis in the mammalian lung," *Current Biology*, vol. 8, no. 19, pp. 1083–1086, 1998.

[15] K. Peters, S. Werner, X. Liao, S. Wert, J. A. Whitsett, and L. Williams, "Targeted expression of a dominant negative FGF receptor blocks branching morphogenesis and epithelial differentiation of the mouse lung," *The EMBO Journal*, vol. 13, no. 14, pp. 3296–3301, 1994.

[16] S. Bellusci, J. Grindley, H. Emoto, N. Itoh, and B. L. M. Hogan, "Fibroblast growth factor 10 (FGF10) and branching morphogenesis in the embryonic mouse lung," *Development*, vol. 124, no. 23, pp. 4867–4878, 1997.

[17] D. Sutherland, C. Samakovlis, and M. A. Krasnow, "*branchless* encodes a *Drosophila* FGF homolog that controls tracheal cell migration and the pattern of branching," *Cell*, vol. 87, no. 6, pp. 1091–1101, 1996.

[18] W. Y. Park, B. Miranda, D. Lebeche, G. Hashimoto, and W. V. Cardoso, "FGF-10 is a chemotactic factor for distal epithelial buds during lung development," *Developmental Biology*, vol. 201, no. 2, pp. 125–134, 1998.

[19] H. Min, D. M. Danilenko, S. A. Scully, et al., "Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to *Drosophila* branchless," *Genes and Development*, vol. 12, no. 20, pp. 3156–3161, 1998.

[20] K. Sekine, H. Ohuchi, M. Fujiwara, et al., "Fgf10 is essential for limb and lung formation," *Nature Genetics*, vol. 21, no. 1, pp. 138–141, 1999.

[21] S. Bellusci, R. Henderson, G. Winnier, T. Oikawa, and B. L. M. Hogan, "Evidence from normal expression and targeted misexpression that bone morphogenetic protein-4 (Bmp-4) plays a role in mouse embryonic lung morphogenesis," *Development*, vol. 122, no. 6, pp. 1693–1702, 1996.

[22] M. Weaver, N. R. Dunn, and B. L. M. Hogan, "Bmp4 and Fgf10 play opposing roles during lung bud morphogenesis," *Development*, vol. 127, no. 12, pp. 2695–2704, 2000.

[23] J. D. Tefft, L. Matt, S. Smith, et al., "Conserved function of *mSpry-2*, a murine homolog of *Drosophila sprouty*, which negatively modulates respiratory organogenesis," *Current Biology*, vol. 9, no. 4, pp. 219–222, 1999.

[24] M. Weaver, J. M. Yingling, N. R. Dunn, S. Bellusci, and B. L. M. Hogan, "Bmp signaling regulates proximal-distal differentiation of endoderm in mouse lung development," *Development*, vol. 126, no. 18, pp. 4005–4015, 1999.

[25] M. Roth-Kleiner and M. Post, "Genetic control of lung development," *Biology of the Neonate*, vol. 84, no. 1, pp. 83–88, 2003.

[26] J. M. Shannon and B. A. Hyatt, "Epithelial-mesenchymal interactions in the developing lung," *Annual Review of Physiology*, vol. 66, pp. 625–645, 2004.

[27] W. V. Cardoso, "Lung morphogenesis revisited: old facts, current ideas," *Developmental Dynamics*, vol. 219, no. 2, pp. 121–130, 2000.

[28] H. Wan, Y. Xu, M. Ikegami, et al., "Foxa2 is required for transition to air breathing at birth," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 40, pp. 14449–14454, 2004.

[29] P. C. Martis, J. A. Whitsett, Y. Xu, A.-K. T. Perl, H. Wan, and M. Ikegami, "C/EBPα is required for lung maturation at birth," *Development*, vol. 133, no. 6, pp. 1155–1164, 2006.

[30] V. Davé, T. Childs, Y. Xu, et al., "Calcineurin/Nfat signaling is required for perinatal lung maturation and function," *Journal of Clinical Investigation*, vol. 116, no. 10, pp. 2597–2609, 2006.

[31] P. H. Burri, "Fetal and postnatal development of the lung," *Annual Review of Physiology*, vol. 46, pp. 617–628, 1984.

[32] H. Boström, K. Willetts, M. Pekny, et al., "PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis," *Cell*, vol. 85, no. 6, pp. 863–873, 1996.

[33] M. Weinstein, X. Xu, K. Ohyama, and C.-X. Deng, "FGFR-3 and FGFR-4 function cooperatively to direct alveogenesis in the murine lung," *Development*, vol. 125, no. 18, pp. 3615–3623, 1998.

[34] T. J. Mariani, "Regulation of alveogenesis by reciprocal proximodistal FGF and retinoic acid signaling," *American Journal of Respiratory Cell and Molecular Biology*, vol. 31, no. 2, pp. S52–S57, 2004.

[35] Y. Kasahara, R. M. Tuder, L. Taraseviciene-Stewart, et al., "Inhibition of VEGF receptors causes lung cell apoptosis and emphysema," *Journal of Clinical Investigation*, vol. 106, no. 11, pp. 1311–1319, 2000.

[36] X. Zeng, S. E. Wert, R. Federici, K. G. Peters, and J. A. Whitsett, "VEGF enhances pulmonary vasculogenesis and disrupts lung morphogenesis in vivo," *Developmental Dynamics*, vol. 211, no. 3, pp. 215–227, 1998.

[37] H.-P. Gerber, K. J. Hillan, A. M. Ryan, et al., "VEGF is required for growth and survival in neonatal mice," *Development*, vol. 126, no. 6, pp. 1149–1159, 1999.

[38] B. Thébaud, F. Ladha, E. D. Michelakis, et al., "Vascular endothelial growth factor gene therapy increases survival, promotes lung angiogenesis, and prevents alveolar damage in hyperoxia-induced lung injury: evidence that angiogenesis participates in alveolarization," *Circulation*, vol. 112, no. 16, pp. 2477–2486, 2005.

[39] V. Compernolle, K. Brusselmans, T. Acker, et al., "Loss of HIF-2α and inhibition of VEGF impair fetal lung maturation, whereas treatment with VEGF prevents fatal respiratory distress in premature mice," *Nature Medicine*, vol. 8, no. 7, pp. 702–710, 2002.

[40] E. A. Boyden and D. H. Tompsett, "The changing patterns in the developing lungs of infants," *Acta Anatomica*, vol. 61, no. 2, pp. 164–192, 1965.

[41] D. Massaro, N. Teich, S. Maxwell, G. D. Massaro, and P. Whitney, "Postnatal development of alveoli. Regulation and evidence for a critical period in rats," *Journal of Clinical Investigation*, vol. 76, no. 4, pp. 1297–1305, 1985.

[42] K. Kida and W. M. Thurlbeck, "The effects of β-aminopropionitrile on the growing rat lung," *American Journal of Pathology*, vol. 101, no. 3, pp. 693–710, 1980.

[43] A. Nagai, M. Katayama, W. M. Thurlbeck, R. Matsui, S. Yasui, and K. Konno, "Effect of indomethacin on lung development in postnatal rats: possible role of prostaglandin in alveolar formation," *American Journal of Physiology - Lung Cellular and Molecular Physiology*, vol. 268, no. 1, part 1, pp. L56–L62, 1995.

[44] Y. Kimura, T. Suzuki, C. Kaneko, et al., "Retinoid receptors in the developing human lung," *Clinical Science*, vol. 103, no. 6, pp. 613–621, 2002.

[45] P. Dolle, V. Fraulob, P. Kastner, and P. Chambon, "Developmental expression of murine retinoid X receptor (RXR) genes," *Mechanisms of Development*, vol. 45, no. 2, pp. 91–104, 1994.

[46] S. Malpel, C. Mendelsohn, and W. V. Cardoso, "Regulation of retinoic acid signaling during lung morphogenesis," *Development*, vol. 127, no. 14, pp. 3057–3067, 2000.

[47] P. Dolle, E. Ruberte, P. Leroy, G. Morriss-Kay, and P. Chambon, "Retinoic acid receptors and cellular retinoid binding proteins—I: a systematic study of their differential pattern of transcription during mouse organogenesis," *Development*, vol. 110, no. 4, pp. 1133–1151, 1990.

[48] C. Wongtrakool, S. Malpel, and J. Gorenstein, "Down-regulation of retinoic acid receptor α signaling is required for sacculation and type I cell formation in the developing lung," *Journal of Biological Chemistry*, vol. 278, no. 47, pp. 46911–46918, 2003.

[49] M. Mark, N. B. Ghyselinck, and P. Chambon, "Function of retinoid nuclear receptors: lessons from genetic and pharmacological dissections of the retinoic acid signaling pathway during mouse embryogenesis," *Annual Review of Pharmacology and Toxicology*, vol. 46, pp. 451–480, 2006.

[50] P. Germain, P. Chambon, G. Eichele, et al., "International union of pharmacology. LX. Retinoic acid receptors," *Pharmacological Reviews*, vol. 58, no. 4, pp. 712–725, 2006.

[51] P. Germain, P. Chambon, G. Eichele, et al., "International union of pharmacology. LXIII. Retinoid X receptors," *Pharmacological Reviews*, vol. 58, no. 4, pp. 760–772, 2006.

[52] P. Kastner, J. M. Grondona, M. Mark, et al., "Genetic analysis of RXRα developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis," *Cell*, vol. 78, no. 6, pp. 987–1003, 1994.

[53] H. M. Sucov, E. Dyson, C. L. Gumeringer, J. Price, K. R. Chien, and R. M. Evans, "RXR α mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis," *Genes and Development*, vol. 8, no. 9, pp. 1007–1018, 1994.

[54] M. Li, H. Chiba, X. Warot, et al., "RXRα ablation in skin keratinocytes results in alopecia and epidermal alterations," *Development*, vol. 128, no. 5, pp. 675–688, 2001.

[55] M. Li, A. K. Indra, X. Warot, et al., "Skin abnormalities generated by temporally controlled RXRα mutations in mouse epidermis," *Nature*, vol. 407, no. 6804, pp. 633–636, 2000.

[56] D. Metzger, M. Li, and P. Chambon, "Targeted somatic mutagenesis in the mouse epidermis," *Methods in Molecular Biology*, vol. 289, pp. 329–340, 2005.

[57] Y.-J. Y. Wan, D. An, Y. Cai, et al., "Hepatocyte-specific mutation establishes retinoid X receptor α as a heterodimeric integrator of multiple physiological processes in the liver," *Molecular and Cellular Biology*, vol. 20, no. 12, pp. 4436–4444, 2000.

[58] P. Kastner, M. Mark, M. Leid, et al., "Abnormal spermatogenesis in RXRβ mutant mice," *Genes and Development*, vol. 10, no. 1, pp. 80–92, 1996.

[59] W. Krezel, V. Dupé, M. Mark, A. Dierich, P. Kastner, and P. Chambon, "RXR γ null mice are apparently normal and compound RXR α+/-/RXR β-/-/RXR γ-/- mutant mice are viable," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 17, pp. 9010–9014, 1996.

[60] N. S. Brown, A. Smart, V. Sharma, et al., "Thyroid hormone resistance and increased metabolic rate in the RXR-γ- deficient mouse," *Journal of Clinical Investigation*, vol. 106, no. 1, pp. 73–79, 2000.

[61] G. D. Massaro, D. Massaro, W. Y. Chan, et al., "Retinoic acid receptor-β: an endogenous inhibitor of the perinatal formation of pulmonary alveoli," *Physiological Genomics*, vol. 4, no. 1, pp. 51–57, 2000.

[62] S. McGowan, S. K. Jackson, M. Jenkins-Moore, H.-H. Dai, P. Chambon, and J. M. Snyder, "Mice bearing deletions of retinoic acid receptors demonstrate reduced lung elastin and alveolar numbers," *American Journal of Respiratory Cell and Molecular Biology*, vol. 23, no. 2, pp. 162–167, 2000.

[63] T. J. Desai, F. Chen, J. Lü, et al., "Distinct roles for retinoic acid receptors α and β in early lung morphogenesis," *Developmental Biology*, vol. 291, no. 1, pp. 12–24, 2006.

[64] R. Mollard, N. B. Ghyselinck, O. Wendling, P. Chambon, and M. Mark, "Stage-dependent responses of the developing lung to retinoic acid signaling," *International Journal of Developmental Biology*, vol. 44, no. 5, pp. 457–462, 2000.

[65] D. Lohnes, P. Kastner, A. Dierich, M. Mark, M. LeMeur, and P. Chambon, "Function of retinoic acid receptor γ in the mouse," *Cell*, vol. 73, no. 4, pp. 643–658, 1993.

[66] E. Ruberte, P. Dolle, A. Krust, A. Zelent, G. Morriss-Kay, and P. Chambon, "Specific spatial and temporal distribution of retinoic acid receptor γ transcripts during mouse embryogenesis," *Development*, vol. 108, no. 2, pp. 213–222, 1990.

[67] J. Luo, P. Pasceri, R. A. Conlon, J. Rossant, and V. Giguere, "Mice lacking all isoforms of retinoic acid receptor β develop normally and are susceptible to the teratogenic effects of retinoic acid," *Mechanisms of Development*, vol. 53, no. 1, pp. 61–71, 1995.

[68] N. B. Ghyselinck, V. Dupé, and A. Dierich, "Role of the retinoic acid receptor β (RARβ) during mouse development," *International Journal of Developmental Biology*, vol. 41, no. 3, pp. 425–447, 1997.

[69] C. Mendelsohn, D. Lohnes, D. Decimo, et al., "Function of the retinoic acid receptors (RARs) during development (II). multiple abnormalities at various stages of organogenesis in RAR double mutants," *Development*, vol. 120, no. 10, pp. 2749–2771, 1994.

[70] P. Kastner, M. Mark, N. Ghyselinck, et al., "Genetic evidence that the retinoid signal is transduced by heterodimeric RXR/RAR functional units during mouse development," *Development*, vol. 124, no. 2, pp. 313–326, 1997.

[71] M. Maden, "The role of retinoic acid in embryonic and post-embryonic development," *Proceedings of the Nutrition Society*, vol. 59, no. 1, pp. 65–73, 2000.

[72] G. D. Massaro and D. Massaro, "Postnatal treatment with retinoic acid increases the number of pulmonary alveoli in rats," *American Journal of Physiology - Lung Cellular and Molecular Physiology*, vol. 270, no. 2, part 1, pp. L305–L310, 1996.

[73] G. C. Massaro and D. Massaro, "Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats," *Nature Medicine*, vol. 3, no. 6, pp. 675–677, 1997.

[74] H. Matsuura, H. Adachi, R. C. Smart, X. Xu, J. Arata, and A. M. Jetten, "Correlation between expression of peroxisome proliferator-activated receptor β and squamous differentiation in epidermal and tracheobronchial epithelial cells," *Molecular and Cellular Endocrinology*, vol. 147, no. 1-2, pp. 85–92, 1999.

[75] J. Berger and D. E. Moller, "The mechanisms of action of PPARs," *Annual Review of Medicine*, vol. 53, pp. 409–435, 2002.

[76] L. Benayoun, S. Letuve, A. Druilhe, et al., "Regulation of peroxisome proliferator-activated receptor γ expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling," *American Journal of Respiratory and Critical Care Medicine*, vol. 164, no. 8, part 1, pp. 1487–1494, 2001.

[77] A. C. Wang, X. Dai, B. Luu, and D. J. Conrad, "Peroxisome proliferator-activated receptor-γ regulates airway epithelial cell activation," *American Journal of Respiratory Cell Molecular Biology*, vol. 24, no. 6, pp. 688–693, 2001.

[78] H. J. Patel, M. G. Belvisi, D. Bishop-Bailey, M. H. Yacoub, and J. A. Mitchell, "Activation of peroxisome proliferator-activated receptors in human airway smooth muscle cells has a superior anti-inflammatory profile to corticosteroids: relevance for chronic obstructive pulmonary disease therapy," *Journal of Immunology*, vol. 170, no. 5, pp. 2663–2669, 2003.

[79] D. S. Calnek, L. Mazzella, S. Roser, J. Roman, and C. M. Hart, "Peroxisome proliferator-activated receptor γ ligands increase release of nitric oxide from endothelial cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 1, pp. 52–57, 2003.

[80] G. Chinetti, S. Griglio, M. Antonucci, et al., "Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages," *Journal of Biological Chemistry*, vol. 273, no. 40, pp. 25573–25580, 1998.

[81] G. Woerly, K. Honda, M. Loyens, et al., "Peroxisome proliferator-activated receptors α and γ down-regulate allergic inflammation and eosinophil activation," *Journal of Experimental Medicine*, vol. 198, no. 3, pp. 411–421, 2003.

[82] P. Gosset, A.-S. Charbonnier, P. Delerive, et al., "Peroxisome proliferator-activated receptor γ activators affect the maturation of human monocyte-derived dendritic cells," *European Journal of Immunology*, vol. 31, no. 10, pp. 2857–2865, 2001.

[83] A.-M. Barlier-Mur, B. Chailley-Heu, C. Pinteur, A. Henrion-Caude, C. Delacourt, and J. R. Bourbon, "Maturational factors modulate transcription factors CCAAT/enhancer-binding proteins α, β, δ, and peroxisome proliferator-activated receptor-γ in fetal rat lung epithelial cells," *American Journal of Respiratory Cell and Molecular Biology*, vol. 29, no. 5, pp. 620–626, 2003.

[84] L. F. Michael, M. A. Lazar, and C. R. Mendelson, "Peroxisome proliferator-activated receptor γ1 expression is induced during cyclic adenosine monophosphate-stimulated differentiation of alveolar type II pneumonocytes," *Endocrinology*, vol. 138, no. 9, pp. 3695–3703, 1997.

[85] D. M. Simon, M. C. Arikan, S. Srisuma, et al., "Epithelial cell PPARγ contributes to normal lung maturation," FASEB j, vol. 20, no. 9, pp. 1507–1509, 2006.

[86] J. Zhang, M. Fu, T. Cui, et al., "Selective disruption of PPARγ2 impairs the development of adipose tissue and insulin sensitivity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 29, pp. 10703–10708, 2004.

[87] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[88] S. Z. Duan, C. Y. Ivashchenko, S. E. Whitesall, et al., "Hypotension, lipodystrophy, and insulin resistance in generalized PPARγ-deficient mice rescued from embryonic lethality," *Journal of Clinical Investigation*, vol. 117, no. 3, pp. 812–822, 2007.

[89] P. Shankaranarayanan and S. Nigam, "IL-4 induces apoptosis in A549 lung adenocarcinoma cells: evidence for the pivotal role of 15-hydroxyeicosatetraenoic acid binding to activated peroxisome proliferator-activated receptor γ transcription factor," *Journal of Immunology*, vol. 170, no. 2, pp. 887–894, 2003.

[90] E. Mueller, P. Sarraf, P. Tontonoz, et al., "Terminal differentiation of human breast cancer through PPARγ," *Molecular Cell*, vol. 1, no. 3, pp. 465–470, 1998.

[91] P. Sarraf, E. Mueller, D. Jones, et al., "Differentiation and reversal of malignant changes in colon cancer through PPARγ," *Nature Medicine*, vol. 4, no. 9, pp. 1046–1052, 1998.

[92] P. Tontonoz, S. Singer, B. M. Forman, et al., "Terminal differentiation of human liposarcoma cells induced by ligands for peroxisome proliferator-activated receptor γ and the retinoid X receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 1, pp. 237–241, 1997.

[93] Y. Bren-Mattison, V. van Putten, D. Chan, R. Winn, M. W. Geraci, and R. A. Nemenoff, "Peroxisome proliferator-activated receptor-γ (PPARγ) inhibits tumorigenesis by reversing the undifferentiated phenotype of metastatic non-small-cell lung cancer cells (NSCLC)," *Oncogene*, vol. 24, no. 8, pp. 1412–1422, 2005.

[94] T.-H. Chang and E. Szabo, "Induction of differentiation and apoptosis by ligands of peroxisome proliferator-activated receptor γ in non-small cell lung cancer," *Cancer Research*, vol. 60, no. 4, pp. 1129–1138, 2000.

[95] L. Yang, D. Yan, C. Yan, and H. Du, "Peroxisome proliferator-activated receptor γ and ligands inhibit surfactant protein B gene expression in the lung," *Journal of Biological Chemistry*, vol. 278, pp. 36841–36847, 2003.

[96] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[97] H. Hammad, H. J. de Heer, T. Soullié, et al., "Activation of peroxisome proliferator-activated receptor-γ in dendritic cells inhibits the development of eosinophilic airway inflammation in a mouse model of asthma," *American Journal of Pathology*, vol. 164, no. 1, pp. 263–271, 2004.

[98] R. B. Clark, D. Bishop-Bailey, T. Estrada-Hernandez, T. Hla, L. Puddington, and S. J. Padula, "The nuclear receptor PPARγ and immunoregulation: PPARγ mediates inhibition of helper T cell responses," *Journal of Immunology*, vol. 164, no. 3, pp. 1364–1371, 2000.

[99] J. Padilla, K. Kaur, S. G. Harris, and R. P. Phipps, "PPAR-γ-mediated regulation of normal and malignant B lineage cells," *Annals of the New York Academy of Sciences*, vol. 905, pp. 97–109, 2000.

[100] T. E. Akiyama, S. Sakai, G. Lambert, et al., "Conditional disruption of the peroxisome proliferator-activated receptor γ gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux," *Molecular and Cellular Biology*, vol. 22, no. 8, pp. 2607–2619, 2002.

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Review Article

PPARs in Alveolar Macrophage Biology

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PPARs, most notably PPAR-γ, play a crucial role in regulating the activation of alveolar macrophages, which in turn occupy a pivotal place in the immune response to pathogens and particulates drawn in with inspired air. In this review, we describe the dual role of the alveolar macrophage as both a first-line defender through its phagocytotic activity and a regulator of the immune response. Depending on its state of activation, the alveolar macrophage may either enhance or suppress different aspects of immune function in the lung. We then review the role of PPAR-γ and its ligands in deactivating alveolar macrophages—thus limiting the inflammatory response that, if unchecked, could threaten the essential respiratory function of the alveolus—while upregulating the cell's phagocytotic activity. Finally, we examine the role that inadequate or inappropriate PPAR-γ responses play in specific lung diseases.

1. INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear-receptor superfamily. Their name derives from the first-discovered member of the class, PPAR-α, whose activation induces proliferation of peroxisomes in the liver; no similar effect is seen with other members of the class, however. These receptors act as transcription factors, forming heterodimers with the retinoid X receptor and then binding to specific response elements (PPREs) in the promoter regions of the genes they regulate. When activated by appropriate ligands, PPARs undergo a conformational change that leads to release of corepressors and binding of coactivator molecules, with consequent increases in transcription of the genes involved. Some evidence suggests that in the absence of activating ligands, PPARs may bind corepressors and downregulate expression of genes with PPRE-containing promoters [1].

There are three PPAR isoforms: PPAR-α, PPAR-γ, and PPAR-β/δ. Each of the isoforms is the product of a different gene. PPAR-β/δ is expressed in almost every tissue of the body. PPAR-α is most commonly thought of in connection with hepatocytes and PPAR-γ with adipocytes, but in fact both are expressed in a variety of cells and tissues. Broadly speaking, PPAR-α regulates lipid metabolism, lipoprotein formation and transport as well as lipid catabolism, whereas PPAR-β/δ promotes lipid oxidation, and PPAR-γ promotes adipogenesis [2]. Each has other functions in specific tissues, however. For example, PPAR-α inhibits proliferation of vascular smooth muscle cells in response to injury [3] and antagonizes the effects of angiotensin II on the vascular wall [4]. In skin, PPAR-β/δ induces terminal differentiation of keratinocytes [5]. Activation of PPAR-γ in endothelial and vascular smooth muscle cells likewise inhibits expression of the angiotensin II receptor [6] and lowers blood pressure in hypertensive mice [7]. PPAR-γ agonists have also been shown to exert antiproliferative effects on a variety of cancer cells [8]. This has led to widespread discussion of their possible usefulness in cancer therapy (e.g., for breast cancer [9]) and even to a few early clinical trials.

All three PPARs have significant anti-inflammatory roles in cells of the immune system. PPAR-γ has been found in monocytes/macrophages [10, 11], neutrophils [12], dendritic cells [13], B [14] and T [15–17] lymphocytes, eosinophils [18], natural killer cells [19], and mast cells [20]. PPAR-γ downregulates expression of a number of proinflammatory mediators while upregulating expression of anti-inflammatory mediators (reviewed in [21]). PPAR-α is found in essentially all the same cells as PPAR-γ with

the notable exception of (mature) dendritic cells and alveolar macrophages [22]. Among the many anti-inflammatory effects of PPAR-α that have been demonstrated is inhibition of airway inflammation induced by lipopolysaccharide [23] and of the inflammatory ear-swelling response to leukotriene B_4 [24]. Furthermore, the acute anti-inflammatory effects of the anticholesterolemic drug simvastatin have been shown to be mediated by PPAR-α [25]. The role of PPAR-β/δ in the immune system has been less extensively investigated and while alveolar macrophages have been found to express PPAR-β/δ [22], no studies to our knowledge have demonstrated the functional importance of this receptor in these cells. PPAR-β/δ has been shown, however, to decrease the expression of proinflammatory mediators by other types of macrophages [26].

Monocytes are produced in the bone marrow but, under the influence of chemoattractant molecules, migrate to various tissues of the body where they differentiate into macrophages and other cells of the immune system. The amount of PPAR-γ in monocytes is relatively low [27] but increases sharply during differentiation [28]. Furthermore, PPAR-γ agonists stimulate monocyte-macrophage differentiation [27, 29]. The resulting macrophages play key roles in regulation of the immune process. Macrophages are best recognized as phagocytes, but their secretion of either anti-inflammatory or proinflammatory mediators, depending on their own state of activation, plays a crucial role in regulation of immune system activity. Phenotypic characteristics of macrophages differ depending upon the tissue in which they differentiate and remain. This is particularly true with macrophages of the alveolus which differ substantially from peritoneal macrophages or macrophages differentiated in vitro from blood-borne monocytes.

In this review, we examine the role of PPARs, focusing predominantly on the PPAR-γ subtype, in regulating the activities of alveolar macrophages, which occupy a pivotal spot both as primary phagocyte and as primary regulator of the immune system's response to pathogens and particulates that reach the alveolus through inspired air. We also examine ways in which inadequate or inappropriate PPAR-γ responses can contribute to diseases of the lung.

2. THE ALVEOLAR MACROPHAGE: PIVOTAL REGULATOR OF IMMUNE RESPONSE

The lung is constantly exposed to noxious agents, both living pathogens and nonliving particulates, that are drawn into the alveolus with inspired air. The alveolar macrophage represents the first line of defense against these agents. Yet the gas-exchange function of the alveolus depends crucially on the thinness and integrity of the structures separating the air space from the pulmonary capillary. An inflammatory response, with edema and perhaps subsequent fibrosis, would severely impact this essential function. Thus, while inflammation may at times be necessary to eradicate invading pathogens, this response must be strictly regulated, as an inflammatory response to every arriving particle or organism would substantially diminish the lung's functional capacity.

2.1. Alveolar macrophages: origin and function

There are two major types of resident immune cells in the alveolus: dendritic cells and macrophages. Neutrophils, eosinophils, lymphocytes, and natural killer cells are also present but tend to be less prominent in the absence of overt inflammation. The dendritic cell, which forms part of the alveolar lining, mediates adaptive immunity. Indeed, it is the dendritic cell that presents antigens to other effector cells of the adaptive immune system and thus induces an antigen-specific response. The macrophage is the primary mediator of the innate immune response that does not require recognition of a specific antigen.

Most alveolar macrophages are derived from circulating monocytes. These monocytes are recruited into the lung, where they differentiate into macrophages under influence of the lung environment. In patients who have received bone marrow transplants, macrophages with the donor genotype replace those with host genotype; kinetics indicate an average macrophage lifespan of 81 days [30]. There is also evidence, however, for proliferation of differentiated macrophages within the alveolus, since replicating macrophages can be observed in bronchoalveolar lavage fluid and are more common in smokers and others with chronic lung inflammation [31]. Observations during acute lung inflammation induced by heat-killed Bacillus Calmette-Guérin indicate that even though local proliferation increased approximately 3-fold, the influx of monocytes was eight times as great [32]. In the normal steady state, greater than 70% of the macrophages are derived from circulating monocytes [33]. Substances known to induce the monocyte-macrophage transition include 1,25-dihydroxycholecalciferol and IL-10, as well as serum factors that remain less well defined [34–37].

The most obvious role of the alveolar macrophage is as a phagocyte. Phagocytes engulf viruses, bacteria, fungal cells, and a variety of appropriately sized nonliving particulates. Once engulfed, these particulates may be degraded if they are susceptible to the enzymes of the lysosomal system, as many (but not all) bacteria and fungi are. Otherwise, the particles will remain encapsulated within the macrophages until the latter either die (probably being engulfed by other macrophages), are transferred to lymph nodes draining the site, or are cleared from the airway by the mucociliary system [38].

Phagocytosis of many pathogens is mediated by the macrophage's Toll-like receptors (named for their sequence similarity to the Toll protein that governs dorsal-ventral patterning in *Drosophila* larvae). As recently reviewed by Akira [39], there are multiple Toll-like receptors; each recognizes a different microbial component or pathogen-associated molecular pattern (PAMP) which initiates signaling pathways through selective utilization of intracellular adaptor molecules. Phagocytosis may also be triggered by receptors for complement and the Fc portion of antibodies, thus targeting pathogens that have been recognized by the adaptive immune system [40]. There are also scavenger receptors that facilitate phagocytosis of particles coated with surfactant proteins A and D, which bind to a wide variety of bacteria and opsonize them (i.e., "tag" them for

phagocytosis) [41–43]. Finally, there are scavenger receptors that target inhaled particulates that have not otherwise been "tagged" by the immune system or surfactant proteins [44].

Alveolar macrophages are also involved in maintenance and remodeling of lung tissue, on the one hand secreting growth factors and cytokines that stimulate fibroblast proliferation and matrix synthesis and on the other hand producing matrix-degrading proteinases. Macrophage-secreted factors supporting matrix production include transforming growth factor-β (TGF-β) [45] and insulin-like growth factor-1 [46]. In addition to stimulating fibroblast proliferation, these cytokines stimulate production of collagen and of tissue inhibitors of matrix metalloproteinases (MMPs) while inhibiting metalloproteinase synthesis. A major matrix-degrading enzyme produced by alveolar macrophages is MMP-1 [47], although other MMPs as well as serine and cysteine proteinases also originate in macrophages.

A crucial nonimmune activity of macrophages is maintenance of pulmonary surfactant homeostasis. Surfactant, which serves to prevent alveolar collapse by reducing surface tension, is a mixture of proteins and lipids (mostly phospholipids) secreted by the epithelial cells of the lung [48]. Newly produced and biologically active surfactant takes the form of relatively large protein-lipid aggregates. Over time, however, the mechanical stresses associated with alveolar motion reduce the aggregates' size until they no longer provide effective surface tension reduction. These small, nonactive aggregates are taken up by both the epithelial cell and the alveolar macrophage [49]; most of those taken up by the epithelial cell are recycled, while those taken up by the macrophage are degraded and eliminated [50]. Hence, the macrophage plays a major role in elimination of excess surfactant.

2.2. *Key role of alveolar macrophages in lung immune system regulation*

Factors expressed by the innate immune system, including antibacterial proteins found in the pulmonary surfactant, are relatively noninjurious to the pulmonary epithelium. Only the generation of bactericidal reactive oxygen species is likely to have toxic effects. The adaptive immune system, on the other hand, relies heavily on inflammatory reactions to fight invading pathogens. Thus, in the lung it is desirable to rely on the innate immune system whenever possible.

When macrophages are stimulated by lipopolysaccharide and other microbial components, these cells respond by elaborating substances that upregulate the innate immune system, including chemoattractant molecules that recruit neutrophils and monocytes. Major chemoattractants produced by alveolar macrophages include leukotriene B_4 [51] and chemokines, particularly CXCL8 (IL-8) and CCL3 [52–54].

Conversely, in most circumstances the alveolar macrophage suppresses adaptive immunity, both through direct actions on the T cell and by inhibiting antigen presentation by dendritic cells. Depletion of alveolar macrophages in mice and rats, followed by antigen challenge, results in a marked increase in production of all antibody classes and in the number of T cells found in the lung and regional lymph nodes [55]. Antigen presenting activity of the dendritic cells is also increased [56]. Macrophages suppress lymphocyte activation via the production of nitric oxide, prostaglandin E_2, and immunosuppressive cytokines including TGF-β and IL-10 [57, 58]. More recently it has been found that these immunosuppressive cytokines are the product of an "alternatively activated," or "M2," macrophage induced by the T_H2 cytokines IL-4 and IL-13 [59, 60].

There will be times when the innate system is overwhelmed and the adaptive system must be activated. A recent elegant paper by Takabayshi et al. explains how the alveolar macrophage becomes activated and in turn able to stimulate the adaptive immune system and how this activation is reversed in time in the absence of continued stimulation [61].

3. PPARs AND THE ALVEOLAR MACROPHAGE

Macrophages differentiated from monocytes in vitro express all three isoforms of PPAR: PPAR-α [28], PPAR-β/δ [62], and PPAR-γ [28]. Activation of PPAR-α, but not of PPAR-γ, increased expression of NADPH oxidase and thereby facilitated production of reactive oxygen species [63]. Expression of some, but not all, proinflammatory molecules is decreased in macrophages isolated from PPAR-β/δ knockout mice and increased in macrophages overexpressing the receptor. However, expression of these molecules was decreased by PPAR-β/δ agonists, suggesting that it is specifically the unliganded receptor that is proinflammatory and that ligands may induce a switch between pro- and anti-inflammatory states [64]. In addition, both PPAR-β/δ and PPAR-γ agonists limit the ability of lipopolysaccharide to induce molecules such as nitric oxide synthase that are associated with inflammation [65].

In liver, liver-type fatty acid binding protein (L-FABP) is required for transport of both PPAR-α and PPAR-γ ligands into the nucleus [66]. Interestingly, alveolar macrophages are the only cells of the myeloid lineage to contain L-FABP [22]. Since the promoter region of the L-FABP gene contains a binding site for PPARs [66], this represents a potential signal-enhancing feed-forward mechanism.

PPAR-γ is known to be highly expressed in alveolar macrophages [11, 67, 68]. This is in contrast to peritoneal macrophages, where the amount is quite low in the macrophages normally resident in the peritoneum but sharply higher in activated macrophages elicited by thioglycolate [11]. The expression of PPAR-γ in alveolar macrophages is further upregulated by IL-4 [68]. Conversely, PPAR-γ is downregulated in activated peritoneal macrophages by interferon-γ and lipopolysaccharide [65]. Interestingly, we found that the predominant isoform in alveolar macrophages is PPAR-γ2, previously considered specific for adipocytes [68].

Although the amount of PPAR-γ in monocytes is markedly lower than in macrophages, its activation in a monocyte-like leukemia cell line has been shown to promote differentiation into cells displaying macrophage markers [27]. However, experiments with stem cells genetically lacking PPAR-γ have shown that this receptor is not essential for development of macrophages [69, 70].

As in many other tissues, exactly which of the many natural ligands are physiologically most important is not entirely clear. One of the highest-affinity natural ligands currently known is 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), but levels of this molecule may be quite low in many tissues and often do not correlate with responses presumed to be mediated by PPAR-γ [71]. On the other hand, this ligand is plentiful in histiocytes and dendritic cells from a variety of tissues [72]. An argument for the importance of 15d-PGJ_2 in alveolar macrophages is that lipopolysaccharide-induced synthesis of secretory type IIA phospholipase A_2 is inhibited by arachidonic acid, a precursor of 15d-PGJ_2 but not by its nonmetabolizable analog 5,8,11,15-tetraynoic acid [73]. Arachidonic acid is converted to 15d-PGJ_2 by a pathway dependent on the cyclooxygenase-2 (COX-2) enzyme, and COX-2 inhibitors blocked the effect of arachidonic acid. Furthermore, the effect of arachidonic acid was mimicked by administration of either 15d-PGJ_2 or the PPAR-γ ligand ciglitazone. Thus, the same effect is produced by synthetic PPAR-γ ligands and a metabolic precursor of 15d-PGJ_2, suggesting that the effects observed result from binding of 15d-PGJ_2 or a closely related compound.

It has also been shown [74] that mice lacking lysosomal acid lipase, and thus deficient in free fatty acids (including arachidonic acid), have an inflammatory phenotype in the lung that is largely eliminated by PPAR-γ agonists. In this case, however, the alteration is too far upstream to clearly identify the specific PPAR-γ ligand involved. Additionally, evidence that a given ligand plays a crucial role in one situation does not rule out involvement of different ligands in other situations.

In addition to 15d-PGJ_2, known ligands for PPAR-γ include 13-hydroxyoctadecadienoic acid (13-HODE) and 15-hydroxyeicosatetraenoic acid (15-HETE), respectively produced from linoleic and arachidonic acids by 12/15-lipoxygenase. In peritoneal macrophages, the anti-inflammatory cytokine IL-4 upregulates expression of both 12/15-lipoxygenase and PPAR-γ, suggesting an important role for those unsaturated fatty acid derivatives in at least that specific type of macrophage [75]. 13-HODE is also found associated with oxidized LDL and is believed to play a role in regulating fatty streak macrophages [29].

Recent studies have revealed relatively large amounts of nitrated fatty acids in human blood and urine, with derivatives of oleic acid being particularly abundant [76]. These substances, which are presumably generated as a result of nitric oxide production during inflammation, have been shown to act as potent PPAR-γ ligands at physiological concentrations [76] and to inhibit lipopolysaccharide-induced secretion of proinflammatory cytokines by macrophages [77]. However, this latter effect was reported to reflect direct alkylation of NF-κB rather than PPAR-γ activation. All of these natural ligands are fatty acid derivatives. The alveolus, including its resident macrophages, is constantly bathed in lipid-rich surfactant. Most of these lipids are phospholipids, but about 10% are neutral lipids including free fatty acids [78]. The essential role of free fatty acids in the production of PPAR-γ ligands has been demonstrated by Lian et al. [74] and by Yan et al. [79]. The former group showed that inflammation and abnormal gene expression in the lungs of lysosomal acid lipase knockout mice could be largely reversed by 9-hydroxyoctadenanoic acid or ciglitazone, while the latter group demonstrated that expression of lysosomal acid lipase specifically in macrophages had the same effect in a variety of tissues throughout the body. These results are compatible with the importance of free fatty acid release specifically within the macrophage, but it has also been shown that addition of exogenous arachidonic acid to macrophages cultured ex vivo had effects that appeared to be mediated by PPAR-γ [73]. Thus, PPAR-γ expressing cells in the alveolus are constantly bathed in precursors for the receptor's ligands. The alveolar microenvironment is immunosuppressive in the absence of specific stimulation—a conclusion supported by the finding that PPAR-γ binds to PPREs in resting alveolar macrophages from healthy controls but the binding is greatly reduced in those from patients with a chronic inflammatory condition such as pulmonary sarcoidosis [80].

3.1. Effects of PPAR-γ agonists on the alveolar macrophage

Early investigations of the role of PPAR-γ in activated peritoneal macrophages demonstrated that 15d-PGJ_2 and rosiglitazone inhibited expression of inducible nitric oxide synthase, gelatinase B, and scavenger receptor A [11]. Similarly, in alveolar macrophages, 15d-PGJ_2 and troglitazone inhibited the ability of lipopolysaccharide to induce synthesis of tumor necrosis factor-α while simultaneously upregulating expression of CD36, a scavenger receptor that mediates phagocytosis of (among other things) apoptotic neutrophils [67]; phagocytosis of apoptotic neutrophils is typical during the resolution of inflammation. In another experiment, treatment with PPAR-γ agonists inhibited the oxidative burst following addition of 4β-phorbol-12-myristate-13-acetate (PMA), expression of inducible nitric oxide synthase following treatment with lipopolysaccharide plus interferon-γ, and production of IL-12 following lipopolysaccharide treatment [68].

Given the role that phagocytosis of apoptotic cells plays in resolution of inflammation, it is interesting that the presence of apoptotic cells inhibits the PMA-induced oxidative burst through a mechanism that requires PPAR-γ [81]. Thus, PPAR-γ inhibits potentially destructive effects associated with inflammation, such as the release of reactive oxygen species, while facilitating the resolution of inflammation. On the other hand, however, recent work has shown that PPAR-γ supports noninflammatory protective effects by upregulating activity of alveolar macrophage Fcγ surface receptor (unpublished results) which is of particular relevance as the Fcγ receptor mediates phagocytosis of bacteria and other particles opsonized by attachment of antibodies belonging to the immunoglobulin G class.

Takabayashi et al. demonstrated that actin polymerization is a crucial step in the change of shape that lifts the alveolar macrophage off the epithelial cell and leads to activation [61]. Although the possible involvement of PPAR-γ in this process has not been directly investigated in alveolar

macrophages, PPAR-γ agonists have been shown to inhibit actin polymerization in vascular monocytes [82].

3.2. PPAR-γ effects in other immune cells of the lung

Macrophages are not the only essential immune cells of the lung. The dendritic cell, which is also derived from monocytes and resides within the alveolar wall, takes up and processes foreign substances into antigenic fragments. These cells then migrate to the draining lymph nodes, where they present these antigens to helper T cells that promote adaptive immune responses.

Emerging data convincingly demonstrate that PPAR-γ agonists influence dendritic cell function. For example, exposure of dendritic cells to PPAR-γ ligands during differentiation in vitro results in a reduction in the ability of these cells to generate an immune response [13]. These effects include a reduction in secretion of proinflammatory cytokines and in expression of molecules essential for migration to regional draining lymph nodes where antigen presentation occurs. Dendritic cell responses to stimulation of Toll-like receptors that constitutively respond to specific molecular stimuli are likewise reduced [83]. In fact, ligands for all three PPAR isoforms reduce expression of costimulatory molecules and the ability of dendritic cells to stimulate T cells in a mixed lymphocyte reaction [84]. Furthermore, treatment of dendritic cells with PPAR-γ ligands during antigen-stimulated maturation (a step following differentiation) has been shown to reduce the eosinophilic response in a murine model of asthma following reinjection of the in vitro-treated cells [85]. In vivo treatment with PPAR-γ ligands has also been shown to inhibit migration of epidermal dendritic cells to the draining lymph nodes [86]. In one study, exposure during and after differentiation in vitro produced dendritic cells with a greatly blunted ability to release proinflammatory chemokines and, even more significantly, to activate T-lymphocytes through antigen presentation [13]. This blunted response was shown to be alleviated by administration of IL-12.

Neutrophils also play an important role in lung inflammatory responses. Although there have been numerous studies demonstrating a reduction in neutrophil-predominant inflammation following administration of PPAR-γ agonists [12], studies assessing direct effects on human neutrophils are limited. Most notably, we showed that PPAR-γ expression in neutrophils was upregulated by tumor necrosis factor-α or IL-4 and that incubation of resting human neutrophils with PPAR-γ agonists reduced chemotactic responses to IL-8 or formylmethionylleucylphenylalanine (FMLP) (unpublished results). Additionally, Imamoto et al. showed that the increased expression of neutrophil CD11b/CD18 surface integrins induced by FMLP was suppressed by pioglitazone [87].

3.3. Mechanisms of PPAR-γ action in alveolar macrophages

The molecular mechanisms through which PPAR-γ acts have been well characterized. What is often not explicitly appreciated is that the coactivators used by PPAR-γ are also used by other nuclear transcription factors, including cAMP response element-binding protein (CREB) [88, 89], activator protein-1 (AP-1) [88], basic helix-loop-helix factors [90], signal transducers and activators of transcription (STATs) [91–93], and nuclear factor-κB (NF-κB) [94]. Because the number of coactivator molecules is limited, PPAR-γ activation may restrict availability of coactivators to other nuclear transcription factors and thereby downregulate expression of genes under their control. There is also evidence that PPAR-γ may directly bind to these and other transcription factors, thus preventing them from binding to DNA and promoting gene transcription [87, 95–98]. Indeed, the initial report of PPAR-γ activation in peritoneal macrophages focused on downregulation of genes controlled by AP-1, STAT-1, and NF-κB [11]. PPAR-γ interference with activity of NF-κB also appears likely in alveolar macrophages, since PPAR-γ agonists inhibit the ability of lipopolysaccharide to induce synthesis of secretory type IIA phospholipase A_2, which is promoted by NF-κB [73].

Additionally, PPAR-γ interacts with liver X receptor-α (LXR-α). In contrast to the interactions with NF-κB, however, those with LXR-α are synergistic [99]. Both PPAR-γ agonists and LXR-α agonists inhibit the ability of lipopolysaccharide to induce an inflammatory phenotype in cultured macrophages. However, when suboptimal concentrations of each agonist type are added simultaneously, the effects are far greater than would be seen with either agonist alone. In this study, these effects were associated with reduced activity of NF-κB. Interestingly, LXR-α agonists increase expression and DNA binding of PPAR-γ [69, 99, 100], while PPAR-γ agonists increase LXR-α expression in mesangial cells [101].

4. PPAR-γ IN LUNG DISEASE

There are several lung diseases in which alveolar macrophages appear to play a crucial role in disease pathogenesis and where PPAR-γ agonists may prove useful as therapy. In other cases the role of the alveolar macrophage may be less clear but studies indicate that PPAR-γ ligands may also prove to be beneficial.

4.1. Pulmonary sarcoidosis

Sarcoidosis is a state of chronic granulomatous inflammation that may affect multiple organs, especially the lungs [102]. The cause of sarcoidosis remains unknown, but the pathology is characterized by greatly enhanced activation of the innate and adaptive immune systems [103, 104]. This is accompanied by increased expression of cytokines such as IL-2, IL-12, IL-18, and interferon-γ, with the alveolar macrophage having been demonstrated as a source for at least some of these molecules. Given the established role of PPAR-γ in maintaining alveolar macrophages in a quiescent state and the macrophage's role in activating other components of the immune system, examination of macrophage PPAR-γ levels appeared warranted. Indeed, alveolar macrophages from patients with sarcoidosis had much lower levels of PPAR-γ and higher levels of NF-κB activity than those from healthy controls [80]. While a causal relationship between this deficiency of PPAR-γ and the heightened state of inflammation that

characterizes sarcoidosis is plausible, the effects of PPAR-γ agonist administration or factors known to upregulate PPAR-γ expression on disease course remain to be investigated.

4.2. Alveolar proteinosis

Alveolar proteinosis is a condition in which excessive amounts of pulmonary surfactant, both phospholipids and proteins, accumulate in the lung airspaces [105]. Phospholipid inclusions are also prominent in alveolar macrophages [106]. Most human alveolar proteinosis (not obviously secondary to other conditions) is associated with autoantibodies to granulocyte-macrophage colony stimulating factor (GM-CSF) [107, 108]; animal models in which either GM-CSF or its receptor is genetically deleted can mimic the disease. The current treatment is removal of excess surfactant through whole-lung lavage under general anesthesia, but preliminary studies support the potential efficacy of subcutaneous GM-CSF treatment in the human disease [109, 110].

GM-CSF, which is produced by a number of cell types in the lung [111], promotes growth, differentiation, and activation of cells of the phagocytic lineage [112, 113] and has been shown to promote accumulation and proliferation of alveolar macrophages [114–116]. However, because its activities overlap those of other cytokines [117], hematopoiesis and myelopoiesis are essentially normal in GM-CSF knockout mice [118–121].

Since GM-CSF has been shown to upregulate PPAR-γ in cell culture [11, 75], Bonfield and colleagues examined PPAR-γ expression in alveolar macrophages from patients with alveolar proteinosis [122]. Not only was PPAR-γ mRNA and protein expression much lower than in alveolar macrophages from healthy controls, but macrophage expression of the PPAR-γ-dependent scavenger receptor CD36 was also lower. Furthermore, treatment with GM-CSF fully restored PPAR-γ to normal levels. The conclusion that GM-CSF acts at least partially through effects on macrophage PPAR-γ is supported by recent observations that GM-CSF also upregulates PPAR-γ in macrophages of the fatty streak [123].

These observations raise interesting but speculative and largely unexplored therapeutic possibilities. It could be worth considering the possibility that PPAR-γ agonists, including the thiazolidinediones, might prove as effective as the subcutaneous GM-CSF currently being investigated as a possible treatment.

4.3. Lung injury

Injury to the lung, by inhaled irritant for example, is characterized by exuberant inflammation, epithelial injury, and often the development of secondary pulmonary fibrosis. An appealing animal model of acute lung injury involves the intratracheal administration of fluorescein isothiocyanate. This insult results in a neutrophil-predominant inflammation accompanied by leakage of protein into the alveolus that is maximal at 3 to 7 days, while patchy fibrosis develops 3 to 4 weeks after exposure to the agent [124]. Using this model, we found that PPAR-γ expression increases in alveolar macrophages and that pretreatment with pioglitazone for 5 days prior to fluorescein isothiocyanate exposure significantly reduced inflammation and reduced the number of neutrophils in bronchoalveolar lavage fluid by 50%, but did not affect expression of proinflammatory cytokines [12]. The lack of effect on cytokine expression led us to postulate that pioglitazone was acting directly on the neutrophil to impair the ability of these cells to migrate in response to secreted chemoattractants.

In humans, alveolar macrophages isolated from patients with acute lung injury express elevated amounts of PPAR-γ and of PPAR-γ ligands such as prostaglandin D_2 and 15-HETE [12]. Indeed, the amount of 15-HETE found in these patient's bronchoalveolar lavage fluid is more than 50 times of that seen in lavage fluid from healthy individuals. This would be expected to reduce the extent of inflammation, and hence may represent a step toward eventual resolution.

4.4. Other lung diseases

4.4.1. Endotoxic shock

Lipopolysaccharide from gram-negative bacteria can produce severe systemic inflammation and multiorgan failure, including lung injury. The ability of PPAR-γ agonists to block lipopolysaccharide-induced inflammatory changes in macrophages in vitro has been well described. In an animal model, Kaplan et al. showed that this observation may have clinical relevance [125]. In this study, intraperitoneal injection of lipopolysaccharide in placebo-treated mice resulted in severe inflammatory changes in the lung, including hemorrhage, infiltration of neutrophils, and reduction of the alveolar space, that were visible within 6 hours. Increased expressions of intercellular adhesion molecule-1, vascular cellular adhesion molecule-1, and E-selectin were associated with activation of NF-κB and decreased expression of PPAR-γ; seventy-two-hour mortality was 91%. Treatment with 15d-PGJ_2, beginning 3 hours after lipopolysaccharide injection and continuing every 12 hours thereafter, downregulated expression of adhesion molecules, reduced neutrophil infiltration, and decreased mortality to 45%. Binding of NF-κB to DNA was decreased, while expression and DNA binding of PPAR-γ was increased, as was expression of the protective heat shock protein 70.

Liu et al. obtained results in rats similar to those seen by Kaplan et al. in mice [126]. Specifically, rosiglitazone treatment began 30 minutes before lipopolysaccharide injection and in some cases the PPAR-γ antagonist GW9662 was administered 20 minutes before rosiglitazone. In the absence of rosiglitazone, lung edema and histological injury were apparent within 4 hours. These were significantly reduced by rosiglitazone, which also produced a 71% reduction of the increase in myeloperoxidase activity (a marker for the presence of neutrophils) and an 84% reduction of the increase in malondialdehyde levels. This was accompanied by a marked decrease in inducible NO synthase mRNA and protein. All these rosiglitazone effects were blocked by the PPAR-γ antagonist GW9662.

Although these are animal studies and do not directly demonstrate involvement of either PPAR-γ or alveolar

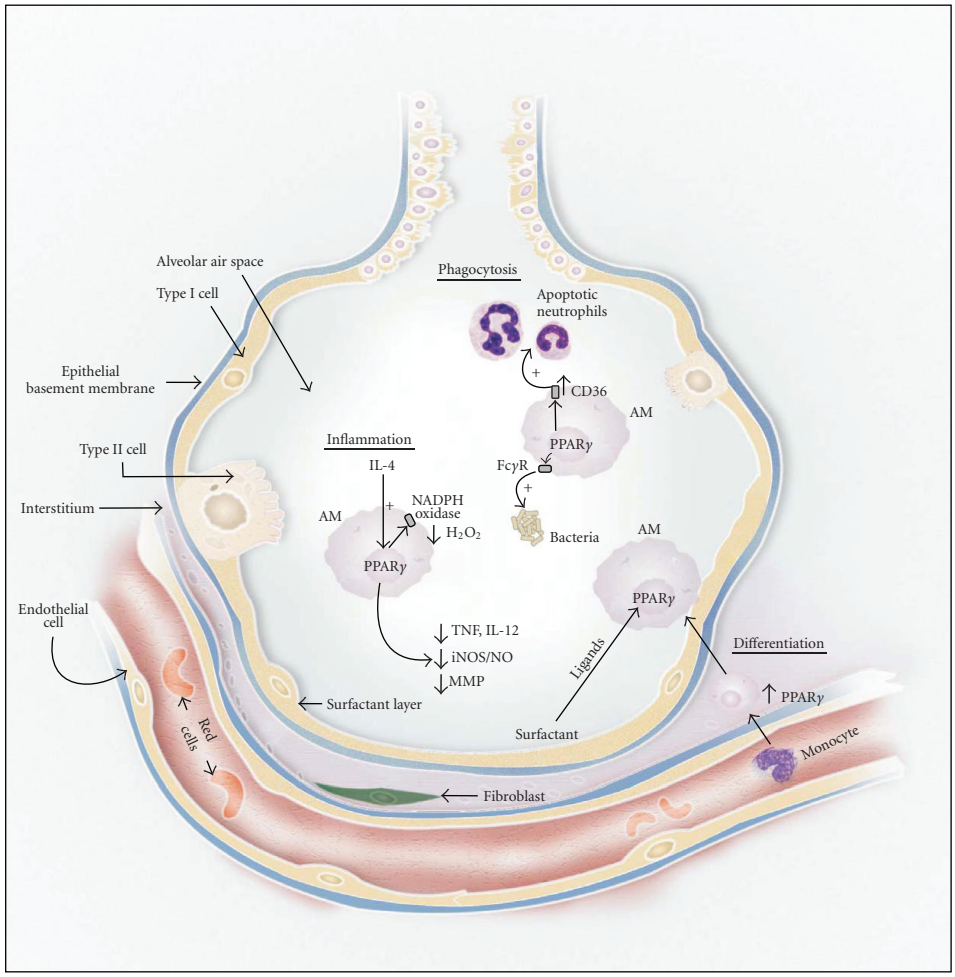

Figure 1: *The alveolar macrophage: role in immunity and effects of PPAR-γ*. PPAR-γ promotes differentiation of monocytes into AMs, then mediates effects that suppress AMs' proinflammatory activities while upregulating phagocytosis through both CD36 and Fcγ surface receptors. Interaction with foreign substances causes the AM to secrete a variety of inflammatory molecules such as TNF-α, IL-12, H_2O_2, nitric oxide, and MMPs. This inflammatory response is suppressed, however, by the production of substances such as IL-4 that reinforce PPAR-γ activity. Alveolar surfactant also contains lipids that can stimulate PPAR-γ, resulting in suppression of AM inflammatory activity. PPAR-γ activation also enhances AM phagocytosis of bacteria and apoptotic neutrophils present in the closing stages of inflammation. Thus, inflammation becomes self-limiting. Abbreviations: AM = alveolar macrophage; FcγR = surface receptor recognizing the Fc portion of immunoglobulin G; iNOS = inducible nitric oxide synthase; MMP = matrix metalloproteinase.

macrophages in the effects observed, they suggest that early treatment with PPAR-γ agonists could ameliorate the effects of endotoxemia, at least in the lung and probably elsewhere.

4.4.2. Asthma

The alveolar macrophage has been described as "the forgotten cell in asthma" [127]. Asthma is an exaggerated response of the lung's adaptive immune system to specific inhaled antigens—a response that the alveolar macrophage downregulates in most circumstances. It is thus not surprising that depletion of alveolar macrophages led to an enhanced response to challenge with an antigen to which mice had previously been sensitized [128]. As discussed by Peters-Golden, alveolar macrophages exert a variety of effects that could lead to suppression of exaggerated asthmatic responses [127] and many of these are precisely the effects that are elicited and maintained by PPAR-γ. Importantly, allergen challenge in asthmatic patients has been found to downregulate PPAR-γ levels in alveolar macrophages [129]. Otherwise, however, the contribution of PPAR-γ to alveolar macrophage responses in asthma has not been explicitly investigated.

5. CONCLUSIONS

Among the many often-overlooked roles of PPAR-γ is its central position in regulating the lung's response to pathogens and other noxious elements drawn in with inspired air. The lung must be able to respond effectively, yet to control the inflammatory response generated in response to foreign agents within the alveolar space. The alveolar macrophage is pivotal in this respect. The ability of these cells to engulf unwanted particles represents the first line of defense, yet when not fully activated (e.g., by interactions with its Toll-likereceptors)

serves to strongly dampen responses by the lung's adaptive immune system. However, when these cells have been sufficiently activated by danger signals within the alveolus, alveolar macrophages release molecules that attract and activate other elements of the innate and adaptive immune systems. Finally, the macrophage's ability to scavenge apoptotic neutrophils is essential for resolution of inflammation once the need has passed.

The central role of PPAR-γ in regulating the activational state of alveolar macrophages is becoming increasingly clear (see Figure 1). Many studies have now shown that macrophage activation is inhibited by PPAR-γ and/or PPAR-γ agonists. This is typically associated with decreases in NF-κB activity, with one likely mechanism being unavailability of that transcription factor's obligate coactivators because they are being used by PPAR-γ instead. Conversely, activation of alveolar macrophages is associated with low levels of PPAR-γ and high levels of NF-κB activity.

Many aspects of alveolar macrophage function and the role of PPAR-γ in regulating these functions remain unclear. Especially uncertain are how these responses contribute to pathological conditions such as asthma and acute lung injury. All of these aspects deserve further investigation, with special attention to the possibility that PPAR-γ agonists may prove therapeutically useful in a variety of lung diseases in which they have not previously been considered.

LIST OF ABBREVIATIONS

13-HODE:	13-hydroxyoctadecadienoic acid
15d-PGJ$_2$:	15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$
15-HETE:	15-hydroxyeicosatetraenoic acid
AP-1:	Activator protein-1
COX-2:	Cyclooxygenase-2
CREB:	cAMP response element-binding protein
FMLP:	Formylmethiolylleucylphenylalanine
GM-CSF:	Granulocyte-macrophage colony stimulating factor
IL-:	Interleukin-
L-FABP:	Liver-type fatty acid binding protein
LDL:	Low-density lipoprotein
LXR:	Liver X receptor
MMP:	Matrix metalloproteinase
NF-κB:	Nuclear factor-κB:
PMA:	4β-phorbol-12-myristate-13-acetate
PPAR:	Peroxisome proliferator-activated receptor
STAT:	Signal transducer and activator of transcription
TGF:	Transforming growth factor

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REFERENCES

[1] C. K. Glass and S. Ogawa, "Combinatorial roles of nuclear receptors in inflammation and immunity," *Nature Reviews Immunology*, vol. 6, no. 1, pp. 44–55, 2006.

[2] R. M. Touyz and E. L. Schiffrin, "Peroxisome proliferator-activated receptors in vascular biology-molecular mechanisms and clinical implications," *Vascular Pharmacology*, vol. 45, no. 1, pp. 19–28, 2006.

[3] F. Gizard, C. Amant, O. Barbier, et al., "PPARα inhibits vascular smooth muscle cell proliferation underlying intimal hyperplasia by inducing the tumor suppressor p16^{INK4a}," *Journal of Clinical Investigation*, vol. 115, no. 11, pp. 3228–3238, 2005.

[4] Q. N. Diep, F. Amiri, R. M. Touyz, et al., "PPARα activator effects on Ang II-induced vascular oxidative stress and inflammation," *Hypertension*, vol. 40, no. 6, pp. 866–871, 2002.

[5] A. D. Burdick, D. J. Kim, M. A. Peraza, F. J. Gonzalez, and J. M. Peters, "The role of peroxisome proliferator-activated receptor-β/δ in epithelial cell growth and differentiation," *Cellular Signalling*, vol. 18, no. 1, pp. 9–20, 2006.

[6] A. Sugawara, K. Takeuchi, A. Uruno, et al., "Transcriptional suppression of type 1 angiotensin II receptor gene expression by peroxisome proliferator-activated receptor-γ in vascular smooth muscle cells," *Endocrinology*, vol. 142, no. 7, pp. 3125–3134, 2001.

[7] M. J. Ryan, S. P. Didion, S. Mathur, F. M. Faraci, and C. D. Sigmund, "PPARγ agonist rosiglitazone improves vascular function and lowers blood pressure in hypertensive transgenic mice," *Hypertension*, vol. 43, no. 3, pp. 661–666, 2004.

[8] H. P. Koeffler, "Peroxisome proliferator-activated receptor γ and cancers," *Clinical Cancer Research*, vol. 9, no. 1, pp. 1–9, 2003.

[9] M. H. Fenner and E. Elstner, "Peroxisome proliferator-activated receptor-γ ligands for the treatment of breast cancer," *Expert Opinion on Investigational Drugs*, vol. 14, no. 6, pp. 557–568, 2005.

[10] C. Jiang, A. T. Ting, and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

[11] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[12] T. J. Standiford, V. C. Keshamouni, and R. C. Reddy, "Peroxisome proliferator-activated receptor-γ as a regulator of lung inflammation and repair," *Proceedings of the American Thoracic Society*, vol. 2, no. 3, pp. 226–231, 2005.

[13] A. Nencioni, F. Grünebach, A. Zobywlaski, C. Denzlinger, W. Brugger, and P. Brossart, "Dendritic cell immunogenicity is regulated by peroxisome proliferator-activated receptor γ," *Journal of Immunology*, vol. 169, no. 3, pp. 1228–1235, 2002.

[14] J. Padilla, K. Kaur, H. J. Cao, T. J. Smith, and R. P. Phipps, "Peroxisome proliferator activator receptor-γ agonists and 15-deoxy-$\Delta^{12,14}$-PGJ$_2$ induce apoptosis in normal and malignant B-lineage cells," *Journal of Immunology*, vol. 165, no. 12, pp. 6941–6948, 2000.

[15] R. B. Clark, D. Bishop-Bailey, T. Estrada-Hernandez, T. Hla, L. Puddington, and S. J. Padula, "The nuclear receptor PPARγ and immunoregulation: PPARγ mediates inhibition of helper T cell responses," *Journal of Immunology*, vol. 164, no. 3, pp. 1364–1371, 2000.

[16] R. Cunard, Y. Eto, J. T. Muljadi, C. K. Glass, C. J. Kelly, and M. Ricote, "Repression of IFN-γ expression by peroxisome proliferator-activated receptor γ," *Journal of Immunology*, vol. 172, no. 12, pp. 7530–7536, 2004.

[17] X. Y. Yang, L. H. Wang, T. Chen, et al., "Activation of human T lymphocytes is inhibited by peroxisome proliferator-activated receptor γ (PPARγ) agonists. PPARγ co-association with transcription factor NFAT," *Journal of Biological Chemistry*, vol. 275, no. 7, pp. 4541–4544, 2000.

[18] G. Woerly, K. Honda, M. Loyens, et al., "Peroxisome proliferator-activated receptors α and γ down-regulate allergic inflammation and eosinophil activation," *Journal of Experimental Medicine*, vol. 198, no. 3, pp. 411–421, 2003.

[19] X. Zhang, M. C. Rodriguez-Galán, J. J. Subleski, et al., "Peroxisome proliferator-activated receptor-γ and its ligands attenuate biologic functions of human natural killer cells," *Blood*, vol. 104, no. 10, pp. 3276–3284, 2004.

[20] H. Sugiyama, T. Nonaka, T. Kishimoto, K. Komoriya, K. Tsuji, and T. Nakahata, "Peroxisome proliferator-activated receptors are expressed in human cultured mast cells: a possible role of these receptors in negative regulation of mast cell activation," *European Journal of Immunology*, vol. 30, no. 12, pp. 3363–3370, 2000.

[21] J. Becker, C. Delayre-Orthez, N. Frossard, and F. Pons, "Regulation of inflammation by PPARs: a future approach to treat lung inflammatory diseases?" *Fundamental & Clinical Pharmacology*, vol. 20, no. 5, pp. 429–447, 2006.

[22] C. Schachtrup, T. E. Scholzen, V. Grau, et al., "L-FABP is exclusively expressed in alveolar macrophages within the myeloid lineage: evidence for a PPARα-independent expression," *The International Journal of Biochemistry & Cell Biology*, vol. 36, no. 10, pp. 2042–2053, 2004.

[23] C. Delayre-Orthez, J. Becker, I. Guenon, et al., "PPARα downregulates airway inflammation induced by lipopolysaccharide in the mouse," *Respiratory Research*, vol. 6, p. 91, 2005.

[24] P. R. Devchand, H. Keller, J. M. Peters, M. Vazquez, F. J. Gonzalez, and W. Wahli, "The PPARα-leukotriene B_4 pathway to inflammation control," *Nature*, vol. 384, no. 6604, pp. 39–43, 1996.

[25] R. Paumelle, C. Blanquart, O. Briand, et al., "Acute antiinflammatory properties of statins involve peroxisome proliferator-activated receptor-α via inhibition of the protein kinase C signaling pathway," *Circulation Research*, vol. 98, no. 3, pp. 361–369, 2006.

[26] T. L. Graham, C. Mookherjee, K. E. Suckling, C. N. A. Palmer, and L. Patel, "The PPARδ agonist GW0742X reduces atherosclerosis in $LDLR^{-/-}$ mice," *Atherosclerosis*, vol. 181, no. 1, pp. 29–37, 2005.

[27] P. Tontonoz, L. Nagy, J. G. A. Alvarez, V. A. Thomazy, and R. M. Evans, "PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL," *Cell*, vol. 93, no. 2, pp. 241–252, 1998.

[28] G. Chinetti, S. Griglio, M. Antonucci, et al., "Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages," *Journal of Biological Chemistry*, vol. 273, no. 40, pp. 25573–25580, 1998.

[29] L. Nagy, P. Tontonoz, J. G. A. Alvarez, H. Chen, and R. M. Evans, "Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ," *Cell*, vol. 93, no. 2, pp. 229–240, 1998.

[30] E. D. Thomas, R. E. Ramberg, G. E. Sale, R. S. Sparkes, and D. W. Golde, "Direct evidence for a bone marrow origin of the alveolar macrophage in man," *Science*, vol. 192, no. 4243, pp. 1016–1018, 1976.

[31] P. B. Bitterman, L. E. Saltzman, S. Adelberg, V. J. Ferrans, and R. G. Crystal, "Alveolar macrophage replication. One mechanism for the expansion of the mononuclear phagocyte population in the chronically inflamed lung," *Journal of Clinical Investigation*, vol. 74, no. 2, pp. 460–469, 1984.

[32] A. Blusse van Oud Alblas, B. van der Linden-Schrever, and R. van Furth, "Origin and kinetics of pulmonary macrophages during an inflammatory reaction induced by intravenous administration of heat-killed bacillus Calmette-Guerin," *Journal of Experimental Medicine*, vol. 154, no. 2, pp. 235–252, 1981.

[33] A. Blusse van Oud Alblas, H. Mattie, and R. van Furth, "A quantitative evaluation of pulmonary macrophage kinetics," *Cell and Tissue Kinetics*, vol. 16, no. 3, pp. 211–219, 1983.

[34] P. Allavena, L. Piemonti, D. Longoni, et al., "IL-10 prevents the differentiation of monocytes to dendritic cells but promotes their maturation to macrophages," *European Journal of Immunology*, vol. 28, no. 1, pp. 359–369, 1998.

[35] S.-I. Hashimoto, M. Yamada, K. Motoyoshi, and K. S. Akagawa, "Enhancement of macrophage colony-stimulating factor-induced growth and differentiation of human monocytes by interleukin-10," *Blood*, vol. 89, no. 1, pp. 315–321, 1997.

[36] R. A. Musson, "Human serum induces maturation of human monocytes in vitro. Changes in cytolytic activity, intracellular lysosomal enzymes, and nonspecific esterase activity," *American Journal of Pathology*, vol. 111, no. 3, pp. 331–340, 1983.

[37] H. Tanaka, E. Abe, C. Miyaura, Y. Shiina, and T. Suda, "1α,25-dihydroxyvitamin D_3 induces differentiation of human promyelocytic leukemia cells (HL-60) into monocyte-macrophages but not into granulocytes," *Biochemical and Biophysical Research Communications*, vol. 117, no. 1, pp. 86–92, 1983.

[38] D. W. H. Riches and M. J. Fenton, "Monocytes, macrophages, and dendritic cells of the lung," in *Murray and Nadel's Textbook of Respiratory Medicine*, R. J. Mason, J. F. Murray, V. C. Broaddus, and J. A. Nadel, Eds., Elsevier Saunders, Philadelphia, Pa, USA, 4th edition, 2005.

[39] S. Akira, "TLR signaling," *Current topics in Microbiology and Immunology*, vol. 311, pp. 1–16, 2006.

[40] H. Y. Reynolds, J. P. Atkinson, H. H. Newball, and M. M. Frank, "Receptors for immunoglobulin and complement on human alveolar macrophages," *Journal of Immunology*, vol. 114, no. 6, pp. 1813–1819, 1975.

[41] Z. C. Chroneos, R. Abdolrasulnia, J. A. Whitsett, W. R. Rice, and V. L. Shepherd, "Purification of a cell-surface receptor for surfactant protein A," *Journal of Biological Chemistry*, vol. 271, no. 27, pp. 16375–16383, 1996.

[42] U. Holmskov, P. Lawson, B. Teisner, et al., "Isolation and characterization of a new member of the scavenger receptor superfamily, glycoprotein-340 (gp-340), as a lung surfactant protein-D binding molecule," *Journal of Biological Chemistry*, vol. 272, no. 21, pp. 13743–13749, 1997.

[43] A. J. Tenner, S. L. Robinson, and R. A. B. Ezekowitz, "Mannose binding protein (MBP) enhances mononuclear phagocyte function via a receptor that contains the 126,000 M_r component of the C1q receptor," *Immunity*, vol. 3, no. 4, pp. 485–493, 1995.

[44] A. Palecanda and L. Kobzik, "Receptors for unopsonized particles: the role of alveolar macrophage scavenger receptors," *Current Molecular Medicine*, vol. 1, no. 5, pp. 589–595, 2001.

[45] N. Khalil, O. Bereznay, M. Sporn, and A. H. Greenberg, "Macrophage production of transforming growth factor β and fibroblast collagen synthesis in chronic pulmonary inflammation," *Journal of Experimental Medicine*, vol. 170, no. 3, pp. 727–737, 1989.

[46] S.-T. Uh, Y. Inoue, T. E. King Jr., E. D. Chan, L. S. Newman, and D. W. H. Riches, "Morphometric analysis of insulin-like growth factor-1 localization in lung tissues of patients with idiopathic pulmonary fibrosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 158, no. 5, part 1, pp. 1626–1635, 1998.

[47] E. J. Campbell, J. D. Cury, C. J. Lazarus, and H. G. Welgus, "Monocyte procollagenase and tissue inhibitor of metalloproteinases. Identification, characterization, and regulation of secretion," *Journal of Biological Chemistry*, vol. 262, no. 33, pp. 15862–15868, 1987.

[48] J. H. Fisher, V. Sheftelyevich, Y.-S. Ho, et al., "Pulmonary-specific expression of SP-D corrects pulmonary lipid accumulation in *SP-D* gene-targeted mice," *American Journal of Physiology*, vol. 278, no. 2, pp. L365–L373, 2000.

[49] A. H. Jobe and M. Ikegami, "Surfactant metabolism," *Clinics in Perinatology*, vol. 20, no. 4, pp. 683–696, 1993.

[50] E. D. Rider, M. Ikegami, and A. H. Jobe, "Localization of alveolar surfactant clearance in rabbit lung cells," *American Journal of Physiology*, vol. 263, no. 2, part 1, pp. L201–L209, 1992.

[51] T. R. Martin, G. Raugi, T. L. Merritt, and W. R. Henderson Jr., "Relative contribution of leukotriene B_4 to the neutrophil chemotactic activity produced by the resident human alveolar macrophage," *Journal of Clinical Investigation*, vol. 80, no. 4, pp. 1114–1124, 1987.

[52] P. C. Carré, R. L. Mortenson, T. E. King Jr., P. W. Noble, C. L. Sable, and D. W. H. Riches, "Increased expression of the interleukin-8 gene by alveolar macrophages in idiopathic pulmonary fibrosis. A potential mechanism for the recruitment and activation of neutrophils in lung fibrosis," *Journal of Clinical Investigation*, vol. 88, no. 6, pp. 1802–1810, 1991.

[53] R. E. Smith, R. M. Strieter, K. Zhang, et al., "A role for C-C chemokines in fibrotic lung disease," *Journal of Leukocyte Biology*, vol. 57, no. 5, pp. 782–787, 1995.

[54] T. J. Standiford, M. W. Rolfe, S. L. Kunkel, et al., "Macrophage inflammatory protein-1 α expression in interstitial lung disease," *Journal of Immunology*, vol. 151, no. 5, pp. 2852–2863, 1993.

[55] T. Thepen, G. Kraal, and P. G. Holt, "The role of alveolar macrophages in regulation of lung inflammation," *Annals of the New York Academy of Sciences*, vol. 725, no. 1, pp. 200–206, 1994.

[56] P. G. Holt, J. Oliver, N. Bilyk, et al., "Downregulation of the antigen presenting cell function(s) of pulmonary dendritic cells in vivo by resident alveolar macrophages," *Journal of Experimental Medicine*, vol. 177, no. 2, pp. 397–407, 1993.

[57] T. Kawabe, K.-I. Isobe, Y. Hasegawa, I. Nakashima, and K. Shimokata, "Immunosuppressive activity induced by nitric oxide in culture supernatant of activated rat alveolar macrophages," *Immunology*, vol. 76, no. 1, pp. 72–78, 1992.

[58] M. D. Roth and S. H. Golub, "Human pulmonary macrophages utilize prostaglandins and transforming growth factor β_1 to suppress lymphocyte activation," *Journal of Leukocyte Biology*, vol. 53, no. 4, pp. 366–371, 1993.

[59] S. Gordon, "Alternative activation of macrophages," *Nature Reviews Immunology*, vol. 3, no. 1, pp. 23–35, 2003.

[60] A. Mantovani, A. Sica, S. Sozzani, P. Allavena, A. Vecchi, and M. Locati, "The chemokine system in diverse forms of macrophage activation and polarization," *Trends in Immunology*, vol. 25, no. 12, pp. 677–686, 2004.

[61] K. Takabayshi, M. Corr, T. Hayashi, et al., "Induction of a homeostatic circuit in lung tissue by microbial compounds," *Immunity*, vol. 24, no. 4, pp. 475–487, 2006.

[62] M. R. Sartippour and G. Renier, "Differential regulation of macrophage peroxisome proliferator-activated receptor expression by glucose: role of peroxisome proliferator-activated receptors in lipoprotein lipase gene expression," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 20, no. 1, pp. 104–110, 2000.

[63] E. Teissier, A. Nohara, G. Chinetti, et al., "Peroxisome proliferator-activated receptor α induces NADPH oxidase activity in macrophages, leading to the generation of LDL with PPAR-α activation properties," *Circulation Research*, vol. 95, no. 12, pp. 1174–1182, 2004.

[64] C.-H. Lee, A. Chawla, N. Urbiztondo, D. Liao, W. A. Boisvert, and R. M. Evans, "Transcriptional repression of atherogenic inflammation: modulation by PPARδ," *Science*, vol. 302, no. 5644, pp. 453–457, 2003.

[65] J. S. Welch, M. Ricote, T. E. Akiyama, F. J. Gonzalez, and C. K. Glass, "PPARγ and PPARδ negatively regulate specific subsets of lipopolysaccharide and IFN-γ target genes in macrophages," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 11, pp. 6712–6717, 2003.

[66] C. Wolfrum, C. M. Borrmann, T. Borchers, and F. Spener, "Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors α- and γ-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 5, pp. 2323–2328, 2001.

[67] K. Asada, S. Sasaki, T. Suda, K. Chida, and H. Nakamura, "Antiinflammatory roles of peroxisome proliferator-activated receptor γ in human alveolar macrophages," *American Journal of Respiratory and Critical Care Medicine*, vol. 169, no. 2, pp. 195–200, 2004.

[68] R. C. Reddy, V. G. Keshamouni, S. H. Jaigirdar, et al., "Deactivation of murine alveolar macrophages by peroxisome proliferator-activated receptor-γ ligands," *American Journal of Physiology*, vol. 286, no. 3, pp. L613–L619, 2004.

[69] A. Chawla, Y. Barak, L. Nagy, D. Liao, P. Tontonoz, and R. M. Evans, "PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation," *Nature Medicine*, vol. 7, no. 1, pp. 48–52, 2001.

[70] K. J. Moore, E. D. Rosen, M. L. Fitzgerald, et al., "The role of PPAR-γ in macrophage differentiation and cholesterol uptake," *Nature Medicine*, vol. 7, no. 1, pp. 41–47, 2001.

[71] L. C. Bell-Parikh, T. Ide, J. A. Lawson, P. McNamara, M. Reilly, and G. A. FitzGerald, "Biosynthesis of 15-deoxy-$\Delta^{12,14}$-PGJ_2 and the ligation of PPARγ," *Journal of Clinical Investigation*, vol. 112, no. 6, pp. 945–955, 2003.

[72] Y. Urade, M. Ujihara, Y. Horiguchi, K. Ikai, and O. Hayaishi, "The major source of endogenous prostaglandin D_2 production is likely antigen-presenting cells. Localization of glutathione-requiring prostaglandin D synthetase in histiocytes, dendritic, and Kupffer cells in various rat tissues," *Journal of Immunology*, vol. 143, no. 9, pp. 2982–2989, 1989.

[73] M. Alaoui-El-Azher, Y. Wu, N. Havet, A. Israël, A. Lilienbaum, and L. Touqui, "Arachidonic acid differentially affects basal and lipopolysaccharide-induced $sPLA_2$-IIA expression

in alveolar macrophages through NF-κB and PPAR-γ-dependent pathways," *Molecular Pharmacology*, vol. 61, no. 4, pp. 786–794, 2002.

[74] X. Lian, C. Yan, Y. Qin, L. Knox, T. Li, and H. Du, "Neutral lipids and peroxisome proliterator-activated receptor-γ control pulmonary gene expression and inflammation-triggered pathogenesis in lysosomal acid lipase knockout mice," *American Journal of Pathology*, vol. 167, no. 3, pp. 813–821, 2005.

[75] J. T. Huang, J. S. Welch, M. Ricote, et al., "Interleukin-4-dependent production of PPAR-γ ligands in macrophages by 12/15-lipoxygenase," *Nature*, vol. 400, no. 6742, pp. 378–382, 1999.

[76] P. R. S. Baker, Y. Lin, F. J. Schopfer, et al., "Fatty acid transduction of nitric oxide signaling: multiple nitrated unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligands," *Journal of Biological Chemistry*, vol. 280, no. 51, pp. 42464–42475, 2005.

[77] T. Cui, F. J. Schopfer, J. Zhang, et al., "Nitrated fatty acids: endogenous anti-inflammatory signaling mediators," *Journal of Biological Chemistry*, vol. 281, no. 47, pp. 35686–35698, 2006.

[78] C. Yan and H. Du, "Alveolus formation: what have we learned from genetic studies?" *Journal of Applied Physiology*, vol. 97, no. 4, pp. 1543–1548, 2004.

[79] C. Yan, X. Lian, Y. Li, et al., "Macrophage-specific expression of human lysosomal acid lipase corrects inflammation and pathogenic phenotypes in $lal^{-/-}$ mice," *American Journal of Pathology*, vol. 169, no. 3, pp. 916–926, 2006.

[80] D. A. Culver, B. P. Barna, B. Raychaudhuri, et al., "Peroxisome proliferator-activated receptor γ activity is deficient in alveolar macrophages in pulmonary sarcoidosis," *American Journal of Respiratory Cell and Molecular Biology*, vol. 30, no. 1, pp. 1–5, 2004.

[81] A. M. Johann, A. von Knethen, D. Lindemann, and B. Brüne, "Recognition of apoptotic cells by macrophages activates the peroxisome proliferator-activated receptor-γ and attenuates the oxidative burst," *Cell Death and Differentiation*, vol. 13, no. 9, pp. 1533–1540, 2006.

[82] N. Singh, R. Webb, R. Adams, et al., "The PPAR-γ activator, Rosiglitazone, inhibits actin polymerisation in monocytes: involvement of Akt and intracellular calcium," *Biochemical and Biophysical Research Communications*, vol. 333, no. 2, pp. 455–462, 2005.

[83] S. Appel, V. Mirakaj, A. Bringmann, M. M. Weck, F. Grünebach, and P. Brossart, "PPAR-γ agonists inhibit toll-like receptor-mediated activation of dendritic cells via the MAP kinase and NF-κB pathways," *Blood*, vol. 106, no. 12, pp. 3888–3894, 2005.

[84] M. A. Jakobsen, R. K. Petersen, K. Kristiansen, M. Lange, and S. T. Lillevang, "Peroxisome proliferator-activated receptor $\alpha, \delta, \gamma 1$ and $\gamma 2$ expressions are present in human monocyte-derived dendritic cells and modulate dendritic cell maturation by addition of subtype-specific ligands," *Scandinavian Journal of Immunology*, vol. 63, no. 5, pp. 330–337, 2006.

[85] H. Hammad, H. J. de Heer, T. Soullié, et al., "Activation of peroxisome proliferator-activated receptor-γ in dendritic cells inhibits the development of eosinophilic airway inflammation in a mouse model of asthma," *American Journal of Pathology*, vol. 164, no. 1, pp. 263–271, 2004.

[86] V. Angeli, H. Hammad, B. Staels, M. Capron, B. N. Lambrecht, and F. Trottein, "Peroxisome proliferator-activated receptor γ inhibits the migration of dendritic cells: consequences for the immune response," *Journal of Immunology*, vol. 170, no. 10, pp. 5295–5301, 2003.

[87] E. Imamoto, N. Yoshida, K. Uchiyama, et al., "Inhibitory effect of pioglitazone on expression of adhesion molecules on neutrophils and endothelial cells," *BioFactors*, vol. 20, no. 1, pp. 37–47, 2004.

[88] J. Arias, A. S. Alberts, P. Brindle, et al., "Activation of cAMP and mitogen responsive genes relies on a common nuclear factor," *Nature*, vol. 370, no. 6486, pp. 226–229, 1994.

[89] R. P. S. Kwok, J. R. Lundblad, J. C. Chrivia, et al., "Nuclear protein CBP is a coactivator for the transcription factor CREB," *Nature*, vol. 370, no. 6486, pp. 223–226, 1994.

[90] R. Eckner, T.-P. Yao, E. Oldread, and D. M. Livingston, "Interaction and functional collaboration of p300/CBP and bHLH proteins in muscle and B-cell differentiation," *Genes and Development*, vol. 10, no. 19, pp. 2478–2490, 1996.

[91] S. Bhattacharya, R. Eckner, S. Grossman, et al., "Cooperation of Stat2 and p300/CBP in signalling induced by interferon-α," *Nature*, vol. 383, no. 6598, pp. 344–347, 1996.

[92] A. E. Horvai, L. Xu, E. Korzus, et al., "Nuclear integration of JAK/STAT and Ras/AP-1 signaling by CBP and P300," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 4, pp. 1074–1079, 1997.

[93] J. J. Zhang, U. Vinkemeier, W. Gu, D. Chakravarti, C. M. Horvath, and J. E. Darnell Jr., "Two contact regions between Stat1 and CBP/p300 in interferon γ signaling," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 26, pp. 15092–15096, 1996.

[94] N. D. Perkins, L. K. Felzien, J. C. Betts, K. Leung, D. H. Beach, and G. J. Nabel, "Regulation of NF-κB by cyclin-dependent kinases associated with the p300 coactivator," *Science*, vol. 275, no. 5299, pp. 523–527, 1997.

[95] V. Bocher, I. Pineda-Torra, J.-C. Fruchart, and B. Staels, "PPARs: transcription factors controlling lipid and lipoprotein metabolism," *Annals of the New York Academy of Sciences*, vol. 967, pp. 7–18, 2002.

[96] F. Chen, M. Wang, J. P. O'Connor, M. He, T. Tripathi, and L. E. Harrison, "Phosphorylation of PPARγ via active ERK1/2 leads to its physical association with p65 and inhibition of NF-$\kappa\beta$," *Journal of Cellular Biochemistry*, vol. 90, no. 4, pp. 732–744, 2003.

[97] G. Chinetti, J.-C. Fruchart, and B. Staels, "Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation," *Inflammation Research*, vol. 49, no. 10, pp. 497–505, 2000.

[98] H. Shu, B. Wong, G. Zhou, et al., "Activation of PPARα or γ reduces secretion of matrix metalloproteinase 9 but not interleukin 8 from human monocytic THP-1 cells," *Biochemical and Biophysical Research Communications*, vol. 267, no. 1, pp. 345–349, 2000.

[99] G. Piraino, J. A. Cook, M. O'Connor, et al., "Synergistic effect of peroxisome proliferator activated receptor-γ and liver X receptor-α in the regulation of inflammation in macrophages," *Shock*, vol. 26, no. 2, pp. 146–153, 2006.

[100] J. B. Seo, H. M. Moon, W. S. Kim, et al., "Activated liver X receptors stimulate adipocyte differentiation through induction of peroxisome proliferator-activated receptor γ expression," *Molecular and Cellular Biology*, vol. 24, no. 8, pp. 3430–3444, 2004.

[101] X. Z. Ruan, J. F. Moorhead, R. Fernando, D. C. Wheeler, S. H. Powis, and Z. Varghese, "PPAR agonists protect mesangial cells from interleukin 1β-induced intracellular lipid accumulation by activating the ABCA1 cholesterol efflux pathway," *Journal of the American Society of Nephrology*, vol. 14, no. 3, pp. 593–600, 2003.

[102] R. P. Baughman, A. S. Teirstein, M. A. Judson, et al., "Clinical characteristics of patients in a case control study of sarcoidosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 164, no. 10, part 1, pp. 1885–1889, 2001.

[103] G. W. Hunninghake, G. N. Bedell, D. C. Zavala, M. Monick, and M. Brady, "Role of interleukin-2 release by lung T-cells in active pulmonary sarcoidosis," *American Review of Respiratory Disease*, vol. 128, no. 4, pp. 634–638, 1983.

[104] K. Shigehara, N. Shijubo, M. Ohmichi, et al., "IL-12 and IL-18 are increased and stimulate IFN-γ production in sarcoid lungs," *Journal of Immunology*, vol. 166, no. 1, pp. 642–649, 2001.

[105] J. F. Seymour and J. J. Presneill, "Pulmonary alveolar proteinosis: progress in the first 44 years," *American Journal of Respiratory and Critical Care Medicine*, vol. 166, no. 2, pp. 215–235, 2002.

[106] D. W. Golde, M. Territo, T. N. Finley, and M. J. Cline, "Defective lung macrophages in pulmonary alveolar proteinosis," *Annals of Internal Medicine*, vol. 85, no. 3, pp. 304–309, 1976.

[107] T. Kitamura, N. Tanaka, J. Watanabe, et al., "Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor," *Journal of Experimental Medicine*, vol. 190, no. 6, pp. 875–880, 1999.

[108] N. Tanaka, J. Watanabe, T. Kitamura, Y. Yamada, S. Kanegasaki, and K. Nakata, "Lungs of patients with idiopathic pulmonary alveolar proteinosis express a factor which neutralizes granulocyte-macrophage colony stimulating factor," *FEBS Letters*, vol. 442, no. 2-3, pp. 246–250, 1999.

[109] M. S. Kavuru, E. J. Sullivan, R. Piccin, M. J. Thomassen, and J. K. Stoller, "Exogenous granulocyte-macrophage colony-stimulating factor administration for pulmonary alveolar proteinosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 161, no. 4, part 1, pp. 1143–1148, 2000.

[110] J. F. Seymour, J. J. Presneill, O. D. Schoch, et al., "Therapeutic efficacy of granulocyte-macrophage colony-stimulating factor patients with idiopathic acquired alveolar proteinosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 163, no. 2, pp. 524–531, 2001.

[111] J. C. Gasson, "Molecular physiology of granulocyte-macrophage colony-stimulating factor," *Blood*, vol. 77, no. 6, pp. 1131–1145, 1991.

[112] D. Metcalf, "Cellular hematopoiesis in the twentieth century," *Seminars in Hematology*, vol. 36, no. 4, supplement 7, pp. 5–12, 1999.

[113] N. A. Nicola and D. Metcalf, "Specificity of action of colony-stimulating factors in the differentiation of granulocytes and macrophages," *Ciba Foundation Symposium*, vol. 118, pp. 7–28, 1986.

[114] J. A. Huffman Reed, W. R. Rice, Z. K. Zsengellér, S. E. Wert, G. Dranoff, and J. A. Whitsett, "GM-CSF enhances lung growth and causes alveolar type II epithelial cell hyperplasia in transgenic mice," *American Journal of Physiology*, vol. 273, no. 4, part 1, pp. L715–L725, 1997.

[115] K. Nakata, K. S. Akagawa, M. Fukayama, Y. Hayashi, M. Kadokura, and T. Tokunaga, "Granulocyte-macrophage colony-stimulating factor promotes the proliferation of human alveolar macrophages in vitro," *Journal of Immunology*, vol. 147, no. 4, pp. 1266–1272, 1991.

[116] S. Worgall, R. Singh, P. L. Leopold, et al., "Selective expansion of alveolar macrophages in vivo by adenovirus-mediated transfer of the murine granulocyte-macrophage colony-stimulating factor cDNA," *Blood*, vol. 93, no. 2, pp. 655–666, 1999.

[117] D. Krumwieh, E. Weinmann, B. Siebold, and F. R. Seiler, "Preclinical studies on synergistic effects of IL-1, IL-3, G-CSF and GM-CSF in cynomolgus monkeys," *International Journal of Cell Cloning*, vol. 8, supplement 1, pp. 229–248, 1990, discussion 247–248.

[118] G. Dranoff, A. D. Crawford, M. Sadelain, et al., "Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis," *Science*, vol. 264, no. 5159, pp. 713–716, 1994.

[119] R. Nishinakamura, A. Miyajima, P. J. Mee, V. L. J. Tybulewicz, and R. Murray, "Hematopoiesis in mice lacking the entire granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 functions," *Blood*, vol. 88, no. 7, pp. 2458–2464, 1996.

[120] L. Robb, C. C. Drinkwater, D. Metcalf, et al., "Hematopoietic and lung abnormalities in mice with a null mutation of the common β subunit of the receptors for granulocyte-macrophage colony-stimulating factor and interleukins 3 and 5," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 21, pp. 9565–9569, 1995.

[121] E. Stanley, G. J. Lieschke, D. Grail, et al., "Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 12, pp. 5592–5596, 1994.

[122] T. L. Bonfield, C. F. Farver, B. P. Barna, et al., "Peroxisome proliferator-activated receptor-γ is deficient in alveolar macrophages from patients with alveolar proteinosis," *American Journal of Respiratory Cell and Molecular Biology*, vol. 29, no. 6, pp. 677–682, 2003.

[123] M. Ditiatkovski, B.-H. Toh, and A. Bobik, "GM-CSF deficiency reduces macrophage PPAR-γ expression and aggravates atherosclerosis in ApoE-deficient mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 10, pp. 2337–2344, 2006.

[124] P. J. Christensen, R. E. Goodman, L. Pastoriza, B. Moore, and G. B. Toews, "Induction of lung fibrosis in the mouse by intratracheal instillation of fluorescein isothiocyanate is not T-cell-dependent," *American Journal of Pathology*, vol. 155, no. 5, pp. 1773–1779, 1999.

[125] J. M. Kaplan, J. A. Cook, P. W. Hake, M. O'Connor, T. J. Burroughs, and B. Zingarelli, "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15D-PGJ_2), a peroxisome proliferator activated receptor γ ligand, reduces tissue leukosequestration and mortality in endotoxic shock," *Shock*, vol. 24, no. 1, pp. 59–65, 2005.

[126] D. Liu, B.-X. Zeng, S.-H. Zhang, et al., "Rosiglitazone, a peroxisome proliferator-activated receptor-γ agonist, reduces acute lung injury in endotoxemic rats," *Critical Care Medicine*, vol. 33, no. 10, pp. 2309–2316, 2005.

[127] M. Peters-Golden, "The alveolar macrophage: the forgotten cell in asthma," *American Journal of Respiratory Cell and Molecular Biology*, vol. 31, no. 1, pp. 3–7, 2004.

[128] C. Tang, M. D. Inman, N. van Rooijen, et al., "Th type 1-stimulating activity of lung macrophages inhibits Th2-mediated allergic airway inflammation by an IFN-γ-dependent mechanism," *Journal of Immunology*, vol. 166, no. 3, pp. 1471–1481, 2001.

[129] M. Kobayashi, M. J. Thomassen, T. Rambasek, et al., "An inverse relationship between peroxisome proliferator-activated receptor γ and allergic airway inflammation in an allergen challenge model," *Annals of Allergy, Asthma & Immunology*, vol. 95, no. 5, pp. 468–473, 2005.

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Review Article

Peroxisome Proliferator-Activated Receptors and Acute Lung Injury

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Peroxisome proliferator-activated receptors are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily. PPARs regulate several metabolic pathways by binding to sequence-specific PPAR response elements in the promoter region of target genes, including lipid biosynthesis and glucose metabolism. Recently, PPARs and their respective ligands have been implicated as regulators of cellular inflammatory and immune responses. These molecules are thought to exert anti-inflammatory effects by negatively regulating the expression of proinflammatory genes. Several studies have demonstrated that PPAR ligands possess anti-inflammatory properties and that these properties may prove helpful in the treatment of inflammatory diseases of the lung. This review will outline the anti-inflammatory effects of PPARs and PPAR ligands and discuss their potential therapeutic effects in animal models of inflammatory lung disease.

1. PPARs: OVERVIEW

PPARs are members of the nuclear hormone receptor superfamily that were initially characterized as molecules that mediated the proliferation of peroxisomes in rodent liver parenchymal cells in response to the hypolipidemic drug clofibrate [1]. Subsequently, PPARs have been shown to regulate the expression of genes involved in a variety of biological processes, including lipid metabolism and insulin sensitivity [2, 3]. Three isotypes of PPAR exist, PPAR-α (alpha), PPAR-β/δ (beta/delta), and PPAR-γ (gamma), which are encoded by three separate genes and display distinctly different tissue distributions and functions. PPAR-γ, like other PPAR isotypes, exists as a heterodimer complexed with the retinoid X receptor and several corepressor molecules that tonically suppress PPAR activity [4]. In the presence of PPAR ligands, corepressor molecules are shed, followed by association of coactivator proteins, binding to specific PPAR-response elements, and transcription of target genes [4] (see Figure 1).

PPAR-α is activated by polyunsaturated fatty acids and synthetic fibrates, and is implicated in regulation of lipid metabolism, lipoprotein synthesis and metabolism, and inflammatory response in liver and other tissues. PPAR-α is highly expressed in tissues with high fatty acid oxidation (such as liver, kidney, and heart muscle), where it controls a comprehensive set of genes that regulate most aspects of lipid catabolism. Like several other nuclear hormone receptors, PPAR-α heterodimerizes with RXR alpha to form a transcriptionally competent complex [5]. In addition, PPAR-α is expressed in vascular endothelial cells, smooth muscle cells, monocyte/macrophages, and T lymphocytes. Activation of PPAR-α in selected cellular systems increases HDL cholesterol synthesis, stimulates "reverse" cholesterol transport, and reduces triglycerides [6].

The biological role of PPAR-β/δ has not been clearly defined. Animal studies revealed that PPAR-β/δ plays an important role in the metabolic adaptation of several tissues to environmental changes. Treatment of obese animals with specific PPAR-β/δ agonists results in normalization of metabolic parameters and reduction of adiposity. PPAR-β/δ was also implicated in the regulation of fatty acid burning capacities of skeletal muscle and adipose tissue by controlling the expression of genes involved in fatty acid uptake, beta oxidation, and energy uncoupling. Moreover, PPAR-β/δ has been

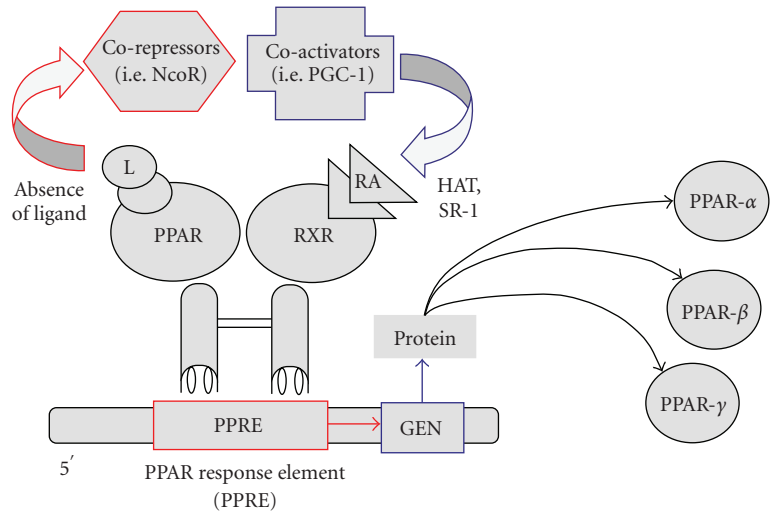

Figure 1: Schematic of PPAR activation events. Like other nuclear hormone receptors, PPAR acts as a ligand-activated transcription factor. PPAR-α, when activated after binding with specific ligand, interacts with RXR and regulates the expression of target genes. These genes are also involved in the catabolism of fatty acids. Conversely, PPAR-γ is activated by different ligands (e.g., prostaglandins, leukotrienes, and antidiabetic thiazolidinediones) and regulates the expression of genes involved in the storage of the fatty acids. PPAR-β is only weakly activated by fatty acids, prostaglandins, and leukotrienes and has no known physiologically relevant ligand. Abbreviations: nuclear corepressor protein: (NcoR); PPAR gamma coactivator 1:(PGC-1); histone acyltransferase: (HAT); steroid receptor coactivator-1: (SR-1); 9-cis retinoic acid: (RA).

shown to mediate the adaptive metabolic response of skeletal muscle to endurance exercise by controlling the number of oxidative myofibers and stimulating fatty acid catabolism in muscular tissue [7]. Recent studies revealed that ligand activation of these receptors is associated with improved insulin sensitivity and elevated HDL levels thus demonstrating promising potential for targeting PPAR-β/δ in the treatment of obesity, dyslipidemias, and type 2 diabetes [8].

PPAR-γ plays an important role in the regulation of proliferation and differentiation of several cell types, including adipose cells. This receptor has the ability to bind a variety of small lipophilic compounds derived from both metabolism and nutrition. These ligands, in turn, direct cofactor recruitment to PPAR-γ, regulating the transcription of genes in a variety of complex metabolic pathways. PPAR-γ is highly expressed in adipocytes, where it mediates differentiation, promotes lipid storage, and, as a consequence, is thought to indirectly improve insulin sensitivity and enhance glucose disposal in adipose tissue and skeletal muscle [9, 10]. Activation by drugs of the glitazone (thiazolidinediones) group results in insulin sensitization and antidiabetic action. Naturally occurring lipids can also activate PPAR-γ, including arachidonic, oleic, and linoleic acid, and the cyclopentenone prostaglandin (PG) 15-deoxy $\text{Delta}_{12,14}$-PGJ_2 (15d-PGJ_2), a metabolite of prostaglandin D_2. Nitosylated oleic and linoleic acid species have more recently been identified as potent PPAR-γ agonists at concentrations present in human tissues. The cellular expression profile of PPAR-γ in pulmonary tissue has not been well characterised, but studies have uncovered abundant expression of PPAR-γ in airway epithelium [11], bronchial submucosa [12], in mononuclear phagocytes such as human alveolar macrophages (AM), human T lymphocytes, and in several pulmonary cell lines, including human bronchial and alveolar epithelial cells (NL20, BEAS, and A549 [13]) and human airway smooth muscle (HASM) cells [14]. The expression of the various isotypes of PPAR is highly cell specific. For instance, HASM cells express PPAR-α and PPAR-γ, but not PPAR-β/δ, whereas primary normal human bronchial epithelial cells and human lung epithelial cell lines BEAS 2B, A549, and NCI-H292 all express PPAR-γ and PPAR-β/δ, but not PPAR-α [15]. Because little is known regarding the role of PPAR-β/δ in regulating inflammation, especially in the context of lung injury, this review will focus on the biology of PPAR-α and PPAR-γ in human and animal models of acute lung injury (ALI).

2. ACUTE LUNG INJURY (ALI)

Injury to the lung can occur in response to a variety of pulmonary and extrapulmonary insults. In humans, ALI and its more severe form, the acute respiratory distress syndrome (ARDS) are syndromes of acute respiratory failure, which are defined clinically on the basis of both radiographical (bilateral lung field infiltrates) and physiological (the ratio of arterial oxygen pressure and the inspiratory oxygen concentration, $P_a/F_i \leq 300$ mmHg for ALI and ≤ 200 mmHg for ARDS) criteria. These syndromes occur as a result of widespread damage to cells and structures of the alveolar capillary membrane and evolve within hours to days [16]. ALI/ARDS can develop as a consequence of critical illness of diverse etiologies, including direct injury to lung such as pneumonia, aspiration, toxic inhalation, near drowning, or lung contusion; as well as indirect mechanisms, such as sepsis, burn injury, pancreatitis, gynecological insults

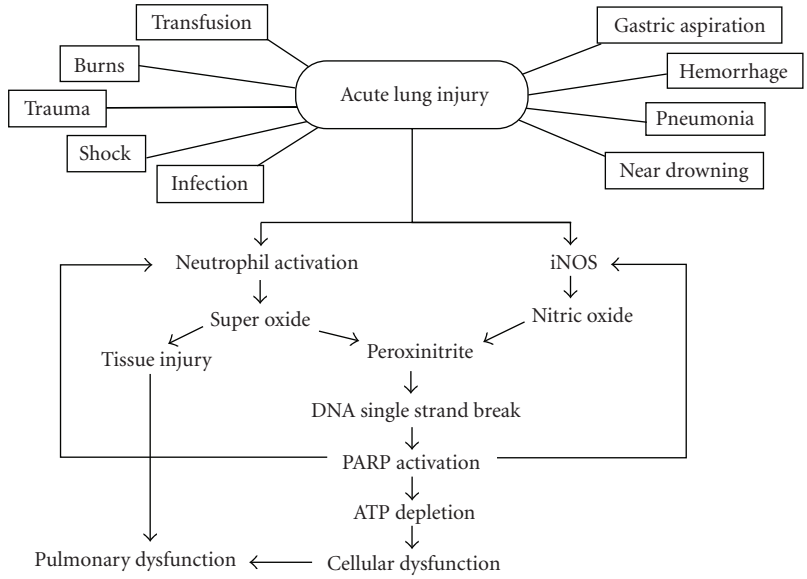

Figure 2: Pathophysiological events in acute lung injury.

(abruption of placenta, amniotic embolism, eclampsia), or massive blood transfusion [17].

The pathophysiological consequences of ALI/ARDS are related to the altered pulmonary capillary permeability and alveolar diffusion capacity, as well as the increased intrapulmonary shunt. Endothelial injury and increased vascular permeability is a central feature of ALI/ARDS, and some but not all studies suggest a role for neutrophils in mediating endothelial injury [17, 18]. Epithelial injury is also important not only in the development but also the repair of the ALI/ARDS [19]. The degree of epithelial injury can predict outcome of ALI/ARDS [20]. Loss of epithelial integrity and injury to type II alveolar cells can disrupt the normal fluid transport, thereby impairing the removal of fluid from the alveolar space. Injury to the type II pneumocytes can reduce the production of surfactant, which contributes to the clinical course of worsening atelectasis and gas exchange. The process of epithelial repair can be dysregulated, leading to proliferation of fibroblasts, exuberant matrix deposition and remodeling, and culminate in fibrosis [21, 22]. There are complex autocrine and paracrine interrelationships of cytokines, as well as proinflammatory mediators that initiate and amplify the inflammatory response in ALI/ARDS. The cellular responses include the expression of endothelial adhesion molecules, as well as the margination and migration of neutrophils and other inflammatory cells. A number of soluble factors are released that contribute to the pathobiology of ALI/ARDS, including cytokines, lipid mediators, proteases, oxidants, growth factors (e.g., transforming growth factors (TGFs)), nitric oxide (NO), and neuropeptides [23] (see Figure 2). This inflammatory state is driven by the activation of several key-signalling pathways including the NF-κB, AP-1 and the mitogen-activated protein kinase (MAPK) pathways.

2.1. PPAR-α and lung injury

Based on both in vivo and in vitro studies in multiple cell systems, PPAR-α ligands have important anti-inflammatory properties. For example, treatment of an activated murine macrophage cell line with the synthetic PPAR-α agonist Wy14643 [peroxisome proliferation-activated receptor-alpha (PPAR-alpha) activator, 4-cholro-6-(2.3-xylidino)-2-pyrimidinaylthio acetic acid] resulted in inhibition of nitric oxide synthase (NOS), whereas LTB_4 and 8(S)-HETE, two natural PPAR-α ligands, stimulated the expression of nitric oxide synthase (NOS) activity in these same cells [24]. The authors have postulated that this disparity resulted from low potency and specificity of the endogenous ligands in comparison with that of synthetic compounds [25]. The in vivo role of PPAR-α in the regulation of inflammatory/immune-related functions is less well studied. The first in vivo evidence for the role of PPAR-α evolved from studies using PPAR-α deficient mice [26]. These mice are viable, but exhibit altered triglyceride and cholesterol metabolism and fail to respond to appropriate PPAR-α ligands. Data generated using PPAR-α knockout mice indicate that this receptor regulates acute inflammation in vivo [27]. For example, PPAR-α-deficient mice have abnormally prolonged responses to different inflammatory stimuli [28]. Furthermore, fibrates have anti-inflammatory properties in vitro [29] and in vivo [30]. In particular, PPAR-α ligands can inhibit the expression of several proinflammatory genes such as IL-6, VCAM, and cyclooxygenase-2, in response to cytokine activation [30]. Moreover, the suppressive effect of PPAR-α ligands is mediated by inhibition of NF-κB activation, in part by enhancing the expression of IκBα [31]. It is important to note that synthetic and natural PPAR-α agonists can exert multiple biologic effects, including some which occur in a

PPAR-α-independent fashion [32]. WY14643, like GW7647, shows excellent selectivity for murine and human PPAR-α.

Recent investigations have addressed the contribution of PPAR-α to the development of acute pleural and pulmonary inflammation and injury. We reported that when compared with wild-type mice, PPAR-α knockout mice experienced more severe pleural inflammation when subjected to intrapleural carrageenan administration. Specifically, the absence of a functional PPAR-α gene resulted in a significant augmentation of several inflammatory parameters (e.g., pleural exudate formation, mononuclear cell infiltration, and histological injury). Furthermore, PPAR-$\alpha^{-/-}$ mice had enhanced the expression of tumor necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), and FAS ligand in the pleural space post carrageenan administration [33].

Agonists for PPAR-α have been shown to reduce lipopolysaccharide (LPS)- and cytokine-induced secretion of matrix metalloproteinase-9 (MMP-9) in human monocytes and rat mesangial cells, suggesting that this nuclear hormone receptor may play a beneficial role in controlling both tissue inflammation and remodeling. Consistent with this notion, Delayre-Orthez showed enhanced airway neutrophil and macrophage infiltration, elaboration of TNF-α, chemokines, and MMP 9 in PPAR-$\alpha^{-/-}$ mice challenged with intranasal LPS, compared to that observed in similarly treated PPAR-$\alpha^{+/+}$ mice. Conversely, pretreatment with the PPAR-α agonist fenofibrate reduced LPS-medicated airway inflammation, cytokine/chemokine expression and MMP-2 and -9 activity in bronchoalveolar lavage fluid [34]. Our laboratory has investigated the role of PPAR-α ligands in acute pulmonary inflammation using an experimental model of acute pancreatitis induced by cerulein. Intraperitoneal administration of cerulein in PPAR-α deficient mice resulted in severe infiltration of pancreatic and lung tissue with neutrophils (as measured by changes in myeloperoxidase activity), and enhanced expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), P-selectin, and growth factors TGF-β and VEGF in lung tissue, as compared to that observed in wild-type animals [35]. Interestingly, Jiang et al. have recently shown that acute lung injury in rats in response to LPS results in a reduced expression of PPAR-α mRNA and protein in the lung, raising the possibility that alterations in PPAR-α expression/activity may contribute to heightened inflammatory response [36].

Similar to effects in other models of pulmonary injury, PPAR-α appears to play a pivotal role in regulating the inflammatory response in experimental models of bleomycin-induced acute lung injury. Intratracheal administration of bleomycin in PPAR-$\alpha^{-/-}$ mice resulted in a significant augmentation of TNF-α, IL-1β, and immunoreactive poly-ADP-ribose, as well as a loss of body weight and increased mortality. The dysregulated expression of poly-ADP-ribose is of particular relevance, as this molecule is synthesized from nicotinamide adenine dinucleotide (NAD) by poly-ADP ribose polymerase (PARP) during periods of oxidative stress, and enhanced PARP activity results in consumption of NAD^+, ATP depletion, and ultimately cellular dysfunction. Conversely, the treatment of wild-type mice with WY14643 (1 mg/kg daily) prior to bleomycin administration significantly reduced the degree of lung injury, attenuated the rise in bleomycin-induced myeloperoxidase activity, and reduced the expression of TNF-α, IL-1β , and poly-ADP-ribose [37].

2.2. PPAR-γ and lung injury

In contrast to genetic models of PPAR-α deficiency, studies evaluating immunomodulatory effects of PPAR-γ have been limited by the absence of mice that are homozygous deficient for PPAR-γ, as these fetuses die in utero. For that reason, most studies assessing the role of PPAR-γ in inflammatory responses in vivo have relied on treatment with PPAR-γ agonists and/or antagonists or the use of mice that are heterozygous PPAR-γ deficient mice (PPAR-$\gamma^{+/-}$), which display reduced but not absence PPAR-γ activity.

As previously noted, the cyclopentenone prostaglandin 15d-PGJ_2 functions as an endogenous ligand for PPAR-γ. We reported that 15d-PGJ_2 (given at 10, 30, or 100 μg/kg IP) in the carrageenan-induced pleurisy model exerted potent anti-inflammatory effects (e.g., inhibition of pleural exudate formation, mononuclear cell infiltration, delayed development of clinical indicators, and histological injury) in vivo. Furthermore, 15d-PGJ_2 reduced the increase in nitrotyrosine and poly (ADP-ribose) polymerase and the expression of inducible nitric-oxide synthase and cyclooxygenase-2, as determined by immunohistochemistry, in the lungs of carrageenan-treated mice [38]. We also observed that rosiglitazone (given at 3, 10, or 30 mg/kg IP 15 minutes before carrageenan administration in the pleurisy model) exerted similar anti-inflammatory effects (e.g., inhibition of pleural exudate formation, mononuclear cell infiltration, and histological injury) in vivo as that observed with 15d-PGJ_2. Furthermore, rosiglitazone reduced: (1) the increase in nitrotyrosine and poly (ADP-ribose) polymerase (PARP); (2) the expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), intercellular adhesion molecules-1 (ICAM-1), and P-selectin in the lungs of carrageenan-treated rats. In order to elucidate whether the protective effect of rosiglitazone was causally related to activation of PPAR-γ, we investigated the effect of a PPAR-γ antagonist, bisphenol A diglycidyl ether (BADGE), on the protective effects of rosiglitazone. BADGE (30 mg/kg IP) administered 30 minutes prior to treatment with rosiglitazone significantly antagonized the suppressive properties of the PPAR-γ agonist [39].

In an animal model of severe haemorrhage and resuscitation, Abdelrahman et al. investigated the effects of 15d-PGJ_2 administration on the development of multiple organ injury/dysfunction. Importantly, PPAR-γ agonist 15d-PGJ_2 abolished the renal dysfunction and largely reduced the liver injury caused by hemorrhagic shock. In addition, 15d-PGJ_2 attenuated lung and intestinal injury (as determined by histology) caused by haemorrhage and resuscitation [40].

We investigated the effects of rosiglitazone on the development of nonseptic shock caused by zymosan in mice. Treatment of mice with rosiglitazone (3 mg/kg IP, 1 and 6 hours after zymosan) attenuated the peritoneal exudation

and the migration of polymorphonuclear cells caused by zymosan. Rosiglitazone also attenuated zymosan-induced lung dysfunction, as well as the increase in myeloperoxidase activity and malondialdehyde concentrations in the lung. To elucidate whether the protective effects of rosiglitazone occurred in a PPAR-γ specific fashion, we investigated the effect of a PPAR-gamma antagonist, GW9662, on the protective effects of rosiglitazone. GW9662 (1 mg/kg administered IP 30 minutes before treatment with rosiglitazone) significantly abolished the protective effect of rosiglitazone [41].

There exists convincing evidence that treatment with PPAR-γ agonists can also modulate pulmonary inflammation and tissue injury in response to systemic LPS administration and ischemia-reperfusion injury. For instance, experimental endotoxemia for 4 hours induced histological evidence of lung injury and edema formation, both of which were significantly attenuated by rosiglitazone pretreatment. The protective effects of rosiglitazone were correlated with the reduction by 71% and 84%, of the increase of myeloperoxidase and malondialdehyde, respectively, in the lung tissue. Furthermore, the pulmonary induction of nitric oxide was reduced by 82% of the increase related to lipopolysaccharide [42]. More recently, it has been shown that preischemic treatment with pioglitazone, a synthetic ligand of PPAR-γ, significantly attenuated ischemia/reperfusion (I/R)-induced lung injury in rats, including reductions in lung microvascular permeability, lipid peroxidation, tissue-associated polymorphonuclear leukocyte infiltration, and proinflammatory cytokine production. These findings can be explained, at least in part, by PPAR-γ-mediated inhibition of transcription factors such as NF-κB [43], resulting in attenuated cytokine, chemokine and eicosanoid production, adhesion molecule expression, and as a consequence reduced inflammatory cell influx and injury to the alveolar capillary [44–47]. Another mechanism of protection afforded by the PPAR-γ agonist troglitazone in I/R lung injury is suppression of transcription factor early growth response gene-1 and its inflammatory gene targets such as interleukin-1β, monocyte chemotactic protein-1, and macrophage inflammatory protein-2 [48].

While the majority of studies have found potent anti-inflammatory properties of PPAR-γ agonists, observations made in several studies challenge this paradigm. Notably, Inoue et al. [49] demonstrated that pretreatment of mice with 15d-PGJ_2 did not reduce pulmonary inflammation induced by intratracheal LPS administration. In fact, at the highest concentrations (1 mg/kg), 15d-PGJ_2 paradoxicallyenhanced LPS-induced alveolar inflammation, pulmonary edema, and inflammatory cytokine expression. One possible explanation for the observed disparity in results may be attributable to PPAR-independent effects of selected agonists, dose-dependent toxicity or differences in the model systems used. The role of PPAR-γ in acute lung inflammation was also investigated in fluorescein isothiocyanate-treated mice. Here, pretreatment with pioglitazone (vehicle by oral gavage daily for 5 days) decreased the number of neutrophils recovered in bronchoalveolar lavage (BAL) by 50% 3 days after intratracheal challenge with fluorescein isothiocyanate. However, the decreased pulmonary inflammation was not associated withinhibition of the expression of inflammatory cytokines (TNF-α, macrophage inflammatory protein-2, *KC*, IL-12, or IFN-γ) in either BAL fluidor whole lung homogenates [50]. The authors speculated that the possible mechanism by which a PPAR-γ ligand suppresses inflammation in the absence of changes in cytokine expressionwas by a direct effect on migration of neutrophils (and possibly other leukocytes) in response to endogenous chemoattractants [50]. In the FITC model, treatment with pioglitazone also had only modest suppressive effects on alveolar-capillary leak or subsequent fibroproliferation. The disparate effects of PPAR-γ agonists on inflammation relative to alveolar capillary injury and repair may be attributable to direct effects of PPAR-γ activation on alveolar epithelial cells. Treatment of A549 alveolar type II-like epithelial cells with 15d-PGJ_2 or TZDs, or forced expression of a constitutively active PPAR-γ has been shown to suppress NF-κB transcriptional activity and decreased inflammatory cytokine and chemokine production. However, incubation of these cells with PPAR-γ ligands also suppressed alveolar epithelial cell proliferative responses. Collectively these data suggest that beneficial anti-inflammatory properties of PPAR-γ in ALI may be partially offset by growth inhibitory effects on alveolar epithelial cells, responses that are necessary for repair of an injured alveolar-capillary membrane.

Orderly lung remodeling is required for restoration of an intact alveolar-capillary membrane after injury. Fibroblasts are one of the key effector cells in this process. However, the differentiation of fibroblasts to myofibroblasts can result in excessive and uncontrolled production of collagen and other extracellular matrix components, leading to fibrosis. Importantly, PPAR-γ agonists have been shown to block two of the most important profibrotic activities of TGF-β on pulmonary fibroblasts; myofibroblast differentiation and production of excess collagen. Both natural (15d-PGJ_2) and synthetic (ciglitazone and rosiglitazone) PPAR-γ agonists inhibited TGF-β-driven myofibroblast differentiation in human lung fibroblasts, as determined by alpha-smooth muscle actin expression. PPAR-γ agonists also potently attenuated TGF-β-induced type I collagen protein production [51]. Transfection with a dominant-negative PPAR-γ construct partially reversed the inhibition of myofibroblast differentiation by 15d-PGJ2 and rosiglitazone, but the irreversible PPAR-γ antagonist GW-9662 did not, suggesting that the antifibrotic effects of the PPAR-γ agonists are mediated through both PPAR-γ-dependent and independent mechanisms.

Observations made in several studies suggest that the activation of PPAR-γ may exert both anti-inflammatory and antifibrotic effects in vivo. Mice subjected to intratracheal administration of bleomycin develop marked lung injury followed by fibrosis. An increase in immunoreactivity to nitrotyrosine, poly (ADP ribose) polymerase (PARP), and inducible nitric oxide synthase as well as a significant loss of body weight and mortality was observed in the lung of bleomycin-treated mice. Administration of the two PPAR-gamma agonists rosiglitazone (10 mg/kg IP) or 15d-PGJ_2 (30 μg/kg IP) significantly reduced: (1) the loss of body weight; (2) mortality rate; (3) infiltration of the lung with

polymorphonuclear neutrophils (myeloperoxidase activity); (4) edema formation; (5) histological evidence of lung injury and fibroproliferation; and (6) nitrotyrosine, PARP, and inducible nitric oxide synthase formation [52]. Pretreatment with the PPAR-gamma competitive antagonist BADGE substantially mitigated the effect of the two PPAR-gamma agonists, indicating a PPAR-γ specific response. Our findings are in agreement with Ando et al. [53], who demonstrated that the intravenous injection of prostaglandin D synthase (PGDS) cDNA-expressing fibroblasts significantly reduced lung edema, BAL leukocytes, and pulmonary collagen 4 weeks after intratracheal instillation of bleomycin. Moreover, this attenuated lung response to bleomycin was quite similar to that seen in animals pretreated with 15d-PGJ_2, the nonenzymatic metabolite of PGD_2, suggesting that these naturally occurring ligands exert relevant effects on the fibroproliferative response in vivo.

3. CONCLUSION

The subsequent tissue response to acute and chronic lung injury involves an intricate series of events including immune cell infiltration, release of injurious host-derived molecules such as reactive oxygen and nitrogen species, and high permeability edema formation. In addition, fibroproliferative repair is characterized by myofibroblast transdifferentiation and the deposition of extracellular matrix proteins. Failure to initiate, maintain, or stop this repair program has dramatic consequences such as cell death or exuberant wound repair. PPARs appear to be critical regulators of host inflammatory and reparative responses, and these transcriptional factors may be activated by lipid mediators produced in response to lung injury. The generation of better transgenic model systems, including conditional and site-specific transgenic mouse models, are required to more precisely define the contribution of PPAR-γ and other PPAR family members to disease pathogenesis in ALI and other inflammatory lung diseases. This class of nuclear hormone receptors may serve as important targets for therapeutic intervention in the treatment of patient with both acute and chronic inflammatory disorders of the lung.

REFERENCES

[1] R. Hess, W. Stäubli, and W. Riess, "Nature of the hepatomegalic effect produced by ethyl-chlorophenoxy-isobutyrate in the rat," *Nature*, vol. 208, no. 13, pp. 856–858, 1965.

[2] J. Vamecq and N. Latruffe, "Medical significance of peroxisome proliferator-activated receptors," *The Lancet*, vol. 354, no. 9173, pp. 141–148, 1999.

[3] J. C. Corton, S. P. Anderson, and A. Stauber, "Central role of peroxisome proliferator-activated receptors in the actions of peroxisome proliferators," *Annual Review of Pharmacology and Toxicology*, vol. 40, pp. 491–518, 2000.

[4] P. Dowell, J. E. Ishmael, D. Avram, V. J. Peterson, D. J. Nevrivy, and M. Leid, "Identification of nuclear receptor corepressor as a peroxisome proliferator-activated receptor α interacting protein," *Journal of Biological Chemistry*, vol. 274, no. 22, pp. 15901–15907, 1999.

[5] J. C. Fruchart, B. Staels, and P. Duriez, "The role of fibric acids in atherosclerosis," *Current Atherosclerosis Reports*, vol. 3, no. 1, pp. 83–92, 2001.

[6] B. Desvergne, L. Michalik, and W. Wahli, "Be fit or be sick: peroxisome proliferator-activated receptors are down the road," *Molecular Endocrinology*, vol. 18, no. 6, pp. 1321–1332, 2004.

[7] A. Fredenrich and P. A. Grimaldi, "PPAR delta: an uncompletely known nuclear receptor," *Diabetes and Metabolism*, vol. 31, no. 1, pp. 23–27, 2005.

[8] A. D. Burdick, D. J. Kim, M. A. Peraza, F. J. Gonzalez, and J. M. Peters, "The role of peroxisome proliferator-activated receptor-β/δ in epithelial cell growth and differentiation," *Cellular Signalling*, vol. 18, no. 1, pp. 9–20, 2006.

[9] C. A. Argmann, T.-A. Cock, and J. Auwerx, "Peroxisome proliferator-activated receptor γ: the more the merrier?" *European Journal of Clinical Investigation*, vol. 35, no. 2, pp. 82–92, 2005.

[10] M. A. Lazar, "PPARγ, 10 years later," *Biochimie*, vol. 87, no. 1, pp. 9–13, 2005.

[11] H. Sundvold, A. Brzozowska, and S. Lien, "Characterisation of bovine peroxisome proliferator-activated receptors γ1 and γ2: genetic mapping and differential expression of the two isoforms," *Biochemical and Biophysical Research Communications*, vol. 239, no. 3, pp. 857–861, 1997.

[12] S. Ueki, Y. Matsuwaki, H. Kayaba, et al., "Peroxisome proliferator-activated receptor γ regulates eosinophil functions: a new therapeutic target for allergic airway inflammation," *International Archives of Allergy and Immunology*, vol. 134, supplement 1, pp. 30–36, 2004.

[13] M. Hetzel, D. Walcher, M. Grüb, H. Bach, V. Hombach, and N. Marx, "Inhibition of MMP-9 expression by PPARγ activators in human bronchial epithelial cells," *Thorax*, vol. 58, no. 9, pp. 778–783, 2003.

[14] H. J. Patel, M. G. Belvisi, D. Bishop-Bailey, M. H. Yacoub, and J. A. Mitchell, "Activation of peroxisome proliferator-activated receptors in human airway smooth muscle cells has a superior anti-inflammatory profile to corticosteroids: relevance for chronic obstructive pulmonary disease therapy," *Journal of Immunology*, vol. 170, no. 5, pp. 2663–2669, 2003.

[15] R. Pawliczak, C. Han, X.-L. Huang, A. J. Demetris, J. H. Shelhamer, and T. Wu, "85-kDa cytosolic phospholipase A_2 mediates peroxisome proliferator-activated receptor γ activation in human lung epithelial cells," *Journal of Biological Chemistry*, vol. 277, no. 36, pp. 33153–33163, 2002.

[16] G. R. Bernard, A. Artigas, K. L. Brigham, et al., "The American-European consensus conference on ARDS: definitions, mechanisms, relevant outcomes, and clinical trial coordination," *American Journal of Respiratory and Critical Care Medicine*, vol. 149, no. 3, part 1, pp. 818–824, 1994.

[17] L. B. Ware and M. A. Matthay, "The acute respiratory distress syndrome," *New England Journal of Medicine*, vol. 342, no. 18, pp. 1334–1349, 2000.

[18] Y. Sivan, C. Mor, S. Al-Jundi, and C. J. Newth, "Adult respiratory distress syndrome in severely neutropenic children," *Pediatric Pulmonology*, vol. 8, no. 2, pp. 104–108, 1990.

[19] M. A. Matthay and J. P. Wiener-Kronish, "Intact epithelial barrier function is critical for the resolution of alveolar edema in humans," *American Review of Respiratory Disease*, vol. 142, no. 6, pp. 1250–1257, 1990.

[20] J. I. Sznajder, "Strategies to increase alveolar epithelial fluid removal in the injured lung," *American Journal of Respiratory and Critical Care Medicine*, vol. 160, no. 5 part 1, pp. 1441–1442, 1999.

[21] W. M. Zapol, R. L. Trelstad, J. W. Coffey, I. Tsai, and R. A. Salvador, "Pulmonary fibrosis in severe acute respiratory failure," *American Review of Respiratory Disease*, vol. 119, no. 4, pp. 547–554, 1979.

[22] J. F. Tomashefski, "Pulmonary pathology of the adult respiratory distress syndrome," *Clinics in Chest Medicine*, vol. 11, no. 4, pp. 593–619, 1990.

[23] A. C. J. Windsor, C. J. Walsh, P. G. Mullen, et al., "Tumor necrosis factor-α blockade prevents neutrophil CD18 receptor upregulation and attenuates acute lung injury in porcine sepsis without inhibition of neutrophil oxygen radical generation," *Journal of Clinical Investigation*, vol. 91, no. 4, pp. 1459–1468, 1993.

[24] P. R. Colville-Nash, S. S. Qureshi, D. Willis, and D. A. Willoughby, "Inhibition of inducible nitric oxide synthase by peroxisome proliferator-activated receptor agonists: correlation with induction of heme oxygenase 1," *Journal of Immunology*, vol. 161, no. 2, pp. 978–984, 1998.

[25] G. Chinetti, J.-C. Fruchart, and B. Staels, "Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation," *Inflammation Research*, vol. 49, no. 10, pp. 497–505, 2000.

[26] R. B. Clark, "The role of PPARs in inflammation and immunity," *Journal of Leukocyte Biology*, vol. 71, no. 3, pp. 388–400, 2002.

[27] A. E. Lovett-Racke, R. Z. Hussain, S. Northrop, et al., "Peroxisome proliferator-activated receptor α agonists as therapy for autoimmune disease," *Journal of Immunology*, vol. 172, no. 9, pp. 5790–5798, 2004.

[28] T. Genovese, E. Mazzon, R. Di Paola, et al., "Role of endogenous ligands for the peroxisome proliferators activated receptors alpha in the secondary damage in experimental spinal cord trauma," *Experimental Neurology*, vol. 194, no. 1, pp. 267–278, 2005.

[29] H. Okamoto, T. Iwamoto, S. Kotake, S. Momohara, H. Yamanaka, and N. Kamatani, "Inhibition of NK-κB signaling by fenofibrate, a peroxisome proliferator-activated receptor-α ligand, presents a therapeutic strategy for rheumatoid arthritis," *Clinical and Experimental Rheumatology*, vol. 23, no. 3, pp. 323–330, 2005.

[30] P. Delerive, K. De Bosscher, S. Besnard, et al., "Peroxisome proliferator-activated receptor α negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-κB and AP-1," *Journal of Biological Chemistry*, vol. 274, no. 45, pp. 32048–32054, 1999.

[31] P. Delerive, P. Gervois, J.-C. Fruchart, and B. Staels, "Induction of IκBα expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-α activators," *Journal of Biological Chemistry*, vol. 275, no. 47, pp. 36703–36707, 2000.

[32] E. Dyrøy, A. Yndestad, T. Ueland, et al., "Antiinflammatory effects of tetradecylthioacetic acid involve both peroxisome proliferator-activated receptor α-dependent and -independent pathways," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 7, pp. 1364–1369, 2005.

[33] S. Cuzzocrea, E. Mazzon, R. Di Paola, et al., "The role of the peroxisome proliferator-activated receptor-α (PPAR-α) in the regulation of acute inflammation," *Journal of Leukocyte Biology*, vol. 79, no. 5, pp. 999–1010, 2006.

[34] C. Delayre-Orthez, J. Becker, I. Guenon, et al., "PPARα downregulates airway inflammation induced by lipopolysaccharide in the mouse," *Respiratory Research*, vol. 6, p. 91, 2005.

[35] T. Genovese, E. Mazzon, R. Di Paola, et al., "Role of peroxisome proliferator-activated receptor-α in acute pancreatitis induced by cerulein," *Immunology*, vol. 118, no. 4, pp. 559–570, 2006.

[36] P. Jiang, J.-C. Wang, Y.-M. Zhao, and G.-S. Qian, "Influence and mechanism of peroxisome proliferation activated receptor-α expression induced by WY14643 in rat lung with acute lung injury," *Zhongguo Weizhongbing Jijiuyixue*, vol. 18, no. 8, pp. 466–469, 2006.

[37] T. Genovese, E. Mazzon, R. Di Paola, et al., "Role of endogenous and exogenous ligands for the peroxisome proliferator-activated receptor α in the development of bleomycin-induced lung injury," *Shock*, vol. 24, no. 6, pp. 547–555, 2005.

[38] S. Cuzzocrea, N. S. Wayman, E. Mazzon, et al., "The cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 attenuates the development of acute and chronic inflammation," *Molecular Pharmacology*, vol. 61, no. 5, pp. 997–1007, 2002.

[39] S. Cuzzocrea, B. Pisano, L. Dugo, et al., "Rosiglitazone, a ligand of the peroxisome proliferator-activated receptor-γ, reduces acute inflammation," *European Journal of Pharmacology*, vol. 483, no. 1, pp. 79–93, 2004.

[40] M. Abdelrahman, M. Collin, and C. Thiemermann, "The peroxisome proliferator-activated receptor-γ ligand 15-deoxy $\Delta^{12,14}$ prostaglandin J_2 reduces the organ injury in hemorrhagic shock," *Shock*, vol. 22, no. 6, pp. 555–561, 2004.

[41] S. Cuzzocrea, B. Pisano, L. Dugo, et al., "Rosiglitazone, a ligand of the peroxisome proliferator-activated receptor-γ, reduces the development of nonseptic shock induced by zymosan in mice," *Critical Care Medicine*, vol. 32, no. 2, pp. 457–466, 2004.

[42] D. Liu, B. X. Zeng, and Y. Shang, "Decreased expression of peroxisome proliferator-activated receptor γ in endotoxin-induced acute lung injury," *Physiological Research*, vol. 55, no. 3, pp. 291–299, 2006.

[43] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[44] S. Ohtsuka, M. Kakihana, H. Watanabe, T. Enomoto, R. Ajisaka, and Y. Sugishita, "Alterations in left ventricular wall stress and coronary circulation in patients with isolated systolic hypertension," *Journal of Hypertension*, vol. 14, no. 11, pp. 1349–1355, 1996.

[45] R. M. Strieter, R. Wiggins, S. H. Phan, et al., "Monocyte chemotactic protein gene expression by cytokine-treated human fibroblasts and endothelial cells," *Biochemical and Biophysical Research Communications*, vol. 162, no. 2, pp. 694–700, 1989.

[46] H. Ishii, M. Ishibashi, M. Takayama, T. Nishida, and M. Yoshida, "The role of cytokine-induced neutrophil chemoattractant-1 in neutrophil-mediated remote lung injury after intestinal ischaemia/reperfusion in rats," *Respirology*, vol. 5, no. 4, pp. 325–331, 2000.

[47] P. L. Khimenko, G. J. Bagby, J. Fuseler, and A. E. Taylor, "Tumor necrosis factor-α in ischemia and reperfusion injury in rat lungs," *Journal of Applied Physiology*, vol. 85, no. 6, pp. 2005–2011, 1998.

[48] M. Okada, S. F. Yan, and D. J. Pinsky, "Peroxisome proliferator-activated receptor-γ (PPAR-γ) activation suppresses ischemic induction of Egr-1 and its inflammatory gene targets," *The FASEB Journal*, vol. 16, no. 14, pp. 1861–1868, 2002.

[49] T. J. Standiford, V. C. Keshamouni, and R. C. Reddy, "Peroxisome proliferator-activated receptor-γ as a regulator of lung inflammation and repair," *Proceedings of the American Thoracic Society*, vol. 2, no. 3, pp. 226–231, 2005.

[50] K.-I. Inoue, H. Takano, R. Yanagisawa, et al., "Effect of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 on acute lung injury induced by lipopolysaccharide in mice," *European Journal of Pharmacology*, vol. 481, no. 2-3, pp. 261–269, 2003.

[51] H. A. Burgess, L. E. Daugherty, T. H. Thatcher, et al., "PPARγ agonists inhibit TGF-β induced pulmonary myofibroblast differentiation and collagen production: implications for therapy of lung fibrosis," *Lung Cellular and Molecular Physiology*, vol. 288, no. 6, pp. L1146–L1153, 2005.

[52] T. Genovese, S. Cuzzocrea, R. Di Paola, et al., "Effect of rosiglitazone and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 on bleomycin-induced lung injury," *European Respiratory Journal*, vol. 25, no. 2, pp. 225–234, 2005.

[53] M. Ando, Y. Murakami, F. Kojima, et al., "Retrovirally introduced prostaglandin D_2 synthase suppresses lung injury induced by bleomycin," *American Journal of Respiratory Cell and Molecular Biology*, vol. 28, no. 5, pp. 582–591, 2003.

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Review Article

Peroxisome Proliferator Activated Receptor Ligands as Regulators of Airway Inflammation and Remodelling in Chronic Lung Disease

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Inflammation is a major component in the pathology of chronic lung diseases, including asthma. Anti-inflammatory treatment with corticosteroids is not effective in all patients. Thus, new therapeutic options are required to control diverse cellular functions that are currently not optimally targeted by these drugs in order to inhibit inflammation and its sequelae in lung disease. Peroxisome proliferator activated receptors (PPARs), originally characterised as regulators of lipid and glucose metabolism, offer marked potential in this respect. PPARs are expressed in both lung infiltrating and resident immune and inflammatory cells, as well as in resident and structural cells in the lungs, and play critical roles in the regulation of airway inflammation. In vitro, endogenous and synthetic ligands for PPARs regulate expression and release of proinflammatory cytokines and chemoattractants, and cell proliferation and survival. In murine models of allergen-induced inflammation, PPARα and PPARγ ligands reduce the influx of inflammatory cells, cytokine and mucus production, collagen deposition, and airways hyperresponsiveness. The activity profiles of PPAR ligands differ to corticosteroids, supporting the hypothesis that PPARs comprise additional therapeutic targets to mimimise the contribution of inflammation to airway remodelling and dysfunction.

1. INTRODUCTION

Current treatment for chronic lung diseases, including asthma, targets the inflammatory response that is a major contributor to disease pathology. Although inhaled corticosteroids are safe and effective in most patients, a significant proportion of patients with asthma fail to obtain the expected benefits of anti-inflammatory treatment or suffer adverse side effects, and these drugs have not been shown to prevent disease progression. The involvement of diverse cell types and mediators in the inflammatory process provides numerous potential therapeutic options in addition to those targeted by corticosteroids. Novel anti-inflammatory agents with different activity profiles to corticosteroids may minimise persistent inflammation and reduce its contribution to airway remodelling and airways hyperresponsiveness (AHR) in asthma and the loss of pulmonary function in other chronic inflammatory lung diseases.

Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors that have recently been implicated as targets for the regulation of inflammation. PPARs are members of the nuclear hormone receptor family with three isoforms, designated PPARα (NR1C1), PPARβ (PPARδ, NR1C2), and PPARγ (NR1C3). Activation of these receptors has been shown to regulate diverse cellular responses including production of immunomodulatory cytokines, chemotaxis, cell differentiation, proliferation, and survival. This review describes the localisation of these receptors in key cells involved in the pathogenesis of inflammatory diseases in the lung, and presents in vitro and in vivo evidence describing the anti-inflammatory efficacy of PPAR ligands. The identification of complementary or additional actions to those exerted by corticosteroids supports further exploration of the therapeutic potential of PPAR ligands in asthma and chronic lung inflammation.

2. PPARs AND RXRs

The name PPAR derives from the identification of PPARα as the molecular target for the fibrate class of drugs that induce

peroxisome proliferation in rodents, a property not shared by the other PPAR isoforms. PPARα, PPARβ, and PPARγ share a common structure of 4 domains consisting of a variable amino terminal activation function-1 domain (AF-1, A/B), a DNA binding domain (C), a hinge region (D), and a highly conserved activation function-2 domain (AF-2, E/F). The large T-shaped ligand-binding domain within the AF-2 region enables PPARs to bind promiscuously to a plethora of structurally diverse endogenous and synthetic ligands [1]. In addition to ligand binding, AF-2 is important for association with coregulators of receptor activity, and for receptor dimerization and nuclear translocation. Unlike glucocorticoid receptors that form homodimers, PPARs exist as heterodimers with retinoid X receptors (RXR). Like PPARs, there are three distinct isoforms of RXR, namely RXRα, RXRβ, and RXRγ, that are all activated by the endogenous ligand 9-cis retinoic acid [2].

3. PROPOSED MECHANISMS OF GENE REGULATION BY PPAR

The molecular mechanisms of gene regulation by PPARs are complex. Heterodimerization of PPAR with RXR may be affected by competition between PPAR isoforms and with other nuclear receptors that are also RXR partners, such as retinoic acid receptors, vitamin D receptors, and liver X receptors. In the absence of ligand, PPAR-RXR forms a complex with corepressor proteins with histone deacetylase activity, including nuclear receptor corepressor (NCoR) and the silencing mediator for retinoid and thyroid hormone receptors (SMRT) that prevents interaction with intracellular targets. Ligand binding causes corepressor dissociation, and ligand-dependent recruitment of coactivators such as steroid receptor coactivator-1 (SRC-1) and the PPAR binding protein (PBP) [3, 4].

Regulation of gene transcription can occur following nuclear translocation of this activated complex. Transcriptional activation or suppression can occur following recognition of PPAR response elements (PPRE) in promoters of target genes and binding to PPRE consensus sequences comprising AGGTCA hexamers separated by a single nucleotide spacer DR-1 (reviewed in [5]). Alternatively, PPAR can negatively regulate gene expression by antagonizing other signal-dependent transcription factors such as nuclear factor κB (NFκB), CAAT/enhancer binding protein (C/EBP), signal transducers and activators of transcription (STAT) or activator protein 1 (AP-1). This may occur via direct binding to cause transrepression [6] or by sequestering coactivators such as the glucocorticoid receptor interacting protein-1/transcriptional intermediary factor (GRIP-1/TIF) required for activity of other transcription factors [7]. PPARγ ligands may also mediate responses via activation of mitogen-associated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K) pathways [8, 9]. The differential tissue distribution of PPARα, PPARβ, and PPARγ as well as competition between these isoforms and with other nuclear receptors for the accessory proteins that regulate their activity may allow the specific recognition of target genes and other transcription factors to modulate cell function [3].

4. PPAR TISSUE DISTRIBUTION AND LIGANDS

PPARα, PPARβ, and PPARγ are all widely expressed and share some common ligands. However, activation of a specific PPAR can be achieved using selective ligands in tissues where all isoforms are present or by targeting tissues where the isoforms are differentially expressed (Tables 1, 2).

PPARα is highly expressed predominantly in liver, kidney, skeletal muscle, and heart, and has a role in the catabolism of fatty acids. Structurally diverse ligands for PPARα include naturally occurring fatty acids and eicosanoids such as 8S-hydroxyeicosatetranoic acid (8S-HETE) and leukotriene B4 (LTB4). Among the synthetic PPARα ligands described are the fibrate class of drugs used clinically for the treatment of dyslipidaemia such as clofibrate and fenofibrate, and pharmacological tools such as Wy14 643 [2].

The physiological role of PPARβ is less certain due to its ubiquitous expression. Fatty acids also activate PPARβ, with prostacyclin among other potential endogenous ligands for PPARβ. Recently developed synthetic ligands such as GW501516 and L165041 have been used to support a role for PPARβ in regulation of fatty acid oxidation and cell differentiation in skeletal muscle and adipose tissue [2, 4].

PPARγ was originally characterised as a regulator of adipocyte differentiation, but also plays key roles in glucose and lipid metabolism. Activation of PPARγ also occurs in response to a wide variety of potential endogenous ligands as well as synthetic agonists, such as the thiazolidinedione (TZD) class of insulin-sensitising drugs. Naturally occurring PPARγ ligands include polyunsaturated fatty acids (PUFAs), such as linoleic acid, arachidonic acid, and eicosapentanoic acids, and oxidised lipids such as 9- and 13-hydroxyoctadecadienoic acid (HODE), and 12- and 15-HETE [10]. The arachidonic acid metabolite 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2 (15-d-PGJ_2) has been widely used experimentally to define PPARγ-dependent responses, despite additional actions mediated through PPARα activation [11]. In this context, it is important to also note that it is still uncertain whether this prostaglandin D_2 metabolite can be generated in vivo at the micromolar concentrations sufficient to mediate potential PPARγ-dependent effects [12].

The TZD or glitazone class of drugs used in the treatment of type 2 diabetes are believed to exert their insulin-sensitising and hypoglycaemic effects through stimulation of PPARγ [4]. Activation of PPARγ by TZDs results in an alteration in the transcription of several genes involved in glucose and lipid utilisation such as GLUT4 glucose transporter and fatty acid transporter protein [13], and their binding affinity to PPARγ closely parallels their in vivo hypoglycaemic potency [14]. These synthetic ligands include rosiglitazone (RGZ), ciglitazone (CGZ), pioglitazone (PGZ), and troglitazone (TGZ), with RGZ reported to be the most potent [15].

5. PPAR EXPRESSION AND ITS REGULATION IN INFLAMMATORY CELLS AND IN THE LUNG

Recent evidence supports a role for PPARs in the regulation of lung inflammation. Differential expression of PPAR isoforms has been demonstrated in different inflammatory,

Table 1: Natural and synthetic ligands for PPAR isoforms.

Isoform	Natural ligands	Synthetic ligands	Antagonists
PPARα	PGD_2	Fibrates	
	PGI_2	Wy14 643	MK866
	LTB_4	GW9578, GW7647	GW6471
	8S-HETE	NSAIDs	
PPARβ	PGA_1	GW501516, GW0742	Sulindac
	PGI_2	L165041	
PPARγ	PGD_2, PGJ_2, 15-d-PGJ_2	TZDs	BADGE
	9-HODE, 13-HODE	GW262570	GW9662
	12-HETE, 15-HETE	NSAIDs	T0070907
PPARα, γ	—	GW2331	—
		Ragaglitazar	
PPARα, β, γ	Saturated FAs	—	—
	PUFAs		

BADGE: bisphenol A diglycidyl ether; FA: fatty acid; HETE: hydroxyeicosatetranoic acid; HODE: hydroxyoctadecadienoic acid; NSAID: nonsteroidal anti-inflammatory drug; PUFA: polyunsaturated fatty acid; TZD: thiazolidinedione.

resident, and structural cells in the airways (see Table 2). Both PPARα and PPARγ are expressed in macrophages and monocytes [16–18], eosinophils [19, 20], with PPARβ also expressed in neutrophils [21, 22]. Dendritic cells express PPARγ but not PPARα [14, 23], but both PPARα and PPARγ are expressed in B and T lymphocytes [24, 25]. PPARβ and PPARγ but not PPARα are expressed in mast cells [26], while all three isoforms have been detected in A549 and BEAS-2B airway epithelial cell lines [27–30]. Mesenchymal expression of PPARs has been demonstrated with PPARγ detected in primary fibroblasts [31], and with PPARα and PPARγ but not PPARβ in airway smooth muscle [13, 32]. The variable patterns of expression of these PPARs in these diverse cell types suggest that receptor activation of different isoforms may specifically modulate both the production of mediators implicated in inflammation and the cellular responses that contribute to tissue remodelling and the development of AHR.

Emerging evidence suggests that PPAR receptor expression is altered in lung disease, with changes in PPARγ levels being the most extensively studied. PPARγ has been localised in mucosal eosinophils and macrophages, airway epithelium and smooth muscle in human airway biopsies, with increased expression in asthmatic patients compared with controls [13]. In addition, in murine models of allergen-induced inflammation, higher levels of PPARγ were evident in total lung extracts [36, 37] and could be localised to airway epithelium and muscle cells, mast cells, and some inflammatory cells [38].

The stimulus for the increased PPARγ levels detected in intact airways is unclear, as is the functional role of this increase. In vitro, PPARγ is inducible by the inflammatory cytokine interleukin-4 (IL-4) in airway epithelial cells and macrophages [27, 39]. PPARγ expression in monocytes is increased with macrophage differentiation and activation [16, 17] and in sensitised mast cells following antigen exposure [26]. Similar changes in the cellular environment in asthmatic airways may contribute to the elevation in PPARγ levels. The lower levels of PPARγ expression in glucocorticoid-treated asthmatics, compared with untreated asthmatics [13], suggest that increased PPARγ expression may be a product of the inflammatory pathways sensitive to steroid therapy. The current hypothesis is that PPARγ expression is upregulated in response to inflammatory cytokines to provide a negative feedback mechanism, whereby endogenous PPARγ ligands could activate these receptors to limit the cellular inflammatory response in the airways. Increases in PPARα and PPARβ during inflammation have not yet been described, although PPARβ expression in the lung has recently been shown to be increased in the lungs of streptozotocin-induced diabetic rats [40].

However, PPAR upregulation does not appear to be a generalised response to inflammation. PPARα and PPARβ were expressed in peripheral blood lymphocytes, monocytes, and neutrophils healthy subjects and in patients with cystic fibrosis (CF). However, relatively lower levels and activity of PPARα, and not PPARβ, were detected in the lymphocytes from CF patients [22]. The authors speculate that this may be associated with changes in levels of endogenous PPARα ligands in CF, but that treatment with synthetic PPARα ligands may increase receptor expression and activity to minimise the immune response [41]. Although ovalbumin sensitisation has been shown to increase PPARγ expression in the lung [36–38], levels of PPARα were decreased in inflamed lungs of allergen-exposed mice [42]. In addition, a recent study has shown that PPARγ mRNA and protein are downregulated in alveolar macrophages following segmental allergen challenge in asthmatic patients, but not healthy controls [43]. It has been suggested that this downregulation could contribute to ongoing pulmonary inflammation, tissue injury, and loss of function. Alternatively, it could accompany the reduction or resolution of inflammation following activation by PPARγ ligands since increases in PPARγ expression induced in a murine model of asthma by allergen sensitisation were inhibited by administration of the synthetic ligand, CGZ [38].

Table 2: PPAR isoforms in inflammatory cells and lung structural cells.

Cell type	PPARα	PPARβ	PPARγ
Macrophage/monocyte	✓ [16, 22]	✓ [22, 33]	✓ [13, 17, 18, 33]
	× [33]	—	× [22]
Eosinophil	✓ [19]	—	✓ [13, 19, 20]
Neutrophils	✓ [22]	✓ [21, 22]	✓ [21]
	—	—	× [22]
Lymphocytes	✓ [22, 24, 25]	✓ [22]	✓ [24, 25]
	—	—	× [22]
Dendritic cells	× [14, 23]	—	✓ [14, 23]
Mast cells	× [26, 34]	✓ [26]	✓ [26]
Epithelial cells	✓ [28]	✓ [28, 29]	✓ [13, 27, 28, 30]
Lung fibroblasts	—	—	✓ [13, 31]
Airway smooth muscle	✓ [32, 35]	× [32, 35]	✓ [13, 32, 35]

These studies suggest a complex interaction between the initiation and resolution of the inflammatory process and changes in PPAR receptor expression that may be regulated by both inflammatory mediators and the levels of endogenous PPAR ligands. It is important therefore to define receptor-mediated responses at both the cellular level and in integrated animal models of disease to elucidate the role for PPARs in lung inflammation.

Against this translational background, the development of selective ligands for PPAR isoforms has been critical. However, there are marked differences between reported binding affinities and receptor activation potencies for PPAR ligands versus the concentrations required to elicit cellular effects. Multiple approaches are therefore required to support claims for PPAR-dependency of these actions. These include confirming receptor expression in cells of interest and the use of pharmacological antagonists. Several irreversible antagonists for PPARγ have been described including bisphenol A diglycidyl ether (BADGE) [44], and GW9662 [45], although the utility of the former may be compromised by its partial agonist activity [46]. Antagonists for other PPAR isoforms have been described (see Table 1), but have not been utilised in studies outlined in this review.

More recently, molecular techniques have been used to characterise potential PPAR-mediated responses. Adenoviral constructs expressing a dominant negative PPARγ gene that binds to the ligand and the PPRE on DNA but does not initiate transcription have been used in lung fibroblasts [31], while the effects of overexpression of functional PPARγ have been assessed in murine models of asthma [36, 37, 47]. In vivo, transgenic approaches have also characterised the regulatory role of PPARα in inflammation using PPARα-deficient mice [19, 48]. A similar approach for PPARγ is not possible, since complete elimination of this isoform results in embryonic lethality. However, heterozygous knockout mice (PPAR$\gamma^{+/-}$) have been generated [49] and used to implicate PPARγ in mast cell proliferation [50]. More recently, a developmental study using mice with specific ablation of PPARγ in the airway epithelium showed that these conditionally PPAR$^{-/-}$-targeted mice had reduced collagen extracellular matrix (ECM) gene expression in the lung [51]. This suggests that PPARγ has a role in the epithelial-mesenchymal interactions necessary for the establishment of normal lung structure [51]. The implications of this finding on inflammation in lung disease have yet to be explored.

6. PPAR FUNCTION IN INFLAMMATORY CELLS AND IN THE LUNG

There is now extensive evidence that PPAR ligands regulate inflammatory and immune processes mediated by cells in which one or more PPAR isoforms is expressed. Many of these actions are in common with corticosteroids, which have been shown to have inhibitory effects on T cell, eosinophil, neutrophil, mast cell/basophil, and macrophage function [52]. PPAR ligands have also been shown to affect cellular responses of resident and structural cells implicated in inflammation and tissue remodelling in chronic lung diseases. Most of these studies have focussed on PPARγ, and some direct comparisons have been made with corticosteroids [32, 53]. In many cases it remains to be determined whether the effects of PPAR ligands are receptor-mediated, to clarify differences in the activities and mechanisms of action of putative endogenous ligands and synthetic agonists, and to determine their potential therapeutic advantages over corticosteroids.

A characteristic feature of airway inflammation in asthma is the predominance of Th2 lymphocytes and their products, which mediate inflammatory cell recruitment of mast cells, eosinophils, and lymphocytes and subsequent release of mediators from these cells. Both 15-d-PGJ_2 and CGZ are reported to inhibit T cell proliferation [24, 54]. 15-d-PGJ_2 but not CGZ or PPARα agonists induced T cell apoptosis [55]. 15-d-PGJ_2 also decreased production of both Th1 and Th2 type cytokines from T cells [56]. In addition, T cells obtained from sensitised mice treated with CGZ showed decreased IFNγ, IL-4, and IL-2 release when exposed to allergen [56]. However, 15-d-PGJ_2 also caused a potentially proinflammatory induction of IL-8 gene expression in human T cells and macrophages via a MAPK and/or NFκB-dependent signalling pathway [8, 57].

In activated monocytes, the PPARγ ligands 15-d-PGJ_2 and TGZ inhibited production of proinflammatory cytokines tumour necrosis factor α (TNFα), interleukin-1β (IL-1β) and IL-6 [58]. In contrast, natural and synthetic agonists for PPARα were ineffective in these cells [58]. 15-d-PGJ_2 and RGZ also reduced TNFα release and the expression of inducible nitric oxide synthase (iNOS) and matrix metalloproteinase (MMP)-9 in activated macrophages, in part by antagonising the activities of AP-1, STAT, and NFκB [17, 33]. Both PPARα and PPARγ agonists induced macrophage apoptosis, in cells stimulated with TNFα and IFNγ [16].

6.1. Eosinophils

Eosinophils are elevated in the airways of asthmatics and can release inflammatory and cytotoxic mediators, cytokines, and growth factors that contribute to tissue remodelling and AHR. IL-5 and eotaxin-induced chemotaxis were reduced by PPARα- and PPARγ-selective ligands Wy14 643 and RGZ at micromolar concentrations, with the effect of RGZ prevented by the PPARγ antagonist, GW9662 [19]. In contrast to these findings, it has recently been shown that both 15-d-PGJ_2 and TGZ prime eotaxin-induced chemotaxis in the picomolar to low nanomolar concentration range, and that this effect is also prevented by GW9662 [59]. The possibility that endogenous ligands may have proinflammatory effects via PPARγ at physiological concentrations, but that exogenous ligands may be negative immunomodulators at higher concentrations, will require further investigation. This explanation may also resolve discrepancies in other in vitro studies examining regulation of cytokine release and expression that ascribe both pro- and anti-inflammatory properties to PPARγ ligands.

6.2. Mast cells

Mast cell infiltration of airway smooth muscle (ASM) in the airway wall is associated with impaired function in asthma [60]. In response to antigen stimulation, mast cells release stored mediators such as histamine, and produce arachidonic acid derivatives such as prostaglandins and leukotrienes, and cytokines such as TNFα, IL-4, and granulocyte macrophage-colony stimulating factor (GM-CSF). Several roles for PPARs in regulation of mast cell function have been proposed. The PPARβ ligand carbaprostacyclin and PPARγ ligands 15-d-PGJ_2 and TGZ suppressed histamine release and TNFα and GM-CSF production by human basophilic KU812 cells and mast cells [26, 61]. In addition, the increase in cell surface expression of the high affinity IgE receptor FcεRI in response to antigen was reduced by selective PPARβ and PPARγ ligands, namely PGA_1 and 15-d-PGJ_2 [61]. PPARα ligands had no effect on cytokine release or FcεRI expression in these human cells [26, 61]. Although fenofibrate, Wy14 643, and CGZ inhibited antigen-induced leukotriene production from rat basophilic leukemia (RBL)-2H3 cells, these effects are likely to be PPAR-independent since PPARα mRNA was below detection [34] and the inhibition by CGZ was not prevented by a PPARγ antagonist or associated with nuclear translocation of the receptor [62]. However, proliferation of bone-marrow derived murine mast cells was increased by RGZ in an apparently PPARγ-dependent manner, since it was prevented by the PPARγ antagonist GW9662 and the effect of RGZ was reduced in cells from PPAR$\gamma^{+/-}$ mice [50].

Further studies are required to explore the mechanisms of action of these selective PPARβ and PPARγ ligands, and to clarify the role of both receptors in the modulation of mast cell function. Previous in vivo studies suggest that corticosteroids have a minimal effect on mast cell degranulation and the appearance of mast cell mediators after segmental antigen challenge in subjects with asthma [63].

6.3. Epithelial cells

Epithelial remodelling has been documented early in the development of asthma. Airway epithelial cells can contribute to persistent inflammation through synthesis and secretion of enzymes and mediators that regulate matrix turnover and inflammatory cell influx. Potential anti-inflammatory actions for PPARγ ligands have been described in various epithelial cell lines. RGZ and PGZ decreased TNFα- and phorbol myristate acetate (PMA)-induced MMP-9 activity levels in NL20 and BEAS cells, associated with inhibition of NFκB activation [30]. In A549 cells, TZDs reduced expression of iNOS [27] and decreased secretion of Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES) and IL-8 [27, 64]. In contrast to these studies, both RGZ and TGZ potentiated TNFα-induced production of proinflammatory cytokines GM-CSF, IL-6 and IL-8 from A549 cells, independently of PPARγ, NFκB or MAPK activation [65].

There is also a complex relationship between PPARγ and cyclo-oxygenase-2 (COX-2) expression in airway epithelial cells. Both RGZ and CGZ inhibited increases in PMA-induced COX-2 expression by inhibiting AP-1 signalling [66]. However, TGZ increased basal and TNFα-induced COX-2 expression independently of PPARγ and NFκB, but dependent on PI3K and ERK MAPK pathways in A549 cells [9]. In this study, neither 15-d-PGJ_2 nor RGZ (PPARγ ligands), GW262570 (PPARγ/α agonist), nor L-165041 (PPARβ agonist) regulated COX-2 expression. Further investigation of these discrepancies will be required to define the effects of PPARγ ligands on airway epithelial cells and the mechanisms by which these pro- and/or anti-inflammatory responses occur.

6.4. Mesenchymal cells

Fibroblasts play an important role in regulation of ECM deposition that contributes to subepithelial fibrosis layer of the airway, and the development of fixed airway obstruction in asthma [67]. Thickening of the ASM layer is another characteristic feature of airway remodelling in asthma. This has been associated with ASM hypertrophy and/or hyperplasia [68, 69] and increased ECM deposition within the ASM bundle [70]. These cells can respond to both mitogens and inflammatory mediators and contribute to further changes in the airway through the production of ECM proteins, cytokines, and chemokines. The PPARγ ligands RGZ, CGZ,

and 15dPGJ$_2$ inhibited the differentiation of human lung fibroblasts into myofibroblasts and reduce collagen I production following TGFβ stimulation, although the PPARα ligand Wy14 643 was ineffective [31]. These potential antifibrotic effects may be only partly mediated by PPARγ, since they were suppressed by expression of a dominant negative PPARγ construct, but not by GW9662 [31]. It has been reported that corticosteroids did not prevent TGFβ-induced collagen I production by ASM cells from individuals with or without asthma [71], and this suggests that PPARγ agonists may have a therapeutic advantage over corticosteroids in the regulation of lung fibrosis.

In ASM, the PPARγ agonists 15-d-PGJ$_2$ and CGZ suppressed both GM-CSF and G-CSF release [32, 53]. Interestingly, the profile of inflammatory mediator inhibition differed between CGZ and corticosteroids, as dexamethasone inhibited GM-CSF but not G-CSF levels [32, 53]. In a separate study, 15-d-PGJ$_2$ and TGZ were shown to inhibit TNFα-induced production of eotaxin and monocyte chemotactic protein-1 (MCP-1) but not IL-8 secretion from ASM [53]. The inhibitory effects of 15-d-PGJ$_2$ were additive with fluticasone, offering the intriguing possibility that PPARγ agonists in combination with corticosteroids may provide additional therapeutic benefit in asthma [53]. Although PPARγ heterodimerisation with the retinoid X receptor is well characterized, this study also described additional complexity in the mechanisms of action of PPARγ agonists, with direct physical interactions between PPARγ and GR [53]. In addition, PPARγ agonists were shown to mediate anti-inflammatory effects directly via GR activation, with RGZ and CGZ stimulating GR nuclear translocation in a PPARγ deficient cell line [72]. Assessment of these potential interactions between PPARγ and GR by receptor translocation studies may provide additional insights into mechanisms underlying the relative activities of nuclear receptor agonists in ASM.

PPARγ agonists CGZ and 15-d-PGJ$_2$ increased COX-2 expression in ASM, by binding to the PPRE in the COX-2 promoter [35]. Despite the proposed proinflammatory role for this enzyme, the increased PGE$_2$ levels following induction of COX-2 may act in an autocrine manner to reduce subsequent production of GM-CSF, and to inhibit proliferation of ASM [73, 74].

PPARγ ligands may also exert direct antimitogenic actions that could inhibit airway remodelling. Both 15-d-PGJ$_2$ and TZDs inhibited proliferation of human cultured ASM cells [32, 75]. The effects of 15-d-PGJ$_2$ and RGZ were mitogen-independent, as each ligand decreased FGF2- and thrombin-mediated proliferation with similar potency [75]. However, only the effects of RGZ were reversed by the selective PPARγ antagonist GW9662 [75]. RGZ caused inhibition of cell-cycle progression in late G1 phase, without decreasing mitogen-stimulated cyclin D1 protein levels, a mechanism that differs from dexamethasone [76]. Of additional interest, the degree of inhibition of serum-induced ASM proliferation was greater for CGZ than dexamethasone [32]. It is critical to extend these comparisons to cells derived from asthmatics where the ability of corticosteroids to inhibit proliferation is reduced [77] to determine whether PPARγ provides an additional or alternative therapeutic target to glucocorticoid receptors to regulate remodelling in asthma.

PPARα was also expressed in ASM, but surprisingly, Wy14 643 did not regulate inflammatory cytokine production [32, 53], COX-2 expression [35], or proliferation [32, 77] of these cells.

7. PPAR REGULATION OF ALLERGEN-INDUCED INFLAMMATION IN VIVO

On the basis of these in vitro findings in inflammatory and structural cells, the role of PPARs has now been explored in murine models of allergen-induced bronchial inflammation. Although these models do not recapitulate all the pathophysiological changes seen in asthma, sensitisation and repeated aerosol challenges with ovalbumin (OVA) induce airway eosinophilia, changes in airway structure and increases in airways reactivity. Numerous studies have now utilised synthetic PPAR ligands, adenoviral constructs carrying PPAR cDNA (AdPPAR) and transgenics to support immunomodulatory roles for PPARs in the regulation of inflammation, airway wall remodelling, and hyperresponsiveness. Although the majority of studies have focussed on PPARγ, some comparisons have been made with the other PPAR isoforms.

7.1. PPARα

In PPARα-deficient Balb/c mice sensitized and challenged with OVA, there were greater increases in lung inflammation, airway eosinophilia, and antigen-specific serum IgE levels than in wild-type OVA-treated mice [19]. This was associated with relatively higher IL-6, IL-13, and eotaxin levels in the lung, although IL-4, IL-5, and soluble vascular and cell adhesion molecule-1 (sVCAM-1) were not different [19]. Critically, the PPARα-deficient Balb/c mice also displayed a greater response to MCh after OVA-sensitization and aerosol challenge than wild-type mice, providing a functional correlate to the cellular and humoral changes [19]. It was suggested that this PPARα deletion worsened eosinophilia and asthma-like symptoms by preventing the anti-inflammatory actions mediated by the endogenous PPARα ligand, LTB4, known to be produced abundantly by mast cells and other cell types in asthma. A separate study utilising a selective PPARα ligand provides support for this explanation, since GW9578 inhibited allergen-induced influx of eosinophils and lymphocytes [28]. In the same study, the selective PPARβ agonist GW501516 had no effect on inflammatory cell influx [28].

There are conflicting reports on the role of PPARα in regulating airway inflammation induced by lipopolysaccharide (LPS), which is characterised by infiltration of neutrophils and macrophages, increased chemoattractant levels, and elevated MMP activity in BALF. Although GW8578 had no effect on neutrophil influx or increased levels of keratinocyte derived-chemokine (KC) or TNFα following LPS treatment [28], fenofibrate reduced the increase in BALF neutrophils and macrophages as well as levels of TNFα, KC, macrophage inflammatory protein-2 (MIP-2), monocyte chemoattractant protein-1 (MCP-1) and both MMP-2

and MMP-9 activities [78]. Further studies are required to confirm if PPARα activation may also have a beneficial effect in acute or chronic inflammatory airway disorders involving neutrophils and macrophages.

7.2. PPARγ

7.2.1. Inflammation

In Balb/c mice sensitized and challenged with OVA, PPARγ ligands reduced levels of proinflammatory mediators in the bronchoalveolar lavage (BAL) fluid and lung [19, 28, 36, 37, 56] (see Figure 1). Significantly, cytokines associated with Th2-driven humoral responses were decreased by treatment with synthetic PPARγ ligands. Increased levels of IL-4, IL-5, IL-13, and eosinophil cationic protein were reduced by the administration of RGZ or PGZ [36, 37]. In addition, in vitro studies of T cell obtained from sensitised mice treated with CGZ showed decreased IL-4 release when exposed to allergen [56].

Although the antigen sensitisation protocol differed, a common finding in these studies in Balb/c mice has been that PPARγ ligands CGZ, RGZ and PGZ, and administration of AdPPARγ reduced the OVA-induced influx of inflammatory cells, specifically eosinophils [19, 28, 36–38, 47] (see Figure 1). However, in C57BL/6 mice, RGZ treatment had no effect on levels of inflammatory cells in the bronchoalveolar lavage fluid despite inhibiting airways hyperresponsiveness [79]. Whether this is a strain difference in sensitivity to regulation of this measure of airway inflammation, or relates to the ligand and/or route of administration used is yet to be clarified.

7.2.2. Airway remodelling

Airway wall remodelling is characterised by goblet cell metaplasia, collagen deposition and subepithelial fibrosis, and smooth muscle hypertrophy and/or hyperplasia. The role of PPARγ in regulating these changes has been explored. Treatment of Balb/c mice with nebulised CGZ was associated with a reduction in mucous production [38] (see Figure 1). Since orally administered CGZ had no effect [56] and oral RGZ treatment had no impact on goblet cell hyperplasia in C57BL/6 mice [79], the route of administration may be critical to regulate this parameter.

There is now evidence that activation of PPARγ may regulate ECM deposition that occurs in airway wall remodelling. CGZ decreased basement membrane thickness and airways collagen deposition in response to antigen sensitization and challenge, associated with a reduction in TGF-β synthesis [38]. Inhibition of TGF-β signalling has also previously been shown to be inhibited by CGZ in cultured lung fibroblasts [31]. In support of a generalised antifibrotic activity for PPAR ligands, both RGZ and Wy14 643 have been shown to reduce bleomycin-induced pulmonary fibrosis in mice [18, 48, 80].

The antifibrotic effect of CGZ seen in vivo may also be related to regulation of the activity of MMPs or their inhibitors. Although an increase in MMP-2 proteolytic activity in the BALF with OVA treatment was not affected by RGZ [79], it has previously been reported that RGZ inhibits MMP-9 expression in bronchial epithelial cell lines [30].

Further studies are required to assess whether the inhibitory effects of TZDs on ASM proliferation translate to the in vivo setting, where antigen sensitization and challenge leads to inflammation, airway fibrosis, and thickening of the ASM layer. This is critical since the ability of corticosteroids to reduce airway structural changes in murine models is variable [81, 82], and airway remodelling persists despite optimal clinical use of corticosteroids in asthmatic patients [83].

7.2.3. Airways hyperresponsiveness

The impact of regulation of these markers of inflammation and tissue remodelling by PPARγ ligands on AHR has also been explored. Using unrestrained plethysmography, the methacholine (MCh)-induced increase in enhanced pause (Penh) has been used as a measure of AHR following allergen sensitisation and challenge. In Balb/c mice, nebulized CGZ, oral RGZ, or oral PGZ completely prevented the increased response to MCh [19, 36–38, 47]. The effects of the synthetic PPARγ ligands were mimicked by AdPPARγ [36, 37, 47] and abrogated by GW9662 [19, 36–38, 47].

Using invasive measurements of respiratory resistance and compliance, RGZ reduced the increase in airways resistance after OVA challenge in C57BL/6 mice, without affecting the decrease in lung compliance, reflecting an effect on the airways rather than the parenchyma of the lung [79]. This finding provides further support for the proposed role of PPARγ in the regulation of AHR, and is important in light of recent criticism of the use of Penh measurements to draw conclusions about the effects of potential therapeutic agents on AHR [84]. However, since this inhibition by RGZ occurred in the absence of a significant effect on the OVA-induced increase in BAL inflammatory cells, MMP-2 activity, and goblet cell number, it is possible that RGZ may modulate AHR by a mechanism that is independent of inhibition of inflammatory cell recruitment to the airway [79]. Dissociation between inhibition of eosinophilia and reduction in AHR have previously been reported, although the dose of dexamethasone required to inhibit AHR was higher than that needed to inhibit eosinophilia in a murine model of allergic airway inflammation [85].

7.2.4. Potential mechanisms for decreased inflammation and AHR by PPARγ ligands

MCh reactivity was unchanged in saline-challenged mice after oral treatment with RGZ for 7 days [79], suggesting that exposure of ASM to RGZ does not directly inhibit contractile responsiveness. In addition, RGZ did not modulate basal airway tone, or the contraction in response to MCh or histamine in isolated guinea pig tracheal rings [79]. However, CGZ and RGZ have been shown to produce concentration-dependent relaxation smooth muscle of isolated mouse trachea [86]. This effect was not prevented by GW9662, but was inhibited by indomethacin, and suggested that TZDs could act independently of PPARγ to inhibit PGE_2 metabolism by

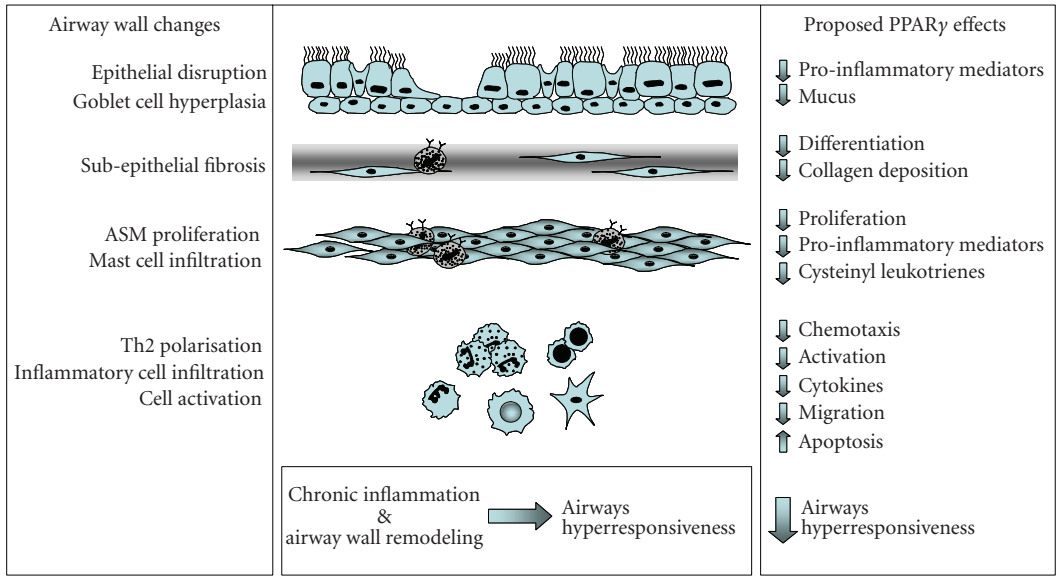

Figure 1: Proposed effects of PPARγ ligands on inflammatory and remodelling changes in the asthmatic airway that contribute to airways hyperresponsiveness.

15-hydroxyprostaglandin dehydrogenase leading to a dilator response [86, 87].

A series of studies have shown that inhibitory effects of TZDs and AdPPARγ on both eosinophilia and AHR were prevented by GW9662 and implicate increases in phosphatase and tensin homologue deleted on chromosome ten (PTEN) and IL-10 in the protective roles of PPARγ-activation [36, 37, 47]. This is in agreement with a separate study, in which RGZ inhibited the migration of antigen-loaded dendritic cells in the mediastinal lymph nodes and increased IL-10 production [88].

8. SUMMARY

On the basis of these in vitro and in vivo findings, substantial evidence has emerged to provide proof-of-concept for the future clinical application of PPARγ ligands to treat airway inflammation. Given their long record of use in type 2 diabetes, TZDs appear to be ideally placed for use in the treatment of chronic lung disease. Like corticosteroids, they appear to have broad anti-inflammatory effects and possess potential anti-remodelling efficacy on multiple cell types in the lung and in animal models of asthma. However, PPARγ agonists including RGZ may offer additional therapeutical advantages to current treatment, if they can be proven to exert control over proinflammatory and proasthmatic pathways that are not susceptible to inhibition by corticosteroids in the clinical setting. Further comparative studies are required to explore these novel complementary or additional actions of PPARγ agonists to those already identified.

The efficacy of TZDs in asthma has not yet been evaluated in human clinical studies, although there has been a case report describing the reduction in asthma symptoms in patients treated with PGZ for diabetes [89]. A limited trial examining the effect of RGZ on lung function in comparison with low dose inhaled corticosteroids in steroid naïve smokers with asthma is currently underway in the United Kingdom to determine whether targeting PPARγ may offer therapeutic benefit in the future treatment of asthma and other inflammatory lung diseases.

ABBREVIATIONS

AHR:	Airways hyperresponsiveness
AP-1:	Activator protein-1
ASM:	Airway smooth muscle
BADGE:	Bisphenol A diglycidyl ether
BALF:	Bronchoalveolar lavage fluid
C/EBP:	CAAT/enhancer binding protein
CF:	Cystic fibrosis
CGZ:	Ciglitazone
COX-2:	Cyclo-oxygenase-2
15-d-PGJ$_2$:	15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$
ECM:	Extracellular matrix
ERK:	Extracellular regulated kinase
FA:	Fatty acid
FGF-2:	Fibroblast growth factor-2
G-CSF:	Granulocyte colony stimulating factor
GM-CSF:	Granulocyte-macrophage colony stimulating factor
GR:	Glucocorticoid receptor
GRIP-1:	Glucocorticoid receptor interacting protein-1
HETE:	Hydroxyeicosatetranoic acid
HODE:	Hydroxyoctadecadienoic acid
IFNγ :	Interferonγ
iNOS:	Inducible nitric oxide synthase
KC:	Keratinocyte-derived chemokine
LPS:	Lipopolysaccharide

LTB4: Leukotriene B4
MAPK: Mitogen-associated protein kinase
MCh: Methacholine
MCP-1: Monocyte chemotactic protein-1
MIP-2: Macrophage inflammatory protein-2
MMP: Matrix metalloprotease
NFκB: Nuclear factor κB
NSAID: Nonsteroidal anti-inflammatory drug
OVA: Ovalbumin
PGZ: Pioglitazone
PI3K: Phosphoinositide-3-kinase
PMA: Phorbol myristate acetate
PPAR: Peroxisome proliferator activated receptor
PPRE: Peroxisome proliferator activated receptor response element
PUFA: Polyunsaturated fatty acid
PTEN: Phosphatase and tensin homologue deleted on chromosome ten
RANTES: Regulated upon activation normal T cell expressed and secreted
RGZ: Rosiglitazone
RXR: Retinoid X receptor
STAT: Signal transducers and activators of transcription
sVCAM-1: Soluble vascular and cell adhesion molecule-1
TGFβ : Transforming growth factorβ
TGZ: Troglitazone
TIF: Transcriptional intermediary factor
TNFα : Tumour necrosis factorα
TZD: Thiazolidinedione

REFERENCES

[1] R. T. Nolte, G. B. Wisely, S. Westin, et al., "Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-γ," *Nature*, vol. 395, no. 6698, pp. 137–143, 1998.

[2] L. A. Moraes, L. Piqueras, and D. Bishop-Bailey, "Peroxisome proliferator-activated receptors and inflammation," *Pharmacology and Therapeutics*, vol. 110, no. 3, pp. 371–385, 2006.

[3] E. S. Tien, D. B. Hannon, J. T. Thompson, and J. P. Vanden Heuvel, "Examination of ligand-dependent coactivator recruitment by peroxisome proliferator-activated receptor-α (PPARα)," *PPAR Research*, vol. 2006, Article ID 69612, 9 pages, 2006.

[4] J. Berger and D. E. Moller, "The mechanisms of action of PPARs," *Annual Review of Medicine*, vol. 53, pp. 409–435, 2002.

[5] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[6] D. S. Straus, G. Pascual, M. Li, et al., "15-deoxy$\Delta^{12,14}$-prostaglandin J_2 inhibits multiple steps in the NF-κB signaling pathway," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 9, pp. 4844–4849, 2000.

[7] P. Gervois, N. Vu-Dac, R. Kleemann, et al., "Negative regulation of human fibrinogen gene expression by peroxisome proliferator-activated receptor α agonists via inhibition of CCAAT box/enhancer-binding protein β," *Journal of Biological Chemistry*, vol. 276, no. 36, pp. 33471–33477, 2001.

[8] S. G. Harris, R. S. Smith, and R. P. Phipps, "15-deoxy-$\Delta^{12,14}$-PGJ_2 induces IL-8 production in human T cells by a mitogen-activated protein kinase pathway," *Journal of Immunology*, vol. 168, no. 3, pp. 1372–1379, 2002.

[9] K. M. Patel, K. L. Wright, P. Whittaker, P. Chakravarty, M. L. Watson, and S. G. Ward, "Differential modulation of COX-2 expression in A549 airway epithelial cells by structurally distinct PPARγ agonists: evidence for disparate functional effects which are independent of NF-κB and PPARγ," *Cellular Signalling*, vol. 17, no. 9, pp. 1098–1110, 2005.

[10] T. M. Willson, P. J. Brown, D. D. Sternbach, and B. R. Henke, "The PPARs: from orphan receptors to drug discovery," *Journal of Medicinal Chemistry*, vol. 43, no. 4, pp. 527–550, 2000.

[11] B. M. Forman, P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans, "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ," *Cell*, vol. 83, no. 5, pp. 803–812, 1995.

[12] L. C. Bell-Parikh, T. Ide, J. A. Lawson, P. McNamara, M. Reilly, and G. A. FitzGerald, "Biosynthesis of 15-deoxy-$\Delta^{12,14}$-PGJ_2 and the ligation of PPARγ," *Journal of Clinical Investigation*, vol. 112, no. 6, pp. 945–955, 2003.

[13] L. Benayoun, S. Letuve, A. Druilhe, et al., "Regulation of peroxisome proliferator-activated receptor γ expression in human asthmatic airways: relationship with proliferation, apoptosis, and airway remodeling," *American Journal of Respiratory and Critical Care Medicine*, vol. 164, no. 8, part 1, pp. 1487–1494, 2001.

[14] C. Faveeuw, S. Fougeray, V. Angeli, et al., "Peroxisome proliferator-activated receptor γ activators inhibit interleukin-12 production in murine dendritic cells," *FEBS Letters*, vol. 486, no. 3, pp. 261–266, 2000.

[15] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[16] G. Chinetti, S. Griglio, M. Antonucci, et al., "Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages," *Journal of Biological Chemistry*, vol. 273, no. 40, pp. 25573–25580, 1998.

[17] M. Ricote, J. Huang, L. Fajas, et al., "Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 13, pp. 7614–7619, 1998.

[18] T. J. Standiford, V. G. Keshamouni, and R. C. Reddy, "Peroxisome proliferator-activated receptor-γ as a regulator of lung inflammation and repair," *Proceedings of the American Thoracic Society*, vol. 2, no. 3, pp. 226–231, 2005.

[19] G. Woerly, K. Honda, M. Loyens, et al., "Peroxisome proliferator-activated receptors α and γ down-regulate allergic inflammation and eosinophil activation," *Journal of Experimental Medicine*, vol. 198, no. 3, pp. 411–421, 2003.

[20] S. Ueki, T. Adachi, J. Bourdeaux, et al., "Expression of PPARγ in eosinophils and its functional role in survival and chemotaxis," *Immunology Letters*, vol. 86, no. 2, pp. 183–189, 2003.

[21] M. E. Greene, B. Blumberg, O. W. McBride, et al., "Isolation of the human peroxisome proliferator activated receptor γ cDNA: expression in hematopoietic cells and chromosomal mapping," *Gene Expression*, vol. 4, no. 4-5, pp. 281–299, 1995.

[22] V. Reynders, S. Loitsch, C. Steinhauer, T. Wagner, D. Steinhilber, and J. Bargon, "Peroxisome proliferator-activated

receptor α (PPARα) down-regulation in cystic fibrosis lymphocytes," *Respiratory Research*, vol. 7, p. 104, 2006.

[23] P. Gosset, A.-S. Charbonnier, P. Delerive, et al., "Peroxisome proliferator-activated receptor γ activators affect the maturation of human monocyte-derived dendritic cells," *European Journal of Immunology*, vol. 31, no. 10, pp. 2857–2865, 2001.

[24] R. B. Clark, D. Bishop-Bailey, T. Estrada-Hernandez, T. Hla, L. Puddington, and S. J. Padula, "The nuclear receptor PPARγ and immunoregulation: PPARγ mediates inhibition of helper T cell responses," *Journal of Immunology*, vol. 164, no. 3, pp. 1364–1371, 2000.

[25] D. C. Jones, X. Ding, and R. A. Daynes, "Nuclear receptor peroxisome proliferator-activated receptor α (PPARα) is expressed in resting murine lymphocytes. The PPARα in T and B lymphocytes is both transactivation and transrepression competent," *Journal of Biological Chemistry*, vol. 277, no. 9, pp. 6838–6845, 2002.

[26] H. Sugiyama, T. Nonaka, T. Kishimoto, K. Komoriya, K. Tsuji, and T. Nakahata, "Peroxisome proliferator-activated receptors are expressed in human cultured mast cells: a possible role of these receptors in negative regulation of mast cell activation," *European Journal of Immunology*, vol. 30, no. 12, pp. 3363–3370, 2000.

[27] A. C. C. Wang, X. Dai, B. Luu, and D. J. Conrad, "Peroxisome proliferator-activated receptor-γ regulates airway epithelial cell activation," *American Journal of Respiratory Cell and Molecular Biology*, vol. 24, no. 6, pp. 688–693, 2001.

[28] A. Trifilieff, A. Bench, M. Hanley, D. Bayley, E. Campbell, and P. Whittaker, "PPAR-α and -γ but not -δ agonists inhibit airway inflammation in a murine model of asthma: in vitro evidence for an NF-κB-independent effect," *British Journal of Pharmacology*, vol. 139, no. 1, pp. 163–171, 2003.

[29] R. Arnold and W. König, "Peroxisome-proliferator-activated receptor-γ agonists inhibit the release of proinflammatory cytokines from RSV-infected epithelial cells," *Virology*, vol. 346, no. 2, pp. 427–439, 2006.

[30] M. Hetzel, D. Walcher, M. Grüb, H. Bach, V. Hombach, and N. Marx, "Inhibition of MMP-9 expression by PPARγ activators in human bronchial epithelial cells," *Thorax*, vol. 58, no. 9, pp. 778–783, 2003.

[31] H. A. Burgess, L. E. Daugherty, T. H. Thatcher, et al., "PPARγ agonists inhibit TGF-β induced pulmonary myofibroblast differentiation and collagen production: implications for therapy of lung fibrosis," *American Journal of Physiology*, vol. 288, no. 6 32-6, pp. L1146–L1153, 2005.

[32] H. J. Patel, M. G. Belvisi, D. Bishop-Bailey, M. H. Yacoub, and J. A. Mitchell, "Activation of peroxisome proliferator-activated receptors in human airway smooth muscle cells has a superior anti-inflammatory profile to corticosteroids: relevance for chronic obstructive pulmonary disease therapy," *Journal of Immunology*, vol. 170, no. 5, pp. 2663–2669, 2003.

[33] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[34] M. Yamashita, "Peroxisome proliferator-activated receptor α-independent effects of peroxisome proliferators on cysteinyl leukotriene production in mast cells," *European Journal of Pharmacology*, vol. 556, no. 1–3, pp. 172–180, 2007.

[35] L. Pang, M. Nie, L. Corbett, and A. J. Knox, "Cyclooxygenase-2 expression by nonsteroidal anti-inflammatory drugs in human airway smooth muscle cells: role of peroxisome proliferator-activated receptors," *Journal of Immunology*, vol. 170, no. 2, pp. 1043–1051, 2003.

[36] S. R. Kim, K. S. Lee, H. S. Park, et al., "Involvement of IL-10 in peroxisome proliferator-activated receptor γ-mediated anti-inflammatory response in asthma," *Molecular Pharmacology*, vol. 68, no. 6, pp. 1568–1575, 2005.

[37] K. S. Lee, S. J. Park, P. H. Hwang, et al., "PPAR-γ modulates allergic inflammation through up-regulation of PTEN," *The FASEB Journal*, vol. 19, no. 8, pp. 1033–1035, 2005.

[38] K. Honda, P. Marquillies, M. Capron, and D. Dombrowicz, "Peroxisome proliferator-activated receptor γ is expressed in airways and inhibits features of airway remodeling in a mouse asthma model," *Journal of Allergy and Clinical Immunology*, vol. 113, no. 5, pp. 882–888, 2004.

[39] J. T. Huang, J. S. Welch, M. Ricote, et al., "Interleukin-4-dependent production of PPAR-γ ligands in macrophages by 12/15-lipoxygenase," *Nature*, vol. 400, no. 6742, pp. 378–382, 1999.

[40] C.-J. Huang, I.-M. Liu, and J.-T. Cheng, "Increase of peroxisome proliferator-activated receptor δ gene expression in the lungs of streptozotocin-induced diabetic rats," *Pulmonary Pharmacology & Therapeutics*, vol. 20, no. 1, pp. 69–74, 2007.

[41] I. Inoue, K. Shino, S. Noji, T. Awata, and S. Katayama, "Expression of peroxisome proliferator-activated receptor α (PPARα) in primary cultures of human vascular endothelial cells," *Biochemical and Biophysical Research Communications*, vol. 246, no. 2, pp. 370–374, 1998.

[42] J. Becker, C. Delayre-Orthez, N. Frossard, and F. Pons, "Regulation of inflammation by PPARs: a future approach to treat lung inflammatory diseases?" *Fundamental and Clinical Pharmacology*, vol. 20, no. 5, pp. 429–447, 2006.

[43] M. Kobayashi, M. J. Thomassen, T. Rambasek, et al., "An inverse relationship between peroxisome proliferator-activated receptor γ and allergic airway inflammation in an allergen challenge model," *Annals of Allergy, Asthma & Immunology*, vol. 95, no. 5, pp. 468–473, 2005.

[44] H. M. Wright, C. B. Clish, T. Mikami, et al., "A synthetic antagonist for the peroxisome proliferator-activated receptor γ inhibits adipocyte differentiation," *Journal of Biological Chemistry*, vol. 275, no. 3, pp. 1873–1877, 2000.

[45] L. M. Leesnitzer, D. J. Parks, R. K. Bledsoe, et al., "Functional consequences of cysteine modification in the ligand binding sites of peroxisome proliferator activated receptors by GW9662," *Biochemistry*, vol. 41, no. 21, pp. 6640–6650, 2002.

[46] D. Bishop-Bailey, T. Hla, and T. D. Warner, "Bisphenol A diglycidyl ether (BADGE) is a PPARγ agonist in an ECV304 cell line," *British Journal of Pharmacology*, vol. 131, no. 4, pp. 651–654, 2000.

[47] K. S. Lee, S. R. Kim, S. J. Park, et al., "Peroxisome proliferator activated receptor-γ modulates reactive oxygen species generation and activation of nuclear factor-κB and hypoxia-inducible factor 1α in allergic airway disease of mice," *Journal of Allergy and Clinical Immunology*, vol. 118, no. 1, pp. 120–127, 2006.

[48] T. Genovese, E. Mazzon, R. Di Paola, et al., "Role of endogenous and exogenous ligands for the peroxisome proliferator-activated receptor α in the development of bleomycin-induced lung injury," *Shock*, vol. 24, no. 6, pp. 547–555, 2005.

[49] P. D. G. Miles, Y. Barak, W. He, R. M. Evans, and J. M. Olefsky, "Improved insulin-sensitivity in mice heterozygous for PPAR-γ deficiency," *Journal of Clinical Investigation*, vol. 105, no. 3, pp. 287–292, 2000.

[50] K. Maeyama, M. Emi, and M. Tachibana, "Nuclear receptors as targets for drug development: peroxisome proliferator-activated receptor γ in mast cells: its roles in proliferation and

differentiation," *Journal of Pharmacological Sciences*, vol. 97, no. 2, pp. 190–194, 2005.

[51] D. M. Simon, M. C. Arikan, S. Srisuma, et al., "Epithelial cell PPARγ contributes to normal lung maturation," *The FASEB Journal*, vol. 20, no. 9, pp. 1507–1509, 2006.

[52] M. G. Belvisi, "Regulation of inflammatory cell function by corticosteroids," *Proceedings of the American Thoracic Society*, vol. 1, no. 3, pp. 207–214, 2004.

[53] M. Nie, L. Corbett, A. J. Knox, and L. Pang, "Differential regulation of chemokine expression by peroxisome proliferator-activated receptor γ agonists: interactions with glucocorticoids and β_2-agonists," *Journal of Biological Chemistry*, vol. 280, no. 4, pp. 2550–2561, 2005.

[54] R. Cunard, M. Ricote, D. DiCampli, et al., "Regulation of cytokine expression by ligands of peroxisome proliferator activated receptors," *Journal of Immunology*, vol. 168, no. 6, pp. 2795–2802, 2002.

[55] S. G. Harris and R. P. Phipps, "Prostaglandin D_2, its metabolite 15-d-PGJ_2, and peroxisome proliferator activated receptor-γ agonists induce apoptosis in transformed, but not normal, human T lineage cells," *Immunology*, vol. 105, no. 1, pp. 23–34, 2002.

[56] C. Mueller, V. Weaver, J. P. Vanden Heuvel, A. August, and M. T. Cantorna, "Peroxisome proliferator-activated receptor γ ligands attenuate immunological symptoms of experimental allergic asthma," *Archives of Biochemistry and Biophysics*, vol. 418, no. 2, pp. 186–196, 2003.

[57] Y. Fu, N. Luo, and M. F. Lopes-Virella, "Upregulation of interleukin-8 expression by prostaglandin D_2 metabolite 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (15d-PGJ_2) in human THP-1 macrophages," *Atherosclerosis*, vol. 160, no. 1, pp. 11–20, 2002.

[58] C. Jiang, A. T. Ting, and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

[59] Y. Kobayashi, S. Ueki, G. Mahemuti, et al., "Physiological levels of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 prime eotaxin-induced chemotaxis on human eosinophils through peroxisome proliferator-activated receptor-γ ligation," *Journal of Immunology*, vol. 175, no. 9, pp. 5744–5750, 2005.

[60] C. E. Brightling, P. Bradding, F. A. Symon, S. T. Holgate, A. J. Wardlaw, and I. D. Pavord, "Mast-cell infiltration of airway smooth muscle in asthma," *New England Journal of Medicine*, vol. 346, no. 22, pp. 1699–1705, 2002.

[61] Y. Fujimura, H. Tachibana, and K. Yamada, "Peroxisome proliferator-activated receptor ligands negatively regulate the expression of the high-affinity IgE receptor FcϵRI in human basophilic KU812 cells," *Biochemical and Biophysical Research Communications*, vol. 297, no. 2, pp. 193–201, 2002.

[62] K. Okuyama, M. Yamashita, Y. Kitabatake, S. Kawamura, M. Takayanagi, and I. Ohno, "Ciglitazone inhibits the antigen-induced leukotrienes production independently of PPARγ in RBL-2H3 mast cells," *European Journal of Pharmacology*, vol. 521, no. 1–3, pp. 21–28, 2005.

[63] M. C. Liu, D. Proud, L. M. Lichtenstein, et al., "Effects of prednisone on the cellular responses and release of cytokines and mediators after segmental allergen challenge of asthmatic subjects," *Journal of Allergy and Clinical Immunology*, vol. 108, no. 1, pp. 29–38, 2001.

[64] A. Momoi, K. Murao, H. Imachi, et al., "Thiazolidinedione inhibits production of RANTES in a cytokine-treated human lung epithelial cell line," *FEBS Letters*, vol. 452, no. 3, pp. 301–304, 1999.

[65] C. Desmet, B. Warzée, P. Gosset, et al., "Pro-inflammatory properties for thiazolidinediones," *Biochemical Pharmacology*, vol. 69, no. 2, pp. 255–265, 2005.

[66] K. Subbaramaiah, D. T. Lin, J. C. Hart, and A. J. Dannenberg, "Peroxisome proliferator-activated receptor γ ligands suppress the transcriptional activation of cyclooxygenase-2. Evidence for involvement of activator protein-1 and CREB-binding protein/p300," *Journal of Biological Chemistry*, vol. 276, no. 15, pp. 12440–12448, 2001.

[67] J. Bousquet, P. Chanez, J. Y. Lacoste, et al., "Indirect evidence of bronchial inflammation assessed by titration of inflammatory mediators in BAL fluid of patients with asthma," *Journal of Allergy and Clinical Immunology*, vol. 88, no. 4, pp. 649–660, 1991.

[68] M. Ebina, T. Takahashi, T. Chiba, and M. Motomiya, "Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma. A 3-D morphometric study," *American Review of Respiratory Disease*, vol. 148, no. 3, pp. 720–726, 1993.

[69] P. G. Woodruff, G. M. Dolganov, R. E. Ferrando, et al., "Hyperplasia of smooth muscle in mild to moderate asthma without changes in cell size or gene expression," *American Journal of Respiratory and Critical Care Medicine*, vol. 169, no. 9, pp. 1001–1006, 2004.

[70] R. J. Thomson, A. M. Bramley, and R. R. Schellenberg, "Airway muscle stereology: implications for increased shortening in asthma," *American Journal of Respiratory and Critical Care Medicine*, vol. 154, no. 3, part 1, pp. 749–757, 1996.

[71] J. K. Burgess, B. G. G. Oliver, M. H. Poniris, et al., "A phosphodiesterase 4 inhibitor inhibits matrix protein deposition in airways in vitro," *Journal of Allergy and Clinical Immunology*, vol. 118, no. 3, pp. 649–657, 2006.

[72] A. Ialenti, G. Grassia, P. Di Meglio, P. Maffia, M. Di Rosa, and A. Ianaro, "Mechanism of the anti-inflammatory effect of thiazolidinediones: relationship with the glucocorticoid pathway," *Molecular Pharmacology*, vol. 67, no. 5, pp. 1620–1628, 2005.

[73] M. A. Saunders, J. A. Mitchell, P. M. Seldon, et al., "Release of granulocyte-macrophage colony stimulating factor by human cultured airway smooth muscle cells: suppression by dexamethasone," *British Journal of Pharmacology*, vol. 120, no. 4, pp. 545–546, 1997.

[74] M. G. Belvisi, M. Saunders, M. Yacoub, and J. A. Mitchell, "Expression of cyclo-oxygenase-2 in human airway smooth muscle is associated with profound reductions in cell growth," *British Journal of Pharmacology*, vol. 125, no. 5, pp. 1102–1108, 1998.

[75] J. E. Ward, H. Gould, T. Harris, J. V. Bonacci, and A. G. Stewart, "PPARγ ligands, 15-deoxy-$\Delta^{2,14}$-prostaglandin J_2 and rosiglitazone regulate human cultured airway smooth muscle proliferation through different mechanisms," *British Journal of Pharmacology*, vol. 141, no. 3, pp. 517–525, 2004.

[76] D. Fernandes, E. Guida, V. Koutsoubos, et al., "Glucocorticoids inhibit proliferation, cyclin D1 expression, and retinoblastoma protein phosphorylation, but not activity of the extracellular-regulated kinases in human cultured airway smooth muscle," *American Journal of Respiratory Cell and Molecular Biology*, vol. 21, no. 1, pp. 77–88, 1999.

[77] P. R. A. Johnson, J. K. Burgess, P. A. Underwood, et al., "Extracellular matrix proteins modulate asthmatic airway smooth muscle cell proliferation via an autocrine mechanism," *Journal of Allergy and Clinical Immunology*, vol. 113, no. 4, pp. 690–696, 2004.

[78] C. Delayre-Orthez, J. Becker, I. Guenon, et al., "PPARα down-regulates airway inflammation induced by lipopolysaccharide in the mouse," *Respiratory Research*, vol. 6, p. 91, 2005.

[79] J. E. Ward, D. J. Fernandes, C. C. Taylor, J. V. Bonacci, L. Quan, and A. G. Stewart, "The PPARγ ligand, rosiglitazone, reduces airways hyperresponsiveness in a murine model of allergen-induced inflammation," *Pulmonary Pharmacology & Therapeutics*, vol. 19, no. 1, pp. 39–46, 2006.

[80] T. Genovese, S. Cuzzocrea, R. Di Paola, et al., "Effect of rosiglitazone and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 on bleomycin-induced lung injury," *European Respiratory Journal*, vol. 25, no. 2, pp. 225–234, 2005.

[81] A. Trifilieff, A. El-Hashim, and C. Bertrand, "Time course of inflammatory and remodeling events in a murine model of asthma: effect of steroid treatment," *American Journal of Physiology*, vol. 279, no. 6, pp. L1120–L1128, 2000.

[82] S. Y. Lee, J. S. Kim, J. M. Lee, et al., "Inhaled corticosteroid prevents the thickening of airway smooth muscle in murine model of chronic asthma," to appear in *Pulmonary Pharmacology & Therapeutics*.

[83] C. Ward and H. Walters, "Airway wall remodelling: the influence of corticosteroids," *Current Opinion in Allergy and Clinical Immunology*, vol. 5, no. 1, pp. 43–48, 2005.

[84] J. Bates, C. Irvin, V. Brusasco, et al., "The use and misuse of Penh in animal models of lung disease," *American Journal of Respiratory Cell and Molecular Biology*, vol. 31, no. 3, pp. 373–374, 2004.

[85] M. A. Birrell, C. H. Battram, P. Woodman, K. McCluskie, and M. G. Belvisi, "Dissociation by steroids of eosinophilic inflammation from airway hyperresponsiveness in murine airways," *Respiratory Research*, vol. 4, p. 3, 2003.

[86] P. J. Henry, A. D'Aprile, G. Self, T. Hong, and T. S. Mann, "Inhibitors of prostaglandin transport and metabolism augment protease-activated receptor-2-mediated increases in prostaglandin E_2 levels and smooth muscle relaxation in mouse isolated trachea," *Journal of Pharmacology and Experimental Therapeutics*, vol. 314, no. 3, pp. 995–1001, 2005.

[87] H. Cho and H.-H. Tai, "Thiazolidinediones as a novel class of NAD^+-dependent 15-hydroxyprostaglandin dehydrogenase inhibitors," *Archives of Biochemistry and Biophysics*, vol. 405, no. 2, pp. 247–251, 2002.

[88] H. Hammad, H. J. de Heer, T. Soullié, et al., "Activation of peroxisome proliferator-activated receptor-γ in dendritic cells inhibits the development of eosinophilic airway inflammation in a mouse model of asthma," *American Journal of Pathology*, vol. 164, no. 1, pp. 263–271, 2004.

[89] Y. Hashimoto and K. Nakahara, "Improvement of asthma after administration of pioglitazone," *Diabetes Care*, vol. 25, no. 2, p. 401, 2002.

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Review Article

The Role of PPARs in Lung Fibrosis

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Pulmonary fibrosis is a group of disorders characterized by accumulation of scar tissue in the lung interstitium, resulting in loss of alveolar function, destruction of normal lung architecture, and respiratory distress. Some types of fibrosis respond to corticosteroids, but for many there are no effective treatments. Prognosis varies but can be poor. For example, patients with idiopathic pulmonary fibrosis (IPF) have a median survival of only 2.9 years. Prognosis may be better in patients with some other types of pulmonary fibrosis, and there is variability in survival even among individuals with biopsy-proven IPF. Evidence is accumulating that the peroxisome proliferator-activated receptors (PPARs) play important roles in regulating processes related to fibrogenesis, including cellular differentiation, inflammation, and wound healing. PPARα agonists, including the hypolidipemic fibrate drugs, inhibit the production of collagen by hepatic stellate cells and inhibit liver, kidney, and cardiac fibrosis in animal models. In the mouse model of lung fibrosis induced by bleomycin, a PPARα agonist significantly inhibited the fibrotic response, while PPARα knockout mice developed more serious fibrosis. PPARβ/δ appears to play a critical role in regulating the transition from inflammation to wound healing. PPARβ/δ agonists inhibit lung fibroblast proliferation and enhance the antifibrotic properties of PPARγ agonists. PPARγ ligands oppose the profibrotic effect of TGF-β, which induces differentiation of fibroblasts to myofibroblasts, a critical effector cell in fibrosis. PPARγ ligands, including the thiazolidinedione class of antidiabetic drugs, effectively inhibit lung fibrosis in vitro and in animal models. The clinical availability of potent and selective PPARα and PPARγ agonists should facilitate rapid development of successful treatment strategies based on current and ongoing research.

1. INTRODUCTION

Pulmonary fibrosis is a potentially fatal disease characterized by accumulation of scar tissue in the lung interstitium, resulting in loss of alveolar function, destruction of normal lung architecture, and respiratory distress [1–3]. Known causes include inhalation of dusts and other particulates such as silica and asbestos, chemo- and radiation therapy, autoimmunity, hypersensitivity pneumonitis, and sarcoidosis [4, 5]. The idiopathic interstitial pneumonias, as the name suggests, are a group of fibrotic diseases of unknown etiology, the commonest of which is the usual intersitial pneumonitis (UIP), also called idiopathic pulmonary fibrosis (IPF) [6–8]. Some types of fibrosis respond to corticosteroids but many are refractory [9–11]. Prognosis is varied, but can be poor. UIP is considered to be the most severe of the idiopathic interstitial pneumonias. However, there is significant variability in the natural history of this disease. For example, the mean survival time after a diagnosis of UIP is less than three years [12], but there are patients who can survive for much longer periods of time with much slower (or rarely no) progression of their lung disease [13]. In contrast, other patients can develop acute exacerbations of their pulmonary fibrosis with the rapid onset of dyspnea, new radiographic abnormalities, respiratory failure, and death in 20%–86% of patients. Histological examination of their lungs reveals diffuse alveolar damage superimposed on a background of UIP [12]. The etiology of these exacerbations is unclear, but factors including infection have been implicated.

At the cellular level, pulmonary fibrosis is characterized by proliferation and accumulation of fibroblasts and scar-forming myofibroblasts in the lung interstitium with increased synthesis and deposition of extracellular matrix proteins including collagen and fibronectin [9, 14]. Although

fibroblasts were previously regarded as simple structural cells, they are now recognized as having important sentinel and regulatory functions and are a rich source of regulatory cytokines and chemokines [15]. Fibroblasts differentiate to myofibroblasts after appropriate stimuli, including transforming growth factor (TGF)-β1 [9, 14, 16]. Myofibroblasts have some of the characteristics of smooth muscle cells, including contractility and expression of α-smooth muscle actin (α-SMA) [14, 17, 18]. The differentiation of fibroblasts to myofibroblasts, along with increased cellular proliferation and matrix deposition, leads to the development of fibroblastic foci similar in appearance to the early stages of normal wound healing. Fibrosis is usually progressive, leading to destruction of the normal lung architecture [2, 14, 17, 18]. Other organs can develop fibrosis, including the skin, liver, kidney, and pancreas, and the cellular events and signals are likely to be similar.

It has been hypothesized that fibrosis is a consequence of abnormal regulation of wound repair [2, 19, 20]. An injury leads to acute inflammation, followed by an initial repair phase in which fibroblasts and myofibroblasts at the injury site replace damaged tissue with scar tissue. Normally, this phase of wound repair is self-limiting, with myofibroblasts eventually undergoing apoptosis, and the scar tissue may be remodeled and reconstructed as relatively normal functional tissue. In fibrosis, the fibroblasts and myofibroblasts do not undergo apoptosis, but continue to proliferate, resulting in progressive scarring. The cellular signals involved in the maintenance of the profibrotic phenotype are unknown, although it is likely that TGF-β is a critical factor [21–24].

2. PPARs AND LUNG DISEASE

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor family, that function to regulate a wide range of physiological activities [25]. Three different isoforms of PPARs have been identified: PPARα (NR1C1), PPARβ/δ (NUC1; NR1C2), and PPARγ (NR1C3), encoded by three separate genes. The PPARs and their obligate coreceptors, the retinoid X receptors (RXRs), bind a variety of ligands. The ligand-activated heterodimeric complexes then induce expression of target genes carrying peroxisome proliferators response elements (PPREs) in their promoters. PPARα was first identified as the mediator of the response to peroxisome proliferators in rodents [26]. Over the past decade, PPARs have been implicated as important regulators of various physiological processes, such as lipid and lipoprotein metabolism, glucose homeostasis, cellular proliferation, differentiation, and apoptosis. PPARα is found in high levels in liver, kidney, heart, and muscle, whereas PPARβ/δ is ubiquitously expressed [26, 27]. PPARγ is found in two main isoforms, PPARγ1 and PPARγ2, derived from different premRNA splice variants that use different transcription start sites. PPARγ is widely expressed, and has been found in blood cells, such as macrophages [28], T and B lymphocytes [29, 30], and platelets [31], as well as in tissues including adipose, colon, spleen, retina, skeletal muscle, liver, bone marrow, and lung [27]. Within the lung, PPARγ is expressed by the epithelium, smooth muscle cells, fibroblasts, endothelium, macrophages, eosinophils, and dendritic cells [32].

The role of the PPARs in lung disease is not yet clear. Both PPARα and PPARγ have been localized in lung tissue, including bronchial epithelial cells, alveolar walls, and alveolar macrophages [27, 32, 33]. A comparison of nonsmokers, smokers with chronic obstructive pulmonary disease (COPD), and smokers without COPD found no statistically significant difference in the number of PPARγ-positive macrophages, but found an increased number of PPARα-positive alveolar macrophages in smokers with COPD [34]. Sarcoidosis and pulmonary alveolar proteinosis are two other disorders in which alveolar macrophages are deficient in PPARγ [35].A causal relationship has not been determined, however, treatment of pulmonary alveolar proteinosis with granulocyte-macrophage colony-stimulating factor (GM-CSF) restores alveolar macrophage PPARγ levels [36].

There is evidence that the PPARs, particularly PPARα and PPARγ, play a role in regulating inflammation. For example, fatty-acid-derived inflammatory mediators, including prostaglandins and leukotrienes, are ligands for PPARα and γ [37]. Although the pathogenesis of fibrosis appears to be distinct from inflammation, and many forms of fibrosis are refractory to anti-inflammatory therapies such as corticosteroids, recent work has supported the hypothesis that fibrosis is a consequence of a dysregulated wound healing process with an initial injury and inflammatory response. Certainly, many important inflammatory signals and mediators, particularly TGF-β, TNF-α, and IL-1β, and prostaglandins, play key roles in fibrosis [21–24]. This review will discuss recent reports examining the link between PPARs and fibrosis, and the possibility of using PPAR ligands as antifibrotic therapies. Because the study of PPARs in lung fibrosis is relatively new, we will also review selected results from fibrotic disease models in other organs.

3. PPARα

PPARα was originally cloned as the molecular target for the hypolipidemic fibrate drugs, although arachidonic acid metabolites (eicosanoids, prostaglandins, and leukotrienes) are also important ligands [38]. PPARα plays a key role in lipid metabolism and is highly expressed in tissues involved in lipid and cholesterol metabolism, including the liver, kidney, and macrophages. PPARα ligands have important anti-inflammatory properties, although some studies have reported proinflammatory effects as well [37, 39]. Little is known about PPARα in lung disease, although other fibrosis models implicate PPARα in regulating fibrosis.

In the liver, the PPARα agonists fenofibrate and WY14643 dramatically reduced fibrosis in the thioacetamide model of cirrhosis [40]. N-3 polyunsaturated fatty acid, another PPARα ligand, reduced hepatic and serum TNF-α levels and reduced the degree of liver injury in a rat model of nonalcoholic steatohepatitis [41]. The synthetic PPARα agonist

WY14643 reduced the severity of steatohepatitis in C57BL/6 mice fed a methionine- and choline-deficient diet, with reductions in hepatic mRNA levels of collagen alpha 1, tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2, and matrix metalloproteinase (MMP)-13 [42].

Fenofibrate also attenuated cardiac and vascular fibrosis in pressure-overloaded rat hearts, with reductions in collagen I and III mRNA [43], and inhibited fibrotic left ventricular remodeling in mineralcorticoid-dependent hypertension [44]. The PPARα agonist gemfibrozil attenuated glomerulosclerosis and collagen deposition in diabetic ApoE-knockout mice [45].

Recent reports have found significantly reduced PPARα mRNA levels in lymphocytes from cystic fibrosis patients [46], while PPARα knockout mice develop more severe carageenan-induced pleural inflammation [47], suggesting a connection between diminished PPARα-dependent gene activation and disease pathology.

The role of PPARα in lung fibrosis was investigated in mice using the bleomycin model of lung injury and fibrosis. Intratracheal instillation of the antineoplastic agent bleomycin causes acute lung inflammation that develops into severe fibrosis, with proliferation of α-SMA-positive myofibroblasts, increased collagen deposition, and loss of normal alveolar architecture [48, 49]. PPARα-knockout mice treated with bleomycin developed more severe inflammation and fibrosis than wild-type mice, with increased immunohistochemical detection of TNF-α and IL-1β, increased apoptosis of interstitial cells, and decreased survival [50]. Treatment of wild-type mice with the PPARα agonist WY-14643 enhanced survival and reduced the severity of fibrosis, as well as reducing the detection of TNF-α and apoptosis by immunohistochemistry. The authors concluded that endogenous PPARα ligands play an important role in limiting the fibrotic response in wild-type mice, and that treatment with PPARα ligands has potential as an antifibrotic therapy.

As yet, there have been no molecular mechanisms proposed to explain these results. Since bleomycin treatment results in an acute inflammatory response that later resolves into fibrosis, it is possible that PPARα agonists act to inhibit fibrosis by moderating the initial inflammatory response. This could be addressed by using a fibrogenic insult that provokes minimal inflammation, such as adenovirus-mediated overexpression of TGF-β [24].

Interestingly, there is some evidence that the effects of PPARα agonists are not entirely dependent on PPARα-dependent transcription [51]. Since the above study did not report treating PPARα-knockout mice with WY-14643, the issue of the PPARα dependence or independence of the effect was not addressed. It should also be noted that WY-14643 is also a weak PPARγ agonist [52], and PPARγ agonists may have antifibrotic activity as well (discussed below). One way to investigate the PPARα dependence or independence of PPARα agonists would be to study their effects in PPARα-knockout fibroblasts in vitro and PPARα-knockout mice in vivo. Studies using additional in vivo models of fibrosis (such as thoracic radiation or inhalation of crystalline silica) should also prove informative.

4. PPARβ/δ

Although little is known about PPARβ/δ in the lung, PPARβ/δ does play a critical role in wound healing in the skin. PPARβ/δ expression is upregulated following skin injury. Further, PPARβ/δ-knockout mice exhibit defective in vivo wound healing, and keratinocytes from PPARβ/δ-knockout mice show decreased adhesion and migration in vitro [53]. It has been suggested that PPARβ/δ is a critical regulator of the transition from the initial inflammatory response to the later wound healing program [54].

An intriguing recent study suggested that PPARβ/δ may be a target of prostacyclin mimetics used in treating pulmonary hypertension. Treprostinil sodium activated a PPARβ/δ reporter gene and inhibited proliferation of lung fibroblasts in vitro. The effect was not seen in lung fibroblasts from PPARβ/δ-knockout mice, demonstrating that the effect was dependent on PPARβ/δ and not on the prostacyclin receptor [55]. Finally, PPARβ/δ agonists enhance the efficacy of PPARγ agonists in mediating adipocyte differentiation in vitro [56], suggesting that PPARβ/δ agonists may also potentiate the antifibrotic effects of PPARγ agonists discussed below.

5. PPARγ

PPARγ is expressed in many types of lung cells including fibroblasts, ciliated airway epithelial cells and alveolar type II pneumocytes, alveolar macrophages, T lymphocytes, and airway smooth muscle cells [57]. Endogenous ligands of PPARγ include 15-deoxy $-\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) [58, 59], lysophosphatidic acid [60], and nitrolinoleic acid [61]. PPARγ can also be activated by synthetic ligands including the thiazolidinedione (TZD) class of clinically used insulin-sensitizing drugs [62] including rosiglitizone and pioglitizone, as well as oleanic acid derivatives known as triterpenoids [63].

The anti-inflammatory properties of PPARγ ligands have been well described [37, 64]. In the lung, PPARγ ligands inhibit LPS-induced neutrophilia [65, 66] and allergic airway inflammation and hyperresponsiveness in a mouse model of asthma [67, 68]. PPARγ ligands also inhibit the release of proinflammatory mediators from airway epithelial cells and alveolar macrophages [69, 70]. In addition, PPARγ plays an important role in regulating cellular differentiation, as PPARγ ligands promote differentiation of preadipocyte fibroblasts to adipocytes [58, 59, 71].

A number of studies have investigated PPARγ ligands as potential antifibrotic agents in vivo. Pioglitazone reduced carbon-tetrachloride-induced hepatic fibrosis in rats, with decreases in hydroxyl proline content, procollagen I mRNA, and α-SMA-positive hepatic stellate cells [72]. A similar effect was observed when fibrosis was induced by a choline-deficient diet [73, 74]. Rosiglitazone inhibits cardiac fibrosis in rats [44] and kidney fibrosis in diabetic mice and rats [45]. Intriguingly, improvements in renal function have been noted in patients with type II diabetes who are treated with TZDs [75, 76].

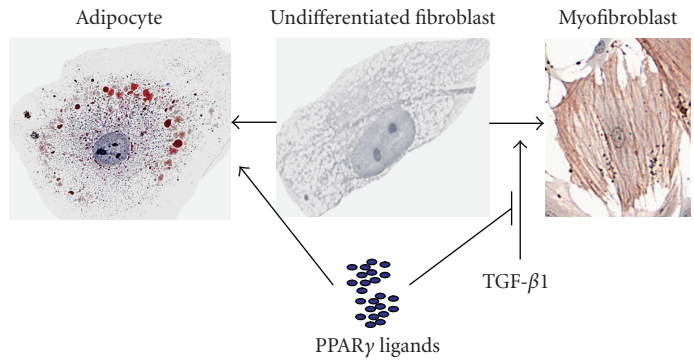

Figure 1: *PPARγ ligands promote fibroblast differentiation to adipocytes and inhibit differentiation to myofibroblasts*. Primary human fibroblasts (center panel) can be differentiated to adipocyte-like cells (left panel) by treatment with 1 μM 15d-PGJ_2 for 8 days. Lipid droplets were visualized with oil red O staining. Alternatively, incubation with 10 ng/mL TGF-β for 3 days will differentiate fibroblasts to myofibroblasts (right panel). α-SMA was detected by immunocytochemistry. Note the long bundles of contractile fibers.

Only a limited amount of data is available on the effects of PPARγ agonists on lung fibrosis in vivo. Ciglitazone administered by nebulization in a mouse model of asthma not only reduced lung inflammation and eosinophilia, but also reduced basement membrane thickening and collagen deposition associated with airway remodeling, as well as synthesis of the profibrotic cytokine TGF-β [68]. This effect was abolished by concomitant use of GW9662, an irreversible PPARγ antagonist. Rosiglitazone and 15d-PGJ_2 significantly reduced mortality, inflammation, cellular infiltrates, and histological fibrosis following intratracheal administration of bleomycin [77]. Studies of the in vivo effects of PPARγ agonists have been hampered by the fact that unlike PPARα, homozygous germline deletion of the PPARγ gene results in embryonic lethality [78]. A conditional knockout mouse, in which exon 2 of the PPARγ gene has been flanked by *loxP* sites, has been developed [78], and strategies to inducibly knock out PPARγ expression in the adult mouse lung prior to fibrotic insult are being explored in a number of laboratories.

The antifibrotic effects of PPARγ ligands have been studied in vitro, leading to new insights into their mechanism of action. As previously discussed, TGF-β drives differentiation of lung fibroblasts to myofibroblasts, a key effector cell in fibrosis [16, 23, 24]. In contrast, PPARγ ligands differentiate fibroblasts to fat-storing adipocytes [58, 59]. This suggests that PPARγ ligands may oppose the fibrogenic effects of TGF-β (Figure 1). We investigated the ability of PPARγ ligands to counter the profibrotic effects of TGF-β on primary human lung fibroblasts. Rosiglitazone and 15d-PGJ_2 efficiently inhibited TGF-β-driven differentiation of human lung fibroblasts to myofibroblasts, with reductions in the expression of α-SMA (a myofibroblast marker) and production of collagen [79].

Similar results have been observed in other cell types. Differentiation of hepatic stellate cells to a myofibroblast phenotype is a key step in liver fibrosis [80–82]. PPARγ agonists suppress proliferation of hepatic stellate cells and chemotaxis in response to platelet-derived growth factor (PDGF) [83], and induce hepatocyte growth factor (HGF), an anti-fibrotic cytokine [84]. PPARγ ligands also block PDGF-dependent proliferation, prolyl4-hydroxylase (α) mRNA, and the expression of collagen and α-SMA by pancreatic stellate cells [85]. Renal cortical fibroblasts treated with glucose induce myofibroblastic markers. Treatment of these cells with pioglitizone decreased collagen IV production, incorporation of proline, fibronectin production, and MMP-9 activity as well as reduced secretion of TIMP-1 and -2 [86, 87].

The molecular mechanisms by which PPARγ ligands inhibit myofibroblast differentiation and effector function are under investigation. Because TGF-β appears to be a key profibrotic cytokine in lung fibrosis [2, 21], several groups have investigated the ability of PPARγ ligands to interfere with TGF-β signaling. TGF-β signaling is mediated by the Smad family of transcription factors [21]. Binding of TGF-β to type 2 TGF-β receptor recruits type 1 TGF-β receptors (TGF-βR-I), forming a heterotetrameric structure that phosphorylates Smad2 and Smad3. Smad2 and Smad3 form heteromeric complexes with Smad4, which translocate to the nucleus and activate transcription of target genes (Figure 2). In human hepatic stellate cells, TGF-β causes a time- and dose-dependent increase in Smad3 phosphorylation, followed by increased collagen production. Cotreatment with either a TGF-βR-I kinase inhibitor or the synthetic PPARγ agonist GW7845 resulted in dose-dependent inhibition of both collagen production and Smad3 phosphorylation [88]. In contrast, the natural PPARγ agonist 15d-PGJ_2 did not inhibit nuclear translocation of Smad2/3 complexes in human renal mesangial cells treated with TGF-β. Instead, 15d-PGJ_2 induced expression of the antifibrotic hepatocyte growth factor (HGF) via a peroxisome proliferator response element in the HGF promoter, and upregulated the Smad corepressor TG-interacting factor (TGIF), leading to inhibition of α-SMA and fibronectin expression [84]. Interestingly, the same study reported that 15d-PGJ_2 did inhibit Smad2/3 nuclear translocation in rat kidney fibroblasts treated with TGF-β, while we have reported that 15d-PGJ_2 does not inhibit TGF-β-stimulated phosphorylation of Smad2 in human lung

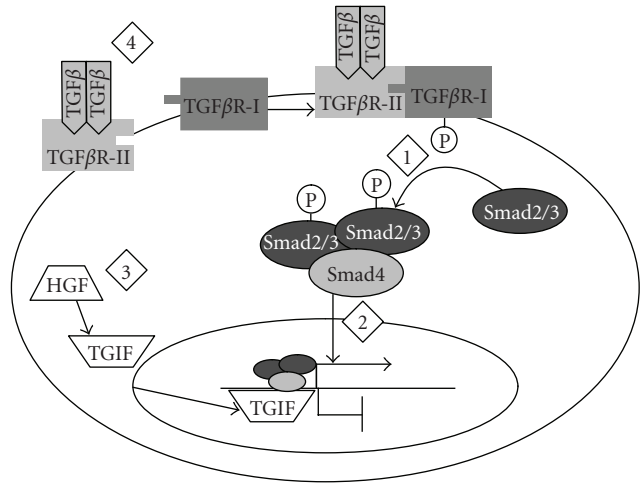

Figure 2: *The TGF-β signaling pathway*. Binding of TGF-β to TGF-β receptor II recruits TGF-β receptor I (TGF-βR-I). The kinase domain of TGF-βR-I phosphorylates Smad2 and 3, which form a heteromeric complex with Smad4 that translocates into the nucleus where it activates transcription of target genes. Numbers indicate points in the pathway where PPARγ ligands have been demonstrated to interfere with TGF-β signaling. (1) GW7845, a PPARγ ligand, inhibited Smad3 phosphorylation in human hepatic stellate cells [88]. (2) 15d-PGJ_2 inhibited nuclear translocation of Smad2/3 in rat kidney fibroblasts [84]. (3) In human renal mesangial cells, 15d-PGJ_2 induced hepatocyte growth factor (HGF), which upregulates the Smad corepressor TG-interacting factor (TGIF) [84]. (4) In mouse L929 fibroblasts, 15d-PGJ_2 or retinoic acid upregulated the phosphatase and tensin homologue deleted on chromosome 10 (PTEN), leading to repression of TGF-β1 transcription [89].

fibroblasts [79]. It is possible that inhibition of myofibroblast differentiation by PPARγ agonists is mediated by different mechanisms in different cell types, or that natural and synthetic agonists act by different mechanisms.

Another candidate mechanism for inhibition of profibrotic effector functions of fibroblasts involves upregulation of the tumor-suppressor phosphatase and tensin homologue deleted on chromosome 10 (PTEN). The PTEN promoter contains a PPRE, and PPARγ ligands upregulate PTEN expression [90]. In vitro studies have shown that PTEN inhibits fibroblast-myofibroblast differentiation and expression of α-SMA and collagen in human and mouse lung fibroblasts [91], while loss of PTEN activity contributes to the migratory/invasive phenotype of lung fibroblasts isolated from IPF patients [92]. It has also been reported that PTEN levels are decreased in the lung tissue of IPF patients, and that PTEN knockout mice aremore susceptible to bleomycin-induced fibrosis [91]. Interestingly, both 15d-PGJ_2 and the RXR ligand 9-*cis*-retinoic acid inhibited transcription of the TGF-β1 gene via PTEN upregulation in mouse L929 fibroblasts [89], providing an additional mechanism by which PPARγ ligands might interfere directly with the profibrotic effects of TGF-β.

One important consideration is that the effects of PPARγ ligands may not all be dependent on PPARγ-dependent transcriptional activation. PPARγ-dependent transcriptional repression has been described in adipogensis, but not in myofibroblast differentiation [93, 94]. Additionally, recent reports have suggested that some of the biological effects of 15d-PGJ_2 are moderated by a PPARγ-independent mechanism involving modification of protein thiols by an electrophilic carbon on the imidazole ring of 15d-PGJ_2 [95, 96]. For example, the ability of troglitazone or 15d-PGJ_2 to inhibit proliferation of hepatic stellate cells was shown to be PPARγ-independent [97], while 15d-PGJ_2 inhibts the proliferation of human breast carcinoma cell lines by covalent modification of the estrogen receptor DNA-binding domain [98]. We examined the PPARγ dependence of the antifibrotic effects of PPARγ ligands on human lung fibroblasts. Neither the irreversible PPARγ antagonist GW9662 nor a dominant-negative PPARγ mutant significantly blocked the ability of 15d-PGJ_2 to inhibit TGF-β-induced α-SMA expression, suggesting that this effect of 15d-PGJ_2 was largely PPARγ-independent [79]. However, the antifibrotic effects of rosiglitizone were rescued significantly by the dominant-negative PPARγ, suggesting that while rosiglitizone was less effective at inhibiting myofibroblast differentiation, the effect was mostly dependent on PPARγ [79].

6. RETINOID X RECEPTOR

The PPARs must form heterodimers with the retinoid X receptor (RXR) in order to initiate gene transcription [99]. Therefore, it has been proposed that the anti-inflammatory and antifibrotic functions of PPARs may be addressed or enhanced by RXR ligands, predominantly the retinoic acids [100, 101]. In the rat liver, endogenous and synthetic retinoic acids (RA) reduced proliferation of HSCs and production of collagen I. In addition, all-*trans* RAs inhibited the synthesis of collagen I/II and fibronectin but did not affect HSC proliferation [102]. Levels of RXR-α and RXR-β were decreased in the HSC of rats with cholestatic liver fibrosis [103]. In addition, there were decreases in all-*trans* RA and 9-*cis*-RA levels and RA binding to the retinoid receptor response element (RARE) in fibrotic liver tissue. Similar

findings have been demonstrated in glomerular mesangial cells where 9-*cis*-RA induced the antifibrotic growth factor HGF and inhibited TGF-β-stimulated induction of α-SMA and fibronectin [104]. Synergistic effects between RXR ligands and PPAR ligands have not yet been reported in lung fibroblasts in vitro or in animal models of lung fibrosis, though this is under investigation.

7. CONCLUSION

Although the role of the PPARs in fibrosing diseases has been less well studied than their role in regulating inflammation, a number of key results have emerged. PPARγ agonists inhibit the differentiation of lung fibroblasts to myofibroblasts in vitro, and also inhibit airway remodeling and fibrosis in animal models [77, 79]. PPARα agonists also attenuated fibrosis in the mouse bleomycin model, while PPARα knockout mice developed more severe disease [50].

Our understanding of the role of PPARs in lung fibrosis is hindered by the relative lack of experiments directly involving the lung or lung cells. However, progress has also been made toward determining the role of the PPARs in fibrosing diseases of the liver, kidney, and pancreas. Hepatic stellate cells and pancreatic stellate cells differentiate to myofibroblast-like cells under the same stimulus as lung fibroblasts, and this differentiation is inhibited by both natural and synthetic PPARγ ligands [83–85]. The TZD class of PPARγ agonists is effective in reducing liver, cardiac, and kidney fibrosis in rats and mice [44, 45, 72]. PPARα agonists, including the fibrate drugs, have also shown promise in attenuating liver, kidney, and cardiac fibrosis [40, 43, 45].

The mechanisms by which PPAR ligands alter fibrosis are not well understood, but appear to involve multiple regulatory pathways (see Figure 3). Natural and synthetic PPARγ agonists inhibit TGF-β-driven myofibroblast differentiation and activation in hepatic stellate cells, kidney fibroblasts, and lung fibroblasts. In human hepatic stellate cells, the PPARγ agonist GW7845 inhibited Smad3 phosphorylation and nuclear translocation [88], while a similar result was seen with 15d-PGJ_2 in rat kidney fibroblasts [84]. However, 15d-PGJ_2 did not alter Smad2 phosphorylation in human lung fibroblasts [79] or human renal mesangial cells, but instead upregulated HGF and TGIF [84]. It is likely that the precise mechanism of action of PPARγ ligands varies depending on the cell type and agonist used. A further complication is that PPARγ agonists appear to have PPARγ-independent effects. Further studies using pharmaceutical inhibitors of PPARγ or PPARγ knockout cell lines may prove useful in further investigations.

A very intriguing recent report found that 15d-PGJ_2 altered transcriptional activity of the estrogen receptor by covalent modification of cysteine residues in its zinc finger DNA-binding domain [98]. Since cysteine is a ready target of covalent modification by 15d-PGJ_2 [95, 96] and many transcription factors use cysteine-rich zinc finger DNA-binding domains, this suggests that one possible mechanism by which PPARγ ligands can affect the regulation of cell differentiation independently of PPARγ itself is via modification of other transcription factors.

(1) PPARα ligands have antifibrotic effects in rodent liver and lung fibrosis models; the mechanism is unknown but may involve downregulation of inflammation.
(2) PPARβ/δ plays a role in regulating the transition from inflammation to normal wound healing.
(3) PPARβ/δ agonists potentiate the antifibrotic activities of PPARγ agonists.
(4) PPARγ ligands upregulate transcription of genes that oppose myofibroblast differentiation (PTEN).
(5) PPARγ ligands interfere with TGF-β signaling via the Smad pathway in some cell types.

Figure 3: Key concepts in the regulation of fibrosis by PPARs.

There are less data available on the mechanism of action of PPARα and β/δ agonists. Although PPARα agonists attenuate animal preclinical fibrosis models, studies of the direct effect of PPARα ligands on myofibroblast activation have not been reported. Treprostinil inhibition of lung fibroblast proliferation is PPARβ/δ-dependent [55], and PPARβ/δ also appears to play a role in keratinocyte maturation and function [53]. It has been hypothesized that fibrosis is a consequence of dysregulated wound healing and tissue remodeling following an initial injury [54]. This may provide the mechanistic link between PPARα and β/δ and fibrosis. Rather than directly acting on fibroblasts and myofibroblasts, PPARα may regulate inflammation, while PPARβ/δ regulates the transition from inflammation to wound healing [54, 105]. Thus, PPARα and β/δ agonists may ameliorate fibrosis by altering the initial inflammatory response and the transition to a fibrogenic milieu, respectively.

The relationship between the PPARs and fibrosis is likely to be complex. As discussed above, PPARα and PPARγ are involved in regulating both inflammation and fibrosis, and some ligands have affinity for more than one PPAR. In addition, because RXR is the obligate dimerization partner for all three PPARs, modulating RXR activity may have multiple overlapping or even conflicting effects. A number of useful tools exist to study these relationships, including highly specific synthetic agonists and antagonists, dominant negative expression constructs, and germline and conditional gene knockouts. Each of these approaches has potential advantages and drawbacks. In particular, genetic ablation of PPAR genes will eliminate their function from both inflammatory and repair processes, making it difficult to determine their role in each process independently. This problem can be addressed by using multiple complimentary approaches to examine PPAR function in both normal and abnormal wound repair and fibrosis.

It must be emphasized that important classes of PPARα (the fibrate drugs) and PPARγ (TZDs) agonists are currently available in the clinic. Although the frequency of lung fibrosis in the general population is not high, it may be possible to perform retrospective studies of long-term users of TZDs and fibrates to determine whether these drugs reduce the incidence or severity of lung fibrosis and other fibrosing diseases. More importantly, the clinical availability of these

drugs means that significant results from animal studies of fibrosis models may be rapidly applied in the clinical setting. Recent advances in drug delivery by inhalation may allow delivery of antifibrotic PPAR agonists directly to the site of fibrosis (as has already been demonstrated with the use of ciglitazone in a mouse model of airway remodeling [68]), achieving higher effective doses at the target site with lower systemic side effects. As most forms of lung fibrosis are refractory to current treatment, the rapid translation of basic research to bedside practice holds great promise for a patient population suffering from a largely untreatable disease.

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REFERENCES

[1] V. J. Thannickal, G. B. Toews, E. S. White, J. P. Lynch III, and F. J. Martinez, "Mechanisms of pulmonary fibrosis," *Annual Review of Medicine*, vol. 55, pp. 395–417, 2004.

[2] P. J. Sime and K. M. A. O'Reilly, "Fibrosis of the lung and other tissues: new concepts in pathogenesis and treatment," *Clinical Immunology*, vol. 99, no. 3, pp. 308–319, 2001.

[3] T. Geiser, "Idiopathic pulmonary fibrosis—a disorder of alveolar wound repair?" *Swiss Medical Weekly*, vol. 133, no. 29-30, pp. 405–411, 2003.

[4] N. Fujimura, "Pathology and pathophysiology of pneumoconiosis," *Current Opinion in Pulmonary Medicine*, vol. 6, no. 2, pp. 140–144, 2000.

[5] B. T. Mossman and A. Churg, "Mechanisms in the pathogenesis of asbestosis and silicosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 157, no. 5, part 1, pp. 1666–1680, 1998.

[6] D. A. Zisman, M. P. Keane, J. A. Belperio, R. M. Strieter, and J. P. Lynch III, "Pulmonary fibrosis," *Methods in Molecular Medicine*, vol. 117, pp. 3–44, 2005.

[7] K. B. Baumgartner, J. M. Samet, D. B. Coultas, et al., "Occupational and environmental risk factors for idiopathic pulmonary fibrosis: a multicenter case-control study. Collaborating Centers," *American Journal of Epidemiology*, vol. 152, no. 4, pp. 307–315, 2000.

[8] K. B. Baumgartner, J. M. Samet, C. A. Stidley, T. V. Colby, and J. A. Waldron, "Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis," *American Journal of Respiratory and Critical Care Medicine*, vol. 155, no. 1, pp. 242–248, 1997.

[9] E. S. White, M. H. Lazar, and V. J. Thannickal, "Pathogenetic mechanisms in usual interstitial pneumonia/idiopathic pulmonary fibrosis," *Journal of Pathology*, vol. 201, no. 3, pp. 343–354, 2003.

[10] P. de Vuyst and P. Camus, "The past and present of pneumoconioses," *Current Opinion in Pulmonary Medicine*, vol. 6, no. 2, pp. 151–156, 2000.

[11] H. R. Collard, J. H. Ryu, W. W. Douglas, et al., "Combined corticosteroid and cyclophosphamide therapy does not alter survival in idiopathic pulmonary fibrosis," *Chest*, vol. 125, no. 6, pp. 2169–2174, 2004.

[12] J. J. Swigris, W. G. Kuschner, J. L. Kelsey, and M. K. Gould, "Idiopathic pulmonary fibrosis: challenges and opportunities for the clinician and investigator," *Chest*, vol. 127, no. 1, pp. 275–283, 2005.

[13] D. S. Kim, H. R. Collard, and T. E. King Jr., "Classification and natural history of the idiopathic interstitial pneumonias," *Proceedings of the American Thoracic Society*, vol. 3, no. 4, pp. 285–292, 2006.

[14] S. H. Phan, "The myofibroblast in pulmonary fibrosis," *Chest*, vol. 122, supplement 6, pp. 286S–289S, 2002.

[15] R. S. Smith, T. J. Smith, T. M. Blieden, and R. P. Phipps, "Fibroblasts as sentinel cells. Synthesis of chemokines and regulation of inflammation," *American Journal of Pathology*, vol. 151, no. 2, pp. 317–322, 1997.

[16] M. B. Vaughan, E. W. Howard, and J. J. Tomasek, "Transforming growth factor-β1 promotes the morphological and functional differentiation of the myofibroblast," *Experimental Cell Research*, vol. 257, no. 1, pp. 180–189, 2000.

[17] G. Gabbiani, "The myofibroblast in wound healing and fibrocontractive diseases," *Journal of Pathology*, vol. 200, no. 4, pp. 500–503, 2003.

[18] S. H. Phan, K. Zhang, H. Y. Zhang, and M. Gharaee-Kermani, "The myofibroblast as an inflammatory cell in pulmonary fibrosis," *Current Topics in Pathology*, vol. 93, pp. 173–182, 1999.

[19] M. Selman, T. E. King Jr., and A. Pardo, "Idiopathic pulmonary fibrosis: prevailing and evolving hypotheses about its pathogenesis and implications for therapy," *Annals of Internal Medicine*, vol. 134, no. 2, pp. 136–151, 2001.

[20] J. Gauldie, M. Kolb, and P. J. Sime, "A new direction in the pathogenesis of idiopathic pulmonary fibrosis?" *Respiratory Research*, vol. 3, p. 1, 2001.

[21] U. Bartram and C. P. Speer, "The role of transforming growth factor β in lung development and disease," *Chest*, vol. 125, no. 2, pp. 754–765, 2004.

[22] M. Kelly, M. Kolb, P. Bonniaud, and J. Gauldie, "Re-evaluation of fibrogenic cytokines in lung fibrosis," *Current Pharmaceutical Design*, vol. 9, no. 1, pp. 39–49, 2003.

[23] H.-Y. Zhang and S. H. Phan, "Inhibition of myofibroblast apoptosis by transforming growth factor β_1," *American Journal of Respiratory Cell and Molecular Biology*, vol. 21, no. 6, pp. 658–665, 1999.

[24] P. J. Sime, Z. Xing, F. L. Graham, K. G. Csaky, and J. Gauldie, "Adenovector-mediated gene transfer of active transforming growth factor- β_1 induces prolonged severe fibrosis in rat lung," *Journal of Clinical Investigation*, vol. 100, no. 4, pp. 768–776, 1997.

[25] C. Blanquart, O. Barbier, J. C. Fruchart, B. Staels, and C. Glineur, "Peroxisome proliferator-activated receptors: regulation of transcriptional activities and roles in inflammation," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 85, no. 2–5, pp. 267–273, 2003.

[26] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[27] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[28] M. Ricote, J. Huang, L. Fajas, et al., "Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human

atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 13, pp. 7614–7619, 1998.

[29] S. G. Harris, J. Padilla, L. Koumas, D. Ray, and R. P. Phipps, "Prostaglandins as modulators of immunity," *Trends in Immunology*, vol. 23, no. 3, pp. 144–150, 2002.

[30] J. Padilla, E. Leung, and R. P. Phipps, "Human B lymphocytes and B lymphomas express PPAR-γ and are killed by PPAR-γ agonists," *Clinical Immunology*, vol. 103, no. 1, pp. 22–33, 2002.

[31] F. Akbiyik, D. M. Ray, K. F. Gettings, N. Blumberg, C. W. Francis, and R. P. Phipps, "Human bone marrow megakaryocytes and platelets express PPARγ, and PPARγ agonists blunt platelet release of CD40 ligand and thromboxanes," *Blood*, vol. 104, no. 5, pp. 1361–1368, 2004.

[32] D. M. Simon, M. C. Arikan, S. Srisuma, et al., "Epithelial cell PPARγ is an endogenous regulator of normal lung maturation and maintenance," *Proceedings of the American Thoracic Society*, vol. 3, no. 6, pp. 510–511, 2006.

[33] D. M. Simon, M. C. Arikan, S. Srisuma, et al., "Epithelial cell PPARγ contributes to normal lung maturation," *The FASEB Journal*, vol. 20, no. 9, pp. 1507–1509, 2006.

[34] E. Marian, S. Baraldo, A. Visentin, et al., "Up-regulated membrane and nuclear leukotriene B4 receptors in COPD," *Chest*, vol. 129, no. 6, pp. 1523–1530, 2006.

[35] D. A. Culver, B. P. Barna, B. Raychaudhuri, et al., "Peroxisome proliferator-activated receptor γ activity is deficient in alveolar macrophages in pulmonary sarcoidosis," *American Journal of Respiratory Cell and Molecular Biology*, vol. 30, no. 1, pp. 1–5, 2004.

[36] T. L. Bonfield, C. F. Farver, B. P. Barna, et al., "Peroxisome proliferator-activated receptor-γ is deficient in alveolar macrophages from patients with alveolar proteinosis," *American Journal of Respiratory Cell and Molecular Biology*, vol. 29, no. 6, pp. 677–682, 2003.

[37] G. Rizzo and S. Fiorucci, "PPARs and other nuclear receptors in inflammation," *Current Opinion in Pharmacology*, vol. 6, no. 4, pp. 421–427, 2006.

[38] T. Sher, H.-F. Yi, O. W. McBride, and F. J. Gonzalez, "cDNA cloning, chromosomal mapping, and functional characterization of the human peroxisome proliferator activated receptor," *Biochemistry*, vol. 32, no. 21, pp. 5598–5604, 1993.

[39] S. Cuzzocrea, "Peroxisome proliferator-activated receptors and acute lung injury," *Current Opinion in Pharmacology*, vol. 6, no. 3, pp. 263–270, 2006.

[40] T. Toyama, H. Nakamura, Y. Harano, et al., "PPARα ligands activate antioxidant enzymes and suppress hepatic fibrosis in rats," *Biochemical and Biophysical Research Communications*, vol. 324, no. 2, pp. 697–704, 2004.

[41] G. Svegliati-Baroni, C. Candelaresi, S. Saccomanno, et al., "A model of insulin resistance and nonalcoholic steatohepatitis in rats: role of peroxisome proliferator-activated receptor-α and n-3 polyunsaturated fatty acid treatment on liver injury," *American Journal of Pathology*, vol. 169, no. 3, pp. 846–860, 2006.

[42] E. Ip, G. Farrell, P. Hall, G. Robertson, and I. Leclercq, "Administration of the potent PPARα agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice," *Hepatology*, vol. 39, no. 5, pp. 1286–1296, 2004.

[43] T. Ogata, T. Miyauchi, S. Sakai, Y. Irukayama-Tomobe, K. Goto, and I. Yamaguchi, "Stimulation of peroxisome-proliferator-activated receptor α (PPARα) attenuates cardiac fibrosis and endothelin-1 production in pressure-overloaded rat hearts," *Clinical Science*, vol. 103, supplement 48, pp. 284S–288S, 2002.

[44] M. Iglarz, R. M. Touyz, E. C. Viel, et al., "Peroxisome proliferator-activated receptor-α and receptor-γ activators prevent cardiac fibrosis in mineralocorticoid-dependent hypertension," *Hypertension*, vol. 42, no. 4, pp. 737–743, 2003.

[45] A. C. Calkin, S. Giunti, K. A. Jandeleit-Dahm, T. J. Allen, M. E. Cooper, and M. C. Thomas, "PPAR-α and -γ agonists attenuate diabetic kidney disease in the apolipoprotein E knockout mouse," *Nephrology Dialysis Transplantation*, vol. 21, no. 9, pp. 2399–2405, 2006.

[46] V. Reynders, S. Loitsch, C. Steinhauer, T. Wagner, D. Steinhilber, and J. Bargon, "Peroxisome proliferator-activated receptor α (PPARα) down-regulation in cystic fibrosis lymphocytes," *Respiratory Research*, vol. 7, p. 104, 2006.

[47] S. Cuzzocrea, E. Mazzon, R. Di Paola, et al., "The role of the peroxisome proliferator-activated receptor-α (PPAR-α) in the regulation of acute inflammation," *Journal of Leukocyte Biology*, vol. 79, no. 5, pp. 999–1010, 2006.

[48] T. H. Thatcher, P. J. Sime, and R. K. Barth, "Sensitivity to bleomycin-induced lung injury is not moderated by an antigen-limited T-cell repertoire," *Experimental Lung Research*, vol. 31, no. 7, pp. 685–700, 2005.

[49] D. H. Bowden, "Unraveling pulmonary fibrosis: the bleomycin model," *Laboratory Investigation*, vol. 50, no. 5, pp. 487–488, 1984.

[50] T. Genovese, E. Mazzon, R. Di Paola, et al., "Role of endogenous and exogenous ligands for the peroxisome proliferator-activated receptor α in the development of bleomycin-induced lung injury," *Shock*, vol. 24, no. 6, pp. 547–555, 2006.

[51] E. Dyrøy, A. Yndestad, T. Ueland, et al., "Antiinflammatory effects of tetradecylthioacetic acid involve both peroxisome proliferator-activated receptor α-dependent and -independent pathways," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 7, pp. 1364–1369, 2005.

[52] J. M. Lehmann, J. M. Lenhard, B. B. Oliver, G. M. Ringold, and S. A. Kliewer, "Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs," *Journal of Biological Chemistry*, vol. 272, no. 6, pp. 3406–3410, 1997.

[53] L. Michalik, B. Desvergne, N. S. Tan, et al., "Impaired skin wound healing in peroxisome proliferator-activated receptor (PPAR)α and PPARβ mutant mice," *Journal of Cell Biology*, vol. 154, no. 4, pp. 799–814, 2001.

[54] N. S. Tan, L. Michalik, B. Desvergne, and W. Wahli, "Peroxisome proliferator-activated receptor-β as a target for wound healing drugs," *Expert Opinion on Therapeutic Targets*, vol. 8, no. 1, pp. 39–48, 2004.

[55] F. Y. Ali, K. Egan, G. A. FitzGerald, et al., "Role of prostacyclin versus peroxisome proliferator-activated receptor β receptors in prostacyclin sensing by lung fibroblasts," *American Journal of Respiratory Cell and Molecular Biology*, vol. 34, no. 2, pp. 242–246, 2006.

[56] K. Matsusue, J. M. Peters, and F. J. Gonzalez, "PPARβ/δ potentiates PPARγ-stimulated adipocyte differentiation," *The FASEB Journal*, vol. 18, no. 12, pp. 1477–1479, 2004.

[57] T. H.-W. Huang, V. Razmovski-Naumovski, B. P. Kota, D. S.-H. Lin, and B. D. Roufogalis, "The pathophysiological function of peroxisome proliferator-activated receptor-γ in lung-related diseases," *Respiratory Research*, vol. 6, p. 102, 2005.

[58] B. M. Forman, P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans, "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ," *Cell*, vol. 83, no. 5, pp. 803–812, 1995.

[59] S. A. Kliewer, J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris, and J. M. Lehmann, "A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation," *Cell*, vol. 83, no. 5, pp. 813–819, 1995.

[60] T. M. McIntyre, A. V. Pontsler, A. R. Silva, et al., "Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARγ agonist," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 1, pp. 131–136, 2003.

[61] F. J. Schopfer, Y. Lin, P. R. S. Baker, et al., "Nitrolinoleic acid: an endogenous peroxisome proliferator-activated receptor γ ligand," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 7, pp. 2340–2345, 2005.

[62] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[63] A. E. Place, N. Suh, C. R. Williams, et al., "The novel synthetic triterpenoid, CDDO-imidazolide, inhibits inflammatory response and tumor growth in vivo," *Clinical Cancer Research*, vol. 9, no. 7, pp. 2798–2806, 2003.

[64] M. G. Belvisi, D. J. Hele, and M. A. Birrell, "Peroxisome proliferator-activated receptor γ agonists as therapy for chronic airway inflammation," *European Journal of Pharmacology*, vol. 533, no. 1–3, pp. 101–109, 2006.

[65] D. Liu, B. X. Zeng, S. H. Zhang, and S. L. Yao, "Rosiglitazone, an agonist of peroxisome proliferator-activated receptor γ, reduces pulmonary inflammatory response in a rat model of endotoxemia," *Inflammation Research*, vol. 54, no. 11, pp. 464–470, 2005.

[66] K.-I. Inoue, H. Takano, R. Yanagisawa, et al., "Effect of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 on acute lung injury induced by lipopolysaccharide in mice," *European Journal of Pharmacology*, vol. 481, no. 2-3, pp. 261–269, 2003.

[67] J. E. Ward, D. J. Fernandes, C. C. Taylor, J. V. Bonacci, L. Quan, and A. G. Stewart, "The PPARγ ligand, rosiglitazone, reduces airways hyperresponsiveness in a murine model of allergen-induced inflammation," *Pulmonary Pharmacology and Therapeutics*, vol. 19, no. 1, pp. 39–46, 2006.

[68] K. Honda, P. Marquillies, M. Capron, and D. Dombrowicz, "Peroxisome proliferator-activated receptor γ is expressed in airways and inhibits features of airway remodeling in a mouse asthma model," *Journal of Allergy and Clinical Immunology*, vol. 113, no. 5, pp. 882–888, 2004.

[69] C. Jiang, A. T. Ting, and B. Seed, "PPAR-γ agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

[70] A. C. C. Wang, X. Dai, B. Luu, and D. J. Conrad, "Peroxisome proliferator-activated receptor-γ regulates airway epithelial cell activation," *American Journal of Respiratory Cell and Molecular Biology*, vol. 24, no. 6, pp. 688–693, 2001.

[71] S. E. Feldon, C. W. O'Loughlin, D. M. Ray, S. Landskroner-Eiger, K. E. Seweryniak, and R. P. Phipps, "Activated human T lymphocytes express cyclooxygenase-2 and produce proadipogenic prostaglandins that drive human orbital fibroblast differentiation to adipocytes," *American Journal of Pathology*, vol. 169, no. 4, pp. 1183–1193, 2006.

[72] I. A. Leclercq, C. Sempoux, P. Stärkel, and Y. Horsmans, "Limited therapeutic efficacy of pioglitazone on progression of hepatic fibrosis in rats," *Gut*, vol. 55, no. 7, pp. 1020–1029, 2006.

[73] K. Kawaguchi, I. Sakaida, M. Tsuchiya, K. Omori, T. Takami, and K. Okita, "Pioglitazone prevents hepatic steatosis, fibrosis, and enzyme-altered lesions in rat liver cirrhosis induced by a choline-deficient L-amino acid-defined diet," *Biochemical and Biophysical Research Communications*, vol. 315, no. 1, pp. 187–195, 2004.

[74] H. Uto, C. Nakanishi, A. Ido, et al., "The peroxisome proliferator-activated receptor-γ agonist, pioglitazone, inhibits fat accumulation and fibrosis in the livers of rats fed a choline-deficient, L-amino acid-defined diet," *Hepatology Research*, vol. 32, no. 4, pp. 235–242, 2005.

[75] R. E. Buckingham, "Thiazolidinediones: pleiotropic drugs with potent anti-inflammatory properties for tissue protection," *Hepatology Research*, vol. 33, no. 2, pp. 167–170, 2005.

[76] M. C. Lansang, C. Coletti, S. Ahmed, M. S. Gordon, and N. K. Hollenberg, "Effects of the PPAR-γ agonist rosiglitazone on renal haemodynamics and the renin-angiotensin system in diabetes," *Journal of the Renin-Angiotensin-Aldosterone System*, vol. 7, no. 3, pp. 175–180, 2006.

[77] T. Genovese, S. Cuzzocrea, R. Di Paola, et al., "Effect of rosiglitazone and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 on bleomycin-induced lung injury," *European Respiratory Journal*, vol. 25, no. 2, pp. 225–234, 2005.

[78] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[79] H. A. Burgess, L. E. Daugherty, T. H. Thatcher, et al., "PPARγ agonists inhibit TGF-β induced pulmonary myofibroblast differentiation and collagen production: implications for therapy of lung fibrosis," *American Journal of Physiology*, vol. 288, no. 6, pp. L1146–L1153, 2005.

[80] T. Miyahara, L. Schrum, R. Rippe, et al., "Peroxisome proliferator-activated receptors and hepatic stellate cell activation," *Journal of Biological Chemistry*, vol. 275, no. 46, pp. 35715–35722, 2000.

[81] M. L. Hautekeete and A. Geerts, "The hepatic stellate (Ito) cell: its role in human liver disease," *Virchows Archiv*, vol. 430, no. 3, pp. 195–207, 1997.

[82] D. M. Bissell, "Connective tissue metabolism and hepatic fibrosis: an overview," *Seminars in Liver Disease*, vol. 10, no. 1, pp. iii–iv, 1990.

[83] F. Marra, E. Efsen, R. G. Romanelli, et al., "Ligands of peroxisome proliferator-activated receptor γ modulate profibrogenic and proinflammatory actions in hepatic stellate cells," *Gastroenterology*, vol. 119, no. 2, pp. 466–478, 2000.

[84] Y. Li, X. Wen, B. C. Spataro, K. Hu, C. Dai, and Y. Liu, "Hepatocyte growth factor is a downstream effector that mediates the antifibrotic action of peroxisome proliferator-activated receptor-γ agonists," *Journal of the American Society of Nephrology*, vol. 17, no. 1, pp. 54–65, 2006.

[85] A. Masamune, K. Satoh, Y. Sakai, M. Yoshida, A. Satoh, and T. Shimosegawa, "Ligands of peroxisome proliferator-activated receptor-γ induce apoptosis in AR42J cells," *Pancreas*, vol. 24, no. 2, pp. 130–138, 2002.

[86] S. Zafiriou, S. R. Stanners, S. Saad, T. S. Polhill, P. Poronnik, and C. A. Pollock, "Pioglitazone inhibits cell growth and reduces matrix production in human kidney fibroblasts," *Journal of the American Society of Nephrology*, vol. 16, no. 3, pp. 638–645, 2005.

[87] U. Panchapakesan, S. Sumual, C. A. Pollock, and X. Chen, "PPARγ agonists exert antifibrotic effects in renal tubular cells exposed to high glucose," *American Journal of Physiology*, vol. 289, no. 5, pp. F1153–F1158, 2005.

[88] C. Zhao, W. Chen, L. Yang, L. Chen, S. A. Stimpson, and A. M. Diehl, "PPARγ agonists prevent TGFβ1/Smad3-signaling in human hepatic stellate cells," *Biochemical and Biophysical Research Communications*, vol. 350, no. 2, pp. 385–391, 2006.

[89] S. J. Lee, E. K. Yang, and S. G. Kim, "Peroxisome proliferator-activated receptor-γ and retinoic acid X receptor α represses the TGFβ1 gene via PTEN-mediated p70 ribosomal S6 kinase-1 inhibition: role for Zf9 dephosphorylation," *Molecular Pharmacology*, vol. 70, no. 1, pp. 415–425, 2006.

[90] R. E. Teresi, C.-W. Shaiu, C.-S. Chen, V. K. Chatterjee, K. A. Waite, and C. Eng, "Increased PTEN expression due to transcriptional activation of PPARγ by Lovastatin and Rosiglitazone," *International Journal of Cancer*, vol. 118, no. 10, pp. 2390–2398, 2006.

[91] E. S. White, R. G. Atrasz, B. Hu, et al., "Negative regulation of myofibroblast differentiation by PTEN (phosphatase and tensin homolog deleted on chromosome 10)," *American Journal of Respiratory and Critical Care Medicine*, vol. 173, no. 1, pp. 112–121, 2006.

[92] E. S. White, V. J. Thannickal, S. L. Carskadon, et al., "Integrin $\alpha 4\beta 1$ regulates migration across basement membranes by lung fibroblasts: a role for phosphatase and tensin homologue deleted on chromosome 10," *American Journal of Respiratory and Critical Care Medicine*, vol. 168, no. 4, pp. 436–442, 2003.

[93] Y. Zuo, L. Qiang, and S. R. Farmer, "Activation of CCAAT/enhancer-binding protein (C/EBP) α expression by C/EBPβ during adipogenesis requires a peroxisome proliferator-activated receptor-γ-associated repression of HDAC1 at the C/ebpα gene promoter," *Journal of Biological Chemistry*, vol. 281, no. 12, pp. 7960–7967, 2006.

[94] S. S. Chung, H. H. Choi, Y. M. Cho, H. K. Lee, and K. S. Park, "Sp1 mediates repression of the resistin gene by PPARγ agonists in 3T3-L1 adipocytes," *Biochemical and Biophysical Research Communications*, vol. 348, no. 1, pp. 253–258, 2006.

[95] J. Atsmon, B. J. Sweetman, S. W. Baertschi, T. M. Harris, and L. J. Roberts II, "Formation of thiol conjugates of 9-deoxy-Δ^9,Δ^{12}(*E*)-prostaglandin D_2 and Δ^{12}(*E*)-prostaglandin D_2," *Biochemistry*, vol. 29, no. 15, pp. 3760–3765, 1990.

[96] T. Shibata, T. Yamada, T. Ishii, et al., "Thioredoxin as a molecular target of cyclopentenone prostaglandins," *Journal of Biological Chemistry*, vol. 278, no. 28, pp. 26046–26054, 2003.

[97] K. Shimizu, K. Shiratori, M. Kobayashi, and H. Kawamata, "Troglitazone inhibits the progression of chronic pancreatitis and the profibrogenic activity of pancreatic stellate cells via a PPARγ- independent mechanism," *Pancreas*, vol. 29, no. 1, pp. 67–74, 2004.

[98] H.-J. Kim, J.-Y. Kim, Z. Meng, et al., "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits transcriptional activity of estrogen receptor-α via covalent modification of DNA-binding domain," *Cancer Research*, vol. 67, no. 6, pp. 2595–2602, 2007.

[99] I. Issemann, R. A. Prince, J. D. Tugwood, and S. Green, "The peroxisome proliferator-activated receptor: retinoid X receptor heterodimer is activated by fatty acids and fibrate hypolipidaemic drugs," *Journal of Molecular Endocrinology*, vol. 11, no. 1, pp. 37–47, 1993.

[100] H. S. Ahuja, A. Szanto, L. Nagy, and P. J. A. Davies, "The retinoid X receptor and its ligands: versatile regulators of metabolic function, cell differentiation and cell death," *Journal of Biological Regulators and Homeostatic Agents*, vol. 17, no. 1, pp. 29–45, 2003.

[101] P. Germain, P. Chambon, G. Eichele, et al., "International union of pharmacology. LXIII. Retinoid X receptors," *Pharmacological Reviews*, vol. 58, no. 4, pp. 760–772, 2006.

[102] K. Hellemans, P. Verbuyst, E. Quartier, et al., "Differential modulation of rat hepatic stellate phenotype by natural and synthetic retinoids," *Hepatology*, vol. 39, no. 1, pp. 97–108, 2004.

[103] M. Ohata, M. Lin, M. Satre, and H. Tsukamoto, "Diminished retinoic acid signaling in hepatic stellate cells in cholestatic liver fibrosis," *American Journal of Physiology*, vol. 272, no. 3, part 1, pp. G589–G596, 1997.

[104] X. Wen, Y. Li, K. Hu, C. Dai, and Y. Liu, "Hepatocyte growth factor receptor signaling mediates the anti-fibrotic action of 9-*cis*-retinoic acid in glomerular mesangial cells," *American Journal of Pathology*, vol. 167, no. 4, pp. 947–957, 2005.

[105] G. Chinetti, J.-C. Fruchart, and B. Staels, "Peroxisome proliferator-activated receptors and inflammation: from basic science to clinical applications," *International Journal of Obesity and Related Metabolic Disorders*, vol. 27, supplement 3, pp. S41–S45, 2003.

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Review Article

The Role of Peroxisome Proliferator-Activated Receptors in Pulmonary Vascular Disease

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily that regulate diverse physiological processes ranging from lipogenesis to inflammation. Recent evidence has established potential roles of PPARs in both systemic and pulmonary vascular disease and function. Existing treatment strategies for pulmonary hypertension, the most common manifestation of pulmonary vascular disease, are limited by an incomplete understanding of the underlying disease pathogenesis and lack of efficacy indicating an urgent need for new approaches to treat this disorder. Derangements in pulmonary endothelial-derived mediators and endothelial dysfunction have been shown to play a pivotal role in pulmonary hypertension pathogenesis. Therefore, the following review will focus on selected mediators implicated in pulmonary vascular dysfunction and evidence that PPARs, in particular PPARγ, participate in their regulation and may provide a potential novel therapeutic target for the treatment of pulmonary hypertension.

1. INTRODUCTION

Originally described in 1990, peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily [1]. PPARs have been implicated in diverse disorders including cancer, diabetes, and atherosclerosis, and activation of these receptors regulates diverse physiological processes ranging from lipogenesis to inflammation. Three distinct PPAR subclasses have been identified; PPARα, PPARβ/δ, and PPARγ. These isotypes are encoded by separate genes and exhibit different tissue distributions and function. PPARα is predominantly expressed in liver, heart, kidney, and muscle where it regulates genes involved in lipid metabolism. PPARβ/δ is a more ubiquitously expressed isoform that stimulates fatty acid oxidation in heart and skeletal muscle [2] and whose diverse functions include cell differentiation [3] and participation in placental development, cancer [4], wound repair [5], and atherosclerosis [6]. PPARγ, expressed in many tissues including adipose, vascular endothelium and smooth muscle, and heart among others, is an important regulator of genes involved in cellular differentiation, particularly adipogenesis, lipid metabolism, and glucose homeostasis. More recently, PPARγ has been shown to play a pivotal role in cell growth, inflammation, apoptosis, and angiogenesis [7–10]. There is limited evidence for the potential roles of PPARα and PPARβ/δ in pulmonary vascular function and disease. However, recent studies have established that pulmonary hypertension in humans is associated with reduced PPARγ expression and that PPARγ ligands can attenuate the development of pulmonary hypertension in several experimental models. This review will summarize recent work implicating PPARγ in pulmonary vascular disease.

2. PPAR BIOLOGY

Ligand binding stimulates the PPAR to form a heterodimer with the retinoid X receptor (RXR) in the cytoplasm [11]. Once activated, the PPAR/RXR heterodimer translocates to the nucleus where the complex binds to PPAR response elements (PPRE) in the promoter region of responsive genes to modulate transcriptional activity. Gene regulation involves ligand-induced conformational changes in the PPAR receptor that mediate interaction with specific coactivator (e.g. steroid receptor coactivator-1 and p300) and corepressor molecules. The coactivator proteins either possess histone acetyltransferase activity or recruit other proteins with this

activity to the transcription start site. Acetylation of histone proteins alters chromatin structure, facilitating the binding of RNA polymerase and the initiation of transcription [12]. PPARs can also repress gene expression by interfering with other signaling pathways and by recruiting corepressors to unliganded PPARs [13].

Structurally diverse ligands activate PPARs. For example, ligands of PPARα include polyunsaturated fatty acids, arachidonic acid metabolites such as leukotriene B_4, and synthetic fibrate compounds used in the treatment of dyslipidemia. Ligands for PPARβ/δ continue to be defined and include prostacyclin suggesting a potential role for PPARβ/δ in regulation of vascular tone, platelet aggregation, and cell proliferation [14, 15]. On the other hand, PPARγ ligands include the thiazolidinedione class of anti-diabetic medications (e.g. pioglitazone, rosiglitazone, and troglitazone), components of oxidized low-density lipoprotein [16], nitrated fatty acids (nitroalkenes), long chain fatty acids and their metabolites, and the PGD_2 metabolite, 15-deoxy-Δ12,14-prostglandin J_2 (15d-PGJ_2). However, despite this promiscuity for activating ligands and broad tissue distribution, specificity of PPAR-mediated tissue effects occurs, in part, through recruitment of ligand-specific populations of coactivator and corepressor molecules [17–19].

3. PATHOGENESIS OF PULMONARY VASCULAR DYSFUNCTION

The appreciation of the potential role of PPARγ in pulmonary vascular disease derives from several basic concepts of vascular disease pathogenesis. Current evidence indicates that endothelial dysfunction and derangements in the balanced production of vasodilatory and vasoconstrictive mediators play a critical role in both systemic [20, 21] and pulmonary vascular [22] diseases. Within the systemic circulation, endothelial dysfunction represents an early step in the pathogenesis of atherosclerotic vascular disease that culminates in coronary, peripheral vascular and cerebrovascular disease.

In contrast, pulmonary hypertension represents the most common manifestation of pulmonary vascular disease. Pulmonary hypertension is characterized by pulmonary vasoconstriction and vascular smooth muscle cell and endothelial cell proliferation. Defined as elevation of mean pulmonary artery pressure above 25 mmHg at rest or above 30 mmHg with exercise, pulmonary hypertension caused 15 668 deaths and 260 000 hospital visits in the United States in 2002 [23]. Pulmonary hypertension is most commonly caused by diverse clinical conditions that produce chronic continuous or intermittent alveolar hypoxia including chronic obstructive pulmonary disease, obstructive sleep apnea, or living at altitude. These conditions promote pulmonary vasoconstriction, vascular remodeling, and pulmonary hypertension. Less commonly, pulmonary hypertension develops secondary to congenital heart defects, autoimmune diseases, left-sided heart failure, or ingestion of certain anorexigen drugs or as a consequence of derangements in bone morphogenetic protein receptor signaling [24, 25]. Existing treatment strategies for patients with pulmonary hypertension are limited by an incomplete understanding of the underlying disease pathogenesis, high cost, and lack of efficacy indicating an urgent need for new approaches to our understanding and treatment of this disorder.

Abundant evidence in humans and animal models indicates that derangements in pulmonary endothelial-derived mediators and endothelial dysfunction play a pivotal role in pulmonary hypertension pathogenesis. Many of these endothelial mediators are also impacted by PPARγ. The following summarizes what is known about the interplay between PPARγ and these mediators.

3.1. Nitric oxide

Nitric oxide (NO) has been studied extensively as an endothelium-derived mediator that plays a critical role in normal vascular function and that promotes a host of vascular protective effects. For example, NO inhibits smooth muscle proliferation [26] and platelet aggregation [27], reduces endothelin-1 (ET-1) production [28], and protects against hypoxia-induced vasoconstriction [29]. Although chronic hypoxia causes pulmonary vasoconstriction through complex mechanisms, compelling evidence indicates that dysregulation of vascular endothelial function constitutes a critical event in the pathogenesis of pulmonary hypertension [22]. These endothelial derangements include alterations in the proliferative capacity of vascular endothelium as well as derangements in endothelium-derived mediators that modulate vascular smooth muscle cell function such as NO, ET-1, serotonin, and prostanoids [30, 31]. While impaired NO bioavailability contributes to pulmonary hypertension [32, 33], the relationship between endothelial nitric oxide synthase (eNOS) expression and pulmonary hypertension is not clear as reports have variously described reduced, unchanged, or increased levels of the enzyme [34–37]. Perhaps this is not surprising given that eNOS-mediated NO production is regulated by complex mechanisms including co-factor availability [38–40], eNOS phosphorylation [41–43], and protein-protein interactions [44–48]. Thus, pulmonary hypertension-associated alterations in these regulatory mechanisms as well as in eNOS expression determine rates of NO production in the pulmonary circulation.

Once NO is produced, its bioavailability can also be regulated by levels of other reactive targets in the surrounding vicinity. For example, superoxide reacts with NO at an extremely rapid, diffusion-limited rate to form the potent oxidant, peroxynitrite [49]. This reaction not only diverts NO from its generally salutary effects on physiological downstream signaling pathways but can simultaneously lead to oxidation of the eNOS cofactor, tetrahydrobiopterin, causing eNOS uncoupling and eNOS-mediated production of superoxide rather than NO [50, 51]. These findings support evidence for impaired endothelium-derived, NO-mediated vasodilation in pulmonary hypertension [52]. The ability of NO inhalation to improve pulmonary hemodynamics and quality of life in selected patients with pulmonary hypertension [53] further suggests the importance of relative NO

deficiency in this disorder. Collectively these and other studies indicate that post-translational alterations in eNOS regulation and/or enhanced NO degradation rather than reduced eNOS expression contribute significantly to pulmonary hypertension pathogenesis [38, 44, 46, 47].

NADPH oxidase is an important source of superoxide in pulmonary vasculature, and its stimulation by hypoxic conditions has been recognized for at least 10 years [54]. Recent publications have confirmed the importance of NADPH oxidase-derived reactive oxygen species in hypoxia-induced pulmonary hypertension. For example, in isolated-perfused lung preparations from wild-type mice, ventilation with 3% oxygen caused acute vasoconstrictor responses whereas hypoxic-induced vasoconstriction was blunted in NADPH oxidase deficient, $p47^{phox}$ knockout mice [55]. Similarly, C57Bl/6 mice exposed to 10% oxygen for 3 weeks demonstrated increased superoxide generation in pulmonary arteries and increased right ventricular pressure and pulmonary arterial medial wall thickness [56]. These hypoxia-induced derangements were completely attenuated in similarly treated NADPH oxidase deficient, $gp91^{phox}$ knockout mice. In a separate report, these same investigators demonstrated that chronic hypoxia enhanced ET-1-stimulated pulmonary arterial vasoconstriction and superoxide generation and that these ET-1 effects were attenuated in $gp91^{phox}$ knockout mice [57]. NADPH oxidase appears to reside in both the endothelial and vascular smooth muscle cell compartments. Hypoxia stimulated superoxide generation in segments of intact pulmonary artery and in pulmonary artery endothelial or vascular smooth muscle cells *ex vivo*, and hypoxia-stimulated superoxide generation was inhibited by pharmacological inhibition of NADPH oxidase (with diphenyliodonium or apocynin) and was associated with enhanced $gp91^{phox}$ expression [47]. Taken together, these reports indicate that NADPH oxidase is an important mediator of pulmonary hypertension in response to hypoxia and that it contributes to enhanced vasoconstrictor responses in the pulmonary circulation following chronic hypoxia.

PPARγ ligands stimulate NO release from endothelial cells through PPARγ-dependent signaling pathways [58, 59]. This enhanced endothelial NO release was not related to increased eNOS expression [58, 59] but was mediated, in part, by alterations in the post-translational regulation of eNOS that increased enzyme activity [58]. PPARγ ligands also produced coordinate reductions in endothelial NADPH oxidase expression and activity and increased CuZn superoxide dismutase expression and activity [60, 61]. Although additional studies will be required to confirm that these effects of PPARγ ligands on superoxide production and degradation are PPARγ-dependent, these findings suggest that PPARγ ligands have great potential for favorably modulating NO bioavailability. Rosiglitazone-induced reductions in NADPH oxidase activity in a rat model of hypertension further support the potential of PPARγ ligands to favorably modulate dysregulated reactive oxygen species production [62]. Taken together, these findings suggest that PPARγ ligands can regulate the balance between endothelial NO and superoxide production and provide insights into potential mechanisms by which PPARγ ligands could reduce pulmonary endothelial dysfunction.

PPARγ ligands also exert a variety of other effects on vascular wall cells that could be mediated, in part, by NO bioavailability. PPARγ ligands inhibit stimulated plasminogen activator inhibitor-1 production [63], inhibit smooth muscle cell migration and proliferation [64], and angiogenesis [65]. Nitroalkenes, the product of NO and unsaturated fatty acids, are potent endogenous PPARγ agonists that modulate PPARγ-regulated signaling events such as adipogenesis and CD36 expression in macrophages [66]. Nitroalkenes also stimulate relaxation of vessel segments in an NO-dependent manner [67] although their role in vascular regulation remains to be defined. Finally, in models of inflammation, PPARγ ligands reduce inducible nitric oxide synthase expression [68], cytokine-induced monocyte chemotactic protein-1 production [69], and endothelial-leukocyte adhesion [70]. Taken together, these reports illustrate that PPARγ plays a central role in regulating NO bioavailability and emphasize the potential relevance of PPARγ biology to both systemic and pulmonary vascular function.

3.2. Endothelin-1

ET-1 is a polypeptide that has been implicated in pulmonary hypertension pathogenesis. ET-1 is a potent vasoconstrictor that promotes platelet aggregation, and its receptors are upregulated in the lung in both animal models [71, 72] and patients with pulmonary hypertension [36]. ET-1, as well as endothelium-derived reactive oxygen species, attenuated NO-dependent pulmonary vasodilation following exposure to chronic hypoxia in isolated rat lungs [73]. ET-1-induced pulmonary vasoconstriction was markedly reduced by administration of Cu/Zn superoxide dismutase and was completely attenuated in $gp91^{phox}$ deficient mice [56]. 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 pulmonary hypertension to improve functional status and other indices of pulmonary hypertension-related morbidity [73], further suggesting that ET-1 is an important mediator of pulmonary vascular dysregulation. Limited evidence suggests that PPAR ligands inhibit ET-1 secretion by vascular endothelial cells [74, 75].

3.3. Prostacyclin

Prostacyclin, another endothelial-derived mediator involved in pulmonary vascular regulation, is a potent vasodilator that inhibits platelet aggregation and exerts anti-inflammatory, anti-thrombotic, and anti-proliferative vascular effects [76]. Overexpression of prostacyclin synthase protected mice from chronic hypoxia-induced pulmonary hypertension [77] whereas prostacyclin-receptor deficient mice were sensitized to hypoxia-induced pulmonary hypertension [78]. Decreased prostacyclin synthase expression has been noted in the pulmonary arteries of patients with severe pulmonary hypertension compared to normal subjects, and the

vascular endothelium was found to be the major site of lung vascular prostacyclin synthase expression [34]. In patients with pulmonary hypertension, prostacyclin derivatives decreased urinary isoprostane metabolites, an index of oxidative stress without altering thromboxane A2 [79]. Currently, this endothelial-derived mediator is a therapeutic target in the treatment of pulmonary hypertension [80], however the precise cellular mechanisms responsible for prostacyclin-mediated benefits remain to be defined.

Several studies have suggested 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 [81–83]. The ability of PPARγ ligands to inhibit COX-2 induction [84] suggests potential relationships between PPARγ and altered prostaglandin metabolism in vascular dysfunction. PPARβ/δ, a putative receptor for prostacyclin, was involved in prostacyclin-induced increases in endothelial cell survival [85] and has been implicated in the anti-thrombotic and anti-proliferative actions of prostacyclin [14, 15].

3.4. Rho/rho kinase

The small GTPase, Rho, and its associated effector, Rho-kinase play a central role in diverse cellular functions including smooth muscle contraction, cell proliferation, and gene expression. Several studies have demonstrated that the Rho/Rho-kinase pathway participates in the pathogenesis of pulmonary hypertension. Rho-kinase activation was involved in hypoxia-induced pulmonary vasoconstriction [86] and increased basal pulmonary vascular tone in chronically hypoxic rats [87]. Rho-kinase inhibition reversed acute hypoxic vasoconstriction [88] and attenuated the development of chronic hypoxia-induced pulmonary hypertension and vascular remodeling in mice [89]. Long-term inhibition of Rho-kinase also prevented or reversed monocrotaline-induced pulmonary hypertension in rats by enhancing apoptosis and reducing proliferation of pulmonary artery smooth muscle cells [90]. Interestingly, inhaled Rho-kinase inhibitors caused selective pulmonary artery pressure reduction in several models of pulmonary hypertension [91]. Hypoxia-induced Rho-kinase activation may also contribute to capillary angiogenesis and sustained vasoconstriction [92]. Collectively, these data suggest that the Rho/Rho-kinase pathway represents an attractive therapeutic target in pulmonary hypertension.

Recent evidence demonstrated that PPARγ activation inhibited the Rho/Rho-kinase pathway through upregulation of the protein tyrosine phosphatase, SHP-2 [93]. The demonstration that PPARγ ligands increased NO production [58, 59] and that NO increased SHP-2 activity and suppressed Rho/Rho kinase activation [94] provides additional evidence that this pathway may be amenable to manipulation with PPARγ ligands. Thus, the role of PPARγ in the regulation of the Rho/Rho-kinase pathway during pulmonary hypertension remains a promising area for continued investigation.

4. PPARγ AND SYSTEMIC VASCULAR DISEASE

To date, a more extensive literature has been devoted to investigation of PPARγ in the systemic than in the pulmonary circulation. In general, PPARγ activation attenuates endothelial dysfunction and the development of atherosclerosis. These findings are reviewed in brief to emphasize common pathways involved in PPARγ-mediated regulation of vascular function. In vivo studies of atherosclerosis in non-diabetic mouse models, including low-density lipoprotein receptor or apolipoprotein E-deficient mice, demonstrated that thiazolidindione PPARγ ligands reduced lesion formation [95–97] consistent with PPARγ-mediated vascular protection in non-diabetic vascular disease. PPARγ activation also inhibited VEGF receptor expression and decreased endothelial tube formation in rats [65] as well as reduced VEGF and leptin-induced migration of human endothelial cells [98]. Another important step in the development of atherosclerosis involves adhesion of inflammatory cells to the endothelium. PPARγ activation decreased expression of several adhesion molecules, specifically VCAM and ICAM in endothelial cells [99] and reduced monocyte-endothelial cell interaction [70].

In addition, a growing body of literature in animal and human subjects indicates that PPARγ ligand therapy is associated with improved endothelial function in vivo [100–103]. For example, pioglitazone and rosiglitazone decreased angiotensin II-induced hypertension and improved endothelium-dependent vasodilation in the rat [104]. Several mechanisms have been proposed for the antihypertensive effects of PPARγ ligands such as increased expression of PPARγ receptors in blood vessels [104], reduced expression of angiotensin II type I receptors [105], and more recently, direct inhibition of the Rho/Rho-kinase pathway [93]. In an ET-1-dependent hypertensive rat model, rosiglitazone restored endothelium-dependent vasodilation, diminished hypertension progression, and prevented vascular remodeling by decreasing ET-1 production and blunting production of reactive oxygen species [62]. Clinical data in diabetic subjects have demonstrated that thiazolidinedione PPARγ ligands: (a) reduced surrogate markers of vascular disease [101], (b) improved flow-mediated, endothelium-dependent vasodilation [102], and (c) reduced carotid intimal thickening [106] and neointimal formation after coronary stent placement [107]. The vascular protective effect of PPARγ ligands in humans was recently extended to nondiabetic subjects with documented coronary disease; rosiglitazone reduced common carotid arterial intima-media thickness progression [108]. Moreover, in healthy, nondiabetic individuals, rosiglitazone significantly increased flow-mediated endothelium-dependent vasodilation as well as reduced inflammatory biomarkers of atherosclerosis [109]. Finally, pioglitazone improved endothelial-dependent dilation in nondiabetic patients with cardiovascular risk factors [110]. Large clinical trials are currently underway that will ultimately determine if thiazolidinediones alter systemic vascular outcomes in patients with and without diabetes.

5. PPARγ AND PULMONARY HYPERTENSION

Several studies have suggested a potential role for PPARγ in the pathogenesis of pulmonary hypertension. For example, PPARγ is abundantly expressed in pulmonary vascular endothelial cells of normal human lung tissue and is significantly reduced in the plexiform lesions of human subjects with pulmonary hypertension [111]. Reduced PPARγ expression was also demonstrated in vascular lesions of a rat model of severe pulmonary hypertension caused by treatment with a VEGF receptor inhibitor in combination with hypobaric hypoxia exposure [111]. Furthermore, loss of PPARγ expression resulted in abnormal proliferation of apoptosis-resistant endothelial cells. The causal link between apoptosis and pulmonary hypertension-associated alterations in PPARγ expression remains to be established. However, additional evidence that vascular endothelial cell apoptosis is induced by overexpression of PPARγ or by treatment with 15d-PGJ_2 suggests fertile areas for future investigation [112]. Hypoxia as well as shear stress were implicated in reduced PPARγ expression in human endothelial-like cell lines [111]. Because oscillatory shear stress downregulates eNOS and upregulates ET-1 [113] and NADPH oxidase [114, 115], these findings suggest that the hemodynamic derangements in pulmonary hypertension may contribute to the development or propagation of vascular dysfunction and that reductions in PPARγ expression during pulmonary hypertension may lead to dysregulated production of a broad variety of vascular mediators that contribute to pulmonary vascular remodeling and pulmonary hemodynamic dysfunction.

Not only does pulmonary hypertension appear to be associated with reduced PPARγ expression, emerging evidence suggests that ligand-induced PPARγ activation attenuates pulmonary vascular dysfunction in animal models of pulmonary hypertension. For example, PPARγ activation with either pioglitazone or troglitazone significantly reduced pulmonary hypertension and pulmonary artery wall thickening in a rat model of monocrotaline-induced pulmonary hypertension [116]. Although the exact mechanisms by which PPARγ exerts its effects in pulmonary hypertension remain to be defined, several studies have shown that PPARγ activation reduced proliferation of vascular smooth muscle cells and promoted apoptosis in several cell lines in vitro [117, 118]. Murine models of pulmonary hypertension are characterized more by medial thickening of the pulmonary vasculature and lack the characteristic plexiform lesions composed of proliferative intraluminal endothelial cells that characterize human pulmonary hypertension [119]. These reports indicate that attenuation of monocrotaline-induced pulmonary hypertension may well be related to the capacity of PPARγ activation to inhibit vascular smooth muscle cell proliferation [116].

PPARγ ligands also attenuated hypoxia-induced pulmonary hypertension. Treatment with rosiglitazone reduced hypoxia-induced pulmonary artery remodeling in Wistar-Kyoto rats [120]. In this study rats were randomized to normoxia or hypobaric hypoxia and treated with rosiglitazone (2.5 mg/kg/day) for 3 weeks. Rosiglitazone decreased right ventricular hypertrophy and pulmonary arterial remodeling. Moreover, these changes were attributed to the inhibition of smooth muscle proliferation and were not associated with increased apoptosis further supporting previous findings in the monocrotaline-induced pulmonary hypertension model.

While little is known about the involvement of PPARβ/δ in pulmonary hypertension, recent data suggest that PPARβ/δ could be a potential therapeutic target. PPARβ/δ was activated by prostacyclin [15] suggesting that the beneficial effects of prostacyclin therapy, the current treatment of choice for many patients with severe pulmonary hypertension, could be mediated in part through activation of PPARβ/δ. Additionally, treprostinil sodium, a prostacylin mimetic, activated PPARβ/δ and inhibited proliferation of human lung fibroblasts at concentrations consistent with a PPAR rather than a prostacyclin receptor-mediated pathway [15]. These limited observations suggest that PPARβ/δ deserves additional study as a potential therapeutic target for treatment of pulmonary hypertension.

6. FUTURE DIRECTIONS AND CONCLUSIONS

In unpublished data, we have observed that exposure to chronic hypoxia (10% oxygen) for 3 weeks reduced lung PPARγ expression and caused pulmonary hypertension in C57Bl/6 mice as indicated by elevation of right ventricular systolic pressure and right ventricular hypertrophy. Treatment with rosiglitazone (10 mg/kg/day) by gavage during the final 10 days of this hypoxia exposure regimen attenuated pulmonary hypertension and right ventricular hypertrophy. Hypoxia-induced pulmonary hypertension was also associated with reductions in serum levels of nitrosylhemoglobin (NO-Hgb), an index of NO bioavailability. Hypoxia-induced alterations in NO bioavailability were not associated with lower eNOS protein levels. These preliminary findings further support the hypothesis that ligand-induced PPARγ activation attenuates hypoxia-induced reductions in NO bioavailability in part by suppressing the generation of reactive oxygen species that inactivate NO such as superoxide [61, 120, 121] and in part by promoting eNOS activity through modification of post-translational regulatory mechanisms [58]. Taken together, these findings suggest that PPARγ may represent a novel potential therapeutic target in pulmonary hypertension that modulates nitroso-redox balance in the vasculature. The relationships between PPARγ and selected aspects of endothelial dysfunction in pulmonary hypertension are schematically presented in Figure 1.

Current evidence strongly suggests that vascular endothelial dysregulation plays a crucial role in the initiation and progression of pulmonary hypertension. Moreover, alterations in endothelium-derived mediators such as NO, ET-1, and prostanoids as well as reactive oxygen species have been established as important mechanisms in the development of vascular remodeling leading to pulmonary hypertension. Our understanding of PPARγ biology has progressed rapidly over the last decade but much remains to be learned about the mechanisms by which these receptors and

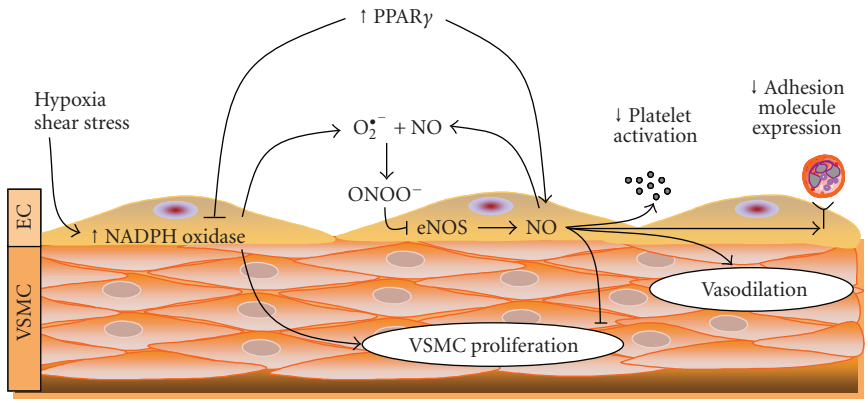

Figure 1: *The effects of PPARγ activation on reactive oxygen species and nitric oxide production in the vascular wall.* Factors including hypoxia and shear stress increase the production of superoxide in the vascular wall by NADPH oxidase. Superoxide ($O_2^{\bullet-}$) rapidly reacts with nitric oxide (NO) generated by endothelial nitric oxide synthase (eNOS) to reduce the bioavailability of NO to stimulate vasodilation and inhibit vascular smooth muscle cell (VSMC) proliferation, platelet activation, and adhesion molecule expression. PPARγ activation inhibits NADPH oxidase expression and activity [61] and stimulates NO production in vascular endothelial cells (EC) [58, 59]. These effects illustrate potential mechanisms by which PPARγ activation may favorably modulate pulmonary endothelial dysfunction and pulmonary hypertension.

their ligands regulate the pulmonary vasculature. Identifying specific downstream targets regulated by PPARs in the pulmonary vasculature will facilitate the development of potential PPAR-related therapeutic strategies for the prevention or treatment of pulmonary hypertension.

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REFERENCES

[1] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[2] A. J. Gilde, K. A. J. M. van der Lee, P. H. M. Willemsen, et al., "Peroxisome proliferator-activated receptor (PPAR) α and PPARβ/δ, but not PPARγ, modulate the expression of genes involved in cardiac lipid metabolism," *Circulation Research*, vol. 92, no. 5, pp. 518–524, 2003.

[3] I. Saluja, J. G. Granneman, and R. P. Skoff, "PPAR δ agonists stimulate oligodendrocyte differentiation in tissue culture," *GLIA*, vol. 33, no. 3, pp. 191–204, 2001.

[4] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor δ on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2002.

[5] N. S. Tan, L. Michalik, B. Desvergne, and W. Wahli, "Multiple expression control mechanisms of peroxisome proliferator-activated receptors and their target genes," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 93, no. 2–5, pp. 99–105, 2005.

[6] C.-H. Lee, A. Chawla, N. Urbiztondo, D. Liao, W. A. Boisvert, R. M. Evans, and L. K. Curtiss, "Transcriptional repression of atherogenic inflammation: modulation by PPARδ," *Science*, vol. 302, no. 5644, pp. 453–457, 2003.

[7] G. Chinetti, S. Griglio, M. Antonucci, et al., "Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages," *Journal of Biological Chemistry*, vol. 273, no. 40, pp. 25573–25580, 1998.

[8] R. A. Daynes and D. C. Jones, "Emerging roles of PPARs in inflammation and immunity," *Nature Reviews Immunology*, vol. 2, no. 10, pp. 748–759, 2002.

[9] V. G. Keshamouni, D. A. Arenberg, R. C. Reddy, M. J. Newstead, S. Anthwal, and T. J. Standiford, "PPAR-γ activation inhibits angiogenesis by blocking ELR+CXC chemokine production in non-small cell lung cancer," *Neoplasia*, vol. 7, no. 3, pp. 294–301, 2005.

[10] V. G. Keshamouni, R. C. Reddy, D. A. Arenberg, et al., "Peroxisome proliferator-activated receptor-γ activation inhibits tumor progression in non-small-cell lung cancer," *Oncogene*, vol. 23, no. 1, pp. 100–108, 2004.

[11] K. L. Gearing, M. Gottlicher, M. Teboul, E. Widmark, and J.-A. Gustafsson, "Interaction of the peroxisome-proliferator-activated receptor and retinoid X receptor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 4, pp. 1440–1444, 1993.

[12] E. D. Rosen and B. M. Spiegelman, "PPARγ: a nuclear regulator of metabolism, differentiation, and cell growth," *Journal of Biological Chemistry*, vol. 276, no. 41, pp. 37731–37734, 2001.

[13] H. E. Xu, T. B. Stanley, V. G. Montana, et al., "Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPARα," *Nature*, vol. 415, no. 6873, pp. 813–817, 2002.

[14] F. Y. Ali, S. J. Davidson, L. A. Moraes, et al., "Role of nuclear receptor signaling in platelets: antithrombotic effects of PPARβ," *The FASEB Journal*, vol. 20, no. 2, pp. 326–328, 2006.

[15] F. Y. Ali, K. Egan, G. A. FitzGerald, et al., "Role of prostacyclin versus peroxisome proliferator-activated receptor β receptors in prostacyclin sensing by lung fibroblasts," *American Journal of Respiratory Cell and Molecular Biology*, vol. 34, no. 2, pp. 242–246, 2006.

[16] P. Tontonoz, L. Nagy, J. G. A. Alvarez, V. A. Thomazy, and R. M. Evans, "PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL," *Cell*, vol. 93, no. 2, pp. 241–252, 1998.

[17] H. S. Camp, O. Li, S. C. Wise, et al., "Differential activation of peroxisome proliferator-activated receptor-γ by troglitazone and rosiglitazone," *Diabetes*, vol. 49, no. 4, pp. 539–547, 2000.

[18] Y. Kodera, K.-I. Takeyama, A. Murayama, M. Suzawa, Y. Masuhiro, and S. Kato, "Ligand type-specific interactions of peroxisome proliferator-activated receptor γ with transcriptional coactivators," *Journal of Biological Chemistry*, vol. 275, no. 43, pp. 33201–33204, 2000.

[19] J. M. Olefsky, "Treatment of insulin resistance with peroxisome proliferator-activated receptor γ agonists," *Journal of Clinical Investigation*, vol. 106, no. 4, pp. 467–472, 2000.

[20] M. A. Haidara, H. Z. Yassin, M. Rateb, H. Ammar, and M. A. Zorkani, "Role of oxidative stress in development of cardiovascular complications in diabetes mellitus," *Current Vascular Pharmacology*, vol. 4, no. 3, pp. 215–227, 2006.

[21] R. E. Schmieder, "Endothelial dysfunction: how can one intervene at the beginning of the cardiovascular continuum?" *Journal of Hypertension*, vol. 24, supplement 2, pp. S31–S35, 2006.

[22] R. Budhiraja, R. M. Tuder, and P. M. Hassoun, "Endothelial dysfunction in pulmonary hypertension," *Circulation*, vol. 109, no. 2, pp. 159–165, 2004.

[23] A. Hyduk, J. B. Croft, C. Ayala, K. Zheng, Z. J. Zheng, and G. A. Mensah, "Pulmonary hypertension surveillance—United States, 1980–2002," *Morbidity and Mortality Weekly Report*, vol. 54, no. 5, pp. 1–28, 2005.

[24] L. Du, C. C. Sullivan, D. Chu, et al., "Signaling molecules in nonfamilial pulmonary hypertension," *The New England Journal of Medicine*, vol. 348, no. 6, pp. 500–509, 2003.

[25] J. H. Newman, L. Wheeler, K. B. Lane, et al., "Mutation in the gene for bone morphogenetic protein receptor II as a cause of primary pulmonary hypertension in a large kindred," *The New England Journal of Medicine*, vol. 345, no. 5, pp. 319–324, 2001.

[26] U. C. Garg and A. Hassid, "Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells," *Journal of Clinical Investigation*, vol. 83, no. 5, pp. 1774–1777, 1989.

[27] H. Azuma, M. Ishikawa, and S. Sekizaki, "Endothelium-dependent inhibition of platelet aggregation," *British Journal of Pharmacology*, vol. 88, no. 2, pp. 411–415, 1986.

[28] A. P. L. Smith, E. A. G. Demoncheaux, and T. W. Higenbottam, "Nitric oxide gas decreases endothelin-1 mRNA in cultured pulmonary artery endothelial cells," *Nitric Oxide*, vol. 6, no. 2, pp. 153–159, 2002.

[29] M. A. Perrella, E. S. Edell, M. J. Krowka, D. A. Cortese, and J. C. Burnett Jr., "Endothelium-derived relaxing factor in pulmonary and renal circulations during hypoxia," *American Journal of Physiology*, vol. 263, no. 1, part 2, pp. R45–R50, 1992.

[30] F. Ichinose, R. Ullrich, A. Sapirstein, et al., "Cytosolic phospholipase A_2 in hypoxic pulmonary vasoconstriction," *Journal of Clinical Investigation*, vol. 109, no. 11, pp. 1493–1500, 2002.

[31] J. Wang, M. Juhaszova, L. J. Rubin, and X.-J. Yuan, "Hypoxia inhibits gene expression of voltage-gated K^+ channel α subunits in pulmonary artery smooth muscle cells," *Journal of Clinical Investigation*, vol. 100, no. 9, pp. 2347–2353, 1997.

[32] S. Adnot, B. Raffestin, S. Eddahibi, P. Braquet, and P.-E. Chabrier, "Loss of endothelium-dependent relaxant activity in the pulmonary circulation of rats exposed to chronic hypoxia," *Journal of Clinical Investigation*, vol. 87, no. 1, pp. 155–162, 1991.

[33] R. H. Steinhorn, J. A. Russell, S. Lakshminrusimha, S. F. Gugino, S. M. Black, and J. R. Fineman, "Altered endothelium-dependent relaxations in lambs with high pulmonary blood flow and pulmonary hypertension," *American Journal of Physiology*, vol. 280, no. 1, pp. H311–H317, 2001.

[34] R. M. Tuder, C. D. Cool, M. W. Geraci, et al., "Prostacyclin synthase expression is decreased in lungs from patients with severe pulmonary hypertension," *American Journal of Respiratory and Critical Care Medicine*, vol. 159, no. 6, pp. 1925–1932, 1999.

[35] L. P. McQuillan, G. K. Leung, P. A. Marsden, S. K. Kostyk, and S. Kourembanas, "Hypoxia inhibits expression of eNOS via transcriptional and posttranscriptional mechanisms," *American Journal of Physiology*, vol. 267, no. 5, part 2, pp. H1921–H1927, 1994.

[36] A. Giaid and D. Saleh, "Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension," *The New England Journal of Medicine*, vol. 333, no. 4, pp. 214–221, 1995.

[37] C. Xue and R. A. Johns, "Endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension," *The New England Journal of Medicine*, vol. 333, no. 24, pp. 1642–1644, 1995.

[38] J. P. Khoo, L. Zhao, N. J. Alp, et al., "Pivotal role for endothelial tetrahydrobiopterin in pulmonary hypertension," *Circulation*, vol. 111, no. 16, pp. 2126–2133, 2005.

[39] M. Nandi, A. Miller, R. Stidwill, et al., "Pulmonary hypertension in a GTP-cyclohydrolase 1-deficient mouse," *Circulation*, vol. 111, no. 16, pp. 2086–2090, 2005.

[40] P. Rosenkranz-Weiss, W. C. Sessa, S. Milstien, S. Kaufman, C. A. Watson, and J. S. Pober, "Regulation of nitric oxide synthesis by proinflammatory cytokines in human umbilical vein endothelial cells. Elevations in tetrahydrobiopterin levels enhance endothelial nitric oxide synthase specific activity," *Journal of Clinical Investigation*, vol. 93, no. 5, pp. 2236–2243, 1994.

[41] S. Dimmeler, I. Fleming, B. Fisslthaler, C. Hermann, R. Busse, and A. M. Zeiher, "Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation," *Nature*, vol. 399, no. 6736, pp. 601–605, 1999.

[42] D. Fulton, J.-P. Gratton, T. J. McCabe, et al., "Regulation of endothelium-derived nitric oxide production by the protein kinase Akt," *Nature*, vol. 399, no. 6736, pp. 597–601, 1999.

[43] T. Murata, M. Hori, K. Sakamoto, H. Karaki, and H. Ozaki, "Dexamethasone blocks hypoxia-induced endothelial dysfunction in organ-cultured pulmonary arteries," *American Journal of Respiratory and Critical Care Medicine*, vol. 170, no. 6, pp. 647–655, 2004.

[44] G. G. Konduri, J. Ou, Y. Shi, and K. A. Pritchard Jr., "Decreased association of HSP90 impairs endothelial nitric oxide synthase in fetal lambs with persistent pulmonary hypertension," *American Journal of Physiology*, vol. 285, no. 1, pp. H204–H211, 2003.

[45] G. García-Cardeña, R. Fan, V. Shah, et al., "Dynamic activation of endothelial nitric oxide synthase by Hsp90," *Nature*, vol. 392, no. 6678, pp. 821–824, 1998.

[46] T. Murata, K. Sato, M. Hori, H. Ozaki, and H. Karaki, "Decreased endothelial nitric-oxide synthase (eNOS) activity resulting from abnormal interaction between eNOS and its

regulatory proteins in hypoxia-induced pulmonary hypertension," *Journal of Biological Chemistry*, vol. 277, no. 46, pp. 44085–44092, 2002.

[47] S. Muzaffar, N. Shukla, G. D. Angelini, and J. Y. Jeremy, "Acute hypoxia simultaneously induces the expression of $gp91^{phox}$ and endothelial nitric oxide synthase in the porcine pulmonary artery," *Thorax*, vol. 60, no. 4, pp. 305–313, 2005.

[48] K. A. Pritchard Jr., A. W. Ackerman, E. R. Gross, et al., "Heat shock protein 90 mediates the balance of nitric oxide and superoxide anion from endothelial nitric-oxide synthase," *Journal of Biological Chemistry*, vol. 276, no. 21, pp. 17621–17624, 2001.

[49] J. S. Beckman and W. H. Koppenol, "Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly," *American Journal of Physiology*, vol. 271, no. 5, part 1, pp. C1424–C1437, 1996.

[50] N. Kuzkaya, N. Weissmann, D. G. Harrison, and S. Dikalov, "Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric-oxide synthase," *Journal of Biological Chemistry*, vol. 278, no. 25, pp. 22546–22554, 2003.

[51] U. Landmesser, S. Dikalov, S. R. Price, et al., "Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension," *Journal of Clinical Investigation*, vol. 111, no. 8, pp. 1201–1209, 2003.

[52] S. Mehta, D. J. Stewart, D. Langleben, and R. D. Levy, "Short-term pulmonary vasodilation with L-arginine in pulmonary hypertension," *Circulation*, vol. 92, no. 6, pp. 1539–1545, 1995.

[53] R. N. Channick, J. W. Newhart, F. W. Johnson, et al., "Pulsed delivery of inhaled nitric oxide to patients with primary pulmonary hypertension: an ambulatory delivery system and initial clinical tests," *Chest*, vol. 109, no. 6, pp. 1545–1549, 1996.

[54] C. Marshall, A. J. Mamary, A. J. Verhoeven, and B. E. Marshall, "Pulmonary artery NADPH-oxidase is activated in hypoxic pulmonary vasoconstriction," *American Journal of Respiratory Cell and Molecular Biology*, vol. 15, no. 5, pp. 633–644, 1996.

[55] N. Weissmann, S. Zeller, R. U. Schäfer, et al., "Impact of mitochondria and NADPH oxidases on acute and sustained hypoxic pulmonary vasoconstriction," *American Journal of Respiratory Cell and Molecular Biology*, vol. 34, no. 4, pp. 505–513, 2006.

[56] J. Q. Liu, I. N. Zelko, E. M. Erbynn, J. S. K. Sham, and R. J. Folz, "Hypoxic pulmonary hypertension: role of superoxide and NADPH oxidase ($gp91^{phox}$)," *American Journal of Physiology*, vol. 290, no. 1, pp. L2–L10, 2006.

[57] J. Q. Liu, E. M. Erbynn, and R. J. Folz, "Chronic hypoxia-enhanced murine pulmonary vasoconstriction: role of superoxide and $gp91^{phox}$," *Chest*, vol. 128, no. 6 supplement, pp. 594S–596S, 2005.

[58] J. A. Polikandriotis, L. J. Mazzella, H. L. Rupnow, and C. M. Hart, "Peroxisome proliferator-activated receptor γ ligands stimulate endothelial nitric oxide production through distinct peroxisome proliferator-activated receptor γ-dependent mechanisms," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 9, pp. 1810–1816, 2005.

[59] D. S. Calnek, L. J. Mazzella, S. Roser, J. Roman, and C. M. Hart, "Peroxisome proliferator-activated receptor γ ligands increase release of nitric oxide from endothelial cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 1, pp. 52–57, 2003.

[60] I. Inoue, S.-I. Goto, T. Matsunaga, et al., "The ligands/activators for peroxisome proliferator-activated receptor α (PPARα) and PPARγ increase Cu^{2+},Zn^{2+}-superoxide dismutase and decrease p22phox message expressions in primary endothelial cells," *Metabolism*, vol. 50, no. 1, pp. 3–11, 2001.

[61] J. Hwang, D. J. Kleinhenz, B. Lassègue, K. K. Griendling, S. Dikalov, and C. M. Hart, "Peroxisome proliferator-activated receptor-γ ligands regulate endothelial membrane superoxide production," *American Journal of Physiology*, vol. 288, no. 4, pp. C899–C905, 2005.

[62] M. Iglarz, R. M. Touyz, F. Amiri, M.-F. Lavoie, Q. N. Diep, and E. L. Schiffrin, "Effect of peroxisome proliferator-activated receptor-α and -γ activators on vascular remodeling in endothelin-dependent hypertension," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 1, pp. 45–51, 2003.

[63] K. Kato, H. Satoh, Y. Endo, et al., "Thiazolidinediones down-regulate plasminogen activator inhibitor type 1 expression in human vascular endothelial cells: a possible role for PPARγ in endothelial function," *Biochemical and Biophysical Research Communications*, vol. 258, no. 2, pp. 431–435, 1999.

[64] R. E. Law, W. P. Meehan, X.-P. Xi, et al., "Troglitazone inhibits vascular smooth muscle cell growth and intimal hyperplasia," *Journal of Clinical Investigation*, vol. 98, no. 8, pp. 1897–1905, 1996.

[65] X. Xin, S. Yang, J. Kowalski, and M. E. Gerritsen, "Peroxisome proliferator-activated receptor γ ligands are potent inhibitors of angiogenesis in vitro and in vivo," *Journal of Biological Chemistry*, vol. 274, no. 13, pp. 9116–9121, 1999.

[66] F. J. Schopfer, Y. Lin, P. R. S. Baker, et al., "Nitrolinoleic acid: an endogenous peroxisome proliferator-activated receptor γ ligand," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 7, pp. 2340–2345, 2005.

[67] T. Cui, F. J. Schopfer, J. Zhang, et al., "Nitrated fatty acids: endogenous anti-inflammatory signaling mediators," *Journal of Biological Chemistry*, vol. 281, no. 47, pp. 35686–35698, 2006.

[68] U. Ikeda, M. Shimpo, Y. Murakami, and K. Shimada, "Peroxisome proliferator-activated receptor-γ ligands inhibit nitric oxide synthesis in vascular smooth muscle cells," *Hypertension*, vol. 35, no. 6, pp. 1232–1236, 2000.

[69] K. Murao, H. Imachi, A. Momoi, et al., "Thiazolidinedione inhibits the production of monocyte chemoattractant protein-1 in cytokine-treated human vascular endothelial cells," *FEBS Letters*, vol. 454, no. 1-2, pp. 27–30, 1999.

[70] S. M. Jackson, F. Parhami, X.-P. Xi, et al., "Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte-endothelial cell interaction," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 19, no. 9, pp. 2094–2104, 1999.

[71] H. F. Frasch, C. Marshall, and B. E. Marshall, "Endothelin-1 is elevated in monocrotaline pulmonary hypertension," *American Journal of Physiology*, vol. 276, no. 2, part 1, pp. L304–L310, 1999.

[72] H. Li, S.-J. Chen, Y.-F. Chen, et al., "Enhanced endothelin-1 and endothelin receptor gene expression in chronic hypoxia," *Journal of Applied Physiology*, vol. 77, no. 3, pp. 1451–1459, 1994.

[73] N. L. Jernigan, B. R. Walker, and T. C. Resta, "Endothelium-derived reactive oxygen species and endothelin-1 attenuate NO-dependent pulmonary vasodilation following chronic hypoxia," *American Journal of Physiology*, vol. 287, no. 4, pp. L801–L808, 2004.

[74] P. Delerive, F. Martin-Nizard, G. Chinetti, et al., "Peroxisome proliferator-activated receptor activators inhibit thrombin-induced endothelin-1 production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway," *Circulation Research*, vol. 85, no. 5, pp. 394–402, 1999.

[75] F. Martin-Nizard, C. Furman, P. Delerive, et al., "Peroxisome proliferator-activated receptor activators inhibit oxidized low-density lipoprotein-induced endothelin-1 secretion in endothelial cells," *Journal of Cardiovascular Pharmacology*, vol. 40, no. 6, pp. 822–831, 2002.

[76] H. Olschewski, F. Rose, R. Schermuly, et al., "Prostacyclin and its analogues in the treatment of pulmonary hypertension," *Pharmacology and Therapeutics*, vol. 102, no. 2, pp. 139–153, 2004.

[77] M. W. Geraci, B. Gao, D. C. Shepherd, et al., "Pulmonary prostacyclin synthase overexpression in transgenic mice protects against development of hypoxic pulmonary hypertension," *Journal of Clinical Investigation*, vol. 103, no. 11, pp. 1509–1515, 1999.

[78] Y. Hoshikawa, N. F. Voelkel, T. L. Gesell, et al., "Prostacyclin receptor-dependent modulation of pulmonary vascular remodeling," *American Journal of Respiratory and Critical Care Medicine*, vol. 164, no. 2, pp. 314–318, 2001.

[79] I. M. Robbins, J. D. Morrow, and B. W. Christman, "Oxidant stress but not thromboxane decreases with epoprostenol therapy," *Free Radical Biology and Medicine*, vol. 38, no. 5, pp. 568–574, 2005.

[80] E. B. Rosenzweig and R. J. Barst, "Pulmonary arterial hypertension: a comprehensive review of pharmacological treatment," *Treatments in Respiratory Medicine*, vol. 5, no. 2, pp. 117–127, 2006.

[81] D. Bishop-Bailey, "Peroxisome proliferator-activated receptors in the cardiovascular system," *British Journal of Pharmacology*, vol. 129, no. 5, pp. 823–834, 2000.

[82] M. E. Burleigh, V. R. Babaev, J. A. Oates, et al., "Cyclooxygenase-2 promotes early atherosclerotic lesion formation in LDL receptor-deficient mice," *Circulation*, vol. 105, no. 15, pp. 1816–1823, 2002.

[83] R. E. Law, S. Goetze, X.-P. Xi, et al., "Expression and function of PPARγ in rat and human vascular smooth muscle cells," *Circulation*, vol. 101, no. 11, pp. 1311–1318, 2000.

[84] K. Subbaramaiah, D. T. Lin, J. C. Hart, and A. J. Dannenberg, "Peroxisome proliferator-activated receptor γ ligands suppress the transcriptional activation of cyclooxygenase-2. Evidence for involvement of activator protein-1 and CREB-binding protein/p300," *Journal of Biological Chemistry*, vol. 276, no. 15, pp. 12440–12448, 2001.

[85] J.-Y. Liou, S. Lee, D. Ghelani, N. Matijevic-Aleksic, and K. K. Wu, "Protection of endothelial survival by peroxisome proliferator-activated receptor-δ mediated 14-3-3 upregulation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 7, pp. 1481–1487, 2006.

[86] Z. Wang, N. Jin, S. Ganguli, D. R. Swartz, L. Li, and R. A. Rhoades, "Rho-kinase activation is involved in hypoxia-induced pulmonary vasoconstriction," *American Journal of Respiratory Cell and Molecular Biology*, vol. 25, no. 5, pp. 628–635, 2001.

[87] T. Nagaoka, Y. Morio, N. Casanova, et al., "Rho/Rho-kinase signaling mediates increased basal pulmonary vascular tone in chronically hypoxic rats," *American Journal of Physiology*, vol. 287, no. 4, pp. L665–L672, 2004.

[88] T. P. Robertson, M. Dipp, J. P. T. Ward, P. I. Aaronson, and A. M. Evans, "Inhibition of sustained hypoxic vasoconstriction by Y-27632 in isolated intrapulmonary arteries and perfused lung of the rat," *British Journal of Pharmacology*, vol. 131, no. 1, pp. 5–9, 2000.

[89] K. A. Fagan, M. Oka, N. R. Bauer, et al., "Attenuation of acute hypoxic pulmonary vasoconstriction and hypoxic pulmonary hypertension in mice by inhibition of Rho-kinase," *American Journal of Physiology*, vol. 287, no. 4, pp. L656–L664, 2004.

[90] K. Abe, H. Shimokawa, K. Morikawa, et al., "Long-term treatment with a Rho-kinase inhibitor improves monocrotaline-induced fatal pulmonary hypertension in rats," *Circulation Research*, vol. 94, no. 3, pp. 385–393, 2004.

[91] T. Nagaoka, K. A. Fagan, S. A. Gebb, et al., "Inhaled Rho-kinase inhibitors are potent and selective vasodilators in rat pulmonary hypertension," *American Journal of Respiratory and Critical Care Medicine*, vol. 171, no. 5, pp. 494–499, 2005.

[92] J.-M. Hyvelin, K. Howell, A. Nichol, C. M. Costello, R. J. Preston, and P. McLoughlin, "Inhibition of Rho-kinase attenuates hypoxia-induced angiogenesis in the pulmonary circulation," *Circulation Research*, vol. 97, no. 2, pp. 185–191, 2005.

[93] S. Wakino, K. Hayashi, T. Kanda, et al., "Peroxisome proliferator-activated receptor γ ligands inhibit Rho/Rho-kinase pathway by inducing protein tyrosine phosphatase SHP-2," *Circulation Research*, vol. 95, no. 5, pp. e45–e55, 2004.

[94] Y. Chang, B. Ceacareanu, M. Dixit, N. Sreejayan, and A. Hassid, "Nitric oxide-induced motility in aortic smooth muscle cells: role of protein tyrosine phosphatase SHP-2 and GTP-binding protein Rho," *Circulation Research*, vol. 91, no. 5, pp. 390–397, 2002.

[95] Z. Chen, S. Ishibashi, S. Perrey, et al., "Troglitazone inhibits atherosclerosis in apolipoprotein E-knockout mice: pleiotropic effects on CD36 expression and HDL," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 21, no. 3, pp. 372–377, 2001.

[96] A. R. Collins, W. P. Meehan, U. Kintscher, et al., "Troglitazone inhibits formation of early atherosclerotic lesions in diabetic and nondiabetic low density lipoprotein receptor-deficient mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 21, no. 3, pp. 365–371, 2001.

[97] A. C. Li, K. K. Brown, M. J. Silvestre, T. M. Willson, W. Palinski, and C. K. Glass, "Peroxisome proliferator-activated receptor γ ligands inhibit development of atherosclerosis in LDL receptor-deficient mice," *Journal of Clinical Investigation*, vol. 106, no. 4, pp. 523–531, 2000.

[98] S. Goetze, A. Bungenstock, C. Czupalla, et al., "Leptin induces endothelial cell migration through Akt, which is inhibited by PPARγ-ligands," *Hypertension*, vol. 40, no. 5, pp. 748–754, 2002.

[99] C. M. Halabi and C. D. Sigmund, "Peroxisome proliferator-activated receptor-γ and its agonists in hypertension and atherosclerosis: mechanisms and clinical implications," *American Journal of Cardiovascular Drugs*, vol. 5, no. 6, pp. 389–398, 2005.

[100] R. Avena, M. E. Mitchell, E. S. Nylen, K. M. Curry, and A. N. Sidawy, "Insulin action enhancement normalizes brachial artery vasoactivity in patients with peripheral vascular disease and occult diabetes," *Journal of Vascular Surgery*, vol. 28, no. 6, pp. 1024–1032, 1998, discussion 1031–1032.

[101] L. Cominacini, U. Garbin, A. Fratta Pasini, et al., "Troglitazone reduces LDL oxidation and lowers plasma E-selectin

concentration in NIDDM patients," *Diabetes*, vol. 47, no. 1, pp. 130–133, 1998.

[102] T. Murakami, S. Mizuno, K. Ohsato, et al., "Effects of troglitazone on frequency of coronary vasospastic-lnduced angina pectoris in patients with diabetes mellitus," *American Journal of Cardiology*, vol. 84, no. 1, pp. 92–94, 1999.

[103] A. B. Walker, P. D. Chattington, R. E. Buckingham, and G. Williams, "The thiazolidinedione rosiglitazone (BRL-49653) lowers blood pressure and protects against impairment of endothelial function in Zucker fatty rats," *Diabetes*, vol. 48, no. 7, pp. 1448–1453, 1999.

[104] Q. N. Diep, M. El Mabrouk, J. S. Cohn, et al., "Structure, endothelial function, cell growth, and inflammation in blood vessels of angiotensin II-infused rats: role of peroxisome proliferator-activated receptor-γ," *Circulation*, vol. 105, no. 19, pp. 2296–2302, 2002.

[105] K. Takeda, T. Ichiki, T. Tokunou, et al., "Peroxisome proliferator-activated receptor γ activators downregulate angiotensin II type 1 receptor in vascular smooth muscle cells," *Circulation*, vol. 102, no. 15, pp. 1834–1839, 2000.

[106] J. Minamikawa, S. Tanaka, M. Yamauchi, D. Inoue, and H. Koshiyama, "Potent inhibitory effect of troglitazone on carotid arterial wall thickness in type 2 diabetes," *Journal of Clinical Endocrinology and Metabolism*, vol. 83, no. 5, pp. 1818–1820, 1998.

[107] T. Takagi, T. Akasaka, A. Yamamuro, et al., "Troglitazone reduces neointimal tissue proliferation after coronary stent implantation in patients with non-insulin dependent diabetes mellitus: a serial intravascular ultrasound study," *Journal of the American College of Cardiology*, vol. 36, no. 5, pp. 1529–1535, 2000.

[108] J. S. Sidhu, Z. Kaposzta, H. S. Markus, and J. C. Kaski, "Effect of rosiglitazone on common carotid intima-media thickness progression in coronary artery disease patients without diabetes mellitus," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 5, pp. 930–934, 2004.

[109] J. Hetzel, B. Balletshofer, K. Rittig, et al., "Rapid effects of rosiglitazone treatment on endothelial function and inflammatory biomarkers," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 9, pp. 1804–1809, 2005.

[110] U. Campia, L. A. Matuskey, and J. A. Panza, "Peroxisome proliferator-activated receptor-γ activation with pioglitazone improves endothelium-dependent dilation in nondiabetic patients with major cardiovascular risk factors," *Circulation*, vol. 113, no. 6, pp. 867–875, 2006.

[111] S. Ameshima, H. Golpon, C. D. Cool, et al., "Peroxisome proliferator-activated receptor gamma (PPARγ) expression is decreased in pulmonary hypertension and affects endothelial cell growth," *Circulation Research*, vol. 92, no. 10, pp. 1162–1169, 2003.

[112] D. Bishop-Bailey and T. Hla, "Endothelial cell apoptosis induced by the peroxisome proliferator- activated receptor (PPAR) ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2," *Journal of Biological Chemistry*, vol. 274, no. 24, pp. 17042–17048, 1999.

[113] M. B. Dancu, D. E. Berardi, J. P. Vanden Heuvel, and J. M. Tarbell, "Asynchronous shear stress and circumferential strain reduces endothelial NO synthase and cyclooxygenase-2 but induces endothelin-1 gene expression in endothelial cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 11, pp. 2088–2094, 2004.

[114] J. Hwang, A. Saha, Y. C. Boo, et al., "Oscillatory shear stress stimulates endothelial production of O_2^- from $p47^{phox}$-dependent NAD(P)H oxidases, leading to monocyte adhesion," *Journal of Biological Chemistry*, vol. 278, no. 47, pp. 47291–47298, 2003.

[115] J. S. McNally, M. E. Davis, D. P. Giddens, et al., "Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress," *American Journal of Physiology*, vol. 285, no. 6, pp. H2290–H2297, 2003.

[116] Y. Matsuda, Y. Hoshikawa, S. Ameshima, et al., "Effects of peroxisome proliferator-activated receptor γ ligands on monocrotaline-induced pulmonary hypertension in rats," *Nihon Kokyuki Gakkai Zasshi*, vol. 43, no. 5, pp. 283–288, 2005.

[117] Y. Lin, X. Zhu, F. L. McIntee, H. Xiao, J. Zhang, M. Fu, and Y. E. Chen, "Interferon regulatory factor-1 mediates PPARγ-induced apoptosis in vascular smooth muscle cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 2, pp. 257–263, 2004.

[118] T. Okura, M. Nakamura, Y. Takata, S. Watanabe, Y. Kitami, and K. Hiwada, "Troglitazone induces apoptosis via the p53 and Gadd45 pathway in vascular smooth muscle cells," *European Journal of Pharmacology*, vol. 407, no. 3, pp. 227–235, 2000.

[119] K. R. Stenmark and I. F. McMurtry, "Vascular remodeling versus vasoconstriction in chronic hypoxic pulmonary hypertension: a time for reappraisal?" *Circulation Research*, vol. 97, no. 2, pp. 95–98, 2005.

[120] J. T. Crossno Jr., K. G. Morris Jr., and D. J. Klemm, "Attenuation of hypoxia-induced pulmonary artery remodeling by a peroxisome proliferator-activated receptor-γ agonist," *Chest*, vol. 128, supplement 6, p. 580S, 2005.

[121] J. Hwang, D. J. Kleinhenz, H. L. Rupnow, et al., "The PPARγ ligand, rosiglitazone, reduces vascular oxidative stress and NADPH oxidase expression in diabetic mice," *Vascular Pharmacology*, vol. 46, no. 6, pp. 456–462, 2007.

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Review Article

Peroxisome Proliferator-Activated Receptors in Lung Cancer

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily. Their discovery in the 1990s provided insights into the cellular mechanisms involved in the control of energy homeostasis; the regulation of cell differentiation, proliferation, and apoptosis; and the modulation of important biological and pathological processes related to inflammation, among others. Since then, PPARs have become an exciting therapeutic target for several diseases. PPARs are expressed by many tumors including lung carcinoma cells, and their function has been linked to the process of carcinogenesis in lung. Consequently, intense research is being conducted in this area with the hope of discovering new PPAR-related therapeutic targets for the treatment of lung cancer. This review summarizes the research being conducted in this area and focuses on the mechanisms by which PPARs are believed to affect lung tumor cell biology.

1. INTRODUCTION

Lung cancer is the leading cause of cancer death in the world for both men and women [1]. Primary malignant cancers of the lung are classified into small cell lung cancer (SCLC) and nonsmall cell lung cancer (NSCLC) [2]. NSCLC accounts for 75% and SCLC constitutes the remainder. Based on the cellular phenotype, NSCLC is further subdivided into squamous cell carcinoma, adenocarcinoma, and large cell carcinomas [2]. Despite advances in understanding the mechanisms involved in carcinogenesis, the development of new surgical procedures, and the use of new radio and chemotherapeutic protocols, the 5-year survival rate for lung cancer patients is poor and remains less than 15% [1]. This underscores the desperate need for novel strategies for early detection, prevention, and treatment of this disease.

Peroxisome proliferator-activated receptors (PPARs) have recently emerged as potential targets for the development of safe and effective therapies for lung cancer [3]. PPARs are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily [4]. They were initially found to be involved in the control of energy homeostasis and cell differentiation, proliferation, apoptosis, and inflammation. This suggested a role for PPARs in several disorders such as diabetes, metabolic syndrome, and atherosclerosis [5]. Early research also linked PPARs to carcinogenesis and, to date, PPARs have been implicated in solid organ cancers like breast, ovary, prostate, bladder, gastric, and colon as well as in leukemias [3]. Similarly, several studies have identified PPARs in lung cancer cells. Few tantalizing studies in animal models of lung cancer showed that modulation of specific PPARs results in decreased tumor burden. Hence, many studies are underway to test the impact of targeting these receptors for therapeutic purposes.

2. PPARS ARE MEMBERS OF THE NUCLEAR RECEPTOR SUPERFAMILY

Nuclear receptors (NRs) are a superfamily of phylogenetically related proteins that are ligand-dependent transcriptional regulators. A total of 48 NR genes have been identified in the human genome [4]. They regulate a diverse range of normal physiological functions such as homeostasis, reproduction, development, differentiation, and metabolism [5]. In addition, ligand-independent actions of several members of the NR superfamily have also been reported, which may

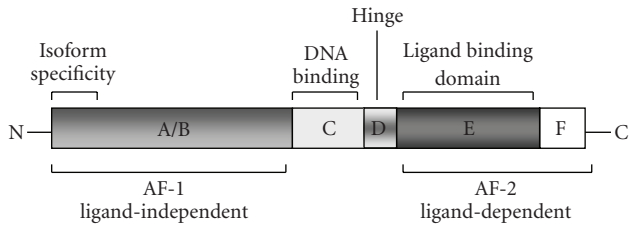

Figure 1: Structural organization of the functional domains of nuclear receptors.

explain their complex range of effects [5]. The NR superfamily includes receptors for classical steroid hormones (estrogens, androgens, progesterone, glucocorticoids, mineralocorticoids, and vitamin D3), bile acids, retinoic acids, and thyroid hormones. In addition, a large number of receptors have been identified through sequence similarity to known receptors, but lacking identified natural ligands. The latter are referred to as nuclear orphan receptors and PPARs fall into this latter category.

Sequence alignment and phylogenetic tree construction resulted in in the classification of the NR family into six evolutionary groups of unequal size with PPARs in group 1 (NR1) along with thyroid and retinoic acid receptors [6]. All nuclear receptors share a common structural organization with multiple distinct functional domains (Figure 1). The N-terminal A/B domain contains at least one constitutively active transactivation region (AF-1) and several autonomous transactivation domains. The C domain is the most conserved region, responsible for DNA-binding specificity and essential for both homo- and heterodimerization of receptors. The D domain is a less conserved flexible hinge region between DNA-binding and the C-terminal ligand-binding domain E. The D domain contains the nuclear localization signal and also serves as docking site for cofactors. The E domain is a moderately conserved domain with a ligand-dependent transactivation function called AF-2. Some members also have a c-terminal F domain, whose sequence is extremely variable, and its structure and function are not known.

NR family members also share a common mode of action to regulate target gene expression. Ligand binding induces a conformational change in the receptor that permits homo- or heterodimerization, dissociation of corepressors, and concomitant association of coactivators. The homo- or heterodimer-coactivator complex binds to specific response elements in the promoter regions of target genes to regulate their transcription. Given the wide range of functions they regulate, it is not surprising that several members of the NR superfamily are implicated in various pathological conditions including the regulation of tumorigenesis. The effects of individual members are either beneficial or detrimental to tumorigenesis depending on the processes regulated by a given receptor and the tissue(s) in which it is expressed.

PPARs represent one of the intensively studied and well-characterized groups of NRs. Three subtypes of PPARs, encoded by three separate genes, have been identified and cloned: PPAR-α (NR1C1), PPAR-β/δ (NR1C2), and PPAR-γ (NR1C3) [6]. PPAR-α is the first member and was identified in the early 1990s in rodents as a receptor for compounds that induce peroxisome proliferation, which explains its name [7]. Subsequently, other two members were identified based on sequence similarity. Since then, PPARs have been recognized as important sensors for cellular fatty acids and fatty acid derivatives and mediate their effects through transcriptional regulation. Through these pathways, PPARs and their ligands are implicated in the regulation of cell proliferation, differentiation, and survival, and, therefore, carcinogenesis [8].

PPARs heterodimerize with retinoid X receptor (RXR) before binding a peroxisome proliferators response element (PPRE) in target genes. In addition to the induction of target gene expression, PPARs also mediate indirect repressive effects through transrepression by inhibiting the activity of key transcription factors via direct protein–protein interactions or by sequestrating cofactors necessary to their activity. In this fashion, PPAR-α and PPAR-γ interfere with NF-κB- and AP-1-mediated gene transcription, whereas PPAR-β/δ represses the expression of target genes induced by PPAR-α and PPAR-γ by binding to PPRE in association with corepressors [9–11].

Cofactors are proteins that can repress (corepressors) or enhance (coactivators) nuclear receptor transcriptional activity by bridging transcription factors to the basic transcription machinery or by specifically modifying chromatin structure. The nuclear receptor corepressor (NCoR), for example, and the silencing mediator of retinoid and thyroid receptors (SMRT) repress nuclear receptor activity. Their repressive effects are thought to occur through the recruitment of histone deacetylases (HDACs), but interactions with the basal transcriptional machinery might also play a role. The importance of corepressor interactions for PPAR-α and PPAR-β/δ action is currently poorly understood. The PPAR-γ interacting protein (PRIP/RAP250) and the PRIP-interacting protein with methyltransferase domain (PIMT) are two coactivators acting as molecular scaffolds which enhance PPAR-γ and RXR-mediated transcription. Importantly, the choice of PPAR/RXR heterodimers for PPAR target gene activation by PPAR agonists are related to the availability of cofactors such as CREB binding protein (CBP) and p300 versus SRC-1. Thus, the relative levels of cofactor expression control the specificity of the physiological response to PPAR or RXR agonists [12].

3. PPARS IN LUNG CANCER

In normal cells, the process of cellular differentiation is typically accompanied by cessation of proliferation, followed by senescence and, eventually, apoptosis. The balance between these events is disrupted in cancer cells. Therefore, the induction and maintenance of a differentiated state have been an important strategy in the search for cancer therapeutics [13]. The use of all-trans retinoic acid for the treatment of acute promyelocytic leukemia represents the first successful application of such an approach [14]. However, this approach has not been successfully exploited for the treatment

of solid tumors. Since PPAR-β/δ and PPAR-γ play a key role in the differentiation of keratinocytes and adipocytes, it has been proposed that drugs capable of activating these receptors might be useful in arresting tumor growth [8, 15, 16]. In contrast, the role of PPAR-α in human carcinogenesis is less clear, but ligands that activate PPAR-α are implicated in the development of hepatocellular carcinoma in rodents [8, 17].

4. PPAR-α

PPAR-α is expressed in several tissues including liver, kidney, heart, skeletal muscle [18–20], vascular smooth muscle cells [21], endothelial cells [22], and monocytes/macrophages [23]. It was the first PPAR to be identified, and was shown to mediate peroxisome proliferators actions [18]. Peroxisome proliferators include several unrelated molecules such as steroids, lipids, hypolipidemic drugs (fibrates), industrial plasticizers, pesticides, and solvents that target the liver, among other organs, where they are known to induce peroxisome proliferation, liver hypertrophy, and hyperplasia, followed by hepatocellular carcinoma in rodents [18]. PPAR-α null mice are resistant to the effects of peroxisome proliferators (e.g., clofibrate) and PPAR-α ligands (e.g., Wy-14,643) as well as to the development of hepatocellular carcinoma in response to peroxisome proliferators [24]. The underlying mechanisms responsible for this effect remain incompletely understood. It has been proposed that peroxisome proliferators induce DNA replication and proliferation in hepatocytes in a PPAR-α-dependent manner [25, 26]. However, there is no direct evidence that PPAR-α effects the transcription of cell-cycle genes. Peroxisome proliferators are also reported to repress apoptosis in hepatocytes both in vitro and in vivo [27, 28]. The involvement of PPAR-α in this process was confirmed in studies using dominant negative PPAR-α in rat primary hepatocytes [29].

Interestingly, humans appear to be resistant to many of the adverse effects of the known peroxisome proliferators, but retain their beneficial effects. For example, epidemiological studies failed to show significant peroxisome proliferation in the liver of patients treated with hypolipidemic drugs [30, 31], and cell culture studies indicate that human cells display a reduced transcriptional response to PPAR-α activation when compared with rat cells [32]. These differences are important, but the mechanisms involved in their manifestation are unknown. Understanding the differences in the range of responses displayed by rodents and humans is one of the challenging aspects of PPAR-α biology. Today, very little is known about the role of PPAR-α in lung cancer biology and, thus, attention should be given to this area.

5. PPAR-β/δ

This PPAR isotype was first named as PPAR-β when isolated from Xenopus oocyte [33]. It was named PPAR-δ when it was subsequently identified in mouse [34], rat [35], and humans [36, 37], as it was not obviously homologous to the Xenopus gene. Nevertheless, it is now clear that both PPAR-β and δ are *bonafide* orthologues and, for clarity, it is referred to as PPAR-β/δ. The expression of PPAR-β/δ is broad since it has been detected in all of the tissues tested, with varied expression levels. It is expressed at relatively higher levels in the brain, adipose tissue, and skin [19, 38]. Several naturally occurring compounds such as saturated and polyunsaturated fatty acids and eicosanoids serve as PPAR-β/δ agonists in the micro molar range. However, similar to other PPARs, true physiological ligands of PPAR-β/δ are yet to be identified. Recently, synthetic agonists with affinities in the nanomolar range have been developed. GW501516 was the first synthetic PPAR-β/δ ligand developed by GlaxoSmithKline [39]. It was followed by Merck's L-165,041 compound [40] and a 1,3,5-trisubstituted aryl compound by Novartis [41]. Unlike PPAR-α and PPAR-γ ligands, none of the PPAR-β/δ ligands are in clinical use, but they are in different stages of clinical testing.

The generation of receptor knock-out mice unveiled multiple developmental and homeostatic abnormalities in PPAR-β/δ null animals including placental defects, defects in myelination, decreased body fat, impaired wound healing, and altered inflammatory responses in skin [42–44]. Studies with high-affinity synthetic ligands revealed a critical role for PPAR-β/δ in glucose and lipid metabolism making it an important therapeutic target for the treatment of insulin resistance, glucose intolerance, hypertension and dyslipidemia (collectively known as metabolic syndrome or syndrome X), and with the potential to control weight gain, enhance physical endurance, improve insulin sensitivity, and ameliorate atherosclerosis [45].

Recent studies with knock-out mice and the treatment of human keratinocytes with high-affinity ligands have demonstrated that PPAR-β/δ plays a crucial role in the control of important cellular functions such as adhesion, proliferation, differentiation, and survival [8, 46]. Its role in lung cancer is less studied. However, in NSCLC cell lines, activation of PPAR-β/δ with GW501515 increased proliferation via stimulation of PI3-kinase/Akt signaling resulting in increased recognition of prostaglandin E_2 via transcriptional upregulation of its EP4 receptor [47]. This contrasts PPAR-β/δ with PPAR-γ whose activation is consistently associated with inhibition of NSCLC proliferation.

6. PPAR-γ

PPAR-γ was discovered based on its similarity to PPAR-α, and it is the most intensively studied NR. By utilizing three different promoters, a single PPAR-γ gene encodes three isoforms namely PPAR-γ1, PPAR-γ2, and PPAR-γ3 [48]. Analysis of PPAR-γ1 and γ3 transcripts revealed that they both translate into the same PPAR-γ1 protein [49]. PPAR-γ2 protein contains an additional 30 amino acids at its N-terminus compared to PPAR-γ1. PPAR-γ is highly expressed in adipose tissue and it is a master regulator of adipocyte differentiation [50, 51]. In addition to its role in adipogenesis, PPAR-γ serves as an important transcriptional regulator of glucose and lipid metabolism, and it has been implicated in the regulation of insulin sensitivity, atherosclerosis, and inflammation [52–54]. PPAR-γ is also expressed in multiple other tissues such as breast, colon, lung, ovary, prostate, and thyroid where it was demonstrated to regulate

cellular proliferation, differentiation, and apoptosis [55–58]. More recently, various leukocyte populations, including monocytes/macrophages, lymphocytes, and dendritic cells, have also been shown to express PPAR-γ suggesting a role for this molecule in the regulation of immune responses [59]. PPAR-γ has been described as a negative regulator of macrophage function since its activation suppresses the production of inflammatory cytokines, chemokines, metalloproteases, and nitric oxide [60, 61]. These PPAR-γ mediated anti-inflammatory effects are not restricted to monocytes, as treatment with PPAR-γ agonists results in inhibition of cytokine/chemokine production in several epithelial and stromal cell populations [62]. As will be discussed later, PPAR-γ activation also inhibits tumor progression in NSCLC [62, 63].

Since its discovery, several natural and synthetic compounds have been identified as activators of PPAR-γ. The insulin sensitizing antidiabetic drugs known as thiazolidinediones (TZDs) were the first compounds identified as PPAR-γ agonists [64]. The TZDs rosiglitazone and pioglitazone are currently in clinical use for the treatment of type-II diabetes, while troglitazone was withdrawn from clinical use because it was linked to idiosyncratic liver toxicity [65]. Other non-TZD synthetic ligands include certain nonsteroidal anti-inflammatory drugs such as isoxzolidinedione JTT-501 [66] and tyrosine-based GW7845 [67]. Naturally occurring compounds that activate PPAR-γ in vitro include polyunsaturated fatty acids, prostaglandin D_2 (PGD_2) and its metabolite 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2(15d-PGJ_2), 12/15 lipoxygenase products 15-hydroxyeicosatetraenoic acid (15-HETE), and 13-hydroxyoctadecadienoic acid [68, 69]. However, none of these compounds activated PPAR-γ at physiologically relevant concentrations. More recently, intact nitroalkenes such as OA–NO_2 (nitrated oleic acid) and LNO_2 (nitrated linoleic acid) were observed to activate PPAR-γ at concentrations well within their detected levels in human plasma and urine making them ideal candidates for long-awaited endogenous ligands [70, 71]. It would be interesting to investigate whether nitroalkenes are present in tumor tissues, and their potential role in tumorigenesis. In addition, compounds from several medicinal plants such as Saurufuran A from *Saururus chinesis* [72], flavonoids such as chrysin and kampferol [73], phenolic compounds from *Glycyryhiza uralensis* [74], and curcumin from *Curcumin longa* [75, 76] are also shown to activate PPAR.

The synthetic ligands and some natural ligands have been used to elucidate the role of PPAR-γ in cellular functions both in vitro and in vivo. However, several caveats should be taken into consideration when interpreting such studies [3]. First, the natural ligands that regulate PPARs in vivo remain incompletely defined. Second, not all PPAR-γ ligands exert their effects through PPAR-γ since there is strong evidence for the activation of PPAR-γ-independent signals, particularly with the natural ligand 15d-PGJ_2. Third, high-affinity ligands for PPAR-γ (e.g., the TZDs) may exert partial agonist/antagonist activity [77]. The latter might be due to the fact that individual TZDs induce different PPAR-γ conformations that influence the recruitment of different coactivator/corepressor molecules. Much information is now available regarding the potential role of PPAR-γ and its ligands in lung cancer and, thus, the rest of the discussion will focus on this topic.

7. PPAR-γ AND PPAR-γ LIGANDS IN LUNG CANCER

PPAR-γ is expressed in many cancers including colon, breast, and prostate, and with few exceptions, PPAR-γ ligands are generally antiproliferative in these settings. Similarly, PPAR-γ is expressed in SCLC and NSCLC [78]. Furthermore, PPAR-γ ligands induce growth arrest and promote changes associated with differentiation as well as apoptosis in a variety of lung carcinoma cell lines, although most of the knowledge available in this area has been generated in NSCLC [3, 62]. The exact mechanisms linking modulation of PPAR-γ with cancer growth inhibition remain incompletely elucidated; however, strong evidence suggests that PPAR-γ ligands modulate the intracellular machinery involved in cell signaling and cell cycle control, and inhibit tumor cell recognition of extracellular mitogenic signals. Yet, other studies suggest that modulation of PPAR-γ affects the expression of angiogenic factors needed for the development of the vascular network responsible for supplying nutrients to tumor cells. These mechanisms are discussed below as they relate to the action of PPAR-γ ligands in lung cancer.

7.1. *PPAR-γ ligands interfere with tumor cell signaling and cell-cycle control*

Several observations point to targets for PPAR-γ ligands in the intracellular machinery responsible for cell-cycle control in tumor cells. For example, PPAR-γ ligands have been found to inhibit the growth of A549 adenocarcinoma cells due to G0/G1 cell cycle arrest through the upregulation of mitogen-activated protein kinases Erk1/2 and the downregulation of G1 cyclins D and E [62]. Troglitazone inhibits NSCLC proliferation in part by stimulating the expression of the GADD 153 (for growth arrest and DNA damage inducible gene-153) [79]. PPARγ ligands can also trigger the activation of the mitogen-activated protein Kinase (MAPK) Erk cascade, which plays a central role in intracellular signaling by many extracellular stimuli. Interestingly, PPARγ itself is a target for Erks, and Erk5 was reported to interact with PPAR-γ, but unlike the other MAPKs, this interaction induces activation rather than inhibition of PPAR-γ transcriptional activity [80]. Troglitazone was found to induce the apoptosis of NCI-H23 cells via a mitochondrial pathway through the activation of Erk1/2 [81]. In that study, the pro-apoptotic effects of troglitazone were clearly mediated via PPAR-γ since PPAR-γ siRNA blocked the response. Others have shown similar results using CRL-202 cells, and further demonstrated that troglitazone downregulated the expression of the pro-apoptotic molecules Bcl-w and Bcl-2, and decreased the activity of SAPK/JNK [82]. PPAR-γ ligands also induce the expression of death receptor 5 (DR5) and increase DR5 distribution at the cell surface in addition to reducing c-FLIP levels in human lung cancer cells. These agents cooperated with TRAIL to enhance apoptosis in human lung carcinoma cells [83].

Tumor suppressor genes are also affected by PPAR-γ ligands. For example, PGJ_2 and ciglitazone stimulated the expression of p21 mRNA and protein expression in NSCLC, and this coincided with a reduction in cyclin D1 mRNA expression [84]. Of note, p21 antisense oligonucleotides significantly blocked lung carcinoma cell growth inhibition observed with PPAR-γ ligands thereby establishing an important role for p21 in this process. These findings are consistent with those of others showing that the proliferation of A549 cells injected subcutaneously into nude mice was inhibited significantly by treatment with ciglitazone, and this coincided with increased expression in tumors of PPAR-γ and p21, and with downregulation of cyclin D1 [85]. A connection between p53, another tumor suppressor gene, and PPAR-γ ligands has also been demonstrated by showing that 15-deoxy-PGJ_2, together with docetaxel, stimulates apoptosis in NSCLC through inhibition of Bcl2 and cyclin D1, and overexpression of caspases and p53 [86].

Recent reports implicate alterations in the mammalian target of rapamycin (mTOR) signaling pathway in the antitumor effects of PPARγ ligands. Rosiglitazone, for example, was reported to reduce the phosphorylation of Akt, an upstream positive modulator of mTOR, and increase PTEN, a negative modulator of mTOR, in NSCLC H1792 and H1838 cells; this resulted in inhibition of cell proliferation [87] (Figure 2). Although the effects of rosiglitazone on Akt and PTEN were blocked by the selective PPAR-γ antagonist GW9662 and restored by transient overexpression of PPAR-γ, cell growth was not entirely restored suggesting the involvement of additional PPAR-γ-independent mechanisms of action. These observations are consistent with the work of others showing similar increases in PTEN expression induced by rosiglitazone [88]. Further work revealed that rosiglitazone increased the phosphorylation of AMPKα, a target of LKB1 and upstream downregulator of mTOR [87]. Rosiglitazone may also activate TSC2, another potential tumor suppressor and upstream downregulator of mTOR. The latter pathway was independent of PPAR-γ since it was not affected by GW9662 or PPAR-γ siRNA. This again highlights the fact that TZDs may act via PPARγ-independent pathways. This is important since TZDs display proinflammatory activities in part via their ability to augment PPAR-β/δ signaling. Thus, some effects of PPAR-γ ligands may be mediated through an off-target effect [89]. These studies emphasize the need for PPAR modulators with increased receptor subtype specificity.

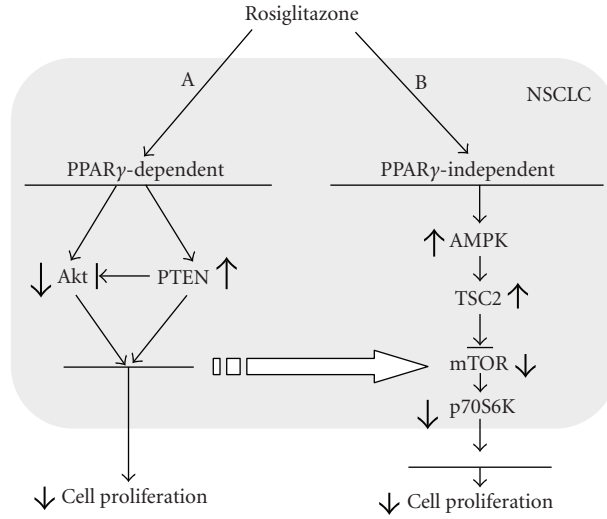

Figure 2: Rosilitazone stimulates NSCLC proliferation by affecting the Akt/mTOR pathway through PPARγ-dependent and PPARγ-independent mechanisms.

7.2. PPAR-γ ligands inhibit tumor cell recognition of extracellular mitogenic factors

Several studies suggest that PPAR-γ ligands exert their antitumor effects by blocking access to mitogenic agents such as PGE_2, a major cyclooxygenase metabolite that plays important roles in tumor biology. The functions of PGE_2 are mediated through one or more of its receptors EP1, EP2, EP3, and EP4 [90]. Human NSCLC cell lines express EP2 receptors, among other EP receptors, and the inhibition of cell growth by PPAR-γ ligands like GW1929, PGJ_2, ciglitazone, troglitazone, and rosiglitazone is associated with a significant decrease in EP2 mRNA and protein expression. Notably, the inhibitory effects of rosiglitazone and ciglitazone, but not PGJ_2, were reversed by a specific PPAR-γ antagonist GW9662, suggesting the involvement of PPAR-γ-dependent and PPAR-γ-independent mechanisms [90].

Other studies suggest that PPAR-γ ligands might prevent the interaction of tumor cells with their surrounding stroma, thereby interfering with host-derived and tumor-derived factors with mitogenic and prosurvival effects. An example of this is fibronectin, a matrix glycoprotein that resides in the lung stroma that is increased in most, if not all, chronic forms of lung disease [91]. This is true for tobacco-related lung disorders and fibrotic disorders, all associated with increased incidence of lung cancer [92]. Several studies suggest that fibronectin serves as a mitogen and survival factor for NSCLC [93], and fibronectin was recently shown to stimulate tumor cell expression of matrix metalloproteinases, proteases implicated in metastatic disease [94]. These observations support the idea that tumor cell interactions with fibronectin through surface integrin receptors are advantageous for tumors since they stimulate proliferation, survival, and metastases [93]. This idea remains to be proven in vivo, but if found to be true, this might unveil a new target for anticancer strategies. In this regard, PPAR-γ ligands were shown to inhibit fibronectin expression in NSCLC cells by inhibiting transcription factors involved in regulation of fibronectin gene expression [95]. PPAR-γ ligands (rosiglitazone and GW1929, but not PGJ_2) were also recently reported to inhibit the expression of the gene encoding for the α5 integrin subunit resulting in reduced expression of the integrin $\alpha5\beta1$, a fibronectin receptor that mediates fibronectin's mitogenic effects in NSCLC cells and nontumor lung cells [96]. Thus, by inhibiting the expression of fibronectin and its integrin $\alpha5\beta1$, PPAR-γ ligands might reduce tumor cell recognition of fibronectin

with consequent changes in cell proliferation and apoptosis.

7.3. PPAR-γ ligands inhibit angiogenesis and tumor vascularization

The idea that PPAR-γ might regulate the generation of the complex vascular network that supplies tumor cells is supported by studies showing significant reduction in blood vessel density in the lung tumors generated by the injection of A549 cells into the flanks of SCID mice treated with PPARγ ligands [97]. In studies in vitro, the treatment of A549 cells with troglitazone or their transient transfection with a constitutively active PPAR-γ construct blocked the production of angiogenic molecules such as ELR+CXC chemokines IL-8 (CXC-8), ENA-78 (CXCL5), and Gro-alpha (CXCL1) [97]. Moreover, conditioned media from untreated A549 cells stimulated human microvascular endothelial cell chemotaxis, whereas the condition media of troglitazone-treated A549 was inhibitory. Of note, PPARγ activation inhibited NF-κB, a transcription factor known to regulate the expression of many of the pro-angiogenic factors mentioned above. Similarly, rosiglitazone was shown to inhibit mouse lung tumor cell growth and metastasis in vivo through direct and indirect anti-angiogenic effects [63].

7.4. PPAR-γ is a novel candidate for targeting tumor microenvironment

In tumors, cancer cells coexist with different cell types including fibroblasts, macrophages, endothelial cells, and multitude of diverse cytokines and chemokines secreted by these cells, constituting a distinct tumor microenvironment. One of the important conceptual advances in tumor biology in recent years has been the appreciation that all major aspects of a cancer cell are influenced by the tumor microenvironment. Interestingly, PPAR-γ is expressed in all major cell types present in the tumor microenvironment, and its ligands have been shown to inhibit several of the pro-tumorigenic functions of these cell types in vitro and, in some cases, in vivo. For example, PPAR-γ ligands were shown to inhibit proliferation, and induce apoptosis, migration, and tube formation in endothelial cells [98]. Also, PPAR-γ ligands can inhibit the transdifferentiation of fibroblasts into myofibroblasts, a phenotype similar to that of tumor-associated fibroblasts, in several fibrotic conditions [99–102]. A recent study demonstrated that PPAR-γ ligands completely reverse the antitumor cytotoxic T-lymphocyte suppressive activity and the M2 phenotype of tumor-associated macrophages [103]. PPAR-γ ligands are also known to inhibit the expression of several cytokines and chemokines produced by all of the major cell types present in the tumor microenvironment (60, 61, 97, 98]. Together with data showing effects on fibronectin matrix expression and recognition in NSCLC [95], the above observations suggest that PPAR-γ might be a novel candidate for targeting the tumor microenvironment.

8. IMPLICATIONS FOR THERAPY AND RESEARCH NEEDS

The studies mentioned above suggest that PPARs are involved in lung cancer cell biology. However, their roles remain uncertain, and much needs to be learned before they are targeted for therapeutic intervention, especially when considering PPAR-α and PPAR-β/δ. Activation of PPAR-γ is strongly associated with decreased lung carcinoma cell proliferation both in vitro and in vivo. Furthermore, in primary NSCLC, the expression of PPAR-γ has been correlated with tumor histological type and grade, and decreased PPAR-γ expression was correlated with poor prognosis [104]. Because of this, and the fact that synthetic agonists of PPAR-γ with good safety profiles are currently in use in the clinical arena, PPAR-γ has emerged as a reasonable target for the development of anti-lung cancer therapies. Synthetic and natural PPAR-γ activators might be useful. For example, arachidonic acid treatment inhibits the growth of A549 cells, and this effect is blocked by the synthetic PPAR-γ inhibitor GW9662 [105]. MK886, a 5-lipoxygenase activating protein-directed inhibitor, stimulates apoptosis and reduces the growth of A549 cells through activation of PPARγ [106]. These and related drugs can be used alone or in combination with other drugs for synergistic effects. This was observed when using low doses of MK886 in combination with ciglitazone and 13-cis-retinoic acid on A549 and H1299 cells [106]. Also, dramatic synergistic anticancer effects have been reported for lovastatin (an HMG-CoA reductase inhibitor) and the PPAR-γ ligand troglitazone in several cell lines including lung cancer cells [107]. An enhancement by rosiglitazone of the antitumor effects of gefitinib on A549 cell growth was recently noted suggesting that combination strategies using selective nuclear receptor activators in conjunction with epidermal growth factor receptor inhibitors might prove effective [108].

Although little information is available in vivo, emerging data are beginning to unveil potential implications to the human condition. In this regard, a retrospective analysis of a cohort of 87 678 individuals identified through the Veterans Integrated Services Network 16 data warehouse revealed a 33% reduction in lung cancer risk among TZD users compared with nonusers after adjusting for confounder variables. Interestingly, a similar risk reduction was not observed for colorectal and prostate cancers [109].

Despite the above, enthusiasm for this approach should be tempered by work showing that the PPAR-γ ligands rosiglitazone, ciglitazone, and PGJ_2 were found to stimulate PPAR-γ transactivation in lung adenocarcinoma cell lines in vitro, but little to no effects were noted in squamous cell or large cell carcinomas suggesting that their anticancer properties might not be shared by all lung tumors, or that important PPAR-γ-independent pathways are at play [108, 110]. Thus, a better understanding of the mechanisms of action of activated PPARs in tumors (and host cells) is required since the dissection of these pathways might unveil better targets for therapy. Nevertheless, the data available to date regarding PPAR-γ is promising and justify engaging in prospective, randomized, clinical studies to determine the true role

of PPAR-γ ligands in lung cancer, while further work is performed to identify more selective and effective strategies.

REFERENCES

[1] R. T. Greenlee, T. Murray, S. Bolden, and P. A. Wingo, "Cancer statistics," *A Cancer Journal for Clinicians*, vol. 50, pp. 7–33, 2000.

[2] Y. Sekido, K. M. Fong, and J. D. Minna, "Progress in understanding the molecular pathogenesis of human lung cancer," *Biochimica et Biophysica Acta—Reviews on Cancer*, vol. 1378, no. 1, pp. F21–F59, 1998.

[3] S. Han and J. Roman, "Peroxisome proliferators-activated receptor gamma: a novel target for cancer therapeutics?" *Anticancer Drugs*, vol. 18, pp. 237–244, 2007.

[4] M. Robinson-Rechavi, A. S. Carpentier, M. Duffraisse, and V. Laudet, "How many nuclear hormone receptors are there in the human genome?" *Trends in Genetics*, vol. 17, no. 10, pp. 554–556, 2001.

[5] H. Laudet VaG, *The Nuclear Receptor Facts Book*, Academic Press, San Diego, Calif, USA, 2002.

[6] J. Auwerx, E. Baulieu, M. Beato, et al., "A unified nomenclature system for the nuclear receptor superfamily," *Cell*, vol. 97, no. 2, pp. 161–163, 1999.

[7] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[8] L. Michalik, B. Desvergne, and W. Wahli, "Peroxisome-proliferator-activated receptors and cancers: complex stories," *Nature Reviews Cancer*, vol. 4, no. 1, pp. 61–70, 2004.

[9] P. Delerive, K. De Bosscher, S. Besnard, et al., "Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NfκB and AP-1," *The Journal of Biological Chemistry*, vol. 274, no. 45, pp. 32048–32054, 1999.

[10] N. Wang, L. Verna, N. G. Chen, et al., "Constitutive activation of peroxisome proliferator-activated receptor-γ suppresses pro-inflammatory adhesion molecules in human vascular endothelial cells," *The Journal of Biological Chemistry*, vol. 277, no. 37, pp. 34176–34181, 2002.

[11] Y. Shi, M. Hon, and R. M. Evans, "The peroxisome proliferator-activated receptor δ, an integrator of transcriptional repression and nuclear receptor signaling," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 5, pp. 2613–2618, 2002.

[12] J. N. Feige, L. Gelman, L. Michalik, B. Desvergne, and W. Wahli, "From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions," *Progress in Lipid Research*, vol. 45, no. 2, pp. 120–159, 2006.

[13] H. Kawamata, M. Tachibana, T. Fujimori, and Y. Imai, "Differentiation-inducing therapy for solid tumors," *Current Pharmaceutical Design*, vol. 12, no. 3, pp. 379–385, 2006.

[14] R. P. Warrell Jr., S. R. Frankel, W. H. Miller Jr., et al., "Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans-retinoic acid)," *The New England Journal of Medicine*, vol. 324, no. 20, pp. 1385–1393, 1991.

[15] D. J. Kim, M. T. Bility, A. N. Billin, T. M. Willson, E. J. Gonzalez, and J. M. Peters, "PPARβ/δ selectively induces differentiation and inhibits cell proliferation," *Cell Death Differentiation*, vol. 13, pp. 53–60, 2006.

[16] N. S. Tan, N. Vinckenbosch, P. Liu, et al., "Selective cooperation between fatty acid binding proteins and peroxisome proliferator-activated receptors in regulating transcription," *Molecular and Cellular Biology*, vol. 22, no. 14, pp. 5114–5127, 2002.

[17] Q. Yan, S. Ito, and F. J. Gonzalez, "Hepatocyte-restricted constitutive activation of PPARα induces hepatoproliferation but not hepatocarcinogenesis," *Carcinogenesis*, vol. 28, no. 6, pp. 1171–1177, 2007.

[18] E. A. Lock, A. M. Mitchell, and C. R. Elcombe, "Biochemical mechanisms of induction of hepatic peroxisome proliferation," *Annual Review of Pharmacology and Toxicology*, vol. 29, pp. 145–163, 1989.

[19] D. Auboeuf, J. Rieusset, L. Fajas, et al., "Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver x receptor-alpha in humans: no alteration in adipose tissue of obese and niddm patients," *Diabetes*, vol. 46, no. 8, pp. 1319–1327, 1997.

[20] O. Braissant, F. Foufelle, C. Scotto, M. Dauca, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat," *Endocrinology*, vol. 137, pp. 354–366, 1996.

[21] Q. N. Diep, R. M. Touyz, and E. L. Schiffrine, "Docosahexaenoic acid, a peroxisome proliferator-activated receptor-alpha ligand, induces apoptosis in vascular smooth muscle cells by stimulation of p38 mitogen-activated protein kinase," *Hypertension*, vol. 36, no. 5, pp. 851–855, 2000.

[22] I. Inoue, K. Shino, S. Noji, T. Awata, and S. Katayama, "Expression of peroxisome proliferator-activated receptor alpha (ppar alpha) in primary cultures of human vascular endothelial cells," *Biochemical and Biophysical Research Communications*, vol. 246, no. 2, pp. 370–374, 1998.

[23] G. Chinetti, S. Griglio, M. Antonucci, et al., "Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages," *The Journal of Biological Chemistry*, vol. 273, no. 40, pp. 25573–25580, 1998.

[24] S. S. Lee, T. Pineau, J. Drago, et al., "Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators," *Molecular and Cellular Biology*, vol. 15, no. 6, pp. 3012–3022, 1995.

[25] J. M. Peters, T. Aoyama, R. C. Cattley, U. Nobumitsu, T. Hashimoto, and F. J. Gonzalez, "Role of peroxisome proliferator-activated receptor alpha in altered cell cycle regulation in mouse liver," *Carcinogenesis*, vol. 19, no. 11, pp. 1989–1994, 1998.

[26] J. M. Peters, R. C. Cattley, and F. J. Gonzalez, "Role of PPAR alpha in the mechanism of action of the nongenotoxic carcinogen and peroxisome proliferator wy-14,643," *Carcinogenesis*, vol. 18, no. 11, pp. 2029–2033, 1997.

[27] A. C. Bayly, R. A. Roberts, and C. Dive, "Suppression of liver cell apoptosis in vitro by the non-genotoxic hepatocarcinogen and peroxisome proliferator nafenopin," *The Journal of Cell Biology*, vol. 125, no. 1, pp. 197–203, 1994.

[28] R. A. Roberts, "Non-genotoxic hepatocarcinogenesis: suppression of apoptosis by peroxisome proliferators," *Annals of the New York Academy of Sciences*, vol. 804, no. 1, pp. 588–611, 1996.

[29] R. A. Roberts, N. H. James, N. J. Woodyatt, N. Macdonald, and J. D. Tugwood, "Evidence for the suppression of apoptosis by the peroxisome proliferator activated receptor alpha (PPAR alpha)," *Carcinogenesis*, vol. 19, no. 1, pp. 43–48, 1998.

[30] P. Bentley, I. Calder, C. Elcombe, P. Grasso, D. Stringer, and H. J. Wiegand, "Hepatic peroxisome proliferation in rodents and its significance for humans," *Food and Chemical Toxicology*, vol. 31, no. 11, pp. 857–907, 1993.

[31] E. Boitier, J. C. Gautier, and R. Roberts, "Advances in understanding the regulation of apoptosis and mitosis by peroxisome-proliferator activated receptors in pre-clinical models: relevance for human health and disease," *Comparative Hepatology*, vol. 2, no. 1, p. 3, 2003.

[32] C. Scotto, J. M. Keller, H. Schohn, and M. Dauca, "Comparative effects of clofibrate on peroxisomal enzymes of human (hep ebna2) and rat (fao) hepatoma cell lines," *European Journal of Cell Biology*, vol. 66, no. 4, pp. 375–381, 1995.

[33] C. Dreyer, G. Krey, H. Keller, F. Givel, G. Helftenbein, and W. Wahli, "Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors," *Cell*, vol. 68, no. 5, pp. 879–887, 1992.

[34] S. A. Kliewer, B. M. Forman, B. Blumberg, et al., "Differential expression and activation of a family of murine peroxisome proliferator-activated receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, pp. 7355–7359, 1994.

[35] E. Z. Amri, F. Bonino, G. Ailhaud, N. A. Abumrad, and P. A. Grimaldi, "Cloning of a protein that mediates transcriptional effects of fatty acids in preadipocytes. Homology to peroxisome proliferator-activated receptors," *The Journal of Biological Chemistry*, vol. 270, no. 5, pp. 2367–2371, 1995.

[36] L. Jow and R. Mukherjee, "The human peroxisome proliferator-activated receptor (PPAR) subtype nuc1 represses the activation of hppar alpha and thyroid hormone receptors," *Journal of Biological Chemistry*, vol. 270, no. 8, pp. 3836–3840, 1995.

[37] H. Matsuo and J. F. Strauss III, "Peroxisome proliferators and retinoids affect JEG-3 choriocarcinoma cell function," *Endocrinology*, vol. 135, no. 3, pp. 1135–1145, 1994.

[38] J. Berger and D. E. Moller, "The mechanisms of action of PPARs," *Annual Review of Medicine*, vol. 53, pp. 409–435, 2002.

[39] M. L. Sznaidman, C. D. Haffner, P. R. Maloney, et al., "Novel selective small molecule agonists for peroxisome proliferator-activated receptor delta (PPAR-delta)—synthesis and biological activity," *Bioorganic & Medicinal Chemistry Letters*, vol. 13, no. 9, pp. 1517–1521, 2003.

[40] C. E. Bassene, F. Suzenet, N. Hennuyer, et al., "Studies towards the conception of new selective PPAR beta/delta ligands," *Bioorganic & Medicinal Chemistry Letters*, vol. 16, no. 17, pp. 4528–4532, 2006.

[41] R. Epple, M. Azimioara, R. Russo, et al., "1,3,5-trisubstituted aryls as highly selective PPARdelta agonists," *Bioorganic & Medicinal Chemistry Letters*, vol. 16, no. 11, pp. 2969–2973, 2006.

[42] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor delta on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2002.

[43] J. M. Peters, S. S. Lee, W. Li, et al., "Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor beta(delta)," *Molecular and Cellular Biology*, vol. 20, no. 14, pp. 5119–5128, 2000.

[44] N. S. Tan, L. Michalik, N. Noy, et al., "Critical roles of ppar beta/delta in keratinocyte response to inflammation," *Genes & Development*, vol. 15, no. 24, pp. 3263–3277, 2001.

[45] G. D. Barish., V. A. Narkar, and R. M. Evans, "Ppar delta: a dagger in the heart of the metabolic syndrome," *The Journal of Clinical Investigation*, vol. 116, no. 3, pp. 590–597, 2006.

[46] A. D. Burdick, D. J. Kim, M. A. Peraza, F. J. Gonzalez, and J. M. Peters, "The role of peroxisome proliferator-activated receptor-beta/delta in epithelial cell growth and differentiation ," *Cellular Signalling*, vol. 18, no. 1, pp. 9–20, 2006.

[47] S. Han, J. D. Ritzenthaler, B. Wingerd, and J. Roman, "Activation of peroxisome proliferator-activated receptor beta/delta (PPARβ/δ) increases the expression of prostaglandin E2 receptor subtype E4. The role of phosphatidylinositol 3-kinase and CCAAAT/enhancer-binding protein beta," *The Journal of Biological Chemistry*, vol. 280, no. 39, pp. 33240–33249, 2005.

[48] L. Fajas, D. Auboeuf, E. Raspe, et al., "The organization, promoter analysis, and expression of the human ppargamma gene," *The Journal of Biological Chemistry*, vol. 272, no. 30, pp. 18779–18789, 1997.

[49] L. Fajas, J. C. Fruchart, and J. Auwerx, "PPAR-gamma3 mRNA: a distinct PPAR-gamma mRNA subtype transcribed from an independent promoter," *FEBS Letters*, vol. 438, no. 1-2, pp. 55–60, 1998.

[50] B. M. Spiegelman, "Ppar-gamma: adipogenic regulator and thiazolidinedione receptor," *Diabetes*, vol. 47, no. 4, pp. 507–514, 1998.

[51] P. Tontonoz, E. Hu, and B. M. Spiegelman, "Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor," *Cell*, vol. 79, no. 7, pp. 1147–1156, 1994.

[52] M. Lehrke and M. A. Lazar, "The many faces of PPAR gamma," *Cell*, vol. 123, no. 6, pp. 993–999, 2005.

[53] R. K. Semple, V. K. Chatterjee, and S. O'Rahilly, "PPAR γ and human metabolic disease," *Journal of Clinical Investigation*, vol. 116, pp. 581–589, 2006.

[54] T. J. Standiford, V. G. Keshamouni, and R. C. Reddy, "Peroxisome proliferator-activated receptor-γ as a regulator of lung inflammation and repair," *Proceedings of the American Thoracic Society*, vol. 2, no. 3, pp. 226–231, 2005.

[55] E. Elstner, C. Muller, K. Koshizuka, et al., "Ligands for peroxisome proliferator-activated receptorgamma and retinoic acid receptor inhibit growth and induce apoptosis of human breast cancer cells in vitro and in BNX mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 15, pp. 8806–8811, 1998.

[56] K. G. Lambe and J. D. Tugwood, "A human peroxisome-proliferator-activated receptor-gamma is activated by inducers of adipogenesis, including thiazolidinedione drugs," *European Journal of Biochemistry / FEBS*, vol. 239, no. 1, pp. 1–7, 1996.

[57] E. Mueller, P. Sarraf, P. Tontonoz, et al., "Terminal differentiation of human breast cancer through PPAR gamma," *Molecular Cell*, vol. 1, no. 3, pp. 465–470, 1998.

[58] P. Sarraf, E. Mueller, D. Jones, et al., "Differentiation and reversal of malignant changes in colon cancer through PPAR gamma," *Nature Medicine*, vol. 4, pp. 1046–1052, 1998.

[59] R. A. Daynes and D. C. Jones, "Emerging roles of PPARs in inflammation and immunity," *Nature Reviews Immunology*, vol. 2, no. 10, pp. 748–759, 2002.

[60] C. Jiang, A. T. Ting, and B. Seed, "PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines," *Nature*, vol. 391, no. 6662, pp. 82–86, 1998.

[61] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[62] V. G. Keshamouni, R. C. Reddy, D. A. Arenberg, et al., "Peroxisome proliferator activated receptor gamma activation inhibits tumor progression in non small cell lung cancer," *Oncogene*, vol. 23, no. 1, pp. 100–108, 2004.

[63] D. Panigrahy, S. Singer, L. Q. Shen, et al., "PPAR γ ligands inhibit primary tumor growth and metastasis by inhibiting angiogenesis," *The Journal of Clinical Investigation*, vol. 110, no. 7, pp. 923–932, 2002.

[64] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPARgamma)," *The Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[65] P. B. Watkins and R. W. Whitcomb, "Hepatic dysfunction associated with troglitazone," *The New England Journal of Medicine*, vol. 338, no. 13, pp. 916–917, 1998.

[66] T. Shibata, K. Matsui, K. Nagao, H. Shinkai, F. Yonemori, and K. Wakitani, "Pharmacological profiles of a novel oral antidiabetic agent, JTT-501, an isoxazolidinedione derivative," *European Journal of Pharmacology*, vol. 364, no. 2-3, pp. 211–219, 1999.

[67] N. Suh, Y. Wang, C. R. Williams, et al., "A new ligand for the peroxisome proliferator-activated receptor-gamma (PPAR-gamma), GW7845, inhibits rat mammary carcinogenesis," *Cancer Research*, vol. 59, no. 22, pp. 5671–5673, 1999.

[68] J. T. Huang, J. S. Welch, M. Ricote, et al., "Interleukin-4-dependent production of PPAR-γ ligands in macrophages by 12/15-lipoxygenase," *Nature*, vol. 400, no. 6742, pp. 378–382, 1999.

[69] L. Nagy, P. Tontonoz, J. G. Alvarez, H. Chen, and R. M. Evans, "Oxidized ldl regulates macrophage gene expression through ligand activation of PPARgamma," *Cell*, vol. 93, no. 2, pp. 229–240, 1998.

[70] F. J. Schopfer, Y. Lin, P. R. Baker, et al., "Nitrolinoleic acid: an endogenous peroxisome proliferator-activated receptor γ ligand," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 7, pp. 2340–2345, 2005.

[71] P. R. Baker, Y. Lin, F. J. Schopfer, et al., "Fatty acid transduction of nitric oxide signaling: multiple nitrated unsaturated fatty acid derivatives exist in human blood and urine and serve as endogenous peroxisome proliferator-activated receptor ligands," *Journal of Biological Chemistry*, vol. 280, no. 51, pp. 42464–42475, 2005.

[72] B. Y. Hwang, J.-H. Lee, J. B. Nam, H. S. Kim, Y. S. Hong, and J. J. Lee, "Two new furanoditerpenes from Saururus chinenesis and their effects on the activation of peroxisome proliferator-activated receptor γ," *Journal of Natural Products*, vol. 65, no. 4, pp. 616–617, 2002.

[73] Y.-C. Liang, S.-H. Tsai, D.-C. Tsai, S.-Y. Lin-Shiau, and J.-K. Lin, "Suppression of inducible cyclooxygenase and nitric oxide synthase through activation of peroxisome proliferator-activated receptor-γ by flavonoids in mouse macrophages," *FEBS Letters*, vol. 496, no. 1, pp. 12–18, 2001.

[74] M. Kuroda, Y. Mimaki, Y. Sashida, et al., "Phenolics with PPAR-gamma ligand-binding activity obtained from licorice (glycyrrhiza uralensis roots) and ameliorative effects of glycyrin on genetically diabetic kk-A^y mice," *Bioorganic & Medicinal Chemistry Letters*, vol. 13, no. 24, pp. 4267–4272, 2003.

[75] J. Xu, Y. Fu, and A. Chen, "Activation of peroxisome proliferator-activated receptor-gamma contributes to the inhibitory effects of curcumin on rat hepatic stellate cell growth," *American Journal of Physiology*, vol. 285, no. 1, pp. G20–G30, 2003.

[76] S. Zheng and A. Chen, "Activation of PPARgamma is required for curcumin to induce apoptosis and to inhibit the expression of extracellular matrix genes in hepatic stellate cells in vitro," *The Biochemical Journal*, vol. 384, pt. 1, pp. 149–157, 2004.

[77] M. J. Reginato, S. T. Bailey, S. L. Krakow, et al., "A potent antidiabetic thiazolidinedione with unique peroxisome proliferator-activated receptor γ-activating properties," *Journal of Biological Chemistry*, vol. 273, no. 49, pp. 32679–32684, 1998.

[78] K. Inoue, Y. Kawahito, Y. Tsubouchi, et al., "Expression of peroxisome proliferator-activated receptor (PPAR)-gamma in human lung cancer," *Anticancer Research*, vol. 21, pp. 2471–2476, 2001.

[79] T. Satoh, M. Toyoda, H. Hoshino, et al., "Activation of peroxisome proliferator-activated receptor-γ stimulates the growth arrest and DNA-damage inducible 153 gene in non-small cell lung carcinoma cells," *Oncogene*, vol. 21, no. 14, pp. 2171–2180, 2002.

[80] E. Burgermeister, D. Chuderland, T. Hanoch, M. Meyer, M. Liscovitch, and R. Seger, "Interaction with MEK causes nuclear export and downregulation of peroxisome proliferator-activated receptor γ," *Molecular and Cellular Biology*, vol. 27, no. 3, pp. 803–817, 2007.

[81] M. Li, T. W. Lee, A. P. Yim, T. S. Mok, and G. G. Chen, "Apopotsis induced by troglitazone is both peroxisome proliferator-activated receptor-γ- and ERK-dependent in human non-small cell lung cancer cells," *The Journal of Cell Physiology*, vol. 209, no. 2, pp. 428–438, 2006.

[82] M. Li, T. W. Lee, T. S. Mok, T. D. Warner, A. P. Yim, and G. G. Chen, "Activation of peroxisome proliferator-activated receptor-gamma by troglitazone (TGZ) inhibits human lung cell growth," *The Journal of Cellular Biochemistry*, vol. 96, no. 4, pp. 760–774, 2005.

[83] W. Zou, X. Liu, P. Yue, F. R. Khuri, and S. Y. Sun, "PPARg ligands enhance TRAIL-induced apoptosis through DR5 up-regulation and c-FLIP downregulation in human lung cancer cells," *Cancer Biological Therapeutics*, vol. 6, no. 1, pp. 99–106, 2007.

[84] S. Han, N. Sidell, P. B. Fisher, and J. Roman, "Up-regulation of p21 gene expression by peroxisome proliferator-activated receptor gamma in human lung carcinoma cells," *Clinical Cancer Research*, vol. 10, no. 6, pp. 1911–1919, 2004.

[85] W. Zhang, H. Zhang, and L. Xing, "Influence of ciglitazone on A549 cells growth in vitro and in vivo and mechanism," *Journal of Huazhong University of Science and Technology. Medical Sciences*, vol. 26, no. 1, pp. 36–39, 2006.

[86] S. V. Fulzele, A. Chatterjee, M. S. Shaik, T. Jackson, N. Ichite, and M. Singh, "15-deoxy-detal 12,14-prostaglandin J2 enhances docetaxel anti-tumor activity against A549 and H460 non small cell lines and xenograft tumors," *Anticancer Drugs*, vol. 18, pp. 65–78, 2007.

[87] S. Han and J. Roman, "Rosiglitazone suppresses human lung carcinoma cell growth through PPARγ-dependent and PPARγ-independent signal pathways," *Molecular Cancer Therapeutics*, vol. 5, pp. 430–437, 2006.

[88] S. Lee, G. Y. Hur, K. H. Jung, et al., "PPAR-gamma agonist increase gefitinib's antitumor activity thorugh PTEN expression," *Lung Cancer*, vol. 51, no. 3, pp. 297–301, 2006.

[89] J. M. Hall and D. P. McDonnell, "The molecular mechanisms underlying the proinflammatory actions of thiazolidinediones in human macrophages," *Molecular Endocrinology*, vol. 21, no. 8, pp. 1756–1768, 2007.

[90] S. Han and J. Roman, "Suppression of prostaglandin E2 receptor subtype EP2 by PPAR-γ ligands inhibits human lung carcinoma cell growth," *Biochemical and Biophysical Research Communications*, vol. 314, no. 4, pp. 1093–1099, 2004.

[91] A. H. Limper and J. Roman, "Fibronectin: a versatile matrix protein with roles in thoracic development, repair and infection," *Chest*, vol. 101, no. 6, pp. 1663–1673, 1992.

[92] J. Roman, J. D. Ritzenthaler, A. Gil-Acosta, H. N. Rivera, and S. Roser-Page, "Nicotine and fibronectin, expression in lung fibroblasts: implications for tobacco-related lung tissue remodeling," *FASEB Journal*, vol. 18, no. 12, pp. 1436–1438, 2004.

[93] S. Han, F. R. Khuri, and J. Roman, "Fibronectin stimulates non-small cell lung carcinoma cell growth through activation of Akt/mammalian target of rapamycin/S6 kinase and inactivation of LKB1/AMP-activated protein kinase signal pathways," *Cancer Research*, vol. 66, no. 1, pp. 315–323, 2006.

[94] S. Han, J. D. Ritzenthaler, S. V. Sitaraman, and J. Roman, "Fibronectin increases matrix metalloproteinase-9 expression through activation of c-Fos via extracellular-regulated kinase and phosphatidylinositol 3-kinase pathways in human lung carcinoma cells," *Journal of Biological Chemistry*, vol. 281, no. 40, pp. 29614–29624, 2006.

[95] S. Han, J. D. Ritzenthaler, H. N. Rivera, and J. Roman, "Peroxisome proliferator-activated receptor-gamma ligands suppress fibronectin gene expression in human lung carcinoma cells: involvement of both CRE and Sp1," *American Journal of Physiology (Lung Cellular Molecular Physiology)*, vol. 289, no. 3, pp. L419–L428, 2005.

[96] S. Han, H. N. Rivera, and J. Roman, "Peroxisome proliferator-activated receptor-gamma ligands inhibit alpha5 integrin gene transcription in non-small cell lung carcinoma cells," *American Journal of Respiratory Cell and Molecular Biology*, vol. 32, no. 4, pp. 350–359, 2005.

[97] V. G. Keshamouni, D. A. Arenberg, R. C. Reddy, M. J. Newstead, S. Anthwal, and T. J. Standiford, "PPAR-gamma activation inhibits angiogenesis by blocking ELR+CXC chemokine production in non-small cell lung cancer," *Neoplasia*, vol. 7, no. 3, pp. 294–301, 2005.

[98] D. Panigrahy, S. Huang, M. W. Kieran, and A. Kaipainen, "PPARgamma as a therapeutic target for tumor angiogenesis and metastasis," *Cancer Biology Therapeutics*, vol. 4, pp. 687–693, 2005.

[99] A. K. Ghosh, S. Bhattacharyya, G. Lakos., S. J. Chen., Y. Mori, and J. Varga, "Disruption of transforming growth factor β signaling and profibrotic responses in normal skin fibroblasts by peroxisome proliferator-activated receptor γ," *Arthritis & Rheumatism*, vol. 50, no. 4, pp. 1305–1318, 2004.

[100] S. Zafiriou, S. R. Stanners, S. Saad, T. S. Polhill, P. Poronnik, and C. A. Pollock, "Pioglitazone inhibits cell growth and reduces matrix production in human kidney fibroblasts," *Journal of the American Society of Nephorology*, vol. 16, pp. 638–645, 2005.

[101] H. A. Burgess, L. E. Daugherty, T. H. Thatcher, et al., "PPARgamma agonists inhibit TGF-beta induced pulmonary myofibroblast differentiation and collagen production: implications for therapy of lung fibrosis," *American Journal of Physiology (Lung Cellular Molecular Physiology)*, vol. 288, pp. L1146–L1153, 2005.

[102] L. Yang, C. C. Chan, O. S. Kwon, et al., "Regulation of peroxisome proliferator-activated receptor-γ in liver fibrosis," *American Journal of Physiology (Gastrointestinal and Liver Physiology)*, vol. 291, pp. G902–G911, 2006.

[103] J. A. Van Ginderachter, S. Meerschaut, Y. Liu, et al., "Peroxisome proliferator-activated receptor γ (PPARγ) ligands reverse CTL suppression by alternatively activated (M2) macrophages in cancer," *Blood*, vol. 15, no. 2, pp. 525–535, 2006.

[104] S. Theocharis, H. Kanelli, E. Politi, et al., "Expression of peroxisome proliferator-activated receptor-gamma in non-small cell lung carcinoma: correlation with histological type and grade," *Lung Cancer*, vol. 36, no. 3, pp. 249–255, 2002.

[105] G. Muzio, A. Trombetta, M. Maggiora, et al., "Arachidonic acid suppresses growth of human lung tumor A549 cells through down-regulation of ALDH3A1 expression," *Free Radicals in Biological Medicine*, vol. 40, no. 11, pp. 1929–1938, 2006.

[106] I. Avis, A. Martinez, J. Tauler, et al., "Inhibitors of the arachidonic acid pathway and peroxisome proliferator-activated receptor ligands have superadditive effects on lung cancer growth inhibition," *Cancer Research*, vol. 65, pp. 4181–4190, 2005.

[107] C. J. Yao, G. M. Lai, C. F. Chan, A. L. Chen, Y. Y. Yang, and S. E. Chuang, "Dramatic synergistic anticancer effect of clinically achievable doses of lovastatin and troglitazone," *International Journal of Cancer*, vol. 118, no. 3, pp. 773–779, 2005.

[108] R. A. Nemenoff and R. A. Winn, "Role of nuclear receptors in lung tumorigenesis," *European Journal of Cancer*, vol. 41, no. 16, pp. 2561–2568, 2005.

[109] R. Govindarajan, L. Ratnasinghe, D. L. Simmons, et al., "Thiazolidinediones and the risk of lung, prostate, and colon cancer in patients with diabetes," *Journal of Clinical Oncology*, vol. 25, no. 12, pp. 1476–1481, 2007.

[110] C. D. Allred and M. W. Kilgore, "Selective activation of PPAR γ in breast, colon and lung cancer cell lines," *Molecular and Cellular Endocrinology*, vol. 235, no. 1-2, pp. 21–29, 2005.

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Research Article

Stimulatory Effects of Peroxisome Proliferator-Activated Receptor-γ on Fcγ Receptor-Mediated Phagocytosis by Alveolar Macrophages

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Alveolar macrophages abundantly express PPAR-γ, with both natural and synthetic agonists maintaining the cell in a quiescent state hyporesponsive to antigen stimulation. Conversely, agonists upregulate expression and function of the cell-surface receptor CD36, which mediates phagocytosis of lipids, apoptotic neutrophils, and other unopsonized materials. These effects led us to investigate the actions of PPAR-γ agonists on the Fcγ receptor, which mediates phagocytosis of particles opsonized by binding of immunoglobulin G antibodies. We found that troglitazone, rosiglitazone, and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 increase the ability of alveolar, but not peritoneal, macrophages to carry out phagocytosis mediated by the Fcγ receptor. Receptor expression was not altered but activation of the downstream signaling proteins Syk, ERK-1, and ERK-2 was observed. Although it was previously known that PPAR-γ ligands stimulate phagocytosis of unopsonized materials, this is the first demonstration that they stimulate phagocytosis of opsonized materials as well.

1. INTRODUCTION

Phagocytosis—engulfment of invading pathogens, particulates, and dying cells—is a crucial homeostatic mechanism in multicellular organisms. Most mammalian phagocytosis is carried out by macrophages or neutrophils. This process begins with adhesion of the material to be phagocytosed to a receptor on the macrophage or neutrophil surface. The receptor then triggers intracellular signals that lead to a zipper-like infolding of the cell membrane, engulfing the receptor and that which is bound to it. Further signals cause transport of the resulting endosome to the lysosome, where enzymes are available to digest commonly phagocytosed materials.

Both oposonin-dependent and-independent classes of cell-surface receptors mediate phagocytosis. Among the former are the Fc receptors that recognize the Fc portion of an immunoglobulin bound through its antigen-recognition site to the target particle or organism [1]. The most important of these is the Fcγ receptor for immunoglobulin G (IgG), but Fcα receptors and Fcε receptors (for the Fc portions of immunoglobulin A and immunoglobulin E, resp.) also exist. Complement receptors also recognize opsonized particles that are bound with complement proteins [2]. The broad class of opsonin-independent receptors involved in immune surveillance and phagocytosis includes the Toll-like and scavenger receptors that recognize apoptotic cells, microbial components, and other unopsonized materials [3, 4].

The nuclear receptor, peroxisome proliferator-activated receptor-γ (PPAR-γ), is expressed in a variety of cells of the immune system, including macrophages, neutrophils, eosinophils, lymphocytes, and mast cells. This receptor is expressed abundantly in alveolar macrophages (AMs) [5–7] but at much lower levels in resident macrophages of the bone marrow and peritoneum [6, 7]. In peritoneal macrophages

(PMs) that have been elicited by activating agents such as thioglycolate, however, PPAR-γ is upregulated significantly [7].

Many aspects of AM function have been found to be modulated by both natural and synthetic PPAR-γ ligands [8]. For example, PPAR-γ ligands inhibit the ability of various stimuli to induce production of proinflammatory mediators, including tumor necrosis factor-α and interleukin-12, expression of inducible nitric oxide synthase, and the production of reactive oxygen species [5, 6]. Conversely, activation of PPAR-γ in AMs has been shown to upregulate phagocytosis of apoptotic neutrophils through increased expression of the CD36 surface receptor [5]. PPAR-γ ligands have also been shown to increase CD36-mediated phagocytosis of senescent neutrophils and fluorescent-labeled latex beads by pancreatic stellate cells [9].

In light of these results, we hypothesized that activation of PPAR-γ could regulate Fcγ receptor-mediated phagocytosis. We therefore performed experiments in both AMs and PMs using IgG-opsonized phagocytic targets and ligands for PPAR-γ.

2. MATERIALS AND METHODS

2.1. Animals

Pathogen-free 129/SvEv mice (The Jackson Laboratory, Bar Harbor, Me, USA) and 125–150 gm female Wistar rats (Charles River Laboratories, Portage, Mish, USA) were utilized. Animals were treated according to National Institutes of Health guidelines for the use of experimental animals with the approval of the University of Michigan Committee on Use and Care of Animals.

2.2. Reagents

O-phenylenediamine dihydrochloride, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), and sodium dodecyl sulfate were obtained from Sigma-Aldrich (St. Louis, Mo, USA). Uniform, superparamagnetic, 2.8 micron polystyrene beads covalently coated with IgG were purchased from Dynal-Invitrogen (Carlsbad, Calif, USA). Troglitazone, rosiglitazone, and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2(15d-PGJ_2) were obtained from Cayman Chemical (Ann Arbor, Mich, USA). These compounds were dissolved in DMSO to a stock concentration of 10 mM and stored at -80°C prior to use. RPMI-1640 and penicillin/streptomycin/amphotericin B solutions were purchased from Gibco-Invitrogen (Carlsbad, Calif, USA). Tryptic soy broth was supplied by Difco (Detroit, Mich, USA). *Klebsiella pneumoniae* 43816, serotype 2, was obtained from the American Type Culture Collection (Rockville, Md, USA); aliquots were grown until mid-log phase in TSB at 37°C under 5% CO_2 atmosphere. The concentration of bacteria in culture was determined spectrophotometrically at 600 nm [10]. Required dilutions of all compounds were prepared immediately before use and equivalent quantities of vehicle were added to the appropriate controls.

2.3. Cell isolation and culture

Resident AMs from mice and rats were obtained via ex vivo lung lavage as previously described [11] and resuspended in RPMI to a final concentration of 2×10^6 cells/mL. Resident peritoneal macrophages (PMs) from mice and rats were harvested by lavage as previously published [12]. Cells were allowed to adhere to tissue-culture-treated slides/plates for 1 hour at 37°C in a 5% CO_2 atmosphere, followed by two washings with warm RPMI to remove nonadherent cells. Prior to use, macrophages were cultured overnight in RPMI containing 10% fetal bovine serum and 1% penicillin/streptomycin/amphotericin B. On the following day, cells were washed again with a warm medium to remove nonadherent cells.

2.4. Microcolorimetric erythrocyte phagocytosis assay

Macrophage phagocytosis of IgG-opsonized sheep red blood cells (SRBCs) was assessed as previously described [13, 14]. Briefly, cells were plated and cultured overnight in 96-well culture-treated dishes (Becton, Dickinson, Franklin Lakes, NJ, USA) at a density of 2×10^5 cells/well and in the presence of PPAR-γ ligands or vehicle controls. SRBCs (ICN, Costa Mesa, Calif, USA) were opsonized with a subagglutinating concentration of polyclonal rabbit anti-SRBC IgG (Organon Teknika-Cappel, Durham, NC, USA). Macrophages were then washed twice with warm RPMI and preincubated for 45 minutes with cytochalasin D (5 μg/mL) or vehicle. Following preincubation, opsonized SRBCs were added at a ratio of 50 : 1 (SRBC : macrophage) and cultures were incubated for an additional 90 minutes at 37°C. Wells were then washed three times with phosphate buffered saline to remove noningested erythrocytes and 100 μL of 0.3% sodium dodecyl sulfate in phosphate buffered saline was added to each well for 10 minutes. A standard curve was derived by adding serial dilutions of known numbers of SRBCs to separate wells followed by addition of sodium dodecyl sulfate solution. Lastly, 100 μL of O-phenylenediamine dihydrochloride solution was added to each well as a chromogen. Following a 30-minute incubation in the dark at 22°C, the absorbance (A) at 450 nm was evaluated with an automated reader (VersaMAX, Molecular Devices, Sunnyvale, Calif, USA). The number of SRBCs per well was derived from A_{450} data using the standard curve prepared as described. The phagocytic index (PI) was defined as the number of SRBCs in an experimental well (ingested + adhered SRBCs) minus the mean number of SRBCs in wells treated with the phagocytosis inhibitor cytochalasin D (adhered SRBCs) and was expressed as the percentage of the control. Independent experiments were performed in septuplet.

2.5. Phagocytosis of IgG-opsonized beads

Phagocytosis of IgG-opsonized beads (IgG-beads) was quantified via light microscopy. Macrophages were cultured on 8-chamber glass slides before the challenge with IgG-beads at a ratio of 40 beads/cell. PPAR-γ ligands or vehicle controls were added before the addition of IgG-beads as described

in Section 3 and/or figure legends. Experiments were terminated and uningested IgG-beads were removed by aspirating supernatants and washing slides three times with cold phosphate buffered saline. Slides were subsequently stained with a modified Wright-Giemsa stain and examined under light microscopy. The PI was determined from 200 cells per well by multiplying the percentage of macrophages containing at least 1 IgG-bead by the mean number of IgG-beads per positive cell [13, 15]. The ability to distinguish intracellular from surface-associated IgG-beads was verified by comparing the PI of untreated cells with that of cells exposed for 30 minutes to the phagocytosis inhibitor cytochalasin D (5 μg/mL) [16]. A minimum of 4 replicate wells per condition was studied in each experiment.

2.6. Phagocytosis of live, serum-opsonized bacteria

Once the Gram-negative pathogen *K. pneumoniae* has been opsonized with immune serum, it is subject to phagocytosis by alveolar macrophages via the Fcγ class of receptors [17]. We assessed phagocytosis of *K. pneumoniae* based on a protocol for bacterial killing that we have previously published [18]. Briefly, rat AMs at a concentration of 2×10^6/mL, prepared as described, were seeded in a 96-well tissue culture dish and exposed to PPAR-γ ligands or vehicle controls for 18 hours. The next day, *K. pneumoniae* were opsonized with 3% anti-*K. pneumoniae* rat-derived immune serum, as previously described [16]. Macrophages were then infected with a 0.1-mL suspension of opsonized *K. pneumoniae* (1×10^7 colony-forming units/mL; multiplicity of infection, 50 : 1) and incubated for 30 minutes to allow phagocytosis to occur. Cells were then washed three times with 100 μL of phosphate buffered saline to remove noningested bacteria, after which the macrophages were lyzed with 100 μL of TSB containing 0.5% saponin (which did not lyze the bacteria). Cultures were incubated for 2 hours at 37°C to amplify bacterial growth prior to the addition of the tetrazolium salt MTT (5 mg/mL in phosphate buffered saline). Plates were held for 30 minutes at 37°C, after which the purple formazan salt was solubilized with a solution of isopropanol/0.1 N HCL and 1% Triton X-100 [19]. The intensity of the absorbance at 595 nm was directly proportional to the number of intracellular bacteria associated with the macrophages [19]. Results are expressed as a percent of the untreated cells.

2.7. Immunoblot analysis

Western blots were performed as previously described [20]. Briefly, the whole cell protein extracts were obtained by lyzing freshly harvested AMs in a buffer [50 mM Tris-HCl (pH 7.4), 25 mM KCl, 5 mM $MgCl_2$, 0.2% Nonidet P-40] supplemented with protease and phosphatase inhibitors (Roche Diagnostics, Mannheim, Germany). Protein samples (40 μg) were resolved on 10% Tris-HCl polyacrylamide gels and subsequently transferred to nitrocellulose membranes. Membranes were probed with commercially available rabbit polyclonal antibodies against phospho-spleen tyrosine kinase (phospho-Syk; Tyr525/526; Cell Signaling Technology, Danvers, Mass, USA; 1 : 500), total Syk (Santa Cruz Biotechnology, Inc., Calif, USA; 1 : 800), total p42/44 (ERK-1/2; Cell Signaling Technology; 1 : 1000), or with mouse monoclonal antibodies against β-actin (Sigma-Aldrich; 1 : 10000) or phospho-p42/44 (Tyr204/Thr202; Cell Signaling Technology; 1 : 1000) followed in either case by horseradish peroxidase-conjugated antirabbit or antimouse, respectively, secondary antibodies, and ECL chemiluminescence detection reagents (Amersham Biosciences, Piscataway, NJ, USA). For experiments involving activation of the Fcγ receptor, AMs were treated for 7 minutes with IgG-SRBCs at a ratio of 33 SRBC per macrophage [21]. Band density from Western blots was determined using Adobe Photoshop 6.0 (Adobe, San Jose, Calif, USA).

2.8. RT-PCR of Fcγ receptors I, IIB, and III

The mRNA expression of Fcγ receptors I, IIB, and III was determined in macrophages treated for 16 hours with troglitazone (5 μM) or with DMSO vehicle. RNA from cultured cells was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RT-PCR was then performed using the Access RT-PCR kit (Promega Corporation, Madison, Wis, USA) according to the manufacturer's directions, with 100 ng of RNA being used for each reaction. The primers used in the reaction were synthesized according to standard methods and displayed in Table 1. The PCR conditions were as follows: 45 minutes at 45°C, 2 minutes at 94°C followed by 30 cycles of 30 seconds at 94°C followed by 1 minute at 58°C, and then 90 seconds at 68°C. All PCRs were performed in a reaction volume of 50 μL.

2.9. Statistical analysis

Data are represented as mean ± SE and were analyzed with the Prism 4.0 statistical program (GraphPad Software, San Diego, Calif, USA). Comparisons between two experimental groups were performed with Student t test. Comparisons among ≥ 3 experimental groups were performed with analysis of variance (ANOVA) followed by Dunnett's adjustment for multiple comparisons. Differences were considered significant if $P < .05$. All experiments were performed on at least three separate occasions unless otherwise specified.

3. RESULTS

3.1. Troglitazone increases Fcγ receptor-mediated phagocytosis in rat AMs but not PMs

Troglitazone is a thiazolidinedione no longer approved for human use but still commonly used experimentally to activate PPAR-γ. An earlier study demonstrated that doses >10 μM decreased Fcγ receptor-mediated phagocytosis in a macrophage-like cell line, although this effect was accompanied by apoptosis [22]. To study Fcγ receptor-mediated phagocytosis in a more biologically relevant system, we employed lower, nonapoptotic inducing doses of this drug using primary AMs. As shown (Figures 1(a) and 1(b)), troglitazone enhanced the ingestion of IgG-SRBCs by rat AMs, with the effect being both dose- and time-dependent. The peak

Table 1: Primer sequences used for RT-PCR.

Gene	Primer	
FcγRI	Forward	5′-GAG CAG GGA AAG AAA GCA AAT TCC-3′
	Reverse	5′-TTA AGA GTT GCA TGC CAT GGT CC-3′ (232 bp)
FcγRIIB	Forward	5′-CCC AAG TCC AGC AGG TCT TTA CC-3′
	Reverse	5′-TTC TGG CTT GCT TTT CCC AAT GCC-3′ (277 bp)
FcγRIII	Forward	5′-GAT CCA GCA ACT ACA TCC TCC ATC-3′
	Reverse	5′-GCC TTG AAC TGG TGA TCC TAA GTC-3′ (333 bp)

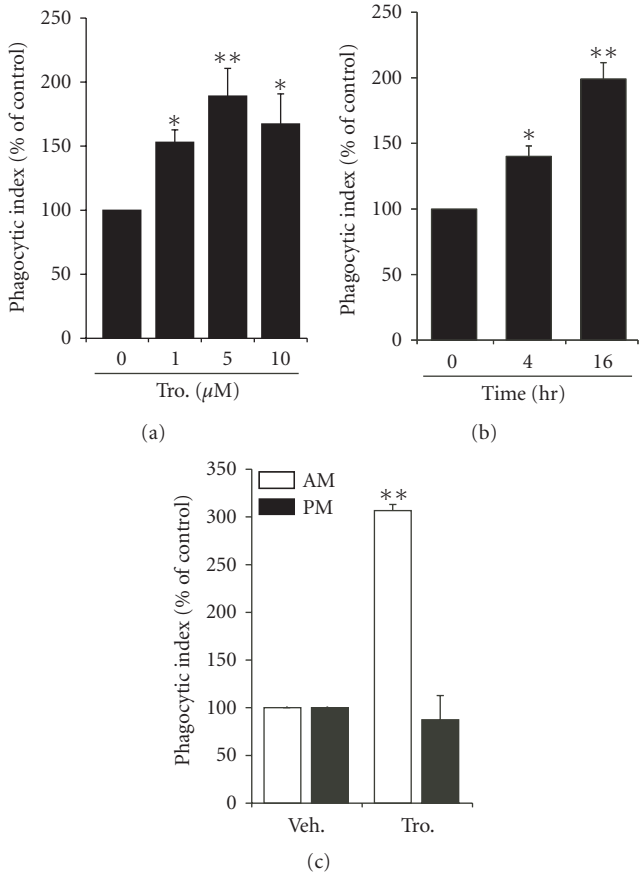

Figure 1: Stimulation of Fcγ receptor-mediated phagocytosis by troglitazone in rat macrophages. Rat alveolar macrophages (AMs) were treated for 16 hours with troglitazone at the doses indicated by (a) or with 5 μM troglitazone for the times indicated by (b) prior to the challenge with IgG-opsonized sheep red blood cells (SRBCs). In (c), both AMs and peritoneal macrophages (PMs) were treated for 16 hours with 5 μM troglitazone before phagocytosis was assessed, as described in Section 2. $^{*}P < .05$ and $^{**}P < .01$ compared to untreated cells.

effect occurred with a 16-hour incubation in the presence of 5 μM troglitazone; this exposure increased phagocytosis to 199 ±12.4% of the untreated value (Figure 1(b)). At this dose of troglitazone, apoptosis was not observed (data were not shown).

Unlike AMs, PMs express little PPAR-γ [6]. We speculated that the effect of troglitazone would be more potent in AMs than PMs, reflecting the differences in PPAR-γ expression. Indeed, we observed no increase in the ingestion of IgG-SRBCs by rat PMs treated with troglitazone (Figure 1(c)).

3.2. Troglitazone enhances Fcγ receptor-mediated phagocytosis in murine AMs but not PMs

To address the generalizability of our initial observation, we repeated our experiments using murine macrophages and a different IgG-opsonized target, an IgG-coated polystyrene bead. Figure 2 demonstrates that troglitazone enhanced Fcγ receptor-mediated phagocytosis by AMs over the same concentration range observed for the rat, while no effects were seen in the PMs.

3.3. Multiple PPAR-γ ligands enhance Fcγ receptor-mediated phagocytosis by AMs

The above studies were limited by (a) the application of a single PPAR-γ ligand with known/suspected PPAR-γ-independent signaling properties [23] and (b) the use of nonphysiological targets of IgG-opsonization. We therefore tested the ability of rat AMs to ingest IgG-opsonized bacteria using the relevant Gram-negative pathogen *K. pneumoniae*. As demonstrated in Figure 3, troglitazone, rosiglitazone, and 15d-PGJ_2 each increased phagocytosis of *K. pneumoniae* by ~20%–25% when administered to the cells at a 10 μM concentration. Thus, distinct PPAR-γ ligands enhance the ingestion of IgG-opsonized pathogens by primary lung macrophages.

3.4. PPAR-γ activation does not modulate Fcγ receptor expression

PPAR-γ ligands have been shown to increase the phagocytosis of apoptotic cells by increasing the cell surface expression of the CD36 receptor [5]. By analogy, we speculated that the observed stimulation of Fcγ receptor-mediated phagocytosis by PPAR-γ ligands might reflect increased expression of that receptor. We therefore performed RT-PCR for the Fcγ receptors I and III using RNA extracted from mouse AMs treated for 16 hours with 5 μM troglitazone. We also considered an alternative possibility that PPAR-γ activation might suppress the expression of the Fcγ IIB receptor, which is an inhibitory Fcγ receptor. However, we did not detect significant differences in the expression of any of these three receptors by RT-PCR (Figure 4), confirming the flow-cytometric results obtained by Kasono et al. using J774.A1 macrophages [22].

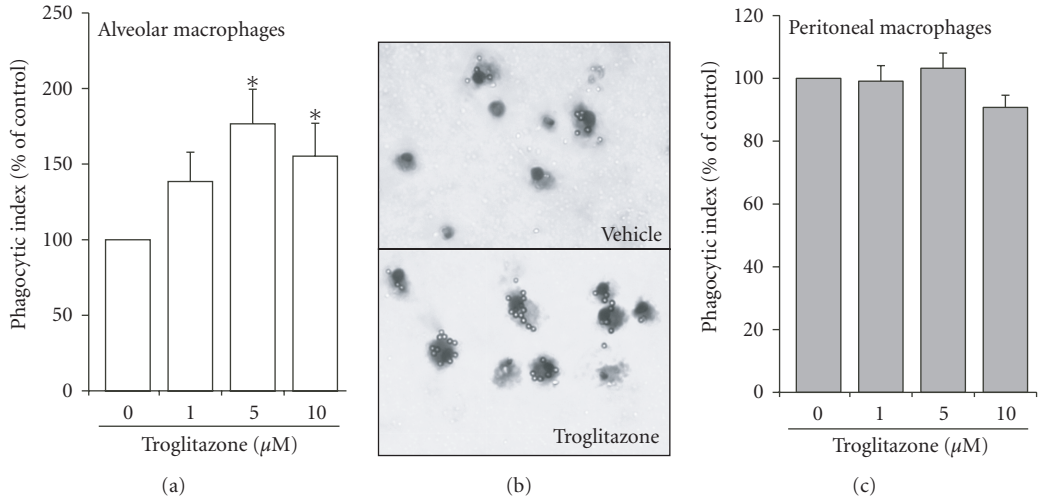

Figure 2: Stimulation of Fcγ receptor-mediated phagocytosis by troglitazone in mouse macrophages. Murine AMs (a) and (b) or PMs (c) were treated for 16 hours with 5 μM troglitazone before phagocytosis of IgG-opsonized beads was assessed, as described in Section 2. Panel (b) is a representative light microscopy field (400x magnification) demonstrating the effect of troglitazone (5 μM, bottom panel) compared to vehicle (top panel). $^*P < .05$ compared to untreated cells.

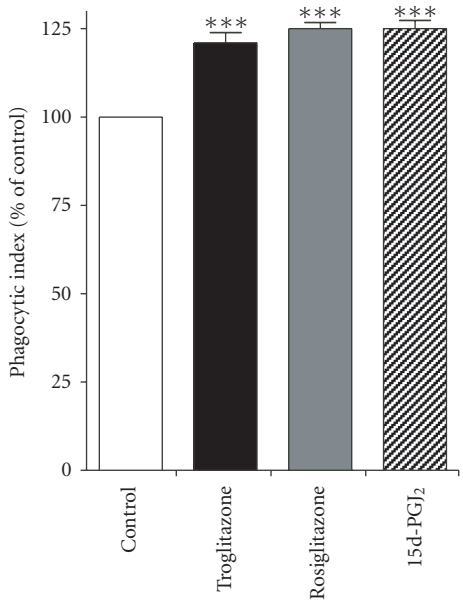

Figure 3: PPAR-γ ligands enhance phagocytosis of opsonized *K. pneumoniae*. Rat AMs were pretreated for 16 hours with troglitazone, rosiglitazone, or 15d-PGJ_2 (each at 10 μM) prior to infection with immune serum-opsonized *K. pneumoniae* at a multiple of infection of 50 : 1. Phagocytosis was determined after 30 minutes, as detailed in Section 2. $^{***}P < .001$ compared to untreated cells.

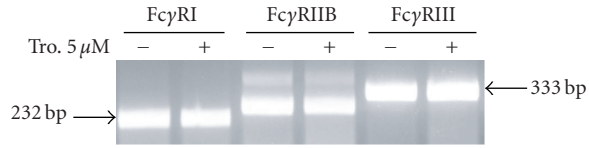

Figure 4: Expression of mRNA for Fcγ receptors is not affected by PPAR-γ ligands. Mouse AMs were plated and treated for 16 hours with either 5 μM troglitazone or DMSO vehicle. RNA was isolated, amplified by RT-PCR, and subjected to electrophoresis. The expected sizes of cDNAs for Fcγ receptors I, IIB, and III, respectively, are 232, 277, and 333 bp.

3.5. *Troglitazone enhances post-Fcγ receptor signaling in AMs*

Because the expression of Fcγ receptors was not altered by troglitazone, we postulated that PPAR-γ activation might be enhancing the intracellular signaling network involved in the internalization of IgG-opsonized targets. We therefore tested the effect of troglitazone (5 μM for 16 hours) on the activation of proximal and distal signaling molecules involved in Fcγ receptor-mediated phagocytosis [24]. As shown in Figure 5, the proximal tyrosine kinase Syk becomes phosphorylated when cells are challenged with IgG-SRBCs; this phosphorylation was significantly enhanced by troglitazone. The extracellular signal-regulated protein kinases (ERK)-1 and -2 (also known as p42/44 proteins) are also important in IgG-mediated phagocytosis [24]. We found that 16-hour administration of troglitazone to AMs stimulated activation of ERK-1 and -2 over and above that triggered by IgG-SRBCs alone. Analysis showed that only prior treatment with troglitazone led to statistically significant increases in the phosphorylation of Syk or ERK proteins in response to opsonized SRBCs (Figure 5).

4. DISCUSSION

In this study, we demonstrate that activation of PPAR-γ enhances the phagocytosis of IgG-opsonized targets via the Fcγ class of receptors in AMs. To our knowledge, this is the first study to demonstrate that PPAR-γ ligands increase

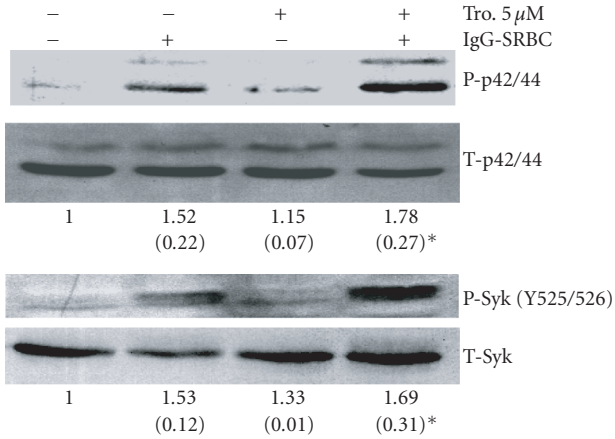

Figure 5: Troglitazone enhances Syk and ERK activation during Fcγ receptor-mediated phagocytosis. Rat AMs were treated with 5 μM troglitazone for 16 hours prior to challenge with IgG-opsonized SRBCs. Unopsonized SRBCs were used as negative controls. Cells were lyzed after 7 minutes and subjected to Western immunoblot analysis. Bands labeled p42 and p44 represent ERK-1 and -2. The phosphorylation of Syk was identified on the tyrosine residues 525 and 526. Representative blots from three independent experiments are shown. Values represent the mean ($\pm$ SE) of the ratio of phosphorylated to total proteins determined by band densitometry from multiple experiments ($n = 3 - 4$), expressed relative to untreated cells. $^*P < .05$ compared to untreated cells.

Fcγ receptor-mediated phagocytosis. Notably, the effects of troglitazone that were seen in AMs were not observed in resident PMs. This result accords with the earlier finding that AMs express significantly more PPAR-γ than PMs do [6].

We hypothesized that PPAR-γ activation might regulate Fcγ receptor-mediated phagocytosis based on the nuclear receptor's known ability to enhance phagocytosis mediated via other receptors on the cell surface. For example, PPAR-γ activation has been shown to increase expression of the cell surface receptor CD36, which is involved in the recognition and internalization of apoptotic cells, and thereby to enhance apoptotic cell uptake by macrophages [5]. The phagocytosis of senescent neutrophils and unopsonized polystyrene beads by pancreatic stellate cells was also enhanced by PPAR-γ-activating agents [9]. This effect was also shown to result from increased expression of the cell-surface receptor CD36, although the receptor(s) involved was not specifically characterized.

Our studies were strengthened by the use of AMs and PMs from both rats and mice and by the use of multiple IgG-opsonized targets, including standard SRBCs and live bacterial pathogens. However, our results appear to differ from the only other published study of PPAR-γ activation and receptor-mediated phagocytosis [22]. Using the macrophage-like cell line J774.A1, Kasono et al. found that troglitazone, pioglitazone, and 15d-PGJ_2 suppressed phagocytosis of IgG-opsonized SRBCs without—as we also found—altering Fcγ receptor expression. However, the authors demonstrated that both troglitazone and pioglitazone induced significant apoptosis in these cells at the same concentrations used to suppress phagocytosis (15d-PGJ_2 was not tested). It therefore seems likely that the inhibition by PPAR-γ ligands of Fcγ receptor-mediated ingestion in J774.A1 cells occurred primarily as a consequence of cell death through apoptosis. It is notable, however, that Kusano et al. found that both the suppression of phagocytosis and the induction of apoptosis occurred at doses of troglitazone >30 μM, whereas a dose of 10 μM caused an increase in phagocytosis that did not reach statistical significance. We also observed inhibition of phagocytosis and cell death in AMs at concentrations of troglitazone >10 μM (data were not shown).

Although we found qualitatively similar, stimulatory effects of troglitazone on Fcγ receptor-mediated phagocytosis using three unique phagocytic targets (erythrocytes, beads, and *K. pneumoniae*), the magnitude of troglitazone's effects differed with regards to the model examined. The reasons for this are not entirely clear. The greatest effect of troglitazone was seen in assays using inert targets (IgG-SRBCs and IgG-beads), as compared to the use of live, serum-opsonized bacteria. We speculate that as yet undefined differences between the interactions of macrophages with live bacteria versus interactions with inert targets might underlie these variabilities.

Azuma et al. demonstrated that the PPAR-γ ligand 15d-PGJ_2 dose dependently inhibited the phagocytosis by glycogen-elicited (activated) PMs from Wistar rats of unopsonized *Escherichia coli* [25] (lack of opsonization implied that phagocytosis was not mediated by the Fcγ receptor). However, since PPAR-γ expression is known to be markedly upregulated in activated compared to resident PMs [7], the disparity between these results and our failure to find an effect of troglitazone on phagocytosis via the Fcγ receptor in resident PMs is not surprising.

The finding of activation of phagocytosis in AMs, rather than the inhibition that Azuma et al. observed in activated PMs, may be attributed to differences in the two cell types [26, 27]. The alveolus is constantly exposed to pathogens and irritant particles drawn in with the inspired air, and the inciting of an inflammatory response to inhaled irritants might impair the ability of the alveolar space to participate in the essential function of gas exchange. Studies have shown that PPAR-γ ligands inhibit AM inflammatory responses, including the production of reactive oxygen species, and the expression of pro-inflammatory cytokines and inducible nitric oxide synthase [5, 6]. Phagocytosis without accompanying inflammatory activity, however, does not threaten alveolar function. There is, thus, no conflict between downregulation of inflammatory responses and simultaneous upregulation of phagocytosis mediated by either CD36 receptors [5, 9] or Fcγ receptors (this study). This point is further supported by the finding of Sutterwala et al. in bone marrow macrophages, in which the binding of materials such as IgG-opsonized SRBCs to the Fcγ receptor promoted the production of the anti-inflammatory cytokine interleukin-10 and the resultant inhibition of the pro-inflammatory cytokine interleukin-12's production [28].

Regulation of Fcγ receptor expression and activity is complex. Granulocyte-macrophage colony stimulating factor is required both for constitutive expression in AMs and

for upregulation of receptor expression by interferon-γ [29]. Mancuso et al. found that leukotrienes B_4 and C_4, as well as 5-hydroxyeicosatrienoic acid (5-HETE), stimulated AM phagocytosis of *K. pneumoniae* [16]. A subsequent study showed that this effect was specific to bacteria opsonized with IgG and due to downstream activation of Fcγ receptor internalization and transport rather than to increased receptor expression [17]. These same leukotrienes stimulate AM bactericidal activity by activating NADPH oxidase and stimulating production of H_2O_2 [18], an effect that in this case is opposite to that of PPAR-γ ligands. Conversely, reflecting the frequent antagonism between leukotrienes and prostaglandins, prostaglandin E_2 has been shown to inhibit Fcγ receptor-mediated phagocytosis in AMs [13].

We found that, just as with leukotrienes, increased Fcγ receptor-mediated phagocytosis induced by PPAR-γ ligands did not result from increased receptor expression. While the PPAR-γ ligands did not alter Fcγ receptor mRNA expression, these studies do not rule out the possibility that the ligands altered phagocytosis by increasing the surface expression of these receptors. Regardless, we infer from our data that PPAR-γ ligands prime cells for an enhanced activation of downstream effectors involved with postbinding internalization and transport, such as Syk, ERK-1, and ERK-2. While our data support a mechanism whereby PPAR-γ ligands stimulate post-Fcγ receptor signaling (rather than receptor expression), our work does not definitively establish the true role of these signaling pathways in this process.

Syk, which is a protein tyrosine kinase, has been shown to be essential for the transport of internalized Fc receptors to lysosomes [30]. Enhancement of Fcγ receptor-mediated phagocytosis in AMs by LTB_4 has also been shown to depend on Syk activation [21]. Our study appears to be the first to demonstrate effects of PPAR-γ ligands on Syk activity. Effects of PPAR-γ ligands on ERK-1/2 activation, however, have previously been established. For example, inhibition of growth and induction of apoptosis by 15d-PGJ_2 in a neuroblastoma cell line was associated with ERK activation [31] as was troglitazone-induced arrest of cell growth in lung adenocarcinoma cells [32]. Similar effects of troglitazone in lung cancer cells were shown to be blocked by inhibition of either PPAR-γ expression or ERK-1/2 activity [33]. In a mouse osteoblastic cell line, induction of apoptosis by ciglitazone was accompanied by increased amounts of phosphorylated ERK, with cell death being blocked by both PPAR-γ and ERK antagonists [34].

It may be questioned whether the effects we saw were necessarily mediated via PPAR-γ, since it is known that 15d-PGJ_2 and thiazolidinediones can act through PPAR-γ-independent mechanisms [35, 36]. Although the evidence is indirect, finding similar effects with troglitazone, rosiglitazone, and 15d-PGJ_2 argues for an effect mediated through their common receptor. This conclusion is further strengthened by the observation that such effects were seen in AMs, where PPAR-γ expression is abundant, but not in resident PMs that express relatively little of this receptor.

In summary, we demonstrate here that PPAR-γ ligands stimulate phagocytosis via the Fcγ receptor in AMs but not in PMs. This effect does not depend on increased expression of the cell-surface receptor, but rather on downstream activation of Syk, ERK-1, and ERK-2. In AMs, PPAR-γ ligands thus stimulate phagocytosis mediated by two quite different classes of cell-surface receptors and do so via quite different mechanisms.

ACKNOWLEDGMENT

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REFERENCES

[1] J. V. Ravetch and S. Bolland, "IgG Fc receptors," *Annual Review of Immunology*, vol. 19, pp. 275–290, 2001.

[2] E. J. Brown, "Complement receptors, adhesion, and phagocytosis," *Infectious Agents and Disease*, vol. 1, no. 2, pp. 63–70, 1992.

[3] S. Akira, "TLR signaling," *Current Topics in Microbiology and Immunology*, vol. 311, pp. 1–16, 2006.

[4] A. Palecanda and L. Kobzik, "Receptors for unopsonized particles: the role of alveolar macrophage scavenger receptors," *Current Molecular Medicine*, vol. 1, no. 5, pp. 589–595, 2001.

[5] K. Asada, S. Sasaki, T. Suda, K. Chida, and H. Nakamura, "Antiinflammatory roles of peroxisome proliferator-activated receptor γ in human alveolar macrophages," *American Journal of Respiratory and Critical Care Medicine*, vol. 169, no. 2, pp. 195–200, 2004.

[6] R. C. Reddy, V. G. Keshamouni, S. H. Jaigirdar, et al., "Deactivation of murine alveolar macrophages by peroxisome proliferator-activated receptor-γ ligands," *American Journal of Physiology*, vol. 286, no. 3, pp. L613–L619, 2004.

[7] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[8] T. J. Standiford, V. C. Keshamouni, and R. C. Reddy, "Peroxisome proliferator-activated receptor-γ as a regulator of lung inflammation and repair," *Proceedings of the American Thoracic Society*, vol. 2, no. 3, pp. 226–231, 2005.

[9] K. Shimizu, M. Kobayashi, J. Tahara, and K. Shiratori, "Cytokines and peroxisome proliferator-activated receptor γ ligand regulate phagocytosis by pancreatic stellate cells," *Gastroenterology*, vol. 128, no. 7, pp. 2105–2118, 2005.

[10] M. J. Greenberger, R. M. Strieter, S. L. Kunkel, J. M. Danforth, R. E. Goodman, and T. J. Standiford, "Neutralization of IL-10 increases survival in a murine model of *Klebsiella pneumonia*," *The Journal of Immunology*, vol. 155, no. 2, pp. 722–729, 1995.

[11] M. Peters-Golden and P. Thebert, "Inhibition by methylprednisolone of zymosan-induced leukotriene synthesis in alveolar macrophages," *American Review of Respiratory Disease*, vol. 135, no. 5, pp. 1020–1026, 1987.

[12] M. Peters-Golden, R. W. McNish, R. Hyzy, C. Shelly, and G. B. Toews, "Alterations in the pattern of arachidonate metabolism accompany rat macrophage differentiation in the lung," *The Journal of Immunology*, vol. 144, no. 1, pp. 263–270, 1990.

[13] D. M. Aronoff, C. Canetti, and M. Peters-Golden, "Prostaglandin E_2 inhibits alveolar macrophage phagocytosis through an E-prostanoid 2 receptor-mediated increase in intracellular cyclic AMP," *The Journal of Immunology*, vol. 173, no. 1, pp. 559–565, 2004.

[14] C. Smacchia, P. Rebulla, F. Drago, F. Morelati, M. Pappalettera, and G. Sirchia, "A micro colorimetric assay using cryopreserved monocytes to evaluate antibody-mediated red cell-monocyte interaction," *Haematologica*, vol. 82, no. 5, pp. 526–531, 1997.

[15] D. M. Aronoff, C. Canetti, and M. Peters-Golden, "Prostaglandin E_2 inhibits alveolar macrophage phagocytosis through an E-prostanoid 2 receptor-mediated increase in intracellular cyclic AMP," *The Journal of Immunology*, vol. 173, no. 1, pp. 559–565, 2004.

[16] P. Mancuso, T. J. Standiford, T. Marshall, and M. Peters-Golden, "5-Lipoxygenase reaction products modulate alveolar macrophage phagocytosis of *Klebsiella pneumoniae*," *Infection and Immunity*, vol. 66, no. 11, pp. 5140–5146, 1998.

[17] P. Mancuso and M. Peters-Golden, "Modulation of alveolar macrophage phagocytosis by leukotrienes is Fc receptor-mediated and protein kinase C-dependent," *American Journal of Respiratory Cell and Molecular Biology*, vol. 23, no. 6, pp. 727–733, 2000.

[18] C. H. Serezani, D. M. Aronoff, S. Jancar, P. Mancuso, and M. Peters-Golden, "Leukotrienes enhance the bactericidal activity of alveolar macrophages against *Klebsiella pneumoniae* through the activation of NADPH oxidase," *Blood*, vol. 106, no. 3, pp. 1067–1075, 2005.

[19] R. Peck, "A one-plate assay for macrophage bactericidal activity," *Journal of Immunological Methods*, vol. 82, no. 1, pp. 131–140, 1985.

[20] J. E. Kolodsick, M. Peters-Golden, J. Larios, G. B. Toews, V. J. Thannickal, and B. B. Moore, "Prostaglandin E_2 inhibits fibroblast to myofibroblast transition via E. Prostanoid receptor 2 signaling and cyclic adenosine monophosphate elevation," *American Journal of Respiratory Cell and Molecular Biology*, vol. 29, no. 5, pp. 537–544, 2003.

[21] C. Canetti, B. Hu, J. L. Curtis, and M. Peters-Golden, "Syk activation is a leukotriene B_4-regulated event involved in macrophage phagocytosis of IgG-coated targets but not apoptotic cells," *Blood*, vol. 102, no. 5, pp. 1877–1883, 2003.

[22] K. Kasono, J. Nishida, H. Tamemoto, et al., "Thiazolidinediones increase the number of platelets in immune thrombocytopenic purpura mice via inhibition of phagocytic activity of the reticulo-endothelial system," *Life Sciences*, vol. 71, no. 17, pp. 2037–2052, 2002.

[23] F. Turturro, R. E. Oliver III, E. Friday, I. Nissim, and T. Welbourne, "Troglitazone and pioglitazone interactions via PPAR-γ-independent and -dependent pathways in regulating physiological responses in renal tubule-derived cell lines," *American Journal of Physiology*, vol. 292, no. 3, pp. C1137–C1146, 2007.

[24] E. García-García and C. Rosales, "Signal transduction during Fc receptor-mediated phagocytosis," *Journal of Leukocyte Biology*, vol. 72, no. 6, pp. 1092–1108, 2002.

[25] Y. Azuma, M. Shinohara, P.-L. Wang, and K. Ohura, "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a negative regulator of macrophage functions," *International Immunopharmacology*, vol. 1, no. 12, pp. 2101–2108, 2001.

[26] S. Gordon, S. Keshav, and L. P. Chung, "Mononuclear phagocytes: tissue distribution and functional heterogeneity," *Current Opinion in Immunology*, vol. 1, no. 1, pp. 26–35, 1988.

[27] D. A. Hume, I. L. Ross, S. R. Himes, R. T. Sasmono, C. A. Wells, and T. Ravasi, "The mononuclear phagocyte system revisited," *Journal of Leukocyte Biology*, vol. 72, no. 4, pp. 621–627, 2002.

[28] F. S. Sutterwala, G. J. Noel, P. Salgame, and D. M. Mosser, "Reversal of proinflammatory responses by ligating the macrophage Fcγ receptor type I," *Journal of Experimental Medicine*, vol. 188, no. 1, pp. 217–222, 1998.

[29] P.-Y. Berclaz, Y. Shibata, J. A. Whitsett, and B. C. Trapnell, "GM-CSF, via PU.1, regulates alveolar macrophage FcγR-mediated phagocytosis and the IL-18/IFN-γ-mediated molecular connection between innate and adaptive immunity in the lung," *Blood*, vol. 100, no. 12, pp. 4193–4200, 2002.

[30] C. Bonnerot, V. Briken, V. Brachet, et al., "Syk protein tyrosine kinase regulates Fc receptor γ-chain-mediated transport to lysosomes," *The EMBO Journal*, vol. 17, no. 16, pp. 4606–4616, 1998.

[31] E. J. Kim, K. S. Park, S. Y. Chung, et al., "Peroxisome proliferator-activated receptor-γ activator 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits neuroblastoma cell growth through induction of apoptosis: association with extracellular signal-regulated kinase signal pathway," *Journal of Pharmacology and Experimental Therapeutics*, vol. 307, no. 2, pp. 505–517, 2003.

[32] V. G. Keshamouni, R. C. Reddy, D. A. Arenberg, et al., "Peroxisome proliferator-activated receptor-γ activation inhibits tumor progression in non-small-cell lung cancer," *Oncogene*, vol. 23, no. 1, pp. 100–108, 2004.

[33] M. Li, T. W. Lee, A. P. Yim, T. S. Mok, and G. G. Chen, "Apoptosis induced by troglitazone is both peroxisome proliferator-activated receptor-γ- and ERK-dependent in human non-small lung cancer cells," *Journal of Cellular Physiology*, vol. 209, no. 2, pp. 428–438, 2006.

[34] S. H. Kim, C. I. Yoo, H. T. Kim, J. Y. Park, C. H. Kwon, and Y. K. Kim, "Activation of peroxisome proliferator-activated receptor-γ (PPARγ) induces cell death through MAPK-dependent mechanism in osteoblastic cells," *Toxicology and Applied Pharmacology*, vol. 215, no. 2, pp. 198–207, 2006.

[35] T. V. Petrova, K. T. Akama, and L. J. Van Eldik, "Cyclopentenone prostaglandins suppress activation of microglia: down-regulation of inducible nitric-oxide synthase by 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 8, pp. 4668–4673, 1999.

[36] B. M. Spiegelman, "PPARγ in monocytes: less pain, any gain?" *Cell*, vol. 93, no. 2, pp. 153–155, 1998.

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Research Article

Rosiglitazone, an Agonist of PPARγ, Inhibits Non-Small Cell Carcinoma Cell Proliferation In Part through Activation of Tumor Sclerosis Complex-2

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PPARγ ligands inhibit the proliferation of non-small cell lung carcinoma (NSCLC) cells in vitro. The mechanisms responsible for this effect remain incompletely elucidated, but PPARγ ligands appear to inhibit the mammalian target of rapamycin (mTOR) pathway. We set out to test the hypothesis that PPARγ ligands activate tuberous sclerosis complex-2 (TSC2), a tumor suppressor gene that inhibits mTOR signaling. We found that the PPARγ ligand rosiglitazone stimulated the phosphorylation of TSC2 at serine-1254, but not threonine-1462. However, an antagonist of PPARγ and PPARγ siRNA did not inhibit these effects. Rosiglitazone also increased the phosphorylation of p38 MAPK, but inhibitors of p38 MAPK and its downstream signal MK2 had no effect on rosiglitazone-induced activation of TSC2. Activation of TSC2 resulted in downregulation of phosphorylated p70S6K, a downstream target of mTOR. A TSC2 siRNA induced p70S6K phosphorylation at baseline and inhibited p70S6K downregulation by rosiglitazone. When compared to a control siRNA in a thymidine incorporation assay, the TSC2 siRNA reduced the growth inhibitory effect of rosiglitazone by fifty percent. These observations suggest that rosiglitazone inhibits NSCLC growth partially through phosphorylation of TSC2 via PPARγ-independent pathways.

1. INTRODUCTION

Lung cancer remains the leading cause of cancer-related mortality in the United States, and 30% to 40% of newly diagnosed patients with non-small cell lung cancer (NSCLC) present with regionally advanced and unresectable stage III disease [1]. Despite recent advances in understanding the molecular biology of lung carcinoma and the introduction of multiple new chemotherapeutic agents for its treatment, the poor five-year survival rate of less than 15% has not changed substantially [2]. This justifies the continuous search for agents with therapeutic potential against NSCLC.

Peroxisome proliferator-activated receptors (PPARs; isotypes α, β/δ, γ) are ligand-inducible nuclear transcription factors that heterodimerize with retinoid X receptors and bind to PPAR response elements (PPREs) located in the promoter region of PPAR target genes [3]. These lipid-sensitive receptors can be activated in a variable isotype-specific manner by natural fatty acids, leukotrienes, prostaglandins, and some synthetic agonists, including antidiabetic drugs such as rosiglitazone, ciglitazone, and pioglitazone which are specific PPARγ ligands. These drugs are also effective in regulating cell activation, differentiation, proliferation, and/or apoptosis [4, 5]. The role of PPARγ, one PPAR isotype, has been extensively studied thanks to the availability of synthetic PPARγ agonists. The anticancer activity of PPARγ agonists has been examined in a variety of cancers including colon, breast, and prostate [6]. These and related studies support a role for PPARγ as a potential tumor suppressor.

Several studies have implicated PPARγ in lung cancer as well. The expression of PPARγ has been demonstrated in NSCLC and was correlated with tumor histological type and grade [7]. Thus, it has been postulated that PPARγ mRNA levels may serve as a prognostic marker in lung carcinoma in addition to playing important roles in lung carcinogenesis. Activation of PPARγ by troglitazone, ciglitazone, and

pioglitazone caused growth inhibition and apoptosis of NSCLC cells [8, 9]. Recently, studies in animal models of tumorigenesis showed that treatment of A549 tumor-bearing SCID mice with troglitazone or pioglitazone inhibited primary tumor growth by 66.7%, and significantly inhibited the number of spontaneous lung metastasis lesions [10]. Together, these observations suggest that PPARγ ligands may serve as potential therapeutic agents in the management of NSCLC, but the mechanisms responsible for these effects remain incompletely elucidated.

We have reported that PPARγ agonists inhibit NSCLC proliferation by inhibiting the mammalian target of rapamycin (mTOR) signaling pathway through PPARγ-dependent and -independent mechanisms [11]. The mTOR subfamily belongs to the phosphatidylinositol 3-kinase (PI3-K)-related kinase family and is partly inhibited by rapamycin, a feature that has facilitated efforts to study its function in eukaryotic cells [12]. mTOR signaling induced by hormones, growth factors, and amino acids regulates the phosphorylation of several proteins including p70 ribosomal protein S6 kinase (p70S6K) and eIF-4E binding protein (4E-BP1), which are key regulators of translation, and are among the most well-characterized targets of mTOR [12].

One of the downregulators of the mTOR pathway is the tumor suppressor protein tuberous sclerosis complex (TSC). TSC is composed of two proteins, TSC1 (also known as hamartin) and TSC2 (known as tuberin), which function to integrate growth factor and cell stress responses [13]. We set out to explore the effects of PPARγ agonists on TSC expression and the contribution of this pathway on inhibition of cell proliferation in NSCLC cells treated with the PPARγ agonist rosiglitazone. We found that PPARγ ligands activate TSC2, which, in turn, inhibits mTOR signaling in NSCLC cells through PPARγ-independent pathways.

2. MATERIALS AND METHODS

2.1. Culture and chemicals

The human NSCLC cell line H2106 was obtained from the American Type Culture Collection (Manassas, Va, USA) and grown in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, HEPES buffer, 50 IU/mL penicillin/streptomycin, and 1 μg amphotericin (complete medium) as previously described [14]. Polyclonal antibodies specific for TSC2, p38 MAPK, p70S6K, and their respective phosphorylated active forms were purchased from Cell Signaling (Beverly, Mass, USA). GW9662 was purchased from Cayman Chemical Co. (Ann Arbor, Mich, USA). The inhibitor of the mitogen-activated protein kinase-activated protein kinase 2 (MAPKAP kinase 2, MK2), a synthetic 13-residue peptide (KKKALNRQLGVAA) corresponding to the phosphorylation site of HSP27, one of the known substrates of MK2, was purchased from Calbiochem (San Diego, Calif, USA). Rosiglitazone, antibodies against PPARγ, SB239063, and other chemicals were purchased from Sigma Aldrich (St. Louis, Mo, USA) unless otherwise indicated.

2.2. Western Blot analysis

Western blotting was performed as previously described [15]. Briefly, protein concentrations were determined by the Bio-Rad protein assay. Equal amounts of protein from whole cell lysates were solubilized in 2x SDS-sample buffer and separated on SDS-8% polyacrylamide gels. Blots were incubated with antibodies raised against TSC2 and phosphorylated TSC2 (1:2000), p38 MAPK and phosphor-p38 MAPK, p70S6K and phosphor-p70S6K (1:1000). The blots were washed and followed by incubation with a secondary goat antibody raised against rabbit IgG conjugated to horseradish peroxidase (1:2000, Cell Signaling, Beverly, Mass, USA). The blots were washed, transferred to freshly made ECL solution (Amersham, Arlington, Ill, USA) for 1 minute, and exposed to X-ray film. In controls, the antibodies were omitted or replaced with a control rabbit IgG.

2.3. Treatment with PPARγ and TSC2 small interfering RNA

The PPARγ (Cat number sc-29455) and TSC2 siRNAs (Cat number sc-36762) and the control siRNA (Cat number sc-37007) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, Calif, USA). For the transfection procedure, cells were grown to 50% confluence and PPARγ, TSC2, or control siRNAs were transfected using the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, Calif, USA) according to the manufacturer's instructions. Briefly, oligofectamine reagent was incubated with serum-free medium for 10 minutes. Subsequently, a mixture of siRNA was added. After incubation for 15 minutes at room temperature, the mixture was diluted with medium and added to each well. The final concentration of siRNA in each well was 100 nM. After culturing for 48 hours, cells were washed and resuspended in new culture media in the presence or absence of rosiglitazone for up to 48 hours for Western blot and cell growth assays.

2.4. [Methyl-^{3}H] thymidine incorporation assay

H2106 NSCLC cells (10^4 cells/well) were cultured with the selective PPARγ antagonist GW9662 (20 μM) for 1 hour, or transfected with TSC2 siRNA (100 nM) for 48 hours before exposing the cells to rosiglitazone (10 μM) followed by incubation with 1 μCi/mL [methyl-^{3}H] thymidine (Amersham, specific activity 250 Ci/mmol) for up to 48 hours. The medium was removed and the attached cells were washed with 1x PBS. Afterwards, the attached cells were treated with ice-cold 6% trichloroacetic acid (TCA) at 4°C for 20 minutes and washed once with 6% TCA. The cells were then solubilized with 0.1 N NaOH and counted in a liquid scintillation counter in 4 mL of scintillation fluid.

2.5. Statistical analysis

All experiments were repeated a minimum of three times. All data collected from Western Blot and [Methyl-^{3}H]-thymidine incorporation assays were expressed as means $\pm$SD. The data presented in some figures are from a representative

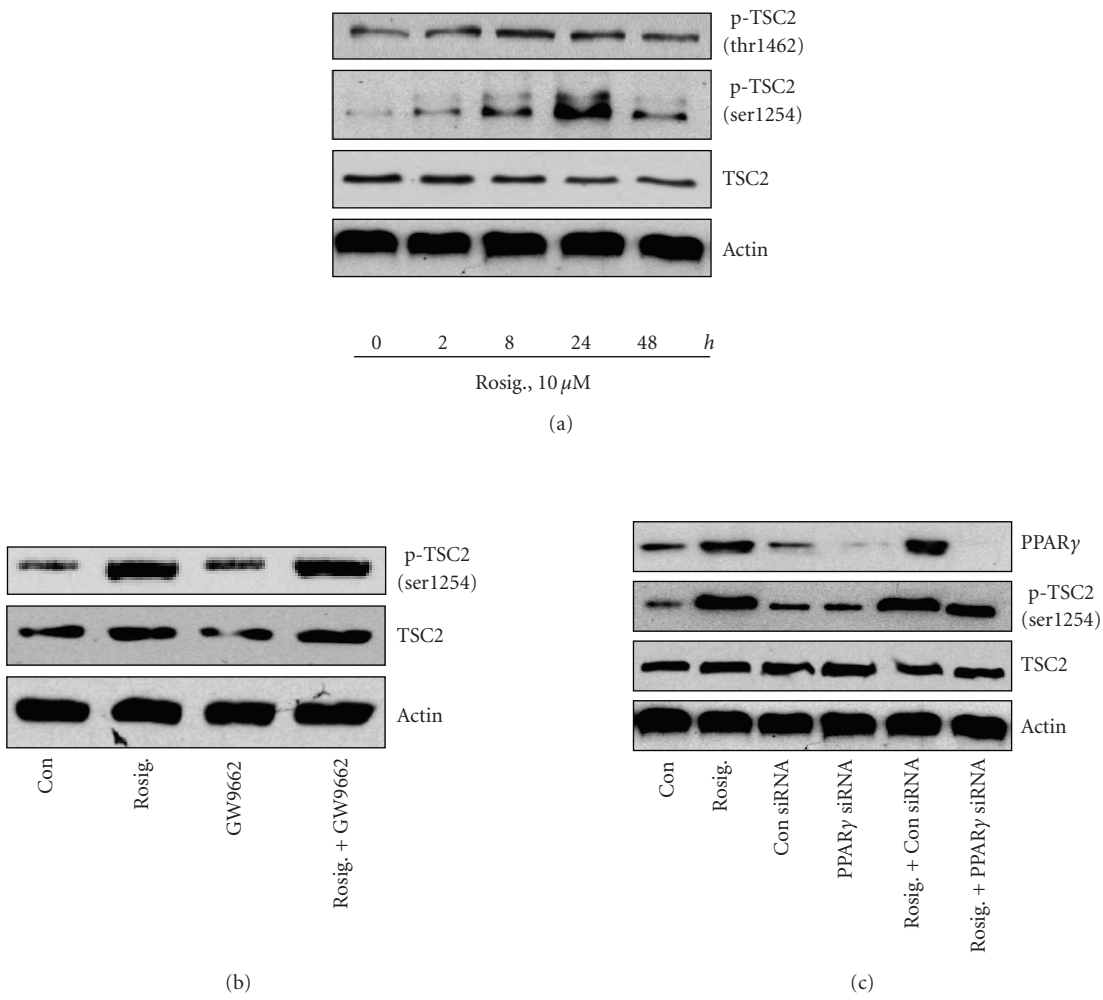

Figure 1: *Rosiglitazone stimulates the activation of TSC2. (a) Time-dependent effect of rosiglitazone on TSC2 phosphorylation*. Cellular protein was isolated from H2106 cells that were cultured with increasing concentrations of rosiglitazone (Rosig.) for 1 hour followed by Western blot analysis with antibodies against total TSC2 and phosphorylated TSC2 (p-TSC2). *(b) Effect of PPARγ antagonists on rosiglitazone-induced TSC2 phosphorylation*. Cellular protein was isolated from H2106 cells cultured for up to 2 hours in the presence or absence of GW9662 (20 μM) before exposure of cells to rosiglitazone (Rosig., 10 μM) for an additional 24 hours, then subjected to Western blot analysis for total TSC2 and phosphorylated TSC2 (p-TSC2). *(c) Effect of PPARγ siRNA on rosiglitazone-induced TSC2 phosphorylation*. H2106 cells were transfected with control or PPARγ siRNA (100 nM each) for 48 hours before exposing the cells to rosiglitazone (Rosig., 10 μM) for up to 24 hours. Afterwards, we performed Western blot analysis for total TSC2 and phosphorylated TSC2 (p-TSC2). Actin served as internal control for normalization purposes (*Con*, indicates untreated control cells).

experiment, which was qualitatively similar in the replicate experiments. Statistical significance was determined with Student's *t* test (two-tailed) comparison between two groups of data sets. Asterisks shown in the figures indicate significant differences of experimental groups in comparison with the corresponding control condition ($P < .05$, see figure legends).

3. RESULTS

3.1. Rosiglitazone stimulates the expression of TSC2 protein

Since rosiglitazone has been found to regulate the PI3-K/Akt/mTOR/p70S6K signaling pathway, we tested if it also affected TSC2, an upstream regulator of that pathway. H2106 cells treated with rosiglitazone for the indicated period of time showed an increase in the phosphorylation of TSC2 at serine-1254, whereas only a slight increase in phosphorylation was detected on threonine-1462 (Figure 1(a)). Total TSC2 protein levels remained unchanged. PPARγ ligands have been shown to exert their effects through pathways dependent and independent of PPARγ. To test if phosphorylation of TSC2 by rosiglitazone was mediated through activation of PPARγ, cells were pretreated with a selective PPARγ antagonist, GW9662, or PPARγ siRNA before exposing them to rosiglitazone. As depicted in Figures 1(b) and 1(c), the inhibitory effect of rosiglitazone on the phosphorylation of TSC2 was not affected by GW9662 (b) or by PPARγ siRNA

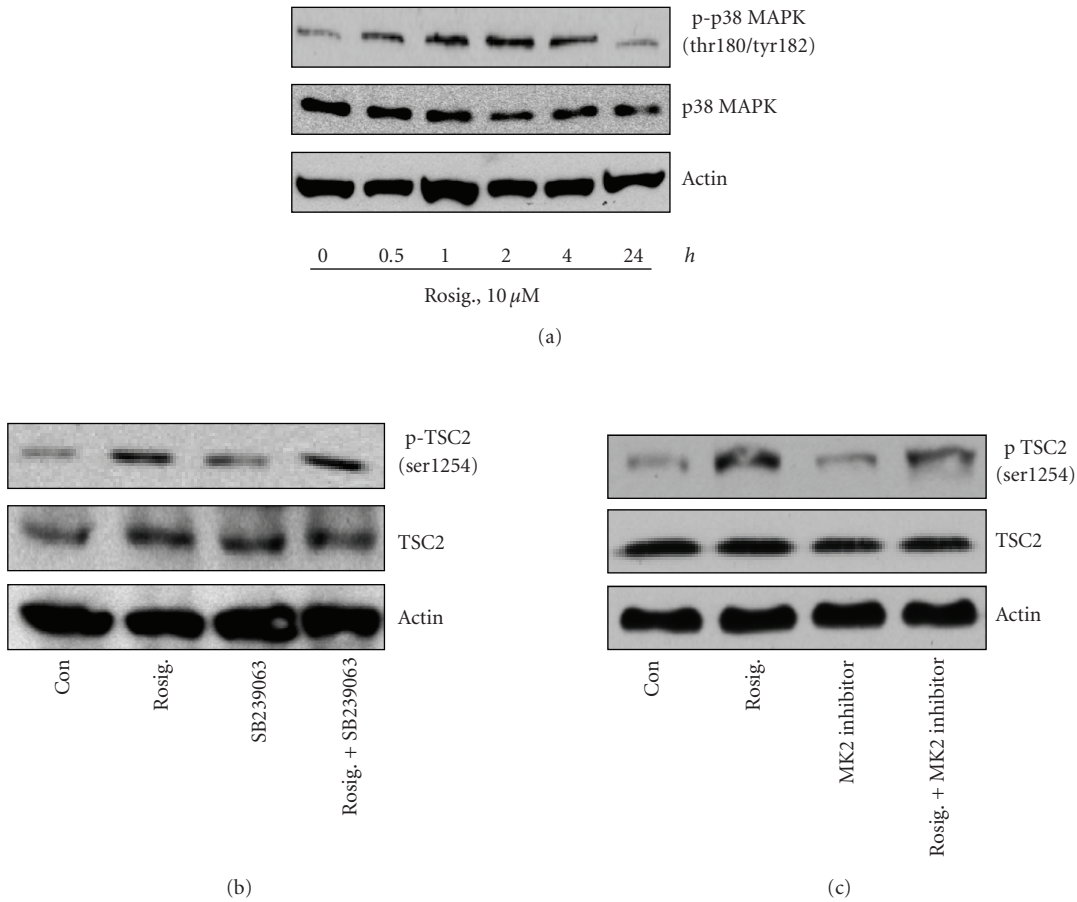

Figure 2: *The role of p38 MAPK signaling cascade in mediating the effect of rosiglitazone on activation of TSC2. (a) Time-dependent effect of rosiglitazone on phosphorylation of p38 MAPK*. Cellular proteins were isolated from H2106 cells treated with rosiglitazone (Rosig., 10 μM) for the indicated time period. Afterwards, Western blot analyses were performed using a polyclonal antibody against phosphor-p38 MAPK (Thr180/Tyr182) and total p38 MAPK. Actin served as internal control for normalization purposes. *(b) Effect of p38 inhibitor on rosiglitazone-induced TSC2 phosphorylation*. Cellular protein was isolated from H2106 cells cultured for up to 2 hours in the presence or absence of SB239063 (10 μM) before exposure of cells to rosiglitazone (Rosig., 10 μM) for an additional 24 hours, then subjected to Western blot analysis for total TSC2 and phosphorylated TSC2 (p-TSC2). Actin served as internal control for normalization purposes (*Con*, indicates untreated control cells). *(c) Effect of MK2 inhibitor on rosiglitazone-induced TSC2 phosphorylation*. Cellular protein was isolated from H2106 cells cultured for up to 2 hours in the presence or absence of MK2 inhibitor (10 μM) before exposure of cells to rosiglitazone (Rosig., 10 μM) for an additional 24 hours, then subjected to Western blot analysis for total TSC2 and phosphorylated TSC2 (p-TSC2). Actin served as internal control for normalization purposes (*Con*, indicates untreated control cells).

(c) suggesting that PPARγ-independent signals mediated this effect. Note that the PPARγ siRNA blocked PPARγ protein production, while the control siRNA had no effect (c).

3.1.1. *Rosiglitazone increases the phosphorylation of p38 MAPK, but blockade of p38 MAPK and its downstream signals had no effect on rosiglitazone-induced activation of TSC2*

PPARγ ligands have been shown to induce the activation of p38 MAPK in different cell systems [16, 17]. Activation of p38 mitogen-activated protein kinase (MAPK) and its downstream kinase MK2 have been associated with the phosphorylation of TSC2 [18]. Similarly, we found that rosiglitazone induced a transient increase in the phosphorylation of p38 MAPK in a time-dependent manner with maximal induction at 2 hours (Figure 2(a)). We next assessed if activation of p38 signals were related to the effect of rosiglitazone on TSC2 activation. As shown in Figures 2(b) and 2(c), SB239063, a selective p38 inhibitor, and KKKALNRQLGVAA, a potent and selective inhibitor of MK2, had no effect on rosiglitazone-induced TSC2 phosphorylation (serine-1254). No effects were noticed with increasing doses of these inhibitors (not shown).

3.1.2. *Silencing TSC2 restored the mTOR-related signal and partially blocked the effect of rosiglitazone on cell growth inhibition*

We next examined if upregulation of TSC2 by rosiglitazone was associated with inhibition of mTOR signaling as deter-

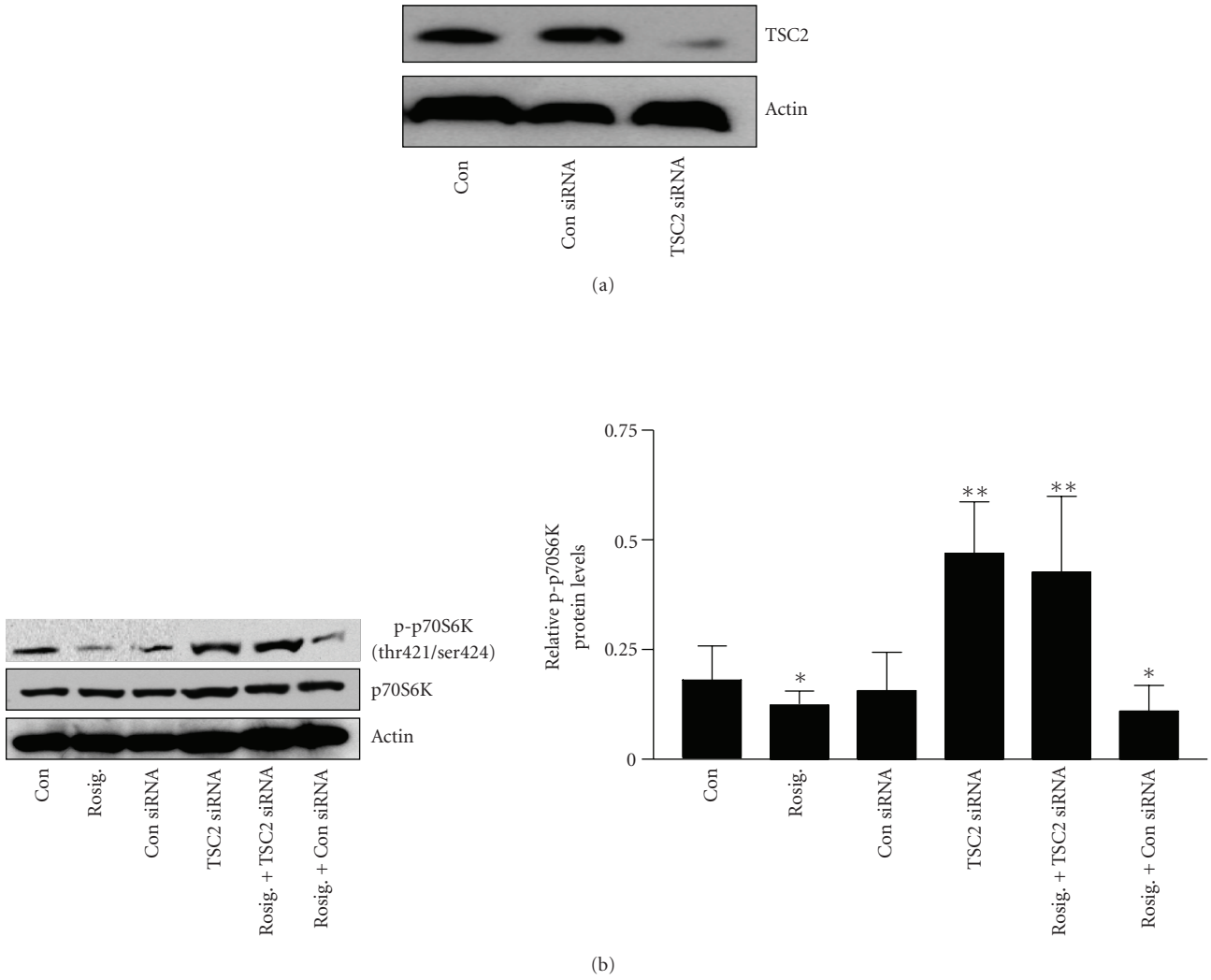

Figure 3: *Silencing TSC2 restored the activity of p70S6K. (a) TSC2 siRNA blocks TSC2 production*. H2106 cells were transfected with control or TSC2 siRNA (100 nM each) for 30 hours. Afterwards, we performed Western blot analysis for TSC2 proteins (Con, indicates untreated cells). *(b) TSC2 siRNA ameliorates the inhibitory effect of rosiglitazone on p70S6K phosphorylation*. H2106 cells were transfected with control or TSC2 siRNA (100 nM each) for 48 hours before exposing the cells to rosiglitazone (Rosig., 10 μM) for up to 24 hours. Afterwards, we performed Western blot analysis for p70S6K proteins (Con, indicates untreated cells). The representative data shown here is obtained from at least three separate experiments. Graphs are densitometry results. (*indicates significant differences as compared to the zero hour or untreated cells ($P < .05$); **indicates significance of combination treatment as compared with rosiglitazone alone ($P < .05$).)

mined by evaluating the phosphorylation state of p70S6K, a downstream target of mTOR. To determine the exact contribution of TSC2, we tested tumor cells transfected with control and TSC2 siRNAs. As shown in Figure 3(a), the TSC2 siRNA blocked TSC2 protein production, while the control siRNA had no effect. Armed with these tools, we tested the effects of rosiglitazone on p70S6K. As expected, upregulation of TSC2 by rosiglitazone coincided with downregulation of phosphorylated p70S6K (Figure 3(b)). Silencing of TSC2 by siRNA induced phosphorylation of p70S6K at baseline and inhibited p70S6K downregulation in the presence of rosiglitazone demonstrating a direct link between TSC2 induction and inhibition of mTOR signaling (Figure 3(b)).

3.2. Rosiglitazone inhibits carcinoma cell proliferation

We next tested the contribution of TSC2 to NSCLC cell proliferation in the setting of rosiglitazone treatment using an [^{3}H] thymidine incorporation assay. As expected, we found that rosiglitazone inhibited NSCLC cell proliferation. This is consistent with our own observations (not shown) and findings by others [10] showing inhibition of tumor growth in vivo in response to rosiglitazone. Interestingly, silencing of TSC2 reduced the growth inhibitory effect of rosiglitazone by approximately 50%, whereas a control siRNA had no effect (Figure 4).

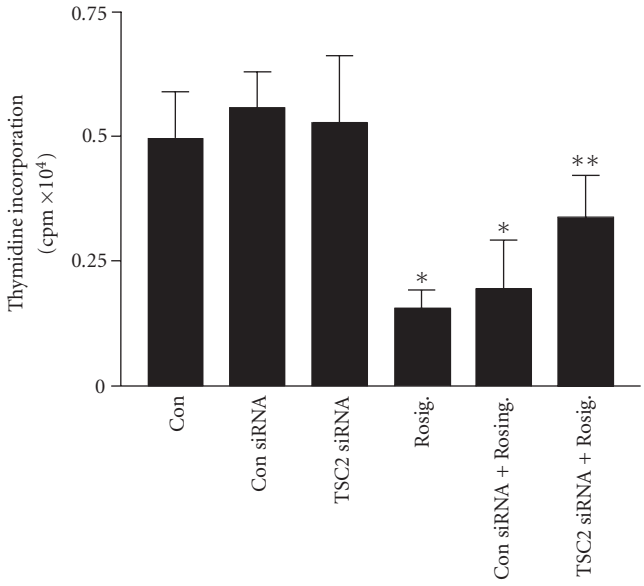

Figure 4: *Silencing TSC2 partly blocked the effect of resiglitazone on cell growth inhibition*. H2106 cells transfected with control or TSC2 siRNA (100 nM each) for 48 hours before exposing the cells to rosiglitazone (Rosig., 10 μM) and incubated with 10 μCi/mL [methyl-^{3}H] thymidine for 48 hours. Afterwards, cell numbers were determined. All data are depicted as means ± SD. (*indicates significant differences as compared to the untreated cells ($P < .05$); **indicates significance of combination treatment as compared with rosiglitazone (Rosig.) alone ($P < .05$).)

4. DISCUSSION

Rosiglitazone, one of the thiazolidinedione derivatives, is the most potent and selective synthetic ligand of PPARγ. It binds to PPARγ with a kD of approximately 40 nM and it is known to have marked adipogenic effects on preadipocyte and mesenchymal stem cells in vitro as well as dramatic antidiabetic effects [19]. However, not all of its cellular effects are mediated via PPARγ [20, 21]. Herein, we show that rosiglitazone increases PPARγ protein expression in NSCLC in a time- and dose-dependent fashion. Note that the concentrations used were consistent with those reported by others. For example, Valentiner et al. found that rosiglitazone inhibited the in vitro growth and viability of human neuroblastoma cell lines in a dose-dependent manner showing considerable effects only at high concentrations (10 μM and 100 μM) [22]. In another study, rosiglitazone inhibited both the proliferation and invasiveness of the human adrenocortical cancer cell line H295R in a dose-dependent manner with maximal effects (about 50% inhibition) noted at 20 μM [23].

We previously demonstrated that rosiglitazone inhibited the activation of the PI3-K/Akt/mTOR signaling pathway in NSCLC cells [11] and, therefore, set out to explore the effects of rosiglitazone on modulators of this pathway. mTOR signaling is induced by hormones, growth factors, and amino acids, and regulates the phosphorylation of several proteins including p70S6K and 4E-BP1, which are key regulators of translation, and are amongst the most well-characterized targets of mTOR [12]. A modulator of the mTOR pathway is the tumor suppressor protein TSC2, which functions to integrate growth factor and cell stress responses [13]. The TSC2 gene is known to be involved in mammalian cell cycle control and its overexpression is thought to exert an antitumor effect on cancer cells [24]. Here, we report that rosiglitazone increased the phosphorylation of TSC2 highlighting the relevance of this tumor suppressor in mediating the effects of rosiglitazone. However, this effect appeared to be independent of PPARγ since the inhibitor of PPARγ, GW9662, and transfection with PPARγ siRNA had no effect on this process.

TSC2 is phosphorylated by multiple kinases, including Akt, RSK1, ERK, CDC2, MK2, and AMPK. Therefore, TSC2 integrates signals from multiple signaling pathways and influences cell growth through regulation of the mTOR pathway [25]. Note that the rosiglitazone-induced phosphorylation of TSC2 occurred at serine-1254, but not at threonine-1462 sites, which are sites different from those phosphorylated by Akt and AMPK [26, 27]. The effect of phosphorylation of TSC2 at serine-1254 site remains unclear [25]. The interaction of TSC2 with 14-3-3 is associated with phosphorylation of serine-1254 and may be independent of Akt. However, TSC2 serine-1254 phosphorylation does not necessarily influence TSC2–14-3-3 interactions [28]. On the contrary, the association between 14-3-3 and TSC2 requires phosphorylation of serine-1210, which is not considered an Akt phosphorylation site [29]. This discrepancy may be due to the different cells studied and the elicitation of mechanisms other than those related to PI3-K, 14-3-3 and p38 pathways. How these multiple phosphorylation events are integrated by TSC2 to regulate cell growth needs to be explored further.

PPARγ ligands have been shown to induce the activation of p38 MAPK in different cell systems [16, 17]. In line with this, we showed that rosiglitazone increased the phosphorylation of p38 MAPK in NSCLC cells. Activation of p38 MAPK and its downstream signal MK2 have been associated with phosphorylation of TSC2 (serine-1210) [18], and the inhibitor of p38 MAPK reduced the phosphorylation of both p38 MAPK and MK2 [30]. However, in the current study, blockade of p38 MAPK and its downstream signal MK2 had no effect on rosiglitazone-induced TSC2 phosphorylation suggesting that the p38 MAPK cascade plays no role in mediating the effect of rosiglitazone on TSC2. The concentrations of these inhibitors were based on other studies which showed significant inhibition of p38 MAPK and its downstream MK2 signaling cascade [30, 31].

TSC1-TSC2 complexes have recently been implicated in cell survival responses. The molecular mechanisms by which TSC2 affects mTOR-related signals remain unclear. We found that knockdown of TSC2 resulted in inhibition of the effect of rosiglitazone on the mTOR downstream target p70S6K suggesting a role for TSC2 in mediating this effect. In cell proliferation assays, we showed that the TSC2 siRNA partially restored NSCLC cell growth in the presence of rosiglitazone, although knockdown of TSC2 alone had no effect

on NSCLC cell proliferation. This suggests that TSC2 does not contribute to NSCLC cell proliferation at baseline, but its phosphorylation partially mediates the growth inhibitory effect of rosiglitazone.

Taken together, our results demonstrate that rosiglitazone inhibits NSCLC cell growth in part through activation of TSC2 with the consequent suppression of mTOR signaling. This effect appeared to be independent of PPARγ and p38 MAPK signaling pathways. This work complements our previous work demonstrating partial inhibition of the mTOR pathway by rosiglitazone through downregulation of Akt and induction of PTEN via PPARγ-dependent pathways [11]. Together, the activation of rosiglitazone-induced PPARγ-dependent and -independent pathways results in inhibition of NSCLC growth. These observations are justifying further work testing the use of rosiglitazone (and perhaps other PPARγ ligands) as potential coadjuvants in the treatment of NSCLC in humans.

ABBREVIATIONS

NSCLC:	Non-small cell lung carcinoma
PPARγ:	Peroxisome proliferator-activated receptor gamma
PPRE:	PPAR response element
PI3-K:	Phosphatidylinositol 3-kinase
p38 MAPK:	p38 mitogen-activated protein kinase
MK2:	MAPKAP kinase 2
mTOR:	Mammalian target of rapamycin
p70S6K:	p70 ribosomal S6 kinase
TSC2:	Tuberous sclerosis complex-2
4E-BP1:	Eukaryotic initiation factor 4E-binding protein 1
siRNA:	Small RNA interference

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REFERENCES

[1] M. A. Socinski, "Combined modality trials in unresectable stage III non-small cell lung cancer: the cancer and leukemia group B experience," *Seminars in Oncology*, vol. 32, supplement 3, pp. S114–S118, 2005.

[2] C. A. Granville and P. A. Dennis, "An overview of lung cancer genomics and proteomics," *American Journal of Respiratory Cell and Molecular Biology*, vol. 32, no. 3, pp. 169–176, 2005.

[3] S. A. Kliewer, K. Umesono, D. J. Noonan, R. A. Heyman, and R. M. Evans, "Convergence of 9-*cis* retinoic acid and peroxisome proliferator signaling pathways through heterodimer formation of their receptors," *Nature*, vol. 358, no. 6389, pp. 771–774, 1992.

[4] S. Schmidt, E. Moric, M. Schmidt, M. Sastre, D. L. Feinstein, and M. T. Heneka, "Anti-inflammatory and antiproliferative actions of PPAR-γ agonists on T lymphocytes derived from MS patients," *Journal of Leukocyte Biology*, vol. 75, no. 3, pp. 478–485, 2004.

[5] N. Hirase, T. Yanase, Y.-M. Mu, et al., "Thiazolidinedione induces apoptosis and monocytic differentiation in the promyelocytic leukemia cell line HL60," *Oncology*, vol. 57, supplement 2, pp. 17–26, 1999.

[6] C. Grommes, G. E. Landreth, and M. T. Heneka, "Antineoplastic effects of peroxisome proliferatoractivated receptor γ agonists," *The Lancet Oncology*, vol. 5, no. 7, pp. 419–429, 2004.

[7] S. Theocharis, H. Kanelli, E. Politi, et al., "Expression of peroxisome proliferator activated receptor-gamma in non-small cell lung carcinoma: correlation with histological type and grade," *Lung Cancer*, vol. 36, no. 3, pp. 249–255, 2002.

[8] T. Satoh, M. Toyoda, H. Hoshino, et al., "Activation of peroxisome proliferator-activated receptor-γ stimulates the growth arrest and DNA-damage inducible 153 gene in non-small cell lung carcinoma cells," *Oncogene*, vol. 21, no. 14, pp. 2171–2180, 2002.

[9] S. Han, N. Sidell, P. B. Fisher, and J. Roman, "Up-regulation of p21 gene expression by peroxisome proliferator-activated receptor γ in human lung carcinoma cells," *Clinical Cancer Research*, vol. 10, no. 6, pp. 1911–1919, 2004.

[10] V. G. Keshamouni, R. C. Reddy, D. A. Arenberg, et al., "Peroxisome proliferator-activated receptor-γ activation inhibits tumor progression in non-small-cell lung cancer," *Oncogene*, vol. 23, no. 1, pp. 100–108, 2004.

[11] S. Han and J. Roman, "Rosiglitazone suppresses human lung carcinoma cell growth through PPARγ-dependent and PPARγ-independent signal pathways," *Molecular Cancer Therapeutics*, vol. 5, no. 2, pp. 430–437, 2006.

[12] X. Wang, A. Beugnet, M. Murakami, S. Yamanaka, and C. G. Proud, "Distinct signaling events downstream of mTOR cooperate to mediate the effects of amino acids and insulin on initiation factor 4E-binding proteins," *Molecular and Cellular Biology*, vol. 25, no. 7, pp. 2558–2572, 2005.

[13] Y. Li, M. N. Corradetti, K. Inoki, and K.-L. Guan, "TSC2: filling the GAP in the mTOR signaling pathway," *Trends in Biochemical Sciences*, vol. 29, no. 1, pp. 32–38, 2004.

[14] S. Han, N. Sidell, S. Roser-Page, and J. Roman, "Fibronectin stimulates human lung carcinoma cell growth by inducing cyclooxygenase-2 (COX-2) expression," *International Journal of Cancer*, vol. 111, no. 3, pp. 322–331, 2004.

[15] S. Han, Z. M. Lei, and C. V. Rao, "Up-regulation of cyclooxygenase-2 gene expression by chorionic gonadotropin during the differentiation of human endometrial stromal cells into decidua," *Endocrinology*, vol. 137, no. 5, pp. 1791–1797, 1996.

[16] J. J. Schlezinger, J. K. Emberley, and D. H. Sherr, "Activation of multiple mitogen-activated protein kinases in pro/pre-B cells by GW7845, a peroxisome proliferator-activated receptor γ agonist, and their contribution to GW7845-induced apoptosis," *Toxicological Sciences*, vol. 92, no. 2, pp. 433–444, 2006.

[17] M. Li, T. W. Lee, T. S. K. Mok, T. D. Warner, A. P. C. Yim, and G. G. Chen, "Activation of peroxisome proliferator-activated receptor-γ by troglitazone (TGZ) inhibits human lung cell growth," *Journal of Cellular Biochemistry*, vol. 96, no. 4, pp. 760–774, 2005.

[18] Y. Li, K. Inoki, P. Vacratsis, and K.-L. Guan, "The p38 and MK2 kinase cascade phosphorylates tuberin, the tuberous sclerosis 2 gene product, and enhances its interaction with 14-3-3," *Journal of Biological Chemistry*, vol. 278, no. 16, pp. 13663–13671, 2003.

[19] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[20] A. Galli, E. Ceni, D. W. Crabb, et al., "Antidiabetic thiazolidinediones inhibit invasiveness of pancreatic cancer cells via PPARγ independent mechanisms," *Gut*, vol. 53, no. 11, pp. 1688–1697, 2004.

[21] C.-W. Shiau, C.-C. Yang, S. K. Kulp, et al., "Thiazolidenediones mediate apoptosis in prostate cancer cells in part through inhibition of Bcl-xL/Bcl-2 functions independently of PPARγ," *Cancer Research*, vol. 65, no. 4, pp. 1561–1569, 2005.

[22] U. Valentiner, M. Carlsson, R. Erttmann, H. Hildebrandt, and U. Schumacher, "Ligands for the peroxisome proliferator-activated receptor-γ have inhibitory effects on growth of human neuroblastoma cells in vitro," *Toxicology*, vol. 213, no. 1-2, pp. 157–168, 2005.

[23] P. Ferruzzi, E. Ceni, M. Tarocchi, et al., "Thiazolidinediones inhibit growth and invasiveness of the human adrenocortical cancer cell line H295R," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 3, pp. 1332–1339, 2005.

[24] S.-I. Kawaguchi, K. Harada, Supriatno, H. Yoshida, and M. Sato, "Overexpression of tuberous sclerosis complex 2 exerts antitumor effect on oral cancer cell lines," *Oral Oncology*, vol. 39, no. 8, pp. 836–841, 2003.

[25] A. Y. Choo, P. P. Roux, and J. Blenis, "Mind the GAP: Wnt steps onto the mTORC1 train," *Cell*, vol. 126, no. 5, pp. 834–836, 2006.

[26] C. J. Potter, L. G. Pedraza, and T. Xu, "Akt regulates growth by directly phosphorylating Tsc2," *Nature Cell Biology*, vol. 4, no. 9, pp. 658–665, 2002.

[27] K. Inoki, T. Zhu, and K.-L. Guan, "TSC2 mediates cellular energy response to control cell growth and survival," *Cell*, vol. 115, no. 5, pp. 577–590, 2003.

[28] M. Nellist, M. A. Goedbloed, and D. J. J. Halley, "Regulation of tuberous sclerosis complex (TSC) function by 14-3-3 proteins," *Biochemical Society Transactions*, vol. 31, no. 3, pp. 587–591, 2003.

[29] S. D. Shumway, Y. Li, and Y. Xiong, "14-3-3β binds to and negatively regulates the tuberous sclerosis complex 2 (TSC2) tumor suppressor gene product, tuberin," *Journal of Biological Chemistry*, vol. 278, no. 4, pp. 2089–2092, 2003.

[30] S. Das, A. Tosaki, D. Bagchi, N. Maulik, and D. K. Das, "Potentiation of a survival signal in the ischemic heart by resveratrol through p38 mitogen-activated protein kinase/mitogen- and stress-activated protein kinase 1/cAMP response element-binding protein signaling," *Journal of Pharmacology and Experimental Therapeutics*, vol. 317, no. 3, pp. 980–988, 2006.

[31] H. Ishida, K. Ohkawa, A. Hosui, et al., "Involvement of p38 signaling pathway in interferon-α-mediated antiviral activity toward hepatitis C virus," *Biochemical and Biophysical Research Communications*, vol. 321, no. 3, pp. 722–727, 2004.