JOURNAL OF SMOOTH MUSCLE RESEARCH

J. Smooth Muscle Res. 2021; 57: 1–7
Published online: March 3, 2021; doi: 10.1540/jsmr.57.1

Invited Review

Anti-inflammatory mechanisms of the vascular smooth muscle PPARγ

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Submitted September 14, 2020; accepted in final form January 22, 2021

Abstract

This review highlights molecular mechanisms of anti-inflammatory and protective effects of the nuclear transcription factor, peroxisome proliferator-activated receptor γ (PPARγ) in vascular tissue. PPARγ is an ubiquitously expressed nuclear factor, and well-studied in adipose tissue and inflammatory cells. Additionally, beneficial effects of vascular PPARγ's on atherosclerosis and vascular remodeling/dysfunction have been reported although the detailed mechanism remains to be completely elucidated. Clinical and basic studies have shown that the synthetic PPARγ ligands, thiazolidinediones (TZDs), have protective effects against cardiovascular diseases such as atherosclerosis. Recent studies utilizing genetic tools suggested that those protective effects of TZDs on cardiovascular diseases are not due to a consequence of improvement of insulin resistance, but may be due to a direct effect on PPARγ's in vascular endothelial and smooth muscle cells. In this review, we discuss proposed mechanisms by which the vascular PPARγ regulates vascular inflammation and remodeling/dysfunction especially in smooth muscle cells.

Key words: PPARγ, vascular dysfunction, smooth muscle, inflammation

Introduction

The PPARγ is an ubiquitously expressed and well-studied nuclear factor in the field of metabolism (1) and immunity (2, 3). For example, PPARγ controls adipogenesis, lipid metabolisms and glucose homeostasis by regulating numerous genes including aP2, C/EBPα, FGF21, and Glut4. PPARγ activation promotes glucose uptake as well as lipid storage (4, 5), and decreases gluconeogenesis (6) resulting in an enhancement of insulin sensitivity (7). Therefore, synthetic PPARγ agonists, thiazolidinediones (TZDs), are used as medication for type 2 diabetes. Although the mechanisms of PPARγ action remain less known in other tissues than adipose tissue, synthesized PPARγ agonists such as pioglitazone have impressive cardiovascular benefits such

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as decreased risk of heart disease, stroke, and atherosclerosis (7–9). It is reported that the protective effect on cardiovascular disease, especially atherosclerosis, was due to a direct effect of PPARγ activation on atheroma and vascular walls, but not a consequence of improvement of insulin resistance (10). Consistently, studies using genetically engineered animal models have shown that PPARγ's, in both vascular endothelium and smooth muscle, plays a crucial role in regulating vascular homeostasis independently of systemic metabolisms. Here, we will review the advances in the studies of protective mechanisms of vascular PPARγ, especially focusing on vascular smooth muscle cells (SMC).

Vascular-PPARγ's and Atherosclerosis

Large clinical studies indicated that TZDs have protective effects on vascular events including coronary disease, atherosclerosis, and stroke in patients with Type 2 diabetes (8, 11, 12). One of the studies has shown that treatment of prediabetes with pioglitazone decreased progression of carotid intima media thickness independently of the effects on blood glucose and lipid, insulin resistance, or blood inflammatory markers (13). Thus, the protection was suggested to be associated with PPARγ activation in immune cells and/or vascular cells such as endothelial cells (ECs) and SMC's. Indeed, there are several reports showing that vascular PPARγ's exert protective roles by regulating initiation and development of atherogenesis. LDL receptor knockout mice with EC-PPARγ deletion showed accelerated initiation of atherosclerotic lesion formation compared to controls or LDLR knockout mice with macrophage-PPARγ deletion when fed with a high-cholesterol diet (14). In transplantation of carotid artery to CBA/CaJ recipient mice, exaggerated development of the lesion formation with increased inflammatory cell infiltration and TNF-α expression was observed in the carotid arteries from SMC-PPARγ deletion mice (15). After 2 weeks of the transplantation, NF-κB activity and VCAM-1 expression in the lesion were strongly elevated in SMC-PPARγ KO mice. Atherosclerotic lesion was also exacerbated in apolipoprotein E (ApoE)-deficient mice crossed with SMC-PPARγ KO mice compared to littermate control fed with a high cholesterol diet (16). Interestingly, this report demonstrated that ApoE- and SMC-PPARγ-deficient mice showed loss of perivascular adipose tissues, resulting in loss of the protective effect on development of atherosclerosis.

P467L- or V290M-mutation of the PPARγ is known to be a loss-of-function mutation causing insulin resistance and blood pressure elevation in human subjects. Mice expressing dominant-negative (DN) mutant PPARγ (P467L or V290M) crossed with ApoE-deficient mice showed increased atheroma formation when fed with a high cholesterol diet (17). In the aortic lesions, expression of NF-κB target genes such as VCAM-1 and MCP1 was increased in EC- or SMC-DN PPARγ mutant mice compared to littermate control. Because proliferation and migration of vascular SMC, which are deeply associated with NF-κB activation, are one of the steps for development of atherosclerosis, PPARγ-NF-κB interaction might be essential for TZD-induced anti-atherosclerotic effects.

PPARγ and NF-κB

Ligand dependent PPARγ activation regulates numerous genes with a heterodimeric partner, retinoid X receptor (RXR) when bound to specific regions on the DNA of target genes termed PPAR response element (PPRE). The PPARγ also functions in protein-protein interaction, and one of the crucial target proteins is NF-κB. PPARγ-NF-κB interaction has been reported in various cell types (Fig. 1). In macrophage, PPARγ's inhibited NF-κB activity and its downstream pro-inflammatory pathway via transrepression mechanisms in

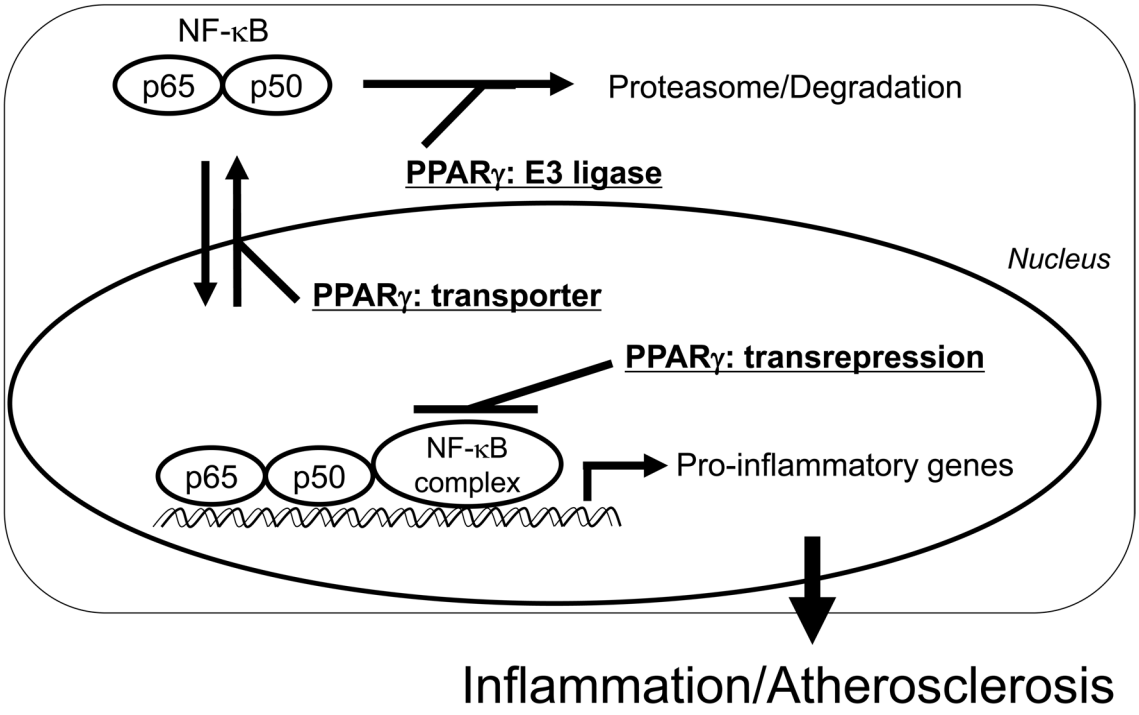

Fig. 1. Schematic view of possible PPARγ-NF-κB interaction in vascular SMC. In the nucleus, PPARγ inhibits NF-κB activity and its downstream pro-inflammatory pathway via transrepression mechanisms in a way that PPARγ directly binds to the corepressor complex on the promoters of NF-κB target genes. PPARγ also binds to NF-κB subunit, p65 which exports it to cytoplasm, resulting in inhibition of NF-κB activity. In addition, PPARγ acts as an E3 ubiquitin ligase and induces degradation of p65, which inhibits NF-κB-mediated inflammation.

a way that the PPARγ directly binds to the corepressor complex on the promoters of NF-κB target genes and inhibited its promoter activity (18). Although transrepression of NF-κB activity by the PPARγ required a ligand activation as its transactivation, it did not require RXR heterodimer and binding to the PPRE. In cancer cells, PPARγ's were reported to bind to the NF-κB subunit, p65 in the nucleus and export it to the cytoplasm, resulting in inhibition of NF-κB activity (19). In addition, it is also reported that the PPARγ acts as an E3 ubiquitin ligase and induces degradation of p65 in cancer cells and fibroblasts (20). The PPARγ has a RING domain and activation by ligands or agonists induces PPARγ binding to p65, which induces ubiquitination of p65 promoting its degradation in a proteasome-dependent manner. In vascular SMC's, the PPARγ was reported to directly bind to p65 and facilitate p65 nuclear export, rather than degradation of p65 (21). In contrast, the DN PPARγ mutation (P467L) completely lost the binding ability to p65 and was retained in the nucleus when treated with TNF-α. Another study reported PPARγ activation in human airway SMC inhibited TNF-α-induced nuclear translocation of p65 and NF-κB/DNA binding activity (22).

SMC-PPARγ and Vascular Remodeling/Dysfunction

Animal studies revealed that agonist-mediated activation of PPARγ's could reverse pulmonary arterial hypertension with improved plasma levels of adiponectin and insulin sensitivity (23). In contrast, SMC-PPARγ deletion mice spontaneously developed pulmonary arterial hypertension, which is characterized as pulmonary arterial remodeling, and elevated right ventricle systolic pressure and hypertrophy (24). The authors also demonstrated that inhibition of PPARγ's in pulmonary arterial SMCs caused activation of TGFβ1 signaling

both *in vivo* and *in vitro* (25). In this context, SMC-PPARγ directly binds to Smad3/Stat3, and inhibits TGFβ1-induced glucose metabolism and pulmonary arterial hypertension. In addition, LDL receptor-related protein 1 (LRP1) in vascular SMC, which was decreased in human pulmonary hypertension, protected pulmonary arterial remodeling and PPARγ activation by pioglitazone reversed pulmonary hypertension caused by LRP1 deficiency in SMC (26). The protective mechanism of SMC-PPARγ was due to inhibition of Smad3, Nox4 and CTGF, TGFβ1 downstream target. Another group has revealed that loss-of-function of PPARγ's in SMC caused systemic hypertension (27). Mice expressing the DN PPARγ mutation (P467L) in vascular SMC showed systolic hypertension and vascular dysfunction via increased Rho kinase (28) and decreased NO sensitivity (29). The PPARγ target gene, RhoBTB1, which acts as an adaptor of the Cullin-3 E3 ring ubiquitin ligase complex (CRL3), was significantly decreased in mutant mice (30). The authors further demonstrated that the mechanisms linking the mutation in SMC-PPARγ and hypertension is critically involved in Cullin-3. In SMC, RhoA and phosphodiesterase 5 are substrates for CRL3 (29, 31) and either the loss-of-function mutation or the deletion of Cullin-3 in SMC caused severe hypertension and vascular dysfunction with increased RhoA kinase and decreased nitric oxide (NO) sensitivity (32, 33). Another study revealed that mice expressing DN PPARγ in SMC exhibited augmented hypertension and vascular remodeling caused by deoxycorticosterone acetate-salt (34). They identified tissue inhibitor of metalloproteinase-4 (TIMP-4) as a new PPARγ target gene in SMC and found TIMP-4 tightly regulated SMC migration and vascular remodeling, which consequently influenced the regulation of systemic blood pressure. These data indicate that SMC-PPARγ plays a crucial role in vascular homeostasis and blood pressure regulation with manipulating several key genes and pathways.

Conclusion and Future Study

The PPARγ has been reported to have plentiful beneficial effects on not only adipose tissue, liver, and immune cell, but also on vascular tissues. On the other hand, TZDs are well-known to have several side effects such as weight gain, fluid retention and edema, bone fractures, and bladder cancer (10, 35–39). Therefore, new drugs without off-target effects or with tissue-selective activation of PPARγ are warranted. Similarly, identifying a new target associated with PPARγ will help us to design a new class of therapies that regulate PPARγ function more selectively.

Conflicts of Interest

None.

Acknowledgment

We sincerely would like to thank Dr. Shuichi Tawara at Okayama University of Science for important suggestions.

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