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Revisiting PPARγ as a target for the treatment of metabolic disorders

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As the prevalence of obesity has increased explosively over the last several decades, associated metabolic disorders, including type 2 diabetes, dyslipidemia, hypertension, and cardiovascular diseases, have been also increased. Thus, new strategies for preventing and treating them are needed. The nuclear peroxisome proliferator-activated receptors (PPARs) are involved fundamentally in regulating energy homeostasis; thus, they have been considered attractive drug targets for addressing metabolic disorders. Among the PPARs, PPARγ is a master regulator of gene expression for metabolism, inflammation, and other pathways in many cell types, especially adipocytes. It is a physiological receptor of the potent anti-diabetic drugs of the thiazolidinediones (TZDs) class, including rosiglitazone (Avandia). However, TZDs have undesirable and severe side effects, such as weight gain, fluid retention, and cardiovascular dysfunction. Recently, many reports have suggested that PPARγ could be modulated by post-translational modifications (PTMs), and modulation of PTM has been considered as novel approaches for treating metabolic disorders with fewer side effects than the TZDs. In this review, we discuss how PTM of PPARγ may be regulated and issues to be considered in making novel anti-diabetic drugs that can modulate the PTM of PPARγ. [BMB Reports 2014; 47(11): 599-608]

INTRODUCTION

The nuclear receptors (NRs) are a unique superfamily of ligand-dependent transcription factors that control various biological processes, such as proliferation, apoptosis, differentiation, and energy homeostasis. They are mostly known for their ability to modulate the transcriptional activity according to small lipophilic molecules, including endocrine compo-

nents (metabolites and hormones) as well as exogenous molecules (drugs and environmental compounds) (1).

The peroxisome proliferator-activated receptors (PPARs) are members of the NRs, comprising a subgroup of three closely homologous genes, PPARα (NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3). In the early 1990s, Issemann et al. first identified a genetic sensor for fats, and named it PPARα because it induced peroxisome proliferation through binding to several chemicals, including fibrate hypolipidemic drugs and certain other xenobiotics (2). While peroxisomes contribute to fatty acid oxidation, their proliferation results in hepatomegaly and carcinogenesis in rodents (2). With subsequent studies, two additional related receptors, now known as PPARβ/δ and PPARγ, were identified (2, 3). Similar to other NRs, PPARs can be activated by dietary fatty acids as well as metabolic derivatives in the body, and they control transcriptional networks involved in metabolism. Thus, they can act as lipid sensors that can markedly redirect metabolic cascades.

Despite structural similarities, the PPAR isoforms exhibit significant differences in tissue distribution, ligands, and functions (4).

PPARα is expressed predominantly in the liver, heart, and brown adipose tissue (BAT), which have high catabolic rates for fatty acids. It is a physiological receptor of the hypolipidemic fibrate drugs, and regulates the expression of genes involved in lipid metabolism (5).

PPARδ/β is ubiquitously expressed in multiple tissues, with an especially high concentration in skeletal muscle, and also plays a central role as a powerful regulator of fatty acid oxidation and energy homeostasis (6). Previous reports have suggested that the PPARδ agonist, GW501516, can improve insulin sensitivity in rodent models; it also lowered plasma triglyceride levels, and reduced atherogenic inflammation and weight, suggesting that PPARγ could be a therapeutic target for treating obesity and metabolic disorders (7-9).

PPARγ is the most well-characterized member of the PPARs as a pharmacological receptor of the insulin-sensitizing agents, the TZDs, that have been used widely to treat insulin resistance associated with T2DM (10). With alternative splicing and differential promoter usage, PPARγ exists as two isoforms, PPARγ1 and PPARγ2; PPARγ2 harbors a 30-amino-acid extension at its N-terminus (11). Additionally, their tissue distribution differs. PPARγ1 is expressed in several tissues, includ-

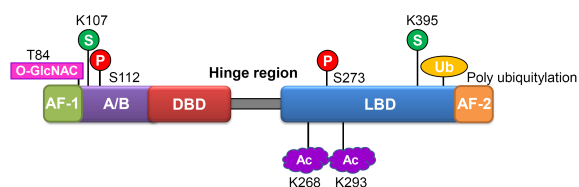*Corresponding author. Tel: +82-52-217-2543; Fax: +82-52-217-5219; E-mail: Janghchoi@unist.ac.kr

ing the lower intestines, macrophages, and adipose tissue (AT), whereas PPARγ2 expression is restricted exclusively to AT. PPARγ acts primarily as a master regulator of metabolic genes, and improves insulin sensitivity through glucose/lipid uptake and storage in peripheral tissues, such as skeletal muscle, liver, and AT (11). In particular, PPARγ is sufficient and necessary for fat cell differentiation. Forced expression of PPARγ can convert fibroblasts into adipocytes, whereas dominant-negative PPARγ mutants in cultured preadipocytes inhibit adipogenesis (12-14). Furthermore, PPARγ knock-out mice fail to develop AT (15-17). Thus, it seems reasonable to consider PPARγ as a key regulator of fat cell biology and AT-related energy homeostasis. For example, inactivation of PPARγ in mature adipocytes leads to insulin resistance through dysregulation of genes associated with insulin signaling, free fatty acid (FFA) uptake, and lipolysis (18, 19). Importantly, mice with increased PPARγ activity are protected from obesity-induced insulin resistance (20), whereas mice lacking PPARγ specifically in fat, muscle, or liver develop hyperlipidemia, hyperglycemia, or hyperinsulinemia (21-24). Consistent with these findings, humans with dominant-negative mutations in a single allele of *PPARG* (the gene encoding PPARγ) have partial lipodystropy and insulin resistance (25-27).

DOMAIN STRUCTURE AND MECHANISM OF ACTION OF PPARs

PPARs consist of distinct functional domains, named A/B, the

Modification	Site	Enzyme	Functions
Phosphorylation	Ser112	MAPK	Decrease of transcriptional activity
	Ser112	Cdk9/cdk7	Increase of transcriptional activity
	Ser273	Cdk5	Dysregulation of gene set associated with insulin resistance
SUMOylation	Lys107	Ubc9	Supression of transcriptional activity
	Lys395	Ubc9	Ligand-dependent repression of inflammatory gene expression in macrophages
Ubiquitination	unknown	E2	Increase of transcriptional activity
O-GlcNAcylation	Thr84	OGT	Decrease of transcriptional activity
Deacetylation	Lys268	Sirt1	Dissociation with NCoR
	Lys293	Sirt1	Ligand-dependent induction of browning and dissociation wiht NCoR

Fig. 1. Domain structure and PTMs of PPARγ. The PPARγ2 protein contains an additional 30 amino acids at the N-terminus, compared with PPARγ1. PPARγ has multiple domains including the A/B domain, ligand-independent activation function 1 (AF-1), DNA-binding domain (DBD), ligand-binding domain (LBD), and ligand-dependent activation function 2 (AF-2). Positions of the phosphorylation (P), SUMOylation (S), ubiquitination (Ub), and acetylation (Ac) sites are marked with the functions and the numbers correspond to amino acid position in PPARγ2.

DNA-binding domain (DBD), the hinge region, and the ligand-binding domain (LBD) (Fig. 1) (28). The N-terminal A/B domain harbors a ligand-independent transcriptional activating function (AF-1). The DBD is highly conserved among PPARs and is formed by two zinc finger-like motifs in a globular structure that can recognize a PPAR-response element (PPRE) in the promoter region of target genes. PPREs are specific DNA sequences formed by repetition of a consensus hexanucleotide sequence (AGGTCA), separated by one or two nucleotides (direct repeat 1 or 2, DR1 or DR2) (28). Additionally, the 5'-AACT extension of this consensus sequence ensures polarity for heterodimer binding to the retinoid X receptor (RXR) (28). The hinge region is involved in DNA recognition due to its structural flexibility (29). The LBD, to which small ligands can bind, is considered a target for drug discovery (30). This domain exhibits a region involved in the dimerization with a partner nuclear receptor, RXR (28, 29). Additionally, the strong C-ligand-dependent transcription activating function (AF-2) in the Cterminus of LBD is responsible for the interaction with co-activators and co-repressors that can regulate the transcriptional activity of PPARs (28). On the promoters of some target genes, unliganded PPARs recruit co-repressors, such as nuclear receptor co-repressor (NCoR) and silencing mediator for retinoic acid receptor and thyroid hormone receptor (SMRT), which are parts of multiprotein complexes containing histone deacetylase activity, and they repress the transcriptional activity of PPARs. When ligands bind to PPARs, conformational changes are induced in this region, and series of molecular events occur, including dissociation of co-repressors, recruitment of co-activators, including SRC1/CBP and TRAP/DRIP/ARC complexes, and PPARs can regulate the expression of target genes involved in adipogenesis, lipid metabolism, inflammation, and metabolic homeostasis (4).

PPARγ

Biological ligands of PPARγ
Although many efforts have been made to identify the endogenous ligands for PPARγ and various molecules have been suggested, this has yet to be clearly resolved. Numerous studies have shown that polyunsaturated fatty acids (PUFAs), certain prostanoids (15-deoxy-Δ12, 14-prostaglandin J2 (15-dPGJ2)), eicosanoids, components of oxidized low-density lipoproteins (9-HODE and 13-HODE), and oxidized alkyl phospholipids (lysophosphatidic acid and nitrolinoleic acid) can activate PPARγ, and lead to increase expression of PPARγ target genes, such as aP2, glucose transporter 4 (Glut4), and adiponectin (31, 32). In particular, 15-dPGJ2 is a well-characterized endogenous PPARγ ligand (33, 34). However, it also seems unlikely that its concentration is ever sufficient to activate PPARγ *in vivo*. Also, the concentrations of 9-HODE and 13-HODE would seem to be too low to work as ligands under normal condition (35). Thus, further studies towards the identification of the specific endogenous ligands for PPARγ are needed.

Synthetic ligands of PPARγ

Synthetic ligands of PPARγ, such as TZDs, the well-known insulin sensitizers, are potent activators of PPARγ. Among them, rosiglitazone and pioglitazone have been used widely for treating type 2 diabetic patients in the clinic (36). Before TZDs were identified as PPARγ ligands, they were known to be effective glucose-lowering agents (11). Rodents treated with TZDs showed significant improvements in systemic insulin resistance in peripheral tissues (37). Kliewer's group demonstrated that PPARγ was the pharmacological target of TZDs with tight binding and high agonistic activity (38). Many subsequent studies have reported biological roles of TZDs/PPARγ, including glucose-lowering and improvement of insulin sensitivity (4). There are other known synthetic ligands that are unrelated to TZDs, and they also bind tightly to PPARγ and show effective insulin-sensitizing activity (39).

Several important issues on TZDs' actions have been raised, such as which tissues are the primary targets influenced by TZDs and how do they control systemic insulin sensitivity. Because PPARγ is highly expressed in AT, that would seem likely to be the primary target for the action of TZDs. Mice lacking PPARγ in AT are markedly deficient in their response to TZD treatment (40, 41). Importantly, TZDs still lower plasma glucose level in tissue-specific PPARγ-knockout models, in the case of liver and muscle, the two main glucose-disposing organs (24, 42). Furthermore, TZD-induced activation of PPARγ controls the insulin signaling pathway in AT directly by increasing the expression of Glut4 and c-Cbl-associated protein (CAP) (43). From these studies, it seems reasonable to conclude that AT is the primary target of TZD action, and improvement of insulin sensitivity in liver and muscle might be secondary effects derived from the effects of TZDs in AT (44, 45).

Although the primary target tissue of TZD action is thought to be AT, that PPARγ is expressed at lower levels in non-adipose tissues and TZDs improve insulin sensitivity in lipodystrophic (fatless) mice, suggest that other tissues may also be targets and contribute to the insulin-sensitizing effects of TZDs (46, 47). Several studies suggest that PPARγ promotes hepatic steatosis, although others studies have shown preventative effects of PPARγ (48-52).

It has been demonstrated that treatment with TZDs decreases the expression of genes involved in gluconeogenesis, and liver-specific disruption of PPARγ in mice results in increased adiposity, hyperlipidemia, and insulin resistance (53), suggesting that the liver may be the primary target for the action of TZDs. In skeletal muscle, PPARγ is expressed at very low levels, and studies analyzing the effect of skeletal muscle-specific deletion of PPARγ have yielded conflicting results. Thus, the effects of TZDs on skeletal muscle seem unlikely to be direct actions (22, 24). Macrophage PPARγ contributes to anti-inflammation and lipid metabolism (54), and the mice lacking PPARγ in macrophages are more prone to systemic insulin resistance (55, 56). Furthermore, these mice have impaired maturation of anti-inflammatory 'M2' macrophages, and

treatment with TZD suppresses pro-inflammatory 'M1' macrophages activation (Fig. 2) (55). Together, TZDs can regulate systemic metabolism mainly through AT, but other non-adipose tissues may also be targets for TZDs.

PPARγ and insulin resistance

What makes TZDs control systemic insulin sensitivity? Two plausible mechanisms have been proposed. The first is 'lipid repartitioning,' by activating PPARγ. Insulin resistance is associated with increased plasma levels of FFAs and accumulation of lipids in peripheral tissues, including the liver and skeletal muscle, other than AT. Because PPARγ has a key role in lipid metabolism, controlling the expression of genes involved in lipogenesis, including aP2, CD36 LPL, FATP-1, glycerol kinase, SREBP-1, and SCD-1 (4), activation of PPARγ by TZDs in AT improves its ability to store lipids. Consequently, the triglyceride content of AT is increased, and FFAs in the circulation, liver, and muscle are lowered. Thus, TZDs can reduce lipotoxicity in muscle and liver, and improve insulin sensitivity (57, 58).

Another potential mechanism is regulating the production and secretion of adipokines, mediated by the TZDs-induced activation of PPARγ in AT (58-64). These adipokines, such as adiponectin, leptin, resistin, and tumor necrosis factor-α (TNF-α), may impact whole-body insulin sensitivity through endocrine signaling pathways. For example, TZDs inhibit the expression of TNF-α, IL-6, and resistin in AT, which promotes insulin resistance in peripheral tissues (58-61). In contrast, acti-
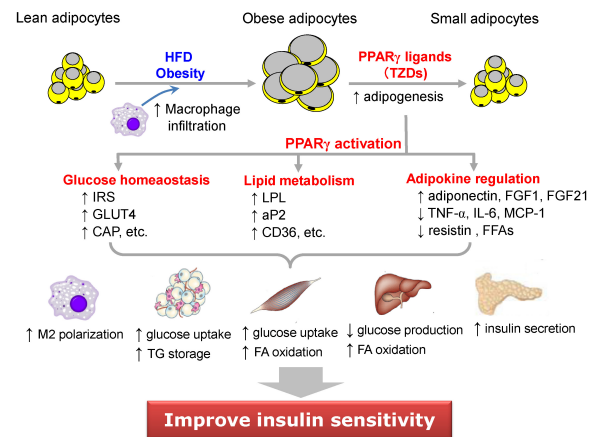

Fig. 2. Mechanism by which of PPARγ ligands regulate insulin sensitivity. In adipose tissue (AT), activation of PPARγ by thiazolidinedione (TZD) modulates glucose and lipid metabolism. PPARγ also regulates the levels of adipokines, such as adiponectin, TNF-α, MCP-1, and resistin. As a result of reduced free fatty acid levels in circulation and changed adipokine profiles, insulin sensitivity is improved, which is also mediated by suppressing glucose production in the liver, stimulating glucose uptake in skeletal muscle and AT, and promoting insulin secretion in the pancreas. Furthermore, activation of PPARγ by TZDs also suppresses macrophage infiltration into AT and induces polarization into an anti-inflammatory M2 phenotype.

vation of PPARγ results in promoting the production of adiponectin, which enhances fatty acid oxidation and insulin sensitivity, resulting in decreased glucose production in the liver and increased glucose usage in muscle, respectively (62-64).

Beyond the beneficial effects of TZDs on insulin sensitivity, they also have positive effects in various metabolic and other disorders, such as cardiovascular disease, Alzheimer's disease, Parkinson's disease, and certain cancers (65). TZDs can also promote browning effects in white adipose tissue (WAT) by activating PPARγ (65). However, many studies have demonstrated that TZDs can have severe side effects, such as weight gain, edema, plasma volume expansion (PVE), increased risk of congestive heart failure, and bone fractures (11, 65). In particular, rosiglitazone (Avandia) has been restricted or withdrawn from the market in the United States or Europe due to the increased incidence of myocardial infarction (65). Pioglitazone (Actos), a less potent PPARγ ligand, does not seem to cause the same cardiovascular risks. However, safety concerns have also been raised about pioglitazone regarding congestive heart failure and bladder cancer (65).

It has been reported that heterozygous *Pparγ*$^{+/-}$ mice have improved insulin sensitivity, and they are resistant to high-fat diet-induced obesity and insulin resistance (17, 66). Based on these findings, "selective PPARγ modulators (SPPARMs)" have become a focus in pharmacological development for type 2 diabetes (67). SPPARMs exhibit potent insulin sensitizing effects of a similar order to the TZDs, while they activate PPARγ partially with less adipogenic effects (68). Studies about SPPARMs mechanistically have created a paradox between PPARγ activity and anti-diabetic efficacy. While TZDs have powerful insulin-sensitizing effects, other compounds with poorer agonist activities, such as MRL24, still retain very good anti-diabetic effects (65). Recent studies showed that PPARγ ligands with almost no agonism also still have robust insulin-sensitizing actions (65). Accordingly, further understanding of the molecular and regulatory mechanisms of PPARγ may extend the application of PPARγ to new and improved therapies for type 2 diabetes.

Regulation of PPARγ by post-translational modifications (PTMs)

In addition to ligand-dependent regulation, PPARγ is also regulated by post-translational modifications (PTMs), including phosphorylation, SUMOylation, ubiquitilation, GlcNAcylation, and acetylation. These modifications regulate both PPARγ expression and its transcriptional activity, contributing to modulating adipocyte development and insulin sensitivity.

Phosphorylation: The most well-described PTM of PPARγ is phosphorylation. Phosphorylation of PPARγ2 at serine 112 (S112; S82 in PPARγ1) in the N-terminal AF-1 domain was identified first (69). As this site is a conserved mitogen-activated protein kinase (MAPK) consensus site, activation of MAPKs leads to phosphorylation of PPARγ at S112 (69-74). Activation of extracellular signal-regulated kinase 1/2 (ERK1/2) by growth factors, including epidermal growth factor (EGF), fibroblast

growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor-β, and prostaglandin PGF2α, leads to phosphorylation of PPARγ (69-74). Furthermore, cellular stresses, including UV and anisomysin, also result in phosphorylation of PPARγ at S112, mediated by the activation of c-Jun N-terminal kinase 1/2 (JNK1/2) and p38 (74). Phosphorylation at S112 represses its transcriptional activity by controlling ligand binding in the LBD or recruitment of co-regulators to the AF-1 region, whereas a non-phosphorylated PPARγ mutant, replacing S112 to alanine (S112A), is transcriptionally more active than wild-type PPARγ (75). In S112A knock-in mice, high-fat diet-induced insulin resistance is protected against with an increase in PPARγ target gene expression even though the mice were obese (76). Conversely, phosphorylation of PPARγ at S112 by cdk9 and cdk7 increased PPARγ activity (77, 78). Thus, the phosphorylation of PPARγ may inhibit or stimulate its transcriptional activity, depending on the cellular contexts and kinases.

Recently, Choi *et al.* identified an additional PPARγ phosphorylation at S273 (S243 in PPARγ1) in PPARγ LBD (79). This phosphorylation is mediated by cdk5, which is activated by pro-inflammatory stimuli and FFAs (79, 80). Cdk5-mediated phosphorylation of PPARγ is linked to high-fat diet-induced obesity. It does not affect the transcriptional activity of PPARγ, but dysregulates the expression of specific genes, including adiponectin and adipsin. Importantly, several anti-diabetic PPARγ ligands, with or without classical agonism, directly block cdk5-mediated PPARγ phosphorylation and restore the expression of gene sets. Additionally, inhibition of PPARγ phosphorylation at S273 in human by rosiglitazone is closely associated with its anti-diabetic effects (79, 80). Li and colleagues demonstrated that adipocyte-specific knock-out of NCoR leads to adipogenesis with reduced inflammation, enhanced systemic insulin sensitivity, and reduced cdk5-mediated PPARγ phosphorylation at S273 (81). Together, these findings suggest that phosphorylation of PPARγ at S273 by cdk5 may play a role in whole-body insulin sensitivity.

SUMOylation: PPARγ activity is also modulated by attachment of SUMO-1 or SUMO-2 ("small ubiquitin-related modifier") at K107 (K77 in PPARγ1) and K395 (K365 in PPARγ1). SUMOylation regulates various cellular processes, including nuclear-cytoplasmic transport, apoptosis, and transcriptional regulation (82). Conjugation of SUMO-1 or SUMO-2 to PPARγ in the N-terminal AF-1 domain (K107) strongly represses the transcriptional activity of PPARγ, and mutation of this site or overexpression of a dominant-negative form of the SUMO E3-ligase Ubc9 increases PPARγ activity (83). These results suggest that SUMO-1 modification at K107 negatively regulates PPARγ activity.

SUMOylation at K107 of PPARγ may be linked to Ser112 phosphorylation. The phosphorylation and SUMOylation sites in the AF-1 domain of PPARγ are in a highly conserved motif that is considered to be a phosphorylation-dependent SUMOylation motif (84). The consensus site consists of the fol-

lowing motif, ψKxExxSP, where ψ is a hydrophobic residue, K is the SUMO acceptor lysine, x is any amino acid, and SP forms a part of the downstream phosphorylation site. Many proteins containing this motif are transcription factors, including the heat-shock factors (HSFs), GATA-1, and myocyte enhancer factor 2 (84). However, among PPAR members, this motif is only found in the AF1 region of PPARγ. In PPARγ, phosphorylation and SUMOylation in the AF-1 domain function to repress its transcriptional activity (85).

A phosphorylation-deficient mutant of PPARγ (S112A) significantly diminished SUMOylation whereas the phospho-mimic mutant (S112D) showed increased SUMOylation at K107 (85), supporting the possibility that phosphorylation of S112 regulates SUMOylation at K107 to repress PPARγ activity (85). SUMOylation of K107 is also regulated by fibroblast growth factor 21 (FGF21), which increases PPARγ activity in adipocytes (86). FGF21 null mice show a lipodystropic phenotype, and have less body fat, decreased expression of PPARγ target genes, and increased PPARγ SUMOylation (86). These results suggest that FGF21 prevents PPARγ SUMOylation at K107, and FGF21 modulates PPARγ transcriptional activity by regulating SUMOylation at K107 and contributes to whole-body insulin sensitivity (86).

PPARγ is also SUMOylated at K395 in the LBD, which is not involved in the regulation of direct PPARγ target genes, but in the transcriptional repression of inflammatory genes in macrophages (87). Ligand-dependent SUMOylation of PPARγ at K395 promotes the interaction of PPARγ with NCoR/histone deacetylase-3 (HDAC3) complexes on NF-κB inflammatory gene promoters, and it prevents ubiquitination and proteosomal degradation of the repressor complex, and sustains repression (87).

Ubiquitination: The PPARγ protein has a short half-life because it is degraded by the polyubiquitin-proteasome system on ligand binding, and interferon-γ exposure in adipocytes augments PPARγ degradation by ubiquitination (88, 89). Although the ubiquitin acceptor sites have yet to be identified, the AF2 domain is required for maximal ubiquitin modification, but is not essential for ligand-dependent recognition by ubiquitin-proteasome system (90). Inhibition of proteasome activity by proteasome inhibitors increases PPARγ activity in adipocytes (90), suggesting ubiquitin modification of PPARγ is required for the activation of PPARγ. Because the ubiquitin acceptor sites have yet to be identified and the mechanism involved in activation of PPARγ by ubiquitination remain unclear, further understanding of the mechanisms for ubiquitination and degradation of PPARγ could eventually offer insights to regulate PPARγ activity.

O-GlcNAcylation: GlcNAcylation, similar to phosphorylation, is the post-translational cycling of a single β-O-linked N-acetylglucosamine (O-GlcNAc) on the hydroxyl groups of serine and threonine residues of target proteins. The only identified O-GlcNAc site in PPARγ is T54 in the AF-1 domain (T84 in PPARγ2), and inhibition of this modification decreases its

transcriptional activity and adipocyte differentiation (91). Consequently, O-GlcNAcylation of PPARγ might influence energy homeostasis and lipid metabolism. Further investigations on the O-GlcNAc modification of PPARγ will provide novel insights on PPARγ-related metabolic regulation.

Acetylation: Acetylation in many transcription factors occurs together with other modifications, such as phosphorylation, SUMOylation, and ubiquitination (92). In PPARγ, 20 potential acetylation sites (93) and two discrete acetylation sites in the ligand binding domain (K238 and K263 in PPARγ1) were identified recently. They play a role in the browning effects associated with TZDs through Sirt1-mediated deacetylation (94). Rosiglitazone promotes the interaction of PPARγ with Sirt1, and reduces acetylation at both K268 and K293 (94). Sirt1-dependent deacetylation of these sites is required for the interaction of PPARγ with PRDM16, a transcriptional co-activator for the browning of WAT (94). High-fat diet-fed mice showed increased acetylation of PPARγ at K268 and K293, leading to reduced Sirt1 binding and decreased insulin sensitivity (94). Additionally, these acetylations were reduced at low temperatures, which causes the browning of WAT (94). While deacetylation of K293 is required for the interaction of PPARγ with PRDM16, deacetylation at K268 had no effect. Acetylation of both lysines was required for the association with NCoR. As described above, an interaction between PPARγ and NCoR leads to cdk5-mediated PPARγ phosphorylation at S273 (81). Thus, it is possible that acetylation of these sites might be linked to phosphorylation at S273. Additionally, lysine residues can be modified by SUMOylation and ubiquitylation, and Qiang *et al.* found acetylation at K107 (94). K107 is also a SUMOylated site, and this SUMOylation is repressed by FGF-21, which promotes PPARγ activity (84, 86). Moreover, inhibition of deacetylation of K268 and K293 blocked the liganddependent degradation of PPARγ, suggesting involvement with ubiquitination. These findings suggest that acetylation may modulate PPARγ activity in collaboration with phosphorylation, SUMOylation, and ubiquitilation.

PTMs and PPARγ ligand
Although TZDs clearly have potent anti-diabetic effects, their use is now limited clinically because of their unwanted side effects. However, recent findings raise the possibility of avoiding the adverse effects of TZDs and pave the way for a reevaluation of PPARγ as a therapeutic target for metabolic disorders.

One possibility is to regulate cdk5-mediated phosphorylation of PPARγ at S273 (79). As mentioned above, this modification does not affect its adipogenic capacity, but alters the expression of a subset of target genes dysregulated in obesity, including insulin-sensitizing adipokines, such as adiponectin and adipsin. PPARγ full agonists, such as rosiglitazone, and the partial agonist MRL24, specifically block phosphorylation of PPARγ at S273, independent of classical transcriptional agonism. The anti-diabetic activity of MRL24 has higher efficacy with fewer side effects than rosiglitazone (95). These find-

ings suggest that the anti-diabetic activity of PPARγ ligands may be associated with their abilities to block phosphorylation of S273, not classical agonism. Based on these findings, SR1664 was developed as a PPARγ ligand (a non-agonist PPARγ ligand) that had no adipogenic capacity or classical transcriptional agonism but blocked the cdk5-mediated phosphorylation in cultured adipocytes and in insulin-resistant mice (96). Moreover, it has potent anti-diabetic activity while not causing the fluid retention and weight gain that are serious side effects of many PPARγ targeted drugs. Furthermore, unlike TZDs, SR1664 does not interfere with bone formation in culture. These findings provide a new possibility that it may be possible to develop a new class of anti-diabetic drugs and eliminate many of the unwanted side effects that occur due to classical agonism of PPARγ (Fig. 3) (65).

In macrophages, SUMOylation is also modulated by PPARγ ligands, including rosiglitazone and GW00072 (87). Ligand-induced SUMOylation of PPARγ at K365 represses NF-κB target genes by inhibiting ubiquitination and degradation of co-repressor complex in NF-κB target gene promoters (87). The SUMOylation-mediated anti-inflammatory effect is strongly associated with the desirable anti-diabetic and anti-atherogenic efficacy of novel drugs.

AT in mammals can be subdivided into two types: WAT to store energy and BAT to dissipate energy through thermogenesis (97). Thus, therapeutic strategies to develop and activate BAT are considered a defense mechanism against obesity. Some TZDs are also capable of promoting browning in WAT (98). Although the mechanism remains unclear, it was recently reported that TZDs increase the half-life of PRDM16 (99). Additionally, rosiglitazone leads to deacetylation of PPARγ at K268 and K293 by Sirt1, and promotes the recruitment of
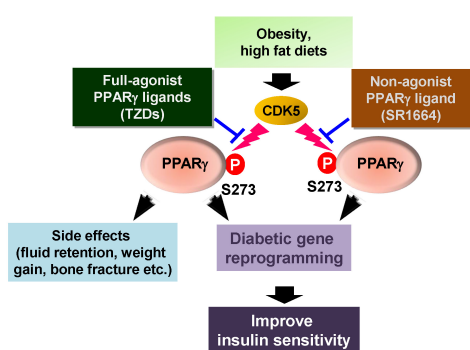
PRDM16 to PPARγ (99). These results suggest novel strategies to prevent obesity through browning of WAT (97). Moreover, deacetylation of either K268 or K293 is necessary for dissociation from NCoR, which enhances the ability of cdk5 to associate with and phosphorylate PPARγ at S273 (81). Thus, compounds that modulate the acetylation of PPARγ with a lack of full agonism may be promising for the treatment of obesity and metabolic disorders.

CONCLUSIONS

The nuclear receptor PPARs play pivotal roles in energy homeostasis and metabolism. Among them, PPARγ has been considered as one of the main therapeutic targets for treating metabolic disorders, and several PPARγ-targeting drugs, such as TZDs, have been developed and used clinically. However, because of unwanted and severe side effects, these drugs have been limited in use. Thus, much effort has been focused on developing new classes of PPARγ-targeting drugs. Recent studies suggest that regulation of specific PTMs of PPARγ may be useful novel approaches for developing anti-diabetic drugs. For example, blocking cdk5-mediated PPARγ phosphorylation by non-agonist PPARγ ligands (e.g., SR1664) is strongly correlated with improved insulin sensitivity without any side effects, such as fluid retention, weight gain, and bone loss. Thus, we have to continue to develop new drugs that can modulate PTMs of PPARγ to improve glucose tolerance and insulin sensitivity, and this work will shed light on drug development for metabolic disorders generally.

ACKNOWLEDGEMENTS

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Fig. 3. Schematic model of novel therapeutic approach to develop anti-diabetic drugs modulating PPARγ phosphorylation. In obesity, the activated cdk5 directly phosphorylates PPARγ at S273 in adipocytes. The anti-diabetic activity of PPARγ ligands is associated with their ability to block phosphorylation of S273, with no classical agonism. Based on these findings, SR1664 was developed as a nonagonist PPARγ ligand that has potent anti-diabetic activity without classical agonism or the severe side effects, such as weight gain, fluid retention, and bone loss.

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