Review Article

Peroxisome Proliferators-Activated Receptor (PPAR) Modulators and Metabolic Disorders

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Overweight and obesity lead to an increased risk for metabolic disorders such as impaired glucose regulation/insulin resistance, dyslipidemia, and hypertension. Several molecular drug targets with potential to prevent or treat metabolic disorders have been revealed. Interestingly, the activation of peroxisome proliferator-activated receptor (PPAR), which belongs to the nuclear receptor superfamily, has many beneficial clinical effects. PPAR directly modulates gene expression by binding to a specific ligand. All PPAR subtypes (α, γ, and δ) are involved in glucose metabolism, lipid metabolism, and energy balance. PPAR agonists play an important role in therapeutic aspects of metabolic disorders. However, undesired effects of the existing PPAR agonists have been reported. A great deal of recent research has focused on the discovery of new PPAR modulators with more beneficial effects and more safety without producing undesired side effects. Herein, we briefly review the roles of PPAR in metabolic disorders, the effects of PPAR modulators in metabolic disorders, and the technologies with which to discover new PPAR modulators.

1. OBESITY, ADIPOCYTES, AND ADIPOKINES

Obesity, which is defined as excess adiposity for a given body size, results from an imbalance between energy intake and energy expenditure. Body mass index (BMI), measured as body weight in kilograms over the square of the height in meters (kg/m^2), represents a widely accepted measure of adiposity. Wealth in industrialized societies, combined with an often-sedentary lifestyle and plentiful, high-calorie diets, creates irreversible weight gain. This social phenomenon can adversely impact well-being. Due to explosive concern for health and well-being, genes associated with human obesity are currently being defined, and whole genome scans will soon unveil its underlying genetic loci. The various causes of obesity are grouped according to behavioral (activity levels, nutrition, smoking status, and socioeconomic status), metabolic (physiological endocrine factors), and biological (genetic, racial, gender, age, and pregnancy status) influences [1]. Obesity has been recognized as a chronic disease since the National Institutes of Health Consensus conference in 1985 [2]. The increase in the prevalence of obesity has led the World Health Organization (WHO) to recently refer to the obesity issue as a "global epidemic".

Chronic disruption of the energy balance due to exceeding energy intake causes hypertrophy and hyperplasia of fat cells, and this is representative of the pathology of obesity. When the intake of energy chronically exceeds energy expenditure, most of the excess energy is stored in the form of triglyceride in adipose tissue (from Greek adip- or adip-, mean fat). Increased adipose tissue mass can arise through an increase in cell size, cell number, or both. Adipocytes are remarkably variable in size, which reflects the amount of stored triglyceride. Mild obesity mainly reflects an increased adipose cell size (hypertrophic obesity), while more severe obesity or obesity arising in childhood typically also involves an increased number of fat cells (hyperplastic obesity) [3]. As a key part of the homeostatic system that controls energy balance, the molecular mechanisms that regulate preadipose cell growth (proliferation), adipose differentiation (adipogenesis), and lipogenesis have been subject to extensive scrutiny. An overview of cell types and molecular events that occur during adipogenesis is presented in Figure 1. Preadipocytes undergo growth arrest, postconfluent mitosis, and clonal expansion following appropriate environmental and gene expression cues. The committed preadipocytes must then withdraw from the cell cycle before
adipose conversion. During the differentiation of adipocytes, the adipocyte phenotypes are characterized by sequential changes in the expression of numerous genes [4, 5]. The study of the cellular and molecular events of adipogenesis was facilitated by the establishment of preadipose cell lines. Among these cell lines, some are derived from embryonic cells such as the 3T3-L1 and 3T3-F442A cell lines, and others such as the Ob17 cell line and its subclones, which originated from adult animals [6]. When maintained in appropriate culture conditions, these cells undergo an adipose conversion characterized by the transcriptional activation of numerous genes. The process of adipose conversion is controlled by external signals, and it has been found that adipogenic cocktails are different depending on the cell systems used. For instance, the 3T3-L1 preadipose cells are induced to differentiate by treatment with high concentrations of cyclic AMP, dexamethasone, and insulin at the preadipose stage. Hormones such as insulin, triiodothyronine, glucocorticoids, and growth hormone exert positive actions on the differentiation of adipose cells. Prostaglandins such as prostacyclin (PGI₂), prostaglandin D₂, and 15-deoxy-D₁₂,1₄·PGJ₂ (15d-PGJ₂) have also been found to be strong activators of adipogenesis. Several transcription factors have been shown to act cooperatively and sequentially to control adipogenesis. These include members of the transcription factors, such as CCAAT/enhancer-binding protein α (C/EBPα) [7], peroxisome proliferator-activated receptor-γ (PPAR-γ), and adipocyte determination, as well as differentiation factor-1 (ADD-1). The last stage of terminal differentiation corresponds to the activation of several genes, including those for proteins involved in triglyceride metabolism [8].

Adipose tissue is partitioned into a few large depots (subcutaneous and visceral locations), and many small depots (heart, epicardium, pericardium, large blood vessels, major lymph nodes, bone marrow, kidney, adrenal glands, and the brain) [9]. All adipocytes secrete a large number of multifunctional molecules, including cytokines, growth factors, enzymes, hormones, complement factors, matrix proteins, and so forth. The proteins that are secreted from adipocytes are designated “adipokines” or “adipocytokines.” Since the isolation of the first-known adipocyte-secreted protein (the serine protease adipin) in 1987 [10], the list of adipokines has been greatly extended. Leptin (from Greek leptos, means thin), encoded by the obese (ob) gene [6, 11], adiponectin (also called Acrp30) [12, 13], Interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) [14], resistin [15], and visfatin [16] are candidates of great interest among the growing number of factors found to be secreted by adipocytes. It has recently been shown that adipokines that are secreted from adipocytes contribute to the development of obesity-associated metabolic disorders, including insulin resistance, cardiovascular disease, and cancer [17].

2. METABOLIC DISORDERS AND THERAPEUTIC TARGETS

Overweight and obesity lead to increased risk for noninsulin-dependent diabetes, hypertension, coronary artery disease, dyslipidemia, gallstones, osteoarthritis, sleep apnea, certain forms of cancer, and degenerative arthritis. As the prevalence of obesity has increased, the heterogeneous clinical disorder strongly associated with abdominal obesity and insulin resistance has been identified as a major risk factor for atherosclerotic cardiovascular disease. This disorder, previously termed “syndrome X” by Reaven, and “insulin-resistance syndrome” by others, is now considered to be metabolic syndrome or metabolic disorder [18]. This disorder shares similar cardiovascular risk factors, including abdominal obesity, impaired glucose regulation/insulin resistance, dyslipidemia, and hypertension. Accordingly, these factors define the
clustering of findings typical of the metabolic disorders, and establish diagnostic criteria. A number of studies have shown that the excess body fat that is stored in the deep abdominal area is associated with metabolic complications [19]. Recently, several molecular drug targets with potential to prevent or treat metabolic disorders have been revealed.

The excess glucocorticoid action by the enzyme 11β-HSD (hydroxysteroid dehydrogenase) type 1 induces obesity and features of metabolic disorders. Transgenic mice which are selectively over-expressing 11β-HSD1 in adipose tissue lead to increased food intake and body weight, as well as the development of visceral obesity. In addition, insulin-resistant diabetes, hyperlipidemia, and hyperphagia were observed in 11β-HSD1 transgenic mice [20]. On the other hand, 11β-HSD1 deficiency causes favorably altered fat distribution and adipose insulin sensitization. Even with high-fat and cholesterogenic diets, lipid profiles are also improved [21]. 11β-HSD1 inhibitors might have beneficial consequences in metabolic disorder. For instance, carbenoxolone, an 11β-HSD1 inhibitor, reduced total cholesterol in healthy subjects, and decreased the glucose production rate during hyperglucagonemia in diabetic patients [22]. AMP-activated protein kinase (AMPK) is a major regulator of lipid and glucose metabolism, and AMPK activation appears as a benefit of exercise in diabetic patients. Activation of AMPK by metformin decreased the level of plasma glucose and plasma triglycerides by promoting muscle glucose uptake and inhibiting hepatic glucose output [23]. SCD-1 (stearyl-CoA desaturase-1) is required for the biosynthesis of the monounsaturated fatty acids from saturated fatty acids, and SCD-1-deficient mice appear visually lean compared to their littermates. SCD-1 deficiency in ob/ob mice ameliorates obesity and completely corrects the excessive hepatic lipid storage and VLDL production of the hypometabolic phenotype in leptin deficiency [24]. An SCD-1 inhibitor that reduces SCD-1 activity may serve as a therapeutic strategy for metabolic disorders, but very few reports are available for the use of SCD-1 inhibitor. IKK kinase β (IKKβ) plays a key role in the activation of NF-κB by phosphorylating IKBa. It has recently been reported to act as a key role in obesity-linked insulin resistance. In obese rodents, increased IKK activity or overexpressed IKK promotes insulin resistance, whereas reduction of IKK activity or IKKβ expression improves insulin sensitivity. In addition, high doses of IKKβ inhibitors such as aspirin and salicylate reverse insulin resistance by sensitizing insulin signaling in obese rodents [25]. Protein tyrosine phosphatase 1B (PTP1B) is closely associated with insulin signaling through the dephosphorylation of activated insulin receptor or insulin receptor substrates. PTP1B deficiency and its heterozygote significantly reduce glucose concentrations in the blood, and PTP1B deficiency causes a significant reduction of circulating insulin concentration compared to wild-type mice. When on a high-fat diet, PTP1B-deficient mice were resistant to diet-induced weight gain, and remained insulin-sensitive [26]. Because PTP1B inhibition provides attractive therapies against metabolic disorders, various studies for the inhibition mechanism of inhibitors against PTP1B, the structure-activity relationship, and synthetic and pharmacological materials have been performed by different groups. Acetyl-CoA carboxylase (ACC) is a key determinant of energy homeostasis because increased malonyl-CoA by ACC activation inhibits mitochondrial fatty acid uptake and oxidation. A lack of malonyl-CoA in the muscle and heart of ACC2-deficient mice show increased oxidation of fatty acid, decreased fat in adipose and liver tissue, and decreased the storage of glycogen in the liver [27]. CP-640186, an isozyme-nonselective ACC inhibitor, inhibits fatty acid and TG synthesis in HepG2 cells, as well as fatty acid synthesis in obese rodents. CP-640186 also stimulates fatty acid oxidation in C2C12 cells [28]. These effects of the ACC inhibitor may provide novel therapeutic potential for treatment of the metabolic disorder. Interestingly, the activation of PPARs by their ligands has many beneficial effects in the improvement of glucose homeostasis and lipid homeostasis.

3. PPARs AND METABOLIC DISORDERS

3.1. PPARs as a nuclear receptor family

Peroxisomes are subcellular organelles that perform diverse metabolic functions, including H2O2-derived respiration, β-oxidation of fatty acids, and cholesterol metabolism. Rodents exposed to peroxisome proliferators lead to hepatocellular hypertrophy, hyperplasia, and transcriptional induction of fatty acid-metabolizing enzymes that are regulated in parallel with peroxisome proliferation [29]. Peroxisome proliferators may activate PPARs by binding directly to the receptors, and the activated PPARs may regulate the expression of genes involved in lipid metabolism and peroxisome proliferation. Recent research on PPARs has moved toward their pivotal roles comprising one family of nuclear receptors [30]. Nuclear receptors, which are present in multicellular organisms, directly control the expression of genes in response to a wide range of developmental, physiological, and environmental signals.

The PPARs of nuclear receptors mainly consist of three subtypes (PPARα, PPARγ, and PPARδ/β). All three PPAR isoforms possess similar structural and functional features. Principally, four functional domains have been identified, and are referred to as A/B, C, D, and E/F. The N-terminal A/B domain contains ligand-independent activation function 1 (AF-1). The ligand-independent activation region can confer constitutive activity on the receptor, and is negatively regulated by phosphorylation [31]. The DNA-binding domain (DBD) or C domain consists of two zinc fingers, and is directly involved with the binding of PPAR to the peroxisome proliferator response element (PPRE) in the promoter regions of target genes. PPREs are direct repeat (DR)-1 elements consisting of two hexanucleotides with the AGGTCA consensus sequence separated by a single nucleotide spacer. Such a sequence, or a similar one, has been found in numerous PPAR-inducible genes, including acyl-CoA oxidase (ACO) and adipocyte fatty acid-binding protein (aP2) [32]. The D site is a hinge region and a docking domain for corepressors. The E/F domain or ligand-binding domain (LBD) is responsible for ligand specificity and the activation of PPAR binding to the PPRE, which increases the expression
of the targeted gene. Upon the binding of a specific ligand to LBD of the E/F domain, the conformation of a PPAR is altered and stabilized. The ligand-bound LBD results in the recruitment of transcriptional coactivators, resulting in gene transcription. Although three of the PPAR isoforms possess similar structures, it is clear that these receptors perform distinct functions according to the specific ligands and their expression patterns in the tissues.

### 3.2. PPARs and their ligands

Ligand-induced activation of the PPAR, by means of low-affinity binding to natural lipid ligands, stimulates an array of molecular responses that aim at maintaining lipid and glucose homeostasis. Ligand-unbound PPAR is associated with chaperon in the cytosol, and the association induces the PPAR to be held in a conformation that allows for high-affinity binding of the ligand [33]. The translocation of a hydrophobic ligand into the cell is facilitated in intra- and extracellular fluids by intracellular lipid-binding proteins (iLBPs) that are members of the family of fatty acid-binding proteins (FABPs). The iLBPs with relatively small sizes (15-16kDa) play important roles in the solubilization and protection of ligands in aqueous spaces. Ligand-loaded iLBP in the cytosol translocates into the nucleus by free diffusion, and they form a short-lived complex with PPAR [34–36]. Ligand is then transferred to the PPAR, and the ligand-bound PPAR forms a heterodimer with the partner nuclear receptor, retinoid X receptor (RXRα). Upon binding to a ligand, the conformation of PPAR is altered and stabilized, and the PPAR-RXR heterodimer then recruits transcriptional coactivators [37–39]. The transcription machinery is bound to PPRE, and directly controls the expression of the target gene (Figure 2) [40].

PPARα was first cloned from the rodent liver in 1990 [41], and PPARβ and PPARγ were first identified in Xenopus [42]. Several groups subsequently reported the cloning of mammalian orthologs of PPARα, PPARδ, and PPARγ. Although PPARα and PPARγ are highly conserved across species, PPARβ varies considerably between Xenopus and
mammals. The murine clone was named PPARδ because of this divergence [43]. PPARα is predominantly expressed in the liver, and is involved in peroxisome proliferation and regulation of fatty acid catabolism. The expression of PPARδ is ubiquitous and abundant in the brain, intestine, skeletal muscle, spleen, macrophages, lung, and adrenals [44]. PPARδ is activated by a large variety of ligands, and has been implicated in developmental and metabolic regulation in several tissues. PPARγ is expressed in adipose tissue, promoting adipogenesis and increasing lipid storage. PPARγ has at least two promoters, and results in the production of two isoforms, 1 and 2. These isoforms are expressed in a tissue-specific pattern. The PPARγ1 isoform is expressed in the spleen, intestine, and white adipose tissue, while the PPARγ2 is preferentially expressed in white and brown fat. PPARγ2 is most abundantly expressed in fat cells, and plays a pivotal role in fat cell differentiation and lipid storage [45]. The distinct physiological roles of each subtype have been shown to be determined by binding to a discrete set of ligands. Although fatty acids could activate PPARs, PPARα activity was induced by eicosanoids [46], cab aprostacyclin [47], and nonsteroidal anti-inflammatory drugs (NSAIDs) [48]. PPARδ was activated by several polyunsaturated fatty acids [49] and eicosanoids [50]. PPARγ specifically binds to thiazolidinediones (TZDs), a class of antidiabetic drugs. Other PPARγ ligands include the natural prostaglandin metabolite 15-deoxy-D12,14-prostaglandin J2 (PGJ2), polyunsaturated fatty acids, and NSAIDs such as ibuprofen and indomethacin [51, 52].

### 3.3. Post-translational regulation of PPARs

PPARs and other nuclear receptors modulate their transcriptional activity via phosphorylation by various kinases, including the mitogen-activated protein kinase (ERK MAPK and p38 MAPK), protein kinase A and C (PKA and PKC), AMP kinase (AMPK), and glycogen synthase kinase 3 (GSK3) [53]. Several mechanisms have been described to explain the modulation of PPAR transcriptional function. First, phosphorylation modulates the affinity of PPARs for their ligand, as well as the coactivator recruitment abilities of PPARs. Although the main phosphorylation site of PPARγ (Ser 112) is located far from the ligand-binding domain, mutated PPARγ (S112D) exhibits a decreased ligand-binding affinity and decreased coactivator recruitment [54]. Second, the phosphorylation of PPARs modulates binding to PPRE. In gel retardation experiments, PPARα phosphorylation via PKA enhances gene expression due to the stabilization of the binding of PPARα to DNA [55]. Finally, phosphorylation plays an important role in ubiquitination and proteasomal catabolism of PPARs. Phosphorylation of the PPARγ AF-1 domain by IFN-γ-ERK-regulated serine phosphorylation promotes the degradation of PPARγ by the ubiquitin-proteasome-dependent degradation in response to ligand activation [56]. PPARα is also degraded by the ubiquitin-proteasome-dependent degradation. However, in contrast to PPARγ, phosphorylation of PPARα induces the stabilization of PPARα by reducing ubiquitination. The phosphorylation and interaction of PPARα with a corepressor stabilize PPARα protein by decreasing its ubiquitination in order to keep a pool of PPARα available for ligand binding and activation [57].

SUMOylation consists of the covalent and reversible conjugation of small ubiquitin-related modifiers (SUMOs) to target protein and regulate biological processes. The number of known SUMO targets is growing, and SUMOylation of PPARγ has recently been reported. SUMOylation of PPARγ mainly occurs at a lysine residue within a ligand-independent activating function domain (AF-1). PPARγ is SUMOylated by SUMO-1 and PIAS proteins that function as E3 ligases [58]. Potential SUMOylation sites of PPARγ include K77 (equivalent to K107 of PPARγ2) and K365. SUMOylation of PPARγ at K77 and K365 occurs in a ligand-dependent manner. SUMOylation of K107 inhibits PPARγ-dependent gene induction, but does not affect transrepression, whereas mutation of K365 eliminates the ability of agonist-activated PPARγ to repress INOS and to be recruited to its promoter [59]. Phosphorylation at S112 of PPARγ2 promotes K107 SUMOylation and exerts more potent repressive effects. The SUMOylation-defective mutation of PPARγ at K77R promotes adipocyte differentiation. The potential SUMOylation site of PPARα has one K185 within the D region, and PPARδ/β has one K104 in the C region, but in vivo SUMOylation is specific for PPARγ among the PPARs [60]. Relatively few studies of post-translational regulation of PPARs have been reported.

### 3.4. Role of PPAR ligands in metabolic disorders

The activation of PPARα upregulates the expressions of several catabolic enzymes that are involved in mitochondrial and peroxisomal β-oxidation and microsomal ω-oxidation, as well as in the transcriptional regulation of genes that are necessary for the maintenance of the redox balance during the oxidative catabolism of fatty acids. The derivatives from fatty acids and fibrates, including gemfibrozil, fenofibrate, cofibrate, bezafibrate, and cipofibrate, can activate PPARα. These fibrates are used in the treatment of hypertriglyceridemia. PPARα agonists fundamentally regulate the β-oxidation of fatty acids, and promote the expression of cytochrome P450 enzymes, which catalyze the ω-hydroxylation of fatty acid [61]. WY14,643, a well-known specific PPARα agonist, increases fatty acid oxidation by increasing the expressions of peroxisomal and mitochondrial fatty acid β-oxidation enzymes. WY14,643 reduces liver insulin resistance more efficiently than muscle insulin resistance by normalizing the circulating triglyceride levels and blood glucose levels in lipaotropic mice [62]. PPARα agonists also activate the expression of apolipoprotein A-1 (ApoA-1) and ATP-binding cassette transporter A1 (ABCA1) [63, 64]. The increased ApoA-1 and ABCA1 proteins enhance cholesterol efflux by the reverse cholesterol transport (RCT) pathway. In addition, PPARα agonists have anti-inflammatory effects in vascular cells. WY14,643 or bezafibrate induces PPARα-mediated inhibition of osteopontin (OPN) expression in human macrophages of atherosclerotic lesions, where they are abundantly synthesized. Bezafibrate significantly decreases OPN plasma levels in type 2 diabetic
patients [65]. Therefore, the PPARα agonist reduces the progression of atherosclerosis and decreases the incidence of coronary heart disease [66]. However, fibrates are contraindicated in patients with renal insufficiency, gallstones, abnormal liver function tests, and pregnancy [67].

The activation of PPARγ promotes the storage of fat by increasing adipocyte differentiation and enhancing the transcription of genes that are important for lipogenesis. The activation of either PPARα or PPARγ in macrophages promotes the cellular efflux of phospholipids and cholesterol in the form of high-density lipoproteins by upregulating the expression of the liver X-receptor (LXR), an oxysterol-activated nuclear hormone receptor that increases expression of the lipid transporter ABCA1 (ATP-binding cassette, subfamily A, member 1) [68]. PPARγ has been the focus of intense research during the past decade because ligands for this receptor have emerged as potent insulin sensitizers that can be used in the treatment of type 2 diabetes [69]. Increased levels of circulating free fatty acids and lipid accumulation in non-adipose tissue have been implicated in the development of insulin resistance. This situation is improved by the PPARγ agonist, which promotes fatty acid storage in fat depots and regulates the expression of adipocyte-secreted hormones that impact glucose homeostasis [70]. The net result of the pleiotropic effects of the PPARγ agonist is improvement of insulin sensitivity, although undesired side effects limit the utility of this therapy. In fact, TZD, a synthetic agonist of PPARγ, appears to be ideally suited for the treatment of this cluster of metabolic abnormalities, which has been termed the insulin resistance or cardiovascular dysmetabolic syndrome as a whole [71]. Two compounds in this class are currently approved for use in the United States. They are Rosiglitazone (Avandia), approved by the US Food and Drug Administration (FDA) in May 1999, and Pioglitazone (Actos), which was approved in July 1999. Historically, the first agent in this class, Troglitazone (Rezulin), was marketed in the United States from March 1997 to March 2000. Troglitazone was banned because the FDA determined that the risk of idiosyncratic hepatotoxicity associated with Troglitazone therapy outweighed its potential benefits [72, 73].

The activation of PPARδ in macrophages also upregulates the expression of the ABCA1 transporter. Recent evidence indicates that PPARδ can also promote cellular lipid accumulation by increasing the expressions of genes that are involved in lipid uptake, and by repressing key genes that are involved in lipid metabolism, inflammation, atherosclerosis, obesity, fertility, and cancer [74, 75]. Several 14- to 18-carbon saturated fatty acids as well as 16- to 20-carbon polyunsaturated fatty acids are screened as PPARδ-binding chemicals in ligand screening and competition binding assays [50, 76, 77]. As physiological ligands of PPARδ, these fatty acids or eicosanoids are unsettled. However, Chawla et al. hypothesized that PPARδ acts as a lipid sensor, where fatty acids derived from very-low-density lipoprotein (VLDL) can activate PPARδ [78]. A PPARδ-specific agonist, GW501516, decreases plasma triglyceride levels in obese monkeys, raises high-density lipoprotein levels, and prompts the initiation of clinical trials to assess its efficacy in hyperlipidemic patients [79]. GW501516 also attenuates weight gain and insulin resistance in mice fed high-fat diets by increasing the expressions of genes that promote lipid catabolism and mitochondrial uncoupling in skeletal muscle, thereby increasing β-oxidation of the fatty acids in skeletal muscle [80]. PPARδ agonists also have anti-inflammatory properties. The PPARδ agonist inhibits LPS-inducible genes, such as Cox-2 and iNOS in murine peritoneal macrophages [81]. These reports indicate that the PPARδ-specific agonist is a potential therapeutic interest for the treatment of metabolic disorder.

4. VARIOUS STRATEGIES FOR SAFER PPAR MODULATORS

Each PPAR subtype regulates a distinct metabolic pathway, and the agonists of each of the PPAR subtypes have distinct effects with undesired side effects such as weight gain, hepatotoxicity, and heart failure. In the case of the TZD class as PPARγ agonists, the major side effect is weight gain. A Pro12Ala substitution in PPARγ2 decreases PPARγ activity, BMI, and insulin resistance [82]. Because of these undesirable effects caused by PPARγ agonists, new therapeutic solutions have been investigated in order to reduce their side effects. Various compounds have been reported to be PPAR antagonists, including Bisphenol A diglycidyl ether (BADGE), PD068235, LG100641, GW9662, SR-202, GW6741, and Compound A and B [83]. A potent selective PPARγ agonist, GW9662, does not recruit PPAR coactivators such as SRC-1 and p300, and it suppresses rosiglitazone-induced adipocyte differentiation in 3T3-L1 adipocytes. GW9662 prevents high-fat diet-induced obesity without affecting food intake, and has no effect on high-fat diet-induced glucose intolerance [84]. The phosphonophosphate SR-202, a PPARγ agonist, inhibits BRL 49653-mediated recruitment of SRC-1 and troglitazone-induced transcriptional activity. SR-202 inhibits PPARγ-induced adipocyte differentiation of 3T3-L1 and prevents weight gain and adipose tissue deposition in mice given a standard diet or high-fat diet. In addition, SR-202 markedly reduces hyperglycemia and hyperinsulinemia in ob/ob mice [85]. A few PPARα antagonists have been reported, but in vivo data have not been disclosed. Several PPARγ antagonists may have therapeutic availability as antiobesity drugs. However, further studies of the molecular effects of PPARγ antagonists are necessary.

The combination agonist strategy, which uses a combination of agonists, has been designed to activate each receptor subtype. In terms of its pharmacological aspects, this strategy may provide more efficacious effects and more safety for undesired side effects. The possible combinations are PPARα/γ dual agonist, PPARγ/δ dual agonist, PPARα/δ dual agonist, and PPARα/γ/δ agonist. The initial combination agonist strategy was focused on the development of PPARα/γ dual agonists. PPARγ agonists such as rosiglitazone and pioglitazone provide undesired side effects of TZDs, including weight gain. By contrast, PPARα agonists such as fibrates decreased adiposity through the stimulation of lipid oxidation. Dual PPARα/γ stimulation with a combination...
of rosiglitazone and fenofibrate in type 2 diabetic patients effectively improved the atherogenic dyslipidemic profile, which plays a key role in the occurrence of cardiovascular mortality [86]. Applications of structurally various PPARα/γ dual agonists have recently been reported. Among these dual PPARα/γ agonists, compounds belonging to the glitazar class have been advanced to clinical development (Phases II and III). These PPARα/γ dual agonists commonly reduce triglycerides and total cholesterol, increase HDL levels, and consequently improve insulin sensitivity. However, the use of a few PPARα/γ dual agonists, including muraglitazar, tesaglitazar, ragaglitazar, farglitazar, TAK559, and KRP297, has been discontinued due to various safety liabilities compared to selective agonists. All glitazars significantly increase weight gain and edema, because of higher PPARγ affinity than PPARα affinity although their affinity for PPARα is higher than fibrates. Muraglitazar increases cardiovascular risks, tesaglitazar impairs glomerular filtration rate, and some have carcinogenic effects in mice [87]. The safety liabilities may be the result of their imbalanced activities on PPARα and PPARγ. Therefore, the best solution would be to screen candidates with appropriate affinity for PPARα and selective PPARγ-modulating activity [88].

Both PPARγ and PPARδ play important roles in glucose and lipid metabolism. A PPARγ/δ dual agonist with a properly controlled γ/δ ratio could attenuate undesired weight gain, improve insulin sensitivity, and stimulate fatty acid oxidation. The dual PPARγ/δ agonist (R)-3-[2-ethyl-4-([3-(4-ethyl-2-pyridin-2-yl-phenoxy)-butoxy]-phenyl]-propionic acid has been shown to lower the glucose level and cause less weight gain than rosiglitazone in hyperglycemic male Zucker diabetic fatty (ZDF) rats [89]. The other dual PPARγ/δ agonist, (R)-3-[4-[4-chloro-2-phenoxy-phenoxy]-butoxy]-2-ethyl-phenyl]-propionic acid, improves insulin sensitivity and reverses diabetic hyperglycemia with less weight gain relative to rosiglitazone in female ZDF rats [90]. PPARα/δ dual agonists (T659 and Compound 24) have recently been reported. T659 has had beneficial effects on HDL-C in experimental primates [91]. Compound 24 has also shown significant effects on HDL-C, TG, and FFA levels in male apoA1 transgenic mice [92]. PPARα/δ dual agonists may improve hyperlipidemia, insulin resistance, and risk of atherosclerosis. The development of PPARα/δ and PPARγ/δ dual agonists is currently being pursued.

Another strategy to reduce the adverse effects of previous PPARγ agonists is the identification of partial agonists, also referred to as selective PPARγ modulators (SPPARMs). SPPARMs are PPARγ ligands with insulin-sensitizing activity and lower stimulation of adipogenesis. Because SPPARMs bind to the ligand-binding pocket of the PPARγ receptor in distinct manners, SPPARM-bound PPARγ induces the displacement of the differential coactivator and the specific gene expression in a tissue-specific manner. Although several PPARγ agonists have been classified as SPPARMs, the majority of these synthetic ligands remain to be characterized at the molecular level, and need to be evaluated in in vivo preclinical models to assess their relationships with weight gain [93]. Halofenate (HA) and PA-082, new SPPARMs, were recently developed. HA causes displacement of corepressors (N-CoR and SMRT), but does not cause efficient recruitment of coactivators (p300, CBP, and TRAP 220). Moreover, HA selectively regulates the expression of multiple PPARγ responsive genes in 3T3-L1 adipocytes, and has acute antidiabetic properties in diabetic ob/ob mice [94]. The isoquinoline derivative PA-082, a prototype of a novel class of non-TZD partial PPARγ agonists, causes preferential recruitment of PPARγ-coactivator-1α (PGC1α) to the receptor compared with rosiglitazone. PA-082 antagonizes rosiglitazone-driven transactivation and TG accumulation in C2H10T1/2 mesenchymal stem cells. However, PA-082 induces mRNAs of genes that encode components of insulin signaling pathways. It also facilitates glucose uptake and insulin signaling in mature adipocytes [95]. The functional study of SPPARMs will provide more information about effective antidiabetic agents to reduce the side effects of weight gain.

The PPARα/γ agonists can activate all three PPAR subtypes, and they can potentially exert various effects on metabolic disorders such as insulin resistance, obesity, dyslipidemia, and hypertension. The well-known lipid-lowering bezafibrate is the first clinically-tested PPAR agonist. Though bezafibrate is a PPAR ligand with a relatively low potency, it considerably raises HDL cholesterol, reduces triglycerides, improves insulin sensitivity, and reduces blood glucose levels [96]. GW677954, a novel PPARγ agonist, is being investigated in Phase II trials for the treatment of metabolic disorders [97]. PLX-204 and GW-625019 are also progressing in Phase I trials for the treatment of metabolic disorders. In addition, LY-465608, DRF-11605, CS-204, and DRL-11605 are under investigation, and may be potent therapeutic agents for the treatment of metabolic disorders [88].

5. TECHNOLOGIES TO DISCOVER NEW PPAR MODULATORS

The development of new technology to discover PPAR modulator is significant in functional study of the nuclear receptors and new potent drug discovery. In general, transactivation and chimeric receptor transactivation assays have been used as cell-based methods employing mammalian cells for the screening of new PPAR modulators. Cell-based assays provide a more physiological relevance, but these assays are costly, time-consuming, and difficult to apply to automated systems used for high-throughput screening (HTS). Recently, Chen et al. introduced a yeast-based method for screening PPAR modulators [98]. Cell-free assays for the screening of PPAR modulators have been developed in numerous forms. The X-ray crystal structure study revealed that the human apo-PPARγ ligand-binding domain (LBD) has a large binding pocket, which may explain the diversity of the PPARγ ligands [99]. When binding to specific ligands in LBD, PPAR changes its conformation. Glutamate and lysine residues that are highly conserved in LBDs of PPAR form a “charge clamp” that contacts the backbone atoms of the LXXL helices of coactivators such as steroid receptor coactivator-1 (SRC-1). In the case of SRC-1, four consecutive LXXL motifs make identical contacts with both subunits of a PPAR-RXR heterodimer [100]. Such allosteric conformational changes
promote the recruitment of nuclear receptor coactivators and effectively stimulate the transcription of their target genes. Different PPAR ligands may elicit distinct downstream biological effects due to unique conformational changes in the nuclear receptor.

A cell-free competition radioreceptor assay using competitive interaction between a recombinant PPAR protein and a radioisotope-labeled ligand in the presence of competitor ligands has been reported previously (Figure 3(a)) [101, 102]. The coactivator-dependent receptor ligand assay (CARLA) has been reported as a cell-free assay based on the interaction between PPAR and the coactivator. CARLA is based on the recruitment of a transcriptional coactivator by changes in the conformation of ligand-bound PPAR (Figure 3(b)). In presence of PPAR ligands, 35S-labeled SRC-1 has stronger interaction with GST-fused PPAR proteins immobilized on glutathione Sepharose beads. Autoradiograms and the quantification of the effect of candidates are dependent on the retention of SRC-1 by the GST-PPAR LBD [76]. The major advantage of CARLA is that it does not require radioactive labeling of candidate modulators, which makes it possible to screen a large number of compounds with this assay; this has simultaneous economic advantages in terms of materials and time.

Scintillation proximity has been developed as a tool for measuring the interaction between a receptor and a ligand. The scintillation proximity assay (SPA) bead is impregnated with a scintillant and coated with a capture molecule such as streptavidin. After preincubation of SPA beads and biotinylated PPAR LBD, radiolabeled ligands were added to a complex of SPA bead-PPAR LBD. Unbound free ligands were eliminated from the SPA-PPAR complex. When SPA bead and radiolabeled ligands come into close proximity, radioactive counts are determined by the β emission from the radioisotope to be absorbed by the scintillant, which will then shift this energy to produce light (Figure 3(c)) [103, 104]. The advantages of the SPA are as follows: low cost, high sensitivity, high reliability, and simplicity, that is, no separation step is required. The simplicity of SPA is an important benefit in its application to HTS. However, unsuitability for kinetic determination and the limited number of useful isotopes were perceived as potential disadvantages.

Fluorescence has been considered as an analytical technique with which to study for the detection and quantitation of interacting molecules. There are several advantages to this technique, including high sensitivity, the relative ease of handling and disposal compared to radioactivity, and the diversity of available fluorophores. Thus, fluorescence resonance energy transfer (FRET) has been applied in the probing of molecular interactions [105]. As shown in Figure 3(d), GST-PPAR LBD was indirectly linked to Eu(K) through an anti-GST antibody, which was covalently linked to Eu(K). Coactivator was also indirectly linked to XL665 through a streptavidin (SA)-biotin adapter. The conformational change of PPAR by the PPAR agonist induces the recruitment of coactivator, and the interaction between PPAR and a coactivator will result in the close proximity of the fluorescence donor and acceptor. Consequently, the fluorescence donor (anti-GST-Eu(K)) is excited, and inputted energy will be transferred to the acceptor (streptavidin-XL665). Homogenous time-resolved fluorescence (HTRF) energy transfer technology takes advantage of fluorescence, as well as the homogenous and time-resolved detection mode. These specificities of HTRF enable it to overcome most of the drawbacks encountered in FRET [106].

Previous cell-free methods with which to screen PPAR agonists have used isotope or fluorescence labeling agonists or proteins. We established very simple ELISA systems based on the ligand-dependent binding between PPAR and coactivators. In brief, the purified recombinant LXXLL motif of coactivators was applied into a 96-well plate, and E. coli lysates containing recombinant PPAR proteins were then added with candidate PPAR agonists. The complex consisting of PPAR and coactivator was then identified with the anti-PPAR antibody (Figure 3(e)). Major advantages of this simple method are its simplicity and its low cost, as these systems do not require any labeling of candidate modulators and proteins. This makes it possible to screen a large number of compounds, with simultaneous economic advantages in terms of materials and time. On the other hand, this method has relatively low sensitivity and has to use a suitable anti-PPAR antibody [107–109].

In the 1980s, surface plasmon resonance (SPR) and related techniques that exploited evanescent waves were applied for the study of biological and chemical interactions. SPR technology has also been successfully employed to study the interactions between ligands and nuclear receptors [110–112], the effects of ligand-binding on nuclear receptor dimerization [113], and ligand screening based on interactions between ligand-bound nuclear receptors and coactivators [114, 115] (Figure 3(f)). In the interaction analysis between PPAR LBD and ligand, PPAR LBD is immobilized on the sensor chip by a standard primary amine-coupling reaction, and the ligand is injected over the immobilized PPAR LBD. In the investigation of ligand binding on receptor dimerization, the partner nuclear receptor is immobilized on the sensor chip by the standard amine-coupling reaction, and the nuclear receptor pre-incubated with its ligand is injected over the immobilized partner nuclear receptor. In ligand screening based on interactions between ligand-bound nuclear receptors and coactivator, the coactivator or LXXLL peptide is immobilized on the sensor using same methods, and the nuclear receptor that was preincubated with a candidate chemical is injected over the immobilized coactivator peptide. The association ($k_a$) and dissociation ($k_d$) rate constants and the dissociation equilibrium constants ($K_{DSS}$) for the bindings were determined using the Biacore biosensor. The binding responses in resonance units (RU) were continuously recorded, and were presented graphically as a function of time. SPR technology has the advantages in that it requires no labeling, can be performed in real-time, and utilizes noninvasive measurements.

6. SUMMARY

Obesity mainly reflects an increased adipose cell size, an increased adipocyte cell number, and an imbalance between
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(a) PPAR against PPAR LBD

(b) CO-activator

(co-activator)

GST

(c) Radiolabeled ligand

Biotin

Streptavidin SPA bead

(d) PPAR against PPAR LBD

α-GST-(Eu)K

Fluorescence resonance Energy transfer at 620 nm

$\nu_{em} (620 \text{ nm})$

$h_{em} (337 \text{ nm})$

Readout (ratio) = $\frac{(665 \text{ nm}/620 \text{ nm}) \times 10,000}$

(e) PPAR against α-PPAR Ab

Weak binding

Strong binding
energy intake and energy expenditure. Excess body fat is associated with metabolic disorders. As a molecular drug target for metabolic disorders, the activation of PPAR by specific ligands has many beneficial clinical effects in the improvement of glucose and lipid homeostasis. The PPARs mainly consist of three subtypes (PPARα, PPARγ, and PPARδ), and all three PPAR isoforms possess similar structural and functional features involving glucose metabolism, lipid metabolism, and energy balance. PPARs directly modulate gene expression upon binding to specific ligands transferred into PPAR via ILBP-mediated translocation. PPAR agonists play an important role in therapeutic aspects of metabolic disorders, whereas undesired effects for the existing PPAR agonists prescribed as therapeutic agents have been reported.

To discover new PPAR modulators with more efficacious effects and more safety against undesired side effects, a novel PPAR antagonist or the combination of agonists such as PPARα/γ dual agonist, PPARγ/δ dual agonist, PPARα/δ dual agonist, and PPARpan (PPARα/γ/δ) agonist has been applied to activate each receptor subtype and selective PPARγ modulators (SPPARγMs). In addition, various technologies have been developed in attempts to discover PPAR modulators as therapeutic agents for the treatment of metabolic disorders. Because cell-based assays have more physiological relevance, the transactivation assay, chimeric receptor transactivation, and yeast two-hybrid methods have also been used. Since cell-free assays are based on direct interaction between PPAR and their specific ligands, a new concept for competing radioreceptor assays has been developed by making the best use of competitive interactions between recombinant PPAR protein and radioisotope-labeled ligands. Later, cell-free assays (CARLA and SPA) were developed based on conformational changes in PPARs caused by their ligands, and the simplicity of SPA permitted application to high-throughput screening (HTS). Radioisotope-free assays like FRET (HTRF), ELISA, and SPR methods are relatively simple in terms of handling and disposal. Thus, HTRF and SPR assays can be applied to a homogenous and time-resolved detection mode.

Interestingly, prior to the discovery of the PPARα, it was reported that Wy-14,643, a well-known synthetic agonist of PPARα, decreased serum cholesterol and triglyceride levels in mice [116]. TZD derivatives, well-known synthetic agonists of PPARγ, were reported as antidiabetes agents prior to the discovery of the PPARγ [117, 118]. An antidiabetes agent,
pioglitazone, a TZD derivative, has recently been shown to bind specifically to a protein named mitoNEET [119]. These studies of PPAR, PPAR modulators, and technologies to discover PPAR modulators will elicit the development of drugs with more efficacious effects and more safety for the treatment of metabolic disorders.

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REFERENCES

[1] E. Ravussin and C. Bouchard, “Human genomics and obesity: finding appropriate drug targets,” European Journal of Pharmacology, vol. 410, no. 2-3, pp. 131–145, 2000.
[2] T. B. Van Itallie, “Health implications of overweight and obesity in the United States,” Annals of Internal Medicine, vol. 103, no. 6, part 2, pp. 983–988, 1985.
[3] M. Lafontan, “Fat cells: afferent and efferent messages define new approaches to treat obesity,” Annual Review of Pharmacology and Toxicology, vol. 45, pp. 119–146, 2005.
[4] F. M. Gregoire, C. M. Smas, and H. S. Sul, “Understanding adipocyte differentiation,” Physiological Reviews, vol. 78, no. 3, pp. 783–809, 1998.
[5] E. D. Rosen and B. M. Spiegelman, “Molecular regulation of adipogenesis,” Annual Review of Cell and Developmental Biology, vol. 16, pp. 145–171, 2000.
[6] C.-S. Hwang, T. M. Loftus, S. Mandrup, and M. Daniel Lane, “Adipocyte differentiation and leptin expression,” Annual Review of Cell and Developmental Biology, vol. 13, pp. 231–259, 1997.
[7] Q.-Q. Tang, J.-W. Zhang, and M. Daniel Lane, “Sequential gene promoter interactions by C/EBPβ, C/EBPα, and PPARγ during adipogenesis,” Biochemical and Biophysical Research Communications, vol. 318, no. 1, pp. 213–218, 2004.
[8] P. A. Grimaldi, “Fatty acid regulation of gene expression,” Current Opinion in Clinical Nutrition & Metabolic Care, vol. 4, no. 5, pp. 433–437, 2001.
[9] B. M. Spiegelman and J. S. Flier, “Adipogenesis and obesity: rounding out the big picture,” Cell, vol. 87, no. 3, pp. 377–389, 1996.
[10] K. S. Cook, H. Y. Min, D. Johnson, et al., “Adipasin: a circulating serum protease homolog secreted by adipose tissue and sciatic nerve,” Science, vol. 237, no. 4813, pp. 402–405, 1987.
[11] M. Otero, R. Lago, F. Lago, et al., “Leptin, from fat to inflammation: old questions and new insights,” FEBS Letters, vol. 579, no. 2, pp. 295–301, 2005.
[12] A. H. Berg, T. P. Combs, and P. E. Scherer, “ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism,” Trends Endocrinology and Metabolism, vol. 13, no. 2, pp. 84–89, 2002.
[13] M. Fasshauer, R. Paschke, and M. Stumvoll, “Adiponectin, obesity, and cardiovascular disease,” Biochimie, vol. 86, no. 11, pp. 779–784, 2004.
[14] J. N. Fain, A. K. Madan, M. L. Hiler, P. Cheema, and S. W. Bahouth, “Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans,” Endocrinology, vol. 145, no. 5, pp. 2273–2282, 2004.
[15] C. M. Stepan and M. A. Lazar, “Resistin and obesity-associated insulin resistance,” Trends in Endocrinology and Metabolism, vol. 13, no. 1, pp. 18–23, 2002.
[16] C. Hug and H. F. Lodish, “Visfatin: a new adipokine,” Science, vol. 307, no. 5708, pp. 366–367, 2005.
[17] G. N. Chalidakos, I. S. Stankulov, M. Hristova, and P. I. Ghenev, “Adipobiology of disease: adipo genes and adipokine-targeted pharmacology,” Current Pharmaceutical Design, vol. 9, no. 12, pp. 1023–1031, 2003.
[18] D. E. Moller and K. D. Kaufman, “Metabolic syndrome: a clinical and molecular perspective,” Annual Review of Medicine, vol. 56, pp. 45–62, 2005.
[19] A. Tremblay and E. Doucet, “Obesity as a disease or a biological adaptation?” Obesity Review, vol. 1, no. 1, pp. 27–35, 2000.
[20] H. Masuzaki, J. Paterson, H. Shinyama, et al., “A transgenic model of visceral obesity and the metabolic syndrome,” Science, vol. 294, no. 5549, pp. 2166–2170, 2001.
[21] N. M. Morton, J. M. Paterson, H. Masuzaki, et al., “Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11β-hydroxysteroid dehydrogenase type 1-deficient mice,” Diabetes, vol. 53, no. 4, pp. 931–938, 2004.
[22] R. C. Andrews, O. Rooyackers, and B. R. Walker, “Effects of the 11β-hydroxysteroid dehydrogenase inhibitor carbenoxolone on insulin sensitivity in men with type 2 diabetes,” Journal of Clinical Endocrinology & Metabolism, vol. 88, no. 1, pp. 285–291, 2003.
[23] G. Zhou, R. Myers, Y. Li, et al., “Role of AMP-activated protein kinase in mechanism of metformin action,” Journal of Clinical Investigation, vol. 108, no. 8, pp. 1167–1174, 2001.
[24] P. Cohen, M. Miyazaki, N. D. Socci, et al., “Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss,” Science, vol. 297, no. 5579, pp. 240–243, 2002.
[25] M. Yuan, N. Konstantopoulos, J. Lee, et al., “Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkβ,” Science, vol. 293, no. 5535, pp. 1673–1677, 2001.
[26] M. Elchebly, P. Payette, E. Michaliszyn, et al., “Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene,” Science, vol. 283, no. 5407, pp. 1544–1548, 1999.
[27] L. Abu-Elheiga, M. M. Matzuk, K. A. Abo-Hashema, and S. J. Wakil, “Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2,” Science, vol. 291, no. 5513, pp. 2613–2616, 2001.
[28] H. J. Harwood Jr., S. F. Peteras, L. D. Shelly, et al., “Isozyme-targeted pharmacology,” Current Pharmaceutical Design, vol. 9, no. 12, pp. 1023–1031, 2003.
and -2 isoforms and influence of insulin,” *Journal of Biological Chemistry*, vol. 272, no. 32, pp. 20230–20235, 1997.

[32] R. A. Graves, P. Tontonoz, K. A. Platt, S. R. Ross, and B. M. Spiegelman, “Identification of a fat cell enhancer: analysis of requirements for adipose tissue-specific gene expression,” *Journal of Cellular Biochemistry*, vol. 49, no. 3, pp. 219–224, 1992.

[33] W. K. Sumanoskera, E. S. Tien, J. W. Davis II, R. Turpey, G. H. Perdew, and J. P. Vanden Heuvel, “Heat shock protein-90 (Hsp90) acts as a repressor of peroxisome proliferator-activated receptor-α (PPARα) and PPARβ activity,” *Biochemistry*, vol. 42, no. 36, pp. 10726–10735, 2003.

[34] A. W. Zimmerman and J. H. Veenkamp, “New insights into the structure and function of fatty acid-binding proteins,” *Cellular and Molecular Life Sciences*, vol. 59, no. 7, pp. 1196–1116, 2002.

[35] N.-S. Tan, N. S. Shaw, N. Vinckenbosch, et al., “Selective cooperation between fatty acid binding proteins and proxi- somal proliferator-activated receptors in regulating transcrip- tion,” *Molecular and Cellular Biology*, vol. 22, no. 14, pp. 5114–5127, 2002.

[36] A. Adida and F. Spener, “Adipocyte-type fatty acid-binding protein as inter-compartmental shuttle for peroxisome pro- liferator activated receptor γ agonists in cultured cell,” *Biochimica et Biophysica Acta*, vol. 1761, no. 2, pp. 172–181, 2006.

[37] Y. Zhu, C. Qi, M. S. Rao, and J. K. Reddy, “Cloning and identification of mouse steroid receptor coactivator-1 (mSRC-1), as a coactivator of peroxisome proliferators-activated receptor γ,” *Gene Expression*, vol. 6, no. 3, pp. 185–195, 1996.

[38] J. J. Voegel, M. J. Heine, C. Zechel, P. Chamhon, and H. Gronemeyer, “TIF2, a 160 kDa transcriptional mediator for the ligand-dependent activation function AF-2 of nuclear receptors,” *The EMBO Journal*, vol. 15, no. 14, pp. 3667–3675, 1996.

[39] J. Torchia, D. W. Rose, J. Inostroza, et al., “The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function,” *Nature*, vol. 387, no. 6634, pp. 677–684, 1997.

[40] B. P. Kota, T. H.-W. Huang, and B. D. Roufogalis, “An overview on biological mechanisms of PPARs,” *Pharmacological Research*, vol. 51, no. 2, pp. 85–94, 2005.

[41] 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.

[42] C. Dreyer, G. Krey, H. Keller, F. Givel, G. Helftenbein, and W. Wahli, “Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors,” *Cell*, vol. 68, no. 5, pp. 879–887, 1992.

[43] S. A. Kliwer, B. M. Forman, B. Blumberg, et al., “Differential expression and activation of a family of murine peroxisome proliferators-activated receptors,” *Proceedings of the National Academy of Science of the United States of America*, vol. 91, no. 15, pp. 7355–7359, 1994.

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

[45] P. Tontonoz, E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman, “mPPARγ 2: tissue-specific regulator of an adipocyte enhancer,” *Genes & Development*, vol. 8, no. 10, pp. 1224–1234, 1994.

[46] K. Yu, W. Bayona, C. B. Kallen, et al., “Differential activation of peroxisome proliferator-activators by eicosanoids,” *Journal of Biological Chemistry*, vol. 270, no. 41, pp. 23975–23983, 1995.

[47] R. Hertz, I. Berman, D. Keppler, and J. Bar-Tana, “Activation of gene transcription by prostacyclin analogues is mediated by the peroxisome proliferators-activated receptor (PPAR),” *European Journal of Biochemistry*, vol. 235, no. 1-2, pp. 242–247, 1996.

[48] J. M. Lehmann, J. M. Lenhard, B. B. Oliver, G. M. Ringold, and S. A. Kliwer, “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.

[49] A. Schmidt, N. Endo, S. J. Rutledge, R. Vogel, D. Shinar, and G. A. Rodan, “Identification of a new member of the steroid hormone receptor superfamily that is activated by a proxi- somal proliferators and fatty acids,” *Molecular Endocrinology*, vol. 6, no. 10, pp. 1634–1641, 1992.

[50] B. M. Forman, J. Chen, and R. M. Evans, “Hypolipidemic drugs, polyunsaturated fatty acid, and eicosanoids are ligands for peroxisome proliferators-activated receptors α and δ,” *Proceedings of the National Academy of Science of the United States of America*, vol. 94, no. 9, pp. 4312–4317, 1997.

[51] B. Desvergne and W. Wahli, “Peroxisome proliferators-activated receptors: nuclear control of metabolism,” *Endocrinology Review*, vol. 20, no. 5, pp. 649–688, 1999.

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

[53] C. Diradourian, J. Girard, and J.-P. Pégourier, “Phosphoryla- tion of PPARs: from molecular characterization to physiologi- cal relevance,” *Biochimie*, vol. 87, no. 1, pp. 33–38, 2005.

[54] D. Shao, S. M. Rangwala, S. T. Bailey, S. L. Krakow, M. J. Reginato, and M. A. Lazar, “Interdomain communication regulating ligand binding by PPAR-γ,” *Nature*, vol. 396, no. 6709, pp. 377–380, 1998.

[55] G. Lazennec, L. Canaple, D. Saugy, and W. Wahli, “Activation of peroxisome proliferator-activated receptors (PPARs) by their ligands and protein kinase A activators,” *Molecular Endocrinology*, vol. 14, no. 12, pp. 1962–1975, 2000.

[56] Z. E. Floyd and J. M. Stephens, “Interferon-γ-mediated activation and ubiquitin-proteasome-dependent degrada- tion of PPARγ in adipocytes,” *Journal of Biological Chemistry*, vol. 277, no. 6, pp. 4062–4068, 2002.

[57] C. Blanquart, R. Mansouri, J.-C. Fruchart, B. Staels, and C. Glineur, “Different ways to regulate the PPARα stability,” *Biochemical and Biophysical Research Communications*, vol. 319, no. 2, pp. 663–670, 2004.

[58] T. Ohshima, H. Koga, and K. Shimotohno, “Transcriptional activity of peroxisome proliferator-activated receptor γ is modulated by SUMO-1 modification,” *Journal of Biological Chemistry*, vol. 279, no. 28, pp. 29551–29557, 2004.

[59] G. Pascual, A. L. Feng, S. Ogawa, et al., “A SUMOylation-dependent pathway mediates transrepression of inflamma- tory response genes by PPAR-γ,” *Nature*, vol. 437, no. 7059, pp. 759–763, 2005.

[60] D. Yamashita, T. Yamaguchi, M. Shimizu, N. Nakata, F. Hirose, and T. Osumi, “The transactivating function of peroxisome proliferator-activated receptor γ is negatively regulated by SUMO conjugation in the amino-terminal domain,” *Genes to Cells*, vol. 9, no. 11, pp. 1017–1029, 2004.
[61] S. Yu, S. Rao, and J. K. Reddy, “Peroxisome proliferators-activated receptors, fatty acid oxidation, steatohepatitis and hepatocarcinogenesis,” Current Molecular Medicine, vol. 3, no. 6, pp. 561–572, 2003.

[62] C. J. Chou, M. Haluzik, C. Gregory, et al., “WY14,643, a peroxisome proliferators-activated receptor α (PPARα) agonist, improves hepatic and muscle steatosis and reverses insulin resistance in lipostatic A-ZIF/F-1 mice,” Journal of Biological Chemistry, vol. 277, no. 27, pp. 24484–24489, 2002.

[63] N. Vu-Dac, S. Chopin-Delannoy, P. Gervois, et al., “The nuclear receptors peroxisome proliferator-activated receptor α and Rev-erba mediate the species-specific regulation of apolipoprotein A-1 expression by fibrate,” Journal of Biological Chemistry, vol. 273, no. 40, pp. 25713–25720, 1998.

[64] B. L. Knight, D. D. Patel, S. M. Humphreys, D. Wiggins, and G. F. Gibbons, “Inhibition of cholesterol absorption associated with a PPARα-dependent increase in ABC binding cassette transporter A1 in mice,” Journal of Lipid Research, vol. 44, no. 11, pp. 2049–2058, 2003.

[65] T. Nakamachi, T. Nomiyama, F. Gizard, et al., “PPARα agonists suppress osteopontin expression in macrophages and decrease plasma levels in patients with type 2 diabetes,” Diabetes, vol. 56, no. 6, pp. 1662–1670, 2007.

[66] G. Chinetti, F. G. Gbaguidi, S. Griglio, et al., “CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors,” Circulation, vol. 101, no. 20, pp. 2411–2417, 2000.

[67] A. C. Li and W. Palinski, “Peroxisome proliferator-activated receptors: how their effects on macrophages can lead to the development of a new drug therapy against atherosclerosis,” Annual Review of Pharmacology and Toxicology, vol. 46, pp. 1–39, 2006.

[68] 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.

[69] R. Pakala, P. Kuchulakanti, S.-W. Rha, E. Cheneau, R. Bafna, and S.-W. Rha, “Ectopic expression of peroxisome proliferator-activated receptor β/δ in epithelial cell growth and differentiation,” Cellular Signalling, vol. 18, no. 1, pp. 9–20, 2006.

[70] G. D. Barish, V. A. Narkar, and R. M. Evans, “PPARδ: a dagger in the heart of the metabolic syndrome,” Journal of Clinical Investigation, vol. 116, no. 3, pp. 590–597, 2006.

[71] G. Krey, O. Braissant, F. L’Horset, et al., “Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay,” Molecular Endocrinology, vol. 11, no. 6, pp. 779–791, 1997.

[72] H. Eric Xu, M. H. Lambert, V. G. Montana, et al., “Molecular recognition of fatty acids by peroxisome proliferator-activated receptors,” Molecular Cell, vol. 3, no. 3, pp. 397–403, 1999.

[73] A. Chawla, C.-H. Lee, Y. Barak, et al., “PPARδ is a very low-density lipoprotein sensor in macrophages,” Proceedings of the National Academy of Sciences of the United States of America, vol. 100, no. 3, pp. 1268–1273, 2003.

[74] W. R. Oliver Jr., J. L. Shenk, M. R. Snaith, et al., “A selective peroxisome proliferator-activated receptor δ agonist promotes reverse cholesterol transport,” Proceedings of the National Academy of Sciences of the United States of America, vol. 98, no. 9, pp. 5306–5311, 2001.

[75] T. Tanaka, J. Yamamoto, S. Iwasaki, et al., “Activation of peroxisome proliferator-activated receptor δ induces fatty acid β-oxidation in skeletal muscle and attenuates metabolic syndrome,” Proceedings of the National Academy of Sciences of the United States of America, vol. 100, no. 26, pp. 15924–15929, 2003.

[76] J. S. Welch, M. Ricote, T. E. Akiyama, F. J. Gonzalez, and C. K. Glass, “PPARδ 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.

[77] S. S. Deeb, L. Fajas, M. Nemoto, et al., “A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity,” Nature Genetics, vol. 20, no. 3, pp. 284–287, 1998.

[78] B. G. Shearer and A. N. Billin, “The next generation of PPAR δ agonists,” Current Opinion in Pharmacology, vol. 6, no. 6, pp. 606–614, 2006.

[79] R. Nakano, E. Kurosaki, S. Yoshida, et al., “Antagonism of peroxisome proliferator-activated receptor γ prevents high-fat diet-induced obesity in vivo,” Biochemical Pharmacology, vol. 72, no. 1, pp. 42–52, 2006.

[80] J. Rieusset, F. Touri, L. Michalik, et al., “A new selective peroxisome proliferator-activated receptor γ antagonist with antiobesity and antidiabetic activity,” Molecular Endocrinology, vol. 16, no. 11, pp. 2628–2644, 2002.

[81] S. Seber, S. Čak, O. Basat, and Y. Altuntas, “The effect of dual PPAR α/γ stimulation with combination of rosiglitazone and fenofibrate on metabolic parameters in type 2 diabetic patients,” Diabetes Research and Clinical Practice, vol. 71, no. 1, pp. 52–58, 2006.

[82] C. Fioret, J.-C. Fruchart, and B. Staels, “PPARγ and PPARδ dual agonists for the treatment of type 2 diabetes and the metabolic syndrome,” Current Opinion in Pharmacology, vol. 6, no. 6, pp. 606–614, 2006.

[83] P. Balakumar, M. Rose, S. S. Ganti, P. Krishan, and M. Singh, “PPAR dual agonists: are they opening Pandora’s Box?” Pharmacological Research, vol. 56, no. 2, pp. 91–98, 2007.

[84] I. C. Gonzalez, J. Lamar, F. Fradier, et al., “Design and synthesis of a novel class of dual PPARα/δ agonists,” Bioorganic & Medicinal Chemistry Letters, vol. 17, no. 4, pp. 1052–1055, 2007.

[85] Y. Xu, G. J. Eitgen, C. L. Broderick, et al., “Design and synthesis of dual peroxisome proliferator-activated receptors γ and δ agonists as novel euglycemic agents with a reduced weight gain profile,” Journal of Medicinal Chemistry, vol. 49, no. 19, pp. 5649–5652, 2006.

[86] J. M. Wallace, M. Schwarz, P. Coward, et al., “Effects of peroxisome proliferator-activated receptor αδ agonists on HDL-cholesterol in vervet monkeys,” Journal of Lipid Research, vol. 46, no. 5, pp. 1009–1016, 2005.
[92] L. Shen, Y. Zhang, A. Wang, et al., “Synthesis and identification of [1,2,4]thiazole derivatives as a new series of potent and orally active dual agonists of peroxisome proliferator-activated receptors α and δ,” *Journal of Medicinal Chemistry*, vol. 50, no. 16, pp. 3954–3963, 2007.

[93] F. Zhang, B. E. Lavan, and F. M. Gregoire, “Selective modulators of PPAR-γ activity: molecular aspects related to obesity and side-effects,” *PPAR Research*, vol. 2007, Article ID 32696, 7 pages, 2007.

[94] T. Allen, F. Zhang, S. A. Moodie, et al., “Halofenate is a selective peroxisome proliferator-activated receptor γ modulator with antidiabetic activity,” *Diabetes*, vol. 55, no. 9, pp. 2323–2333, 2006.

[95] E. Burgermeister, A. Schnoebelen, A. Flamment, et al., “A novel partial agonist of peroxisome proliferator-activated receptor-γ (PPARY) recruits PPARY-coactivator-1a, prevents triglyceride accumulation, and potentiates insulin signaling in vitro,” *Molecular Endocrinology*, vol. 20, no. 4, pp. 809–830, 2006.

[96] A. Tenenbaum, M. Motro, and E. Z. Fisman, “Dual and pan-peroxisome proliferator-activated receptors (PPAR) co-agonism: the bezafibrate lesson,” *Cardiovascular Diabetology*, vol. 4, article 14, 2005.

[97] U. Ramachandran, R. Kumar, and A. Mittal, “Fine tuning of PPAR ligands for type 2 diabetes and metabolic syndrome,” *Mini Reviews in Medicinal Chemistry*, vol. 6, no. 5, pp. 563–573, 2006.

[98] Q. Chen, J. Chen, T. Sun, J. Shen, X. Shen, and H. Jiang, “A yeast two-hybrid-based technology-based system for the discovery of PPARY agonist and antagonist,” *Analytical Biochemistry*, vol. 335, no. 2, pp. 253–259, 2004.

[99] 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.

[100] S. Chen, B. A. Johnson, Y. Li, et al., “Both coactivator LXXLL motif-dependent and -independent interactions are required for peroxisome proliferator-activated receptor γ (PPARY) function,” *Journal of Biological Chemistry*, vol. 275, no. 6, pp. 3733–3736, 2000.

[101] B. M. Forman, P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans, “15-deoxo-Δ12,14-prostaglandin J2 is a ligand for the adipocyte differentiation factor PPARY,” *Cell*, vol. 83, no. 5, pp. 803–812, 1995.

[102] P. W. Young, D. R. Buckle, B. C. C. Cantello, et al., “Identification of high-affinity binding sites for the insulin sensitizer rosiglitazone (BRL-49653) in rodent and human adipocytes using a radiiodinated ligand for peroxisomal proliferator-activated receptor γ,” *Journal of Pharmacology and Experimental Therapeutics*, vol. 284, no. 2, pp. 751–759, 1998.

[103] J. S. Nichols, D. J. Parks, T. G. Consler, and S. G. Blanchard, “Development of a scintillation proximity assay for peroxisome proliferator-activated receptor γ ligand binding domain,” *Analytical Biochemistry*, vol. 257, no. 2, pp. 112–119, 1998.

[104] A. Elbrecht, Y. Chen, A. Adams, et al., “L-764406 is a partial agonist of human peroxisome proliferator-activated receptor γ: the role of Cys311 in ligand binding,” *Journal of Biological Chemistry*, vol. 274, no. 12, pp. 7913–7922, 1999.

[105] G. Zhou, R. Cummings, Y. Li, et al., “Nuclear receptors have distinct affinities for coactivators: characterization by fluorescence resonance energy transfer,” *Molecular Endocrinology*, vol. 12, no. 10, pp. 1594–1604, 1998.

[106] G. Zhou, R. Cummings, J. Hermes, and D. E. Moller, “Use of homogeneous time-resolved fluorescence energy transfer in the measurement of nuclear receptor activation,” *Methods*, vol. 23, no. 1, pp. 54–61, 2001.

[107] M.-C. Cho, H.-S. Lee, J. H. Kim, et al., “A simple ELISA for screening ligands of peroxisome proliferator-activated receptor γ,” *Journal of Biochemistry and Molecular Biology*, vol. 36, no. 2, pp. 207–213, 2003.

[108] H.-S. Lee, M.-C. Cho, T.-W. Baek, et al., “Epitope analysis of PPARγ monoclonal antibody Py48.34A and its application for screening PPARγ ligands,” *Journal of Immunological Methods*, vol. 296, no. 1–2, pp. 125–134, 2005.

[109] M.-C. Cho, H.-E. Yoon, J.-W. Kang, et al., “A simple method to screen ligands of peroxisome proliferator-activated receptor δ,” *European Journal of Pharmaceutical Sciences*, vol. 29, no. 5, pp. 355–360, 2006.

[110] M. A. Cooper, “Optical biosensors in drug discovery,” *Nature Reviews Drug Discovery*, vol. 1, no. 7, pp. 315–328, 2002.

[111] B. Chesiks and L. P. Freedman, “Modulation of nuclear receptor interactions by ligands: kinetic analysis using surface plasmon resonance,” *Biochemistry*, vol. 35, no. 10, pp. 3309–3318, 1996.

[112] C. Yu, L. Chen, H. Luo, et al., “Binding analyses between human PPARγ-LBD and ligands: surface plasmon resonance biosensor assay correlating with circular dichroic spectroscopy determination and molecular docking,” *European Journal of Biochemistry*, vol. 271, no. 2, pp. 386–397, 2004.

[113] L. Yue, F. Ye, C. Gui, et al., “Ligand-binding regulation of LXR/RXR and LXR/PPAR heterodimerizations: SPR technology-based kinetic analysis correlated with molecular dynamics simulation,” *Protein Science*, vol. 14, no. 3, pp. 812–822, 2005.

[114] T. Fujino, Y. Sato, M. Une, et al., “In vitro farnesoid X receptor ligand sensor assay using surface plasmon resonance and based on ligand-induced coactivator association,” *Journal of Steroid Biochemistry and Molecular Biology*, vol. 87, no. 4–5, pp. 247–252, 2003.

[115] T. Kanayasu-Toyoda, T. Fujino, T. Oshizawa, et al., “HX531, a retinoid X receptor antagonist, inhibited the 9-cis retinoic acid-induced binding with steroid receptor coactivator-1 as detected by surface plasmon resonance,” *Journal of Steroid Biochemistry and Molecular Biology*, vol. 94, no. 4, pp. 303–309, 2005.

[116] J. K. Reddy, D. E. Moody, D. L. Azarnoff, and R. M. Tomarelli, “Hepatic effects of some [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio] acetic acid (WY-14,643) analogs in the mouse,” *Archives Internationales de Pharmacodynamie et de Therapie*, vol. 225, no. 1, pp. 51–57, 1977.

[117] T. Solha, K. Mizuno, H. Tawada, Y. Sugiyama, T. Fujita, and Y. Kawamatsu, “Studies on antidiabetic agents. I. Synthesis of 5-[4-(2-methyl-2-phenylpropoxy)-benzyl]thiazolidine-2,4-dione (AL-321) and related compounds,” *Chemical and Pharmaceutical Bulletin*, vol. 30, no. 10, pp. 3563–3573, 1982.

[118] T. Fujita, Y. Sugiyama, S. Taketomi, et al., “Reduction of insulin resistance in obese and/or diabetic animals by 5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878, U-63,287, ciglitazone), a new antidiabetic agent,” *Diabetes*, vol. 32, no. 9, pp. 804–810, 1983.

[119] S. E. Wiley, A. N. Murphy, S. A. Ross, P. van der Geer, and J. E. Dixon, “MitoNEET is an iron-containing outer mitochondrial membrane protein that regulates oxidative capacity,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 13, pp. 5318–5323, 2007.