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PPAR γ Signaling and Emerging Opportunities for Improved Therapeutics

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Abstract

Peroxisome proliferator-activated receptor gamma (PPAR γ) is a ligand-activated nuclear receptor that regulates glucose and lipid metabolism, endothelial function and inflammation. Rosiglitazone (RGZ) and other thiazolidinedione (TZD) synthetic ligands of PPAR γ are insulin sensitizers that have been used for the treatment of type 2 diabetes. However, undesirable side effects including weight gain, fluid retention, bone loss, congestive heart failure, and a possible increased risk of myocardial infarction and bladder cancer, have limited the use of TZDs. Therefore, there is a need to better understand PPAR γ signaling and to develop safer and more effective PPAR γ -directed therapeutics. In addition to PPAR γ itself, many PPAR γ ligands including TZDs bind to and activate G protein-coupled receptor 40 (GPR40), also known as free fatty acid receptor 1. GPR40 signaling activates stress kinase pathways that ultimately regulate downstream PPAR γ responses. Recent studies in human endothelial cells have demonstrated that RGZ activation of GPR40 is essential to the optimal propagation of PPAR γ genomic signaling. RGZ/GPR40/p38 MAPK signaling induces and activates PPAR γ co-activator-1 α , and recruits E1A binding protein p300 to the promoters of target genes, markedly enhancing PPAR γ -dependent transcription. Therefore in endothelium, GPR40 and PPAR γ function as an integrated signaling pathway. However, GPR40 can also activate ERK1/2, a proinflammatory kinase that directly phosphorylates and inactivates PPAR γ . Thus the role of GPR40 in PPAR γ signaling may have important implications for drug development. Ligands that strongly activate PPAR γ , but do not bind to or activate GPR40 may be safer than currently approved PPAR γ agonists. Alternatively, biased GPR40 agonists might be sought that activate both p38 MAPK and PPAR γ , but not ERK1/2, avoiding its harmful effects on PPAR γ signaling, insulin resistance and inflammation. Such next generation drugs might be useful in treating not only type 2 diabetes, but also diverse chronic and acute forms of vascular inflammation such as atherosclerosis and septic shock.

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Graphical abstract

Keywords

Peroxisome proliferator-activated receptor gamma (PPAR γ); Thiazolidinediones (TZDs); G protein-coupled receptor 40 (GPR40); p38 mitogen-activated protein kinase (p38 MAPK); PPAR γ co-activator-1alpha (PGC-1 α); E1A binding protein p300 (EP300)

1. Introduction

1.1. Peroxisome proliferator-activated receptor gamma (PPAR γ) as a target for treating type 2 diabetes and thiazolidinediones (TZDs)

In 2010, an estimated 257 million people worldwide had type 2 diabetes mellitus (T2DM) and the number is projected to rise to 395 million by 2030 (1). TZDs, commonly called glitazones, have been widely used to treat this disease (2). The anti-diabetic effects of TZDs were originally discovered in 1982 (3). Ciglitazone was the first TZD shown to normalize hyperglycemia, hyperinsulinemia, and hypertriglyceridemia in mouse models of T2DM (4). Later, troglitazone (5) and pioglitazone (6) were also shown to decrease insulin resistance by increasing insulin-stimulated glucose utilization and reducing hepatic glucose production. Rosiglitazone (RGZ), synthesized in 1988, was a more selective and potent insulin sensitizer than ciglitazone in rodent models (7, 8). In 1997, troglitazone was the first TZD approved for clinical use, but was soon withdrawn worldwide because of liver toxicity (9). The U.S. Food and Drug Administration subsequently approved RGZ and pioglitazone in 1999 for the treatment of T2DM (10). RGZ was later withdrawn in Europe and its use restricted in the United States due to an increased risk of myocardial infarction (11, 12). Pioglitazone does not appear to share this risk, but otherwise has the same adverse profile common to TZDs and has been associated with bladder cancer (13, 14).

Named for its activation by fibrates and other peroxisome proliferators (15), peroxisome proliferator-activated receptor α (PPAR α /NR1C1) was first identified in 1990. Two other genes belonging to the same family, PPAR β / δ (NR1C2) and PPAR γ (NR1C3), were cloned in *Xenopus* two years later (16). Human homologues of three PPAR isoforms, α (17), β (18) and γ (19), were soon identified. PPAR γ has two isoforms, PPAR γ 1 and PPAR γ 2, with the latter harboring an additional 30 amino acids at its N-terminus (20). PPAR γ 1 is expressed in many tissues including leukocytes and endothelial cells, while PPAR γ 2 is normally restricted to adipose tissue, but can be induced elsewhere (20, 21). In 1994, PPAR γ was found to be a major adipogenic transcription factor in mice (22). Long after the anti-diabetic effects of TZDs were discovered in 1982 (3), PPAR γ was identified as the receptor target of TZDs in 1995 (23, 24). As noted above, this was less than two years before the first TZD was approved for clinical use (9, 25, 26). Besides synthetic TZDs, the endogenous arachidonate 15-deoxy-D^{12, 14}-prostaglandin J₂ (15d-PGJ₂) and related metabolites also activate PPAR γ and induce adipogenesis (24), but at concentrations above that found in cells (27). In addition some unsaturated fatty acids activate PPAR γ , such as the dietary polyunsaturated eicosapentanoic acid, linolenic acids, linoleic acid, and oxidized low-density lipoprotein (28). Therefore, PPAR γ may under some circumstances function as a general fatty acid sensor, with affinity K_D values of 2–50 μ M (29). Also two linoleic acid oxidation products detected in significant amounts in oxidized low-density lipoprotein particles, 9-HODE and 13-HODE, were previously identified as endogenous ligands and activators of PPAR γ (30).

As noted above, TZDs including RGZ and pioglitazone have been associated with a number of adverse effects. These include weight gain (31), fluid retention (31, 32), and reduction in bone mineral density (33). Besides these class effects, RGZ has been associated with excess myocardial infarctions and pioglitazone with bladder cancer. These undesirable, “off-target” effects of TZDs have driven research to better understand PPAR γ signaling and to develop new agents with improved efficacy and safety. TZD-induced weight gain has recently been linked to activation of PPAR γ in the brain, rather than in adipose tissue (34, 35). Intraventricular TZD administration or overexpression of PPAR γ in the brain of normal rats promote sustained increases in feeding and body weight (34). Conversely, mice with selected ablation of brain PPAR γ consumed less food and gained less weight than controls in response to TZD treatment during high-fat feeding. These mice also showed increased physical activity and energy expenditure (35). TZD-induced fluid retention and peripheral edema has been attributed to increased sodium and water reabsorption in the distal collecting ducts of the kidney. Collecting duct-specific knockout of PPAR γ blocked TZD-associated increases in plasma volume and body weight (36). How TZDs exert this action remains unclear, as findings about the role of epithelial sodium channels in this phenomenon are contradictory (36, 37). Besides weight gain, fluid retention associated with TZDs may also contribute to adverse cardiovascular events, such as congestive heart failure (20). Consistent with reductions in bone mineral density and a higher rate of fractures, TZDs caused bone loss in rodents by inhibiting osteoblastogenesis (bone formation) and enhancing osteoclastogenesis (bone resorption). TZDs were proposed to exert these effects through PPAR γ -dependent induction of c-fos, β -catenin, and ERR α (38–40).

1.2. PPAR γ as a therapeutic target in atherosclerosis, pulmonary arterial hypertension, adult respiratory distress syndrome, and septic shock

The direct binding of TZDs and other ligands to PPAR γ activates two distinct signaling pathways. *Cis*-activation drives transcription through agonist-dependent conformational changes in the activation function 2 (AF-2) domain of PPAR γ , recruitment of co-activators such as PPAR γ co-activator-1 α (PGC-1 α), PPAR γ dimerization with the retinoid X receptor (RXR) (41), and the binding of this complex to peroxisome proliferator response elements (PPREs) in the promoters of target genes. This signaling pathway is closely linked to the essential roles of PPAR γ in adipogenesis and glucose homeostasis. Alternatively, ligand-bound PPAR γ has also been shown to suppress inflammation via a mechanism called *trans*-repression. *Trans*-repression is independent of DNA binding by PPAR γ as demonstrated by the PPAR γ C126A/E127A mutant, which remains capable of repressing lipopolysaccharide-induced genes while being rendered incapable of *cis*-activation (42). This mode of repression also does not require RXR dimerization, but does require sumoylation of PPAR γ 2 at K395 by the small ubiquitin-like modifier and subsequent tethering of PPAR γ , nuclear receptor co-repressor and histone deacetylase to NF κ B and AP-1 complexes that regulate the transcription of inflammatory response genes (43). Together, these two distinct, PPAR γ -dependent signaling pathways may provide benefits beyond the treatment of T2DM. Despite their adverse effects, TZDs lower blood pressure (44, 45), improve lipid profiles (46), and inhibit vascular inflammation (47). These effects in non-adipose tissue support the potential of a newer generation of PPAR γ ligands in diverse conditions such as atherosclerosis, pulmonary arterial hypertension, adult respiratory distress syndrome, ulcerative colitis and septic shock. Endothelial PPAR γ disruption was found to accelerate diet-induced atherosclerosis (48), while the PPAR γ ligand 15d-PGJ₂ reduced atherosclerotic lesion in apoE knockout mice (49). PPAR γ deletion in the arterial smooth muscle cells of mice resulted in the development of pulmonary arterial hypertension (50), whereas the PPAR γ ligand RGZ was beneficial in a murine model of this disease (51). Heterozygous mutation of valine to methionine at 290 (V290M) or proline to leucine at 476 (P467L) of PPAR γ was linked to high blood pressure in human subjects (52). PPAR γ expression and activation was found to protect animals from acute respiratory distress syndrome (53). In endotoxin challenge models of septic shock, 15d-PGJ₂ reduced mortality in mice (54) and RGZ reduced organ injury and cytokine release in rats (55). Therefore, safer, more effective PPAR γ activators may have broad applicability in a variety of acute and chronic inflammatory diseases.

2. Cross talk between nitric oxide (NO) and PPAR γ signaling

2.1. Endogenous PPAR γ ligands formed by NO nitration of unsaturated fatty acids

NO is a free radical messenger, synthesized from L-arginine via three nitric oxide synthase isoforms, that plays a central role in vascular health. The biological effects of NO are diverse and many result from the oxidization, nitrosylation, nitrosation, and/or nitration of target molecules (56). While, fatty acids are important sources of energy, covalent modifications can transform some fatty acids into potent signaling molecules (57). For example, nitro-linoleic acid is a potent PPAR γ ligand with a K_i value of 133 nM, which rivals that of RGZ (K_i = 53 nM) and is much lower than that of unmodified linoleic acid (K_i >1 μ M) (58).

Nitro-linoleic and nitro-oleic acids, but not their unmodified forms, induced PPAR γ -driven adipogenesis in preadipocytes (57, 58). *In vivo* administration of nitro-oleic acid, but not parental oleic acid was also shown to ameliorate diabetic symptoms in rats (59).

Nitrated fatty acids are one of the largest pools of active NO derivatives detected in human plasma (56, 57, 60). Under certain circumstances, nitrated fatty acid concentrations in human blood may reach $> 1 \mu\text{M}$ (57). *In vitro*, PPRES reporter gene studies have shown that PPAR γ is very sensitive to nitro-oleic acid with significant activation at 100 nM, while PPAR α and PPAR β/δ were activated at concentrations three times higher (57). Additional evidence suggests that nitrated (61) and oxidized (62) fatty acids can covalently bind to PPAR γ at C285 via Michael addition and thereby activate PPAR γ genomic signaling. Importantly, nitro derivatives of unsaturated fatty acids given to leptin-deficient *ob/ob* mice lower insulin and glucose levels without causing the weight gain associated with RGZ (61).

While the exact identity of biologically relevant natural ligands for PPAR γ remain uncertain, nitro- and nitrohydroxy-fatty acid derivatives are among the most likely candidates (56). Furthermore, these fatty acid adjuncts may also have therapeutic applications. Nitro-oleic acid at physiological concentrations in blood decreased endotoxin-induced endothelial inflammation and neutrophil transmigration in a PPAR γ -dependent manner (47). Direct lung delivery of nitro-oleic acid in a mouse model of acute lung injury significantly decreased pulmonary inflammation and injury, including capillary leak, lung edema, neutrophil infiltration, oxidant stress, and plasma cytokine levels (63). In addition, nitro-oleic acid suppressed murine allergic airway disease at least partially through PPAR γ activation, and unlike the steroid drug fluticasone, induced robust apoptosis and phagocytosis of neutrophils (64). Nitro-oleic acid-mediated PPAR γ activation has also been shown to attenuate colitis in experimental inflammatory bowel disease (65).

2.2. NO induces p38 mitogen-activated protein kinase (MAPK) phosphorylation in endothelium, thereby activating PPAR γ signaling

Besides activation by nitro-fatty acids, NO has been demonstrated to activate PPAR γ via a p38 MAPK-dependent mechanism in human endothelial cells (21). In both endothelial cells and monocytes, low-dose NO caused a rapid dose-dependent increase in PPAR γ binding to a consensus PPRES sequence (21, 66). NO-induced PPAR γ signaling and target gene expression was directly linked to p38 MAPK phosphorylation. Blockade of p38 MAPK with a specific inhibitor or siRNA knockdown abolished the ability of NO to increase PPAR γ DNA binding or to induce PPAR γ target genes (21).

An extensive literature has previously connected p38 MAPK to PPAR γ activation. PPAR γ -dependent adipogenesis in mesenchymal cells, 3T3-L1 pre-adipocytes, and white adipocytes have all been associated with p38 MAPK activation (67–70). In brown fat, p38 MAPK has been shown to activate PGC-1 α and induce the expression of PPAR γ target genes including PGC-1 α itself, and uncoupling protein 1 (71, 72). Notably, p38 MAPK directly phosphorylates PGC-1 α (71, 73–75) and E1A binding protein p300 (EP300) (76), which facilitates co-activator recruitment to PPAR γ target genes, chromatin remodeling and PPAR γ -dependent gene transcription. In addition to NO, carbon monoxide, another low molecular weight, endogenous messenger that activates p38 MAPK (77, 78), has also been

shown to activate PPAR γ (78, 79). Moreover, TZDs have been long known to activate p38 MAPK independent of PPAR γ in a variety cell types, including adipocytes (75), astrocytes (80), cardiomyocytes (81), and epithelial cells (82). The well-documented role of p38 MAPK in PPAR γ signaling and the ability of TZDs to activate both p38 MAPK and PPAR γ suggest that p38 MAPK is an unrecognized facilitator of TZD-mediated PPAR γ activation.

3. Post-translational modifications (PTMs) that regulate or shape PPAR γ genomic signaling

3.1. Obesity, insulin resistance, diabetes and PPAR γ

Obesity has become a major health problem worldwide with a prevalence of 36.9% in men and 38.0% in women (83). The failure of adipose tissue in obesity to store excess energy appropriately leads to ectopic lipid deposition, insulin resistance and ultimately T2DM (84). Four different fat depots play contrasting physiological and pathophysiological metabolic roles in humans: brown (BAT), subcutaneous (SAT), and visceral white adipose tissue (VAT), and ectopic lipid (85, 86). BAT contains numerous mitochondria and expresses uncoupling protein 1, a mitochondrial protein that uncouples oxidative phosphorylation, resulting in inefficient production of ATP and release of energy as heat, thereby acting as a thermogenic organ (85, 86). SAT, the largest fat depot, stores triglycerides that can be readily released during times of energy demand. SAT also secretes adiponectin and leptin that have largely beneficial effects on lipid oxidation, energy utilization, insulin action, and inflammation (85, 86). VAT together with ectopic lipid, are harmful and associated with insulin resistance and increased cardiovascular risk (85).

T2DM is characterized by hyperglycemia due to insulin resistance. While the precise pathogenesis of insulin resistance is not completely clear, intra-abdominal VAT, deficiency of adiponectin and/or leptin, inflammation, and mitochondrial dysfunction have all been identified as important contributors (85, 87). Intra-abdominal fat leads to labile fatty acid release with direct delivery to the liver, inflammatory cell accumulation, reduced adiponectin levels, and decreased PPAR γ activity (85). Lipodystrophy, syndromes are characterized by a partial or near-complete absence of SAT, a relative increase in VAT, and marked insulin resistance (85). Beneficial effects of BAT and SAT on insulin sensitivity may primarily be attributed to increased fatty acid oxidation (85). Mitochondrial dysfunction increases reactive oxygen species production, activating serine/threonine kinases including IKK, JNK, and PKCs, which phosphorylate insulin receptor substrate (IRS). Phosphorylation of IRS-1/2 inhibits signaling pathways downstream from insulin including PI3K, Akt, and PKC ζ , which decreases glucose uptake, increases glucose production, and reduces vasodilation and insulin secretion (87).

PPAR γ is a key regulator of insulin sensitivity that improves insulin resistance in T2DM via multiple mechanisms. Specifically, PPAR γ : 1) induces adipogenesis in beneficial SAT, but not in harmful VAT (88–90); 2) enhances uncoupling protein 1 expression in BAT (71, 72); 3) elevates serum adiponectin levels (91); 4) suppresses inflammation (43); and 5) promotes mitochondrial biogenesis and reduces mitochondrial production of reactive oxygen species (92). While TZDs are full agonists of PPAR γ , they have undesirable adverse effects that

prevent or restrict their clinical application as discussed earlier. An emerging understanding of PPAR γ signaling has the potential to create a new generation of agonists with a better safety profile and broader applicability.

3.2. PPAR γ PTMs associated with obesity and insulin resistance

Similar to other nuclear receptors, PPAR γ activity is tightly regulated by PTMs. *In vitro* assays demonstrate that serine 112 (S112) of mouse PPAR γ 2, corresponding to S82 of mouse and S84 of human PPAR γ 1, can be phosphorylated by either ERK or JNK (93–95). Phosphorylation at these sites is an inactivating event that inhibits both ligand-dependent and -independent PPAR γ transcriptional activity (93, 94). Expression of PPAR γ mutants (S112A) that cannot be phosphorylated increased ligand-induced adipogenesis and eliminated the ability of mitogens to inhibit differentiation (93, 94). Mice homozygous for this S112A mutation are protected from diet-induced obesity and insulin resistance, effects associated with the increased secretion of adiponectin and leptin (96). Cdk7 has also been shown to phosphorylate PPAR γ 2 at S112 and Cdk7 knockdown in mouse embryonic fibroblasts induces adipogenesis and adiponectin expression (97).

In the obese state, mouse PPAR γ 2 is also highly phosphorylated at serine 273 (S273) by both ERK and cyclin-dependent kinase Cdk5 (84, 98). This modification of PPAR γ does not change its *in vitro* transcriptional activity and adipogenic capacity, but *in vivo* reshapes the transcriptional repertoire of PPAR γ . Serine 273 phosphorylation of PPAR γ dysregulates genes whose expression is altered in obesity, reducing the expression of adipokines and adiponectin, insulin-sensitizing hormones secreted by adipose tissue (84, 98). Pro-inflammatory cytokines produced in obesity are known to activate ERK and Cdk5. ERK inhibition or PPAR γ ligands that block S273 accessibility both prevent S273 phosphorylation and reduce insulin resistance in obese wildtype and *ob/ob* mice (84, 98). Several high affinity PPAR γ ligands, such as MRL24 and Mbx-102 that are poor PPAR γ agonists, effectively inhibit S273 phosphorylation and show strong anti-diabetic activity *in vivo* (98). These results suggest that blockade of S273 phosphorylation alone has insulin-sensitizing effects independent of classical notions of PPAR γ activation.

In addition to phosphorylation, PPAR γ acetylation has been reported to regulate its function. EP300 and Tip60 acetylate, while SIRT1 de-acetylates PPAR γ , respectively enhancing and repressing PPAR γ transcriptional activity and its adipogenic potential in 3T3-L1 preadipocytes (99–101). Conversely, K268 and K293 of PPAR γ are highly acetylated in obesity and ligand-induced de-acetylation by SIRT1 was shown to induce BAT and repress VAT genes, potentially reducing insulin resistance (102). As such, the regulation of PPAR γ by acetylation is complex with many possible lysine-site combinations and as yet inadequately explored functional consequences.

Lysine site sumoylation adds another layer of control over PPAR γ genomic signaling. Two functional sumoylation sites have been identified for PPAR γ 2, K107 and K395 (20, 43, 103). K107 sumoylation blocks the *cis*-transcriptional activity of PPAR γ , possibly by promoting co-repressor recruitment (20, 103, 104). Recent findings in fibroblast growth factor 21-knockout mice show that this factor increases PPAR γ activity in adipocytes by preventing K107 sumoylation, enhancing insulin sensitivity (105). Interestingly, K107 is

close to S112 in position and together they form a consensus sumoyl-phospho motif (106). K107 sumoylation is to some extent linked to S112 phosphorylation and ablation of S112 phosphorylation significantly diminished K107 sumoylation (103). Sumoylation at K395 recruits PPAR γ monomers to NF- κ B and AP1 DNA-binding sites, preventing clearance of co-repressor complexes, thereby *trans*-repressing inflammatory response genes (20, 43). Sumoylation at this residue has also been reported to influence the ability of carbon dioxide to decrease pro-inflammatory signaling in macrophages (78). Additionally, the binding of ligands to PPAR γ and interferon- γ exposure were both found to induce PPAR γ ubiquitination. Although ubiquitination residues are yet to be identified, PPAR γ ubiquitination is determined by the ligand binding/AF-2 domains and leads to degradation of PPAR γ via the 26S proteasome (107). Regulation of PPAR γ protein levels through this mechanism likely also plays an important role in obesity.

4. GPR40 and PPAR γ , an integrated two-receptor signaling pathway

4.1. Biological function of GPR40

GPR40, also known as free fatty acid receptor 1 (FFAR1), was orphanized in 2003 as a medium-to-long chain FFA (both saturated and unsaturated) receptor (108). GPR40 is highly expressed in pancreatic β cells, and also, albeit to a lesser degree, in other tissues, including the intestinal tract, bone cells, lung epithelial cells, brain, and monocytes (109, 110). A well-documented function of GPR40 is to mediate effects of FFAs, such as linoleic acid and linolenic acid, on glucose-stimulated insulin secretion in pancreatic β cells (109, 111, 112). GPR40 loss-of-function via siRNA in β cells (112), or gene deletion in *Gpr40*^{-/-} mice (113) consistently resulted in a significant reduction in FFA-induced insulin secretion. Conversely, transgenic overexpression of GPR40 prevented the development of hyperglycemia in mice fed a high-fat diet, and improved insulin secretion and glucose tolerance in genetically diabetic mice (114). Furthermore, GW1100, a GPR40 antagonist, was shown to inhibit GPR40-mediated insulin secretion in the MIN6 β -cell line (115). In humans, a natural variant of GPR40 (G180S) diminishes the ability of pancreatic β cells to sense lipids and impairs FFA-induced insulin secretion (116). GPR40-mediated effects on glucose-stimulated insulin secretion were associated with increased Ca²⁺ influx and were not altered by inhibition of ERK, which was also activated when FFAs bound to GPR40 (112, 117). Food intake activates GPR40 expressed on enteroendocrine cells of the intestinal tract, mediating the secretion of incretin hormones (109), which also stimulate β cell insulin release (111). GPR40 full agonists increased incretin levels in a mouse model of T2DM, normalizing blood glucose levels (118). *Gpr40*^{-/-} mice have reduced incretin and insulin secretion in response to fat, and are not protected from insulin resistance induced by a high-fat diet (119).

4.2. Ligands that activate both GPR40 and PPAR γ

Many FFAs and their derivatives, including lauric acid, myristic acid, palmitic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, and 9-HODE have been shown to be endogenous ligands of both GPR40 and PPAR γ (28, 112, 120, 121). TZDs, including ciglitazone, troglitazone, RGZ, and pioglitazone, all bind to and activate GPR40 with subsequent signal transduction that includes stress kinase pathways (122). RGZ

compared with pioglitazone causes a more robust and sustained activation of ERK (122), a stress kinase often linked to inflammation. These differences in ERK activation could possibly explain some of the efficacy and safety differences among existing synthetic TZDs. Recently, several PPAR γ ligands have been found that promote insulin sensitization with fewer side effects in T2DM rodent models (123, 124). Whether these ligands can activate GPR40 or/and ERK has not been reported. Importantly, it is not entirely clear whether adverse effects associated with TZDs are attributable to the activation of GPR40 or PPAR γ or are entirely independent of both receptors. TZD-induced osteoblast and osteocyte apoptosis has been directly linked to GPR40. The activation of ERK with subsequent recruitment of the pro-apoptotic factor Bax to the outer membrane of the mitochondria was posited as the cause of TZD-related bone loss (125). In opposition to this mechanism, GW9508, a GPR40 agonist that blocks osteoclast differentiation *in vitro*, was found to prevent ovariectomy-induced bone loss in wildtype, but not *Gpr40*^{-/-} mice (126).

4.3. GPR40 and PPAR γ function together as a two-receptor signaling pathway

We recently demonstrated that GPR40 and PPAR γ crosstalk functions as an integrated two-receptor signal transduction pathway in human endothelium (127). RGZ was found to require GPR40 activation with downstream p38 MAPK phosphorylation to optimally propagate PPAR γ nuclear signaling (Fig. 1). The role of p38 MAPK in PPAR γ transcriptional activation also further explains how NO/p38 MAPK signaling, independent of GPR40, induces PPAR γ target genes (21, 127). GPR40 activation of p38 MAPK induced PGC-1 α expression, and directly phosphorylated both PGC-1 α and EP300, thereby promoting their recruitment to PPAR γ response elements. EP300, a histone acetyltransferase that docks with PGC-1 α , remodels chromatin to optimize the transcription of PPAR γ target genes. In human primary pulmonary artery endothelial cells, knockdown of GPR40, p38 MAPK, PGC-1 α , SIRT1 (an essential PGC-1 α activator) or EP300 all substantially reduced the ability of RGZ to induce PPAR γ regulated genes. GPR40 and PPAR γ were seen to cooperate at least additively and sometimes synergistically to initiate PPAR γ genomic responses, depending on the transcriptional context (127). Collectively, this work demonstrated that p38 MAPK, PGC-1 α , and EP300 link GPR40 to downstream PPAR γ genomic signaling. Binding to and activating both GPR40 and PPAR γ appears to be a common feature of many PPAR γ agonists. This connection between GPR40 signaling and PPAR γ transcriptional activation argues that the effects of TZDs on human endothelium might be best understood as a cognate two-receptor system, integrated by p38 MAPK, PGC-1 α and EP300.

In the classical signaling pathway, rosiglitazone (RGZ) binds directly to and activates PPAR γ . In human endothelial cells, RGZ requires GPR40 activation with downstream p38 MAPK phosphorylation to optimally propagate PPAR γ nuclear signaling. GPR40 activation of p38 MAPK induces PGC-1 α expression, and directly phosphorylates both PGC-1 α and EP300, thereby activating them. EP300, a histone acetyltransferase that docks with PGC-1 α , remodels chromatin to optimize the transcription of PPAR γ target genes.

4.4. PPAR γ agonism without GPR40 activation or GPR40/PPAR γ agonism without ERK1/2 activation

PPAR γ agonists that do not bind to or activate GPR40 have not yet been characterized in a systematic fashion. The natural PPAR γ ligand 15d-PGJ2 has been reported to selectively activate PPAR γ , but not GPR40 in human bronchial epithelial cells (110). However, 15d-PGJ2 does appear to activate stress kinases in other cell types, which could be a signature for unrecognized GPR40 activation. Stress kinase activation by 15d-PGJ2 includes ERK in renal epithelial cells, vascular smooth muscle cells and mesangial cells (128–130), ERK and p38 MAPK in osteosarcoma cells (131), and ERK, p38 MAPK and JNK in astrocytes (80). Nonetheless, the possibility that 15d-PGJ2 or other agonists might activate PPAR γ without binding to or activating GPR40 remains an open question that requires further investigation. PPAR γ ligands that can strongly activate PPAR γ genomic responses without interacting with GPR40 or other G-protein coupled receptors would avoid the harmful effects of concomitant stress kinase activation.

For ligands that activate both receptors, some downstream signaling from GPR40 is clearly useful (p38 MAPK) while other aspects (ERK1/2) are undesirable in regards to PPAR γ activation. While GPR40/p38 MAPK activation augments the genomic effects of PPAR γ , ERK1/2 phosphorylates and thereby inactivates PPAR γ (93, 94), and separately has inflammatory and proliferative effects in the vasculature. Furthermore, insulin resistance has been associated with ERK1/2 phosphorylation of PPAR γ at S273 (84). ERK inhibition and PPAR γ ligands both block PPAR γ phosphorylation at S273, providing anti-diabetic benefits in rodent models of T2DM (84, 98, 132). Importantly, GPR40-mediated enhancement of glucose-stimulated insulin secretion is not dependent on ERK1/2 activation (112). Compared to existing drugs, PPAR γ ligands that demonstrate no or substantially reduced GPR40/ERK signaling might be more potent agonists of PPAR γ and have a better safety profile.

5. Concluding remarks

RGZ and pioglitazone, TZD PPAR γ agonists, have been widely used to treat T2DM (2, 133). In addition, PPAR γ agonists also have potent anti-inflammatory effects that may be beneficial in diverse conditions including coronary artery disease (44, 46), pulmonary arterial hypertension (50), acute respiratory distress syndrome (53, 134) and septic shock (54, 55). However, adverse effects, such as weight gain (2, 133), fluid retention (31, 32), congestive heart failure (13), bone fractures (31, 32) and importantly a paradoxical increase in the risk of myocardial infarctions (11, 12) have limited the usefulness of currently available PPAR γ -targeted drugs. Recent efforts have focused on PPAR γ ligands that block ERK/Cdk5-mediated phosphorylation of PPAR γ at S273 (123, 124, 135, 136). GPR40, a FFA and TZD receptor that stimulates insulin secretion has also been identified as a potential target for new T2DM therapeutics (109, 111). As such, GPR40 agonists with little or no PPAR γ binding activity have been synthesized (137, 138), but none of these agents has yet made to the bedside. Furthermore, selective GPR40 agonists, like TZDs, are likely to activate ERK1/2 in the vasculature.

In the endothelium, RGZ and pioglitazone bind to and activate both GPR40 and PPAR γ , which function together through p38 MAPK to optimally propagate PPAR γ genomic

responses. However, GPR40 activation by these drugs also turns on ERK1/2, a stress kinase pathway that suppresses PPAR γ signaling and promotes inflammation. Knowledge of this crosstalk could be used to screen for small molecules that do not bind to GPR40, but yet strongly activate PPAR γ signaling. Exclusive PPAR γ agonists that do not require GPR40/p38 MAPK signaling would circumvent the counterproductive and potentially harmful effects of GPR40/ERK activation. Alternatively ligands of GPR40 and PPAR γ could be sought that activate GPR40 in a biased manner. Ideal ligands would retain the ability to activate p38 MAPK, but not ERK1/2. Activating p38 MAPK would enhance downstream PPAR γ signaling through effects on PGC-1 α and EP300. Bypassing ERK1/2 would avoid its inactivation of PPAR γ and its deleterious impact on insulin resistance and inflammation. The feasibility of either of these approaches requires further investigation.

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Figure 1.
Crosstalk between GPR40 and PPAR γ signaling in human endothelium.