Minireview

PPARγ: a Nuclear Regulator of Metabolism, Differentiation, and Cell Growth*

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The peroxisome proliferator-activated receptors (PPARs)† comprise an important subgroup of the nuclear hormone receptor (NHR) superfamily. These ligand-activated transcription factors have been intensively studied for more than a decade and have been implicated in such diverse pathways as lipid and glucose homeostasis, control of cellular proliferation, and differentiation. The name PPAR derives from the initial cloning of one isoform as a target of various xenobiotic compounds that were observed to induce proliferation of peroxisomes in the liver (1). This protein was called the peroxisome proliferator-activated receptor, now known as PPARγ. Within a few years, the group of PPARs was expanded to include PPARα and PPARβ (also referred to as PPARβ, NUC1, and FAAR) (2–6). This review will focus on PPARγ.

How Do PPARs Work at the Molecular Level?

PPARs possess the canonical domain structure of other NHR superfamily members (see Fig. 1). This includes a poorly characterized N-terminal region that contains a potential trans-activation function known as AF-1, followed by a DNA binding domain that includes two zinc fingers. At the carboxy terminus is a dimerization and ligand binding domain that molecular modeling reveals to be a large hydrophobic pocket and which contains a key, ligand-dependent trans-activation function called AF-2 (7, 8). PPARs bind to cognate DNA elements called PPAR response elements (PPREs) in the 5′-flanking region of target genes. Like many other NHRs, they bind DNA as obligate heterodimers by partnering with one of the retinoid X receptors (RXRs). Known PPREs are direct repeats of an AGC nucleotide sequence separated by a 1-base pair spacer. A short sequence located immediately upstream of the first half-site confers polarity on the PPRE, with the PPAR moiety binding 5′ to the RXR half of the heterodimer (9, 10). Many cell types express more than one PPAR isoform, which begs the question of how isoform-specific targets are regulated. Most likely this occurs through a combination of subtle cis sequence differences flanking the core response element, the presence of specific or selective coactivator proteins, and regulation of endogenous ligands.

PPARγ, like other NHRs, form protein-protein interactions with a variety of nuclear proteins known as coactivators and corepressors, which mediate contact between the PPAR-RXR heterodimer, chromatin, and the basal transcriptional machinery and which promote activation and repression of gene expression, respectively. Coactivator proteins, which include members of the p160/CBP/p300 and DRIP/TRAP families, are general coactivators for NHRs and indeed many non-NHR transcription factors. There are no known receptor-specific coactivators or corepressors, although selectivity for one or another NHR has been illustrated in certain cases (11, 12). Coactivator proteins either possess or recruit histone acetyltransferase (HAT) activity to the transcription start site. Acetylation of histone proteins is believed to relieve the tightly packed structure of the chromatin, allowing the RNA polymerase II complex to bind and initiate transcription. Coactivators also recruit the chromatin remodeling SWI/SNF complex to target promoters (13, 14).

What Are the Physiological Roles Played by PPARγ?

PPARγ is the most intensively studied PPAR isoform. Studies have shown that this receptor participates in biological pathways of intense basic and clinical interest, such as differentiation, insulin sensitivity, type 2 diabetes, atherosclerosis, and cancer. PPARγ exists in two protein isoforms that are created by alternative promoter usage and alternative splicing at the 5′ end of the gene; PPARγ2 contains 30 additional amino acids at the N terminus compared with PPARγ1 (6). Whereas many tissues express a low level of PPARγ1, PPARγ2 is fat-selective and is expressed at very high levels in that tissue.

PPARγ Ligands—Because of its involvement in so many critical physiological and pathologic functions (see below), great effort has been spent in trying to identify an endogenous, high affinity ligand for PPARγ. This has led to a variety of fatty acids and their derivatives that are found to bind to PPARγ with relatively low affinity, but most investigators believe that their relevant concentrations in the nuclei of target cells are likely to be too low for them to be bona fide ligands. Certain eicosanoids have been shown to bind and activate PPARγ with higher affinity (15, 16). 15-Deoxy-12,14-prostaglandin J2, for example, binds to PPARγ with a Kd in the low micromolar range and can activate PPARγ target genes at concentrations of or near the Kd (17, 18). 15-Deoxy-12,14-prostaglandin J2, however, has never been definitively proven to exist in vivo, nor are its effects specific to PPARγ. Many actions of this compound, which have been ascribed to PPARγ activation, have actually been shown to be mediated through inhibition of the NF-κB pathway (19, 20). Other eicosanoids, such as 13-HODE and 15-HETE, have been suggested to act as PPARγ ligands (21), a notion supported by the requirement for 12/15-lipoxygenase in some PPARγ responses in vitro (22).

Despite the paucity of information on true endogenous ligands, several high affinity synthetic PPARγ ligands have been generated. These include the thiazolidinedione (TZD) class of drugs, which are used clinically as insulin sensitizers in patients with type 2 diabetes (23) and were developed without knowledge of their molecular target. Other novel agents, including aryl-tyrosine derivatives, have been developed and are likely to show promise in both the laboratory and the clinic (24).

PPARγ and Adipogenesis—PPARγ was cloned as a transcription factor important in fat cell differentiation; it was also isolated in screens seeking new members of the PPAR family. In the former case, PPARγ was identified as a protein that bound to an enhancer in the 5′-flanking region of the aP2 gene, which encodes a fat cell-selective fatty acid-binding protein (6). This discovery was rapidly followed up by experiments showing that ectopic expression of PPARγ could dramatically promote adipogenesis in nonadipogenic, fibroblastic cells such as NIH-3T3 cells (25). When combined with an appropriate agonist and the pro-adipogenic protein C/EBPα, even myoblasts could be “trans-differentiated” to adipocytes (26). PPARγ plays a crucial role in the function of many, and perhaps most, fat cell-specific genes. PPARγ binding is absolutely required for the function of the fat-selective enhancers for the aP2 gene.
and PEPCK genes in cultured fat cells (27). This analysis of the PEPCK gene has recently been extended in vivo, where activation of this promoter in fat was shown to be dependent on a PPARγ binding site, whereas expression in other tissues was not (28). The role of PPARγ in adipogenesis is also illustrated in studies that have deleted this gene in mice. The homozygous null mutation is lethal relatively early in gestation (embryonic days 10–10.5) secondary to a defect in placental development (29, 30), forcing investigators to use alternative means to investigate whether PPARγ is required for fat cell differentiation. Chimeric mice derived from both wild-type ES cells and cells with a homozygous deletion of PPARγ showed exclusion of null cells from white adipose tissue, but not several other tissues (31). Another group succeeded in bringing a single PPARγ−/− mouse to term by making tetraploid chimeric placentas; although the animal died shortly after birth it was found to lack brown adipose stores (30). In vitro, it has also been shown that PPARγ is required for the differentiation of adipose cells from ES cells and from embryonic fibroblasts (29, 31). The results of these genetic studies have been complemented by experiments using pharmacological inhibitors and dominant negative alleles of PPARγ (32, 33). These approaches have primarily been used to demonstrate a loss of PPARγ agonist-induced adipogenesis in vitro, although one study has shown a reduction in differentiation induced by the usual hormonal stimuli (34).

The CCAAT/enhancer binding proteins C/EBPα, -β, and -δ have also been shown to be important in adipogenic differentiation. A transcriptional cascade exists in which C/EBPδ and -δ induce the formation of PPARs and C/EBPα almost simultaneously (reviewed in Ref. 35). These latter two proteins then go on to promote the fully differentiated phenotype. In a manner analogous to the situation with PPARγ, ectopic expression of C/EBPα in pre-adipocytes is able to drive adipogenesis to completion. Studies on fibroblasts engineered to lack C/EBPα show that they are deficient in PPARγ but can still become adipocytes (albeit without full insulin sensitivity) if PPARγ is added back (36). Conversely, ES cells or fibroblasts that lack PPARγ are deficient in C/EBPα (29, 31). This raises the possibility that induction of PPARγ and C/EBPα represent redundant pathways for fat cell development. We have recently obtained data, however, that this is not the case, as fibroblasts that lack PPARγ are incompetent to undergo adipogenesis even when functional C/EBPα is added back at high levels. The role of C/EBPα in adipogenesis, therefore, is ancillary to the role of PPARγ (see Fig. 2).

PPARγ and Type 2 Diabetes—A role for PPARγ in type 2 diabetes is clearly suggested by the efficacy of TZD ligands in ameliorating insulin resistance, an effect observed by over a million patients currently taking these drugs (37). Several lines of evidence converge to prove that PPARγ is the relevant target of these drugs, including the finding that novel ligands designed to bind the PPARγ ligand binding domain with high affinity are very potent insulin sensitizers in vitro (24).

Additionally, mutations have been discovered in a few patients with severe insulin resistance (38). The protein product of these mutated alleles behaves in a dominant negative fashion in vitro, suggesting a role for PPARγ in the maintenance of basal insulin sensitivity. Interestingly, animals heterozygous for PPARγ exhibit increased insulin sensitivity relative to wild-type controls and also show resistance to diet-induced obesity (29, 39). This may result from elevated serum leptin levels and decreased food intake in these mice (29). Regardless, there exists a discrepancy between the human and rodent situations that requires further explication. To make matters more confusing, a common polymorphism in the PPARγ gene (P12A) has been associated with protection from type 2 diabetes, despite the fact that this allele generates a weaker PPARγ in heterologous transcription assays (40, 41).

Despite intensive investigation and years of clinical use of TZDs, much still remains unclear about the mechanisms by which PPARγ promotes insulin sensitivity. For example, the specific target tissue(s) of TZDs remain unknown. Adipose tissue is one likely target, and a recent study has shown that “fatless” mice expressing a dominant negative C/EBP allele do not show improvement in insulin sensitivity when treated with TZDs (42). An earlier paper (43) on a milder rodent model of lipodystrophic diabetes did not agree with this result, however. Other candidate sites for TZD action include skeletal muscle, liver, and pancreatic beta cells, and tissue-specific conditional knockouts of PPARγ are now being used to address these questions.

Uncertainty also surrounds the key transcriptional events by which PPARγ reduces insulin resistance (see Fig. 3). PPARγ activation in fat increases levels of Glut4, the insulin-stimulated glucose transporter (44), and may have other direct effects on important genes involved in glucose homeostasis. Unbiased target analysis of PPARγ in metabolically important tissues has revealed changes in gene expression that would have the net effect of translocating triglycerides and fatty acids from muscle and liver and promoting their storage in adipose tissue (45). This activity would theoretically improve glucose utilization in muscle and liver, although it must be remembered that similar effects could be equally explained as a consequence of improved insulin signaling in those tissues as well as a cause of insulin sensitization. Repression of genes involved in the promotion of insulin resistance could also explain the effects of TZDs and PPARγ. In fact, TNF-α and IL-6 have been implicated in the development of the insulin resistance associated with obesity; PPARγ activation reduces levels of these cytokines in fat (46, 47). Recently, a small secreted protein called resistin was discovered to be produced by fat cells and to promote systemic insulin resistance, and there is evidence that TZDs may repress expression of this factor as well (48), although recent data call this point into question (49). Finally, a recently discovered protein secreted by adipocytes, known alternatively as adiponectin, aP2, adiponectin, and aP1M1, has been found to be both a TZD target as well as a humoral mediator of insulin sensitivity (50, 51).

PPARγ and Atherosclerosis—The discovery that PPARγ was expressed at relatively high levels in monocytes and macrophages led to studies showing that PPARγ agonists could promote macrophage differentiation and directly induce the scavenger receptor CD36 (52). These findings, coupled with the identification of
PPARγ in “foam cell” macrophages within human atherosclerotic lesions (53, 54), led to fears that TZDs could be promoting atherosclerosis in humans taking these drugs. Endogenous ligands of PPARγ were identified in atherogenic oxidized low density lipoprotein particles in serum, and it was shown that these particles could induce expression of PPARγ itself (21). A pathological cycle was proposed in which these particles induced their own uptake through activation of PPARγ and expression of CD36, leading to foam cell formation.

Other evidence, however, suggested that PPARγ might be beneficial in atherosclerosis (reviewed in Ref. 55). TZDs, for example, have shown to reduce blood pressure in several mammalian models. Other atherogenic pathways are also inhibited by TZDs, including proliferation and migration of vascular smooth muscle cells and suppression of proinflammatory signals within macrophages in the vessel wall, such as IL-6, IL-1β, TNF-α, gelatinase, and scavenger receptor A (56, 57). PPARγ also induces the expression of proteins involved in reverse cholesterol transport, presumably leading to a net reduction of cholesterol in atherosclerotic lesions. These transporters, ABCA1 and ABCG1, are actually induced by the orphan NHR LXRs, which is itself a target of PPARγ (58–60). Reassuringly, TZDs administered to LDL receptor knockout mice reduced atherosclerotic lesion number and size in males and had no adverse effect in females (61).

Interestingly, recent genetic studies show that PPARγ is not required for the formation of macrophages from monocytes, although macrophages lacking PPARγ have greatly reduced basal expression of CD68 (62, 63).

**PPARγ and Cancer**—The activity of PPARγ in inhibiting the proliferation of fibroblasts during adipose differentiation first suggested that this receptor might be capable of reducing malignant behavior. This was examined in human liposarcoma, a malignancy of the adipose lineage. Most liposarcomas have been found to express much higher levels of PPARγ than other sarcomas, and cells grown from liposarcomas were found to have a dramatic differentiation response to PPARγ ligands, including lipid accumulation, cessation of growth, and expression of mRNAs characteristic of fat differentiation (64). A small clinical trial of TZD administration in liposarcoma showed that activation of PPARγ caused signs of adipose differentiation including changes in tissue morphology and gene expression, although the ultimate clinical outcome in these patients remains to be determined (65).

PPARγ is also expressed in a number of epithelial tissues that are important in human cancer, including breast, prostate, and colon. The colonic mucosa has been of special interest because PPARγ is expressed at very high levels here, comparable with the levels of expression in adipose tissue (66). Application of synthetic ligands brings about a marked reduction in cell growth in large numbers of human colon cancer cell lines, and PPARγ activation results in alterations in patterns of gene expression favoring a more mature, less malignant phenotype (67). Additionally, ligand administration to nude mice slows the growth of tumors derived from human colon cancer cells. Finally, mutations of PPARγ in tumor tissue have been detected in some patients with adenocarcinoma of the colon (68). All mutations were heterozygous, and all displayed loss of function, suggesting that PPARγ has tumor suppressor function in the human colon.

Paradoxically, administration of PPARγ ligands caused an increase in colon tumor number in Min mice, a mouse model of APC deficiency (69, 70). No increases in polyp number were seen in wild-type mice, nor have there been reports of PPARγ ligands causing increased tumor formation in humans. Nevertheless, these observations are interesting and suggest that the role of PPARγ in the biology of the colon may be complex.

PPARγ in the prostate may also play an important role in tumor suppression. Up to 30% of patients with prostate cancer have heterozygous deletions of the 3p25 region containing PPARγ, although these deletions are rather large and include many genes. In cultured prostate cell lines, TZDs have been shown to halt cell growth and to reduce secretion of the tumor marker PSA (prostate-specific antigen), and an encouraging response has been seen in some men with metastatic prostate cancer taking TZDs (71).

An interesting observation has also placed PPARγ in the spotlight in follicular thyroid carcinoma. In some cases of this disease, a fusion oncogene is formed by a chromosomal translocation between PAX8, deleted in its C-terminal activation domain, and full-length PPARγ, (72). The resulting fusion protein, the expression of which in the thyroid is presumably driven by the PAX8 promoter, has an extremely powerful dominant negative activity on the transcriptional activity of wild-type PPARγ. The addition of ligand does not relieve this dominant negative activity. This translocation is not observed in benign follicular adenomas, suggesting that it is associated with carcinogenesis. Although the contribution of both the PAX8 and PPARγ components is likely to be important, the crucial role of PPARγ as a tumor suppressor moiety in this oncogene is shown by the fact that other cases of this disease have a fusion protein formed between PPARγ and as yet unidentified partners.

**Conclusions**

The last few years have seen an explosion of information about PPARγ, implicating this NHR in biological processes as diverse as the regulation of metabolism, gene expression, differentiation, regulation of metabolism, control of cellular proliferation, and maintenance of insulin sensitivity. The fact that PPARγ is a ligand-activated transcription factor has opened the door for pharmacological manipulation, allowing rapid application of basic discoveries to the clinical arena. One area of intense focus is the development of selective PPARγ activators, which could activate the receptor in some tissues but not in others. This will hopefully result in the development of drugs that provide the glucose-lowering benefit of TZDs, for example, without the dose-limiting toxicity or the promotion of unwanted adipogenesis. Similarly, agents that exploit the growth-inhibiting effects of PPARγ in cancer cells without inducing metabolic sequelae would be useful. The amount and breadth of research effort devoted to these proteins ensures that more discoveries are certain to emerge.

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