

Minireview

Peroxisome Proliferator-activated Receptor Modulators As Potential Chemopreventive Agents

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Abstract

Peroxisome proliferator-activated receptors (PPARs), members of the superfamily of nuclear steroid hormone receptors, have traditionally been studied for their role in lipid, glucose, and energy homeostasis. Recent evidence suggests that pharmacological activation of PPAR γ and PPAR α , and inhibition of PPAR δ , may prevent cancer. PPAR γ agonists induce differentiation, inhibit the growth of established tumor cells *in vitro* and *in vivo*, and have chemopreventive effects in animal models. PPAR α has anti-inflammatory and differentiating activity and protects against the oxidative damage associated with aging. In contrast, PPAR δ expression may be a factor in colorectal carcinogenesis. PPAR δ is normally repressed by the adenomatous polyposis coli tumor suppressor gene, and impaired adenomatous polyposis coli is strongly associated with human colorectal cancer risk. This review presents a rationale for using PPAR modulators as cancer chemopreventive drugs.

Introduction

PPARs² are ligand-activated transcription factors that heterodimerize with the RXRs and bind to peroxisomal proliferator response elements in the promoter region of target genes. Three PPAR isoforms have been cloned (α , γ , and δ), each exhibiting distinct patterns of tissue distribution and ligand specificity. PPAR α regulates numerous aspects of fatty acid catabolism, whereas PPAR γ controls adipocyte differentiation, systemic glucose levels, and lipid homeostasis (reviewed in Refs. 1 and 2). PPAR δ is involved in development, embryo implantation, myelination of the corpus callosum, lipid metabolism, and epidermal cell proliferation (3, 4). Significant species differences in response to PPAR α , but

not PPAR γ , have been noted. Specifically relevant to cancer, PPAR α agonists increase peroxisomes and induce hepatomegaly and liver cancer in rodents. However, humans are refractory to the hepatotoxic actions of these drugs (reviewed in Ref. 5). In the following sections, evidence is presented for each PPAR class and the specific PPAR agonists that may play a role in cancer chemoprevention.

PPAR γ and Cancer

The observation that PPAR γ stimulates adipose differentiation in cultured mouse fibroblasts generated interest in the receptor's potential anticancer effects (6). Subsequent studies showed that activation of the receptor inhibits proliferation, and in some cases induces differentiation and/or apoptosis, in a variety of tumor cell lines (Table 1). Some of the most extensive work has been conducted in the colon, where PPAR γ is expressed at high levels in normal tissue (7), and in both well- and poorly differentiated colon cancers (8). PPAR γ -selective ligands inhibit proliferation and induce differentiation in colon cancer cell lines (8, 9) and diminish growth of human colon tumor xenografts (Ref. 8; Table 2). Importantly, for the purposes of chemoprevention, these ligands prevent the development of carcinogen-induced preneoplastic aberrant crypt foci in rats (10). Loss-of-function somatic mutations in the PPAR γ gene are found in sporadic human colon cancers, suggesting that PPAR γ may function directly as a tumor suppressor (11). PPAR γ agonists also suppress macrophage activation and inflammatory cytokine production *in vitro* (12, 13) and reduce inflammation (10, 14) and neoplastic lesion development (10) in rodent models of inflammatory bowel disease. The latter finding is particularly relevant to chemoprevention, because the risk of colorectal cancer is increased in patients with this disease (reviewed in Ref. 15).

In contrast, other studies indicate that PPAR γ activation promotes colon cancer in mice carrying a nonsense mutation in the APC tumor suppressor gene; defective APC is highly associated with human colorectal cancer risk (16). The TZDs troglitazone and rosiglitazone enhance colon tumorigenicity in these genetically predisposed mice; the more potent PPAR γ agonist rosiglitazone induces more malignant tumors. However, pharmacological doses of troglitazone are not tumorigenic in wild-type mice, suggesting that increased risk is limited to animals harboring a mutant APC gene (17, 18).

Data available from other target sites suggest that PPAR γ activation has chemopreventive potential. The receptor is expressed in human breast cancer cell lines and breast adenocarcinomas, and TZDs inhibit growth of breast cancer cells *in vitro* and *in vivo*, inducing differentiation in some cases (19, 20). However, cell lines expressing the highest levels of PPAR γ are relatively unresponsive to TZD treat-

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² The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; AOM, azoxymethane; APC, adenomatous polyposis coli; COX, cyclooxygenase; RXR, retinoic X receptor; TZD, thiazolidinedione.

Table 1 Antiproliferative effects of PPAR γ ligands *in vitro*^a

Ligand	Cell Line
Troglitazone	Bladder [39], breast [19, 20], colon [8, 9], lung [78], hematopoietic system [79], pancreas [36], prostate [24, 25], stomach [80]
Rosiglitazone	Colon [8, 9], prostate [24, 25, 81]
Pioglitazone	Breast [19], colon [8], liposarcoma [82], hematopoietic system [79], stomach [80]
Ciglitazone	Lung [31], prostate [26]
GW 1929	Neuroblastoma [83]
15d-PGJ ₂ ^b	Breast [84], colon [8], lung [31, 78], prostate [24, 25, 26], neuroblastoma [83]

^a Growth inhibitory effects of PPAR γ ligands in cancer cell lines, sometimes accompanied by induction of differentiation, apoptosis, and/or cell cycle arrest.

^b 15d-PGJ₂, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂.

Table 2 Anticancer effects of PPAR γ ligands in animal studies^a

Target organ	Ligand	Results	Comments	Reference
Mammary gland	GW 7845	Prevented MNU-induced tumor development in rats	Additive effects with tamoxifen; no increase in body weight noted	[21]
	Troglitazone	Prevented DMBA-induced preneoplastic lesions in mouse mammary organ culture	Significant synergistic effects with RXR ligand	[22]
	Troglitazone	Inhibited growth of human breast cancer cells in immunodeficient mice		[20]
Colon	Troglitazone	Inhibited growth of human colon carcinoma cells in immunodeficient mice		[8]
	Troglitazone	Prevented development of AOM- and AOM/DSS-induced aberrant crypt foci in rats		[10]
	Pioglitazone	Prevented development of AOM/DSS-induced aberrant crypt foci in rats		[10]
Prostate	Troglitazone	Inhibited growth of human prostate cancer cells in immunodeficient mice	No additive effects with ATRA; necrotic and apoptotic changes noted. Similar changes, associated with troglitazone treatment of primary prostate tumors cultured <i>in vitro</i>	[24]

^a MNU, *N*-methyl-*N*-nitrosourea; DMBA, 7,12-dimethylbenz(a)anthracene; AOM, azoxymethane; DSS, dextran sodium sulfate; ATRA, all-*trans*-retinoic acid.

ment. Adding a mitogen-activated protein kinase inhibitor overcomes this insensitivity, suggesting that phosphorylation prevents PPAR γ activation (19).

The relevance of PPAR γ activation for chemoprevention has been demonstrated in rodent mammary cancer models. The highly potent and specific PPAR γ ligand GW 7845 significantly reduces tumor incidence, number, and weight in estrogen receptor-positive mammary tumors when fed to rats after carcinogen administration. Additive effects are observed on coadministration with suboptimal doses of the antiestrogen tamoxifen (21). Likewise, troglitazone prevents development of carcinogen-induced early neoplastic lesions in mouse mammary gland organ culture when administered during initiation or promotion stages. Synergism was noted with RXR-selective ligands (22), although similar effects were not observed in human breast cancer cell lines induced to differentiate in response to PPAR γ activation (19). PPAR γ ligands also diminish expression of aromatase (23), the rate-limiting enzyme in estrogen biosynthesis, which may contribute to chemopreventive effects in estrogen-responsive tissues.

In the prostate, PPAR γ is expressed in primary cancers and cell lines, and receptor agonists display antiproliferative effects *in vitro* (24–26) and in xenografts in nude mice (24); effects *in vivo* are associated with necrotic, as well as apoptotic changes (24). Additionally, a subset of human prostate tumors carry hemizygous deletions of the PPAR γ gene (25). In preliminary clinical studies, 20% (8 of 41) of prostate

cancer patients treated with troglitazone showed decreased serum prostate-specific antigen levels, and 39% showed prolonged stabilization (25). Consistent with these findings, troglitazone decreases prostate-specific antigen levels in prostate cancer cell lines (24, 25). The actions of PPAR γ ligands in the prostate may be associated with decreased activation of the androgen receptor (27) or a reduction in circulating estrogens (reviewed in Ref. 28), secondary to reduced aromatase expression (23).

PPAR γ ligands also inhibit proliferation of human lung, urinary bladder, pancreatic, neuroblastoma, gastric, and liposarcoma cell lines (Table 1) and induce differentiation and inhibit growth of liposarcomas in patients (29). The activated form of matrix metalloproteinase-2, strongly associated with tumor growth, metastasis, and angiogenesis (30), markedly decreases in lung cancer cells treated with PPAR γ agonists (31). Furthermore, PPAR γ activators are direct inhibitors of angiogenesis both *in vitro* and *in vivo* (32, 33). A role for PPAR γ in thyroid follicular carcinoma is suggested by the presence in these tumors of a PAX8-PPAR γ 1 fusion oncoprotein, which functions as a dominant negative suppressor of wild-type PPAR γ (34).

Recent investigations have provided clues about the signaling pathways used by PPAR γ agonists to suppress neoplasia. Inhibition of tumor cell growth has been associated with G₁ cell cycle arrest (9, 35, 36), which in some cases is linked to loss of DNA binding activity of the transcriptional regulator E2F/DP (37). Other mechanisms that may be involved include up-

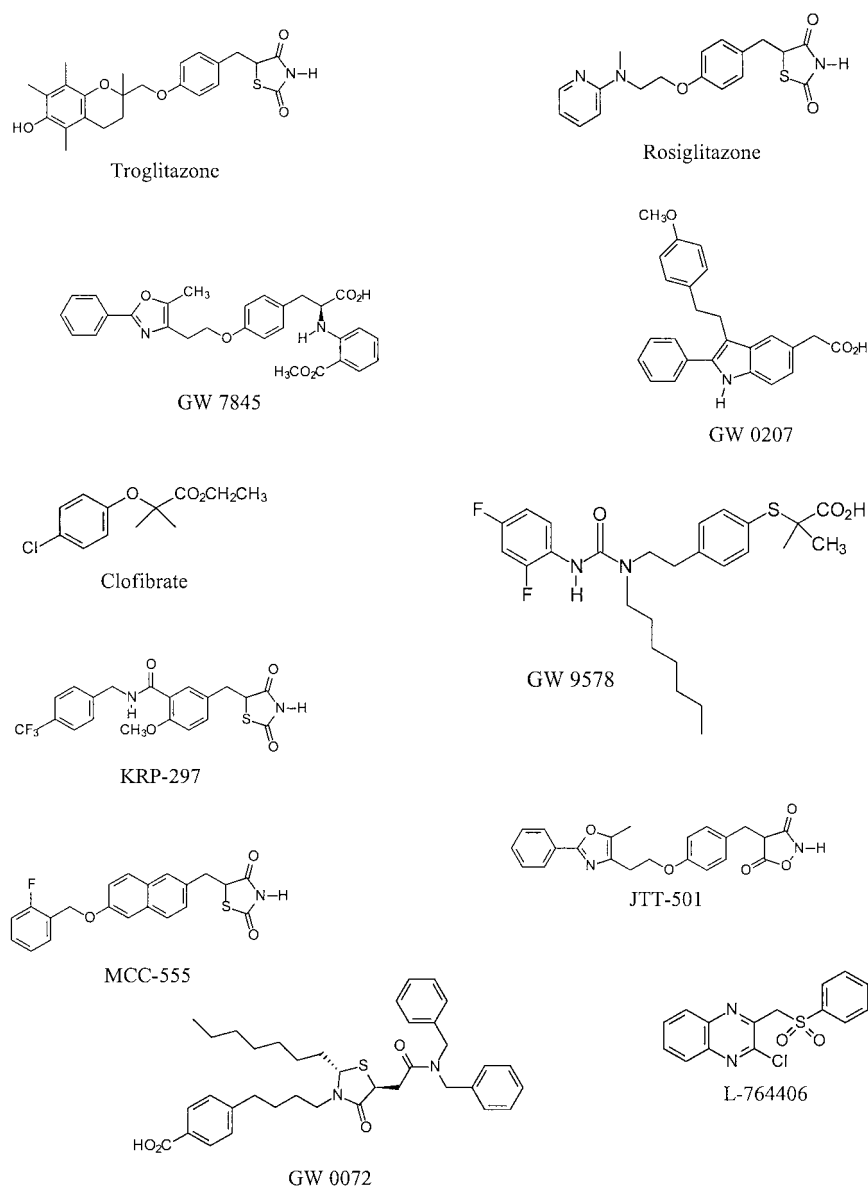


Fig. 1. Chemical structures of PPAR modulators.

regulation of the cyclin-dependent kinase inhibitors p18^{INK4c} and p21^{Waf1/Cip1} and reduced expression of cyclin D1 (31, 38–40). Recent studies showed that the antiproliferative and anti-inflammatory activities of PPAR γ ligands are mechanistically distinct. PPAR γ -dependent antiproliferative effects involve repression of cyclin D1 via sequestration of p300 and interference with *c-fos*-directed transcription. Anti-inflammatory effects, independent of this receptor, are mediated via inhibition of the intracellular kinase intracellular κ B kinase, involved in nuclear factor κ B activation (40). Additionally, PPAR γ agonists up-regulate the phosphatase and tension homologue (PTEN) tumor suppressor, suggesting that phosphoinositide signaling pathways are also associated with PPAR γ -mediated growth suppression (41).

PPAR α and Cancer

Several studies have established a link between PPAR α activation and epidermal differentiation. Fibrates, which are PPAR α ligands, induce differentiation and inhibit proliferation of human keratinocytes *in vitro* (42), and in normal (43) and hyperproliferating (44) mouse skin *in vivo*, but are inactive in PPAR α -deficient mice (43). Farnesol also stimulates PPAR α -dependent differentiation in epidermal keratinocytes (45). Topical PPAR α agonists have weak preventive effects on tumor promotion in mouse skin, despite up-regulation of PPAR α in untreated tumors compared with normal epidermis (46). In this regard, PPAR α expression is also up-regulated in human prostate adenocarcinomas (47). The significance of this observation is as of yet unknown.

Some anticancer actions of phenylacetate may be mediated by PPAR α activation. The relative potencies of phenylacetate and its analogues as PPAR α activators correlate strongly to their growth inhibitory effects in human cancer cell lines (48). Phenylacetate also activates PPAR γ (49), making it difficult to ascribe growth-inhibitory effects specifically to activation of the α isoform. It is important to note that PPAR α ligands are inactive in tumor cell lines in which PPAR γ ligands are effective antiproliferative agents (8, 9, 25); however, the status of PPAR α expression in these tumor cell lines is unknown.

In rodents, PPAR α activation has been associated with both anti- (50, 51) and proinflammatory (52) actions. Opposing effects have also been described in humans. PPAR α agonists diminish expression of inflammatory markers in human cells and patients treated with fibrates (53, 54). In contrast, expression of the inflammatory mediator COX-2 in human breast and colon cancer cells is up-regulated by PPAR α agonists (55). The latter finding is particularly troublesome, given that increased COX-2 expression has been specifically linked to enhanced colon cancer risk (reviewed in Ref. 28).

Other possible connections between PPAR α and cancer prevention come from the inverse association of PPAR α activation with decreased oxidative stress and aging, both linked with tumorigenesis (reviewed in Ref. 56). PPAR α agonists administered to aged PPAR α -replete, but not aged PPAR α -null mice, decreased tissue levels of lipid peroxides and the oxidant-stress-activated transcription factor nuclear factor κ B (51). Additionally, reduced expression of PPAR α and peroxisome-associated genes is observed in aged mice; PPAR α agonists restore expression to levels seen in young animals (57).

PPAR δ and Cancer

In contrast to the γ and α isoforms, activation of PPAR δ is associated positively with tumorigenesis. PPAR δ transcription is normally suppressed by wild-type APC but is up-regulated in colorectal cancer cells, which have an inactivating APC mutation through enhanced β -catenin/Tcf-4 binding to TCF-4-responsive elements in the PPAR δ promoter (58). Xenografts of PPAR δ -null colon cancer cell lines display decreased tumorigenicity in nude mice (59). Consistent with these findings, PPAR δ is overexpressed in human and rodent colorectal tumors, as well as preneoplastic colonic mucosa (58, 60, 61). Although initial experiments suggested that nonsteroidal anti-inflammatory drug-induced apoptosis is mediated in part by PPAR δ inhibition (58), this was not confirmed in additional studies (59). However, additional experiments in PPAR δ -null mice indicate that nonsteroidal anti-inflammatory drug-mediated anti-inflammatory response, which may also be chemopreventive, is at least partially dependent on PPAR δ (3).

PPAR Modulators

As suggested by the data presented above (Fig. 1), agonists of PPAR γ and PPAR α , and antagonists of PPAR δ , may find utility as chemopreventive agents. Although no selective

PPAR δ inhibitors have been identified, a number of agonists for these isoforms has been described. Selective PPAR γ agonists include classic TZDs (troglitazone, rosiglitazone, pioglitazone, and ciglitazone; Refs. 62 and 63) and non-TZD-type agonists. Representatives of the latter include *N*-(2-benzoylphenyl)-*L*-tyrosine derivatives, such as GW 1929, GI 262570, and GW 7845, which are among the most potent and selective PPAR γ agonists identified to date (64, 65). GW 0207, a 2,3-disubstituted indole-5-carboxylic acid, is also a potent and selective PPAR γ agonist (66).

Fibrates are weak PPAR α agonists used to treat hyperlipidemia (2). Newer PPAR α agonists, such as the ureidofibrate GW 9578, are significantly more potent (67) and can be used to study the effects of PPAR α in neoplasia. If both PPAR α and PPAR γ are involved in tumorigenesis, dual receptor agonists, such as the TZD KRP-297 (68) and the related isoxazolidinedione derivative JTT-501 (69), may also find utility as chemopreventive agents.

The following compounds can act as partial PPAR γ agonists/antagonists. Although TZD MCC-555 is a low-affinity PPAR γ ligand, it is an effective PPAR γ activator in animals (70). L-764406, a sulfonyl quinoxaline derivative, is a potent and specific PPAR γ ligand and partial agonist that covalently modifies the receptor (71). The thiazolidine acetamide GW 0072 is a high-affinity PPAR γ ligand and weak partial transcriptional activator of the receptor; it is also a potent inhibitor of rosiglitazone-induced adipocyte differentiation (72).

These partial agonists/antagonists display unique biological properties that may translate to distinct therapeutic and/or toxicological profiles, similar to selective estrogen receptor modulators (partial estrogen receptor agonists/antagonists; reviewed in Ref. 28). This is significant, because the selective PPAR γ agonist troglitazone can have rare but serious hepatotoxic consequences (73). Moreover, it is possible that the cancer-promoting effects of PPAR γ ligands, as observed in animals predisposed to colon cancer, can be mitigated using partial agonists.

Conclusions

The PPAR system's relevance to carcinogenesis is just beginning to be unraveled. Additional research is needed to characterize expression patterns of the various PPAR isoforms in cancerous and precancerous tissue and to determine their precise roles in the carcinogenic process. Numerous fatty acids and their metabolites activate PPAR receptors (74, 75). Thus, PPARs may also provide the missing molecular link between high-fat diets and nutritionally sensitive cancers (76). Although the physiological function of the PPAR was once thought to be restricted to lipid metabolism, it is now clear that these receptors are involved in numerous biological processes. Confirming a crucial role for PPARs in tumorigenesis will foster development of a novel class of cancer preventive drugs (77).

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