Peroxisome proliferator-activated receptor γ: a novel target for cancer therapeutics?

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Peroxisome proliferator-activated receptors are ligand-activated intracellular transcription factors that have been implicated in important biological processes such as inflammation, tissue remodeling and atherosclerosis. Emerging information also implicates peroxisome proliferator-activated receptors in oncogenesis. Peroxisome proliferator-activated receptor γ, the best studied of the peroxisome proliferator-activated receptors, modulates the proliferation and apoptosis of many cancer cell types, and it is expressed in many human tumors including lung, breast, colon, prostate and bladder. Natural and synthetic agents capable of activating peroxisome proliferator-activated receptor γ have been found to inhibit cancer cell growth *in vitro* and in animal models. These agents, however, are not specific and both peroxisome proliferator-activated receptor γ-dependent and peroxisome proliferator-activated receptor γ-independent pathways involved in their effects have been identified. Together, these studies, coupled with a few clinical trials, suggest that peroxisome proliferator-activated receptor γ is a novel target for the development of new and effective anticancer therapies. *Anti-Cancer Drugs* **18:237–244**

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Introduction

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated intracellular transcription factors belonging to the nuclear hormone receptor superfamily [1]. Their discovery during the past decade unveiled novel pathways involved in the control of cellular differentiation, proliferation and apoptosis, which have been implicated in the regulation of important biological processes such as inflammation, tissue remodeling and vascular biology [2]. Data exploring the role of PPARs are so tantalizing that PPARs are now considered targets for new interventions in clinical entities such as diabetes, the metabolic syndrome and atherosclerosis, among other disorders [3]. Their many functions have not escaped the attention of cancer researchers who have documented important roles for PPARs in oncogenesis, thereby identifying a new target for cancer therapeutics. The latter represents the focus of this review, with particular attention to PPARγ, the best studied of the PPARs. We will succinctly review the molecular structure of PPARγ and its cellular mechanisms of action, followed by a discussion of current knowledge regarding its role in cancer. The review is not meant to be exhaustive. Instead, key observations generated *in vitro*, in animal models and in clinical studies are discussed with the hope of encouraging further work in this area.

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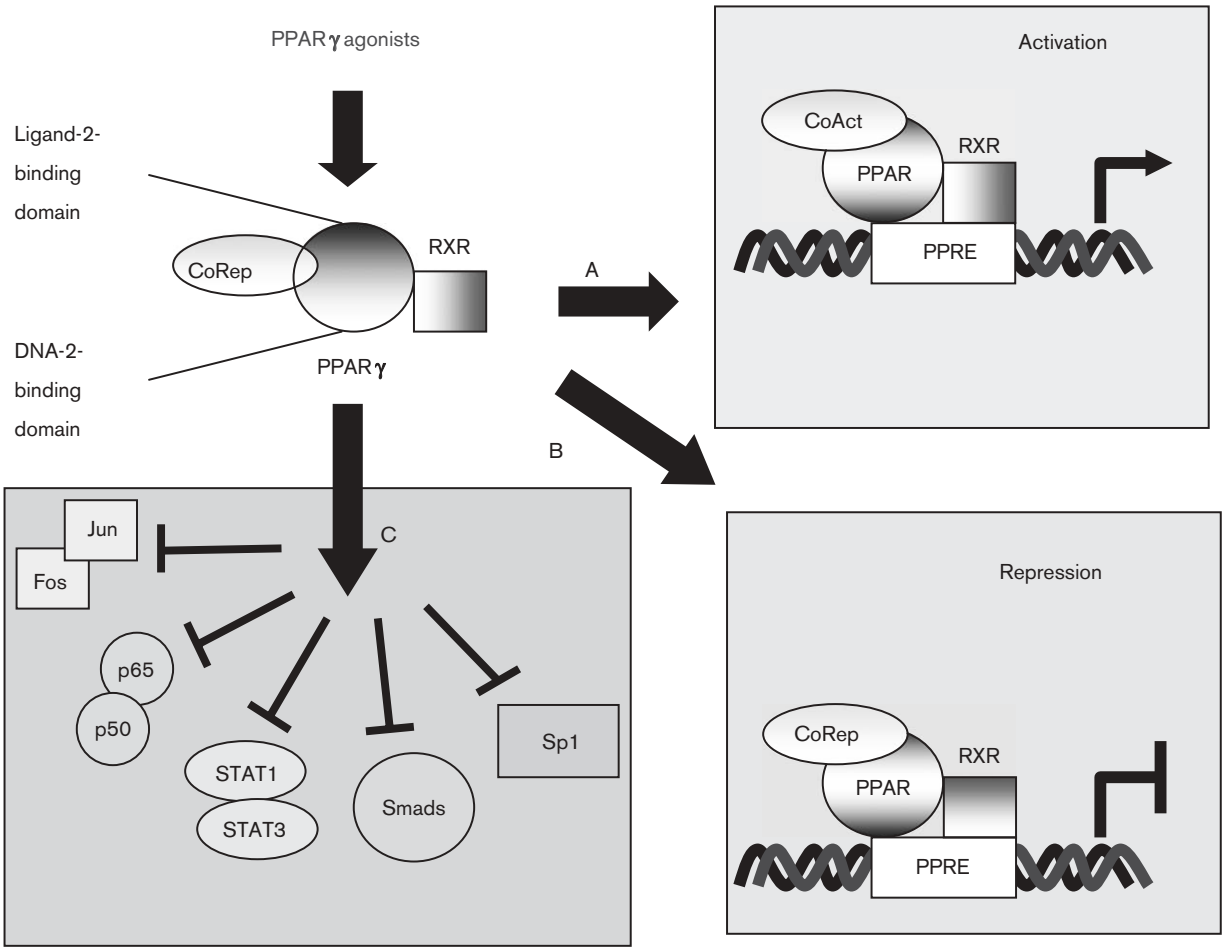
Structural features of peroxisome proliferator-activated receptor γ and mechanisms of peroxisome proliferator-activated receptor γ-dependent gene transcription

PPARs are ligand-inducible transcription factors belonging to the nuclear hormone receptor superfamily that includes receptors for estrogen, thyroid, glucocorticoid and vitamin D. The PPAR subfamily consists of distinct genes that code for several PPAR isoforms denoted PPARα, β/δ and γ, which share about 60–80% homology in their ligand-binding and DNA-binding domains [4]. Amongst the three subtypes (α, β/δ and γ), PPARγ has been the most intensively investigated. In humans, the gene encoding for PPARγ is located on chromosome 3 at position 3p25, close to the retinoic acid (RA) receptor β and the thyroid hormone receptor β. The human PPARγ gene extends over more than 100 kb of genomic DNA and gives rise to three mRNA isoforms (PPARγ1, PPARγ2 and PPARγ3) that differ at their 5′-end as a consequence of alternate promoter usage and splicing. Both the PPARγ1 and γ3 RNA transcripts translate into PPARγ1 protein; PPARγ2 contains an additional 28 amino acids. PPARγ1 is expressed in relatively low abundance in many tissues such as skeletal muscle, prostate, kidney, breast, and gastrointestinal and reproductive tracts, among

others, whereas PPARγ2 is predominantly expressed in adipocytes [4]. The differences in the actions of these distinct PPARγ isoforms remain unclear.

Like other members of this nuclear receptor superfamily, PPARγ is characterized by three general functional domains: the *N*-terminal domain (a site for functional regulation by phosphorylation), the DNA-binding domain and the ligand-binding domain [4]. Upon activation in the cytoplasm, PPARs heterodimerize with retinoid-Xreceptors (RXR), and form a complex that translocates to the nucleus and regulates gene expression (Fig. 1, pathway A). This heterodimeric complex comprises the functional transcription factor that then binds to peroxisome proliferator response elements located within the promoter regions of target genes that consist of a direct repetition of the consensus AGGTCA half-site spaced by one or two nucleotides (DR1 or DR2). In addition to the heterodimer complex, it has been reported that a host of accessory proteins, named 'coactivators' or 'corepressors', bind to the nuclear receptors PPARγ/RXRα in a ligand-dependent manner and impact the transcriptional process by either remodeling chromatin structure and/or acting as adapter molecules that link the nuclear receptor complex to key transcriptional machinery (Fig. 1) [5]. Ligand binding to PPARs appears to trigger conformational changes that permit their dissociation from corepressors and favor their association with coactivators (e.g. steroid receptor coactivator-1 and p300). The coactivator proteins possess or recruit histone acetyltransferase activity

Fig. 1



Differential regulation of gene expression by peroxisome proliferator-activated receptor (PPAR) γ. PPAR γ, requires heterodimerization with a second member of the nuclear receptor family, retinoic X receptor (RXR), and forms a complex that translocates to the nucleus and regulates gene expression. Binding of ligands to PPARγ triggers a conformation change that attracts transcriptional coactivators. The heterodimer–coactivator complex binds to specific response elements [termed peroxisome proliferator response elements (PPREs)] in the promoter regions of target genes (pathway A). In the absence of ligand, PPARγ has the potential to silence genes to which it is bound by recruiting transcriptional corepressor complexes and repress gene expression (pathway B) or in a DNA-binding-independent manner by interfering with key transcription factors (pathway C). The transcriptional coactivators and corepressors exist in multiprotein complexes including histone-modifying enzymes, such as histone acetyltransferases (notably p300/CBP) and histone deacetylases. The activity of these enzymes affects gene transcription by altering chromatin structure.

to the transcription start site. There, acetylation of histone proteins alters chromatin structure, thereby facilitating the binding of RNA polymerase and the initiation of transcription. In addition to their stimulatory effects on gene transcription, PPARs can repress gene expression in a DNA binding-dependent manner through the recruitment of corepressors to unliganded PPARs (Fig. 1, pathway B) or in a DNA binding-independent manner by interfering with key transcription factors (Fig. 1, pathway C).

Elucidating the functions of peroxisome proliferator-activated receptor γ

The functions of PPARγ were initially studied with the aid of synthetic PPARγ activators (also known as PPARγ ligands) termed thiazolidinediones (TZDs). TZDs are a class of insulin-sensitizing agents that includes troglitazone, rosiglitazone and pioglitazone. To stimulate PPARγ there are also several nonsteroidal anti-inflammatory drugs (e.g. indomethacin, ibuprofen, fenoprofen and flufenamic acid). Natural ligands for PPARγ include prostanoids, prostaglandin D_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) and certain polyunsaturated fatty acids. These reagents have been used to elucidate the role of PPARγ in cellular functions both *in vitro* and *in vivo* [6,7]. Several caveats should, however, be taken into consideration when interpreting such studies. First, the natural ligands that regulate PPARs *in vivo* have not been determined. Second, not all PPARγ ligands exert their effects through PPARγ. In fact, there is strong evidence for the activation of PPARγ-independent signals by these agents, particularly in the case of the natural ligand 15d-PGJ_2, and these explain many of effects of PPARγ ligands [8]. Third, high-affinity ligands for PPARγ (e.g. the TZDs) may exert partial ligand/antiligand activity [9]. The latter might be due to the fact that individual TZDs induce different PPARγ conformations that influence the recruitment of different coactivator/corepressor molecules. Thus, the activity of the PPARγ transcriptional complex is influenced by the context of a given gene and its promoter, and by the relative availability of pertinent coactivator/corepressor molecules in the cell or tissue of interest. Regulation of PPARγ can occur at the level of gene expression, ligand availability (both endogenous and pharmacological ligands) and PPARγ activity (phosphorylation status).

Early studies revealed that PPARγ is an important regulator of adipogenic differentiation and glucose homeostasis [10]. Besides those fundamental functions, activation of PPARγ has also been demonstrated to play important roles in several key biological processes including cellular proliferation and differentiation, inflammation, apoptosis, and angiogenesis. Furthermore, PPARγ ligands exert antineoplastic properties in different cell types [3]. Unfortunately, genetic deletion of the PPARγ gene in mice results in embryonic lethality at approximately day 10 of gestation owing to placental insufficiency [11]. This has hindered efforts to elucidate the exact role of PPARγ *in vivo*.

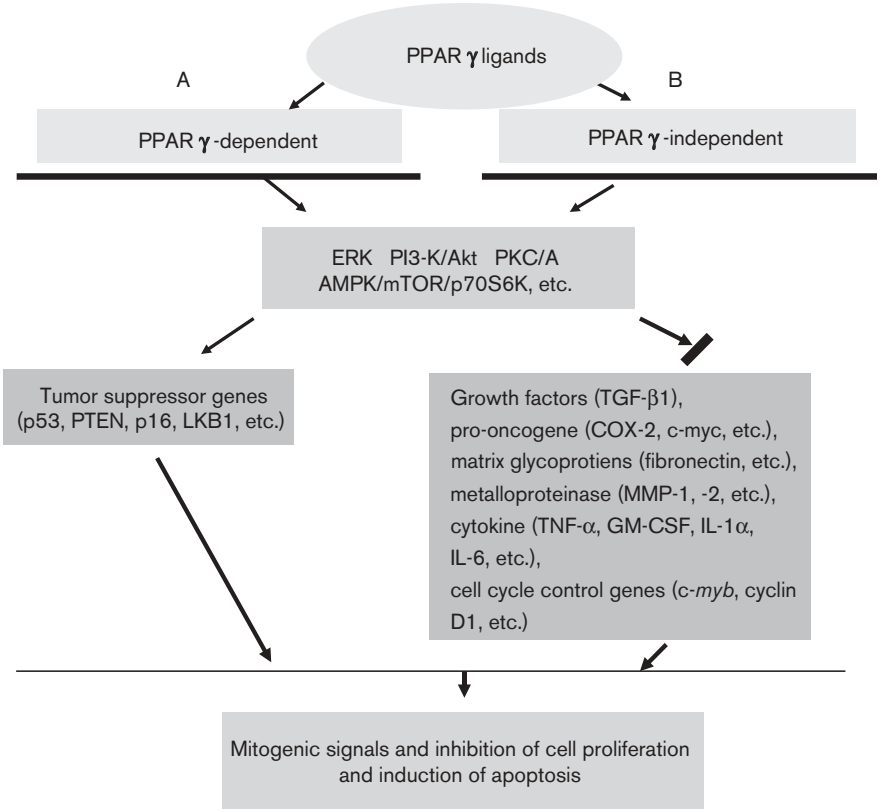
Peroxisome proliferator-activated receptor γ in cancer biology

In view of the ability of PPARγ ligands to inhibit cellular proliferation and promote differentiation, researchers have postulated a role for PPARγ in carcinogenesis. PPARγ is expressed in many cancers including the colon, breast, lung and prostate, and PPARγ ligands are generally antiproliferative in these settings. Specifically, PPARγ ligands inhibit the proliferation of human breast, prostate, colon and pituitary cancer cells *in vitro*. Interestingly, naturally occurring somatic mutations in the gene encoding PPARγ have been found in sporadic colorectal carcinomas. These findings support a role for PPARγ as a potential tumor suppressor, although several murine models suggest that, under certain circumstances, PPARγ ligands may stimulate cancer formation [12]. Despite these paradoxical findings, PPARγ is considered to have potential antineoplastic effects both in solid cancers and in leukemia through inhibition of cell proliferation, induction of apoptosis and terminal differentiation or through inhibition of angiogenesis [13]. In view of the above, PPARγ ligands may represent a promising, novel therapeutic approach for certain human malignancies. This is unlikely to be a common feature of all PPARs in view of the fact that PPARβ/δ agonists have recently been shown to stimulate non-small cell lung carcinoma (NSCLC) cell proliferation [14]. Below, we summarize some of the data available that implicate PPARγ and its ligands in the control of tumor growth.

Peroxisome proliferator-activated receptors γ in lung cancer

PPARγ is expressed in small cell lung carcinomas and NSCLC. In a variety of lung cancer cell lines, PPARγ ligands induce growth arrest, and induce changes associated with differentiation and apoptosis (Fig. 2). For example, PPARγ ligands have been found to inhibit the growth of A549 adenocarcinoma cells owing to G_0/G_1 cell cycle arrest through the downregulation of G_1 cyclins D and E; this was related to stimulation of Erk1/2 activation [15]. Of note, the treatment of NSCLC tumor-bearing severe combined immunodeficiency mice with a PPARγ ligand, troglitazone, inhibited tumor growth and metastasis [15]. PPARγ ligands have also been shown to inhibit lung carcinoma cell proliferation through increased expression of phosphatase and tensin homolog (PTEN) and p21 [16,17]. They also reduce the expression of fibronectin, an extracellular matrix glycoprotein that serves as a mitogen/survival factor for NSCLC [18], and decrease its receptor, the integrin α5β1, through activation of Erk signals via PPARγ-dependent and PPARγ-independent pathways [19]. PPARγ ligands also reduce the expression of the prostaglandin E_2 receptor [20]. The latter is important in view that prostaglandin E_2 is a major product of

Fig. 2



Effects of peroxisome proliferator-activated receptor (PPAR) γ ligands on cancer cell inhibition and induction of apoptosis. PPARγ is expressed in most cancer cell types. Through PPARγ-dependent and PPARγ-independent signals, PPARγ ligands activate or inactivate kinase-signaling pathways (e.g. PI3-K, Erk1/2, AMPK/mTOR/p70S6K, PKC, etc.) resulting in the inhibition of expression of growth factors (e.g. TGF-β1), pro-oncogenes (e.g. cyclooxygenase), matrix glycoproteins with mitogenic activity (e.g. fibronectin), metalloproteinases implicated in metastasis (e.g. MMP-1, -2, etc.), cytokines (e.g. TNF-α, GM-CSF, IL-1α, IL-6), and cell cycle control genes (e.g. c-*myc*, c-*myb*, and cyclin D1), and the induction of expression of tumor suppressor genes (e.g. p53, PTEN, etc.). Together, these processes serve to inhibit cell growth and promote apoptosis in most tumor cell types studied. AMPK, AMP-activated protein kinase; mTOR, mammalian target of rapamycin; PKC, protein kinase C; COX, cyclooxygenase; TGF, transforming growth factor; MMP, matrix metalloproteinase; TNF, tumor necrosis factor; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; PTEN, phosphatase and tensin homolog.

cyclooxygenase-2 that promotes the proliferation of lung carcinoma cells [17,21].

The dramatic synergistic anticancer effects of lovastatin (a 3-hydroxy-3-methylglutaryl-coenzymeA reductase inhibitor) and troglitazone (a PPARγ ligand) have recently been reported in several cancer cell lines including lung cancer cells [22]. The combination of low-dose 5-lipoxygenase activating protein-directed inhibitor, MK886, ciglitazone and 13-*cis*-retinoic acid synergistically inhibit the growth of lung cancer cell lines A549 and H1299, suggesting that targeting PPARγ and arachidonic acid actions is a promising approach in inhibiting lung cancer growth with a favorable therapeutic index [23].

We recently found that the PPARγ ligand rosiglitazone reduced the phosphorylation of Akt and increased PTEN protein expression in NSCLC cells, and this was associated with inhibition of tumor cell proliferation through PPARγ-dependent signals [24]. In addition, rosiglitazone increased the phosphorylation of AMP-activated protein kinase α, a downstream kinase target for LKB1, whereas it decreased phosphorylation of p70 ribosomal protein S6 kinase (p70S6K), a downstream target of mammalian target of rapamycin, through PPARγ-independent signals. These findings suggest that rosiglitazone, via upregulation of the PTEN/AMP-activated protein kinase and downregulation of the Akt/mammalian target of rapamycin /p70S6K signal cascades, inhibits NSCLC cell proliferation through PPARγ-dependent and PPARγ-independent signals [24].

In primary NSCLC, the expression of PPARγ has been correlated with tumor histological type and grade, and decreased PPARγ expression was correlated with poor prognosis [25]. Thus, it has been postulated that PPARγ mRNA levels may serve as a prognostic marker in lung carcinoma.

Peroxisome proliferator-activated receptor γ in gastrointestinal cancer

The expression of PPARγ has been documented in colon cancer in several studies [26,27] and conjugated linoleic acid, which is a natural PPARγ ligand, inhibits peritoneal metastasis in human gastrointestinal cancer cells [28]. Studies using animal models have raised concerns about the potential for PPARγ modulation to affect tumor growth, as both the suppression and enhancement of tumor growth have been observed in different animals treated with activators of PPARγ. The growth of cultured human colon tumor cells and of transplanted tumors in nude mice is inhibited by activators of PPARγ including troglitazone (rezulin), rosiglitazone (avandia) and 15d-PGJ_2 [29–32]. The inhibition of growth is due, in part, to the induction of apoptosis upon PPARγ activation. In contrast, in *min* mice predisposed to the development of intestinal polyps, a condition caused by a mutation in the adenomatous polyposis coli gene, treatment with troglitazone or rosiglitazone leads to an increase in the number and size of intestinal polyps. This suggests that the suppressive effect of PPARγ ligands may be mediated through activation of some other genes such as adenomatous polyposis coli. Studies performed in human colon cancer are also paradoxical. One study suggests that human colon cancer is associated with loss-of-function mutations in PPARγ [16], whereas another failed to detect a mutation in the PPARγ gene in human colon tumor samples [17]. Thus, the role of PPARγ in colon cancer remains controversial.

In an attempt to elucidate the role of PPARγ in gastric carcinogenesis, a group from Japan found that troglitazone suppressed gastric carcinogenesis through activation of PPARγ [33]. Here again, PPARγ ligands acted through both PPARγ-dependent and PPARγ-independent signals [29–37]. With regard to the latter, several PPARγ-independent signals have been identified in relationship with gastrointestinal cancers. For example, 15d-PGJ_2-induced growth inhibition of colon cancer cells is mediated, at least in part, through upregulation of KLF4 expression. This induction is unlikely to be mediated through PPARγ, but may involve the mitogen-activated protein kinase kinase/extracellular signal-regulated protein kinase pathway, and is signal transducers and activators of transcription dependent [37]. Clearly, recommendations for the treatment of gastrointestinal cancer with PPARγ ligands would be premature, but certainly worthy of further investigation.

Peroxisome proliferator-activated receptor γ in breast cancer

In the US, breast cancer accounts for approximately 30% of all cancers diagnosed in women and is the second leading cause of cancer death in women. It has been found that PPARγ activated by various ligands (thiazolidinedione, linoleic acid, ω-6 fatty acid) causes growth arrest and stimulates the terminal differentiation of breast cancer cells *in vitro*. Of concern is the observation that in transgenic mice, increased PPARγ signaling served as a tumor promoter in the mammary gland, suggesting that PPARγ signaling may exacerbate mammary gland tumor development [38]. The PPARγ ligands, 15d-PGJ_2 and ciglitazone, have been shown to inhibit the proliferation and induce the apoptosis of MB-MDA-231 breast cancer cells through activation of PPARγ [39]. Of note, a favorable impact of PPARγ expression on disease-free survival of patients with ductal breast carcinoma and its possible cooperation with ERβ in exerting that favorable effect has been reported [40]. The exact mechanism(s) for this association, however, remains unclear [41–44]. Despite the above, the only published clinical trial using a PPARγ ligand in patients with metastatic breast cancer failed to show any clinical benefits [45].

Peroxisome proliferator-activated receptor γ in prostate cancer

Prostate cancers express abundant and higher constitutive levels of PPARγ than do normal prostate cells, and these cells are growth-inhibited by ligand activation of PPARγ. In contrast, normal prostate epithelial cells do not express either PPARγ1 or PPARγ2 protein and do not appear to be sensitive to growth inhibition by the PPARγ ligand 15d-PGJ_2 [46]. Thiazolidenediones mediate apoptosis in prostate cancer cells in part through inhibition of Bcl-x_L/Bcl-2 functions independently of PPARγ [47]. In clinical trials of patients with advanced prostate cancer, treatment with troglitazone has resulted in prolonged stabilization of prostate-specific antigen levels [48,49].

Peroxisome proliferator-activated receptor γ in other malignancies

PPARγ ligands have been shown to inhibit growth and induce apoptosis and redifferentiation in cancer cell lines and in animal models of thyroid cancer [50–52]. These experiments suggest that strategies targeting PPARγ may be useful in the diagnosis and treatment of thyroid carcinoma [53].

PPARγ has also been detected in bladder tumors and bladder cancer cell lines [54–57]. Furthermore, PPARγ ligands troglitazone and 15d-PGJ_2 inhibit the growth of bladder tumor cells. Of note, the antitumorigenic activity of PPARγ ligands was reported recently in an in-vivo model of bladder cancer [58]. Bacillus Calmette–Guerin, which is considered to be one of the most effective therapeutic reagents for superficial and in-situ bladder cancer treatment, induces functional PPARγ in bladder tumor cells *in vivo* and *in vitro* [59]. Recently, PPARγ-independent induction of growth arrest and

apoptosis in bladder carcinoma cells by troglitazone has also been reported [60].

PPARγ is also expressed in normal ovaries and different pathological grades of ovarian tumors of serous, mucinous, endometrioid, clear cell and mixed subtypes, and its expression is significantly higher in malignant tumors than benign tumors and normal ovaries [61]. PPARγ is also expressed in human endometrial cancer cells, and the treatment of cultured endometrial carcinoma cells with 15d-PGJ_2 significantly reduced their proliferation and increased cell death [62,63].

PPARγ is expressed at high levels in each of the major histologic types of human liposarcoma. Moreover, primary human liposarcoma cells can be induced to undergo terminal differentiation by treatment with the PPARγ ligand pioglitazone, suggesting that the differentiation block in these cells can be overcome by maximal activation of the PPARγ pathway [63]. Initial clinical trials with PPARγ ligand troglitazone reported promising results in liposarcoma [64].

Normal and malignant B lineage cells also express PPARγ and die by apoptosis after PPARγ ligand exposure. The RXR ligand 9-*cis*-retinoic acid in combination with PPARγ ligands greatly enhanced multiple myeloma cell killing [65]. PPARγ also appears to have potential antineoplastic effects in leukemia through inhibition of cell proliferation, induction of apoptosis and terminal differentiation, as well as inhibition of angiogenesis [66]. PPARγ was expressed in acute lymphocytic leukemia cells and its ligands, such as the synthetic TZD-class ligand (pioglitazone) and 15d-PGJ_2 also potently inhibited growth and induced apoptosis of human acute lymphocytic leukemia cells including Ph^+ cells [67–69].

Conclusion

In summary, although their exact role in controlling tumor growth and apoptosis in humans remains undefined, PPARγ has been implicated as a tumor suppressor in most cancers. Only in very few cancers (e.g. colon and breast) have there been concerns about its tumor promoter potential. Clearly, a better understanding of the mechanisms of action of activated PPARγ in tumors is required. Hence, many studies are underway to test the effects of targeting this receptor for therapeutic purposes. Down-regulation of this receptor pathway while minimizing side-effects will be challenging, but it appears that selective PPARγ modulation of desired gene sets is possible through targeting corepressor and coactivators, among other mechanisms. The availability of PPARγ ligands in the clinical arena will aid in investigations directed at studying the true role of PPARs in malignant tumors.

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