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Review Article

Potential of Peroxisome Proliferator-Activated Receptor Gamma Antagonist Compounds as Therapeutic Agents for a Wide Range of Cancer Types

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PPARγ is a therapeutic target that has been exploited for treatment of type II diabetes mellitus (T2DM) with agonist drugs. Since PPARγ is expressed by many hematopoietic, mesodermal and epithelial cancers, agonist drugs were tested and shown to have both preclinical and clinical anticancer activity. While preclinical activity has been observed in many cancer types, clinical activity has been observed only in pilot and phase II trials in liposarcoma and prostate cancer. Most studies address agonist compounds, with substantially fewer reports on anticancer effects of PPARγ antagonists. In cancer model systems, some effects of PPARγ agonists were not inhibited by PPARγ antagonists, suggesting noncanonical or PPARγ-independent mechanisms. In addition, PPARγ antagonists, such as T0070907 and GW9662, have exhibited antiproliferative effects on a broad range of hematopoietic and epithelial cell lines, usually with greater potency than agonists. Also, additive antiproliferative effects of combinations of agonist plus antagonist drugs were observed. Finally, there are preclinical in vivo data showing that antagonist compounds can be administered safely, with favorable metabolic effects as well as antitumor effects. Since PPARγ antagonists represent a new drug class that holds promise as a broadly applicable therapeutic approach for cancer treatment, it is the subject of this review.

1. INTRODUCTION

PPARγ is one of the three known peroxisome proliferator-activated receptors and is a member of the nuclear receptor (NR) superfamily. Since it has a predominantly nuclear location, regardless of whether cognate ligands are present, it is classified as a type II NR. It functions as a transcription factor by heterodimerizing with the retinoid X receptor (RXR), after which this complex binds to specific DNA sequence elements called peroxisome proliferator response elements (PPREs) [1]. In order to become fully active as a transcription factor, PPARγ must be bound by ligand. RXR can be affected by binding its own cognate ligands, usually resulting in incremental increases in transcriptional activity. After the PPARγ/RXR heterodimer binds to PPREs in promoter regions of target genes, coactivator proteins, such as p300 (CBP), SRC-1, and Drip205 (or TRAP220) family members, are recruited to this complex to modulate gene transcription [2–4]. Different PPARγ ligands appear to be able to recruit different coactivators, which may explain differences in the biological activity between ligands [5].

The cardinal biologic activity of PPARγ is the induction of differentiation of adipocytes, the cell type that expresses the highest levels of PPARγ amongst normal tissues. Lower levels of PPARγ are, however, found in other normal tissues and cell types such as skeletal muscle, liver, breast, prostate, colon, type 2 alveolar pneumocytes, some endothelial cells as well as monocytes, and B-lymphocytes. There are three *PPARγ* mRNA isoforms (γ1, γ2, and γ3) and two major protein species (γ1 and γ2). The mRNA isoforms are generated by alternate promoter usage, resulting in an additional 28 amino acids at the N-terminus of PPARγ2 compared with PPARγ1. Most tissues express PPARγ1, whereas the PPARγ2 isoform is expressed mostly by adipocytes. The longer N-terminal domain of PPARγ2 may affect function, since this isoform was shown to confer a higher level of ligand-independent transcriptional activity, which was further increased by physiologic concentrations of insulin [6]. High

levels of PPARγ expression by fat and its role in adipogenesis led to the recognition that agonistic PPARγ ligands have antidiabetic effects. The chemical class of PPARγ agonists known as thiazolidinediones (TZDs) demonstrated high-affinity binding to PPARγ [7] as well as favorable therapeutic properties, and such drugs were eventually registered for the treatment of type II diabetes mellitus (T2DM). Three TZD drugs have been registered in the U.S.: rosiglitazone (Avandia), pioglitazone (Actos), and troglitazone(Rezulin). Subsequent to its marketing and widespread use, troglitazone was associated with idiosyncratic and, in rare cases, fatal hepatic toxicity, and, thus, was withdrawn from the market. The former two drugs, however, have remained as safe and effective therapeutic options for the management of T2DM.

Not long after reports of the cloning of PPARγ and its expression in normal tissues [8, 9], PPARγ expression was observed in an array of primary cancers and derivative cell lines. Its expression was reported initially in liposarcoma [10], and soon thereafter in colon, breast, and prostate carcinomas and additional cancer types [11–14]. In addition to the in vitro and preclinical in vivo anticancer effects of TZDs, pilot clinical studies using troglitazone showed antitumor activity in patients with liposarcoma and prostate cancer [15, 16]. Compounds from other chemical classes were also shown to bind PPARγ and to have antiproliferative effects in cancer models, such as the naturally occurring eicosanoid, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2(15-d-PGJ_2), the N-aryl tyrosine derivative, GW1929 [17], and the triterpenoid, 2-cyano-3,12-dioxooleana-1,9-diene-28-oic acid, CDDO [18]. While compounds that exhibit PPARγ agonist activity, such as TZDs, have PPARγ-dependent antiproliferative effects, they have also been shown to have antiproliferative effects in cell types that are genetically PPARγ-null [19]. Also, uncertainty about mechanisms of anticancer effects of PPARγ ligands has resulted from variability in the classification of some compounds (e.g., bisphenol A diglycidyl ether [BADGE], which has been shown to have both agonist and antagonist activities) [20, 21].

2. EFFECTS OF PPARγ ANTAGONIST COMPOUNDS IN EPITHELIAL CANCER MODEL SYSTEMS: CELL GROWTH AND APOPTOSIS

The initial report of Fehlberg et al. [22] showed an inhibitory effect of this class of agents on a colon cancer and a lymphoma cell line using the compound, BADGE, which as noted has been classified as both an agonist and antagonist. This initial study did not examine effects on proliferation, but showed that apoptotic effects, such as increases in annexin-V binding and reductions in DNA content as assessed by propidium iodide staining, required 50–100 μM concentrations of BADGE, which would tend to increase off-target effects. Subsequently, Seargent et al. [23] showed that a higher affinity, selective PPARγ antagonist, GW9662, had direct antiproliferative effects on three breast cancer cell lines of differing phenotypes (ER+, ER−, and p53-null). This antagonist compound was somewhat more potent in its effects than an agonist (rosiglitazone). In this report, the role of PPARγ in mediating growth inhibition was addressed, but not fully elucidated. All three cell lines expressed it and the predicted, canonical PPARγ-related transactivation effects were demonstrated, with the agonist inducing transactivation and the antagonist suppressing it, thus excluding PPARγ-mediated transactivation as the mechanism of this effect. There are data, however, that suggest that antagonist-type compounds may also act via other PPARγ-dependent pathways. Lea et al. reported similar results using a range of agonist and antagonist compounds on both murine and human cell lines [24]. Schaefer et al. showed that the antiproliferative effect of the PPARγ antagonist, T0070907, on hepatocellular carcinoma cell lines was attenuated by knockdown of PPARγ by siRNA [25]. These data are consistent with a PPARγ-mediated transrepression mechanism, which has been demonstrated with respect to anti-inflammatory effects of PPARγ ligands mediated by the NF-κB signaling pathway. Pascual et al. showed similar effects of a pure agonist (rosiglitazone) and a mixed agonist/antagonist (GW0072) on the repression of a NF-κB-regulated gene, *iNOS*, suggesting that pure antagonists may also be capable of mediating this effect [26].

There are also data that PPARγ ligands (both agonist and antagonist) exert PPARγ-independent effects suggesting other cellular targets of these compounds. This was demonstrated clearly by Palakurthi et al., who demonstrated in vitro and in vivo growth inhibition of two agonist compounds, troglitazone and ciglitazone, in experiments utilizing PPAR$\gamma^{-/-}$ and PPAR$\gamma^{+/+}$ embryonic stem cell lines (ES), both of which exhibited very similar sensitivity to these compounds [19]. This effect was shown to be mediated in part by the inhibition of the initiation of protein translation, since these TZD compounds increased the phosphorylation and consequent inactivation of elongation-initiation factor 2 (eIF2) both in cells that expressed and were null for PPARγ. The effect of antagonist compounds on this pathway has not been reported. As noted, BADGE had similar proapoptotic effects in a colon cancer line expressing PPARγ and a T-lymphoma line that showed no detectable expression of it (by immunoblotting and RT-PCR) of this target [22]. But, given the variable classification of this compound as both an antagonist and agonist, the mechanism underlying this effect and its attribution are unclear.

3. OTHER EFFECTS OF PPARγ ANTAGONIST COMPOUNDS

PPARγ antagonist compounds have also been shown to affect cell shape, adhesion, and invasiveness of cancer cell lines. Masuda et al. evaluated the effects of the PPARγ antagonists, BADGE, GW9662 and T0070907, on four squamous carcinoma cell lines derived from tumors of the oral cavity. Antiproliferative effects were shown for the three antagonists, but not for the agonist, pioglitazone [27]. Effects of these agents on adhesion and anoikis were also evaluated. Antagonists were found to inhibit adhesion and induce cell death related to loss of adhesion (known as anoikis) under normal tissue culture conditions on untreated plastic dishes. T0070907 induced similar inhibition of adhesion to fibronectin-coated plates, and this was significantly reversed

by coincubation of cells with this antagonist and the agonist, pioglitazone, suggesting a PPARγ-dependent effect. Since adhesion and detachment are related to cytoskeletal structure and function, this was assessed by fluorescent staining of F-actin. Using confocal microscopy, T0070907 was shown to cause dose-dependent disruption of F-actin, associated with rounding of the cells. Additional experiments showed inhibition of FAK and MEK-ERK signaling pathways, as well as decreased expression of integrin α5 and CD151, both of which are adhesion proteins that have been implicated in cancer cell invasion and metastasis. Schaefer et al. showed similar effects of PPARγ antagonists on hepatocellular carcinoma cell lines including inhibition of adhesion, induction of anoikis, and inhibition of phosphorylation and activation of FAK [25]. These effects were shown to be dependent on the degree of PPARγ inhibition, and could be mediated by the antagonist or knockdown of PPARγ via specific, cognate siRNA. T0070907 was also shown to have substantially greater growth inhibitory effects on the HepG2 line compared with the agonist drugs, troglitazone, and rosiglitazone. Takahashi et al. demonstrated anti-invasive and growth inhibitory effects of the antagonists, GW9662 and T0070907, on esophageal cancer cell lines. The anti-invasive effects were observed at levels substantially lower than those required for growth inhibition [28]. In summary, all of these studies addressing anticancer effects of PPARγ antagonist compounds have show effects on cell growth, adhesion, and invasion in multiple epithelial cancer models.

Some of these effects are PPARγ-dependent, but the potential role of other targets is suggested by the similar effects of BADGE on a PPARγ+ colon cancer line and a PPARγ-negative T-lymphoma line. Also, the substantially different concentrations of PPARγ antagonists needed to induce anti-invasive effects versus growth inhibition in esophageal cancer lines suggest different mechanisms with differing degrees of PPARγ dependence or lack of involvement of the PPARγ-signaling pathway for some effects. A PPARγ-independent effect of antagonists on colorectal cancer cell lines and in an in vivo tumor xenograft derived from one of the lines was shown in a more recent report by Schaefer et al. [29]. A decrease in tubulin levels was observed that was independent of PPARγ, PPARδ, and proteasome function. This downregulation of tubulins α and β may explain the antimigratory, anti-invasive, and antimetastatic effects that were observed. Thus, in summary, PPARγ antagonist compounds with varying chemical structures (though GW9662 and T0070907 are similar) have several significant anticancer effects in vitro and in vivo in epithelial cancer model systems including breast, colon, aerodigestive squamous cell, and hepatocellular.

4. EFFECTS OF PPARγ ANTAGONISTS IN HEMATOPOIETIC CANCER MODEL SYSTEMS

Studies were conducted in our lab to assess the effects of PPARγ antagonists on hematopoietic cell lines. Initial screening showed that several myeloma (MM) cell lines had the greatest sensitivity to the antiproliferative effects of the antagonists, GW9662 and T0070907. Thus multiple MM lines were tested, including one that is IL-6-dependent, for sensitivity to these compounds as well as to the agonist, pioglitazone. MM lines as well as non-Hodgkin lymphoma (NHL) lines showed significantly greater sensitivity to the growth inhibitory effects of the two antagonist drugs compared with the agonist [30]. As a group, the MM lines were more sensitive than the other groups of cancer cell lines to the antiproliferative effects of the antagonists, particularly T0070907. Other goals were to directly compare the sensitivity of previously tested epithelial cancer types (breast and colon) to hematopoietic lines (MM and NHL) as well as to evaluate a chemoresistant epithelial cancer type (renal cell). These experiments showed that in all the epithelial and hematopoietic cell lines tested, the antagonists were significantly more potent in their growth inhibitory effects compared with the agonist drug.

The IC_{50} values for the panel of 16 cell lines tested in these studies are shown in Table 1. For each of the cell lines in the panel, significant differences in the IC_{50} values of the antagonist compounds and the agonist drug, pioglitazone, were observed (P values ranging from <.04 to <.001, with 12 of 16 lines at <.001). While the MM lines showed the greatest sensitivity to the antagonists, similar degrees of sensitivity to the antagonists were also seen in the subset of breast cancer lines, which included two lines that are estrogen receptor-negative. Though not quite as sensitive as a subset, significant differences between the antagonists and the agonist were also observed in the renal cell lines, which are among the most chemoresistant epithelial lines. The differential sensitivities within and across cell lines did not appear to be related to the levels of PPARγ expression. Also, neither the agonist nor the antagonist induced significant upregulation of PPARγ as has been reported in some studies with PPARγ ligands. Consistent with prior reports, combinations of the agonist and with each of the antagonists did not result in attenuation of growth inhibitory effects. In fact, schedule-dependent increases in growth inhibition were observed, particularly when the antagonists were added to cells 24 hours prior to the agonist. Aspects of the mechanisms of cytotoxicity of the antagonists and agonists were also compared. It was shown that both classes of PPARγ ligand-induced apoptotic effects, but this effect was found to be caspase-independent for the agonist, pioglitazone [30].

Another question that was addressed was the impact of IL-6 on the responses of the MM lines to PPARγ antagonists, since this is a cytokine that plays a central role in the pathogenesis and progression of MM, as well as other cancer types. For these studies, 4 of the 5 MM lines that were utilized were IL-6-independent in order to follow up on a previous report of Wang et al. that analyzed the responses of three MM lines to the PPARγ agonists, 15-d-PGJ_2 and troglitazone. This report showed that growth inhibition and certain downstream signaling events were PPARγ-dependent, and also that two IL-6-dependent MM lines expressed PPARγ while an IL-6-independent line did not [31]. Also, GW9662 was reported to block the effects of the agonists, and had no antiproliferative activity on its own. We utilized five different MM lines, of which four are IL-6 independent (CAG, KMS12-BM, KMS12-PE, and OPM-6)

Table 1: Mean IC_{50} values (μM) for the PPARγ ligands.

Cell lines	Pioglitazone	T0070907	GW9662
Colon			
Moser[#]	26.5 ± 2.6	15.9 ± 1.0	20.1 ± 0.3
HT29[§]	53.0 ± 4.7	11.2 ± 0.0	14.1 ± 0.5
LS174T[#]	38.7 ± 7.4	7.8 ± 1.9	9.5 ± 0.5
HCT-15[§]	53.1 ± 2.5	13.0 ± 0.5	19.0 ± 0.8
RCC			
A498[#]	38.9 ± 4.9	24.3 ± 0.7	29.1 ± 0.3
ClearCa-2[§]	56.4 ± 3.1	20.8 ± 1.9	21.5 ± 0.7
Breast			
ZR75-30[§]	77.9 ± 7.0	3.9 ± 0.3	10.6 ± 0.9
MCF7[§]	54.8 ± 3.9	10.2 ± 1.9	16.6 ± 2.4
MDA-MB-231[§]	78.7 ± 3.5	20.1 ± 1.1	26.8 ± 1.0
MM			
CAG*	62.4 ± 9.9	12.2 ± 1.2	13.8 ± 0.1
KMS12-BM[§]	33.2 ± 5.1	3.2 ± 0.6	11.8 ± 1.6
KMS12-PE[§]	56.4 ± 1.5	4.3 ± 0.3	9.5 ± 0.9
OPM6[§]	48.9 ± 1.8	4.1 ± 0.3	11.5 ± 0.1
U266B1[§]	56.6 ± 1.3	9.9 ± 0.2	29.7 ± 1.5
NHL			
Ramos[§]	66.5 ± 7.4	12.7 ± 0.7	15.1 ± 0.1
SU-DHL6[§]	53.1 ± 1.4	11.8 ± 0.4	14.8 ± 0.3

Mean IC_{50} values from replicate experiments with this panel of cells for each of the three PPARγ ligands are shown above, expressed in μM ± SEM. Cell lines are grouped according to cancer type. IC_{50} values from each cell line were compared by single factor ANOVA analysis, with all lines showing significant differences as indicated: [§]$P < .0001$; *$P < .005$; [#]$P < .04$.

as well as a fifth that is dependent on an IL-6 autocrine loop (U266B1). In contrast to the prior report cited above, of the lines analyzed, CAG expressed PPARγ, while the autocrine IL-6-dependent line, U266B1, did not express PPARγ by immunoblotting. Also, three of the four of IL-6-independent MM lines were more sensitive to the growth inhibitory effects of both of the two PPARγ antagonist compounds compared with the IL-6-dependent line, U266B1 (see Table 1).

In MM cell lines, which are more often IL-6 dependent compared with other B cell lines, the strict dependence on exogenous IL-6 is indicative of ongoing requirement for this signaling pathway, which in pathophysiologic states, such as MM, usually depends on production of this cytokine by stromal cells. In MM, clinically more aggressive or treatment-resistant disease is associated with production of IL-6 by the myeloma cell themselves as opposed to the bone marrow stroma [32]. MM lines show a spectrum of IL-6 dependence, with some being dependent on exogenous IL-6, others being dependent on its autocrine production, and yet others being IL-6-independent for their growth. Even those MM lines that are not strictly dependent on IL-6 for their growth (exogenous or autocrine) can still be affected by the addition of exogenous IL-6 [33] (also shown in one of the lines tested, OPM-6, [34]). Addition of IL-6 to such MM lines has been shown to induce either incremental stimulation of proliferation or induction of resistance to various agents such as dexamethasone, standard chemotherapy drugs such as melphalan and other agents. Thus the interaction of IL-6 and PPARγ antagonist compounds were examined in two MM lines (KMS12-PE and OPM-6). MTT assays were performed in the presence and absence of exogenous IL-6 (5 ng/mL). For both of these MM lines, addition of IL-6 did not induce resistance, but instead appeared to increase the sensitivity of these lines to T0070907, with a similar trend observed with GW9662 [30].

5. DOSE-RESPONSE EFFECTS OF PPARγ ANTAGONIST COMPOUNDS AND INTERACTION WITH OTHER AGENTS

The PPARγ antagonist compounds, GW9662 and T0070907, differ in their antiproliferative dose-response effects compared with the agonist as well as other agents. Not only are the corresponding IC_{50} values for the antagonists significantly lower than the agonist, pioglitazone, but a greater degree of growth inhibition (85–97% versus 50–80%) was observed with the former compounds. Also, of note was that the maximal effects of these agents were seen at concentrations that were only 2- to 3-fold greater than the IC_{50} across the entire panel of cell lines tested that included cell lines with relative and very high levels of chemoresistance (colon and renal cell, resp.). The dose-response curves were much steeper with the antagonist compounds compared to the agonist, pioglitazone, and also much steeper than what is observed with most other agents, including standard chemotherapy drugs and other agents (see Figure 2). This dose-response relationship suggests either a positive cooperative effect, potentially via increased, cooperative recruitment of corepresssors, thereby increasing transrepression. The alternate possibility is that different targets are being engaged with gradually increasing concentrations, which together exhibit additive or supra-additive interactions.

Since MM lines as a group were the most sensitive of the cell lines we tested, interaction with other novel agents for therapy of MM were evaluated. One such agent is anti-CD74 monoclonal antibody (mAb). CD74 was shown to be strongly expressed by the malignant plasma cells in the vast majority of clinical MM specimens as well as the majority of MM lines [35]. It was also shown that this mAb in unlabeled (cold) form exhibited in vitro growth inhibitory effects on both NHL and MM lines [36]. The anti-CD74 mAb used in these studies, LL1, also showed significant therapeutic effects in two preclinical murine NHL xenograft models. In preliminary in vitro studies, the humanized anti-CD74 mAb was combined with T0070907 in two MM lines. These studies also evaluated a sixth MM line (KMS11), which is IL-6 independent, expresses CD74 and is useful as a murine MM xenograft model. This line showed similar sensitivity to T0070907 as the other IL-6-independent lines, with an (unpublished observations, J Burton). Another IL-6-independent MM line that was used in initial studies, KMS12-PE, was also used to evaluate interactions between T0070907 and the hLL1 mAb. While KMS11 line showed moderate sensitivity to hLL1 (maximum growth inhibition

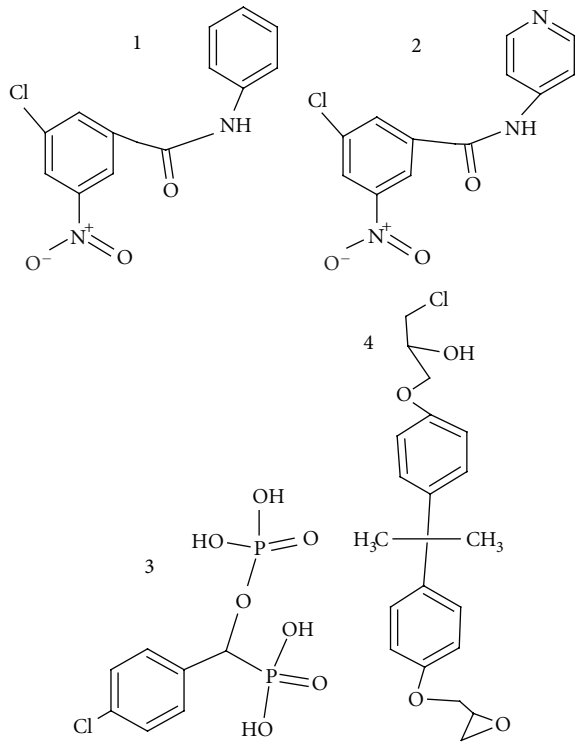

Figure 1: The chemical structures of four PPARγ antagonists: (1) GW9662, (2) T0070907, (3) SR-202, and (4) BADGE.

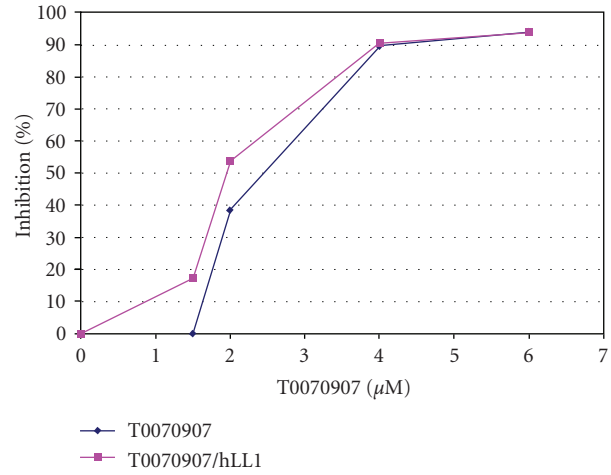

Figure 2: Dose-response curves for the MM line, KMS12-PE, to T0070907, both in the presence and absence of the hLL1 mAb. Square symbols represent the dose-response curve in the presence of hLL1, and diamond symbols represent the curve in the absence of LL1. The ordinate shows percent growth inhibition values and the abscissa the concentration of T0070907 in micromolar.

of 50–70%), the KMS12-PE line was resistant to single-agent hLL1 (<10% inhibition). However, in combination with T0070907, there was a sizable shift to the left of the dose-response curve, as is shown in one representative experiment in Figure 2. Current data indicate that the IC_{50} value decreases by from a mean value of ~4.1 μM for T0070907 alone versus ~3.0 μM with T0070907 in combination with hLL1, suggestive of a supra-additive effect (25–30% observed versus <8% expected based effect of hLL1 alone). This is a promising initial preclinical lead given that hLL1 is now being evaluated in several phase I/II clinical trials in B-cell cancers such as NHL and MM, and appears to be safe and well tolerated.

6. OVERVIEW OF MECHANISMS OF ACTION OF PPARγ AGONIST AND ANTAGONIST COMPOUNDS

The studies reviewed above have shown that the effects of PPARγ ligands are mediated by various mechanisms. Some studies show or suggest canonical PPARγ-mediated effects (i.e., via transactivation), as exemplified by early in vitro studies with agonist compounds that showed fat accumulation, a major PPARγ-mediated effect, in both breast cancer and liposarcoma cell lines [10, 12]. This was also demonstrated in liposarcoma patients in whom increased fat content within tumors was demonstrated by serial CT scanning before and after treatment with an agonist drug [10]. The studies of Wang et al. showed that the growth-inhibitory effects of PPARγ agonist compounds on MM lines was seen only in lines expressing PPARγ and that these effects were reversed by cotreatment with an antagonist compound [31]. In contrast, completely PPARγ-independent effects were demonstrated for both agonist and antagonist compounds in reports from Palakurthi et al. [19] and Schaefer et al. [29]. This was clearly shown for the agonist compounds, troglitazone and ciglitazone, which showed similar antiproliferative effects in PPARγ-wild type and PPARγ-null (knockout) embryonic stem cell lines, both in vitro and in vivo [19]. PPARγ-independent growth inhibitory and antimetastatic effects of several antagonist compounds were shown in both in vitro and in vivo studies using three colon carcinoma cell lines. These effects were associated with reductions in tubulin levels and were also shown to be independent of PPARδ and proteasome function. The PPARγ-independent effect of agonist compounds was shown to be associated with inhibitory effects on the protein translation pathway. The mechanism of PPARγ-independent effects of antagonist compounds on tubulin levels has not been elucidated.

The mechanism of PPARγ-mediated transrepression may explain some of the effects of antagonist compounds. This was suggested by the attenuation of the effects of antagonist compounds by PPARγ knockdown by siRNA in hepatocellular carcinoma cell lines [25]. Also, the observation that combinations of PPARγ agonist and antagonist compounds result in additive antiproliferative effects in various cancer cell lines [24, 30] is consistent with this mechanism. This mechanism is plausible, as it has been shown to inhibit the NF-κB signaling pathway, which is central to inflammation and to the proliferation and survival of multiple cancer types including hepatocellular and colon carcinomas as well as multiple myeloma. The potential role of this and other mechanisms remain to be determined.

7. SUMMARY OF PRECLINICAL STUDIES OF PPARγ ANTAGONIST COMPOUNDS AND THEIR CLINICAL POTENTIAL

The studies reviewed above have shown that PPARγ antagonists have in vitro and preclinical in vivo anticancer effects that are as broad and potent as agonist compounds. These effects have been demonstrated in a wide range of epithelial cancer cell lines as well as hematopoietic cancer cell lines. Exploration of the underlying mechanisms of action for antagonist compounds has shown either involvement of PPARγ or a PPARγ-independent effect. One study suggested the involvement of the canonical transactivation mechanism in that antagonist effects were antagonized by coincubation with an agonist compound, pioglitazone [27]. In another study, where knockdown of PPARγ affected responses to antagonist compounds, the effect was not consistent with the canonical transactivation mechanism, but may be consistent with a transrepressive mechanism [25]. Another study showed that anticancer effects were associated with reductions in tubulin levels (a validated cancer-related target), but this was not mediated by PPARγ, PPARδ, or the proteasome [29].

While there have been numerous preclinical in vivo studies in cancer models with PPARγ agonists, there have been relatively few with antagonist compounds. Also agonists have been tested clinically. Some studies with antagonists have been conducted in noncancer models at low doses ($\leq$1 mg/kg), which were not toxic and biologically active [37, 38]. A chemically distinct, but selective PPARγ antagonist, SR-202, has been synthesized and evaluated in preclinical models (Figure 1). It was given at a dose of 400 mg/kg for periods of up to 10 weeks with favorable metabolic effects such protection against diet-induced hyperinsulinemia and reduction in hyperinsulinemia and hyperglycemia in genetically predisposed (ob/ob) mice [39]. In pilot studies, we have administered moderate doses of GW9662 (15 mg/kg) and T0070907 (7.5 mg/kg) daily for 3 weeks by the intraperitoneal route to immunodeficient mice. These doses and schedules were well tolerated and resulted in no signs of toxicity (unpublished observations). These data indicate that the doses of these antagonists that may be sufficient for anticancer therapy are well tolerated, paving the way for further development of these agents for treatment of cancer.

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