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## Minireview

# Peroxisome Proliferator-activated Receptor $\gamma$ and Cancers<sup>1</sup>

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### Abstract

The peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) ligands have anticancer activity against a wide variety of neoplastic cells *in vitro*. Animal studies have chronicled their *in vivo* anticancer effects and chemopreventive capabilities. In addition, moderate anticancer activities of PPAR $\gamma$  ligands with minimal toxicities have been observed in patients with liposarcomas and prostate cancers. These compounds can slow growth and induce partial differentiation of selected cancer cells. They can decrease levels of cyclin D<sub>1</sub> and E, inflammatory cytokines, and nuclear factor  $\kappa$ B and increase expression of p21<sup>waf1</sup> and p27<sup>kip1</sup>. Surprisingly, some or many of these effects may occur independently of PPAR $\gamma$ . Other data suggest that PPAR $\gamma$  may behave as a tumor suppressor gene, although several compelling murine models, paradoxically, suggest that under selected circumstances, PPAR $\gamma$  ligands may stimulate cancer formation. Nevertheless, the bulk of studies showed that PPAR $\gamma$  ligands do have antiproliferative activity against many transformed cells and may be helpful in the setting of adjuvant and chemopreventive treatments of several common tumors, including colon, prostate, and breast cancers.

### Introduction

The PPAR $\gamma$ <sup>3</sup> is a member of the NHR superfamily (1–4). It functions as a transcription factor after it heterodimerizes with the RXR and binds to specific response elements called peroxisome proliferating response elements (5). To act as a transcription factor, PPAR $\gamma$  requires activation by binding its ligand. Furthermore, the RXR member of this complex can also bind

simultaneously with its ligand, which can result in enhanced transcriptional activity (6). The activated PPAR $\gamma$ /RXR heterodimer attaches to the peroxisome proliferating response element of a target gene, and coactivator proteins are recruited such as p300 (CBP), SRC-1, as well as the Drip205 family of proteins (also known as TRAP 220) to modulate gene transcription (7). Different ligands of PPAR $\gamma$  appear to be able to recruit different coactivators (8, 9), which might provide specificity of biological activity of PPAR $\gamma$ .

Adipocyte tissue has some of the highest levels of PPAR $\gamma$ . However, the receptor is expressed in many other tissues and cell types throughout the body, including monocytes and macrophages (10), liver (11–13), skeletal muscle (14), breast (15), prostate (16), colon, and type 2 alveolar pneumocytes. Two PPAR $\gamma$  isoforms are derived by alternate promoter usage. Most tissue express the PPAR $\gamma$  1 isoform, whereas the PPAR $\gamma$  2 isoform is specific to adipocytes. Although a formal study has not been reported, cancer cells probably express an equivalent amount of PPAR $\gamma$  as their normal counterparts.

The 15-deoxy- $\Delta^{12,14}$ -prostaglandin J<sub>2</sub> is a naturally occurring PPAR $\gamma$  ligand. This eicosanoid can activate PPAR $\gamma$  at micromolar concentrations. Some unsaturated fatty acids also are natural ligands of the receptor. Synthetic PPAR $\gamma$  ligands, known as TZDs, include rosiglitazone (Avandia), pioglitazone (Actos), and troglitazone (Rezulin). The latter causes a severe idiosyncratic liver problem and thus has been discontinued. The TZDs are a breakthrough in the therapy of type II diabetes mellitus because they decrease insulin resistance. TZDs enhance insulin action by increasing glucose uptake in the peripheral tissue as well as reducing the hepatic glucose output, and a strong correlation occurs between the ability of the ligand to activate PPAR $\gamma$  and the antidiabetic potency of the PPAR $\gamma$  ligand. A recently synthesized triterpenoid [2-cyano-3,12-divoaleana-1,9-diene-28-oic acid] can bind to PPAR $\gamma$  and can induce differentiation and inhibit proliferation of a variety of cancer cells; and it has anti-inflammatory activity (17, 18).

Most of the known target genes that are transcriptionally activated by PPAR $\gamma$  belong to the pathway of metabolism and transport of lipids, including lipoprotein lipase, adiponectin, fatty acid binding protein, Acyl-CoA synthase, and fatty acid transport protein. Furthermore, the PPAR $\gamma$  can mediate differentiation of the preadipocyte to adipocytes (19–21). The ligands of PPAR $\gamma$  can also repress transcription. For example, in monocytes and macrophages, they can lower the expression of cytokines such as IL-4 as well as proinflammatory products, including TNF, IL-1, as well as inducible nitric oxide synthetase (22–26). The proinflammatory transcription factors such as AP1, Stat, and NF- $\kappa$ B can also be inhibited. The mechanism by which this occurs is unclear. PPAR $\gamma$  can form weak interactions with the corepressors NCOR and SMRT (27). On the other hand, the repression produced by these compounds may be independent of acting as a ligand of PPAR $\gamma$  (discussed later). The target genes that mediate the anticancer activity of the activated PPAR $\gamma$  are unclear.

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<sup>3</sup> The abbreviations used are: PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; NHR, nuclear hormone receptor; RXR, retinoid X receptor; TZD, thiazolidinedione; TNF, tumor necrosis factor; IL, interleukin; NF- $\kappa$ B, nuclear factor  $\kappa$ B; APC, adenomatous polyposis coli; TCF, T-cell factor; COX-2, cyclooxygenase 2; PSA, prostate-specific antigen.

Table 1 Cancers with growth that is decreased by PPAR $\gamma$  ligands

Names
Liposarcoma
Colon cancer
Breast cancer
Prostate cancer
Myeloid leukemia
Neuroblastoma
Glioblastoma
Lymphoma
Lung cancer
Cervical cancer
Bladder cancer
Head and neck cancer
Esophageal cancer
Gastric cancer
Pancreatic cancer
Choriocarcinoma

### Cancer and PPAR $\gamma$

Cancer cells often obtain their proliferative advantage over their normal counterparts at least, in part, because of their inability to undergo terminal differentiation. They remain in the proliferative pool providing themselves with a growth advantage. Activation of NHRs has been identified as an approach to induce differentiation and inhibit proliferation of cancer cells. The best example of this paradigm is the induction of remission of patients with acute promyelocytic leukemia using all-*trans*-retinoic acid (28, 29). All-*trans*-retinoic acid has also been used to prevent recurrence of head and neck cancers. Because normal preadipocytes can be induced to undergo terminal differentiation in the presence of ligands for PPAR $\gamma$ , investigators were encouraged to use TZDs to attempt to induce differentiation of human liposarcoma cells *in vivo* (30). Successes *in vitro* encouraged these same physician-scientists to give troglitazone to a series of patients with liposarcoma, which resulted in a retardation of growth and induction of differentiation of these tumor cells (31). The long-term effect of TZD on liposarcomas requires further study; nevertheless, these pioneer studies have spurred the examination of the effect of TZDs on a number of cancers both *in vitro* and *in vivo*. Table 1 provides a list of cancers in which PPAR $\gamma$  ligands have been shown to have an antiproliferative activity.

The genes regulated by PPAR $\gamma$  ligands in cancer cells that provide the antiproliferative and prodifferentiation activity are unclear. Often, the expression of the cyclin-dependent kinase inhibitors p21<sup>Waf1</sup> and p27<sup>Kip1</sup> increase in the cancer cells exposed to PPAR $\gamma$  ligands, perhaps by inhibition of the ubiquitin-proteasome protein degradation pathway (32, 33). Perhaps blunting of expression or activity of a variety of inflammatory cytokines and transcription factors (*i.e.*, TNF, IL-1, IL-4, NF- $\kappa$ B) may slow growth of the transformed cells. Also, the PPAR $\gamma$  ligand can induce apoptosis of cells with the activation of caspase-3; this is associated in some cancer cells with a decreased expression of the antiapoptotic proteins, BCL-2 and BCL-X<sub>L</sub>. DNA microarray technology was used to attempt to identify target genes of PPAR $\gamma$  in colon cancer cells using PPAR $\gamma$  agonists and antagonists. Many of the PPAR $\gamma$  selected targets were linked to the growth regulatory pathways (34).

### Colon Cancer

Approximately 5% of individuals will develop colorectal carcinoma during their lifetime. It typically progresses from adenomatous polyps, dysplastic polyps to invasive carcinoma (35, 36). Colon cancer cells usually have a very high expression of PPAR $\gamma$  (13, 37, 38). Table 2 provides several key observations concerning colon cancer and PPAR $\gamma$  and its ligands. Several epidemiological studies suggest that PPAR $\gamma$  ligands have an anticolonic cancer activity. Polyunsaturated fatty acids such as those present in fish oil, as well as nonsteroidal anti-inflammatory drugs, can activate PPAR $\gamma$ , and their consumption is associated with prevention of colon cancer (39, 40). Also, more directly, TZDs ( $5 \times 10^{-6}$ – $10^{-5}$  M) decreased the growth of colon cancer cells *in vitro* as well as when growing as xenografts in nude mice (41). These levels can be achieved clinically (42). A rough correlation exists between sensitivity of the colon cancer cells to their inhibition of proliferation by TZDs and their level of expression of PPAR $\gamma$  (13).

Mutations of PPAR $\gamma$  have been reported in colon cancers (36). In a large collection of 55 colon cancer samples, four somatic PPAR $\gamma$  mutations were found, three in exon 5 and one in exon 3. These mutations included one nonsense, two missense, and one frameshift mutation. The alterations affected the function of PPAR $\gamma$  and were not found in normal leukocytes of the same individuals, showing that these changes were not hereditary (36). The paradigm for most tumor suppressor genes is mutation of one allele and loss of the second allele, which is not the case for PPAR $\gamma$  in colon cancer. Each of the cancers with a mutant PPAR $\gamma$  also had a normal PPAR $\gamma$  allele. This suggests that the altered protein could either exert a dominant negative effect on the normal PPAR $\gamma$  gene product, or loss of 50% expression of PPAR $\gamma$  enhances the transformation to colon cancer. The approximate 50% loss of protein expression as a result of loss of an allele is known as haplo-insufficiency. Other examples of haplo-insufficiency include monoallelic germ-line RET mutations in Hirschsprung disease (43), monoallelic germ-line mutations of PTEN leading to Cowden Syndrome associated with a risk of development of breast and thyroid tumors (44), and monoallelic germ-line mutations of the AML1 gene associated with familial thrombocytopenia progressing to myelodysplastic syndrome and eventually to acute myeloid leukemia (45).

The frequency of PPAR $\gamma$  mutations in colon cancer is unclear. We examined 380 tumors and cell lines, including 55 colon cancers, and were unable to find any samples that had a PPAR $\gamma$  mutation (46). This suggests that PPAR $\gamma$  mutations may occur in cancers, but they are exceedingly rare.

A murine model does point to the protective role of PPAR $\gamma$ . Mice with heterozygous germ-line deletions of PPAR $\gamma$  (PPAR $\gamma^{+/-}$ ) have an increased proclivity to develop carcinogen-induced colon cancer compared with wild-type mice (47). After injection of azoxymethane, all of the PPAR $\gamma^{+/-}$  mice, but only 50% of the wild-type mice, were dead of colon cancer by 36 weeks.

In contrast to the above investigations and observations, data from several groups draw into question the anticolonic cancer activity of PPAR $\gamma$  ligands. Two studies have shown that administration of a TZD to Mim mice resulted in these mice developing more frequent colon cancers than those animals

Table 2 PPAR $\gamma$  and colon cancer

PPAR $\gamma$ : role in preventing and treating colon cancer	PPAR $\gamma$ : association with colon cancer
<ol style="list-style-type: none"> <li>1. Highly polyunsaturated fats and nonsteroidal anti-inflammatory drugs activate PPAR<math>\gamma</math> and their consumption is associated with a lower incidence of colon cancer.</li> <li>2. PPAR<math>\gamma</math> ligands can inhibit growth, induce apoptosis, and cause differentiation of colon cancer cells <i>in vitro</i> and in xenografts.</li> <li>3. PPAR<math>\gamma^{+/-}</math> mice are more susceptible than wild-type mice to carcinogen-induced colon cancer.</li> </ol>	<ol style="list-style-type: none"> <li>1. Inactivating mutations of PPAR<math>\gamma</math> occur in a subset of colon cancers.</li> <li>2. Min (APC<math>^{-/-}</math>) mice develop colon cancer more frequently when given a PPAR<math>\gamma</math> ligand.</li> </ol>

which did not receive this PPAR $\gamma$  ligand (48, 49). Mim mice have a germ-line mutation of the APC gene resulting in an increased frequency of small and large intestinal adenocarcinomas. In one of these studies, the Mim mice that received troglitazone had an average of three colonic cancers/mouse, whereas those that received diluent had a mean of one colonic cancer/mouse (48). The Mim mice in the second study received two different PPAR $\gamma$  ligands; and these mice also had more colon tumors, which were also larger than the similarly treated wild-type mice. The number of small intestinal cancers was unchanged between the two groups, consistent with very little expression of PPAR $\gamma$  in the small intestine (49).

Why do Mim mice receiving a TZD have more colon cancers and what does it mean for humans? The answers to both queries are unclear. The APC protein normally binds to  $\beta$ -catenin, retaining the latter in the cytoplasm and enhancing its degradation. Mutant APC loses this ability, and thus, the colons of Mim mice have high levels of  $\beta$ -catenin, which enters the nucleus, and binds and increases the activity of the family of transcription factors, called TCFs. This leads to the transcriptional activation of a number of cell cycle-related proteins, including *c-myc* and cyclin D1 (50, 51). The treatment of Mim mice with TZDs, for unclear reasons, increases the expression of  $\beta$ -catenin in their colons (49). In contrast, the normal colonic epithelium, as it undergoes differentiation from the crypts to the tip of the villi, decreases its expression of  $\beta$ -catenin, which dampens the growth promoting effects of TCFs. Thus, Mim mice appear to have a dysregulation of the normal differentiation process. This has relevance for humans because mutation of the APC gene is often the initiating event for ~80–90% of sporadic colorectal tumors (52).

Evidence has implicated PPAR $\delta$  in contributing to the development of colon cancer. Colon cancer cells often have high expression of PPAR $\delta$  (53), perhaps because inactivating mutations of APC leads to an active  $\beta$ -catenin/TCF4 transcription complex, which can enhance transcription of PPAR $\delta$  (36, 51, 53, 54). PPAR $\delta$  can have an antiapoptotic effect on colon cancer cells, thus providing these cells a growth advantage over their normal counterparts (54). PPAR $\delta$  may bind to the same target genes as PPAR $\gamma$ , but it also binds tightly to corepressors. Therefore, PPAR $\delta$  may silence PPAR $\gamma$  target genes similar to how retinoic acid receptor responsive genes are repressed by PML-retinoic acid receptor  $\alpha$  in acute promyelocytic leukemia.

Furthermore, the COX-2 gene is highly expressed in ~70–80% of human colorectal carcinomas (55, 56). These high levels of COX-2 can result in elevated production of prostaglandin H2

that is rapidly converted to prostaglandin PGI<sub>2</sub>, PGF<sub>2</sub> $\alpha$ , PGD<sub>2</sub>, and thromboxane A<sub>2</sub>. Prostaglandin I<sub>2</sub> can activate PPAR $\delta$  (57, 58). Thus, colon cancer cells can have stimulation of PPAR $\delta$  from two directions, APC mutations causing hyperactivity of  $\beta$ -catenin/TCF4 and high levels of COX-2.

Interestingly, when COX-2 knockout mice were mated with Mim mice, the number and size of intestinal polyps markedly decreased consistent with COX-2, playing an important role in promoting the development of tumors in the APC mutant genetic background (59–61). Likewise, treatment of Mim mice with selective COX-2 inhibitors can reduce the number of polyps that develop (59). Clearly, additional studies are required to determine the role of ligands of PPAR $\gamma$  in both the prevention and treatment of colon cancer. Also, the importance of PPAR $\delta$  in colon cancer requires additional investigation.

### Breast Cancer

Breast cancer is one of the most frequent malignancies in females, with one woman in eight or nine developing breast cancer in her life. Breast cancer cells often express prominent levels of PPAR $\gamma$ . Table 3 provides several salient observations concerning breast cancer and PPAR $\gamma$ . A couple of studies have shown that  $10^{-6}$ – $10^{-5}$  M TZDs can inhibit proliferation and induce differentiation-like changes in breast cancer cell lines both *in vitro* and growing in nude mice (15, 62).

Additional studies have found that the combination of a retinoid with a PPAR $\gamma$  ligand synergistically inhibited the growth and induced apoptosis of a series of breast cancer cell lines both *in vitro* and in xenografts growing in nude mice (62). This was associated with a decreased number of cells in S phase and an increased percentage of cells in G<sub>0</sub>–G<sub>1</sub>. Up to 30–40% of the breast cancer cells cultured with a TZD and a retinoid became apoptotic with a fall in their levels of bcl-2. This apoptosis could be overcome by forced overexpression of bcl-2 in these cells (62).

*In vivo* experiments have shown that administration of a PPAR $\gamma$  ligand (GW7845) inhibited the development of carcinogen-induced breast cancer in rats (63). Another *in vivo* study showed troglitazone also prevented carcinogen (7,12-dimethylbenz(*a*)anthracene)-induced transformation of murine breast tissues (64). Furthermore, the addition of a retinoid to the PPAR $\gamma$  ligand greatly enhanced their abilities to prevent the transformation of the breasts (64). Of interest, mice who have a heterozygous germ-line deletion of PPAR $\gamma$  (PPAR $\gamma^{+/-}$ ) have a greater susceptibility to develop breast and ovarian cancers after their exposure to 7,12-dimethylbenz(*a*)anthracene, suggesting

Table 3 PPAR $\gamma$  and breast cancer

PPAR $\gamma$ : role in preventing and treating breast cancer	PPAR $\gamma$ : association with breast cancer
<ol style="list-style-type: none"> <li>1. TZDs decrease growth and induce differentiation and apoptosis of breast cancer cell lines <i>in vitro</i> and in nude mice. Retinoids enhance the effect.</li> <li>2. PPAR<math>\gamma</math> agonist (GW7845) blocks <i>N</i>-nitroso-<i>N</i>-methylurea-induced breast cancer in Sprague-Dawley rats.</li> <li>3. PPAR<math>\gamma</math> ligands plus retinoids decrease mammary gland transformation in mice.</li> <li>4. PPAR<math>\gamma^{+/-}</math> mice are more susceptible than wild-type mice to carcinogen-induced breast cancer.</li> </ol>	<ol style="list-style-type: none"> <li>1. Transgenic mice having a constitutively active PPAR<math>\gamma</math> in their breast tissue mated with mouse mammary tumor virus transgenics (breast cancer prone) result in mice with accelerated kinetics of breast cancer development.</li> </ol>

that PPAR $\gamma$  has a protective role against development of these cancers (65).

In a series of interesting experiments with somewhat quizical results, mice were genetically modified so that they constitutively expressed high levels of PPAR $\gamma$  in their breast tissue. These transgenic mice were mated with mouse mammary tumor virus polyoma middle T-expressing transgenic mice that are prone to develop breast cancer. Paradoxically, these mice had accelerated kinetics of development of breast cancer, suggesting that a ligand activated PPAR $\gamma$  under certain circumstances can actually enhance development of breast cancer (66). These experiments suggest that once an initiation event has occurred in breast tissue, increased PPAR $\gamma$  signaling might promote tumor progression in mammary gland tissue.

In summary, the overall role PPAR $\gamma$  plays in breast cancer is somewhat murky. *In vitro* and *in vivo* data show that PPAR $\gamma$  ligands can suppress breast cancer growth; and this can be enhanced by retinoids. However, two genetically modified murine models led to contradictory conclusions. Forced overexpression of PPAR $\gamma$  in the breasts of transgenic mice hastens their development of breast tumors; in contrast, loss of one PPAR $\gamma$  allele (PPAR $^{+/-}$ ), supposedly with reduced PPAR $\gamma$  expression, increased the risk of breast cancer in these mice after their exposure to a carcinogen compared with similarly treated control mice. Clearly, the role of PPAR $\gamma$  and its ligands in prevention and treatment of breast cancer needs clarification.

### Prostate Cancer

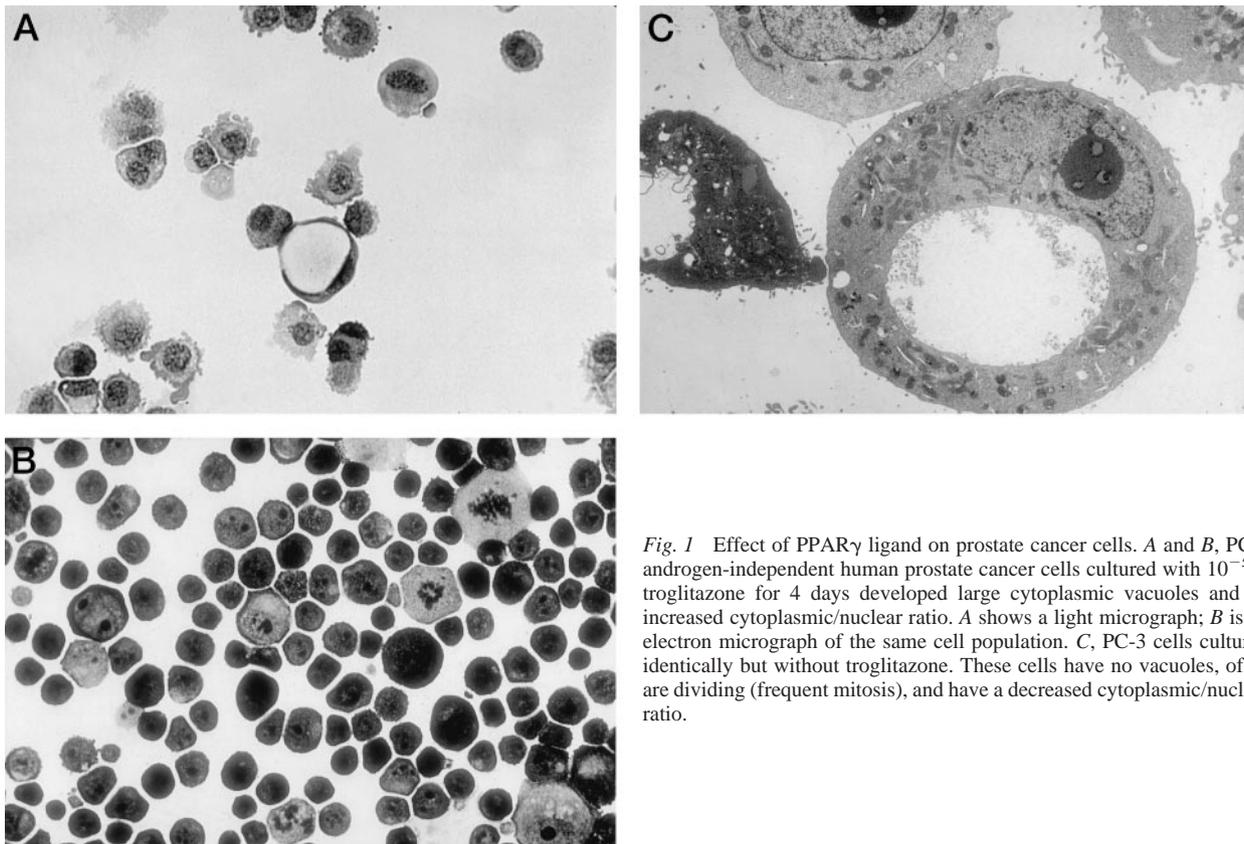
Prostate cancer is the second leading cause of cancer-related deaths among men in the United States, with 40,000 men a year in America dying from this malignancy (67, 68). Although surgical resection and radiotherapy are both potentially curative for localized disease, advanced prostate cancer is associated with a poor prognosis. The blockade of androgen stimulation can lead to either partial or full remission, but the disease reemerges within several years in a poorly differentiated, androgen-independent form.

Prostate cancers often express fairly abundant PPAR $\gamma$  (69, 70). Furthermore, the PPAR $\gamma$  does not appear to be mutated in prostate cancers (15). *In vitro* studies showed that an androgen-independent prostate cancer cell line, PC-3, could be inhibited in its proliferative growth and undergo profound morphological changes when cultured with a ligand of PPAR $\gamma$  (Fig. 1). These cells develop prominent enlargement of the cytoplasm with

numerous vacuoles. In some of the cells, the vacuoles are finely dispersed, whereas others have huge vacuoles that displace the nuclei, almost resembling adipocytes or signet ring cells. These vacuoles, however, are negative for lipids and thus do not stain with oil red O. The cytoplasmic lumens often reveal short microvillous processes, suggesting an adenocarcinomatous differentiation. The cell surface also has short microvillous processes.

In additional investigations, TZDs, as well as prostaglandin J2, can down-regulate androgen-stimulated PSA production in the androgen responsive LNCaP prostate cancer cells (71). Reporter gene studies showed that TZDs inhibited androgen activation of an androgen response element in the regulatory region of the PSA gene. The mechanism by which TZDs inhibits the androgen signaling pathway is unclear, but it may be modulating key transcriptional cofactors.

Individuals with prostate cancer have received troglitazone as part of several clinical trials (15, 72). In the biggest study, 41 patients received 800 mg/day of troglitazone after they had radical prostatectomy with curative intent and subsequently developed a rising serum PSA without demonstrable prostate cancer. These patients, therefore, had a recurrence with a low tumor burden. They received TZD for 18–20 weeks, and the investigators believed that the speed of rise of the serum PSA for many of these individuals diminished while on treatment. However, the number of measurements of PSA before beginning troglitazone was few, complicating the calculation of the rate of rise of the serum PSA while on therapy as compared with before beginning of therapy. Furthermore, while receiving troglitazone, only 1 of 41 patients had a 50% decrease in his serum PSA, and an additional 7 of 41 patients had less than a 50% decrease in their serum PSA levels. Of those 7 individuals, 3 had androgen-dependent and 4 had androgen-independent prostate cancer. We also treated an individual who began having a rising serum PSA  $\sim 2\frac{1}{2}$  years after receiving a radical prostatectomy (Fig. 2). After beginning troglitazone, the PSA stabilized for 1 $\frac{1}{2}$  years; at which time, the individual discontinued the TZD because of concerns about potential liver toxicity from the TZD. Thus, although the results are not dramatic, ligands of PPAR $\gamma$  do appear to have moderate antiprostata cancer activity even against androgen-independent tumors, and because of the low toxicity of most TZDs, additional studies of these agents are probably warranted.



**Fig. 1** Effect of PPAR $\gamma$  ligand on prostate cancer cells. *A* and *B*, PC-3 androgen-independent human prostate cancer cells cultured with  $10^{-5}$  M troglitazone for 4 days developed large cytoplasmic vacuoles and an increased cytoplasmic/nuclear ratio. *A* shows a light micrograph; *B* is an electron micrograph of the same cell population. *C*, PC-3 cells cultured identically but without troglitazone. These cells have no vacuoles, often are dividing (frequent mitosis), and have a decreased cytoplasmic/nuclear ratio.

### Troglitazone: Therapy for Prostate Cancer



**Fig. 2** Treatment of recurrent prostate cancer with PPAR $\gamma$  ligand. Patient had a radical prostatectomy in March 1995 with curative intent. In January 1996, his serum PSA began to rise. In May 1998, patient began oral troglitazone (600–800 mg every day). PSA serum levels stabilized through October 1999, when he elected to discontinue the PPAR $\gamma$  ligand because of its reported liver toxicity. Data as shown (72).

### Acute Myeloid Leukemia

Macrophages and myelomonocytic leukemic cells express abundant PPAR $\gamma$  (73), and PPAR $\gamma$  ligands can induce acute myelomonocytic leukemic cells (THP1) to differentiate toward macrophages with an increased expression of the CD36 scavenger receptors, as well as other surface markers

associated with differentiation including CD11b, CD14, and CD18 (73). Furthermore, other studies have shown that PPAR $\gamma$  ligands can inhibit the clonal proliferation of the U937 myeloid monocytic leukemic cells, and a PPAR $\gamma$  ligand plus 9-*cis*-retinoic acid was capable of synergistically inhibiting the clonal proliferation of HL-60, U937, and THP1 human myeloid leukemic cell lines. In general, however, the effect of PPAR $\gamma$  ligands on myeloid leukemic growth and differentiation is modest (74).

### Is PPAR $\gamma$ a Tumor Suppressor Gene?

Conflicting data garnered in different model systems leaves open the question of whether PPAR $\gamma$  is a tumor suppressor gene (Table 4). Ligands of PPAR $\gamma$  can inhibit proliferation and induce differentiation and apoptosis of a wide-range of neoplastic cells, suggesting that PPAR $\gamma$  is a tumor suppressor gene. Indirect evidence is consistent with this premise. Many follicular thyroid carcinomas have the chromosomal fusion of the transcription factors PPAR $\gamma$  and PAX-8 (70). *In vitro* studies suggest that the PAX-8/PPAR $\gamma$  fusion protein can act in a dominant negative fashion inhibiting the activity of the normal PPAR $\gamma$  allele (70). Thus, this indirect evidence would suggest that inhibition of function of the wild-type PPAR $\gamma$  is associated with the development of thyroid cancer. Also, PPAR $\gamma$  heterozygous knockout mice (PPAR $\gamma^{+/-}$ ) have an increased susceptibility to develop colon, mammary, and ovarian tumors, as well as skin papillomas after administration of a carcinogen (65). In

Table 4 PPAR- $\gamma$  may be a tumor suppressor gene

Yes	No
<ol style="list-style-type: none"> <li>1. PPAR-<math>\gamma</math> ligands can inhibit proliferation, induce differentiation, and apoptosis of a wide range of neoplastic cell types.</li> <li>2. PPAR-<math>\gamma</math> is mutated in sporadic colon cancers.</li> <li>3. PPAR-<math>\gamma</math><sup>+/-</sup> mice more susceptible than wild-type mice to mammary, colon, ovarian, and skin tumors after exposure to carcinogens.</li> <li>4. PAX-8-PPAR-<math>\gamma</math> fusion occurs in follicular thyroid cancer: PAX-8 is a thyroid transcription factor fused to PPAR-<math>\gamma</math>. <i>In vitro</i> studies suggest PAX-8-PPAR-<math>\gamma</math> acts in a dominant negative fashion toward wild-type PPAR-<math>\gamma</math>.</li> </ol>	<ol style="list-style-type: none"> <li>1. Mim mice fed TZD have an increased incidence and size of colon cancers.</li> <li>2. Transgenic mice overexpressing PPAR-<math>\gamma</math> and with a genetic proclivity to developing breast cancer have an increased frequency of breast cancer compared with appropriate controls.</li> <li>3. Mice engineered to have deletion of PPAR-<math>\gamma</math> in several organ systems, including breasts, do not have an increased incidence of cancer at those sites.</li> <li>4. An extensive study of ~400 clinical samples and cell lines, including colon cancers, did not detect PPAR-<math>\gamma</math> mutations.</li> <li>5. Families with several different types of germ-line mutations of PPAR-<math>\gamma</math> do not appear to have increased incidence of cancer.</li> </ol>

addition, mutations of PPAR $\gamma$  have been reported in primary colorectal carcinomas (75).

In contrast, other data suggest that PPAR $\gamma$  is not a tumor suppressor gene, including experiments with Mim mice fed a TZD, as well as transgenic mice who overexpressed PPAR $\gamma$ . These models suggest that PPAR $\gamma$  can act in a cancer-permissive fashion. Also, mice engineered to be PPAR $\gamma$ <sup>-/-</sup> in a variety of organ systems did not have an increased incidence of cancer in these tissues (76). In addition, an extensive study of ~400 clinical samples and cell lines from various human cancers did not detect PPAR $\gamma$  mutations (77). Furthermore, families having one of several different germ-line mutations of PPAR $\gamma$  have been identified. These individuals often have abnormalities of either fat metabolism or glucose homeostasis, but thus far, they have not been reported to have an increased proclivity to develop cancers (78–81).

In summary, the picture is unclear. Several *in vitro* and *in vivo* model systems suggest that PPAR $\gamma$  may behave as a tumor suppressor gene. In contrast, a couple of murine models are consistent with an activated PPAR $\gamma$ -enhancing tumor formation. Clearly, a better understanding of the mechanism of action of activated PPAR $\gamma$  is required. Adding to this complexity, a number of activities mediated by PPAR $\gamma$  ligands may not require PPAR $\gamma$ , as discussed in the next section.

#### Some of the Actions of PPAR $\gamma$ Ligands Can Occur Independent of PPAR $\gamma$

Several pieces of indirect and direct evidence suggest that select anticancer activity of PPAR $\gamma$  ligands could occur independently of PPAR $\gamma$  (Table 5). Indirect evidence includes several studies that found that selective TZDs, including troglitazone but not pioglitazone or rosiglitazone, could inhibit cholesterol biosynthesis or affect cholesterol efflux from cells (82, 83). This selective response by some but not other PPAR $\gamma$  ligands suggests that the inhibition may not be mediated through PPAR $\gamma$ . Additional indirect evidence follows from the observation that pharmacological concentrations ( $10^{-6}$ – $5 \times 10^{-5}$  M) of the PPAR $\gamma$  ligands are required to produce an anticancer activity or inhibit cytokines. In contrast, modulation of lipid-related genes and induction of differentiation of preadipocytes to adi-

Table 5 Evidence that some actions of PPAR $\gamma$  ligands occur independently of PPAR $\gamma$ 

Evidence
Some ligands of PPAR- $\gamma$ (troglitazone) but not others (pioglitazone, rosiglitazone) affect cholesterol biosynthesis and transport.
Pharmacological concentrations of PPAR- $\gamma$ ligands inhibit inflammatory cytokines and inhibit growth and growth-related proteins in cells with no PPAR- $\gamma$ .

pocytes occurs at several logs lower concentrations, closer to the  $K_d$  of PPAR $\gamma$ .

More direct evidence emanates from several studies. We (74) and others (84) noted that proliferation of several acute myeloid leukemia cell lines (KG-1 and KU812) was markedly inhibited by PGJ<sub>2</sub>. Several other studies used embryonic stem cells, which were made homozygously null for PPAR $\gamma$  (PPAR $\gamma$ <sup>-/-</sup>). In the first study, these cells were induced to differentiate down the macrophage pathway (85). Exposure of normal, activated macrophages to either PGJ<sub>2</sub> or TZDs decreased their release of inflammatory cytokines such as TNF, IL-1 (IL-1 $\beta$ ), IL-6, inducible nitric oxide synthase, and COX-2 (23, 26, 86). Surprisingly, exposure of the PPAR $\gamma$ <sup>-/-</sup> macrophages to either PGJ<sub>2</sub> or TZDs also lowered their production of these anti-inflammatory molecules (85). In the second series of experiments, TZD inhibited proliferation, DNA synthesis, and caused a G<sub>1</sub> cell cycle arrest of embryonic stem cells from both PPAR $\gamma$ <sup>-/-</sup> and PPAR $\gamma$ <sup>+/+</sup> mice (87). Likewise, TZDs inhibited both the expression of cyclin D1 and the growth of PPAR $\gamma$ <sup>-/-</sup> embryonic stem cell tumors growing in syngeneic mice (88).

How PPAR $\gamma$  ligands can act independent of PPAR $\gamma$  is under active study. Several hypotheses have been put forth. One group of investigators found that TZDs depleted calcium stores in PPAR $\gamma$ <sup>-/-</sup> ES cells leading to activation of protein kinase R that phosphorylated the  $\alpha$  subunit of the eukaryotic initiation factor 2, causing its inactivation (87). Many cell cycle regulatory proteins as well as oncogenes contain highly structured GC rich 5' untranslated regions, which can make them very dependent on the activity of the translation initiation factors such as

eukaryotic initiation factor 2 $\alpha$ , and their inhibition by TZDs could explain the anticancer effects of these compounds. Several other investigations noted that the PPAR $\gamma$  ligand, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J<sub>2</sub> potently inhibited the NF- $\kappa$ B pathway and this could occur in the absence of PPAR $\gamma^{-/-}$  in the target cells (88, 89). This PPAR $\gamma$ -independent inhibition may occur by covalent modifications of key cysteines in the inhibitor of nuclear factor  $\kappa$ B kinase and the DNA binding domains of NF- $\kappa$ B subunits, preventing them from binding and transactivating cytokine- and growth-related genes.

In summary, PPAR $\gamma$  ligands do have antiproliferative, prodifferentiation, and proapoptotic effects on cancer cells. In combination with retinoids, these activities are enhanced. Although this class of nuclear hormone receptor ligands are unlikely to have a primary role in cancer therapy, their very low toxicity makes them inviting candidates for use in the adjuvant setting in combination with other drugs and for chemoprevention of selected cancers. At this time, anticancer efficacy of PPAR $\gamma$  ligands is being examined in liposarcomas (Dr. George Demetri, Boston, MA) and prostate cancer (Dr. Eric Small, San Francisco, CA). Prostate cancer studies are also planned at the Center for Cancer Research at the National Cancer Institute (Joseph A. Tangrea, Rockville, MD). A Phase IIa trial of the PPAR $\gamma$  agonist, pioglitazone, in oral leukoplakia is also being carried out at the National Cancer Institute, Division of Cancer Prevention. A breast cancer study is to be initiated shortly by Charite (Berlin, Germany).

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