Peroxisome Proliferator-Activated Receptor γ Pathway Targeting in Carcinogenesis: Implications for Chemoprevention

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Abstract The peroxisome proliferator-activated receptor (PPAR) γ is one member of the nuclear receptor superfamily that contains in excess of 80 described receptors. PPARγ activators are a diverse group of agents that range from endogenous fatty acids or derivatives (linolenic, linoleic, and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2) to Food and Drug Administration-approved thiazolidinedione drugs [pioglitazone (Actos) and rosiglitazone (Avandia)] for the treatment of diabetes. Once activated, PPARγ will preferentially bind with retinoid X receptor α and signal antiproliferative, antiangiogenic, and prodifferentiation pathways in several tissue types, thus making it a highly useful target for down-regulation of carcinogenesis. Although PPAR-γ activators show many anticancer effects on cell lines, their advancement into human advanced cancer clinical trials has met with limited success. This article will review translational findings in PPARγ activation and targeting in carcinogenesis prevention as they relate to the potential use of PPARγ activators clinically as cancer chemoprevention strategies.

Over the past 20 years, strides have been made in treatment of several solid tumor malignancies resulting in measurable increases in survival, but a firm challenge remains to establish improved cancer prevention strategies for most cancers. Strategies focusing on abrogating global events during human carcinogenesis [e.g., epigenetic events with histone deacetylase (HDAC) inhibitors, carcinogenesis with natural products, or drugs affecting transcription factor pathways] coexist with focused strategies that seem highly specific such as individual receptor inhibition (e.g., epidermal growth factor receptor) or single-enzyme inhibition (e.g., cyclooxygenase). Presently, antiestrogen strategies for breast carcinoma, topical agents such as Aldara and Effudex for skin carcinoma, and cyclooxygenase-2 inhibitors (celecoxib) for familial polyposis are some of a very limited number of Food and Drug Administration-approved strategies for chemoprevention. Other promising therapies (e.g., retinoids and cyclooxygenase-2 targeting) have fallen from favor due to unacceptable toxicity (1, 2) or inefficacy (3, 4). There is a demand for safe agents that target high-risk conditions such as preexisting intraepithelial neoplasia, a high-risk cancer precursor (ref. 5; Table 1). Peroxisome proliferator-activated receptor γ (PPAR-γ) activation strategies may help fulfill this demand.

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Nuclear Receptor Peroxisome Proliferator-Activated Receptor Activators Broadly Target Preneoplastic Processes

The successful exploitation of nuclear receptor targeting for cancer prevention have thus far included antiestrogen strategies for breast carcinoma prevention. In this scenario, the estrogen receptor, which is linked to breast cancer growth, is successfully antagonized by tamoxifen or raloxifene. Conversely, the agonism of other nuclear receptors (e.g., PPARs) that are more often associated with differentiation is another strategy to target this receptor class.

Peroxisome proliferator-activated receptors α, β (or δ), and γ are 3 of ~100 nuclear receptors in the orphan receptor class (6, 7). This receptor class consists of a variety of steroid, retinoid, thyroid, vitamin D, and other receptors that dimerize and regulate a multitude of downstream metabolic processes when activated or inhibited. The original discovery and cloning of the PPARs leads to a somewhat confusing explanation of their functions because of their tissue distribution and differential effects in humans versus animal models and the fact that they diffusely influence basic metabolic processes, such as energy storage. For example, PPARα is the downstream receptor for a variety of peroxisome proliferators (including industrial plasticizers) associated with hepatocellular carcinogenesis in rats but not in humans (8, 9). Conversely, in humans, natural ligands for PPARα include a variety of fatty acids responsible for lipid storage and are clearly not carcinogens. Synthetic agents, such as fibrates, are approved for the treatment of hyperlipidemia, target PPARα, and are also not carcinogenic in humans. However, at high doses, combined agonists of the PPARα/PPARγ class are associated with bladder carcinogenesis in animals and this has affected their utility clinically (10). The complex nature of these signaling pathways in different species creates these paradoxes.

PPARγ was originally described as a differentiation transcription factor for adipose tissue (11). Its natural ligands are felt to

Table 1. Drugs affecting PPARγ signaling

Agent	Receptor	Drug class	Food and Drug Administration approved
Pioglitazone	PPARγ	Thiazolidinedione	Yes
Rosiglitazone	PPARγ	Thiazolidinedione	Yes
Ciglitazone	PPARγ	Thiazolidinedione	No
Troglitazone	PPARγ	Thiazolidinedione	Yes/withdrawn
Farglitizar	PPARγ	Thiazolidinedione	No
Muraglitizar	PPARα,γ	Thiazolidinedione	No
GW7845	PPARγ	Unknown	No
GW7875	PPARγ	Unknown	No
GW501516	PPARδ	Unknown	No
LG10068	RXRα	Retinoid	No
Clofibrate	PPARα	Fibrate	Yes
Prostaglandin J_2	PPARγ	Prostaglandin	No

be products of the eicosanoid cascade and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 has a particular affinity for this receptor. The thiazolidinedione drugs are used for the treatment of type II diabetes and specifically target PPARγ (Table 1). PPARδ has been associated with colon carcinogenesis in some models and was originally felt to not be particularly related to any logical metabolic processes. Recently, a broader understanding of these receptors has been placed into context in an atlas of nuclear receptors whereby the PPARs are understood as being principally associated with nutrient uptake, storage, and utilization during the circadian rhythm.[1,2] Therefore, an elevated importance is ascribed to these receptors and the utility of targeting them in conditions such as obesity, diabetes, and other metabolic processes such as exercise is emerging. Currently, Food and Drug Administration-approved agents are available to target PPAR α and γ.

Preferential binding partners for PPAR-γ include receptors of the retinoic acid class. The retinoic acid receptors consist of two families and six members: the retinoic acid receptors (α, β, and γ) and the retinoid X receptor (RXR; α, β, and γ). By themselves, this class of receptors can heterodimerize, but in the past several years, a transcriptome consisting of RXR/retinoic acid receptor heterodimers is linked to HDAC has been identified (12–14). This is derepressed on activation with 9-*cis* retinoic acid binding to RXR elements. There are a large number of natural and synthetic retinoids that target these receptors, and some retinoid ligands can bind to either class of receptors (e.g., all-*trans* retinoic acid), whereas others are class restricted (e.g., 9-*cis* retinoic acid and the RXR family). Dysfunction within this signaling complex may therefore be targeted with a large number of drugs affecting either retinoid or HDAC receptors to down-regulate carcinogenesis. This axis, which putatively also involves PPARs, could therefore be dually targeted with drugs that affect retinoid or HDAC receptors, or PPAR and HDAC receptors, once the key dysregulated points are identified. Additionally, simply the presence of functional receptors would allow for exploitation within this axis. One example would be the modulation of receptors of the retinoic acid receptor β class in aerodigestive chemoprevention, which has been shown to be up-regulated in successful aerodigestive chemoprevention (15–18).

[1] Margolis RN, Evans RM, O'Malley BW, NURSA Atlas Consortium. The Nuclear Receptor Signaling Atlas: development of a functional atlas of nuclear receptors. Mol Endocrinol 2005;19:2433–6.

[2] Yang X, Downes M, Yu RT, et al. Nuclear receptor expression links the circadian clock to metabolism. Cell 2006;126:801–10.

Molecularly, it is understood that PPARγ will form a heterodimer with RXRα and transcriptionally activate downstream genes (Fig. 1; ref. 19). The PPARγ/RXRα complex can be phosphorylated at either protein (20–22) and the transcription factor will preferentially bind direct repeat sequences in downstream genes. The consequences of phosphorylation by mitogen-activated protein kinase or other factors (e.g., insulin) is not well studied but in general leads to attenuation of receptor-dependent and receptor-independent effects, such as attenuation of adipose lineage genes in preadipocytes. Interactions occurring in the transcription factor complex with other cofactors (such as SMRT and NCoR), which further modulate PPARγ activity, have been best described in adipogenesis models (23–26).

In cancer, the interactions between PPARγ/RXRα have been the principal targets for exploitation compared with the study of metabolic diseases or adipose metabolism and obesity. The drugs utilized for cancer therapy studies (that would also be appropriate for chemoprevention studies) have included the preclinical activators of PPAR (such as prostaglandins of the J series) and dietary additions (such as linoleic and linolenic acid derivatives). Thiazolidinediones available either preclinically (such as ciglitazone) or as Food and Drug Administration approved drugs for the treatment of diabetes (such as pioglitazone and rosiglitazone) have been used. Additionally, a large number of natural and synthetic retinoid receptor targeting agents have been studied with the focus on RXR/retinoic acid receptor interactions in cancer. There has been less focus on molecular events occuring along the PPAR-γ/RXR-α axis. Further, the more recently described interactions of the RXR with HDAC could additionally be studied with the focus of PPARγ and downstream events. Finally, drugs affecting PPARγ or RXR-α phosphorylation may also be useful (MAPK inhibitors).

Clinical-Translational Advances

PPARs and cancer treatment. The literature is replete with a large volume of studies showing efficacy of PPAR treatment of cell lines and animal models. It is well established that activating each subclass of receptors can result in antiproliferative, proapoptotic, prodifferentiation, and antiangiogenic effects in cell lines or animal models (27). These data, collectively, have resulted in several completed clinical trials in solid tumor malignancies in breast (28), prostate (29), and liposarcoma

(30). Unfortunately, there is a lack of clinical effect in advanced refractory malignancies when PPARγ agonists are used as targeted agents alone. Because the agents available are pleiotropic in nature and given at therapeutic and pharmacologic doses in publications, their effects in a given target organ may vary; both PPARγ-dependent and PPARγ-independent effects could be occurring simultaneously in the same tissue or cell line. This makes it difficult to form general consensus statements regarding their effects in human cancers. In light of clinical inefficacy of the agents, perhaps these agents should be thought of as reasonably safe adjuncts to standard chemotherapy in further testing.

Organ site-specific carcinogenesis and chemoprevention studies of PPARγ. Because the original discovery of the PPAR activators was potentially associated with carcinogenesis, careful evaluation of these agents and metabolic pathways is required to delineate doses and conditions whereby untoward effects may occur (8, 31). Several studies have investigated PPARγ as therapy for carcinogenesis prevention preclinically or preneoplasia, with other support for specific mechanisms of action in target organs (e.g., receptor mutations, allelic variation, etc.).

Thyroid. In human thyroid follicular carcinoma, PPARγ can form a fusion oncogene with the PAX8 promoter (32). This fusion oncogene has been described in several studies and confirmed by numerous techniques and is detectable in up to 50% of follicular thyroid carcinomas. Additionally, it has also been discovered in thyroid follicular adenomas (33), which are typically surgically removed as carcinoma precursors. The discovery of this fusion oncogene is potentially concerning but multiple studies have shown both increased and more differentiated phenotypes of the tumors, which seem to be associated with the t(2;3)(q13;p25) translocation responsible for the PAX8/PPARγ protein. It has been established that this fusion oncoprotein can activate the PPAR response element when transfected into Rat FRTL-5 thyroid cells and also bind the PPAR response element on electromobility shift assay and this resulted in overall increased cell proliferation (34). However, in this analysis, some PPARγ-responsive genes were activated by the protein and others were inhibited. Therefore, this mutation can act by both gain and loss of function in thyroid carcinoma, the balance of which favors differentiated tumor formation in affected individuals. It is possible in instances where this oncoprotein is associated with more aggressive tumors, the fusion gene may possess a greater degree of gain of function attributable to transcription factor and tumor-specific PPARγ cofactor function and regulation. In contradistinction, in the $TR\beta^{PV/PV}$ mouse (which contains a mutated thyroid hormone receptor and develops follicular neoplasms spontaneously), the TRβ receptor acts as a dominant-negative on normal PPARγ transactivation (35). Currently, follicular thyroid neoplasms are the only known neoplasms to be associated with PPARγ fusion gene products and PAX 8/PPARγ is the only known PPARγ-associated fusion gene.

Gastrointestinal system. Earlier studies in colon carcinogenesis demonstrated a potential role for PPARγ in colorectal tumor growth. On PPARδ principally based on early work that suggested a role for PPARδ in colorectal tumor growth (36, 37). In colon carcinoma, experimental evidence is somewhat ambiguous in that PPARγ may play a protective or tumor-suppressive role against cancer development as well as tumorigenesis. Original experiments in the Min mouse model showed enhanced noninvasive polyp formation in the colons of mice given pharmacologic concentrations of troglitazone in the diet for 7 weeks (38). Histologically, the polyps showed dysplasia and hyperplasia without invasion. No alterations were observed in the small intestinal rate of polyp formation with troglitazone. Lefebvre et al. performed a second set of experiments with troglitazone and a second thiazolidinedione, BRL49,653, with similar findings in both the large and the

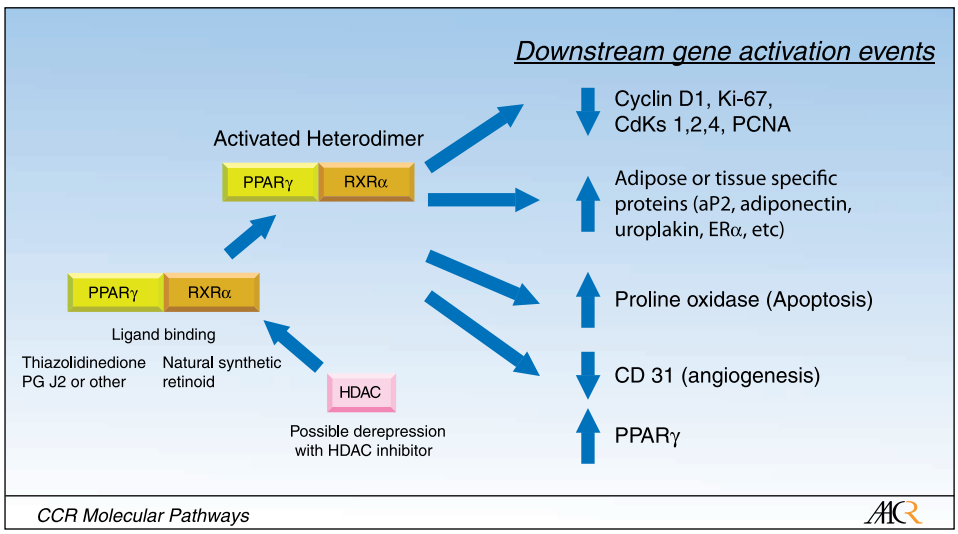

Fig. 1. Evidence-based PPARγ targeting in carcinogenesis. PPARγ will form a heterodimer with RXRα. It is possible that the RXRα can be derepressed from an association with HDAC to facilitate this new binding. Once associated, the PPARγ may be activated with a thiazolidinedione drug to become an "activated" heterodimer, which, in the nucleus, can trigger several downstream gene activation events. These include decreases in proliferation through either cyclin D1 or cyclin-dependent kinases 1, 2, and 4 as well as Ki-67 and proliferating cell nuclear antigen. Other families of either adipose or tissue lineage-specific differentiation proteins are up-regulated, contributing to a more differentiated cell. Also, genes contributing to events, such as CD31 and angiogenesis, may be down-regulated by the activation of PPARγ. Apoptosis-associated proteins such as proline oxidase can also be activated by PPARγ. Finally, PPARγ can target further expression of itself, creating a positive feedback loop, thus amplifying the original effect of the drug-mediated targeting of the receptor.

small bowel (39). Similarly, Yang et al. described troglitazone-induced colon tumorigenesis when given at 0.2% in the diet in C57BL/6J normal mice as well as in $Apc^{1638N/+}$ $Mlh1^{+/-}$ mice (40). More recently, Linsalata et al. found enhanced expression of PPARγ and ornithine decarboxylase pathway products in human tumors compared with normal mucosa, and this was more prevalent in colon cancers with ras mutations (41). These four studies support a role for PPARγ potentially playing a role in colon carcinogenesis albeit primarily in transgenic mouse models.

Conversely, other studies have provided evidence for PPARγ activation as a way to abrogate colon carcinogenesis. Ulcerative colitis is a disease associated with chronic inflammation and increased large bowel cancers. It has recently been discovered that the anti-inflammatory agent 5-aminosalicylic acid, used to treat ulcerative colitis, stimulates PPARγ and is anticarcinogenic. Ulcerative colitis-associated colon cancers are modeled with oral dextran sulfate/intraperitoneal azoxymethane (42). With 100 mg/kg/d 5-aminosalicylic acid administration for 7 weeks, tumorigenesis was decreased by 50% and the tumors were statistically smaller. With 25 mg/kg (low dose) administration of pioglitazone or rosiglitazone, there was no decrease in tumorigenesis over controls. Others have shown that PPARγ mRNA is induced by 5-aminosalicylic acid treatment in humans and PPARγ is deficient in patients afflicted with ulcerative colitis (43). Another potentially useful chemopreventive therapeutic, 9,11-conjugated linoleic acid, has also been tested as a potential PPARγ-activating treatment for the prevention of colon carcinogenesis. This agent is derived from bitter melons, and when administered in the diet of azoxymethane-treated Fischer rats, 50% decreases in tumor incidence and size were observed in the 9,11-conjugated linoleic acid-treated rats, and this was accompanied by an increase in the level of PPARγ in colonic mucosa (44). Others have shown PPARγ activation with 9,11-conjugated linoleic acid (45, 46), and because PPARγ is known to induce itself, this is a simple explanation for why elevated PPARγ levels were observed. In another study that supports an anticarcinogenic role for PPARγ in colon carcinogenesis, immortalized rat intestinal IEC6 cells experience reduced proliferation with linoleic acid (47). These changes are reversible when PPARγ expression is inhibited by RNA knockdown techniques, thus suggesting a tumor-suppressive role for PPARγ constitutive expression. Finally, it has recently been pointed out that thiazolidinedione treatment enhances apoptosis in colon cancer cells through the activation of proline oxidase (48). In other gastrointestinal system malignancies, thiazolidinedione administration with RXR agonists in rat hepatocyte cell lines induces chemopreventive glutathione *S*-transferase 2α (49). In *N*-methyl-*N*-nitrosourea-induced gastric carcinoma, $PPAR\gamma^{+/+}$ transgenic mice developed fewer tumors than their heterozygous ($PPAR\gamma^{+/-}$) counterparts, but troglitazone was only effective in preventing tumors in the homozygotes (50). These data, taken together, show that troglitazone, given at pharmacologic doses, can promote tumor formation in the Min mouse model and in one study using wild-type mice. This agent is no longer on the market. However, other studies show that the induction of PPARγ may be protective against the development of these colon and other gastrointestinal malignancies and promote apoptosis. Finally, agents such as 5-aminosalicylic acid and 9,11-conjugated linoleic acid may be chemopreventive for colon carcinogenesis. Further studies with FDA-approved PPARγ activators and intestinal carcinogenesis are lacking.

Breast. The evidence for utility of PPARγ activators in the prevention of breast cancer is perhaps more straightforward than observed in the conflicting reports in colon cancer development. Two studies (one in mice and one in rats) conducted in the late 1990s showed efficacy in the prevention of mammary carcinogenesis with troglitazone and GW7845. Mehta et al. (51) employed a BALB/c 7,12-dimethylbenz[*a*]anthracence organ culture, which showed a 50% to 100% decrease in gland lesions after treatment with troglitazone or troglitazone and the RXR-selective agonist, LG10068. In this model, the best treatment was a combination of troglitazone and LG10068. Suh et al. (52) used GW7845 in a rat *N*-methyl-*N*-nitrosourea model of breast carcinogenesis and identified decreases in tumor incidence, tumors per animal, and tumor weight after 2 months of treatment. More recently, both PPARγ and PPARδ activators were used to study 7,12-dimethylbenz[*a*]anthracence-treated FVB mice (53). Interestingly, the PPARγ activator GW7875 inhibited tumorigenesis; the tumors that formed were ductal in origin and expressed estrogen receptor-α. The PPARδ activator GW501516 actually increased the incidence of tumors over baseline and the histology was either adenosquamous or squamous carcinomas. Badawi et al. tested an experimental PPARγ activator, Fmoc-L-Leu and celecoxib, alone or in combination, in a *N*-methyl-*N*-nitrosourea model of rat breast carcinogenesis (54). Both agents were effective in reducing the incidence of tumors by ~30%; however, the combination of both agents was responsible for a 65% decrease in tumor incidence. These treatments separately decreased cyclooxygenase-2 and increased PPARγ in tissues, and decreases in prostaglandin E_2 were also observed. Apoptosis was increased and measures of proliferation including proliferating cell nuclear antigen and cyclin-dependent kinase 1 were decreased. In a population study of PPARγ allelic variants isoforms and breast cancer incidence in a Danish population, a variant splice allele of the PPARγ2 gene was associated with decreased risk in women identified as alcohol users (55). These studies point out class differences in the effect of PPAR activators on breast carcinogenesis, with PPARγ inhibiting tumor formation. The potential for combination chemoprevention of breast carcinoma with PPARγ and RXRα activation is also shown. Because PPAR activators are not always pure agonists for a specific subclass of receptors, we are to be cautioned that results from experiments of mixed agonists may not allow straightforward interpretation.

Skin. PPARγ focused studies have been conducted to examine skin squamous malignancies. The original skin carcinogenesis prevention studies occurred in mouse models. He et al. published both troglitazone and ciglitazone decreased proliferation in primary mouse newborn keratinocytes and the CD50 mouse cell line (56). Cell cycle G_1-phase arrest and suppression of cyclin D1 and cyclin-dependent kinases 4 and 2 occurred as well. However, these effects were found to be PPARγ independent, as transfection with dominant-negative PPARγ produced no changes in results of troglitazone treatment. This study was followed with an animal study of 7,12-dimethylbenz[*a*]anthracence-induced carcinogenesis where both rosiglitazone and troglitazone had no effect on either 7,12-dimethylbenz[*a*]anthracence/TPA- or UV-induced

carcinogenesis (57). Further, PPARγ could not be identified in either normal or transformed tissues. However, when PPARδ is targeted, its activation is accompanied by a protein kinase C mediated down-regulation of kinases, which ultimately down-regulate extracellular signal-regulated kinase (58). These studies do not support a hypothesis-driven role for PPARγ activator use in the prevention of skin carcinogenesis.

Aerodigestive carcinogenesis. In aerodigestive squamous carcinoma, the scenario is different than in squamous cancer. In the rat 4-nitroquinolone model of oral carcinogenesis showed ~40% decreases in the incidence of oral squamous carcinoma with decreased multiplicity in rats treated with 500 ppm pioglitazone (59) was observed. Forty-percent reductions in the incidence of rat tongue carcinomas were also observed in a separate study with troglitazone (60). Further support of these preclinical findings can be derived from studies of Govindarajan et al. (61), who were able to show decreased incidence of both head and neck (>40%) and lung (>30%) neoplasms in a Veterans' Administration population of diabetics aged >40 years who were taking thiazolidinedione agents for at least a year. Preclinical studies in lung cancer treatment with PPARγ agonists were done by Chang and Szabo (62), who showed that proliferative indices were decreased and apoptosis and differentiation markers were increased in non-small cell lung cancer cell lines treated with ciglitazone. These data show that skin and oral squamous carcinoma models respond differently to treatment with PPARγ activators, with the thiazolidinediones being ineffective in skin squamous carcinogenesis animal models but significantly altering aerodigestive carcinogenesis. The data from lung cancer experiments give further support for hypothesis-driven biomarkers in proliferation, differentiation, and apoptosis for potential chemoprevention studies with these agents. Finally, the combined data from health-care registries of glitazone use combined with animal models provides a stronger rationale to move forward clinically in aerodigestive premalignancy than animal study evidence alone.

Renal system. Another area of investigation has involved the kidney and related organs and PPARγ effects. The principal experiments in this area have occurred in the bladder urothelial epithelium. In normal urothelial cell cultures, troglitazone is able to induce markers of urothelial differentiation while decreasing squamous metaplasia (63, 64). However, the largest focus on renal epithelial malignancies has been with regard to testing of dual PPARα/γ agonists in animal models before Food and Drug Administration approval. In this setting, the combined agonists at pharmacologic concentrations result in bladder and/or kidney carcinogenesis with at least five dual agonists (10). One detailed pathologic study provides evidence that chronic effects of urolithiasis contribute to this effect (65). This has resulted in withdrawal of most of these agents from further testing. Interestingly, these studies point out a paradox because high pharmacologic doses in animals result in tumor formation with dual agonists, yet PPARγ activation promotes a panel of differentiation markers in renal epithelial cells.

Challenge of incorporating PPARγ agents into prevention clinical trials. One principal issue in any cancer chemoprevention strategy is the safety of the agent. The cardiotoxicity revealed in a recent meta-analysis of rosiglitazone may make this agent less attractive in the prevention realm (66), but pioglitazone does not seem to share this toxicity in a large study of diabetics prospectively analyzed for cardiac risks (67, 68). Other PPARγ activators are currently under development but may not gain industry support. Pioglitazone has been used for a phase II cancer prevention trial of preneoplastic aerodigestive lesions[3] and could be an important available agent for further testing in intraepithelial neoplasia. Anti-inflammatory agents such as 5-aminosalicylic acid would be other potential agents for PPARγ-targeted chemoprevention perhaps in high-risk inflammatory bowel disease patients. Also, natural product derivatives such as 9,11-conjugated linoleic acid from bitter melons might be useful in the clinical chemoprevention setting. It is clear that PPARγ signaling represents a powerful influence in cells where it is metabolically active. It forms a transcriptional complex with RXRα and other cofactors such as NCoR and SMRT. Therefore, combination chemoprevention with agents targeting PPARγ, RXRs, and related metabolic processes would be reasonable approaches as long as the toxicity of such combinations was acceptable.

Secondly, potential hypothesis-driven surrogate markers in clinical trials emerge from the myriad cell processes affected by PPARγ activators (Fig. 1). First, it is notable PPARγ was often induced in tissues as a result of treatment. Therefore, examination of modulation of PPARγ in tissue samples before and after treatment would be one potentially useful tissue marker of an organ-specific effect in a chemoprevention clinical trial. Additionally, modulation of PPARγ binding partners (such as RXRα) could also be assayed by immunohistochemistry in tissue specimens before and after PPARγ activator treatment. The intracellular location or phosphorylation status of the proteins may also be significant, as phosphorylated forms of PPARγ and RXRα located in the nucleus are probably the most likely isoforms to be found in an active state, thus signifying a tissue-specific treatment effect. Next, several studies examined markers of both proliferation and apoptosis after treatment. Clearly, cyclin D1, proliferating cell nuclear antigen, Ki-67, and cyclin-dependent kinases represent a second class of putative surrogate endpoints, which may undergo change during treatment with PPARγ activators. Markers of apoptosis including apoptosis index, caspase activation, or proline oxidase induction would also be reasonable biomarkers. Perhaps the most interesting biomarkers might be those associated with cellular differentiation often promoted by PPARγ activator treatment. These would potentially represent PPARγ-dependent genes in a variety of target tissues and may provide further insight into nuances of PPARγ function. They might also represent adipose lineage markers, as several studies indicate that markers of adipose lineage differentiation can occur with PPARγ treatment (69–71). The antiangiogenic properties represent yet another target for PPARγ effects and the use of endothelial markers to measure angiogenesis has been advocated (72).

PPARs and current clinical trials. There are several PPARγ agonists in the pipeline, undergoing clinical trials primarily for metabolic diseases such as diabetes. GSK has at least three novel agents that are PPAR activators in metabolic disease clinical trials (376501, farglitazar, and rosiglitazone XR; GSK product development pipeline, February 2008). One Daiichi Sankyo compound, CS-7017, an oral PPARγ agonist, is undergoing a single arm phase I evaluation[4] for advanced metastatic cancer.

[3] Unpublished data.

[4] www.clinicaltrials.gov NCT00408434.

A single-arm combination therapy phase I/II clinical trial employing a taxane and CS-7017, for anaplastic thyroid cancer, is also ongoing (NCT00603941). There are two recently completed single-arm phase IIa leukoplakia reversal clinical trials, one employing rosiglitazone and the other employing pioglitazone.[5] The pioglitazone clinical trial showed leukoplakia reversal in most patients and a randomized phase II clinical trial with pioglitazone in leukoplakia patients is planned.[6] These ongoing, or recently completed studies, show considerable ongoing interest in the clinical study of PPARγ agonists for cancer as well as diabetes.

Future directions. It is clear that the road to safe, effective chemoprevention is arduous and agents in routine use are limited. However, substantial evidence on dosing, potential endpoints in clinical trials, and success in animal models of carcinogenesis provide significant preclinical evidence for how to approach use of these agents in early-phase clinical trials. Thiazolidinediones and natural products discussed that activate PPARγ may be lead candidate agents for such studies. The risk benefit ratio of the agents needs to be assessed, and this may be further assisted by logical incorporation of biomarkers into the trials. Of great benefit is that many of the biomarkers for proliferation and apoptosis as well as standard pathology from biopsied lesions are now available at most community hospitals and are standardized on automated immune histochemistry. It is easy to become deterred from considering this strategy, particularly in light of the evidence from some transgenic mouse models and the animal studies employing PPARα/γ combined activators, but it is important to note the antiestrogens commonly in use are associated with endometrial carcinoma development (73). Clearly, risk stratification and the targeting of these agents to specific intraepithelial neoplastic conditions will be important in the future testing of these promising chemoprevention drugs.

[5] www.clinicaltrials.gov
[6] Unpublished data.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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