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Review

Peroxisome proliferator-activated receptor γ in bladder cancer

A promising therapeutic target

Jose J. Mansure, Roland Nassim and Wassim Kassouf*

Division of Urology; McGill University Health Center; Montreal, Canada

Abbreviations: BCG, bacillus calmette-guerin therapy; CDK, cyclin-dependent kinase; CIG, ciglitazone; MAPK, mitogen-activated protein kinase; N-CoR, nuclear receptor corepressor; NOS, nitric oxide synthase; NSAIDs, non steroidal anti-inflammatory drugs; NSCLC, non small-cell lung cancer; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; PTEN, phosphatase and tensin homolog; RXR, retinoid X receptor; TCC, transitional cell carcinoma; TGZ, troglitazone; TZD, thiazolinidinedione; VEGF, vascular endothelial growth factor

Key words: bladder cancer, peroxisome proliferation-activated receptor γ, targeted therapy, cancer biology, molecular therapy

Peroxisome Proliferator-Activated Receptors (PPARs) are ligand-activated intracellular transcription factors, members of the nuclear hormone receptor superfamily. The PPAR subfamily consist of three subtypes encoded by distinct genes denoted PPARα, PPARβ/δ and PPARγ. The peroxisome proliferator-activated receptor γ (PPARγ) is the most extensively studied subtype of the PPARs. Over the last decade, research on PPARγ unveiled its role in important biological processes, including lipid biosynthesis, glucose metabolism, anti-inflammatory response and atherosclerosis. Recently, PPARγ has been shown to be expressed in many cancers including, lung, prostate, bladder, colon, breast, duodenal, thyroid and has been demonstrated to potentially play an important role in carcinogenesis. In bladder cancer, PPARγ ligands such as troglitazone and 15d-PGJ_2 have shown to inhibit tumor growth. We have recently published the first report to show that a new class of PPARγ agonists, PPARγ-active C-DIMs, which are more potent than the previous generation of drugs, exhibit anti-tumorigenic activity against bladder cancer cells in vitro and bladder tumors in vivo. The following review will discuss the molecular structure of PPARγ, its function, and its role in cancer biology and how it is emerging as a promising therapeutic target in bladder cancer.

Structural Features of Peroxisome Proliferator-Activated Receptor γ and Mechanisms of Regulation

Peroxisome Proliferator-Activated Receptors (PPARs) are ligand-activated intracellular transcription factors, members of the nuclear hormone receptor superfamily (NR),[1,2] that include estrogen, thyroid hormone receptors, retinoic acid, Vitamin D3 as well as retinoid X receptors (RXRs). The PPAR subfamily consist of three subtypes encoded by distinct genes denoted PPARα (NR1C1), PPARβ/δ (NR1C2) and PPARγ (NR1C3),[3,4] which are activated by selective ligands. The three subtypes of PPAR exhibit distinct tissue distribution reflecting their biological functions. PPARα is predominantly expressed in hepatocytes, cardiomyocytes, proximal tubule cells of kidney, while PPARβ/δ is more abundantly expressed.[5-8] PPARγ is highly expressed in adipocytes[3] but it is also found in endothelial cells[9] and cells of the immune system.[10] It is also widely expressed in many tumors including, lung, prostate, colon, breast, duodenal, thyroid and bladder. In humans, PPARγ is the most extensively studied subtype of the PPARs that, like other nuclear hormone receptors, possesses a modular structure composed of six defined regions (A–F) in four functional domains (Fig. 1). Upon activation, PPARγ form heterodimers with the retinoid X receptor (RXR) and the complex binds to specific recognition sites, named the peroxisome proliferator response elements (PPRE), located within the promoter regions of PPAR-responsive genes. In addition to the heterodimer complex, binding of agonist ligands to PPARγ triggers a conformation change that attracts transcriptional coactivators, including members of the steroid receptor coactivator (SRC) family that modify chromatin structure and facilitate assembly of the general transcriptional machinery to the promoter.[11,12]

PPARγ can also negatively regulate gene expression in a ligand-dependent manner by inhibiting the activities of other transcription factors, such as members of NFκB and AP-1 families, which has been termed ligand-dependent transrepression (Fig. 2).[13] In contrast, to transcriptional activation and repression, transrepression activity, does not involve binding to typical receptor specific response elements.[14,15] Additionally, in the absence of ligands, PPARγ has the potential to repress the transcription via ligand-independent repression. In this condition, PPARγ and RXR are associated to transcriptional corepressor complexes such as nuclear receptor corepressor (N-CoR) or silencing mediator of retinoid and thyroid receptors (SMRT),[16,17] that function to antagonize the actions of coactivator complexes.[18,19] The transcriptional coactivators and corepressors possess or recruit multiprotein complexes

*Correspondence to: Wassim Kassouf; Division of Urology; L8-315; McGill University Health Center; 1650 Cedar Ave; Montreal, Quebec H3G 1A4 Canada; Tel.: 514.934.8246; Fax: 514.934.8297; Email: wassim.kassouf@muhc.mcgill.ca

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including histone-modifying enzymes, such as histone acetyltransferases (notably p300/CBP) and histone deacetylases (notably HDAC 3), respectively. The activity of these histone-modifying enzymes affects gene transcription by altering chromatin structure, thereby regulating the binding of RNA polymerase.[12]

Natural ligands of PPARγ include fatty acids and eicosanoids,[20] components of oxidized low-density lipoproteins,[21] and oxidized alkyl phospholipids including lysophosphatidic acid[22] and nitrolinoleic acid.[23] The prostaglandin J2 derivative, 15-PGJ2 is the most potent endogenous ligand for the PPARγ receptor and is the most commonly used naturally occurring PPARγ-agonist.[24] Other synthetic compounds that can function as ligands include the anti-diabetic thiazolinidinedione (TZD) class of drugs including troglitazone (TGZ), rosiglitazone (BRL49653), pioglitazone, ciglitazone (CIG) and certain non steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, ibuprofen, flufenamic acid and fenoprofen.[10]

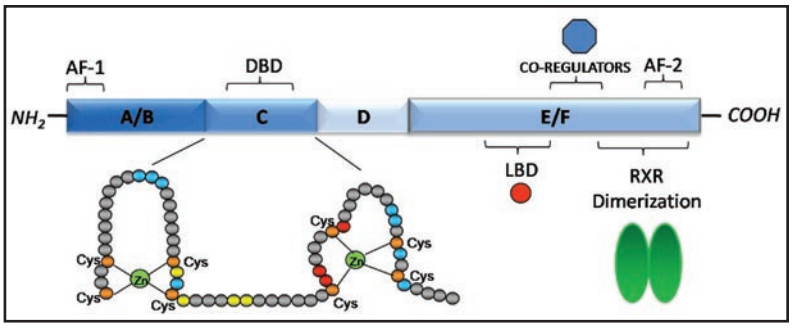

Figure 1. Schematic representation of functional domains of PPAR. PPARs have six defined regions (A–F) in four functional domains: The N-terminal (A/B) region contains the putative ligand-independent transactivation domain (AF-1). The (C) region has two zinc fingers and contains the DNA binding domain (DBD) that target the receptor to specific DNA sequences. The (D) domain is important for co-factor docking. The COOH-terminal (E/F) region contains the ligand binding domain (LBD), a dimerization interface, and the ligand-dependent activation domain (AF2-2) which is also involved in mediating ligand-induced interactions with transcriptional coactivators.

Similar to other nuclear receptors, the PPARs are phosphoproteins and their transcriptional activity is affected by cross-talk with kinases and phosphatases, in a ligand-dependent or -independent manner.[25-27] These effects of phosphorylation on receptor activity depend on several aspects such as, PPAR isotype, modified residue, stimulus and the kinase. Ligand binding by PPARγ is regulated by intramolecular communication between its amino-terminal A/B domain and its carboxy-terminal ligand binding domain (LBD). Modification of the A/B domain, for example by physiological phosphorylation by MAP kinase, reduces ligand-binding affinity, thus negatively regulating the transcriptional and biological functions of PPARγ.[28,29] Extracellular signals such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) stimulate PPARγ phosphorylation at serine 112 in the A/B domain through MAPK signalling, thereby decreasing the ligand-dependent transcriptional activity of PPARγ.[30,31] Alternatively, insulin treatment increases the ligand-independent transcriptional activity of PPARγ via phosphorylation mediated by MAPK.[32] Regulation of PPAR activity through phosphorylation is a complex and still a new area of study. For example, in some cases, in vitro assays demonstrate that mutation of the main MAPK site of phosphorylation in PPARγ2 (S112D) exhibits a decreased ligand-binding affinity[27,28] while activated MAP Kinase Kinase (MEK) has only a small effect on the S112A mutant of PPARγ.[30] It has been proposed that phosphorylation-mediated inhibition of transcriptional activity of nuclear receptors is an important "off-switch" of ligand-induced activity.[33] Alternatively, the phosphorylation status could control interactions between PPARs and corepressors and/or coactivators of transcription. Altogether, this context-specific action of phosphorylation reflects the complexity and intricacy of the signalling pathways involved in gene activation by PPARs.

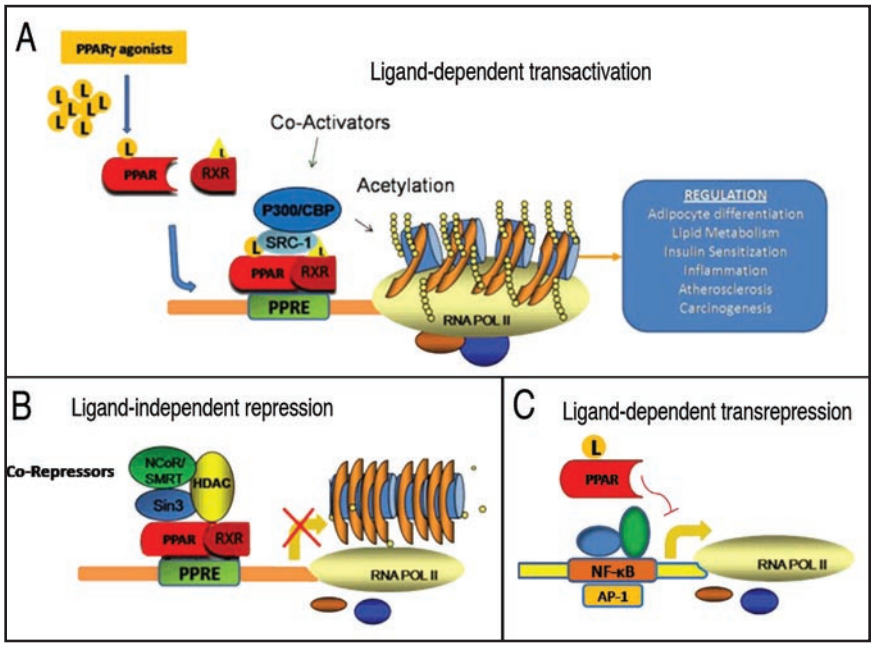

Figure 2. (A) PPAR activation pathway and transcriptional regulation of target genes. Upon activation, PPARγ form heterodimers with the retinoid X receptor (RXR) and form a complex that binds to specific recognition sites, named the peroxisome proliferator response elements (PPRE), located within the promoter regions of PPAR-responsive genes (ligand-dependent transactivation. Heterodimer complex, binding of agonist ligands to PPARγ triggers a conformation change that attracts transcriptional coactivators. (B) In the absence of ligand, PPARγ and RXR are associated with transcriptional corepressor complexes such as nuclear receptor corepressor (N-CoR) or SMRT (silencing mediator of retinoid and thyroid receptors) which contains deacetylation activity (HDAC) and mediate transcription repression (ligand-independent repression). (C) PPARγ represses transcription by inhibiting the activities of other transcription factors, such as members of NFκB and AP-1 families (ligand-dependent transrepression).

Function of PPARγ

The high levels of PPARγ expression in adipose tissue,[34,35] led to the characterization of its important role in adipocyte differentiation and regulation of lipid metabolism. Much of what is known about this role of PPARγ followed the discovery that thiazolidinedione (TZD) anti-diabetic drugs are actually high-affinity agonist ligands

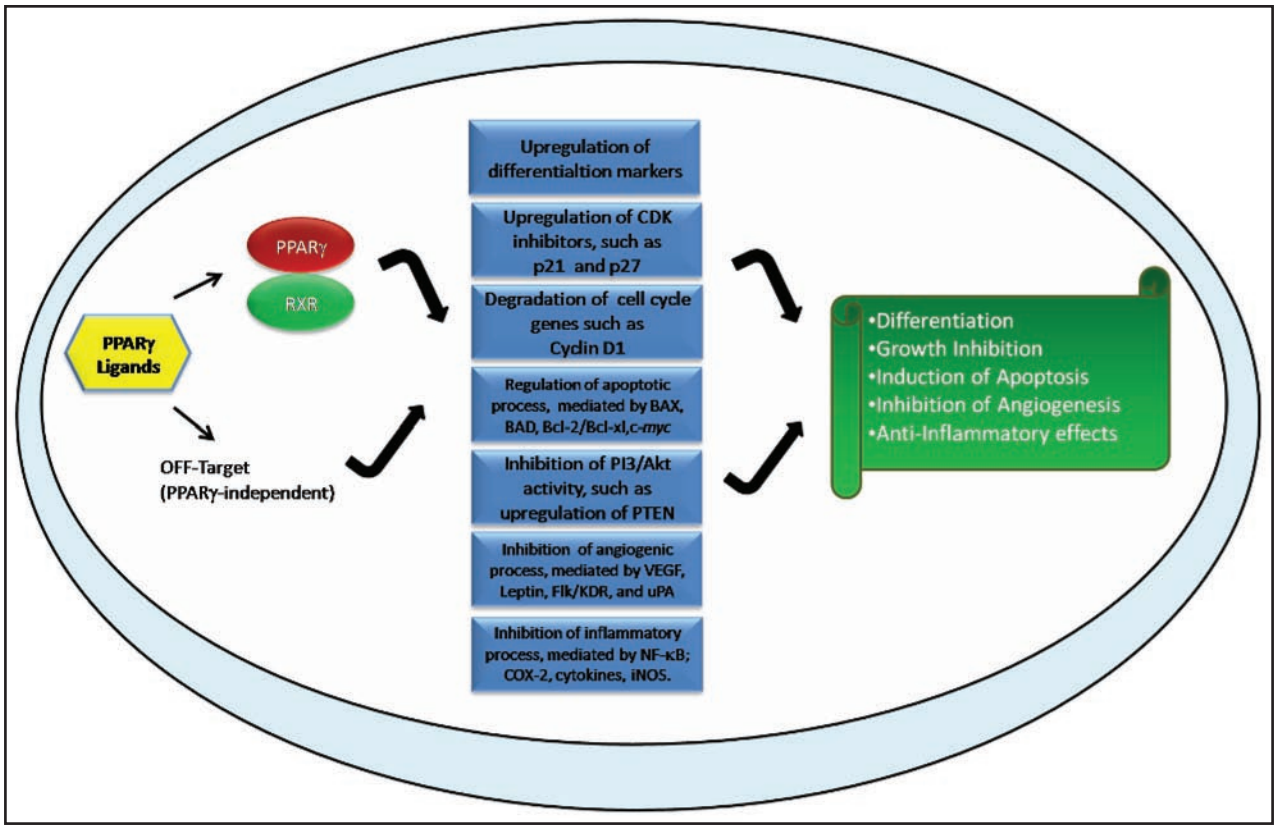

Figure 3. PPARγ-dependent and -independent effects of PPARγ agonists on carcinogenesis. The figure shows selected examples of potential molecular targets of PPARγ agonists that mediate differentiation, anti-proliferation, anti-angiogenic and anti-inflammatory effects.

for PPARγ.[36] In rat adipose tissue, TZDs appear to regulate expression of target genes such as lipoprotein lipase (LPL).[37] Its activation favours adipocyte uptake of circulating fatty acids, leading to an increase in net lipid partitioning into adipocytes. Although the role of PPARγ in adipocyte development suggested a possible connection between PPARγ activity and insulin sensitivity, the discovery that PPARγ as the biologic target for the thiazolidinedione provided the first definitive link as it directly reduced the systemic insulin resistance of peripheral tissues.[38] Activation of PPARγ results in significant decrease of serum glucose concentration in patients with diabetes, which led PPARγ agonists to be widely used in the clinical setting as anti-diabetic medication.[39] Currently, two TZD compounds, rosiglitazone and pioglitazone, are prescribed clinically for this purpose.

Furthermore, PPARγ agonists ablate the actions of TNFα in adipocytes in vivo by inhibition of tumor necrosis factor-α (TNFα) expression,[40-42] a pro-inflammatory cytokine that is expressed by adipocytes, and has also been associated with insulin resistance and diminished insulin signal transduction.[43,44] The inhibitory effects of PPARγ activation on TNFα action led several research groups to examine the anti-inflammatory properties of PPARγ agonists. In fact, PPARγ ligands inhibit not only the production of TNFα, but also of other cytokines, like interleukine (IL)-1b, IL-6 and induce a resting phenotype in macrophages with downregulation of its nitric oxide synthase (NOS) production.[45] Thus, PPARγ may be implicated in multiple other inflammatory processes as it is expressed throughout the immune system and suggest a potential target for treating inflammatory diseases such as atherosclerosis, arthritis and inflammatory bowel disease.[46,47] Lastly, PPARγ agonists have an important role in vascular biology. PPARγ agonists (thiazolidinediones or glitazones) antagonize angiotensin II effects in vivo and in vitro and have cardiovascular anti-oxidant and anti-inflammatory actions. Studies have shown that PPARγ agonists reduce blood pressure and correct vascular structure and endothelial dysfunction in experimental models of hypertension.[48]

Role in Cancer Biology

Although PPARγ function has been explored mostly in studies of adipocyte differentiation, insulin sensitization, inflammation, lipid metabolism and atherosclerosis, others roles such as different aspects of cellular development, differentiation and physiology have been implicated in PPARγ's activity. Moreover, the wide expression of PPARγ in many tumours and the ability of PPARγ ligands to inhibit cellular proliferation, promote differentiation, induce apoptosis and inhibit angiogenesis, lead researchers to postulate that PPARγ may play an important role in carcinogenesis (Fig. 3).

Anti-proliferative mechanisms. The Phosphatase and Tensin Homolog (*PTEN*) tumor suppressor gene modulates several cellular functions, including cell migration, survival and proliferation by antagonizing phosphatidylinositol 3-kinase (PI-3K)-mediated signaling cascades.[49] Previous studies have reported that the activation of PPARγ by rosiglitazone upregulates PTEN expression in human macrophages, Caco2 colorectal cancer cells, MCF7 breast cancer cells, 3T3-L1 adipocytes and C2C12 skeletal muscle cells.[50,51] This upregulation correlated with decreased PI-3K activity[50] and the effects of PPARγ-agonists on PTEN expression disappeared by either

pretreatment with a PPARγ antagonist or knockdown of PPARγ expression.[51] PPARγ ligands have also been shown to inhibit lung carcinoma cell proliferation through increased expression of PTEN and p21.[52,53] In other studies, rosiglitazone reduced phosphorylation of Akt and increased PTEN protein expression in non small-cell lung cancer (NSCLC) cells, and this was associated with inhibition of tumor cell proliferation through PPARγ-dependent signals.[54] PPARγ agonists have also been shown to target cyclin-dependent kinase (CDK) inhibitors such as p18, p21 and p27 during adipogenesis in normal cells and hepatocellular carcinoma cell lines.[55,56] CDK inhibitors block progression of the cell cycle by inactivating the formation of cyclin/CDK complexes, which are crucial for phosphorylation and inactivation of the retinoblastoma protein.[57] It has been demonstrated that glitazones and troglitazone induced p21 with cell cycle arrest in G_1 phase in pancreatic tumor cell lines.[58,59] Itami et al.[60] and Motomura et al.[61] described upregulation of p27 in pancreatic tumors after treatment with PPARγ agonists. Furthermore, PPARγ agonists promote cell cycle arrest by the downregulation of cyclin D1 in several tumour cell lines, including those derived from pancreatic cancer,[60] breast tumours,[62] hepatocellular carcinoma[56] and NSCLC.[63] PPARγ ligands inhibit cyclin D1/Cdk-mediated retinoblastoma (pRb) phosphorylation and thereby maintain pRb in its active form, which prevents G_1 to S phase transition in normal cells, such as adipocyte differentiation and breast cancer cells.[64-66]

Pro-apoptotic mechanisms. Anti-neoplastic effects of PPARγ agonists might also be mediated by induction of cellular apoptosis. Ohta et al.[67] reported that in thyroid cancer cells, expression of PPARγ correlated with the sensitivity of TDZ and 15d-PGJ_2. Thyroid cancer cells that did not express PPARγ showed no growth inhibition with TDZ and 15d-PGJ_2, compared with PPARγ-positive thyroid carcinoma cells. The cell death observed in the thyroid cancer cells seems to be due to apoptosis, since ligands for PPARγ induced condensation of the nucleus and fragmentation of chromatin into nucleosome ladders. In another study on thyroid cancer, Martelli et al.[68] showed that ciglitazone was effective in reducing the growth of thyroid cancer cells that expressed PPARγ, but not in cancer cells that did not express it. However, introduction of wild-type PPARγ into PPARγ-deficient cells turned these cells responsive to ciglatizone. Moreover, overexpression of PPARγ significantly increased apoptosis compared to cells transfected with empty or non-functional PPARγ cDNA. Altogether, these findings suggest a PPARγ-dependent induction of apoptosis by PPARγ ligands in thyroid carcinoma cells.

Recently, Bonofiglio et al.[69] have described a new molecular mechanism by which rosiglitazone induces apoptosis in breast cancer cells. In this study, they have shown rosiglitazone enhanced FasL expression, a trans-membrane protein that induces apoptosis by crosslinking with the Fas receptor. The role of PPARγ and Fas/FasL pathways in rosiglitazone-induced apoptotic events was assessed by caspase 8 cleavage in the presence of specific PPARγ antagonist GW9662 as well as PPARγ and FasL respective RNA interferences. These findings indicate that PPARγ positively regulates FasL expression, in response to rosiglitazone. Moreover, it appears to be a common mechanism in breast cancer cells since it occurs in different types of breast carcinoma cells. Nevertheless, many of the underlying mechanism of the apoptotic properties of PPARγ-agonists remain unknown. Several studies have revealed induction of apoptosis by PPARγ-agonists are cell type-dependent and cannot always be attributed to PPARγ activation. Therefore, careful consideration should be given to individual tumor types and their response to PPARγ-agonist employed.

Inhibition of angiogenesis mechanism. PPARγ knock-out mice embryos die on day 10 of life because of interference with the terminal differentiation pattern of trophoblasts and the vascularisation of the placenta, suggesting that PPARγ has a crucial role in angiogenesis.[70] PPARγ is expressed in normal and tumor endothelial cells and influences angiogenesis at multiple steps. PPARγ agonists inhibit fibroblast growth factor-2 (FGF2) and vascular endothelial growth factor (VEGF)-stimulated proliferation.[9,71,72] PPARγ activation by glitazones induces PPARγ expression in tumor endothelial cells and inhibits angiogenesis both in vitro and in vivo.[71] Additionally, ligand-mediated PPARγ activation results in potent inhibition of endothelial differentiation into tube-like structures in vitro and suppression of VEGF-induced angiogenesis in vivo. Furthermore, PPARγ activation by 15d-PGJ2 also inhibits the expression of at least three important genes in the angiogenic process, the VEGF receptors 1 (Flt-1), 2 (Flk/KDR) and the urokinase plasminogen activator (uPA).[72] Further studies have found that PPARγ also influences angiogenesis by modulation of leptin proliferation effect on human endothelial cells (ECs). Leptin, the product of *ob* gene, functions as a potent angiogenesis inducer, which stimulates EC proliferation and cell survival, through activation of the PI3K/Akt/NOS. It has been reported that PPARγ activation by TZDs downregulates leptin level, in vitro and in vivo[73] and block leptin-stimulated EC migration by inhibition of Akt and eNOS.[74]

Anti-tumorigenic effects mediated by PPARγ-independent pathways. A large number of studies have reported the diverse effects on tumor growth, progression and metastasis exerted by PPARγ agonists. Nonetheless, emerging data have indicated that some of these antitumor effects are not totally or partially PPARγ-dependent, but rather are PPARγ-independent. As previously mentioned, rosiglitazone inhibits NSCLC cell proliferation through PPARγ-dependent signals.[54] However, it also occurs by PPARγ-independent signals, as increase in the phosphorylation of AMPKα, which is mediated by rosiglitazone, is not affected by treatment with PPARγ antagonist, GW9662.[54] Recently, Chaffer et al.[75] have demonstrated that both PPARγ agonists, TGZ and 15dPGJ2, inhibited prostate and bladder cancer cell growth in a PPARγ-independent fashion. These findings suggest that these anti-tumor effects induced by the PPARγ agonists are attributed to regulation of other various cellular signaling pathways, independently from the PPARγ receptor pathways. Further studies have identified some pathways that are activated by TZDs in a PPARγ-independent manner. For example, high levels of prostaglandin E2 (PGE2) as consequence of induction of cyclooxygenase 2 (COX-2) promotes tumor growth and progression through a number of pathways.[76] In addition to their ability to promote gene transcription in a PPARγ-dependent manner, PPARγ-agonists have recently been shown to induce mitogen-activated protein kinase (MAPK) phosphorylation, suggesting the effects of PPARγ agonist may also be mediated by a nongenomic effect.[77] It implies that numerous other target genes may be subject to transcriptional control by PPAR ligands. In the example of TGZ, it induces apoptosis in NSCLC through both "on-target" and "off-target" pathways and activates the ERK MAP kinase family in NSCLC through a PPARγ-independent pathway.[78,79] Studies with TZD derivatives lacking PPARγ activity

have been used to elucidate whether a TZD is signalling through PPARγ-dependent mechanisms. Most notably, a study by Shiau et al.[80] showed that the pioglitazone, troglitazone and ciglitazone derivatives (Δ2-PG, Δ2-TG, Δ2-CG) which cannot activate PPARγ were more effective in suppressing growth in prostate cancer cell lines. These results suggest that TZDs can induce apoptosis independent of PPARγ activation and in this study it appears to be partly due to the inhibition of the antiapoptotic function of Bcl-2 and Bcl-x_L. Similar conclusions by others authors,[81-83] were also made with regards to induction of apoptosis by inhibition of Bcl-2 and BcL-x_L when breast cancer cells, pituitary tumor xenografts animal models, and glioma cells, were treated with TZDs. Furthermore, several others alternative apoptotic pathways can lead to cell death in response to PPARγ agonists. Shimada et al.[84] found that 15d-PGJ2 or TGZ induced apoptosis in colon cancer cells with downregulation of c-*myc* expression, as well as upregulation of c-*jun* and *gadd*153 expression. However, no visible changes in mRNA levels of bcl family genes were detected. Other mediators of apoptosis in PPARγ ligand-induced cell death include activation of tumor-necrosis factor (TNF) and the transcription factor NFκB, which promotes apoptosis.[85] Moreover, separate studies demonstrated that TZDs can also induce apoptosis through TNF-related apoptosis-inducing ligand (TRAIL)-dependent pathways and through PPARγ-independent mechanisms.[86-88] Lastly, a review article recently published by Elrod et.al.[89] describes the role and mechanisms of PPARγ-agonists, on cellular apoptosis. Interestingly, the report highlights that specific types of tumors and unique tumor microenvironments behave differently to PPARγ activation or inhibition. Therefore, a close examination of individual tumor types and their response to PPAR stimulation will be critical for successful cancer therapy targeting PPARγ.

PPARγ and PPARγ-Agonists in Bladder Cancer

Among several solid tumors, PPARγ is commonly expressed in bladder cancer and its level of expression is correlated with tumor grade and stage. Yoshimura et al.[90] have reported a marked expression of PPARγ in bladder cancer tissue compared with normal bladder urothelium. Furthermore, higher levels of expression were associated with higher grade and advanced stage, suggesting that PPARγ agonists may mediate more potent anti-tumor effects in the more aggressive types of bladder cancer.

Early studies have reported that PPARγ agonists play an important role in inducing TCC differentiation and survival by increasing expression of adipocyte-type fatty acid binding proteins (A-FABP).[91] PPARγ interacts directly with A-FABP and loss of PPARγ has been demonstrated to be associated with progression of bladder cancer.[92,93]

Inflammatory process might produce favourable microenvironments for latent DNA damage to proceed to carcinogenesis which include releasing growth and survival factors, promoting angiogenesis, evading apoptosis, subverting the host immune response, and remodelling the microenvironment to facilitate tumor migration and metastasis. In bladder cancer, polymorphism in genes that regulate inflammatory process such as IL-6 and PPARγ are associated with recurrence risk, progression and survival.[94] Patients with PPARγ variant alleles (Single Nucleotide Polymorphism-Pro12Ala) show decreased PPARγ receptor activity and are associated with an increased risk of bladder cancer recurrence and progression.[94] The decreased PPARγ activity, due to variant alleles, leads to reduced anti-inflammatory and anti-proliferative activity and thus may provide favourable conditions for tumor growth.[94] Recent studies have demonstrated that in bladder tumor cells, which PPARγ expression was weak or absent, standard therapy with Bacillus Calmette-Guerin therapy (BCG), induced cytoplasmic expression of PPARγ.[95] Additionally, it is noteworthy that the inhibition of cell viability by 15-d-PGJ_2 was only detected in the presence of BCG, and the effect of 15-d-PGJ_2 was PPARγ-dependent, since BADGE, a specific PPARγ antagonist, reversed the BCG-mediated cell cytotoxicity. BADGE also directly reversed BCG-mediated cell death, confirming that PPARγ is involved in BCG cytotoxic activity.[95] These findings suggest BCG has the ability to induce functional PPARγ which can respond only to the endogenous ligand. Moreover, the promising mechanism of Bacillus Calmette-Guerin (BCG) treating superficial bladder carcinoma has been investigated. In this study, Saban et al.[96] determined the differential gene expression in mouse bladder following chronic intravesical BCG therapy. Interestingly, they found that BCG treatment-specific genes networks overlapped with the several canonical signaling pathways including PPARγ.

Lastly, PPARγ may also target vascular neogenesis in bladder cancer, a disease whose vascular phenotype has previously been shown to respond well to anti-angiogenic drugs.[97] Possati et al.[98] analyzed the expression of PPARγ and angiogenic factors in 75 human bladder tumor specimens and the results were compared to the clinical and pathological characteristics of the disease. They found that the expression of platelet-derived endothelial cell growth factor (PDECGF), an angiogenic factor, is significantly associated with tumor recurrence and poor prognosis. However, the concomitant expression of PPARγ was associated with significantly low incidence of tumor recurrence or progression suggesting a protective effect of PPARγ against PDECGF.[98]

Although PPARγ have shown important role in carcinogenesis, a better understanding of its mechanism of action in bladder tumors is needed. As previously mentioned, Chaffer et al.[75] investigated the effects of a range of endogenous and synthetic PPARγ ligands on proliferation, growth arrest and apoptosis in a series of transitional cell carcinoma (TCC) of the bladder with increasing metastatic potential. They found that TGZ and 15dPGJ2 induced growth inhibition in all bladder carcinoma cell lines although via different mechanism. TGZ induced G_0/G_1 growth arrest whilst 15dPGJ2 induced apoptosis. However, the induction of growth arrest and apoptosis were not reverse by the selective PPARγ antagonist GW9662 indicating the effects of both agents are PPARγ-independent.

Another important factor that needs to be further investigated is the possible carcinogenic effects of some PPARγ-agonists inducing bladder tumors in rodent (www.fda.gov/cder/present/DIA/2004/Elhage.ppt). Lately, Long et al.[99] investigated in rats, the effect of Naveglitazar, a γ-dominant peroxisome proliferator-activated receptor (PPAR)α/γ dual agonist, in carcinogenicity. After 2 years, a significant increase in neoplasms of the bladder occurred only in females of the high-dose group, but no evidence for urolithiasis, as incident event was observed. In another study to determine if rosiglitazone had chemopreventive activity, Lubet et al.[100] have shown that when female rats were treated with different doses of rosiglitazone plus hydroxybutyl(butyl)nitrosamine (OH-BBN), a urinary bladder specific carcinogen, large cancer were developed as compared with

rats treated with OH-BBN alone. However, no apparent activity as a complete carcinogen was observed, which might imply that it is only a tumor promoter that may be highly specific for the OH-BBN urinary bladder cancer model in rats. Additionally, the effects were observed only in females rather than males and could be due to chemical irritant effects. Nonetheless, these effects were observed quite rapidly (8 weeks) when rosiglitazone was administered late, arguing against a constant long-term irritant effect. These findings highlight the need to critically evaluate the involvement of PPARγ-agonists in bladder cancer initiation. Moreover, it is noteworthy to mention that many of the carcinogenic effects of the PPARγ receptor agonists are highly species specific; i.e., observed in rodents but not in humans or primates, which have already been extensively tested in use for various indications.

Recently, Dr. Stephen Safe (Texas A&M University, Houston) has developed a more potent class of PPARγ agonists from a series of 1,1-bis(3V-indolyl)-1-(*p*-substitutedphenyl)methanes. These PPARγ active compounds contain p-trifluromethyl (DIM-C-pPhCF3), p-t-butyl (DIM-C-pPhtBu) and p-phenyl (DIMC-pPhC6H5) substituents. Several studies have shown that these compounds activate PPARγ in different cancer cell lines, such as colon, pancreatic, prostate, bladder, breast, endometrial and kidney.[101-106] Structure-activity studies show induction of PPARγ-dependent transactivation in breast cancer cell lines by these compounds, whereas treatment with the PPARγ-specific antagonist *N*-(4'aminopyridyl)-2-chloro-5-nitrobenzadine inhibited this effect.[101] In pancreatic cancer cell lines, ligand-dependent activation of PPARγ was observed in cells transfected with PPRE-luciferase and treated with DIM-C and troglitazone alone, whereas co-transfection of small inhibitory RNA for PPARγ, significantly inhibited transactivation by DIM-C and its effects on cell proliferation.[101,102] In bladder cancer cells in vitro and in bladder tumor in vivo, we demonstrated that PPARγ active C-DIMs showed significant anti-tumorigenic activity and were highly more potent inhibitors of bladder cancer growth when compared with rosiglitazone, the currently used synthetic PPARγ agonist.[105] In our study, PPARγ-active C-DIMs decreased cell survival in bladder cancer cells and inhibited tumor growth in animal models. The anti-tumorigenic activity of PPARγ-active C-DIMs was associated with induction of caveolin-1 (a terminal differentiation marker) and p21 expression. However, even though these effects were not shared by the well-characterized PPARγ-agonist rosiglitazone, the induction of caveolin-1 was significantly downregulated after co-treatment with the PPARγ antagonist GW9662, which suggest the tumor suppressor activity of C-DIM occurs through PPARγ activation. Additionally, these results are consistent with other studies showing that modulation of cell cycle genes (p21) and the induction of caveolin-1 have been linked to PPARγ-dependent inhibition of pancreatic and colon cancer cell growth after treatment with C-DIMs.[102,103] Nonetheless, not all effects of C-DIMs are promoted by PPARγ activation, but instead, in other responses, the C-DIMs induce pro-apoptotic and growth inhibitory effects also in a PPARγ-independent manner.[107]

Currently, combined therapy has become a breakthrough in treating cancer. In a range of tumor entities, such approach has produced impressive results. Combination therapy of PPARγ agonists and other agents has been shown to be more effective than using either agent alone.[108,109] Moreover, some studies suggest a cross-talk between PPARγ and EGFR signalling pathways.[77,110,111] Our previous work had shown that modulation of glycogen synthase kinase-3 beta (GSK-3β) and cyclin D1 might be a predictor of response to EGFR inhibitors in bladder cancer.[112] Taken together, combined targeting of both EGFR and PPARγ axes can reveal promising molecules to target in bladder cancer. Preliminary results in our laboratory have shown that combined EGFR inhibitors and PPARγ-active C-DIMs provide synergistic inhibitory effects on the growth of bladder tumors (unpublished data).

In summary, these results suggest that PPARγ-agonists may be a potential therapy in human bladder cancer, but a better understanding of its mechanisms of action need to be elucidated prior to clinical exploration

Safety Issues and PPARγ Agonists in Clinical Development

Despite the therapeutic importance, the side effects associated with PPARγ agonist must be tested in large outcome-based clinical trials. Several such trials have been initiated to examine their role in primary as well as secondary prevention of cardiovascular events.[113] The first of these was the Prospective Pioglitazone Clinical Trial in Macrovascular Events Study (PROactive).[114] Although the PROactive study show no statistically significant reduction in the risk of primary composite endpoint, which consisted of mortality, nonfatal myocardial infarction, stroke and acute coronary syndrome, PROactive met its principal secondary endpoint demonstrating that pioglitazone can reduce by 16%, the combined risk of heart attacks, stroke and death in a high-risk patient population with type 2 diabetes and established macrovascular disease. Diabetes Reduction Approaches with ramipril and rosiglitazone Medications (DREAM) study[115] demonstrated that rosiglitazone prevents the development of type 2 diabetes in non diabetic patients suffering from insulin resistance and the metabolic syndrome. However, the incidence of newly diagnosed cardiac insufficiency was higher in the TZD-treated group than in the placebo group. While some studies suggest TZDs may have the propensity to cause peripheral edema and congestive heart failure (CHF) due to an increased cardiac workload resulting from plasma volume expansion,[116] clinical studies in type 2 diabetics have demonstrated no troublesome effects on cardiac performance and there are even some trends toward improved function associated with long term TZD therapy.[117,118] However, new PPARγ agonists, which do not increase fluid retention, should be more cardioprotective than actual TZDs by limiting the potential risk of cardiac insufficiency and thus a new generation of safer PPARγ drug should be of great interest. Recently, a meta-analysis of the three large randomized trials (RECORD, DREAM and ADOPT) shows that rosiglitazone appears to be associated with an increased risk of myocardial infarction and heart failure, but not death due to cardiovascular causes.[119]

Some preclinical studies have suggested that ligand activation of PPARγ can promote carcinogenesis. However, these effects are controversial and the data are not equivocal depending on different parameters such as PPARγ subtype, animal model (rodents, non-rodents, non-human primate) and cancer type (liver, colorectal, urinary tract, etc.,). In 2004 the FDA reviewed the extent of pre-clinical carcinogenesis data on PPARγ agonists and stated that a mechanism of action to explain tumor formation is not available and the mechanism mediated by the receptor cannot be excluded. Nevertheless, it is noteworthy that the PROactive and DREAM studies, which are

the longest clinical trial published to date, show no evidence of any change in number of malignant neoplasm.[120,121]

Several early-phase clinical studies have been performed to evaluate the efficacies of TZDs in various types of cancers. Recently, a phase II clinical trial of rosiglitazone was conducted in 12 patients with liposarcoma to evaluate clinical response. Histologic appearance shows no significant change in differentiation of the liposarcomas by the treatment. Additionally, levels of gene expression of PPARγ and fatty acid binding protein (FABP) induced after 12-week rosiglitazone therapy show that disease in these patients progressed similarly to the others, suggesting increased PPARγ activity did not correlate with the clinical evolution.[122] The efficacy of troglitazone was also evaluated in 25 patients with metastatic colorectal carcinoma but all patients had progressive disease.[123] In breast cancer, two human trials have been conducted. In one study, no objective response to troglitazone after 8 weeks of treatment was observed in 22 women with refractory breast cancer.[124] In a pilot trial of short-term (2–6 weeks) therapy, treatment with rosiglitazone in 38 women with early-stage breast cancer did not elicit significant effects on breast tumor cell proliferation.[125] An early phase II trial in 41 patients with metastatic prostate cancer showed a decreased in levels of prostate specific antigen (PSA) in 20% of the patients and prolonged stabilization of PSA in 39% of patients.[126] However, these results were not reproduced in a large double-blind, randomized, placebo-controlled trial of rosiglitazone in 106 patients with recurrent prostate cancer.[127] Recently, a very interesting and large epidemiologic study was conducted with 87,678 men with diabetes to evaluate whether TZDs have the potential to act as chemopreventive agent.[128] In this retrospective study, risk of lung, prostate and colon cancer development were compared in TZDs and non TZDs users. Risk of lung cancer was reduced by 33 % among TZDs users compared with non TZDs users although the risk reduction for colorectal and prostate cancers did not reach statistical significance. Taken together, clinical trials with PPARγ agonists demonstrated contradictory results since some showed beneficial effects with PPARγ agonists while others did not. These ambivalent findings could be based partly on the selection of pretreated refractory cancers, lack of assessment of the PPARγ protein levels before patients were included, inadequate potency of the agonist, and suboptimal dosing since serum drug concentrations were not evaluated. Nevertheless, the numbers of clinical trials are limited and none examined response rates in patients with bladder cancer.

Conclusion

PPARγ is highly expressed in several solid malignancies, including bladder cancer. Its activation inhibits cancer cell proliferation, angiogenesis, induces apoptosis and plays an important role in carcinogenesis. PPARγ agonists are commonly used in the clinical setting as anti-diabetic medication and have been proven to be associated with acceptable toxicity. However, despite the promising therapeutic target of PPARγ agonists for cancer therapy, there are safety concerns, such as dose limiting side effects associated with PPARγ drug treatments, potential carcinogenicity in rodents, and increased risk of cardiac failure. The effects of PPARγ agonists are complex and do not always correlate with PPARγ activation; the PPARγ-independent effects highlight that these drugs' mechanisms of action need to be further elucidated and careful consideration should be given to the ligand employed. Newer and more potent class of PPARγ agonists, PPARγ-active C-DIMs, have shown great potential for clinical treatment of different cancers, including bladder cancer therapy. We are currently investigating ligand-dependent and ligand-independent pathways responsible for the anti-cancer activities of these compounds in bladder cancer. Clearly, only a better understanding of the mechanism of action of activated PPARγ and PPARγ-agonists will allow us to improve prediction of outcome and selection of patients that could benefit from such therapy in future clinical applications.

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