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# EXPRESSION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs) IN HUMAN URINARY BLADDER CARCINOMA AND GROWTH INHIBITION BY ITS AGONISTS

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**Recent studies have demonstrated that peroxisome proliferator activator-receptors(PPAR)-γ is expressed in various cancer tissues and its ligand induces growth arrest of these cancer cells through apoptosis. In our study, we investigated the expression of PPAR-α, β and γ in human bladder tumor (BT) and normal bladder (NB) tissues as well as the effects of PPAR-γ ligands. Specimens were obtained from 170 patients with BT and 20 with NB. The expressions were investigated using RT-PCR and immunohistochemical methods. We also investigated the inhibitory effect of PPAR-γ ligands on BT-derived cell line. Immunoreactive PPAR-α and -β were significantly apparent in both BT and NB tissues. Although no marked expression of PPAR-γ was observed in NB tissue, significant expression was found in BT tissue. The extent and intensity of immunoreactive PPAR-γ polypeptides in BT cells were statistically much greater than those of NB cells. Correlation between PPAR-γ expression and tissue type or progression of bladder cancer was observed; PPAR-γ expression was higher in G3 of bladder cancer than in G1 and was higher in advanced than in early cancer. PPAR-γ agonists, troglitazone and 15-deoxy-$\Delta^{12,\ 14}$-prostaglandin $J_2$ inhibited the growth of the BT cells. PPAR-γ is expressed in bladder tumor, and results suggest that PPAR-γ ligands may mediate potent antiproliferative effects against BT cells. Thus, PPAR-γ has the ability to become a new target in treatment of bladder tumor.**


**Key words:** *peroxisome proliferator activator-receptors (PPAR)-γ; bladder carcinoma; immunohistochemistry*

Angiogenetic factors play important roles in bladder tumors as well as in other cancers. In recent years, the expression of angiogenic factors in solid human tumors has been widely reported.[1] Growth factors secreted by tumor cells such as fibroblast growth factor and transforming growth factor have increased neovascularization *in vivo* and *in vitro*.[2] Reports demonstrate that peroxisome proliferator-activated receptor (PPAR)- γ ligands inhibit growth of cancer cells *in vitro* and *in vivo*.[3] PPAR-γ, a nuclear hormone receptor, provides a strong link between lipid metabolism and the regulation of gene transcription.[4] PPAR-γ acts in the adipose tissue and promotes lipogenesis under anabolic conditions. Recently, the receptor has also been implicated in inflammation and tumorigenesis. Significant evidence from many experimental systems suggest that PPAR-γ is important in carcinogenesis. PPAR-γ is upregulated in malignant tissue, and PPAR-γ ligands induce terminal differentiation in human breast, colon and lung cancer cells[5–7] and inhibit the growth of human breast, prostate, gastric and lung cancer cells.[5–8] Three distinct PPARs (PPAR-α, PPAR-βand PPAR-γ) have been identified, and PPAR-γ is found in adipose tissue, regulating adipocyte differentiation.[9] Orphan nuclear hormone receptor LXRαand thyroid receptor (TR) act as antagonists of PPARα/RXRα binding to PPAR-response elements (PPREs).[10]

In our study, we determined whether PPAR mRNAs are expressed in NB, and BT tissues by RT-PCR. Next, we examined whether PPARs are expressed in bladder tissues by immunohistochemistry, and we also demonstrated whether these expressions correlate with tumor grade and invasion. Finally, we investigated the inhibitory effect of PPAR-γ ligands, troglitazone and 15-deoxy-$\Delta^{12,\ 14}$-prostaglandin $J_2$ (15dPGJ$_2$) on BT-derived cell lines.

## MATERIAL AND METHODS

### *Tumor specimens*

Tumor specimens were obtained from 170 patients with bladder cancer (BT), of which 87 patients underwent total cystectomy and 83 patients underwent transurethral resection of bladder tumor (TUR-bt). Specimens were obtained from 20 patients having normal bladder tissues (NB) who underwent total prostatectomy because of prostate cancer. Tumor tissues, nontumor tissues, vascular endothelium and interstitial tissues from the subjects were preserved in 10% formalin and embedded in paraffin, serially sectioned onto microscope slides at a thickness of 4 μm.

### *Antibodies*

PPAR-α, β and γ are affinity-purified goat polyclonal antibodies. These antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). They demonstrated that the source of these antibodies, PPAR-α and γ, are affinity-purified goat polyclonal antibodies raised against a peptide mapping at the amino terminus of PPAR-α and -γ of human origin (α differs from the corresponding mouse sequence by amino acids; γ is identical to the corresponding mouse sequence). PPAR-β is an affinity-purified goat polyclonal antibody raised against a peptide mapping at the carboxy terminus of PPAR-β of human origin (differs from the corresponding mouse sequence by 2 amino acids). About specificity of these antibodies, PPAR-α and β react with those of mouse, rat and human origin by Western blotting and immunohistochemistry. PPAR-γ also reacts with PPARγ1 and PPARγ2 of mouse, rat and human origin by Western blotting and immunohistochemistry. These specific antibodies do not cross-match each other, nor do they cross-react with each other.

### *RT-PCR*

Synthetic oligonucleotides were obtained from Nippon Flour Mills (Kanazawa, Japan). We used G3PDH mRNA as a control.

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The primers used were as follows: PPAR-α: sense 5′-CCAGTATTTAGGACGCTGTCC-3′ and antisense 5′AAGTTCTTCAAGTAGGCCAGC-3′; PPAR-β: sense 5′-AACTGCAGATGGGCTGTAAC-3′ and antisense 5′-GTCTCGATGTCGTGGATCAC-3′; PPAR-γ: sense 5′-TCTCTCCGTAATGGAAGACC-3′ and antisense 5′-GCATTATGAGACATCCCCAC-3′; human G3PDH: sense 5′-CCACCCATGGCAAATTCCATGGCA-3′ and antisense 5′-TCTAGAGGGCAGGTCAGGTCCACC-3′.

The primer sets yield PCR products of 492, 484, 474 and 598 base pairs for PPAR-α, PPAR-β, PPAR-γ or G3PDH, respectively. Reactions were incubated in an automatic heat-block for 30 cycles of denaturation for 40 sec, 94°C; annealing for 50 sec, 50°C; extension for 50 sec, 72°C.[11] PCR products were run on 2% agarose gel in TAE buffer (40 mM Tris acetate, 1 mM EDTA) and visualized by ethidium bromide staining.

*Immunohistochemistry*

Tissues sections (3 μm thick) were incubated with anti-PPAR-α, -β, -γ antibody (2 μg/ml) or purified normal goat IgG (2 μg/ml) in a humid chamber for 24 hr and further incubated with biotinylated rabbit anti-goat IgG (Vector Laboratories, Burlingame, CA) for 30 min. After washing with PBS, the sections were incubated with the Vectastatin avidin-biotin peroxidase complex kit (Vector)[12] for 45 min. Color was developed by immersing the sections in a solution of 0.05% wt/vol 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma, St. Louis, MO). The sections were counterstained with hematoxylin (Sigma).

*Statistical analysis*

The extent and intensity of staining with PPAR-α, -β and -γ antibodies were graded on a scale of 0–4+ by 2 blind observers on 2 separate occasions using coded slides, and an average score was calculated.[13] Staining was classified into 5 grades from 0–4+ according to the intensity of staining and the number of positive cells. The observers assessed all tissues on the slides to assign the score. A 4+ grade implies that all staining was maximally intense throughout the specimen, whereas 0 implies that staining was absent throughout the specimen. The microanatomical sites of staining were also recorded. To quantify the expressions of PPAR-α ,-β and -γ, the same 2 pathologists made assessments throughout the study, staining control specimens simultaneously. This method, therefore, increases the credibility of data. In addition, all specimens were reassessed, which also contributed to the exclusion of any subjective variability.

Analyses of data were made by analysis of variance (ANOVA).[14]

*Cell cultures*

Human BT cell lines (transitional cell carcinoma; TCC) T24 were provided by Health Science Research Resources Bank (Osaka, Japan). Normal bladder membrane cell lines (normal transitional cell; NTC) were obtained from the patients with NB.

Cells were grown in culture flask (Nunc, Roskilde, Denmark) in RPMI 1640 supplemented with 10% FBS, 100 U/ml of penicillin and 100 μg/ml of streptomycin in a humidified 5% $CO_2$ atmosphere at 37°C. The media were changed every 3 days and the cells were separated via trypsinization, using trypsin/EDTA when they reached subconfluence.

*Cell proliferative studies*

Troglitazone was obtained from Sankyo Pharmaceuticals (Tokyo, Japan). 15dPGJ$_2$ was purchased from Cayman Chemical Company (St. Louis, MO). Approximately $2.5 \times 10^4$ cells placed onto 8 × 8 mm diameter multichamber slides (Nunc, Copenhagen, Denmark) were treated with troglitazone and 15dPGJ$_2$ (5–40 μM) dissolved in ethanol. The final concentration of ethanol was <0.05%. Cell viability was measured on day 1 by a microplate reader using a modified 3-[4, 5-dimethylthiazol-2-thiazolyl]-2, 5-diphenyltetrazolium bromide (MTT) assay (WST-1 assay; Dojindo, Kumamoto, Japan) and presented as the percentage of control-culture conditions.

## RESULTS

The 170 patients with BT (117 males and 53 females) were aged 37–85 years (mean age, 68.2 ± 17.4 years). Tumor histopathologies were classified by pathologists as transitional cell carcinoma (TCC) in 168 patients (pathologic stage distribution was G1 for 59 patients, G2 for 65 patients and G3 for 44 patients) and squamous cell carcinoma (SCC) in 2 patients. Tumor T factor was pTis for 22 patients, pTa for 31 patients, pT1 for 47 patients, pT2 for 34 patients, pT3 for 30 patients and pT4 for 6 patients. The 20 patients with normal bladder were an average age of 63.9 ± 15.2 years (range, 56–74 years) (Table I).

*PCR of reverse-transcribed RNAfrom BT and NB tissues*

To check PPAR-α, -β, -γ messenger RNA (mRNA) variation, RT-PCR was performed with total RNA extracted from BT and NB specimens. In all, subjects from patients with BT (2 of 59 TCC-G1, 2 of 65 -G2, 2 of 44 -G3, 2 of 2 SCC) and NB (2 of 20) total RNA was purified. Using specific primers for PPAR-α, -β and -γ and G3PDH, the amplification predicted, respectively, fragments of 492 base pair (bp), 484 bp, 474 bp and 598 bp in length. The PPAR-α and -β mRNA were also detected in samples from all specimens (PPAR-α, Fig. 1*a*; PPAR-β, Fig. 1*b*). However, we detected a specific band of PPAR-γ mRNA in the samples from BT (Fig. 1*c*, lane 1: TCC-G1; lane 2: -G2; lane 3: -G3; lane 4: SCC), whereas samples from NB displayed no band (Fig. 1*c*, lane 5). The mRNA of the G3PDH was expressed in all specimens (Fig. 1*d*).

*PPAR-α,-β and -γ immunostaining of bladder tissues from patients with BT and NB*

To assess the tissue distribution of PPAR-α, -β and -γ polypeptides, we stained paraffin-embedded samples with the affinity-purified PPAR-α, -β and -γ antibodies that recognize specifically PPAR-α, -β and -γ. The specificity of this antibody was proven by previous experiments.[9] PPAR-α and -β were expressed in tissues from all specimens, both bladder cancer and normal bladder tissues (not shown).

Although very weak expression of PPAR-γ was found in a few BT tissues, PPAR-γ was strongly expressed in almost all slides from cancer specimens, TCC-G1, -G2, -G3 or SCC (Fig. 2*c–f*). However, no expression of immunoreactive PPAR-γ was found in NB cases (Fig. 2*a*).

**TABLE I.** – PATIENT DATA INCLUDING CELL AND STAGE CHARACTERISTICS

| Characteristics | Normal bladder | Bladder cancer |
|---|---|---|
| No. of patients | 20 | 170 (100%) |
| Male : female | 8 : 12 | 117 : 53 |
| Mean age ± SD | 63.9 ± 15.2 | 68.2 ± 17.4 |
| Histology and grade | | |
| Transitional cell cancer | | 168 (98.8%) |
| Grade 1 | | 59 (35.1%) |
| Grade 2 | | 65 (38.7%) |
| Grade 3 | 44 (26.2%) | |
| Squamous cell cancer | | 2 (0.12%) |
| Stage | | |
| pTis | | 22 (13.0%) |
| pTa | | 31 (18.2%) |
| pT1 | | 47 (27.6%) |
| pT2 | | 34 (20.0%) |
| pT3 | | 30 (17.7%) |
| pT4 | | 6 (3.5%) |

SD, standard deviation.

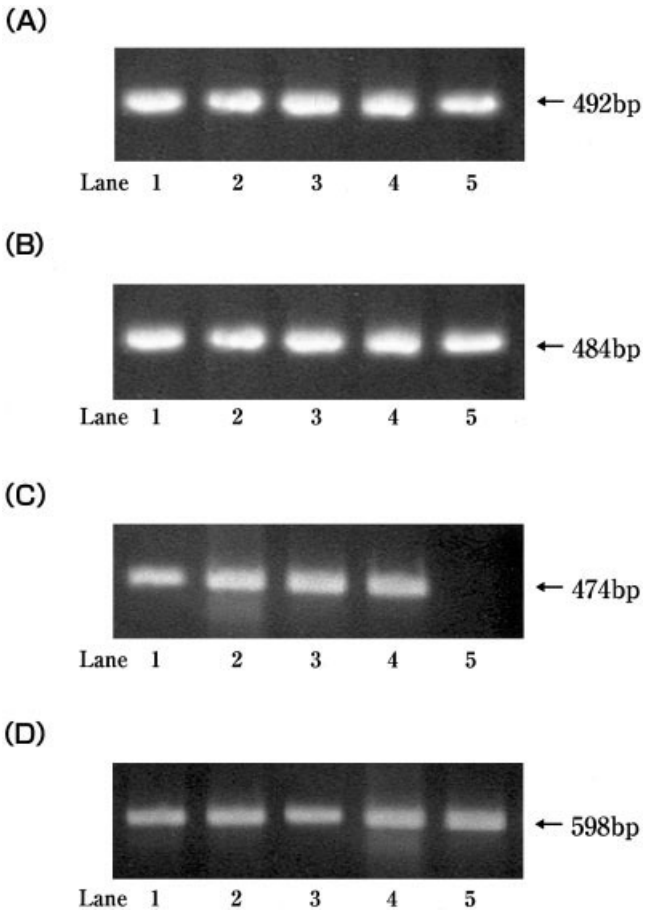


**FIGURE 1** – Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of peroxisome proliferator activator-receptors (PPAR-α, -β and -γ) in bladder tissues from patients with bladder tumor (BT) and normal bladder (NB) samples. Total RNA was purified from BT (2 of 59 TCC-G1, 2 of 65 -G2, 2 of 44 -G3, 2 of 2 SCC) and 2 NB. Amplification of cDNA with PPAR-α, -β, -γ and G3PDH primers used in our study predicted a fragment of 492 base pair (bp), 484 bp, 474 bp and 598 bp in length, respectively. The PPAR-α and -β mRNA were detected in samples from all specimens [*(a)* PPAR-α; *(b)* PPAR-β]. However, we detected specific bands of PPAR-γ mRNA in the samples from BT [*(c)* TCC-G1, lane1; -G2, lane 2; -G3, lane 3; and SCC, lane 4], whereas samples from NB displayed no band [*(c)* lane 5]. *(d)* G3PDH.

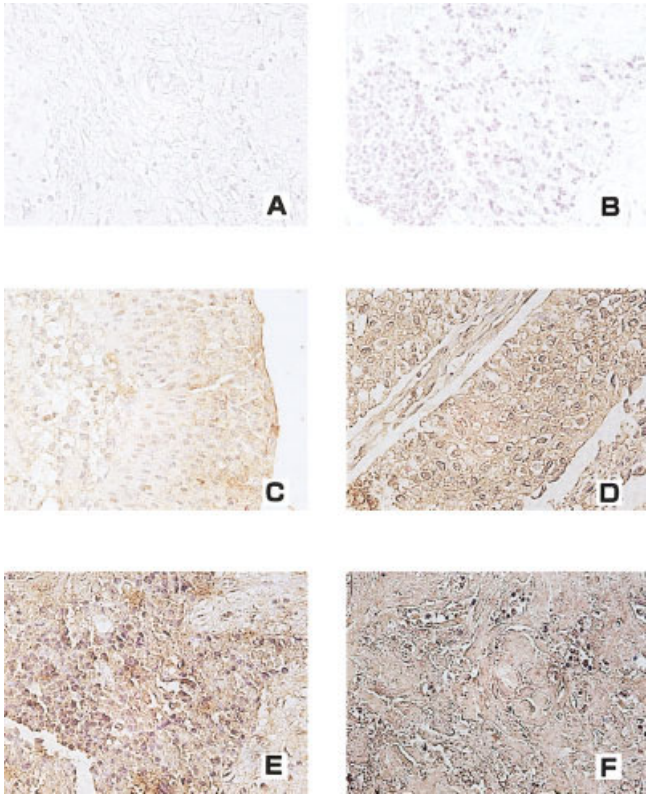


**FIGURE 2** – Representative immunostaining for PPAR-γ in bladder tissues. Very weak expression of immunoreactive PPAR-γ was found in NB [*(a)* ×400] cases. In contrast, we found significant expression of immunoreactive PPAR-γ in cancer cells in all BT groups [*(c)* TCC-G1; *(d)* -G2; *(e)* -G3; *(f)* SCC; ×400] Immunostaining with PBS was completely negative in all subjects with TCC [*(b)* ×400].

*Statistical analysis of PPAR-α, -β and -γ immunostaining*

The extent and intensity of staining with PPAR-α, -β and -γ antibody was graded 0 to 4 (+) by 2 blind observers on 168 TCC, 2 SCC and 20 NB specimens.

There was no difference of the intensity of PPAR-α, -β staining between NB and all groups, nor between grades or between stages (Table II). However, PPAR-γ immunostaining was significantly more extensive and intense in epithelial cells of BT (Ave: G1, 2.2 ± 0.9; G2, 2.7 ± 1.2; G3, 3.4 ± 1.1; SCC, 3.5 ± 0.5, $p < 0.01$) than in epithelial cells of NB (Ave: NB, 0.5 ± 0.6) as shown in Table II. PPAR-γ expression was higher in G3 cancer than in G1 cancer. Moreover, the expression was higher in advanced cancer (pT2 or higher) than in early stage cancer (pT1 or lower) (Table II).

We investigated possible differences in the expression of PPAR-γ among grades (G1, G2, G3) in bladder cancer (TCC) and between early and advanced stages (Tables III and IV). A significant difference was seen only in epithelium between G1 and G2, between G1 and G3 and between G2 and G3, showing that staining was intensified as the grade increased (Table III). Conversely, no difference was seen in blood vessels and stromal tissues between 3 grades (TCC-G1, -G2 and -G3) (Table III). The expression of the PPAR-γ in the blood vessels and stromal tissues from NB was at a basic level (approx. 0.4–0.5) (Table II).

Another comparison between early and advanced stages shows a significant difference in all categories: epithelium, blood vessel and stromal tissue (Table IV). The expression was higher in advanced cancer (pT2 or higher) than in early stage cancer (pT1 or lower).

*Inhibition of BT proliferation by PPAR-γ ligands*

To investigate the effects of PPAR-γ ligands on both NB and BT cells proliferation, we analyzed cell viability *in vitro* using a modified MTT assay. As shown in Figure 3*a*, although agents (troglitazone and $15dPGJ_2$) had no effect on NB cells proliferation, these agents induced the reduction of cell viability with the half-maximal concentration of growth inhibition in the range of 5–40 μM in BT cells. Furthermore, cell counting at days 1, 2 and 3 clearly showed marked inhibition of cell proliferation by treatment with 20 μM of troglitazone and $15dPGJ_2$ (Fig. 3*b*).

## DISCUSSION

Reports have demonstrated that PPAR-γ expression and the effects of PPAR-γ ligands are involved in cell proliferation in several cancers.[3,5–8,15–17] However, little is known regarding PPAR-γ expression in human BT or the effects of PPAR-γ ligands on human BT cells. In our study, we examined the above-mentioned situations.

**TABLE II** – STATISTICAL ANALYSIS OF PPAR-α, β AND γ IMMUNOSTAINING

| Tumor type | Av. ± SD | | |
|---|---|---|---|
| | Epithelium | Blood vessel | Stromal tissue |
| PPAR-α immunostaining | | | |
| Transitional cell cancer | | | |
| Grade 1 | 2.0 ± 0.7 | 1.9 ± 0.7 | 1.8 ± 0.9 |
| Grade 2 | 1.9 ± 0.8 | 1.9 ± 0.8 | 2.0 ± 1.0 |
| Grade 3 | 2.3 ± 1.0 | 2.1 ± 0.9 | 2.0 ± 1.0 |
| Early stage | 2.0 ± 1.0 | 1.8 ± 0.7 | 1.9 ± 1.0 |
| Advanced stage | 2.1 ± 0.9 | 2.0 ± 0.9 | 2.0 ± 1.0 |
| Squamous cell cancer | 2.0 ± 0.3 | 2.0 ± 0.3 | 1.8 ± 0.3 |
| Normal bladder tissue | 2.0 ± 0.9 | 2.0 ± 1.0 | 1.9 ± 0.9 |
| PPAR-β immunostaining | | | |
| Transitional cell cancer | | | |
| Grade 1 | 2.2 ± 0.8 | 2.1 ± 0.6 | 2.2 ± 0.8 |
| Grade 2 | 2.1 ± 0.9 | 2.1 ± 0.8 | 2.3 ± 0.9 |
| Grade 3 | 2.5 ± 1.1 | 2.4 ± 1.0 | 2.5 ± 0.8 |
| Early stage | 2.2 ± 0.9 | 2.2 ± 0.9 | 2.0 ± 1.0 |
| Advanced stage | 2.4 ± 1.1 | 2.4 ± 0.9 | 2.3 ± 0.8 |
| Squamous cell cancer | 1.8 ± 0.3 | 1.7 ± 0.3 | 1.7 ± 0.3 |
| Normal bladder tissue | 1.9 ± 1.0 | 1.9 ± 0.8 | 2.0 ± 1.0 |
| PPAR-γ immunostaining | | | |
| Transitional cell cancer | | | |
| Grade 1 | 2.2 ± 0.9 | 2.2 ± 0.8 | 2.3 ± 1.0 |
| Grade 2 | 2.7 ± 1.2 | 2.6 ± 1.1 | 2.5 ± 1.4 |
| Grade 3 | 3.4 ± 1.1 | 2.7 ± 1.0 | 2.7 ± 0.9 |
| Early stage | 2.2 ± 1.2 | 2.1 ± 0.7 | 2.2 ± 1.0 |
| Advanced stage | 3.4 ± 1.4 | 3.3 ± 1.1 | 3.4 ± 1.3 |
| Squamous cell cancer | 3.5 ± 0.5 | 3.5 ± 0.5 | 3.5 ± 0.5 |
| Normal bladder tissue | 0.5 ± 0.6 | 0.4 ± 0.4 | 0.4 ± 0.4 |

Graded 0 to 4 on the coded sections by 2 blind observers. 0, no staining; 4+, maximum intensity. Statistical analysis was performed using the analysis of variance. Except for PPAR-γ immunostaining, no difference was seen in epithelial cells, blood vessels and stromal tissues between NB and all groups.

**TABLE III** – COMPARISON OF PPAR-γ AMONG GRADES 1, 2 AND 3 TRANSITIONAL CELL CARCINOMA

| Bladder cancer | Grade 1 (*p*-value) | Grade 2 (*p*-value) | Grade 3 (*p*-value) |
|---|---|---|---|
| Epithelium | | | |
| Grade 1 | | <0.05* | <0.05* |
| Grade 2 | 0.035 | | <0.05* |
| Grade 3 | <0.001 | <0.001 | |
| Blood vessels | | | |
| Grade 1 | | Not significant | Not significant |
| Grade 2 | 0.214 | | Not significant |
| Grade 3 | 0.877 | 0.674 | |
| Stromal tissue | | | |
| Grade 1 | | Not significant | Not significant |
| Grade 2 | 0.245 | | Not significant |
| Grade 3 | 0.532 | 0.311 | |

Statistical analysis was performed using the analysis of variance (*p*-value). A significant difference was seen only in epithelium between G1 and G2, between G1 and G3 and between G2 and G3 showing that staining was intensified as the grade increased. No difference was seen in blood vessels and stromal tissues between 3 grades (TCC-G1, -G2 and -G3). $*p < 0.05$.

**TABLE IV** – COMPARISON OF PPAR-γ BETWEEN EARLY AND ADVANCED STAGE

| Bladder cancer stage | Early stage (*p*-value) | Advanced stage (*p*-value) |
|---|---|---|
| Epithelium | | |
| Early stage | | <0.05* |
| Advanced stage | 0.0150 | |
| Blood vessels | | |
| Early stage | | <0.05* |
| Advanced stage | 0.0280 | |
| Stromal tissue | | |
| Early stage | | <0.05* |
| Advanced stage | 0.0370 | |

Statistical analysis was performed using the analysis of variance (*p*-value). Comparison between early and advanced stages shows a significant difference in all categories: epithelium, blood vessel and stromal tissue. $*p < 0.05$.

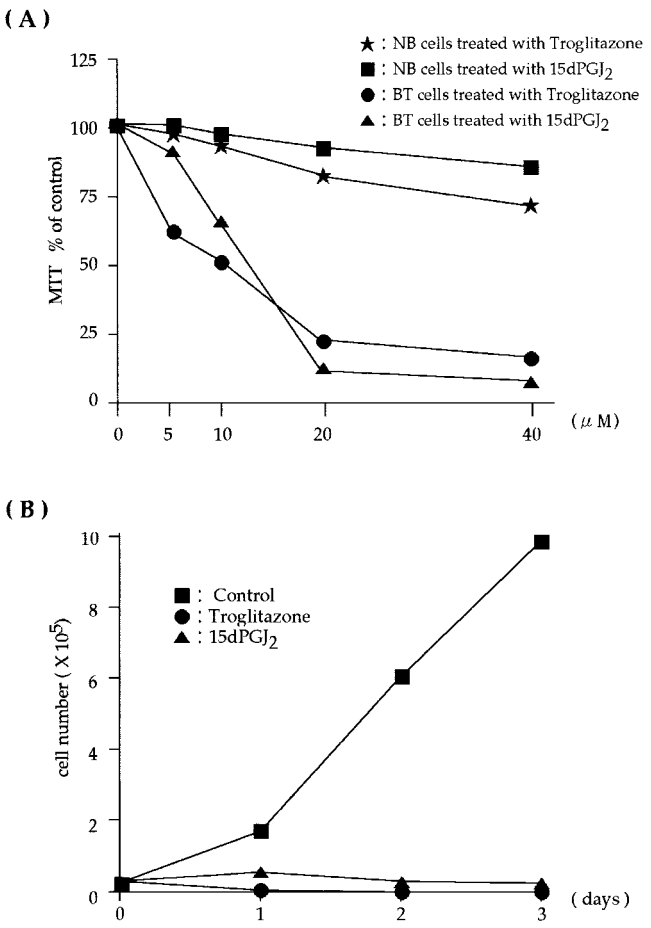


**FIGURE 3** – (a) Effects of PPAR-γ ligands in human bladder cells. The dose-response analysis of human bladder cells treated with troglitazone and $15dPGJ_2$ (5–40 μM, 24 hr) was measured by the MTT assay and expressed as percentage of control culture conditions. *(b)* Effects of PPAR ligands in cell growth of human bladder cancer cells. Human BT cells were treated with troglitazone and $15dPGJ_2$ (10 μM) for 3 days. Cell numbers were determined at days 1, 2, 3.

Peroxisome proliferator activator-receptors (PPARs) are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors, which include steroid hormone, vitamin D3, retinoid and thyroid hormone receptors.[18] After heterodimerization with the retinoid X receptor (RXR), PPAR binds to peroxisome proliferator response elements (PPREs), thereby governing the expression of many target genes.[19,20] Thus far, 3 distinct PPARs, termed PPAR-α, PPAR-β (also called PPAR-δ, NUC-1 or FAAR) and PPAR-γ have been identified. Each PPAR is encoded by a separate gene and shows a distinct tissue distribution. PPAR-α is found in the liver, retina, mucosa, proximal tubules of the kidney, heart, muscle and brown adipose tissue,[9] in which fatty acids are predominantly catabolized. PPAR-α is activated by long-chain fatty acids, polyunsaturated fatty acid (PUFA) such as eicosapentaenoic acid (EPA) and a variety of fibrates.[21,22] PPAR-γ is widely expressed, and its function remains obscure. Recently, He *et al.* have demonstrated

that PPAR-β can be an APC-regulated target of nonsteroidal anti-inflammatory drugs (sulindac) in human colorectal cancer.[23]

Conversely, PPAR-γ is found primarily in adipose tissue, where it plays a critical role in the differentiation of preadipocytes into adipocytes. It is well established that PPAR-γ is a factor capable of promoting differentiation or transdifferentiation of cells. PPAR-γ ligands induce growth arrest through apoptosis in macrophages and endothelial cells.[24] Also, it has been reported that thiazolidinediones, which are PPAR-γ ligands including troglitazone, can inhibit the growth of several cancer cells such as prostate and breast cancer cells *in vitro* and *in vivo*.[3,25] Thiazolidinediones, a new class of antidiabetic drugs as a specific ligand for PPAR-γ, and RXR agonists can regulate differentiation of cancer cells.[3,17] In fact, combination administration of thiazolidinediones and RA receptor-specific nuclear hormone receptor ligand (all-transretinoic acid) induces apoptosis and fibrosis of breast cancer cells.[5] Moreover, thiazolidinediones have a necrotic effect on human prostate cancer and gastric cancer cells.[3,18] Regarding lung cancer, Tsubouchi *et al.* found that PPAR-γ ligands such as thiazolidinedione compounds (troglitazone) and the endogenous PPAR-γ ligand, 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ (15d-$PGJ_2$) inhibited the growth of human lung cancer cells through apoptosis.[17] Chang *et al.* proved that 2 distinct PPAR-γ ligands also induce differentiation and apoptosis in nonsmall cell lung cancer *in vitro*.[7] Moreover, Inoue *et al.* also demonstrated that PPAR-γ agonist thiazolidinedione compounds inhibited the growth of the renal cell carcinoma.[26] Such reports prompted us to examine the detailed PPAR-γ expression in cancer tissues to confirm targets of PPAR-γ ligands in these tissues. Therefore, treatment of cancer cells with thiazolidinedione caused the nuclear translocation of PPAR-γ and the induction of apoptosis.

Angiogenesis plays a significant role in tumorigenesis. Antiangiogenetic therapy is highly promising because it does not induce acquired anticancer drug resistance and is focused on by several researchers. Drevs *et al.* demonstrated the effect of PTK787/ZK 222584, a specific inhibitor of vascular endothelial growth factor (VEGF) receptor tyrosine kinases, on primary tumor, metastasis, vessel density and blood flow in an animal model of renal cell carcinoma.[27] PPAR-γ agonists induce apoptosis in endothelial cells and inhibit VEGF-induced angiogenesis in rats. Therefore, PPAR-γ may become a targeting receptor for useful treatment of angiogenesis in carcinoma.

In our study, we demonstrated for the first time to our knowledge that human BT (TCC) tissues express PPAR-γ. We have shown that PPAR-γ mRNA is detected in bladder cancer tissues by RT-PCR and the expression of PPAR-γ was strongly detected in BT tissues whereas the expression of PPAR-γ in NB tissues was at the basic level by immunohistochemistry.

Although PPAR-γ was strongly expressed in almost all slides from cancer specimens, very weak expression of PPAR-γ was found in a few BT tissues. Because some tumor tissues may have not been expressed, PPAR-γ and some tumor tissues from the subjects may have not been preserved in good condition. However, if we could examine non-PPAR-γ-expressing carcinoma, we suspect the any response of PPAR-γ agonists for cancer cells because PPAR-γ promotes the invasion of cancer. This should be performed in the future.

In our study, we also investigated the inhibitory effect of PPAR-γ ligands on BT-derived cell lines. Our study has also clearly revealed that PPAR-γ ligands significantly inhibited the cell growth of a TCC cell line in a dose- and time-dependent manner. Although we used only one kind of human BT cell line, this cell line was provided by Health Science Research Resources Bank and was stated as control BT cell lines. Thus, we investigated the inhibitory effect on this BT-derived cell line 10 times and an average score was calculated. However, further studies using more cell lines should be performed.

These results indicate that thiazolidinediones and 15d$PGJ_2$ have a growth inhibitory action on TCC cells. Reports show that PPAR-γ is closely associated with the cell cycle to alter cell growth.[28] Because thiazolidinediones and 15d$PGJ_2$ are specific and are high-affinity agonists, we suggest that PPAR-γ activation by such agonists may lead to growth inhibition of TCC cells. Therefore, our study supports the possibility that PPAR-γ ligands may be a novel therapeutic treatment of bladder tumor. These findings suggest that inhibition of PPAR-γ development may lead to both inhibition of the proliferation and metastasis of bladder tumor and to inhibition of bladder carcinogenesis.

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