

Differential Peroxisome Proliferator-Activated Receptor- γ Isoform Expression and Agonist Effects in Normal and Malignant Prostate Cells

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Abstract

Peroxisome proliferator-activated receptor- γ (PPAR- γ) is being studied intensively for its role in carcinogenesis and in mediating the effects of prostate cancer treatment and prevention drugs. Prostate cancers express abundant and higher constitutive levels of PPAR- γ than do normal prostate cells and are growth inhibited by ligand activation of PPAR- γ . However, little is known about the role of PPARs in tumorigenesis or in normal prostate epithelial cells (EC). We examined the expression, phosphorylation patterns, and functions of the human PPAR (hPPAR)- γ 1 and hPPAR- γ 2 isoforms in normal prostate ECs to determine if activation of the receptor is sufficient for PPAR- γ ligand activity in prostate cells. We found that ECs did not express either PPAR- γ 1 or PPAR- γ 2 protein and were not sensitive to growth inhibition by the PPAR- γ ligand 15-deoxy- Δ 12,14-prostaglandin J₂ (15d-PGJ₂). In contrast, prostate cancer cells (PC-3), which express PPAR- γ 1 receptor isoform, are growth inhibited by PPAR- γ ligand. Forced

expression of hPPAR- γ 1 or hPPAR- γ 2 made ECs sensitive to 15d-PGJ₂ and led to reduced cellular viability. The direct repeat-1 promoter containing PPAR response elements was transactivated in ECs expressing exogenous PPAR- γ 1 or PPAR- γ 2, indicating that either isoform can be active in these cells. 15-Lipoxygenase-2, expressed at high levels in ECs, was down-regulated by transfecting PPAR- γ expression construct (either γ 1 or γ 2 isoform) into ECs. Addition of PPAR- γ ligand 15-hydroxyicosatetraenoic acid in the presence of PPAR- γ expression caused further down-regulation of 15-lipoxygenase-2. Our data illustrate that a PPAR- γ ligand (15d-PGJ₂) activates PPAR- γ 1 and selectively induces cell death in human prostate cancer cells but not in normal prostate ECs. These findings have important implications for the development of PPAR- γ -targeting agents that prevent or treat prostate cancer and spare normal prostate cells. (Cancer Epidemiol Biomarkers Prev 2004;13(11):1710-6)

Introduction

Peroxisome proliferator-activated receptors (PPAR) are a family of nuclear transcription factors that interact with PPAR response elements in controlling growth-regulatory gene expression (1). PPARs belong to the steroid hormone receptor superfamily and are involved in ligand-inducible lipid metabolism (2). Prostanoids and their synthetic analogues and other long-chain fatty acids and their metabolites act as ligands for the three PPARs, α , β , and γ (2, 3). The PPAR- γ -selective ligand 15-deoxy- Δ 12,14-prostaglandin J₂ (15d-PGJ₂) is produced in large amounts in the prostate gland via spontaneous conversion from prostaglandin D₂ (4). Another PPAR- γ ligand, 15-hydroxyicosatetraenoic acid (15-HETE), is produced in normal epithelial cells (ECs) by 15-lipoxygenase-2

(15-LOX-2; refs. 5, 6). These PPAR- γ ligands, 15d-PGJ₂ and 15-HETE, are likely to be involved in prostate carcinogenesis.

There are two PPAR- γ isoforms, PPAR- γ 1 and PPAR- γ 2. Some cancers have been shown to express PPAR- γ (7-10) and prostate cancer expresses significantly more PPAR- γ than normal cells (7, 8, 10). Investigators also have reported the inhibitory effects of PPAR- γ agonists on prostate cancer cells (10-13). Kubota et al. (10) found that PPAR- γ agonists caused growth inhibition of PC-3 in a clonogenic assay and immunodeficient mouse xenografts and that a PPAR- γ agonist selectively induced cell death of malignant but not normal prostate cells in short-term cultures of biopsy specimens. This article reports the PPAR- γ patterns of expression and PPAR- γ ligand effects in normal prostate ECs as compared with prostate cancer cells.

Materials and Methods

Cell Culture. Primary EC and PC-3 cells were grown in cell culture as described previously (14).

Northern Blot Analysis. Northern blot analysis was done on 20 μ g of total RNA electrophoretically separated

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on 1% formaldehyde agarose gel as described previously (14). hPPAR- γ cDNA probes labeled with $\alpha^{32}\text{P}$ -dCTP (3000 Ci/mmol, Amersham Life Sciences, Piscataway, NJ) were used to analyze total PPAR- γ and the same blot probed with 36B4 was used as a loading control.

Reverse Transcription-PCR. RNA (1 μg) was analyzed by reverse transcription-PCR (RT-PCR) as described previously (14). Primer pairs were used for RT-PCR analysis of total PPAR- γ sense (5' primer: 5'-TCTCTCCGTAATGGAAGACC-3') and antisense (3' primer: 5'-GCATTATGAGCATCCCCAC-3') and PPAR- γ 2 sense (5'-GCGATTCTTCACTGCTAC-3') and the same antisense used for PPAR- γ (10). 36B4 primers were used as reported previously (15). RT-PCR amplification was done for each set of primers at 25, 30, and 35 cycles. The PCR products were electrophoretically separated, transferred onto a nylon membrane, and probed with total PPAR- γ (5'-GAGTACCAAAGTGCAATCAA-3') or PPAR- γ 2-specific (5'-GTCTGCAAACATATCACA-3') or 36B4 ^{32}P -radiolabeled oligonucleotide probes and after stripping were reprobed for 36B4. RT-PCR DNA products were subcloned using the TOPO TA system (Invitrogen, Carlsbad, CA), sequenced by an automated system (Seqwright, Houston, TX), and subjected to a BLAST analysis for sequence verification.

Human PPAR- γ 1 and Human PPAR- γ 2 Transfection and Treatment with PPAR- γ Ligand. Cells were transiently transfected with human PPAR- γ 1 (pSG/hPPAR- γ 1) and human PPAR- γ 2 (pSG/hPPAR- γ 2) cDNA expression vectors, provided by Dr. Alex Elbrecht, or with 15-LOX-2 expression vector. Transfection was carried out with 500 ng of either plasmid or empty pSG5 expression vector (16). Cells were harvested 48 hours after transfection or were incubated with 10 $\mu\text{mol/L}$ 15d-PG $_2$ (Biomol Research Laboratories, Inc., Plymouth Meeting, PA) or 10 $\mu\text{mol/L}$ 15-HETE for 12 to 16 hours and prepared for assays (17). The transfection efficiency was determined by transfecting either isoform of PPAR- γ expression constructs in combination with green fluorescent protein, an excess ratio of 3:1, and dividing total cell number by the green fluorescent protein-expressing cells that were counted using fluorescence microscopy.

Western Blot Analysis. Cell lysates (100-150 μg of total cellular protein or nuclear lysates) were prepared and analyzed by Western blot analysis as described previously (14). Blots were probed with a primary antibody against PPAR- γ (SC-7196, 1:1,000 dilution, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), 15-LOX-2 (Oxford Biomedical Research Labs, Oxford, MI), or β -actin (1:1,000 dilution, New England Biolabs, Inc., Beverly MA). The anti-PPAR- γ polyclonal SC-7196 antibody has been shown previously to detect phosphorylated and unphosphorylated forms of PPAR- γ (18). Confirmation testing of PPAR- γ doublet migration was confirmed in COS-1 and NIH3T3 cells transfected with PPAR- γ 1 and PPAR- γ 2.³

Alkaline Phosphatase Treatment. The phosphorylation status of PPAR- γ 1 and PPAR- γ 2 was verified by incubating PPAR- γ transfected PC-3, EC, NIH3T3, or COS-1 cell extracts with calf intestine alkaline phosphatase for 5, 30, 60, and 240 minutes as described previously by Hu et al. (19) and analyzed by Western blot using the anti-PPAR- γ SC-7196 antibody. The film was scanned on a laser densitometer (Molecular Dynamics, San Diego CA), and signals were quantified using NIH Image J software. Data analyses were done using the Statview software program (SAS Institute, Inc., Cary, NC). Paired calf intestine alkaline phosphatase-treated and untreated samples were analyzed and the decrease in density of the upper (phosphorylated) band was calculated.

Transactivation Activity Assessment. Transactivation of the PPAR- γ response element was examined by cotransfecting 250 ng of a pDR-1LUC (firefly luciferase) reporter construct containing 3 \times PPAR response element (3) and 100 ng of ptkLUC plasmid (*Renilla* luciferase) either with 500 ng of hPPAR- γ 1 or hPPAR- γ 2 cDNA (cloned into pSG5 expression vector) or with the pSG5 vector alone (control). This dual LUC system has been described previously (16).

Cell Viability and DNA Condensation. Cell viability was determined in the presence or absence of the PPAR- γ ligand 15-PG $_2$. The assay was done using the vital dye calcein MA (CAM) ester (Molecular Probes, Eugene, OR) as described previously (16). Cells transfected with the hPPAR- γ expression vectors or the control vector were plated in 96-well plates and incubated with or without 10 $\mu\text{mol/L}$ 15-PG $_2$ ligand and then with CAM ester in HEPES-buffered saline solution for 15 minutes at 25°C. Cells were subsequently incubated with 4',6-diamidino-2-phenylindole (1 $\mu\text{mol/L}$) and propidium iodide (PI; 1 $\mu\text{mol/L}$; Molecular Probes). Nuclear morphology, DNA dye uptake, and cellular staining were assessed by fluorescence microscopy using an image analysis system (Scanalytics, Inc., Fairfax, VA) and quantified using a Biolumin 9600-well plate reader. Cells lysed with 1% NP40 detergent were used as a negative control for CAM ester conversion and as a positive control for DNA intercalating dye uptake.

Statistical Analyses. Data and statistical analyses were done using the Statview software program (SAS Institute). Student's *t* tests were used to determine the significance (*P*) between mean group values.

RNA Secondary Structure Analysis. PPAR- γ 2 mRNA secondary structure was determined using the Genbank PPAR- γ 2 cDNA sequence (NM 015869) and M-fold RNA structural prediction software (version 3.1) from the Rensselaer Polytechnic Institute Web site (<http://www.bioinfo.rpi.edu/applications/mfold/old/rna/form1.cgi>; ref. 20).

Results

mRNA Expression Profile of PPAR- γ Isoforms in Normal and Malignant Prostate Cells. Constitutive expression of PPAR- γ was determined in EC and PC-3 cells by Northern blot analysis and RT-PCR. Total PPAR- γ message was not detectable in ECs and was strongly

³ A.L. Sabichi et al. Peroxisome proliferator-activated receptor- γ suppresses cyclooxygenase-2 expression in human prostate cells, this issue.

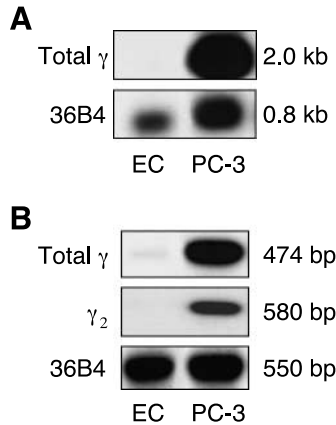


Figure 1. A. Northern blot analysis of total PPAR- γ mRNA expression in normal human prostate ECs and the prostate cancer cell line PC-3. For loading control, the same blot was probed for 36B4 expression (*bottom*). B. PPAR- γ cDNA expression in EC and PC-3 by RT-PCR analysis for PPAR- γ 1 (25 cycles; *top*), PPAR- γ 2 (35 cycles; *middle*), and 36B4 (loading control; same number of amplification cycles as done for each PPAR- γ isoform; *bottom*).

expressed in PC-3 (Fig. 1A). RT-PCR detected trace levels of total PPAR- γ . The level of PPAR- γ 2 was below the level of detection in ECs using this assay. In contrast, RT-PCR detected total PPAR- γ and PPAR- γ 2 message in PC-3 cells (Fig. 1B).

Expression and Phosphorylation Status of Endogenous PPAR- γ 1 and PPAR- γ 2 Proteins in Prostate Cells. PPAR- γ 1 and PPAR- γ 2 are identified as doublet proteins by Western blot analysis; the unphosphorylated form of the protein migrates farther (lower band) and is active (Fig. 2, *open circles*), and the phosphorylated form (upper band) is inactive (Fig. 2, *closed circles*). Western blot analysis showed that EC did not express steady-state 1 or 2 isoform (Fig. 2, *lane 1*). PC-3 expressed PPAR- γ 1 protein but not PPAR- γ 2 protein (Fig. 2, *lane 4*), although RT-PCR analysis showed that PPAR- γ 2 mRNA was present. Western blot of isolated nuclear protein showed the same protein expression patterns (data not shown).

Although PPAR- γ 1 was adequately expressed in EC after transfection of hPPAR- γ 1 (Fig. 2, *lane 2*), we did not observe this same increase in expression of PPAR- γ 1 protein in PC-3 (Fig. 2, *lane 5*) even after several attempts. Control transfections done concurrently included NIH3T3 and COS-1 cells, which showed the expected increase in exogenous protein PPAR- γ 1 expression after transfection. Exogenous PPAR- γ 2 protein was expressed in roughly equal proportions of phosphorylated and unphosphorylated states (Fig. 2, *lane 6*). Transfection efficiency was determined to be $41.3 \pm 1.9\%$ in ECs after cotransfecting green fluorescent protein and PPAR- γ .

PPAR- γ Phosphorylation Analysis. The phosphorylation status of PPAR- γ was verified by Western analysis of calf intestine alkaline phosphatase-treated cell extract. The reduction in density of the upper band of the protein doublet over time was maximal at 240 minutes of calf intestine alkaline phosphatase treatment, at which time

the mean pixel density was reduced 43.7 ± 1.2 -fold compared with this density in the untreated sample (data not shown). We similarly verified phosphorylation results in transfected COS-1 and NIH3T3 cells (data not shown).

Effects of PPAR- γ 1 and PPAR- γ 2 on Direct Repeat-1 Transactivation in Prostate Cells. We further examined the effects of hPPAR- γ 1 and PPAR- γ 2 on transactivation of direct repeat-1 (DR-1). We measured LUC activity in ECs that were cotransfected with a direct PPAR response element driving a LUC reporter plasmid and either a hPPAR- γ 1 or a hPPAR- γ 2 expression plasmid in the absence or presence of PPAR- γ agonist 15d-PGJ₂. ECs transfected with PPAR- γ 1 or PPAR- γ 2 expression plasmids had significantly higher LUC activity versus activity in the control ECs (transfected with control expression plasmid; γ 1, $P = 0.03$; γ 2, $P = 0.05$; Fig. 3). 15d-PGJ₂ stimulated transactivation and significantly increased LUC activity in PPAR- γ 1- or PPAR- γ 2-transfected EC versus in the untreated cells (γ 1, $P = 0.0001$; γ 2, $P = 0.02$) but not in control transfected ECs ($P = 0.15$; Fig. 3). It is notable that constitutive PPAR response element transactivation was >20 -fold greater in untreated PC-3 cells than in ECs, and transactivation activity was significantly increased by 15d-PGJ₂ ($P = 0.04$; Fig. 3). This finding is consistent with endogenous PPAR- γ expression in PC-3 cells.

Effects of Exogenous hPPAR- γ 1 and hPPAR- γ 2 on Prostate Cell Viability. To explore the roles of hPPAR- γ 1 and hPPAR- γ 2 in ligand-activated cell death, the cell viability of hPPAR- γ 1- or hPPAR- γ 2-transfected ECs compared with that of PC-3 was determined by analyzing cellular retention of CAM fluorescent vital dye after exposure to 15d-PGJ₂. CAM is converted intracellularly to a membrane-impermeable product by nonspecific esterases and is retained within intact cells. Both viable cells and cells in the early stages of apoptosis retain CAM, whereas cells in the latter stages of cell

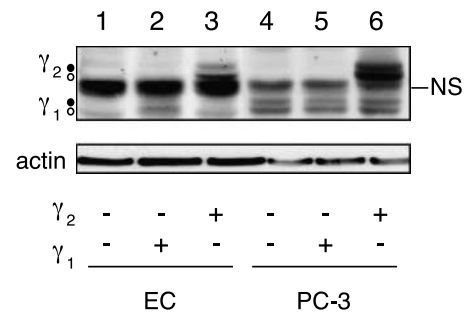


Figure 2. Western blot analysis of constitutive PPAR- γ 1 and PPAR- γ 2 protein expression and phosphorylation status in normal human prostate ECs (*lane 1*) and the prostate cancer cell line PC-3 (*lane 4*) using an antibody that recognizes both PPAR- γ isoforms. Protein expression also was analyzed in cells transfected (+) with hPPAR- γ 1 (*lanes 2 and 5*) or hPPAR- γ 2 (*lanes 3 and 6*) expression vectors. *Closed circles*, inactive phosphorylated PPAR proteins; *open circles*, active nonphosphorylated forms; NS, nonspecific band. β -actin was used as the loading control.

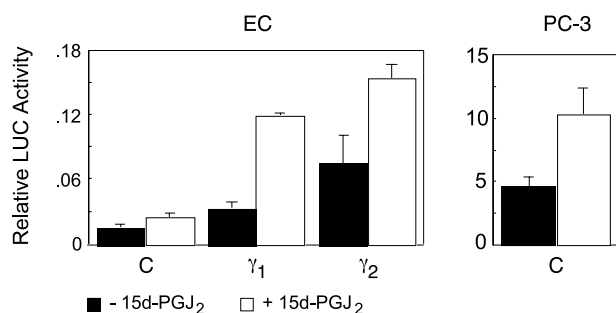


Figure 3. Transcriptional activation of PPAR- γ was measured by LUC activity in ECs coexpressing hPPAR- γ 1 or hPPAR- γ 2 and the DR-1-driven LUC reporter plasmid containing a PPAR response element. Cells cotransfected with DR-1 LUC reporter plasmid and the expression vector (pSG5) alone served as a control (C).

death fail to effectively activate and retain CAM because of disrupted plasma membrane integrity. Control PC-3 cells, which express endogenous PPAR- γ 1 protein, exhibited morphologic evidence of cell death and did not retain CAM vital dye after treatment with 10 μ mol/L 15d-PGJ₂ ligand for 12 hours (Fig. 4A, PC-3 control). Conversely, control vector-transfected ECs remained viable after ligand exposure as shown by the retention of intracellular CAM (Fig. 4A, EC control). However, forced expression of the hPPAR- γ 1 or hPPAR- γ 2 transgene in ECs conferred sensitivity to the ligand and resulted in loss of CAM at 12 hours after ligand exposure. PI uptake in the 15d-PGJ₂-treated PPAR- γ 1- and PPAR- γ 2-transfected cells approached 40% and 65%, respectively (Fig. 4B). PI uptake in PPAR- γ -expressing cells treated with ligand approximated the transfection efficiency, indicating that cell death measured by PI uptake was largely dependent on the presence of PPAR- γ . These findings suggest that cellular sensitivity to ligand was directly related to receptor expression. Uptake of 4',6-diamidino-2-phenylindole (Fig. 4A) and PI (Fig. 4B) into condensed nuclear DNA occurred in conjunction with the loss of vital dye in ligand-treated PC-3 and transfected ECs but not in the treated parental ECs. A significant increase in cell death-associated DNA condensation (as measured by nuclear uptake of PI) was induced by 15d-PGJ₂ in PC-3 cells ($P = 0.002$) and hPPAR- γ -transfected ECs (hPPAR- γ 1, $P < 0.0001$; hPPAR- γ 2, $P < 0.004$) compared with the untreated cells (Fig. 4B). The increase in PI uptake corresponded with the increase in DNA condensation and cell death. Control ECs did not respond to ligand treatment (Fig. 4B).

Expression Patterns of 15-LOX-2 and PPAR- γ . The expression profiles of 15-LOX-2 and PPAR- γ in normal and malignant prostate cells were determined. EC expressed high levels of 15-LOX-2, whereas PC-3 had undetectable levels (Fig. 5; data not shown). The inverse protein expression profiles suggest that a relationship may exist between these proteins. We further examined this relationship by overexpressing hPPAR- γ in EC. The overexpression of hPPAR- γ 1 or hPPAR- γ 2 in EC was associated with a dose-dependent down-regulation of 15-LOX-2 protein expression (Fig. 5, lanes 2 and 3); dose-

dependent data not shown) and down-regulation was augmented by treatment with 15-HETE ligand (Fig. 5, lanes 4 and 5).

Discussion

PPAR- γ has been implicated in the pathogenesis of several human malignancies, including prostate cancer, and exhibits a variety of biological effects depending on the tissue-specific context in which this nuclear receptor is expressed. PPAR- γ isoforms have been shown to have different expression profiles in various tissues (3, 10, 21-24). Expression patterns of PPAR- γ in malignant prostate cells or tissue have been examined by several investigators, but relatively few such studies examine normal prostate cells or compare normal tissue with cancer, and the data are conflicting. Furthermore, the effect of PPAR- γ ligands on normal prostate EC behavior has not been examined. In the present study, we assessed the expression of PPAR- γ isoforms and their functional role in response to PPAR- γ ligand in prostate ECs.

One analysis of PPAR- γ expression in prostate cancer and adjacent normal tissue by immunohistochemistry showed strong PPAR- γ expression in prostate cancer tissues in contrast to low expression in the normal tissue (7). Mueller et al. (9) found that prostate cancer tissue expressed lower levels of PPAR- γ message than adjacent normal prostate tissue. In one study using RT-PCR, PPAR- γ was detected in human prostate cancers and prostatic intraepithelial neoplasia but not in normal prostate tissue (7), whereas in another study using RT-PCR it was detected in both benign and tumor tissues from radical prostatectomy specimens (5).

The only *in vitro* study to evaluate the PPAR- γ expression in primary prostate EC was reported by Nwankwo et al. (8) who found lack of constitutive total PPAR- γ expression in prostate ECs (but found that it may be up-regulated by γ -linoleic acid). Our results in ECs are consistent with the findings of Nwankwo et al. in that normal EC do not express either isoform of PPAR- γ . We also found that PC-3 constitutively expressed both PPAR- γ 1 and PPAR- γ 2 messages (by total PPAR- γ mRNA and RT-PCR) but only PPAR- γ 1 protein (by Western blot). These findings agree with the findings of Butler et al. (12) who also reported constitutive expression of PPAR-1 in PC-3, and other prostate cancer cell lines, after exposure to 15d-PGJ₂. However, in contrast to the findings of Butler et al. (12), we did not observe the up-regulation of PPAR- γ 2 on incubation with 15d-PGJ₂ in PC-3 and also did not observe induction of either PPAR- γ isoform in ECs. The reason for differences in the findings of Butler et al. and our findings are not apparent and are discussed further elsewhere.³

Our present results are the first to highlight differences between PPAR- γ isoforms and normal and malignant prostate cells in response to PPAR- γ ligand. Unlike PC-3, which underwent nonapoptotic cell death upon ligand exposure, ECs did not respond to ligand but were rendered sensitive to ligand effects after being engineered to express PPAR- γ . When either PPAR- γ 1 or hPPAR- γ 2 was expressed in ECs, we observed similar

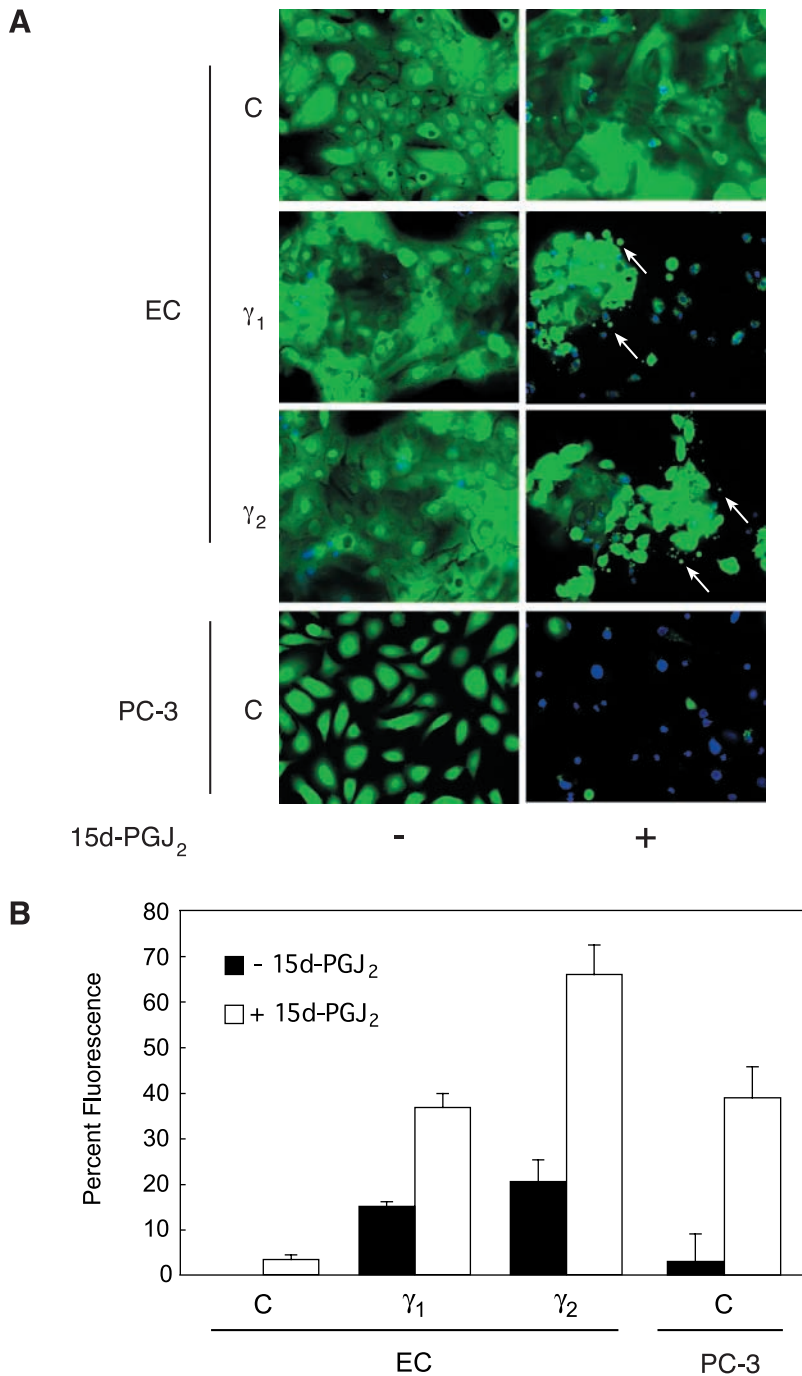


Figure 4. Cell viability after treatment with the PPAR- γ ligand 15d-PGJ₂. Effect of ligand on the prostate cancer cell line PC-3 and ECs transfected with an empty expression vector (C) or hPPAR- γ 1 or hPPAR- γ 2 expression vectors. **A.** Cells were stained with a membrane-permeable CAM ester, which is intracellularly converted to a membrane-impermeable fluoro-chrome by nonspecific esterases (green). After exposure to PPAR- γ ligand, ECs transfected with either hPPAR- γ 1 or hPPAR- γ 2 developed reduced cell viability, apoptotic bodies (arrows), and pyknotic nuclei. Loss of membrane integrity in the later phases of cell death led to leakage of CAM and DNA intercalation of 4',6-diamidino-2-phenylindole (blue). **B.** Cells were also stained with PI and quantitated after incubation with (white bars) or without (black bars) 15d-PGJ₂. PI fluorescence is presented as a percentage of total DNA uptake.

responses to ligand activation (as measured by transactivation of the DR-1 promoter and cell viability), indicating that the two PPAR- γ isoforms have similar biological effects in the prostate cell milieu. Our findings are supported by data showing that PPAR- γ 1 and PPAR- γ 2 regulate similar genes (3, 9, 10, 21-24). Together, these data support the hypothesis that the major ligand effects of 15d-PGJ₂ in prostate cells involve a receptor-dependent mechanism and that the effect can be mediated through either PPAR- γ 1 or PPAR- γ 2.

Although EC transfected with PPAR- γ 1 expressed approximately the same level of unphosphorylated (active form) PPAR- γ 1 protein as did PC-3, transactivation of DR-1 LUC in EC was >20-fold less than that seen in the PC-3 control. This suggests that either the artificially expressed protein is not as active as the endogenous protein or the cellular milieu in EC does not support transactivation to the extent seen in PC-3. It will be important in future studies to establish what cofactors contribute to the up-regulation or suppression

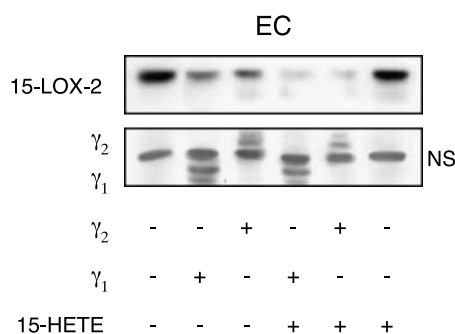


Figure 5. A. 15-LOX-2 was down-regulated by transfecting PPAR- γ expression construct (either γ_1 or γ_2 isoform) into ECs. Addition of PPAR- γ ligand 15-HETE in the presence of PPAR- γ expression caused additional suppression of 15-LOX-2 protein expression.

of transactivation in EC compared with that in PC-3 cells. These factors may include coactivators, corepressors, or retinoid X receptor proteins (25).

We tested the biological effects of the PPAR- γ agonist 15d-PGJ₂ on normal prostate cells and found that EC were resistant to growth-inhibitory effects of 15-PGJ₂, whereas PC-3 were sensitive. On incubation with 15d-PGJ₂, PPAR- γ -induced transfected EC cells undergo cell death with morphologic features consistent with apoptosis, whereas PC-3 died by other mechanisms. The findings agree with those of Butler et al. (12) who found that PPAR- γ_1 was constitutively expressed in PC-3 (and other human prostate cancer cell lines) and caused PC-3 to undergo S-phase arrest and nonapoptotic cell death.

The overexpression of PPAR- γ would be expected to reduce the survival of these malignant cells, because this nuclear receptor induces cell death when ligand activated. One condition that may allow for the high level of PPAR- γ in PC-3 cells may relate to the loss of the tumor suppressor 15-LOX-2, an enzyme that produces the PPAR- γ ligand 15-HETE (26, 27). We and others have shown that 15-LOX-2 is expressed at very high levels in normal EC but is lost in PC-3 and in other malignant prostate cells LNCaP and DU145 (13, 27). 15-LOX-2 has been shown to act as a prostate cancer tumor suppressor *in vitro* (27) and in mice (26). We observed that forced expression of 15-LOX-2 in PC-3 causes down-regulation of PPAR- γ expression (data not shown) and that overexpression of PPAR- γ in EC reduces expression of 15-LOX-2 (Fig. 5).

The inverse expression profiles of 15-LOX-2 and PPAR- γ in normal and malignant prostate cells suggest that a relationship may exist between these proteins. The 15-LOX-2 product 15-HETE has been shown to suppress prostate cancer cell growth by acting as a ligand for PPAR- γ (6, 13, 27). Therefore, the molecular basis for this may involve feedback signals of 15-HETE production by 15-LOX-2 acting as a ligand for PPAR- γ and suppressing growth of prostate cancer cells. We postulate that the loss of 15-LOX-2 tumor suppressor in prostate cells during progression to cancer may create a cellular milieu in which PPAR- γ is up-regulated but does not cause apoptosis. The high expression levels of PPAR- γ in

PC-3 may also result from the loss of a potential suppressive feedback mechanism involving antagonistic properties of 15-LOX-2.

Although we observed PPAR- γ_2 mRNA expression in PC-3, there was little or no PPAR- γ_2 protein expression. Because of this unusual pattern of expression, we evaluated whether the translational machinery was intact by transfecting PPAR- γ_2 cDNA into PC-3 cells, which resulted in a high expression of PPAR- γ_2 protein and so indicated that the machinery was intact. There are several possible explanations for the differential PPAR- γ_2 mRNA and protein expressions in PC-3, including altered endogenous PPAR- γ_2 message, post-transcriptional processing, and translational regulation. PPAR- γ_2 mRNA is known to have both PPAR- γ_1 and PPAR- γ_2 translational start sites (28). For the purposes of theoretical discussion, a review of the stem loop structure was done. The structure indicated that the γ_1 start site was more structurally exposed than was the γ_2 start site, which may account for the preferential translation of PPAR- γ_1 in PC-3. A similar mechanism was described recently for the translational regulation of JunD isoforms (29). Furthermore, when PPAR- γ_2 expression constructs were transfected into EC, COS-1, or NIH3T3 control cells, PPAR- γ_1 was preferentially expressed. The significance of preferential expression of PPAR- γ_1 over PPAR- γ_2 will require further analysis.

In summary, we found that the PPAR- γ agonist 15d-PGJ₂ was selectively active in prostate cancer (which express PPAR- γ_1) versus in normal prostate cells (which do not express PPAR- γ) and that expression of exogenous PPAR- γ in EC conferred sensitivity to ligand, indicating that ligand activity is at least in part receptor mediated. In addition, the expression patterns of PPAR- γ are inversely related to the expression of at least one enzyme (15-LOX-2) that is capable of producing a highly active ligand (15-HETE). Our data also suggest that a feedback loop that may regulate PPAR- γ expression through 15-HETE production in normal EC, which is lost during prostate cancer carcinogenesis and creates conditions whereby PPAR- γ can be overexpressed. These findings have critical implications for the use of PPAR- γ agonists as potential therapeutic or preventive agents that will spare normal tissue while acting on malignant or premalignant tissue. These results further our understanding and have important implications for the development of PPAR- γ -targeting agents (30) for prostate cancer prevention and treatment.

Acknowledgments

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