Suppression of Prostate Tumor Cell Growth by Stromal Cell Prostaglandin D Synthase–Derived Products

Jeri Kim, Peiyang Yang, Milind Suraokar, Anita L. Sabichi, Norma D. Llansa, Gabriela Mendoza, Vemparalla Subbarayan, Christopher J. Logothetis, Robert A. Newman, Scott M. Lippman, and David G. Menter

Departments of 1Genitourinary Medical Oncology, 2Experimental Therapeutics, and 3Clinical Cancer Prevention, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Abstract

Stromal-epithelial interactions and the bioactive molecules produced by these interactions maintain tissue homeostasis and influence carcinogenesis. Bioactive prostaglandin synthases produced by prostaglandin synthases and secreted by the prostate into seminal plasma are thought to support reproduction, but their endogenous effects on cancer formation remain unresolved. No studies to date have examined prostaglandin enzyme production or prostaglandin metabolism in normal prostate stromal cells. Our results show that lipocalin-type prostaglandin D synthase (L-PGDS) and prostaglandin D2 (PGD2) metabolites produced by normal prostate stromal cells inhibited tumor cell growth through a peroxisome proliferator–activated receptor γ (PPARγ)–dependent mechanism. Enzymatic products of stromal cell L-PGDS included high levels of PGD2 and 15-deoxy-Δ12,14-PGD2 but low levels of 15-deoxy-Δ12,14-prostaglandin J2. These PGD2 metabolites activated the PPARγ ligand-binding domain and the peroxisome proliferator response element reporter systems. Thus, growth suppression of PPARγ-expressing tumor cells by PGD2 metabolites in the prostate microenvironment is likely to be an endogenous mechanism involved in tumor suppression that potentially contributes to the indolence and long latency period of this disease. (Cancer Res 2005; 65(14): 6189-98)

Introduction

Dynamically balanced molecular mechanisms in the prostate microenvironment mediate stromal-epithelial function during the development and homeostatic maintenance of the prostate gland. Perturbation of these molecular dynamics can have a negative or positive influence during prostate carcinogenesis.

Among the many products generated by support tissues in the prostate gland, those most likely to profoundly affect the growth of prostate cancers are prostaglandins. Prostaglandins are essential to male reproduction (1, 2), and high levels of prostaglandins are found in semen as products of both prostate and seminal vesicles (3–7).

Unique among glandular epithelial tissues, the prostate is one of the few tissues other than the heart, the brain, and some adipose tissues that make lipocalin-type prostaglandin D synthase (L-PGDS), which synthesizes prostaglandin D2 (PGD2 refs. 8–11). Both L-PGDS protein and PGD2 are prominently found in normal seminal fluid (10). Once PGD2 is made, it forms derivative compounds, most of which can transactivate the peroxisome proliferator–activated receptor γ (PPARγ). One PGD2 derivative, 15-deoxy-Δ12,14-prostaglandin J2 (15-d-PGJ2), can slow the growth and induce the partial differentiation of selected cancer cells (12). Another PGD2 derivative, 15-deoxy-Δ12,14-PGD2 (15-d-PGD2), has also been shown to stimulate PPARγ transactivation in RAW 264.7 cell macrophage cultures as effectively as 15-d-PGJ2 (13). L-PGDS also binds tritiated testosterone and may play a role in androgen transport (14). In castrated rats, testosterone propionate induces L-PGDS synthesis in the epididymis (15). Although multiple studies have shown a strong correlation between elevated L-PGDS expression in the male reproductive tract and male fertility (14, 16, 17), the mechanistic role L-PGDS plays in normal reproductive homeostasis and activity remains unknown.

In previous studies, we observed that PPARγ was highly expressed in malignant cells, which promoted selective growth suppression in tumor cells by PPARγ ligands when compared with normal cells that did not express PPARγ (18, 19). Because high levels of L-PGDS and PGD2 have been found in normal seminal plasma and reproductive tissue (10), we hypothesized in the present study that these products stimulate the PPARγ expressed primarily by prostate tumor cells, resulting in specific growth suppression. As a corollary to this hypothesis, we assumed that normal epithelia not expressing PPARγ would remain unaffected by L-PGDS and PGD2. To test our hypothesis, we examined the expression of L-PGDS and its metabolic products in normal prostate cells and their biological effects on normal prostate epithelial cells and prostate tumor cells.

Materials and Methods

Cell culture. Normal prostate epithelial cells, prostate stromal cells, and prostate smooth muscle cells isolated from young trauma victims were obtained from Clonetics Corp. (San Diego, CA). The PC-3, LNCaP, and DU145 cell lines were obtained from American Type Culture Collection (Manassas, VA). Control RAW 264.7 cells were provided by Dr. B. Su (Department of Immunology, The University of Texas M.D. Anderson Cancer Center, Houston, TX) and HU78 cells by Dr. D. Jones (Department of Hematopathology, The University of Texas M.D. Anderson Cancer Center). Primary cell cultures were maintained in defined culture medium according to the manufacturer’s instructions as described previously (20). All other cell lines were maintained in DMEM and F-12 low-glucose medium mixed at a ratio of 1:1 (Life Technologies, Bethesda, MD) supplemented with 10% fetal bovine serum.

Reverse transcription-PCR. The RNA STAT-60 reagent (Tel-Test, Inc., Friendswood, TX) was used to extract the total RNA, which was treated with DNase I before use in a reverse transcription-PCR (RT-PCR) analysis. RNA (1 μg) was reverse transcribed with mouse mammary tumor virus RT (Life Technologies, Inc., Rockville, MD). L-PGDS (600 bp) was amplified by the

Requests for reprints: David G. Menter, Department of Clinical Cancer Prevention, The University of Texas M.D. Anderson Cancer Center, Box 1360, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-792-0626; Fax: 713-794-4403; E-mail: dmenter@mdanderson.org.

© 2005 American Association for Cancer Research.
primer set 5’-CTGCTCGGTGCAAGAAATGGTCTACTAATCAC-3’ and 5’-TGGGAGATCCTTGGTTCGATCTGACTTA-3’, and PGDS (321 bp) was amplified by the primer set 5’-CAGCACTTATGGCATGC-3’ and 5’-GATGCCCTGCGGCGCTCCT-3’ as described previously (21). DP1 receptor (387 bp) was amplified by the primer set 5’-CCTCTGTG GCCCAAGGCACCACATGTCGGC-3’ and 5’-CAGGCGGAAAGA- GTTAGGTGAAAGG-3’ as described by Nagata et al. (23). Primer pairs (5’-CAGCTCTGGAAACTGTCG-3’ and 5’-GTGATCTACTGGCTTCAAC-3’) were used in RT-PCR to detect 36B4 mRNA (24). The RT-PCR DNA products were subcloned into a topoisomerase PCR system (Invitrogen, Carlsbad, CA) and sequenced by automated sequencing (SeqWright, Houston, TX) to verify the insert DNA.

PCR DNA products were subcloned using a topoisomerase PCR system (Invitrogen, Carlsbad, CA) and sequenced by automated sequencing (SeqWright, Houston, TX) to verify the insert DNA. After sequencing occurred, a 600-bp product was then subcloned into three vectors, a pLRESneo2 selectable vector, a pCMVH-tagged vector, and a pCMVsync-tagged vector, to yield pLPGDNeos, pLPGDSHA, and pLPGDMyc, respectively.

**Determination of prostaglandin D2 metabolites in prostate cells.** Various cell lines were plated in 100-mm tissue culture dishes to attain a confluence of 70% to 75%. Cells were then incubated with 10 μmol/L arachidonic acid for 30 minutes and then 1 hour. The culture medium was collected at each time point, and cells were harvested at 1 hour by trypsinization and subjected to PGD2 extraction.

**Intracellular prostaglandin D2.** The intracellular PGD2 was extracted by using the modified method of Kempen et al. (25). Briefly, cells were resuspended in 500 μL PBS, and 20 μL aliquots were treated with 1 N citric acid and 10% butylated hydroxytoluene (2.5 μL). PGD2 was extracted thrice with 2 mL hexane/ethyl acetate solution (1:1, v/v). The upper organic phases were pooled and evaporated under a stream of nitrogen at 25°C with 2 mL hexane/ethyl acetate solution (1:1, v/v). The upper organic phases were then resuspended in 500 μL methanol. The eluate was evaporated under a stream of nitrogen, and the residue was dissolved in 10 mmol/L ammonium acetate buffer solution (750×, v/v; pH 8.5).

**Liquid chromatography tandem mass spectrometry.** LC/MS/MS analysis was done with a Quattro Ultima tandem mass spectrometer (Micromass, Beverly, MA) equipped with a HP1100 binary pump high-performance liquid chromatography inlet (Agilent Technologies, Inc., Palo Alto, CA). Prostaglandins were separated by using a Luna 3 μm phenyl-hexyl 150 × 3-mm HPLC column (Waters Corp., Milford, MA), and PGD2 metabolites were eluted with 1 mL methanol. The eluate was evaporated under a stream of nitrogen. The residue was dissolved in 10 μmol/L ammonium acetate buffer solution (750×, v/v; pH 8.5).

**Western blot analysis.** Whole cell lysates were prepared as described previously (20). Specifically, protein (100 μg) was loaded in each lane and run on a 7.5% SDS-PAGE and transferred to a nitrocellulose membrane (Schleicher & Schuell Biosience, Inc., Keene, NH). After blocking with 3% bovine serum albumin, the blots were exposed to rabbit primary anti-L-PGDS antibody (Cayman Chemical Co., Ann Arbor, MI) followed by anti-rabbit secondary antibody (Pierce Chemical Co., Rockyford, IL). The signals were detected by using an enhanced chemiluminescence system (Pierce Chemical).

**Transactivation of the peroxisome proliferator response element.** PC-3 cells were cotransfected with [acyl-CoA oxidase-peroxisome proliferator response element (PPRE)-β-thymidine kinase-luciferase reporter (250 ng; ref. 27) and pGal cDNA (100 ng); reporter assay analysis followed as described previously (19). Luciferase activity was normalized to βGal activity that was cotransfected along with the appropriate reporter.

**Peroxisome proliferator–activated receptor γ–specific ligand-binding domain transactivation.** Recombinant pcDNA3 plasmids that contained cDNA inserts encoding either a fusion protein containing GalH DNA-binding domain (amino acids 1-147) coupled to a PPARy ligand-binding domain (LBD; amino acids 174-475) fusion protein or just a GalH DNA-binding domain as a control (28) were used to transfect prostate cancer cells in six-well tissue culture plates. Either plasmid (0.5 μg) plus (GalH)3-ACTGACGCGTCGACGACG-3’ that contained a PPARy sequence shown previously to be an effective small interfering RNA (siRNA) against the PPARy message (31) were cloned into the U6-tetO construct. Additional oligomers (5’-GCCCTTCCTACTGTGGAGCAGCT-3’) and (5’-CGTCAACAGTGT- GAAGGC-3’) that contained a PPARy sequence shown previously to be an effective small interfering RNA (siRNA) against the PPARy message (31) were cloned into the U6-tetO construct. Additional oligomers (5’- CGTCAACAGTGTGGAGGCGCTTTTGGGAC-3’ and (5’-CAAAGGGCCCTTCACACTTGTTAGCAGCT-3’) containing a complementary sequence to the earlier oligos and a T7 transcriptional termination signal to ensure termination were subsequently cloned into the modified U6-tetO construct. The resulting plasmid contained a hairpin siRNA against the PPARy message. The clones were confirmed as being positive for hairpin siRNA insertion after restriction digestion, PCR analysis, and direct sequencing. A control vector containing a previously reported siRNA against the enhanced green fluorescent protein (EGFP; ref. 32) was made in a similar fashion, resulting in a short hairpin (shRNA) that was directed against the EGFP.

In control experiments, a digital image analysis was done after cells were transfected with a plasmid that expressed EGFP either alone or in combination with an EGFP-shRNA-expressing plasmid followed by treatment with 10 μmol/L PGD2. Phase-contrast images were obtained to observe total cell fluorescence and epifluorescence to determine the level of EGFP protein. Similar experiments were done using either EGFP shRNA or PPARy shRNA followed by treatment of cells with 10 μmol/L PGD2. Staining with calcein AM (CAM) and 4,6-diamidino-2-phenylindole (DAPI) dyes was used to evaluate cell viability.

**Cell proliferation assay.** Quantification was done by treating cells with 40 μL of a PBS solution containing 2.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide followed by formazan solubilization in 100 μL DMSO and reading absorbance at a wavelength of 540 nm on a 96-well plate reader (33).

**Statistical analyses.** Data were analyzed statistically using the Statview software program (SAS Institute, Inc., Cary, NC). Student’s t tests were used to determine the significance between mean group values.

**Results**

**Lipocalin-type prostaglandin D synthase is expressed by normal but not by malignant prostate cells.** In normal prostate cells, PCR and Western blot analyses indicated that levels of L-PGDS RNA and protein, respectively, were greatest in stromal cells, less prominent in smooth muscle cells, and lowest in epithelial cells.
(Fig. 1A and B). The intracellular distribution of L-PGDS was in the cytoplasm and endoplasmic reticulum of normal cells (Fig. 1C). In contrast, tumor cells did not exhibit observable intracellular levels of L-PGDS.

Prostate cells lack G protein–activating transmembrane receptors DP1 and DP2. We used a RT-PCR assay to examine normal and tumor-derived prostate cells for the presence of the G protein–activating transmembrane receptors DP1 and DP2. Multiple sets of intron-spanning PCR primers were used to analyze DNase-treated total RNA isolated from these cells. No DP1 or DP2 cDNA amplimers were observed at 25, 30, or 35 thermocycles in any of the prostate cells (Fig. 1D). In contrast, HU78 cells in which DP1 and DP2 expression had been characterized previously showed both PCR products (Fig. 1E).
Arachidonic acid–conditioned stromal cell medium enhances normal cell growth but suppresses cancer growth. We reported previously on the expression, phosphorylation patterns, and functions of the human PPARγ1 and PPARγ2 isoforms in prostate cells (18). We found that prostate epithelial cells did not express either the PPARγ1 or the PPARγ2 protein and were not as sensitive as PC-3 tumor cells to growth inhibition by the PPARγ ligand 15d-PGJ2 in the absence of the expression of either PPAR isoform. In contrast, PC-3 prostate cancer cells, which express high levels of the PPARγ1 receptor isoform, were significantly growth inhibited by 15d-PGJ2. In another study, we showed that PPARγ was expressed by tumor cells (PC-3 > DU145 > LNCaP), but they did not express 15-lipoxygenase-2, an arachidonic acid–metabolizing enzyme that also produces the PPARγ ligand 15-hydroxyeicosatetraenoic acid (19). In contrast, prostate epithelial cells that did not express PPARγ expressed high levels of 15-lipoxygenase-2 (19).

In the present study, we determined the effect of normal stromal cell arachidonic acid metabolites on epithelial cell and tumor cell growth (Fig. 2A). The growth of cells containing the highest levels of the PPARγ receptor (i.e., PC-3 and DU145 cells) was most inhibited by arachidonic acid metabolites, whereas LNCaP cells, which express lower levels of PPARγ, were the least inhibited. In contrast, the growth of epithelial cells, which lack the PPARγ receptor, was slightly stimulated.

Depletion of prostaglandin D2 by antibodies decreases the effects of arachidonic acid–conditioned stromal cell medium. We next verified that PGD2 production was responsible for the effects produced by stromal cell–conditioned medium (Fig. 2B). In these experiments, stromal cell monolayers were treated with arachidonic acid followed by the lowering of PGD2 levels with a specific antibody. The removal of PGD2 from stromal cell–conditioned medium abrogated the stimulation of epithelial cells and the growth suppression of tumor cells (Fig. 2B). These PGD2 depletion effects were verified by fluorescence analysis of monolayers after staining with the CAM vital dye and the DNA-intercalating dye DAPI. Viable epithelial cells elicited bright green fluorescence and excluded DAPI uptake, whereas dying PC-3 cells lost the green fluorescence and allowed the uptake of DAPI because of a loss of membrane integrity (Fig. 2C).

Measurement of prostaglandin D2 metabolites. Total ion chromatography was used to evaluate the retention times of the various PGD2 metabolites and to achieve critical separation profiles expressed by stromal cell–conditioned medium. A, effects of stromal cell–conditioned medium on growth of normal prostate epithelial cells and prostate tumor cells (LNCaP, PC-3, and DU145). Stromal cells were established as monolayers and incubated in the absence or presence (St/AA) of arachidonic acid. Controls consisted of stromal cells incubated with medium alone (St/M), medium alone incubated in plastic tissue culture dishes (M), or medium containing arachidonic acid incubated in plastic tissue culture dishes (AA). The conditioned medium was placed into monolayers of PC-3, LNCaP, and DU145 tumor cells and normal epithelial cells, and total propidium iodide staining was then measured as relative fluorescence units. Representative of two experiments. B, anti-PGD2 antibody depletion of PGD2 from prostate stromal cell–conditioned medium and its effects on both normal epithelial cells and PC-3 tumor cell growth. Stromal cell monolayers were incubated in the presence of arachidonic acid and protein A/G-agarose beads in the presence or absence of anti-PGD2 antibody (anti-PGD2 Ab). Total propidium iodide staining was then measured as in (A). Significance was determined by performing a Student’s t test comparing the conditions with protein A/G alone with those with anti-PGD2 antibody; all results were significant (P < 0.001). Representative of two experiments. C, analysis of cells treated with PGD2-depleted medium conditioned by stromal cell medium alone, stromal cell/arachidonic acid–conditioned medium, protein A/G-agarose beads alone (St/AA Protein A/G), or stromal cell/arachidonic acid-agarose beads in the presence of an anti-PGD2 antibody (St/AA Protein A/G anti-PGD2 Ab). Viable cells have green fluorescence, whereas dead cells have lost the green fluorescence and taken up the blue DAPI dye (arrows). Representative of two experiments.
Mass spectroscopy was then developed using deuterium-labeled standards to distinguish between each of the PGD2 metabolites (PGD2 < 15d-PGD2 < 15d-PGJ2; Fig. 3A). The high expression levels of L-PGDS in normal stromal cells, smooth muscle cells, and epithelial cells corresponded to high levels of PGD2 and were uniformly restricted to normal cells as determined by LC/MS/MS analysis (Fig. 3C).

**Arachidonic acid is converted to prostaglandin D2 metabolites by stromal cells.** The conversion of arachidonic acid to PGD2 was examined in culture medium and in whole cells at 15, 30, 60, and 120 minutes. Similar amounts of PGD2 were produced in both cells and medium, reaching a plateau between 1 and 2 hours (Fig. 3D). The conversion of arachidonic acid to PGD2 metabolites has been shown previously in cultured RAW 264.7 macrophages (13). Using cultured RAW 264.7 macrophages as controls, we determined the level of arachidonic acid conversion by stromal cells to PGD2, 15d-PGD2, and 15d-PGJ2 in the LC/MS/MS analysis. The production of 15d-PGD2 and 15d-PGJ2 at 2 hours by both RAW 264.7 macrophages and stromal cells was examined by LC/MS/MS analysis (Fig. 3E).
Figure 4. PGD₂ compounds affect prostate cancer cell growth. PC-3 cell monolayers were treated with multiple concentrations of PGD₂ compounds and stained at various times with CAM and DAPI. A, digital image analysis was done after 72-hour treatments using epifluorescence microscopy; viable cells have green fluorescence, whereas dead cells have lost the green fluorescence and taken up the blue DAPI dye (arrows). B, quantification using a fluorescence microplate reader showed the suppression of prostate cancer cell growth by these compounds with a relative effectiveness of 15-d-PGJ₂ > 15-d-PGD₂ > PGD₂. C, epifluorescence microscopy of CAM- and DAPI-stained cells that included diluent (control) cells or cells treated with 11Me15KetoD₂ or 15KetoD₂. D, no growth suppression occurred in cells treated with 11Me15KetoD₂ or 15KetoD₂. Concentrations used were as follows: open black squares, control samples; open red diamonds, 1 μmol/L; open green circles, 2.5 μmol/L; open blue diamonds, 5 μmol/L; cross in cyan squares, 7.5 μmol/L; cross in magenta diamonds, 10 μmol/L. Representative of duplicate experiments.
stromal cells was <50% of the production of PGD₂ (Fig. 3F). To evaluate how effectively PGD₂ metabolites were converted in tumor cells, we treated these cells with various concentrations of PGD₂. Nearly 40% of the PGD₂ were converted to 15d-PGD₂ in all cell lines, whereas only ~1% of the PGD₂ was converted to 15d-PGJ₂ (Fig. 3F). Similar conversion rates of PGD₂ to 15d-PGD₂ and 15d-PGJ₂ were observed when 5 and 10 μmol/L PGD₂ were used.

**Prostaglandin D₂ compounds suppress prostate cancer cell growth.** We compared the effects of PGD₂, 15-d-PGD₂, and 15-d-PGJ₂ on PC-3 prostate cancer cells that express the highest level of PPARγ for their effects on growth and death by adding the vital dyes CAM and DAPI, the latter of which was excluded by intact plasma membranes. Fluorescence microscopy of PC-3 prostate cancer cells grown in 96-well plates treated with PGD₂, 15-d-PGD₂, or 15-d-PGJ₂ metabolites for 72 hours showed a concentration-dependent reduction in cell number and a corresponding increase in DAPI incorporation into cell nuclei (Fig. 4). Quantification of CAM conversion by viable cells in 96-well plates was examined after treating prostate cancer cells with PGD₂, 15-d-PGD₂, or 15-d-PGJ₂ metabolites for 6, 24, or 72 hours on a multiwell fluorescence plate reader (Fig. 4A and B). Treatment of prostate cancer cells with PGD₂ or 15-d-PGJ₂ caused a concentration- and time-dependent decrease in the level of CAM fluorescence over 72 hours. The relative treatment effectiveness of these ligands in suppressing prostate cancer cell growth was determined (15-d-PGJ₂ > 15-d-PGD₂ > PGD₂). In contrast, control samples treated for 72 hours with diluent or the DP1 or DP2 receptor-activating metabolite 11Me15KetoD₂ or 15KetoD₂ showed high numbers of viable cells and little or no DAPI incorporation into cell nuclei (Fig. 4C and D).

**Transcriptional activation of the peroxisome proliferator–activated receptor γ response element.** The first enzyme of the peroxisomal β-oxidation pathway, acyl-CoA oxidase, contains upstream cis-acting regulatory regions called PPREs (34). A reporter construct (acyl-CoA oxidase-PPRE)3-thymidine kinase-luciferase was highly induced by PGD₂, 15-d-PGD₂, and 15-d-PGJ₂ in PC-3 cells that expressed high levels of PPARγ (Fig. 5A).

**Peroxisome proliferator–activated receptor γ ligand-binding domain–specific luciferase reporter activation.** The various PGD₂ metabolites were used to activate a chimeric Gal4-PPARγ LBD luciferase reporter system in PC-3 cells, which provided a measure of their relative effectiveness for such activation: PGD₂ was less effective than 15-d-PGD₂, which was less effective than 15-d-PGJ₂ (Fig. 5B). In contrast, DP1 and DP2 receptor ligands had no effect on Gal4-PPARγ-LBD activation.

**Knockdown of peroxisome proliferator–activated receptor γ expression abrogates the suppression of PC-3 cell growth by prostaglandin D₂.** Transfection of PC-3 cells with an EGFP shRNA-containing plasmid had no effect on the inhibition of
growth by PGD2 (Fig. 6A-C). In contrast, the same plasmid that contains a PPARγ shRNA interfered with the ability of PGD2 to inhibit PC-3 cell growth (Fig. 6B and C). In parallel experiments, the transfection of the EGFP shRNA plasmid had no effect on PPARγ protein expression in PC-3 cells, whereas PPARγ shRNA plasmid caused a decrease in PPARγ protein levels (Fig. 6D). These data indicated that growth suppression of PC-3 cells by PGD2 was partly dependent on the presence of the PPARγ receptor.

Discussion

In the present study, we showed that normal prostate cells taken from young trauma victims expressed high levels of L-PGDS and synthesized PGD2. In contrast, prostate tumor cells lost the ability to make L-PGDS but up-regulated the PPARγ gene. PGD2 and its metabolites activated the PPARγ LBD in the tumor cells. Our data suggest that suppression of prostate cancer growth can involve stromally derived prostaglandin metabolism that is unique to the prostate, which may help explain the indolent nature of prostate cancer development.

Besides binding to nuclear receptors, prostaglandins can also bind to membrane receptors. Membranous PGD2 receptors are coupled to G protein and occur as two isoforms, DP1 (35) and DP2 (36). G protein receptors can activate adenylate cyclase, leading to cyclic AMP synthesis, or can antagonize this process (37). They can also increase the phosphatidylinositol turnover that elevates the free intracellular calcium level. In the present study, we did not observe either DP1 or DP2 expression in any prostate cells (Fig. 1D and E), suggesting that the receptor-dependent responses in these cells primarily involve PPARγ.

Various PPARγ-specific ligands that arise from PGD2 can mediate their effects through PPARγ transactivation and the suppression of cell growth (13, 28, 38, 39). Other PPARγ ligands also suppress xenograft tumor development in the prostates of immunocompromised mice (40) and the growth of prostate cancer cell lines in vitro (41, 42). To our knowledge, we are first to show that, in addition to 15-d-PGJ2, PGD2 and 15-d-PGD2, can also transactivate transcription through the PPARγ LBD in prostate cancer cells and suppress their growth (Fig. 5). L-PGDS may also lead to growth inhibition of PPARγ-expressing cells through the production of 15d-PGJ2. In the present study, we observed that very little PGD2 was converted to 15d-PGJ2 by tumor cells; the bulk of the PGD2 was converted to 15d-PGD2 (Fig. 3F).

When tumor cells gain PPARγ expression while losing L-PGDS expression, they are likely to become susceptible to influences from the microenvironment. Stromal-epithelial interactions are dynamically balanced and rely on a sensitive equilibrium among cell proliferation, differentiation, and apoptosis through interactions between normal cells or cancer cells and their microenvironments (43). This feedback between stroma and developing prostate tumor epithelial cells or metastases in other microenvironments may present opportunities for targeting both the tumor cells and the stromal cells (43, 44). Most studies have examined the growth-promoting effects of stromal factors on prostate cancer, which involve the bidirectional exchange of support factors, including androgen and tumor growth factor-β1 (43–47). Changes in the support tissue and generation of reactive stroma can also occur as part of tumor formation (45, 48). Clinical studies have shown that stromal factors can be used as predictors of cancer recurrence (49). In contrast, we know very little about potential growth-suppressive factors present in stroma that affect normal homeostasis and tumor development.
The present study is the first to show that growth-suppressive factors that inhibit tumor cell growth and induce differentiation properties in tumor cells are present in stroma (Figs. 2 and 4). In general, the stromal cells examined in previous growth promotion studies were isolated from samples taken from patients with prostate cancer, benign prostatic hyperplasia, or bladder cancer before undergoing cystoprostatectomy. These stromal cell sources are likely to have different biological properties compared with the stromal cells used in our study, which were obtained from young trauma victims. These potential differences may be age or disease state related, possibilities that are currently under investigation in our laboratory.

The ability of stromally derived L-PGDS factors to suppress tumor growth also remains to be confirmed in vivo. Specifically, in vivo studies must be done to determine if the L-PGDS produced by normal tissue is gradually lost during the later stages of progression leading to outgrowth of the tumor. Such studies should also attempt to determine whether tumor cells can evade the suppressive influence of the prostate microenvironment by forming metastases. Shifting the equilibrium between stromal-epithelial interactions selectively toward growth suppression of prostate tumor cells by prostaglandins or PPARγ agonists may therefore be useful in future designs of cancer therapeutic agents, adjuvant therapy, or preventive measures.

Gene-targeting studies illustrate the importance of PPAR receptors to fatty acid and lipoprotein homeostasis and the need for tissue-specific targeting models to understand the unique role of individual receptors in each particular organ site (50). PPARγ is critical to survival because null embryos die at 10 days' gestation (51). When PPARγ (+/-) heterozygous mice (52) were crossed with mice that had transgenic adenocarcinoma of the mouse prostate (TRAMP; ref. 52), there was no effect on the development of prostate cancer (53). The TRAMP model, however, incorporates a minimal probasin promoter driving large T SV40 virus antigen, which is not highly expressed in all lobes of the mouse prostate and may bypass some of the signaling pathways associated with typical prostate cancer progression that are not mediated by viral proteins. In other mouse studies not involving up-regulated viral proteins, crosses between ARRB2P composite promoter-driving Cre-recombinase (54) and a floxed PPARγ (55) generated knockout mice characterized by a high incidence of prostatic intraepithelial neoplasia (PIN). PIN involvement that favored the development of invasive prostate cancer was consistent with the normal progression of prostate cancer (56). Because the ARRB2P is a second-generation promoter that is efficiently expressed in all lobes of the mouse prostate, it is probably more representative of how tissue-specific loss of PPARγ contributes to cancer progression in these animals (54). These prostate-specific gene-targeting findings are consistent with our present studies that show how normal stromal cell–derived prostaglandins from L-PGDS contribute to growth-suppressive responses in PPARγ-expressing prostate tumor cells (Figs. 2, 3, and 4). Our own PPARγ shRNA data showing that the suppression of PC-3 cell growth by PGD2 is partly dependent on PPARγ expression (Fig. 6) strengthen the argument that PPARγ can act as a tumor suppressor in the presence of a ligand.

Our study shows that prostatic stromal cells generate L-PGDS and PPARγ ligands, which suppress the growth of tumor cells that express PPARγ. We suggest that L-PGDS and PPARγ are promising molecular targets for the development of chemopreventive or chemotherapeutic agents that can specifically affect prostate cancer cells, while sparing normal prostate cells, and the tumor-suppressive effects of L-PGDS and PPARγ.

Acknowledgments

References

Suppression of Prostate Tumor Cell Growth by Stromal Cell Prostaglandin D Synthase–Derived Products

Jeri Kim, Peiying Yang, Milind Suraokar, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/14/6189

Cited articles
This article cites 54 articles, 18 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/14/6189.full#ref-list-1

Citing articles
This article has been cited by 9 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/65/14/6189.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/65/14/6189.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.