Differential Expression of Cyclooxygenase-2 and Its Regulation by Tumor Necrosis Factor-α in Normal and Malignant Prostate Cells\(^1\)

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ABSTRACT

Cyclooxygenase (COX)-2 expression is elevated in some malignancies; however, information is scarce regarding COX-2 contributions to the development of prostate cancer and its regulation by inflammatory cytokines. The present study compared and contrasted the expression levels and subcellular distribution patterns of COX-1 and COX-2 in normal prostate [prostate epithelial cell (PrEC), prostate smooth muscle (PrSM), and prostate stromal (PrSt)] primary cell cultures and prostatic carcinoma cell lines (PC-3, LNCaP, and DU145). The basal COX-2 mRNA and protein levels were high in normal PrEC and low in tumor cells, unlike many other normal cells and tumor cells. Because COX-2 levels were low in prostate smooth muscle cells, prostate stromal cells, and tumor cells, we also examined whether COX-1 and COX-2 gene expression was elevated in response to tumor necrosis factor-α (TNF-α), a strong inducer of COX-2 expression. Northern blot analysis and reverse transcription-PCR demonstrated different patterns and kinetics of expression for COX-1 and COX-2 among normal cells and tumor cells in response to TNF-α. In particular, COX-2 protein levels increased, and the subcellular distribution formed a distinct perinuclear ring in the normal cells at 4 h after TNF-α exposure. The COX-2 protein levels also increased in cancer cells, but the subcellular distribution was less organized; COX-2 protein appeared diffuse in some cells and accumulated as focal deposits in the cytoplasm of other cells. TNF-α induction of COX-2 and prostaglandin E\(_2\) correlated inversely with induction of apoptosis. We conclude that COX-2 expression may be important to PrEC cell function. Although it is low in stromal and tumor cells, COX-2 expression is induced by TNF-α in these cells, and this responsiveness may play an important role in prostate cancer progression.

INTRODUCTION

COX (also called PGH\(_2\) synthase) enzymes catalyze the formation of PGH\(_2\) from arachidonic acid, a process that limits the rate of PG\(_3\) synthesis (1). The COX isoenzymes are encoded on separate genes with distinct expression patterns and functional characteristics. For example, COX-1 is considered a “housekeeping gene” that frequently regulates normal cell activity and key physiological or homeostatic functions (e.g., platelet aggregation; Refs. 1–3). COX-2 is expressed at low levels in most cells and exhibits an “immediate early” response to proinflammatory stimuli, including interleukin-1 and TNF-α (1, 2). Although the role of COX-2 in the development of prostate cancer is relatively unknown, it has been implicated in the development of numerous other cancers including those arising in the colon (4, 5) and breast (6). In various histopathological studies involving human tissues, PGs are frequently overexpressed in cancer tissues compared with levels in adjacent normal tissues (7). Overexpression of COX-2 in colonic epithelial cells inhibits apoptosis (8), increases adhesion and invasiveness of tumor cells (8, 9), and enhances angiogenesis (10). In human prostate cancer cells, PGE\(_2\) treatment increased COX-2 expression, which suggests the importance of prostate-COX-2 regulation by PGs in vitro (11). The regulation of angiogenesis by thromboxane-A2 potentially released from prostate cancer cells may also promote tumor growth (12).

In the study reported here, we examined the expression and subcellular distribution patterns of COX-1 and COX-2 in normal prostate cells and prostate cancer cells. In particular, COX-2 expression patterns in primary cultures of PrEC, PrSM, and PrSt cells were compared with those in three prostatic tumor cell lines. The cancer cell types, which differed in their malignant potentials, were androgen-responsive LNCaP and androgen-unresponsive PC-3 and DU145. Of particular note was our finding that, in contrast to COX-2 expression patterns seen in cancer cells derived from other tissues (e.g., colon and breast), we observed high basal COX-2 levels in normal prostatic epithelial cells as opposed to lower steady-state levels of COX-2 in prostatic carcinoma cells. This led us to question whether COX-2 gene expression was impaired in prostate cancer cells. To answer this question, we studied the effect of TNF-α on COX-2 gene regulation. TNF-α was chosen because it is a strong inducer of COX-2 gene expression and because TNF-α-induced expression may be driven by nuclear factor κB-binding motifs in the COX-2 promoter (13). We determined that although basal COX-2 levels were undetected by Northern blot and RT-PCR analysis in normal PrSM and PrSt cells as well as in those tumor cell lines studied, all of these cells responded to TNF-α by up-regulating COX-2 mRNA. Immunofluorescence studies showed that the COX-2 protein levels increased in all of the cells except the LNCaP cells after treatment with TNF-α. TNF-α also caused the COX-2 protein to redistribute to the perinuclear region in normal cells, but this redistribution pattern was less distinct in the tumor cells. These data indicate that COX-2 may have an important role in prostate cancer progression that involves a response to cytokines.

MATERIALS AND METHODS

rhTNF-α was purchased from R&D Systems, Inc. (Minneapolis, MN). Primary antibodies to COX-1 and COX-2 peptides were purchased from Santa Cruz Biotechnology, Inc. (goat polyclonal IgGs, sc-7950 and sc-7951, respectively; Ann Arbor, MI).

Prostate Cells and Culture Conditions. Primary cultures of normal human PrEC, PrSt, and PrSM were purchased from Clonetics (San Diego, CA). These primary cell cultures were maintained in defined culture medium according to the manufacturer’s instructions. PrECs were grown in prostate epithelial cell growth medium containing 0.4% bovine pituitary extract, 5 μg/ml hydrocortisone, 0.5 ng/ml recombinant human epithelial growth factor, 0.5 μg/ml epinephrine, 10 μg/ml transferrin, 5 μg/ml insulin, 0.1 ng/ml retinoic acid, and 6.5 ng/ml triiodothyronine. PrSM cells were grown in smooth muscle growth medium-2 containing 0.5 ng/ml human epithelial growth factor, 5 μg/ml insulin, 2 ng/ml recombinant human fibroblast growth factor-B, and 5% fetal bovine serum. PrSt cells were grown in stromal cell growth medium containing 1 ng/ml human fibroblast growth factor, 5 μg/ml insulin, and 5% fetal bovine serum. Normal cells were passaged using 0.025% trypsin and 0.01% EDTA in HEPES-buffered saline followed by trypsin-neutralizing solution (Clonetics).
Human prostate carcinoma cell lines consisted of two types: androgen responsive and androgen unresponsive. The LNCaP cell line (androgen responsive) was isolated from a lymph node metastasis. The PC-3 cell line (androgen unresponsive) was isolated from bone metastasis of a patient with grade IV prostate adenocarcinoma, and the DU145 prostate adenocarcinoma cell line (androgen unresponsive) was isolated from a brain metastasis. These cells were maintained in DMEM:Hams’s F-12 low-glucose medium (mixed 1:1; Life Technologies, Inc., Bethesda, MD) supplemented with 10% fetal bovine serum. All of the cells were depleted for at least 12 h and treated with 20 ng/ml rhTNF-α.

RT-PCR. Total RNA (>1 μg after DNase I treatment) was reverse-transcribed with 15 units of MMLV reverse transcriptase (Life Technologies, Inc.) for 15 min at 50°C. PCR reaction (94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and an extension 72°C for 10 min) was carried out with 100 μM primers and 2.5 u of Taq polymerase (Life Technologies, Inc.) in 50 μL. The COX-1 primer pairs consisted of 5′-CAATGCCACCTTCATCGGA-3′ and 5′-GAGCCCGAGTTGACTGTA-3′ (14) that generated a 430-bp COX-1 amplification product. COX-2 primer pairs were 5′-CGAGGTGTATGATGAGTGTT-3′ and 5′-TCTAGCCAGGATGTCGTA-3′ (15) and generated a 540-bp product. The normalization control used was acidic ribosomal protein, 36B4, the primers for which (5′-CAGCTCTGGAGAAACTGCTG-3′; 5′-GAGCCGCAGTTGATACTGA-3′) were used to hybridize with the membrane at 42°C, washed with 2 × SSC containing 0.1% SDS at 37°C for 20 min, and processed by autoradiography. Autoradiographs were digitized and analyzed using a laser densitometer (Molecular Dynamics, Sunnyvale, CA) and printed on an Epson 740 color printer.

Northern Blot Analysis. Total RNAs were extracted using RNA STAT-60 reagent (Tel-Test “B”, Inc.). Northern blot analysis was performed using 20-μg total RNA, run on 1% formaldehyde agarose gel, and transferred overnight to nylon membrane (Gene Screen Plus; New England Nuclear Life Science, Boston, MA). Kinase-labeled oligonucleotides (COX-1, 5′-AGTCTTGGACCAAGTCTGG-3′; COX-2, 5′-GAAGCTTCTAACCTCTCCTAT-3′; and 36B4, 5′-GTGTACTCATCTCCACAGA-3′) generated a 550-bp product (16). One fifth of the volume of the RT-PCR products was separated on a 1.5% agarose gel and transferred onto a nylon membrane (Gene Screen Plus; New England Nuclear Life Science, Boston, MA). This image acquisition equipment is attached to an IX70 fluorescence microscope, and data were acquired using digital image analysis. Our image analysis system uses a Quantix air-cooled black-and-white charge-coupled-device CCD camera that is driven by IP lab software (Scanalytics, Inc., Fairfax, VA). This image acquisition equipment is attached to an IX70 inverted research light microscope equipped with epi-illumination objectives (Olympus America, Inc., Lake Success, NY).

RESULTS

Basal COX mRNA Expression Patterns in Normal and Prostate Cancer Cells. Northern blot analysis showed very high steady state levels of COX-2 mRNA in normal PrEC primary cultures (Fig. 1A,
gradually decreased back to uninduced levels by 4 h (Fig. 1A). PrSt and PrSM cells at 1 h but decreased again at 4 h (Fig. 2, Lanes 1–4). Lanes 1–4).

The finding of high mRNA levels in normal but not prostate tumor cells led us to question whether the inflammatory cytokines such as interleukin-1 and TNF-α gene expression in normal prostate cells was significantly increased at 1 h (Fig. 1B, Lanes 1–4). In comparison, levels were significantly increased in PrSt and PrSM cells at 1 h but decreased again at 4 h (Fig. 2A, Lanes 5–8 and 9–12, respectively). Untreated PC-3, LNCaP, and DU145 cells expressed low levels of COX-2 (Fig. 2B, Lanes 1–5, and 9).

However, there was a gradual increase in COX-2 expressed by the Pr-3 cells up to 4 h (Fig. 2B, Lanes 1–4) compared with a sharp increase in the DU145 cells at 1 h followed by a subsidence at 4 h (Fig. 2B, Lanes 9–12). There was only faint expression in the LNCaP cells at 4 h (Fig. 2B, Lanes 5–8). These results showed that COX-2 was present in all of the cells but that the expression levels and the kinetic response of the cells varied between normal and tumor cells.

COX-1 expression levels did not change significantly in most of the primary cultures in response to TNF-α. We also observed COX-1 gene products after increasing the PCR cycle number to 30 cycles in the prostatic tumor cell lines (Fig. 1B, Lanes 1–12).

**TNF-α Stimulation of COX Protein Synthesis.** Protein expression profiles in prostate cells were analyzed by Western transfer analysis of PAGE-resolved detergent lysates after TNF-α stimulation. The protein expression profiles (Fig. 3, A and B) support what was observed for mRNA production (Figs. 1 and 2). Untreated PC-3 and DU145 cells expressed low levels of COX-2 protein (Fig. 3B, Lanes 9 and 12, respectively). COX-2 protein expression increased gradually and was highest at 4 h in the PC-3 and DU145 cells (Fig. 3B, Lanes 4 and 12, respectively). We were not able to demonstrate COX-2 protein expression in the LNCaP cells by Western analysis under conditions that were comparable with the other cell lines examined in this study (Fig. 3B, Lanes 5–8). COX-1 protein expression was significantly higher in most of the primary cultures compared with COX-1 expression in the prostatic carcinoma cells (Fig. 3A, Lanes 1–12). The PrSM cells expressed the highest levels of COX-1 protein of all of the cells examined (Fig. 3A, Lanes 8–12), followed by PrSt and PrEC (Fig. 3A, Lanes 5–8 and 1–4, respectively). The prostatic carcinoma cells expressed the least amount of COX-1 protein (Fig. 3B, Lanes 1–12). There was no observable change in COX-1 protein in any cell lines after TNF-α treatment.

**Basal Expression Patterns of COX-2 in Normal Cells and Cancer Cells.** COX-1 and COX-2 are integral membrane proteins that localize to the luminal membrane of the endoplasmic reticulum and to the inner and outer membranes of the nuclear envelope proteins (17). Membrane localization is mediated by amino acid sequences that modulate membrane binding and ER targeting (17–20). Although COX-1 and COX-2 associate with both ER and NE membranes,
COX-1 associates predominantly with ER, whereas COX-2 distributes extensively in both the ER and NE membrane (21). The basal expression pattern of COX-2 shown by immunofluorescence studies was high in PrEC but low to undetectable in all of the other cells examined (Fig. 4).

Expression and Intracellular Distribution of COX-1 and COX-2 Protein after TNF-α Stimulation. We examined whether there were alterations in expression and intracellular distribution of COX-1 and COX-2 after TNF-α treatment in normal prostate cells (PrEC, PrSt, and PrSM) and tumor cells (PC-3, LNCaP, and DU145) using immunofluorescence analysis.

There was a remarkable change in the fluorescence intensity and distribution of COX-2 protein in all of the normal cell lines in response to TNF-α stimulation. Specifically, COX-2 protein labeling increased in intensity and formed a sharp perinuclear ring in PrEC, PrSt, and PrSM at 4 h (Fig. 5, insets). In the PC-3, LNCaP, and DU145 prostate carcinoma cells, there was also an increase in COX-2 staining; however, COX-2 was distributed more diffusely or tended to form subcellular clusters (Fig. 5, insets).

COX-1 Protein Expression in Prostate Cells. We also compared COX-1 expression and intracellular distribution using immunofluorescence analysis of normal primary cell and prostate carcinoma cell cultures (data not shown). COX-1 expression was most intense in the PrSM and PrSt cells, but a perinuclear distribution pattern was not apparent (data not shown). Increases in the staining or distribution of COX-1 protein after TNF-α stimulation were barely detectable in either normal prostate cells or prostate carcinoma cells (data not shown).

TNF-α Stimulation of PGE2. To determine whether the induction of COX-2 synthesis was associated with PG production, we measured PGE2 production by prostate cells using EIA. TNF-α stimulation caused an increase in PGE2 production in all of the cell lines except the LNCaP cells. The increase after TNF-α treatment was higher in the normal cells (PrEC, 3.6-fold; PrSt, 1.9-fold; and PrSM, 2.0-fold increases) and lower in the tumor cell lines (LNCaP, 1.1-fold; PC3, 1.5-fold; and DU145, 1.8-fold increases). The PrECs, which expressed the most COX-2, showed the highest relative increase (3.6-fold) in PGE2 production. In contrast, the LNCaP cells, which did not express appreciable changes in the COX-2 levels after TNF-α treatment, had the lowest relative increase (1.1-fold) in PGE2 production.

Apoptosis. Apoptosis was assessed by DAPI and PI. There was an inverse association between the TNF-α induction of PGE2 and apoptosis. In the normal prostate cells, TNF-α produced high relative increases of PGE2 (see above) but very low levels of apoptosis (<1.03-fold increase in apoptosis after TNF-α in all of the three normal cell types, PrEC, PrSt, and PrSM). Among the cancer cell

![Fig. 4. Immunofluorescence analysis of basal COX-2 (green) expression profiles. Basal COX-2 expression in PrEC, PrSt, and PrSM primary cell cultures were compared with that in PC-3, LNCaP, and DU145 prostate carcinoma cells. DAPI-counterstained DNA in nuclei is blue, and Alexa 594-phalloidin-counterstained actin is red. Basal COX-2 (green) expression is high in PrEC but low in all of the other cells (see insets).](image)
lines, TNF-α produced the following effects: the greatest increase in PGE₂ (1.8-fold) and the lowest increase in apoptosis (1.04-fold by PI and 1.02-fold by DAPI) in DU145 cells; the lowest increase in PGE₂ (1.1-fold) and the highest increase in apoptosis (1.43-fold by PI and 2.21-fold by DAPI) in LNCaP cells; and a 1.4-fold increase in PGE₂ and 1.2-fold (PI) and 1.6-fold (DAPI) increases in apoptosis in PC-3 cells (in between the effects in DU145 and LNCaP cells).

**DISCUSSION**

COX seems to play a particularly important role in the physiology of the prostate. Among the human tissues examined (i.e., lung, uterus, testis, brain, pancreas, kidney, liver, thymus, prostate, mammary gland, stomach, and small intestine), the highest levels of COX-2 expression was detected in the prostate (22). PGs have long been known to play an essential role in male reproduction (23). Extremely high levels of PGs are found in semen (~200 µg/ml) as a product of both prostate and seminal vesicles (24). PGs and other substances in semen may help maintain the viability of sperm and aid in fertilization by modulating the female immune response (25). Thus, the constitutive expression of COX-2 is likely to be important in maintaining normal homeostasis and function of the prostate.

Prostate cancer occurs at the highest frequency of all of the cancers identified in American men and is the second leading cause of male cancer mortality (26). Prostate cancer is particularly sensitive to the effects of dietary fats and bioactive PGs (27); e.g., in cultured explants from human BPH and prostate cancer tissue, arachidonic acid metabolism and PG production were profoundly affected by varying the source of fatty acids (28). When the eicosanoid production in BPH and prostate cancer was examined using tritiated arachidonic acid, the only eicosanoid produced in significant amounts by either tissue was tritiated PGE₂, although tritiated PGE₂ was produced at almost 10 times the rate in prostate cancer cells compared with BPH cells (27). Similarly, when nude mice were injected with human DU145 tumors, tumor growth was affected after exposure to PG-inhibitory dietary lipids (28). These studies illustrate the potential importance of fatty acids and PGs in the development of prostate cancer.

Levels of COX-2 are frequently higher in many types of tumor tissue compared with adjacent normal tissue (for review, see Refs. 1, 4, 5). However, our findings in prostate cancer were the inverse of what was noted for these other cancers. In particular, our studies showed very high steady-state levels of COX-2 mRNA and protein in untreated control PrEC cells as compared with a lack of basal expression by PC-3, LNCaP, and DU145 cells. Similar findings were recently reported showing a high basal expression of COX-2 in PrEC and a BPH-1 cell line by Western blot analysis (29). These investigators also noted low basal levels of COX-2 in LNCaP and PC-3 cells.
but did not examine basal COX-2 production in PrSM or PrSt cells (29). When Hong et al. (30) examined the basal expression of COX-1 and COX-2 using RT-PCR, they found COX-1 to be present in all of the three prostate carcinoma cell lines (LNCaP, PC-3, and DU145) but observed COX-2 in only the DU145 cells.

In the present study, the PrSM cells exhibited the highest level of COX-1 mRNA (see Fig. 1A and Fig. 2A) of all of the primary cell cultures we examined, and levels were low to barely detectable in other cell lines as shown by Northern blot analysis. We also demonstrated immediate-early responses in COX-2 gene expression by PrEC cells or the PrSM and PrSt cells as well as increased expression in prostatic carcinoma cells. Furthermore, elevations in either COX-2 or high basal levels of COX-1 in normal cells including PrEC, PrSM, and PrSt lead to increased PGE2 production, which correlated inversely with the induction of apoptosis. Notably, PGE2 can amplify the production of COX-2 in PC-3 and LNCaP tumor cells (11). Accordingly, PGs supplied to developing tumors from surrounding normal cells could help establish a PG feedback loop that causes cancer cell COX-2 levels to increase and enhance tumor progression.

LNCaP cells are one of the few androgen-responsive prostate carcinoma lines and have been the focus of much research, but there are discrepancies in the literature regarding COX-2 mRNA expression by LNCaP cells that data presented here may help to resolve. To elaborate, Tjandrawinata et al. (11) demonstrated that COX-2 mRNA expression levels increase after treatment with dimethyl-PGE2 but observed basal COX-2 expression using RT-PCR after growing cells in 1% serum overnight before dimethyl-PGE2 treatment. In contrast, LNCaP cells in our studies were grown in serum-free medium overnight before stimulation with TNF-α, and the resultant differences in prestimulation serum levels between our studies and those of Tjandrawinata et al. (11) may help explain why we did not see significant basal levels of COX-2 RNA expression in these cells by either Northern blot analysis or RT-PCR (see Fig. 1B and Fig. 2B, Lane 5). However, it is also possible that COX-2 mRNA stability varies among different cell lines. Nevertheless, our findings do agree with findings in other studies that examined COX-2 expression in LNCaP cells. Specifically, as mentioned previously, other investigators did not observe basal COX-2 RNA (30) or protein (29), which is consistent with our observations (Fig. 1B and Fig. 2B, Lane 5).

We also observed differences in the staining intensity and subcellular distribution of COX-2 in prostate cells after treatment with TNF-α (Fig. 5). In particular, there was a remarkable change in the fluorescence intensity and distribution of COX-2 protein in the TNF-α-stimulated PrEC, PrSt, and PrSM (Fig. 5) that was similar to that observed in serum-treated NIH 3T3 fibroblasts and phorbol-myristate acetate-treated endothelial cells (21). Specifically, in the PrEC, PrSt, and PrSM cells there was an increase in the perinuclear distribution of COX-2 that was manifested as a sharp perinuclear ring after 4 h of TNF-α treatment. This pattern of perinuclear distribution has also been observed by others after the transfection of both COX-1 and COX-2 expression vectors into COS-1 cells (20) or membrane binding domain-green fluorescent protein chimeric protein (19). In contrast, COX-2 levels in tumor cells also increased, but the subcellular distribution was less organized. The COX-2 protein appeared diffuse in many cells, accumulated as focal deposits in the cytoplasm of other cells (Fig. 4), and was similar to that observed in COS-1 cells that had been transfected with a variety of mutated COX genes (18, 20). When mutated, the COX-1 and COX-2 constructs in these earlier studies revealed the importance of certain amino acid charges that were essential for targeting to the ER and NE membranes (20) and for insertion to occur into the membrane leaflet (18). These data suggest that targeting efficiency decreases because of a mutation at either the COX-2 COOH-terminal or the NH2-terminal membrane binding domain that disrupts localization to ER and NE of cells and that may be important for the subcellular distribution of COX-2 in normal prostate cells but is aberrant in prostate carcinoma cells. In addition, the organization of the cytoskeleton is important to COX-2 expression, because disruption with microtubule or actin inhibitors has profound effects on COX-2 enzyme levels (31).

In conclusion, these data suggest that COX-2 is important to prostate cancer development by showing that prostate cancer cells exhibit an unusually low level of basal COX-2 expression and an aberrant subcellular COX-2 distribution compared with normal primary cell cultures. The importance of decreased COX-2 expression and the aberrant subcellular distribution in the development of prostate cancer may lead to altered availability of PGE2 precursors for the production of PGs that bind to cytoplasmic PG receptors (1) or nuclear PG receptors (1). In turn, these disruptions in PG availability may shift the balance between cell survival and cell death during carcinogenesis. Notably, the association of high COX-2 levels we observed in normal tissues, if related to any changes in COX-2 expression during preinvasive lesions such as prostatic intraepithelial neoplasia, may be particularly important to prostate carcinogenesis and chemoprevention.

Notwithstanding our findings of reduced endogenous COX-2 expression in (nonstimulated) prostate cancer cells, our findings of cytokine-induced increases in COX-2 expression support the hypothesis that COX-2-selective nonsteroidal anti-inflammatory drugs may be important in preventing prostate cancer by a unique mechanism. This mechanism may depend on the presence of stromal and immunological cytokines or growth factors released by other cells in the tumor microenvironment that may enhance tumor growth via COX-2 induction. Also, it may be that our reduced endogenous COX-2 expression findings suggest that other mechanisms/targets of nonsteroidal anti-inflammatory drugs and other drugs will be important in preventing prostate cancer. Certain reports suggest that COX-2 may be overexpressed in prostate cancer, but this may depend on inflammatory or stromal cell interactions (32).

Additional studies using human biopsy specimens are required to confirm the variable COX-2 expression in individual cell types and stromal contributions during various stages of prostate cancer development, such as prostatic intraepithelial neoplasia, BPH, and PC. Additional studies are also needed to establish the importance of COX-2 responses to cytokines during the development of prostate cancer.

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REFERENCES

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