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# A new prostate cancer therapeutic approach: Combination of androgen ablation with COX-2 inhibitor

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**Prostate cancer is initially responsive to hormonal therapy, but cancers inevitably progress in an androgen-independent fashion with virtually all tumors evolving into more aggressive androgen refractory disease. Immunohistological comparisons of cyclooxygenase 2 (COX-2) expressions in 3 pairs of prostate cancer patients before and after the combined androgen blockade (CAB) therapy show elevated COX-2 expressions. This observation from clinical specimens is further supported by *in vitro* laboratory data using human prostate cancer cells in which the antiandrogen hydroxyflutamide (HF) induced COX-2 expression, and androgen suppressed COX-2 expression. By applying knockdown and overexpression strategies to modulate AR expression in prostate cancer cells, we confirmed that androgen/AR signal suppressed, and HF induced COX-2 expression at both protein and mRNA levels. COX-2 promoter reporter assay indicated that the suppression of COX-2 by androgen/AR is at the transcriptional level *via* modulation of NF-κB signals. Treatment of LNCaP and LAPC4 cells with 1 μM HF in the presence of 1 nM DHT, which mimics the CAB therapy condition, promotes cell growth, and this growth induction can be suppressed *via* adding the COX-2 specific inhibitor, NS398. This suggests that HF promoted prostate cancer cell growth is COX-2 dependent and this HF-COX-2 activation pathway can account for one reason of CAB therapy failure. Together, these findings provide a possible explanation how CAB with antiandrogen HF therapy might fail and provide a potential new therapeutic approach to battle prostate cancer *via* combination of CAB therapy with COX-2 inhibitor(s).**


**Key words:** prostate cancer; combined androgen blockade; cyclooxygenase 2

Prostate cancer is the most common malignancy and the second leading cause of cancer death among men in the United States.[1,2] Ever since Huggins and Hodges[3] first demonstrated that androgen ablation could control prostate cancer growth, combined androgen blockade (CAB) therapy, which is based on suppression of androgen signaling, has played a major role in the management of this disease. CAB strategy usually utilizes luteinizing hormone releasing hormone (LHRH) agonists and antiandrogens to suppress the circulating androgen level and block the function of the androgen receptor (AR).[4] Although 85–90% of advanced prostate cancer patients respond to CAB therapy, it is not curative, with the response lasting for less than 2 years. Then, virtually all cancers inevitably progress into an androgen-independent state.[5,6] The mechanisms by which prostate cancer cells survive after CAB therapy remain unclear.

Derived from arachidonic acid, prostaglandins (PGs) are a family of biologically potent fatty acids, which regulate various pathophysiological processes such as inflammatory reaction, gastro-intestinal cytoprotection and ulceration, and hemostasis.[7] Cyclooxygenase 1 and 2 (COX-1 and -2) are the rate-limiting enzymes in the PG synthesis.[8] COX-1 is constitutively expressed in most tissues, whereas COX-2 is generally expressed at very low levels, but can be induced by growth factors, tumor promoters, and proinflammatory cytokines.[9,10] Compelling data from basic and clinical research suggest that overexpression of COX-2 has been implicated in the initiation and progression of a large number of human cancers.[11–15] The application of nonsteroidal anti-inflammatory drugs (NSAIDs), as well as COX-2 specific inhibitors in cancer clinical practice, has drawn much attention in the past few years as inhibition of COX-2 might provide an effective strategy in the prevention and treatment of cancer.

The role of COX-2 in prostate cancer has been controversial. Several studies showed greater immunostaining of COX-2 in prostate cancer as compared with benign prostatic tissue,[16,17] and the intensity of immunostaining was positively correlated with high tumor grade (Gleason score 8 and 9 *vs.* 5 to 7).[16] In contrast, another study found COX-2 expression was not up-regulated in established prostate cancer and high grade PIN, as compared with adjacent normal prostate tissue.[18] The controversy requires further studies to elucidate the roles of COX-2 in prostate cancer. However, previous studies have never addressed the correlation between COX-2 and CAB therapy and their roles in prostate cancer progression. In our current study, we found 1 nM DHT plus 1 μM HF enhanced COX-2 expression in prostate cancer patients' samples as well as in LNCaP cells, and androgen alone inhibits COX-2 expression in LNCaP cells through mediating the NF-κB pathway. NS398, a COX-2 specific inhibitor, significantly blocked LNCaP cell proliferation stimulated by androgen ablation therapy. These data suggest that overexpression of COX-2 after androgen ablation might be involved in the progression from an androgen-dependent to an androgen-independent phenotype and combination of androgen ablation with COX-2 inhibitors could become a new potential approach to battle the prostate cancer.

## Material and methods

### *Reagents and antibodies*

Primary antibodies to COX-2 peptides were purchased from Cayman Biochemical. (Rabbit polyclonal IgGs; Ann Arbor, MI). The specificity of this COX-2 antibody was verified by other reports by COX-2 blocking peptide.[19–21] COX-2-selective inhibitor NS398 was purchased from Cayman BioChemical (Ann Arbor, MI).

### *Immunohistochemical (IHC) staining*

COX-2 IHC staining was performed using the ABC kit (Vectastain Elite ABC kit, Vector Laboratories, Burlingame). Briefly, sections were rinsed with methyl alcohol-hydrogen peroxide and then

*Abbreviations*: AR, androgen receptor; CAB, combined androgen blockade; COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2; DHT, 5α-dihydrotestosterone; HF, hydroxyflutamide; NSAIDS, non-steroidal anti-inflammatory drugs; PG, prostaglandins.

The first three authors contributed equally to this work.

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microwaved in citrate buffer (pH 6.0) to induce epitope retrieval. Diluted COX-2 primary antibody (1:100) was incubated on slides at 4°C overnight and then incubated with biotinylated secondary antibody at room temperature. For localization, avidin–biotin complex was applied at room temperature for 30 min followed by 3,30-diaminobenzidine tetrahydrochloride as the choursomagen. Slides were counterstained with Mayer hematoxylin. As a negative control for nonspecific staining, COX-2 blocking peptide was added to the diluted COX-2 antibody at a final concentration of 10 mg/ml and incubated for 1 hr at room temperature before the application of the COX-2 antibody to the slides. Then the manual IHC staining was performed as described above.[21]

*Cell culture and plasmids*

LNCaP, PC-3, and COS-1 cell lines were purchased from the American Type Culture Collection (Rockville, MD). PC-3(AR-2) cell line was a gift from Dr. Theodore J. Brown (Department of Zoology, The University of Toronto, Toronto, Ontario, Canada). LAPC-4 cell line is a gift from Dr. Sawyers (Department of Medicine, Jonsson Comprehensive Cancer Center, University of California at Los Angeles, CA) and maintained in Iscove's modified Dubecco's medium. The LNCaP, PC-3, PC-3AR2, CWR22R, and CWR22R-AR↓ cell lines were maintained in RPMI medium containing 10% fetal bovine serum (FBS) and penicillin/streptomycin (Gibco-BRL, Grand Island, NY), and COS-1 monkey kidney cells were maintained in phenol red-free Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS and penicillin/streptomycin all at 5% $CO_2$, and 37°C. The plasmids pGL3-COX-2 promoter and its serial deletion mutant luciferase (*Luc*) reporter gene were gifts from Dr. Rama Natarajan (Gonda Diabetes Center, Beckman Research Institute of City of Hope, Duarte, CA).

*Transfections and reporter gene assays*

Transfections were performed by using SuperFect according to the manufacturer's suggested procedures (Qiagen).[22] After transfection, cells were treated with charcoal-dextran-stripped FBS medium containing either ethanol or ligands for 24 hr. Cell lysates were prepared, and the luciferase (Luc) activity was normalized for transfection efficiency using pRL-CMV as an internal control. Luc assays were performed using the dual-luciferase reporter system (Promega, Madison, WI). Briefly, 1–4 $\times$ $10^4$ cells were plated on 12-well plates 24 hr before adding the precipitation mix containing Luc reporter genes. The medium was changed 24 hr after transfection, and the cells were treated with 1 nM DHT for 24 hr, followed by various treatments for another 16 hr. The cells were then harvested, and whole cell extracts were used for the Luc assay. Luc activity was determined using a Dual-Luciferase Reporter Assay System (Promega) and measured with a luminometer.

*RT-PCR and real-time PCR*

Total RNA was extracted from prostate cancer cells using Trizol (Invitrogen). We carried out reverse transcription with the Super Script II kit (Invitrogen) and PCR amplifications with SYBR Green PCR Master Mix on an iCycler IQ multi-color real-time PCR detection system (Bio-Rad). The COX-2 primer pairs were 5′-CCCTGAGCATCTACGGTTTG-3′ and 5′-CATCGCATACTCT GTTGTGTTC-3′. The AR primer pairs were 5′-CCTGGCTTCCGC AACTTACAC-3′ and 5′-GGACTTGTGCGT GCG GTACTCA-3′. The normalization control used was β-actin, and the primers were 5′-CAGCTCTGGAGAAACTGCTG-3′; and 5′-GTGTACTCAG TCTCCAC AGA-3′. ΔCT values were calculated by subtracting the threshold (CT) value from the corresponding β-actin CT (internal control) value from each time point. Then ΔΔCT values were calculated by subtracting the ΔCT value of untreated controls from the ΔCT value of treated samples. The absence of non-specific amplification products was confirmed by agarose gel electrophoresis.

*Western blot analysis*

Whole cell lysates were made by a standard method, and protein concentrations were measured with the BCA protein reagent (Pierce Chemical, Rockford, IL). Approximately 100 μg of protein/lane was loaded and run on a 10% polyacrylamide gel with a Tris/glycine running buffer system and then transferred onto a polyvinylidene difluoride membrane. The blots were probed with primary AR and COX-2 antibodies with dilutions of 1:250 to 1:1,000 and incubated at room temperature for 2 hr. The secondary antibody [rabbit antigoat IgG, 1:1,000 dilution (Santa Cruz Biotechnology) or rabbit antimouse IgG, 1:1,000 dilution (Pierce Chemical, Rockford, IL)] was used at room temperature for 1 hr. Immunoblot analysis was performed with horseradish peroxidase-conjugated antirabbit and antimouse IgG antibodies using enhanced chemiluminescence Western blotting detection reagents (Amersham Biosciences).

*Cell proliferation assay* in vitro

Cell medium was replenished and cell proliferation was determined by MTT assay (Sigma). Serum-free medium containing MTT (0.5 mg/ml) was added into each well. After 4 hr incubation at 37°C, the stop solution was added to solubilize the formazan product and the absorbance was recorded. Data are expressed as the mean ±S.D. of triplicate samples.

*Colony formation assay*

LNCaP or LAPC4 cells that were under various treatments for 24 hr, then cells were trypsinized, counted, and seeded at 500 cells/dish in 60-mm tissue culture dishes. Cells were fed with fresh growth media every 4–5 days for 2–3 weeks until the colonies were well formed. Crystal violet stain was used to visualize the colonies.

*Immunofluorescence staining*

LNCaP cells were seeded on two-well Lab Tek Chamber slides (Nalge) in RPMI 1640 medium containing 10% FBS. Cells were cultured with 10% charcoal-dextran-stripped FBS for 24 hr, and then were treated with 1 nM DHT, 10 nM DHT, 1 μM HF, 1 nM DHT plus 1 μM HF, or vehicle for 24 hr. Then cells were fixed with fixation solution (3% paraformaldehyde and 10% sucrose in phosphate-buffered saline) for 20 min on ice, followed by permeabilization with methanol for 10 min at −20°C. Slides were washed and blocked with 2% bovine serum albumin in phosphate-buffered saline for 15 min at room temperature. Then the cells were stained with 1 μg/ml of rabbit polyclonal anti-COX-2 antibody at room temperature for 1 hr. After the first antibody incubation, cells were washed and incubated with Texas Red-conjugated goat anti-rabbit IgG. Stained slides were coverslipped and visualized with a fluorescence microscope.

## Results

*Androgen/androgen receptor suppressed COX-2 expression involves NF-κB binding signals*

To investigate the roles of COX-2 expression in the prostate cancer progression during CAB therapy, we examined the DHT effect on COX-2 expression in the androgen-sensitive prostate cancer cell line LNCaP. The basal level of COX-2 mRNA in LNCaP cells was almost undetectable by semi-quantitative PCR analysis, similar to other studies.[23] Therefore, real-time PCR assays were used to compare the relative expression level of COX-2 upon the DHT treatment. The results showed that COX-2 mRNA expression (Fig. 1*a*), as well as protein amounts (Fig. 1*b*), were decreased when the androgen/AR signals were activated by both 1 and 10 nM DHT treatment in LNCaP cells.

To study if AR is involved in this DHT-mediated COX-2 suppression, we applied both knockdown and overexpression of AR strategies. As shown in Figure 1*c*, AR expression was increased in PC-3 stably transfected functional AR cells (PC-3AR2) and reduced in CWR22R-AR↓ cells in which AR was knocked down by homologous recombinant gene targeting,[24] and both cells were maintained in 10% FBS culture medium to sustain androgen level.

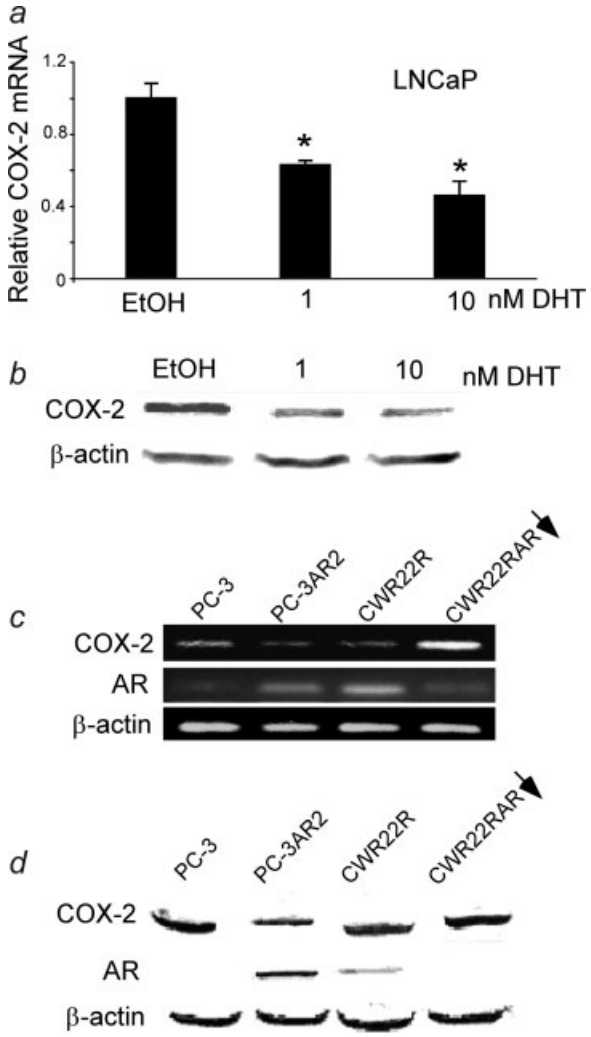


**FIGURE 1** – Androgen/androgen receptor suppresses COX-2 expression. (*a*) The mRNA level of COX-2 was quantified with real-time PCR in LNCaP cells treated with androgen (1 and 10 nM of DHT). (*b*) Western blot was performed to analyze COX-2 protein in LNCaP cells. The $5 \times 10^5$ LNCaP cells were seeded on 60-mm Falcon dishes with RPMI medium-10% FBS and penicillin/streptomycin. Cells were treated with ethanol, 1 nM DHT or 10 nM DHT for 2 days. 100 μg of total protein from LNCaP cells was applied onto a 10% sodium dodecylsulfate-polyacrylamide gel and subjected to electrophoresis followed by electrotransfer to a membrane. Immobilized protein was detected with anti-COX-2 antibody, (*c*) and (*d*) endogenous levels of mRNA and protein of COX-2 and AR in CWR22R, CWR22R-AR↓, PC-3AR2, and PC-3 cells. Semiquantitative RT-PCR and Western Blot analyses were performed.

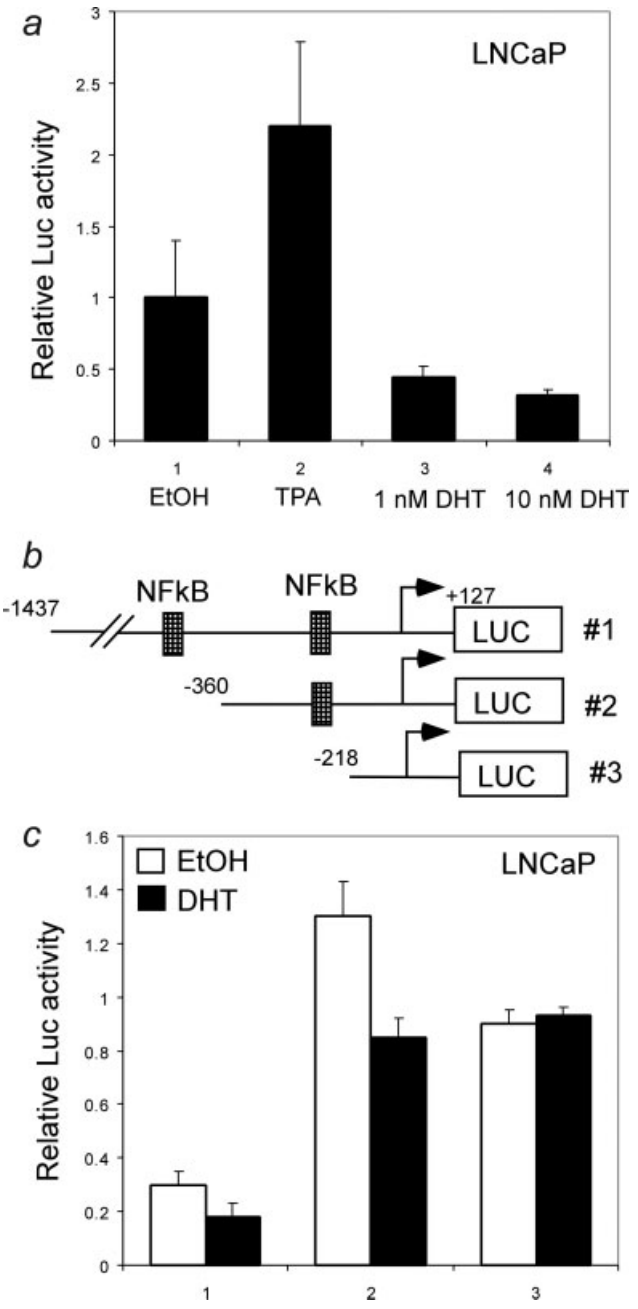


**FIGURE 2** – Androgenic regulation of COX-2 is mediated by NF-κB binding sites in the COX-2 promoter. (*a*) Effects of 1 and 10 nM DHT on COX-2 transactivation in LNCaP cells. The $2 \times 10^4$ LNCaP cells were seeded onto 12-well plates. Cells were transfected with luciferase reporter plasmid, pGL3-COX-2, and pRL-CMV plasmid as an internal control. After 4 hr, the medium was changed to CD medium and incubated overnight. Then cells were treated with ethanol, 1 nM DHT or 10 nM DHT. After 24 hr, cells were lysed with luciferase lysis buffer, and luciferase activity of the cell lysates was determined using a dual-luciferase reporter assay system and measured with a luminometer. (*b*) Diagram of serial deletion mutants (# 1, 2, and 3) of the human COX-2 promoter (from −1,437 and +127 bp relative to the start of transcription (+1 bp)) luciferase plasmid (upper panel). Three deletion mutants constructs: # 1 (−1,437 to +127) construct contains 2 NF-κB binding sites, # 2 (−360 to +127) construct contains one NF-κB binding sites, and # 3 (−218 to +127) construct contains no NF-κB binding site were transfected into LNCaP cells, then cells were treated with 1 nM DHT 24 hr after transfection. The Luc reporter assay was performed to determine the DHT-mediated COX-2 transactivation activity.

Consistent with the data shown in Figures 1*a* and 1*b*, COX-2 mRNA expression was reduced when AR expression increased in PC-3AR2 and COX-2 mRNA expression was increased when AR expression decreased in CWR22R-AR↓ cells (Fig. 1*c*). The same tendency was further confirmed in the COX-2 protein levels (Fig. 1*d*) in those two prostate cancer cell lines.

DHT/AR suppresses COX-2 expression at both RNA and protein levels indicating that regulation of COX-2 by DHT/AR is at the transcriptional level. To confirm this, DHT/AR effect on COX-2 5′ promoter activity was studied. As shown in Figure 2*a*, the activity of COX-2 5′ promoter containing *Luc* reporter was suppressed in the presence of 1 or 10 nM DHT, while TPA, as a positive control, can

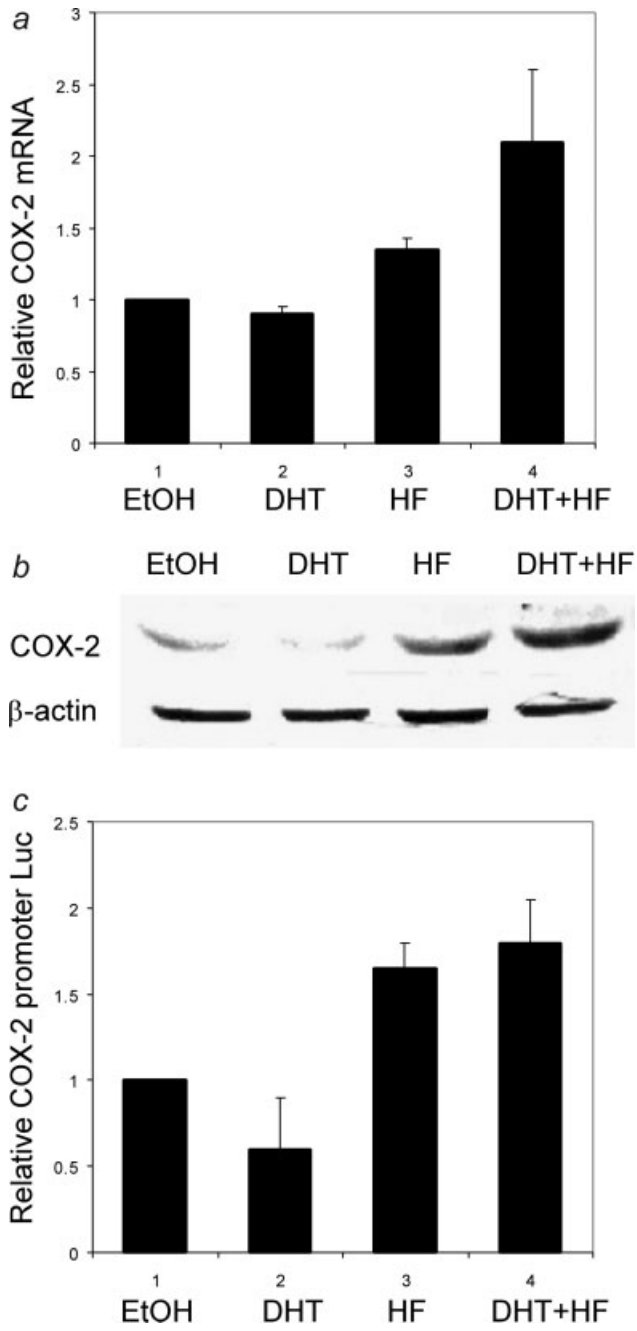


**Figure 3** – CAB with HF therapy stimulates COX-2 expression. (*a*) The expression level of COX-2 mRNA in LNCaP cells treated with 1 μM HF or 1 nM DHT alone, or in combination with both reagents with real-time PCR. (*b*) Western blot was performed to analyze COX-2 protein in LNCaP cells that were treated with ethanol, 1 μM HF or 1 nM DHT, or in combination with both reagents for 2 days. (c) Luciferase assay was used to determine effects of 1 μM HF or 1 nM DHT alone, or in combination with both reagents on COX-2 promoter transactivation activity.

activate COX-2 promoter activity. As sequence analysis of the COX-2 gene 5′ promoter region found no classical androgen response element, we hypothesized that DHT/AR suppresses COX-2 gene expression through modulation of NF-κB, a known DHT/AR regulated gene[25] and upstream modulator for COX-2 gene.[26] To identify the DHT/AR corresponding region, we used the COX-2 5′ promoter into three regions: # 1, 2, and 3 constructs which contains one, two, and no NF-

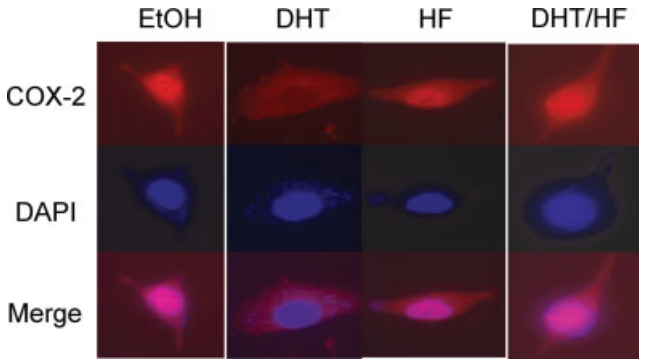


**Figure 4** – CAB with HF therapy promotes COX-2 nuclear translocation. LNCaP cells were treated with vehicle, 1 nM DHT, 1 μM HF, and 1 nM DHT plus 1 μM HF, and COX-2 cellular localization was determined by Immunofluorescence staining.

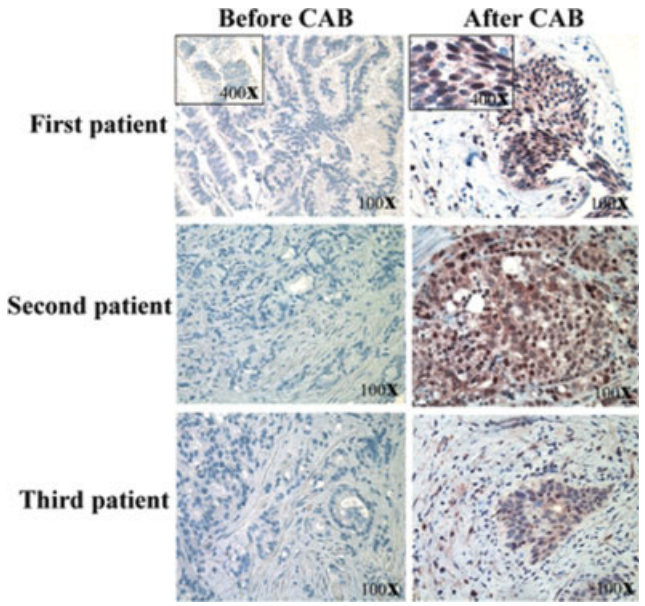


**Figure 5** – COX-2 expression in prostate cancer specimens before and after CAB therapy as detected by immunohistochemistry. Very weak immunoreactivity in prostate cancer patient specimens before CAB therapy was observed in the cytoplasm of epithelial cells (left panel) and strong immunoreactivity was observed in both cytoplasm and nuclei of the epithelial cells in specimens from the same patients after CAB therapy (right panel). Pictures were taken under ×100 or ×400 magnification.

κB binding sites, respectively. As shown in Figure 2*a*, we found that DHT at 1 and 10 nM, suppressed COX-2 gene expression, and 1 nM TPA, an NF-κB inducer, promoted COX-2 gene expression. However, this suppression of COX-2 gene expression was lost in the # 3 COX-2 reporter construct in which the NF-κB binding site was deleted (Fig. 2*b*), suggesting that DHT/AR transcriptionally suppresses COX-2 gene expression through regulating the NF-κB signal pathway.

*CAB with HF therapy stimulates COX-2 gene activity in prostate cancer LNCaP cells*

The above data suggested that DHT/AR could suppress COX-2 gene expression at the transcriptional level; therefore blockage of androgens by CAB therapy might then release the suppression of COX-2 gene expression in prostate cancer cells and result in elevated COX-2 expression. According to the report by Dr. Mohler the mean total prostate DHT concentration before *vs*. after CAB

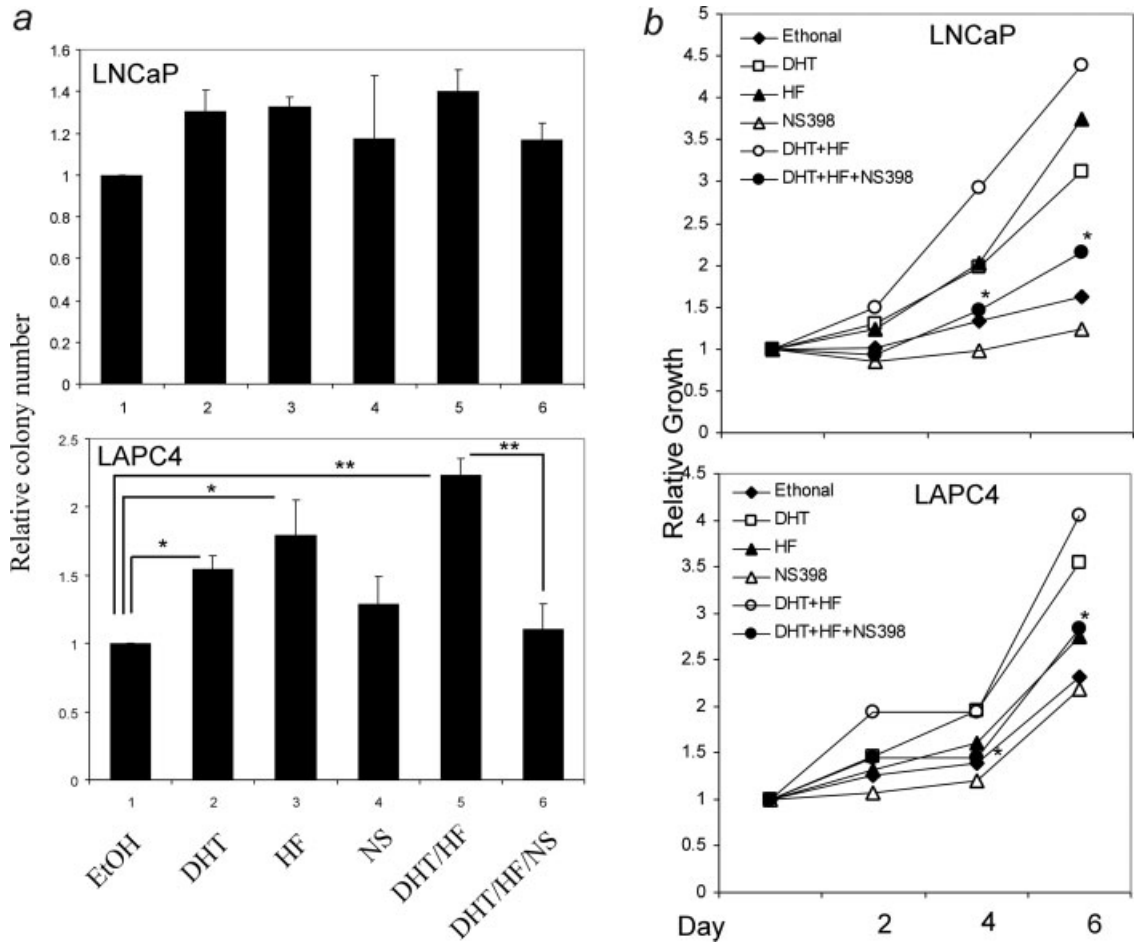


**FIGURE 6** – NS398 suppresses DHT + HF-induced prostate cancer cells growth. (*a*) LNCaP and LAPC4 cells were treated with 1 nM DHT, 1 μM HF, 25 μM NS398, and in various combinations for 24 hr, then cells were trypsinized, counted, and seeded at 500 cells/dish in 60-mm tissue culture dish. Cells were fed with fresh growth media every 4–5 days for 2–3 weeks until the colonies were well formed. Crystal violet stain was used to visualize the colonies. (*b*) $5 \times 10^4$ LNCaP cells and LAPC4 were seeded onto 12-well plates for 24 hr in cultured medium. Cells were then changed to culture medium with 10% CD FBS that contained ethanol, 1 nM DHT or 1 μM HF, or the combination of 1 nM DHT and 1 μM HF with 25 μM NS398. Cells were harvested every 2 days to determine cell proliferation rate by MTT assay. **, $p > 0.01$; *, $p > 0.05$ statistics were examined by student *t* test.

therapy are 2–10 nM DHT *vs*. 1-3 nM DHT, respectively.[27] To mimic the clinical CAB therapy scenario, we treated LNCaP cells with 1 nM DHT and 1 μM HF, and found that COX-2 gene expressions were increased at both mRNA (Fig. 3*a*) and protein levels (Fig. 3*b*). Similarly, the COX-2 5′ promoter activity was also stimulated by the combination of DHT and HF (Fig. 3*c*). We also found that 1 μM HF alone and in combination with DHT induced COX-2 expression, while DHT alone suppressed COX-2 expression suggesting that HF might not only induce COX-2 expression *via* antagonism of androgen/AR-suppressive COX-2 effect, but also *via* androgen/AR-independent pathways.

COX-2 is a key enzyme involved in the production of prostaglandins (PG), which requires the condensation of COX-2 into the nuclear envelope.[28] To test if increased COX-2 gene expression after CAB with HF therapy could also result in increased functional COX-2 within the nucleus, we performed immunofluorescence staining of COX-2 in LNCaP cells. As shown in Figure 4, COX-2 proteins were mainly located in the nucleus when cells were treated with 1 μM HF or vehicle control. In contrast, COX-2 proteins were retained in the cytoplasm when cells were treated with 1 nM DHT, the androgen concentration after CAB therapy, and HF+DHT treatment can reverse the DHT-induced COX-2 cytoplasmic retention where COX-2 was located in the nucleus and its expression was restored to the level similar to vehicle-treated cells. Together, these data clearly demonstrate that CAB with HF therapy would increase COX-2 gene expression as well as promote its nuclear translocation, thus resulting in increasing functional nuclear COX-2 protein in prostate cancer cells.

### *Treatment with CAB and HF therapy results in the increased COX-2 expression in prostate cancer patients*

To further confirm the above *in vitro* findings, we examined COX-2 protein expression in clinical specimens from prostate cancer patients before and after CAB therapy. Three pairs of prostate tumor specimens obtained from patients before and after CAB therapy were subjected to the COX-2 immunostaining. As shown in Figure 5, very weak COX-2 expression was detected mainly in the cytoplasm of luminal epithelial cells in all 3 specimens before androgen ablation therapy. In contrast, an enhanced and strong COX-2 expression was detected in both the cytoplasm and nuclei of epithelial cells in all specimens from the same patients after CAB therapy. Therefore, these observations from clinical specimens support our above findings in prostate cancer cell lines.

### *A new potential therapy* via *combination of CAB with HF therapy and anti-COX-2 inhibitor*

Our studies in both *in vitro* prostate cancer cells and *in vivo* clinical data suggested that CAB with HF therapy, the currently

used therapy to treat prostate cancer, might result in the increased COX-2 expression in the prostate tumor. COX-2 has been implicated to play important roles in the cancer progression and invasion,[29,30] therefore, we were interested to know the cellular response to elevated COX-2 expression. Colony formation assays were used to examine the cell response to treatments in LNCaP and LAPC4 cells. As shown in Figure 6*a*, a treatment with 1 nM DHT alone or 1 μM HF alone, and a combination of both, mimicking prostate patients' serum level after CAB with HF therapy, promotes cell growth. Importantly, treatment with NS398, a COX-2 inhibitor, results in the significant suppression of DHT/HF-induced prostate cancer cell growth. As a control, cell growth was slightly inhibited when LNCaP cells were treated with NS398 alone. Note that LAPC4 cells, which contain wild type AR respond better to treatment than LNCaP cells, which contain mutated AR. To further confirm these results, we applied the MTT assay to examine LNCaP and LAPC4 cell growth upon the treatments. As shown in Figure 6*b*, DHT, HF, and DHT+HF can promote, and NS398 alone suppress, cell growth. The combination of DHT and HF promoted cell growth significantly, starting at Day 4, and adding NS398 reverses the DHT+HF-induced cell growth significantly. Together, the data from 2 AR positive prostate cancer cell lines suggested that the elevated COX-2 expression due to CAB with HF therapy stimulates cell growth, which might contribute to the failure of CAB therapy. And applying COX-2 inhibitor(s), such as NS398, might be able to reverse this adverse effect derived from CAB therapy.

## Discussion

Prostate cancer is one of the most commonly occurring cancers with a high mortality rate among American men. In patients with advanced prostate cancer, CAB therapy using currently available antiandrogens is a standard treatment option, but it almost always results in the emergence of androgen-independent disease and eventual mortality. The precise molecular events that lead from androgen-sensitive prostate cancer to androgen-refractory prostate cancer are, therefore, critical for decoding prostate cancer progression as well as developing specific therapies that can interfere with these pathways to stop disease progression.

It has been proposed that there are many pathways involved in the development of androgen-independent prostate cancer, *via* an AR-dependent or -independent pathway.[31–33] The mechanisms that involve the AR directly include mutations in, or amplification of, the AR gene in a manner that allows the AR to respond to low doses of androgens, other steroids, or antiandrogens.[34,35] In a less direct manner, AR coactivators might increase the sensitivity of the AR to androgens and even other nonandrogenic substances through a number of mechanisms.[34,36] Additional indirect mechanisms that do not result from mutations of the AR might involve activation of the AR by peptide growth factors or cytokines.[37,38] The AR-independent pathways that bypass AR involve neuroendocrine differentiation of prostate cancer cells,[39] deregulation of apoptotic genes,[40] and unknown mechanisms related to down-regulation of AR[41] or the HF activated MAP kinase pathway.[42]

COX-2 has been shown to be involved in prostate cancer, several other human cancers, and inflammatory diseases. Therefore, the potential use of NSAIDs, well known COX inhibitors, as chemopreventive or therapeutic agents for a variety of malignancies, including prostate cancer, is being intensely investigated. Potential mechanistic roles of COX-2 in tumorigenesis and tumor progression include decreased apoptosis and immune surveillance, increased angiogenesis, and tumor invasiveness.[29,43] There have been strong preclinical, epidemiologic, and clinical data supporting an association between NSAID use and a reduced incidence of and mortality from cancer.[44] Based on results from laboratory and clinical studies, it has been suggested that inhibition of COX-2 might be a useful chemopreventive/therapeutic option for prostate cancer.[45] Our studies demonstrated that androgen/AR suppressed, and that 1 μM HF plus 1 nM DHT, which mimics the clinical CAB therapy condition, enhanced COX-2 expression. These results strongly suggest that CAB therapy might induce COX-2 expression, an unwanted CAB side effect, which might then lead to the transition of androgen-independent disease, as well as cancer progression. Our *in vitro* proliferation assay further confirmed that cotreatment with COX-2 inhibitor NS398 significantly suppressed 1 μM HF/1 nM DHT stimulated LNCaP cell growth. Therefore, a combination of a specific COX-2 inhibitor and CAB therapy, which might delay progression of prostate cancer from an androgen-dependent to an androgen-independent stage, could be a useful therapeutic strategy in prostate cancer.

The clinical use of COX-2 inhibitors has recently become controversial because of the cardiovascular complications associated with the use of high doses of COX-2-selective NSAIDs for prolonged periods of time.[46] In comparison with the COX-2-selective inhibitors, the use of a nonselective NSAID, such as naproxen, has been shown to be associated with decreased cardiovascular adverse effects.[47] As shown by our study, a growth inhibition was seen with a COX-2 inhibitor while cells were stimulated by CAB therapy; therefore, it is worthy to apply the clinical utility of a nonselective NSAID, such as naproxen, to eliminate the cancer cell growth induced by CAB therapy, thereby restraining prostate cancer progression.

In conclusion, androgen and its receptor play an inhibitory role in COX-2 expression in prostate cancer cells. COX-2 overexpression in prostate cancer is due to androgen ablation, and might result in the failure of CAB therapy. Our findings suggest that cotreatment with COX-2 inhibitor(s) could diminish prostate cancer progression induced by androgen ablation therapy.

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