

Curcumin Blocks the Activation of Androgen and Interlukin-6 on Prostate-Specific Antigen Expression in Human Prostatic Carcinoma Cells

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ABSTRACT: Curcumin, a naturally occurring compound, exhibits anticancer chemopreventive effects. We evaluated the effects and mechanisms of curcumin on the gene expression of prostate-specific antigen (PSA) in human androgen-sensitive prostatic carcinoma cells. LNCaP cells were used to determine the effect of curcumin on PSA expression. Quantitative PSA expression was assessed by reverse transcription polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), and immunoblot assay. The modulation of androgen, interleukin-6 (IL-6), and prostate-derived Ets factor (PDEF) on the PSA gene was identified by transient gene expression assay with the use of a PSA reporter vector. The effect of curcumin on the activity of androgen receptors was evaluated by electrophoretic mobility shift assay (EMSA). Immunoblot assays, RT-PCR, and ELISA indicated that curcumin treatments blocked the

stimulation of methyltrienolone (R1881) and IL-6 on PSA gene expression in LNCaP cells. The effects of curcumin appear to be mediated via the androgen response element of PSA gene. Results from immunoblot assay and EMSA revealed the modulation of curcumin on the expression of androgen receptor and androgen receptor binding activity on androgen response element of PSA gene. Although overexpression of PDEF dramatically enhanced PSA gene expression, the results of immunoblot assays and transient reporter assays indicated that curcumin treatments did not affect the gene expression of PDEF. Curcumin inhibits R1881- and IL-6-mediated PSA gene expression in LNCaP cells through down-regulation of the expression and activity of androgen receptors.

Key words: IL-6, androgen receptor, PSA, PDEF, R1881.

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Prostate cancer is the most prevalent cancer and the second leading cause of cancer deaths in American men (Ahmedin et al, 2006). The prostate-specific antigen (PSA), a 30- to 33-kDa glycoprotein expressed in all stages of prostate cancer and primarily regulated by androgen, is produced almost exclusively by the luminal epithelial cells of the human prostate. Furthermore, serum PSA is a well-known biomarker for diagnosing and evaluating the status of prostate cancer (Edwards and Bartlett, 2005).

Curcumin (diferuloylmethane), a yellow curry pigment obtained from the plant *Curcuma longa* Linnaeus, has been well documented as a potent anti-inflammatory, anticarcinogenic, and antioxidant agent (Bemis et al,

2006). The evidence from epidemiological and preclinical perspectives has been carried out with respect to the effects of curcumin in cancer chemoprevention (Thomasset et al, 2007). Serious studies have revealed the therapeutic potential of curcumin in androgen-dependent and androgen-independent prostatic carcinoma cells (Dorai et al, 2000, 2001, 2004). Studies suggested that curcumin, which acts as the inhibitor of nuclear factor kappa B (NF- κ B) and nuclear transcription factor activator protein-1 (AP-1), down-regulated cell survival mechanisms in human prostate cancer in vitro (Chen and Tan, 1998; Mukhopadhyay et al, 2001; Deeb et al, 2004). Other studies indicated that curcumin and curcumin analogs down-regulated the gene expression of androgen receptors in androgen-sensitive prostate carcinoma LNCaP cells (Nakamura et al, 2002; Ohtsu et al, 2002).

Several in vitro studies have revealed factors including androgen, interleukin-6 (IL-6), and adriamycin that modulated PSA gene expression (Jia et al, 2004; Tsui et al, 2004a,b). Some transcriptional factors such as the androgen receptor (AR), p53, NF- κ B, prostate-derived Ets factor (PDEF), and Sp family genes are involved in the gene modulation of PSA in LNCaP cells, and most of the transcriptional factor interacted with androgen receptor in the process of PSA promoter regulation

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(Oettgen et al, 2000; Chen and Sawyers, 2002; Tsui et al, 2004a).

Our objectives for this study were to determine the regulatory mechanisms of curcumin on gene expression of PSA with or without the stimulation of androgen. We also evaluated the mechanisms of curcumin on the gene expression of PSA by interacting with other transcriptional factors other than the androgen receptor in the human prostatic carcinoma LNCaP cells.

Materials and Methods

Materials, Cell Lines, and Cell Culture

LNCaP cells were obtained and maintained as described before (Hsieh and Juang, 2005). Curcumin and charcoal (dextran-coated) were purchased from Sigma (St Louis, Missouri) and the bicinchoninic acid (BCA) protein concentration assay kit was purchased from PIERCE (Rockford, Illinois). Recombinant human IL-6 was purchased from Cytolab (Rebovot, Israel), and methyltrienolone (R1881) was purchased from NEN Life Science (Boston, Massachusetts). Fetal calf serum (FCS) was purchased from HyClone (Logan, Utah), and RPMI 1640 and RPMI 1640 phenol red-free (RPMI-PRF) media were purchased from Life Technologies (Rockville, Maryland). The FCS was charcoal-dextran-treated (CD-FCS) to remove steroids as described before (Juang et al, 2004).

Cell Proliferation Assay

Cell proliferation in response to curcumin was measured with a nonradioactive assay as described before (Hsieh and Juang, 2006). Cells (5000 cells/well) were grown in 100 mL of RPMI-PRF medium with 5% CD-FCS for 2 days. Cells were incubated with 100 mL of 0, 5, 10, 20, 40, and 80 μ M curcumin in the same medium for 24, 48, and 72 h. Cells were then incubated with freshly prepared 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS)/phenazine methosulfate (ratio 1:1 by volume) solution (Promega Biosciences, San Luis Obispo, California) for 3 hours at 37°C in a humidified 5% CO₂ atmosphere.

Immunoblot Assay

LNCaP cells were incubated in the RPMI-PRF medium with 5% CD-FCS and different drugs for a period of 24 hours. Equal quantities of cell extract (40 mg) were loaded onto a 12% sodium dodecyl sulfate polyacrylamide gel and analyzed by the electrochemiluminescent detection system as described before (Tsui et al, 2006). The rabbit anti-human PDEF serum was prepared from our laboratory. The pcDNA3-PDEF (see below) was digested with *EcoRI* and *NotI*, and the linearized plasmid DNA was ligated with the pET32a (Novogen, La Jolla, California). The fusion protein, after lysis of the bacterial pellet in IB buffer (20 mM Tris-HCl, 10 mM EDTA, 1% Triton X-100, and 100 μ g/mL lysozyme), was purified through Ni-NTA beads (Qiagen, Hilden, Germany) and eluted following the manufacturer's instruction. Rabbits (New Zea-

Table. Primers used in this study for RT-PCR

Name	Sequence	PCR size, bp
β -actin-P	5'-GAAGATCAAGATCATTGCTCCTCC-3'	720
β -actin-R	5'-GAAGATCAAGATCATTGCTCCTCC-3'	
AR-P	5'-CTGGCTCCGCAACTTACAC-3'	405
AR-R	5'-TGGTAGAAGCGTCTTGAGCA-3'	
PDEF-P	5'-TGACCTTGGGCTCTGGAAGGTCAG-3'	500
PDEF-R	5'-GACCAGTGAGGAGAGCTGGACCGA-3'	

land White) were immunized with the PDEF-His-Tag fusion protein (57 kDa). The fusion protein was emulsified with an equal volume of complete Freund adjuvant for the first injection, and with incomplete Freund adjuvant for 3 subsequent booster injections. The results of immunoblot assay for the whole-cell extract of LNCaP cells revealed 1 major band at 37 kDa and 1 weak band at 50 kDa, which represented the glycosylation form of PDEF from this rabbit polyclonal antibody raised against the human PDEF. The blotting membranes were probed with 1:200 diluted polyclonal PSA antiserum (DAKO), 1:500 diluted human androgen receptor antiserum (N-19; Santa Cruz Biotechnology, Santa Cruz, California), 1:1000 diluted β -actin antiserum (C11, Santa Cruz Biotechnology), or 1:5000 dilute PDEF antiserum. The intensity of different bands were analyzed by GeneTools of ChemiGenius (Syngene, Cambridge, United Kingdom).

Reverse Transcription Polymerase Chain Reaction

The total RNA was isolated with Trizol reagent, and cDNA was synthesized as described before (Hsieh and Juang, 2006). The primers were used for the amplification of sequences specific to the androgen receptor (Juang et al, 2004) and PDEF as shown in the Table. The cDNA quality was verified by performing controlled reactions with primers derived from β -actin-P and β -actin-R (Table). The polymerase chain reaction (PCR) was carried out in a thermal cycler (Thermolyne, Dubuque, Iowa), and the parameters were 30 cycles of 94°C for 0.5 minutes, 55°C for 1 minute, and 72°C for 1 minute. The PCR products were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining under the image capture and analysis system of ChemiGenius (Syngene).

Reporter Vector Constructs

The promoter of the PSA gene was isolated from the PAC clone (LLNLF-214C7; Human Genome Mapping Project Resource Centre, Hinxton, Cambridge, United Kingdom). The PAC clone was digested with *HindIII*, and one 6-kbp DNA fragment was subcloned into the pGL3 reporter vector (Promega Bioscience). The reporter vectors (pPSAH, -41 to -5874; pPSABHE, -4801 to -3933 and -41 to -589; pPSAKH, -41 to -1557; pPSABH, -41 to -589) containing the 5' flanking region of the human PSA gene were cloned by 5' deletion or PCR as described before (Tsui et al, 2004b). The promoter of the PDEF gene was isolated from the BAC clone (RP11-375E1; Invitrogen, Carlsbad, California). The BAC clone was digested with *SacI*, and one 3.7-kbp DNA fragment was subcloned into the pGEM11 vector (pGEM11-PDEF;

Promega Bioscience). The human PDEF promoter/enhancer DNA fragment was synthesized by PCR with 2 primers (T7 and PDEFpromR 5'-AAGCTTAGCCGCGAGATGAA-GAGTTGGC-3') from the pGEM11-PDEF vector. The DNA fragment (-1 to -3280), which was digested with *HindIII* and *SacI*, was ligated with the reporter vector pbGL3 (Promega Bioscience). Proper ligation and orientation were confirmed by extensive restriction mapping and sequencing.

Expression Vector Constructs and Transient Overexpression

The full-length human PDEF cDNA vector (MGC: 4546840) was purchased from Invitrogen. A DNA fragment containing the coding region of human PDEF cDNA was synthesized by reverse transcription (RT)-PCR with 2 primers (PDEFp, 5'-GAATTCATGGGCAGCGCCAGCCCCGGGTC-3', and PDEFr, 5'-GCGGCCGCTCAGATGGGGTGCACGAAC-TGG-3') from the PDEF cDNA vector. The overexpression vector, pcDNA3 (Invitrogen), was digested with *EcoRI* and *NotI*, and the linearized plasmid DNA was ligated with the human PDEF cDNA fragment after digestion with *EcoRI* and *NotI*, resulting in the insertion of the full-length PDEF cDNA into the polyadenylate region that was controlled by the CMV promoter (pcDNA3-PDEF). Proper ligation and orientation were confirmed by extensive restriction mapping and sequencing. The expression vector was introduced into LNCaP cells by electroporation. Ten million cells were suspended in 20 μ L of pcDNA3-PDEF and pcDNA3, respectively, and in 500 μ L of RPMI medium without serum in a 4-mm gap cuvette. Electroporation was conducted with the ECM 830 (BTX, San Diego, California) set at 170 V with a 70-ms pulse length and with the use of a single pulse setting. Cells were maintained in RPMI medium with 10% FCS for 3 days and then in RPMI-PRF medium with 5% CD-FCS for another 2 days before further experiments.

Transient Transfections and Reporter Assay

LNCaP cells (1×10^4 cells/well) were plated onto 24-well plates 1 day before transfection. Cells were transiently transfected with TransFast transfection reagent as described before (Tsui et al, 2006). To eliminate the variable degrees of efficiency of reporter activity, cells were transfected with same amount of plasmid in each well by adding the same concentration of pcDNA3 vector. Reporter vector-transfected LNCaP cells were then treated with different drugs in RPMI-PRF medium with 5% CD-FCS as indicated for an additional 24 hours.

Enzyme-Linked Immunosorbent Assay

LNCaP cells were incubated with 1 mL of different concentrations of drugs as indicated in RPMI-PRF medium with 5% CD-FCS in a 6-well plate (2×10^5 cells/well) for a period of 24 hours. After incubation, the culture media from each well were collected for PSA assays. Cell pellets were washed twice with ice-cold phosphate-buffered saline (PBS) and then dissolved in 500 μ L of PBS. After sonication for 10 seconds, the cell extracts were centrifuged at $23\,000 \times g$ for 20 minutes. The PSA levels in 20 mL of the cell supernatant or culture

media were measured by PSA enzyme-linked immunosorbent assay (ELISA), as described previously (Tsui et al, 2004a). The PSA level in each sample was adjusted by the concentrations of protein in the whole-cell extract, which was measured by a BCA protein assay kit.

Electrophoretic Mobility Shift Assay

An electrophoretic mobility-shift assay (EMSA) was performed as previously described (Tsui et al, 2008). The double strands of DNA fragment containing the putative androgen response element (ARE) of the PSA gene (5'-TGCAGAA-CAGCAAGTGCTAGC-3') was 5' end-labeled with γ - P^{32} -adenosine triphosphate with the use of T4 polynucleotide kinase. The nuclear material of LNCaP cells after treatment with different concentrations of curcumin for 12 hours was extracted with the NE-PER nuclear and cytoplasmic extraction reagents as described by the manufacturer (PIERCE). The 5' end-labeled ARE (ARE probe; 5 nM) was incubated with 2 μ L of nuclear extract (NE) from LNCaP cells in 20 μ L of binding buffer (25 mM HEPES buffer, pH 7.9, 50 mM KCl, 0.05 mM EDTA, 0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, and 10% glycerol) containing 0.5 μ L of poly(dI-dC)-poly(dI-dC). Protein-DNA complex formation was analyzed in 4% polyacrylamide gel by autoradiography.

Statistical Analysis

Results are expressed as $\bar{x} \pm SE$ of at least 3 independent replications of each experiment. Statistical significance was determined by a paired *t* test analysis with the SigmaStat program for Windows version 2.03 (SPSS Inc, Chicago, Illinois).

Results

To determine that the block effect of curcumin on PSA expression was not due to cytotoxic effect, cell proliferation in the experiments compared with the proliferation rates of cells in response to curcumin treatment was measured by MTS assay. Results indicated cell viability decreased less than 10% when cells were treated with 20 μ M or more of curcumin within 24 to 72 hours (Figure 1A). The results from immunoblot assays revealed that the up-regulation of PSA protein amount in R1881 treatment was significantly attenuated when LNCaP cells were treated with different concentrations of curcumin (Figure 1B). Moreover, curcumin also blocked AR and PSA gene expression without the stimulation of androgen (Figure 1B). When LNCaP cells were treated with or without 1 nM R1881 and curcumin (0–20 μ M) in RPMI-PRF medium with 5% CD-FCS for 24 hours, the results from ELISA revealed that R1881 (1 nM) treatment increased about 2.5-fold of the intracellular PSA level and about 10-fold of the PSA secretion; however, curcumin (20 μ M) completely blocked the stimulation of R1881 treatment. The

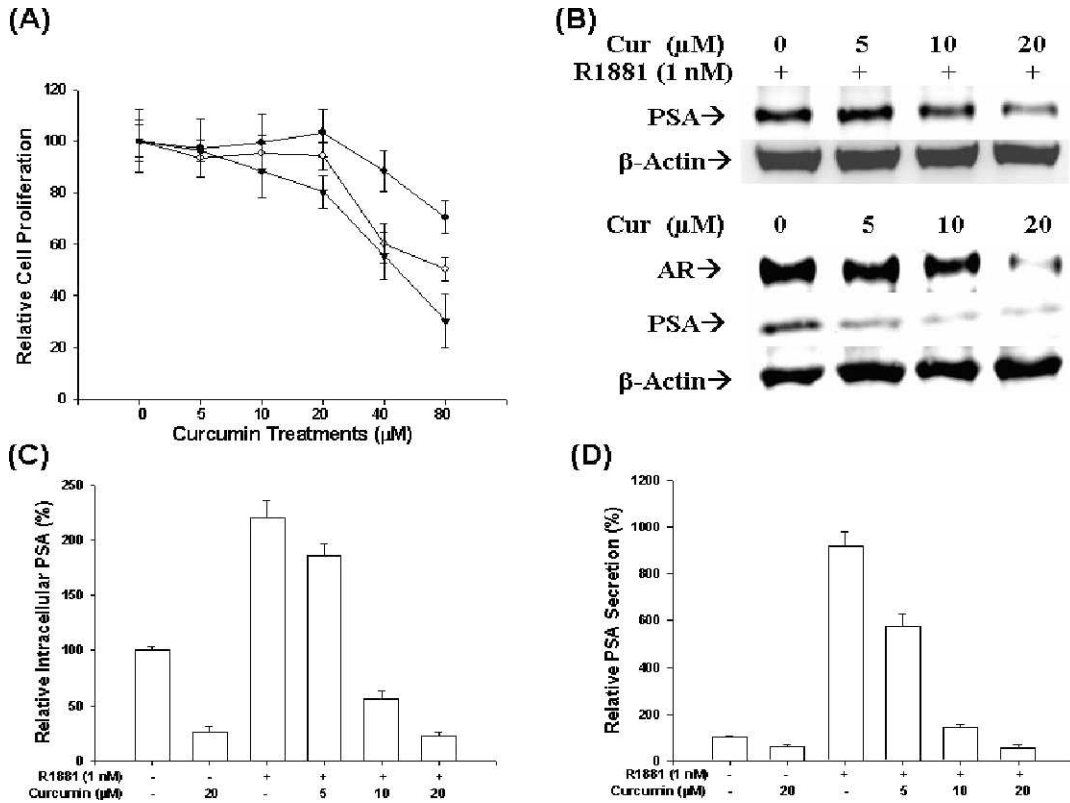


Figure 1. Modulation of prostate-specific antigen (PSA) expression of LNCaP cells after curcumin and androgen treatments. **(A)** Cell proliferation of LNCaP cells after various curcumin treatments, as indicated for 24 (black circle), 48 (white circle), and 72 hours (black triangle), were determined by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Each point on the curve represents the $\bar{x} \pm SE$ (%; $n = 8$) relative to the control group without curcumin treatment. **(B)** LNCaP cells were treated with **(top)** or without **(bottom)** 1 nM R1881 and different concentrations of curcumin (Cur), as indicated for 24 hours. Cells were harvested and lysed to extract protein for the immunoblot assay. LNCaP cells were treated with 1 nM R1881 and different concentrations of curcumin, as indicated for 24 hours. The cells **(C)** and media **(D)** were collected for PSA assays with enzyme-linked immunosorbent assay. Data are expressed as $\bar{x} \pm SE$ (% stimulation; $n = 6$) of PSA levels induced by the different treatments relative to the control treatment.

curcumin (20 µM) treatment also significantly down-regulated the intracellular PSA level and PSA secretion, even when cells were without the stimulation of androgen (Figure 1C and D).

Transient gene expression assay with the use of the PSA reporter vector (pPSABHE), which contained the ARE DNA fragment, indicated that 1 nM R1881 enhanced about 40-fold of the reporter activity; however, 20 µM curcumin treatment significantly blocked the stimulation of the androgen (Figure 2A). Results from 5' deletion reporter assays indicated that the effects of curcumin appeared to be mediated via a region located at -4801 to -3911 bp, a region containing the ARE, upstream of the translational starting point of the PSA gene. The EMSA assay indicated that curcumin treatments blocked the effect of androgen on the gene expression of PSA by decreasing the binding activity of the androgen receptor on the ARE of the PSA gene (Figure 2B).

The reporter assay revealed that curcumin treatments significantly ($P < 0.05$) blocked the reporter activity of the PSA reporter vector in a dosage-dependent way when cells

were treated with curcumin in RPMI-PRF medium with 5% CD-FCS (Figure 2C). These results are similar to those in Figure 1B, which indicated that curcumin could affect PSA gene expression in the androgen bypass condition. Because curcumin has been regarded as the antagonist of NF-κB, our study also determined the effect of NF-κB on the gene expression of PSA. Our strategy was to use an inhibitor of the tumor necrosis factor alpha-induced transcription factor NF-κB (IKκB) overexpression vector to block NF-κB nuclear translocation in the cells. When LNCaP cells were cotransiently transfected with the inhibitor of kappa B (IkB) overexpression vectors and PSA reporter vector (pPSABHE), which contains both the putative ARE and the NF-κB response element, the reporter assay indicated that overexpression of the NF-κB inhibitor alpha (IkBα) vector decreased the promoter activities in a dose-dependent manner. Moreover, adding 20 µM curcumin further inhibited the promoter activities of PSA reporter vector (Figure 2D).

Transient gene expression assays revealed that forced overexpression of PDEF significantly enhanced the

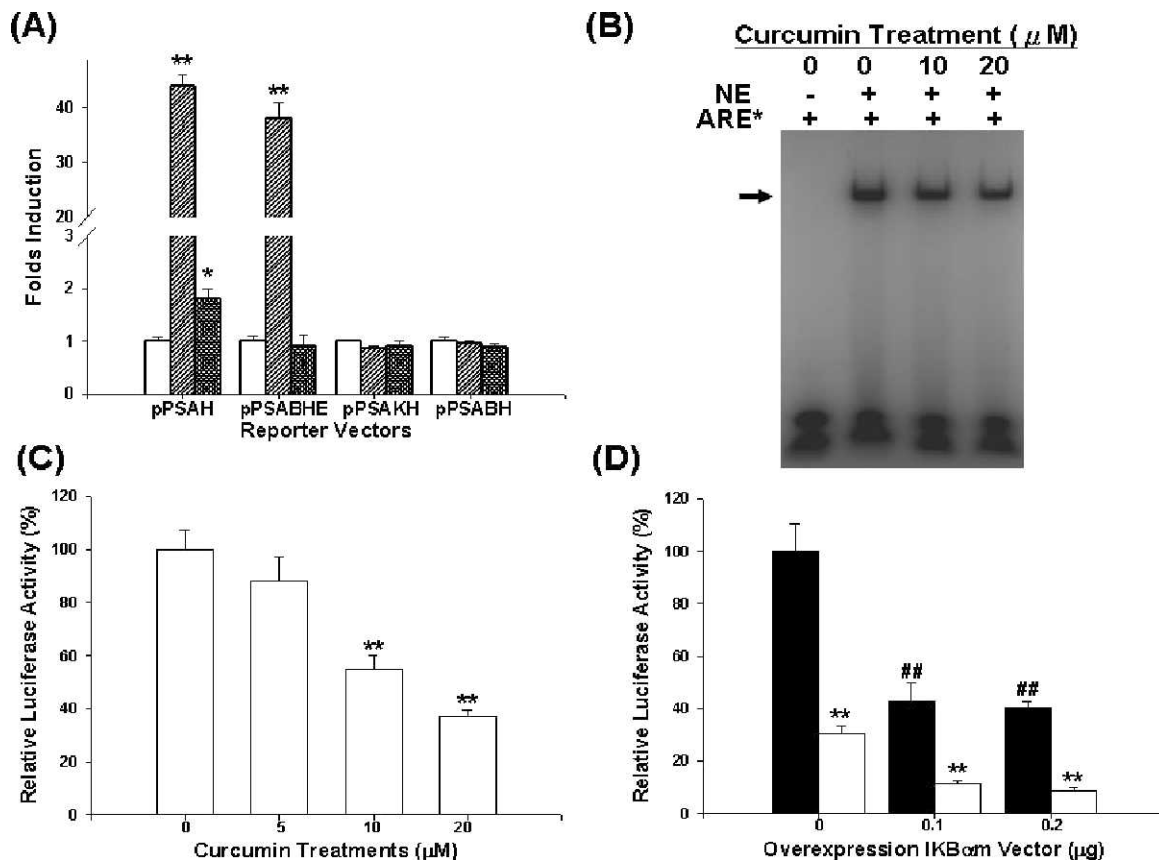


Figure 2. Modulation of curcumin on prostate-specific antigen (PSA) expression is dependent on the androgen response element of the PSA gene in LNCaP cells. **(A)** PSA reporter vectors of transfected LNCaP cells were given a mock treatment (white bars) or treated with 1 nM R1881 (gray bars) or R1881 plus 20 μ M curcumin (black bars). Data are expressed as \bar{x} -fold \pm SE of 6 preparations of luciferase activity in R1881 or curcumin treatments relative to mock-treated groups. **(B)** Activities of androgen receptor after curcumin treatments were determined by electrophoretic mobility shift assay (arrow indicates the binding shift). **(C)** PSA reporter vector (pPSABHE)-transfected LNCaP cells were treated with different concentrations of curcumin. **(D)** LNCaP cells were cotransiently transfected with PSA reporter vector (pPSABHE) and different concentrations of $\text{I}\kappa\text{B}$ overexpression vectors as indicated. White boxes represent the curcumin-treated group, and black boxes represent the control mock-treatment groups. Data are expressed as $\bar{x} \pm \text{SE}$ (%) of 6 preparations of luciferase activity of the $\text{I}\kappa\text{B}$ overexpression group relative to the mock-transfected group (### $P < .01$) and the curcumin-treated group relative to the mock-treated group (** $P < .01$). NE indicates nuclear extract; *, $P < .05$; **, $P < .01$; ###, $P < .01$.

promoter activity with the use of a PSA reporter vector (pPSABHE) in LNCaP cells (Figure 3A). The results of RT-PCR and immunoblot assays indicated that transiently overexpressed PDEF induced PSA gene expression (Figure 3B). Results from ELISA revealed that transiently overexpressed PDEF significantly up-regulated both intracellular PSA levels and PSA secretion (Figure 3C). The results of immunoblot assays indicated that curcumin itself did not block gene expression of PDEF; moreover, the reporter assay with a PDEF reporter vector also confirmed that curcumin treatments did not affect the gene expression of PDEF in LNCaP cells (Figure 3D).

Results from RT-PCR revealed that curcumin blocked the stimulation of 20 ng/mL of IL-6 treatment on the expression of the AR gene; moreover, results from the immunoblot assay also revealed that curcumin treatment blocked the stimulation of IL-6 on AR and PSA gene expression (Figure 4A). Results from ELISA indicated that

when LNCaP cells were treated with 20 ng/mL of IL-6, the intracellular PSA levels increased about 2-fold; however, curcumin treatment (20 μ M) blocked PSA biosynthesis by stimulation of IL-6 in LNCaP cells (Figure 4B). The transient gene expression assay also showed similar results, demonstrating that curcumin treatment blocked up-regulation of PSA promoter activity by IL-6 (Figure 4C).

Discussion

Epidemiological, experimental, and preclinical studies have provided evidence that several dietary substances, including curcumin, that were recommended as chemopreventive agents for prostate cancer are effective (Bemis et al, 2006; Thomasset et al, 2007). Recent studies have indicated that curcumin treatment resulted not only in a significant reduction of invasive ability in

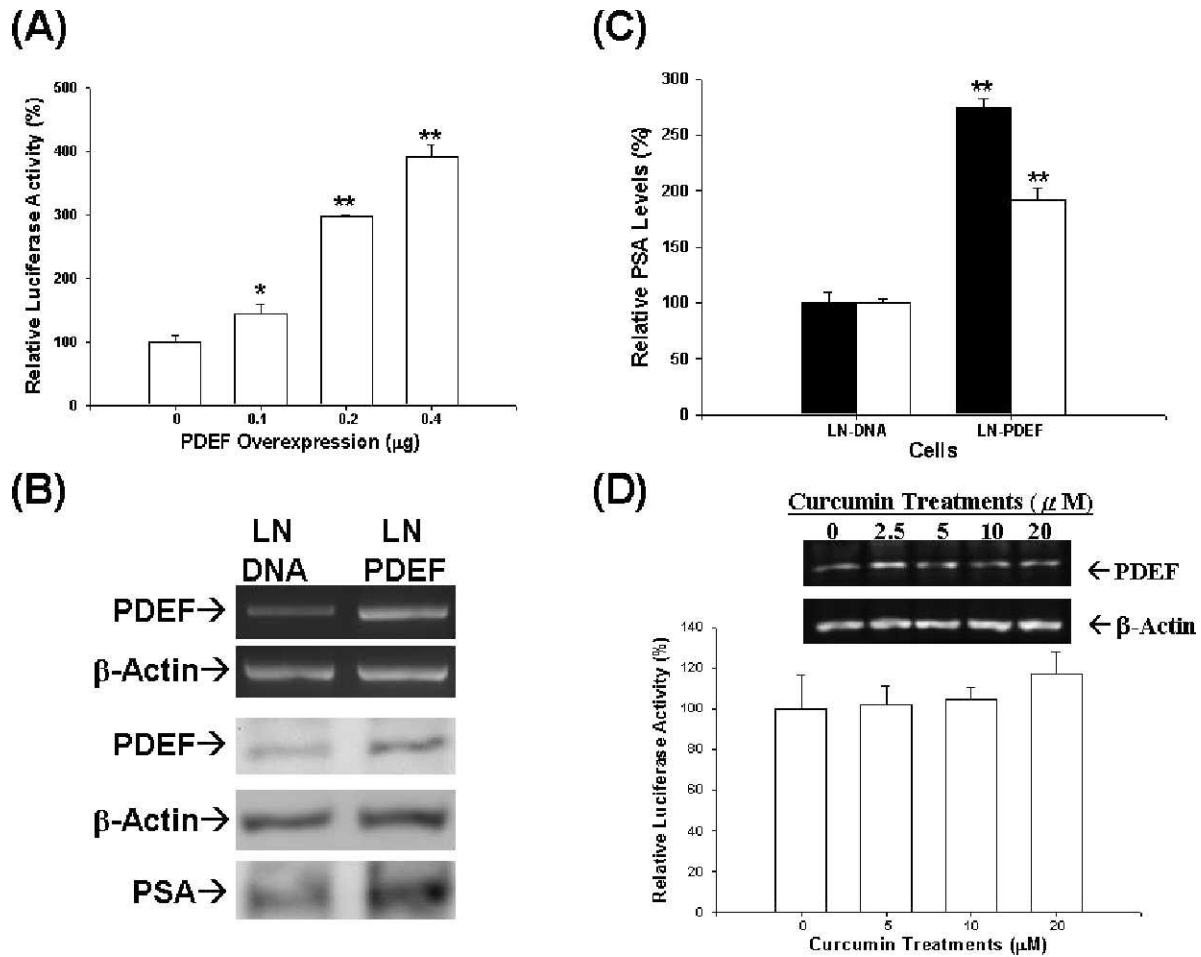


Figure 3. Modulation of curcumin on prostate-specific antigen (PSA) gene expression is independent on the prostate-derived Ets factor (PDEF) signal pathway. **(A)** PSA reporter vector (pPSABHE)-transfected LNCaP cells were cotransiently transfected with different concentrations of the PDEF overexpression vector as indicated. **(B)** The mock-transfected (LN-DNA) and PDEF transiently transfected (LN-PDEF) cells were subsequently cultured in RPMI-phenol red-free (PRF) medium with 5% charcoal-dextran-treated fetal calf serum (CD-FCS) for 48 h. Cells were harvested and lysed to extract RNA and protein for the reverse transcription polymerase chain reaction (**top**) and immunoblot assays (**bottom**). **(C)** Quantitative intracellular PSA levels (white boxes) and PSA secretion (black boxes) of LNCaP cells, after transient overexpression of PDEF, were determined by enzyme-linked immunosorbent assay. Data are expressed as $\bar{x} \pm SE$ (%) of 6 preparations of PSA levels of PDEF-transfected (LN-PDEF) cells in relative to mock-transfected (LN-DNA) cells. **(D)** LNCaP cells were treated with different concentrations of curcumin as indicated in the RPMI-PRF medium with 5% CD-FCS for 24 hours. Cells were harvested and lysed to extract protein for the immunoblot assays (**top**). LNCaP cells were transiently transfected with the PDEF reporter vector. Promoter activity was determined after treatment with different concentrations of curcumin, as indicated in RPMI-PRF medium with 5% CD-FCS for 24 hours (**bottom**). Data are expressed as $\bar{x} \pm SE$ (%) stimulation of 6 preparations relative to the control treatment. * indicates $P < .05$; **, .01.

vitro but also tumor metastasis in vivo of prostate carcinoma cells (Hong et al, 2006; Khor et al, 2006; Kim et al, 2006; Lin et al, 2006). Besides antitumor characteristics, the predominant function of curcumin was found to be antiandrogenic activity (Nakamura et al, 2002; Ohtsu et al, 2002; Lin et al, 2006). Our study revealed that curcumin treatments attenuated the gene expression of PSA in androgen stimulation and androgen-free conditions; however, the precise mechanism of curcumin on the regulation of PSA expression in the androgen bypass condition is still unclear.

Curcumin also was found to induce apoptosis in LNCaP cells through suppression of NF-κB activity

(Mukhopadhyay et al, 2001). Therefore, it was speculated that curcumin might block gene expression of PSA by suppressing NF-κB activity. Previous studies have indicated that NF-κB activates PSA expression in LNCaP cells and NF-κB binding sites found in the PSA core enhancer, which is also a region containing the ARE (Chen and Sawyers, 2002). In our study, we used the superrepressor IκBα (S32A/S36A), a dominant negative inhibitor of NF-κB, to abolish NF-κB activity, indicating that overexpression of IκBα inhibited PSA promoter activity in a dose-dependent way; however, even when NF-κB activity in the LNCaP cells was blocked by overexpression of IκBα, curcumin treatment

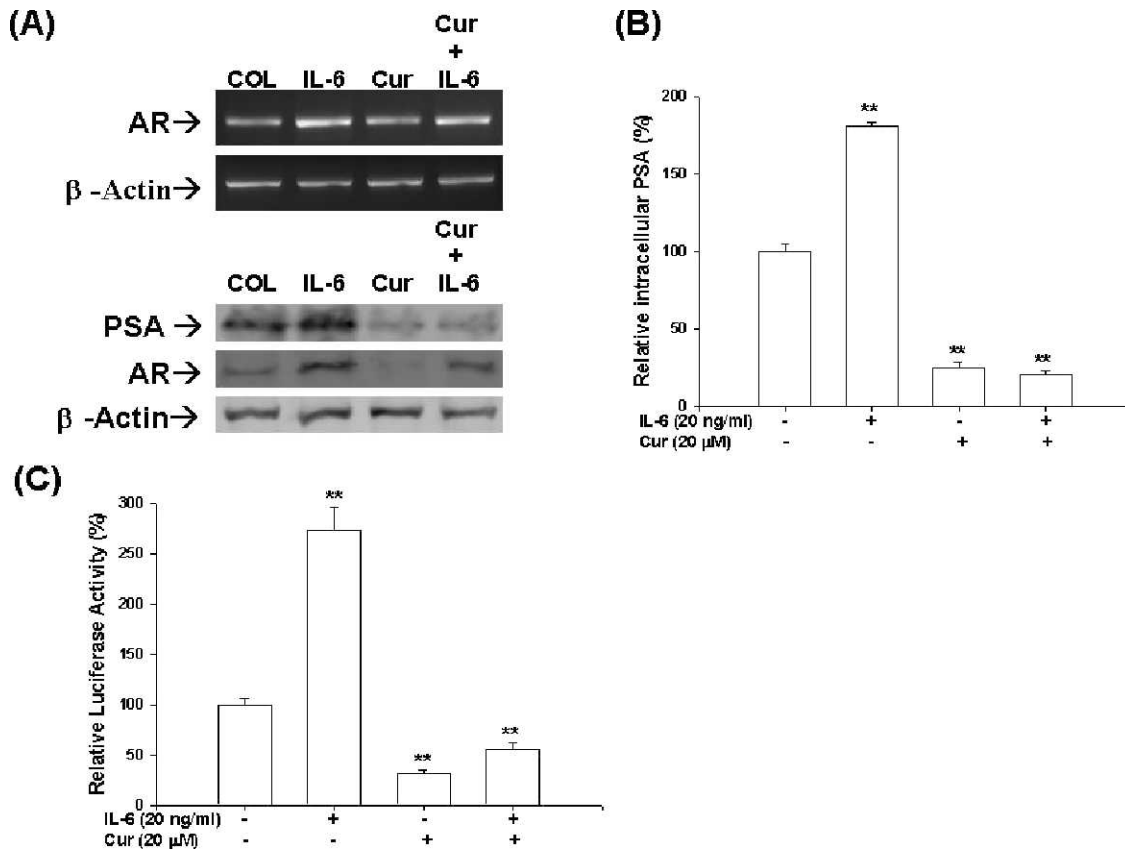


Figure 4. Curcumin blocks the stimulation of IL-6 on prostate-specific antigen (PSA) gene expression. **(A)** LNCaP cells were treated with 20 ng/mL of IL-6, 20 μ M curcumin in RPMI-phenol red-free (PRF) medium with 5% CD-FCS, or both for 24 hours. Cells were harvested and lysed to extract RNA for reverse transcription polymerase chain reaction (RT-PCR) or to extract protein for the immunoblot assays. Gene expression of the androgen receptor (AR) and β -actin was determined by RT-PCR (top), and the protein levels of PSA, AR, and β -actin were determined by immunoblot assay (bottom). **(B)** LNCaP cells were treated with 20 ng/mL of IL-6, 20 μ M curcumin, as indicated in RPMI-PRF medium with 5% charcoal-dextran-treated fetal calf serum (CD-FCS), or both for 24 hours. The cells were collected for PSA assays with enzyme-linked immunosorbent assay. Data are expressed as $\bar{x} \pm$ SE (%; $n = 6$) of PSA levels induced by the different treatments in relative to control mock-treatment. **(C)** LNCaP cells were transiently transfected with PSA reporter vector (pPSABHE) and then treated with 20 ng/mL of IL-6, 20 μ M curcumin as indicated in RPMI-PRF medium with 5% CD-FCS, or both for 24 hours. Data are expressed as the $\bar{x} \pm$ SE (%; $n = 6$) of luciferase activity induced by the different treatments relative to control mock treatment. COL indicates control mock treatment; Cur, curcumin treatment; ** $P < .01$.

still down-regulated the PSA promoter activity. Therefore, it seems that curcumin blocked PSA promoter activity through signal pathways more than NF- κ B.

PDEF, a novel prostate epithelium-specific Ets transcription factor, not only acts as an androgen-independent transcriptional activator of the PSA promoter but also directly interacts with the DNA binding domain of androgen receptors and enhances androgen-mediated activation of the PSA promoter (Oettgen et al, 2000). Recent studies also indicated that silibinin and tectorigenin treatments decreased PSA secretion in LNCaP cells through down-regulation of the gene expression of PDEF (Thelen et al, 2004, 2005). Our study revealed that overexpression of PDEF enhanced PSA gene expression at transcriptional and translational levels under androgen-free conditions. However, curcumin treatments blocked PSA gene expression in the androgen bypass condition, but did not inhibit PDEF gene expression.

Several lines of evidence suggested that high levels of circulating IL-6 correlate with advanced prostate cancer. IL-6 signaling can activate androgen receptors in a ligand-independent manner and might play an important role in hormone-refractory prostate cancer progression (Hobisch et al, 1998; Jia et al, 2004; Edwards and Bartlett, 2005). IL-6 increases PSA and androgen receptor expression through a STAT3-dependent pathway in the absence of androgen in LNCaP cells (Chen et al, 2000; Lin et al, 2001). Our results agreed with those of an earlier study that indicated that IL-6 induced expression of the androgen receptor, which up-regulated PSA promoter activity in the androgen-independent pathway (Hobisch et al, 1998). Moreover, curcumin blocked stimulation of IL-6 on the androgen receptor, which attenuated PSA gene expression in a ligand-independent manner.

With this study, we demonstrated that curcumin inhibits the gene expression and activity of the androgen

receptor that attenuates the stimulation of R1881- and IL6-mediated PSA gene expression in LNCaP cells. Overexpression of PDEF or IL-6 stimulated PSA gene expression in the androgen bypass pathways. Our results suggested that curcumin down-regulates PSA gene expression in androgen-dependent and androgen-independent pathways through blockage of gene expression of the androgen receptor but not of PDEF in LNCaP cells.

References

- Ahmedin J, Rebecca S, Elizabeth W, Taylor M, Jiaquan X, Carol S, Michael JT. Cancer statistics. *Calif Cancer J Clin*. 2006;56:106–130.
- Bemis DL, Katz AE, Buttyan R. Clinical trials of natural products as chemopreventive agents for prostate cancer. *Expert Opin Investig Drugs*. 2006;15:1191–1200.
- Chen CD, Sawyers CL. NF- κ B activates prostate-specific antigen expression and is upregulated in androgen-independent prostate cancer. *Mol Cell Biol*. 2002;22:2862–2870.
- Chen T, Wang LH, Farrar WL. Interleukin 6 activates androgen receptor-mediated gene expression through a signal transducer and activator of transcription 3-dependent pathway in LNCaP prostate cancer cells. *Cancer Res*. 2000;60:2132–2135.
- Chen Y-R, Tan T-H. Inhibition of the c-Jun N-terminal kinase (JNK) signaling pathway by curcumin. *Oncogene*. 1998;17:173–178.
- Deeb D, Jiang H, Gao X, Hafner MS, Wong H, Divine G, Chapman RA, Dulchavsky SA, Gautam SC. Curcumin sensitizes prostate cancer cells to tumor necrosis factor-related apoptosis-inducing ligand/Apo2L by inhibiting nuclear factor-kappaB through suppression of IkappaBalpha phosphorylation. *Mol Cancer Therap*. 2004;3:803–812.
- Dorai T, Cao YC, Dorai B, Buttyan R, Katz AE. Therapeutic potential of curcumin in human prostate cancer. III. Curcumin inhibits proliferation, induces apoptosis, and inhibits angiogenesis of LNCaP prostate cancer cells in vivo. *Prostate*. 2001;47:293–303.
- Dorai T, Dutche JP, Dempster DW, Wiernik PH. Therapeutic potential of curcumin in prostate cancer—V: interference with the osteomimetic properties of hormone refractory C4-2B prostate cancer cells. *Prostate*. 2004;60:1–17.
- Dorai T, Gehani N, Katz A. Therapeutic potential of curcumin in human prostate cancer. II. Curcumin inhibits tyrosine kinase activity of epidermal growth factor receptor and depletes the protein. *Mol Urol*. 2000;4:1–6.
- Edwards J, Bartlett JMS. The androgen receptor and signal-transduction pathways in hormone-refractory prostate cancer. Part 2: androgen-receptor cofactors and bypass pathways. *BJU Int*. 2005;95:1327–1335.
- Hobisch A, Eder IE, Putz T, Horninger W, Bartsch G, Klocker H, Culig Z. Interleukin-6 regulates prostate-specific protein expression in prostate carcinoma cells by activation of the androgen receptor. *Cancer Res*. 1998;58:4640–4645.
- Hong JH, Ahn KS, Bae E, Jeon SS, Choi HY. The effects of curcumin on the invasiveness of prostate cancer in vitro and in vivo. *Prostate Cancer Prostatic Dis*. 2006;9:147–152.
- Hsieh M-L, Juang HH. Cell growth effects of triiodothyronine and expression of thyroid hormone receptor in prostate carcinoma cells. *J Androl*. 2005;26:422–428.
- Jia L, Choong CS, Ricciardelli C, Kim J, Tilley WD, Coetzee GA. Androgen receptor signaling: mechanism of interleukin-6 inhibition. *Cancer Res*. 2004;64:2619–2626.
- Juang HH, Hsieh M-L, Tsui KH. Testosterone modulates mitochondrial aconitase in the full-length human androgen receptor-transfected PC-3 prostatic carcinoma cells. *J Mol Endocrinol*. 2004;33:121–132.
- Khor TO, Keum YS, Lin W, Kim JH, Hu R, Shen G, Xu C, Gopalakrishnan A, Reddy B, Zheng X, Conney AH, Kong AN. Combined inhibitory effects of curcumin and phenethyl isothiocyanate on the growth of human PC-3 prostate xenografts in immunodeficient mice. *Cancer Res*. 2006;66:613–621.
- Kim JH, Xu C, Keum YS, Reddy B, Conney A, Kong AT. Inhibition of EGFR signaling in human prostate cancer PC-3 cells by combination treatment with beta-phenylethyl isothiocyanate and curcumin. *Carcinogenesis*. 2006;27:475–482.
- Lin DL, Whitney MC, Yao Z, Keller ET. Interleukin-6 induces androgen responsiveness in prostate cancer cells through up-regulation of androgen receptor expression. *Clin Cancer Res*. 2001;7:1773–1781.
- Lin L, Shi Q, Nyarko AK, Bastow KF, Wu CC, Su CY, Shih CC, Lee KH. Antitumor agents 250. Design and synthesis of new curcumin analogues as potential anti-prostate cancer agents. *J Med Chem*. 2006;49:3963–3972.
- Mukhopadhyay A, Bueso-Ramos C, Chatterjee D, Pantazis P, Aggarwal BB. Curcumin downregulates cell survival mechanisms in human prostate cancer cell lines. *Oncogene*. 2001;20:7597–7609.
- Nakamura K, Yasunaga Y, Segawa T, Ko D, Moul JW, Srivastava S, Rhim JS. Curcumin down-regulates AR gene expression and activation in prostate cancer cell lines. *Int J Oncol*. 2002;21:825–830.
- Oettgen P, Finger E, Sun Z, Akbarali Y, Thamrongsak U, Boltax J, Grall F, Dube A, Weiss A, Brown L, Quinn G, Kas K, Endress G, Kunsch C, Libermann TA. PDEF, a novel prostate epithelium-specific Ets transcription factor, interacts with the androgen receptor and activates prostate-specific antigen gene expression. *J Biol Chem*. 2000;275:1216–1225.
- Ohtsu H, Xiao Z, Ishida J, Nagai M, Wang HK, Itokawa H, Su CY, Shih C, Chiang T, Chang E, Lee Y, Tsai MY, Chang C, Lee KH. Antitumor agents 217, curcumin analogues as novel androgen receptor antagonists with potential as anti-prostate cancer agents. *J Med Chem*. 2002;45:5037–5042.
- Thelen P, Jarryu H, Ringert RH, Wuttke W. Silibinin down-regulates prostate epithelium-derived Ets transcription factor in LNCaP prostate cancer cell. *Planta Med*. 2004;70:397–400.
- Thelen P, Scharf JG, Burfeind P, Hemmerlein B, Wuttke W, Spengler B, Christoffel V, Ringert RH, Seidlova-Wuttke D. Tectorigenin and other phytochemical extracted from leopard lily *Belamcanda chinensis* affect new and established targets for therapies in prostate cancer. *Carcinogenesis*. 2005;26:1360–1367.
- Thomasset SC, Berry DP, Garcea G, Marczylo T, Steward WP, Gescher AJ. Dietary polyphenolic phytochemicals—promising cancer chemopreventive agents in humans? A review of their clinical properties. *Int J Cancer*. 2007;120:451–458.
- Tsui KH, Chang PL, Juang HH. Zinc blocks gene expression of mitochondrial aconitase in human prostatic carcinoma cells. *Int J Cancer*. 2006;118:609–615.
- Tsui KH, Chang PL, Lin HT, Juang HH. Down-regulation of the prostate specific antigen promoter by p53 in human prostate cancer cells. *J Urol*. 2004a;172:2035–2039.
- Tsui KH, Hsieh WC, Lin MH, Chang PL, Juang HH. Triiodothyronine modulates cell proliferation of human prostatic carcinoma cells by downregulation of the B-cell translocation gene 2. *Prostate*. 2008;68:610–619.
- Tsui KH, Wu L, Chang PL, Hsieh ML, Juang HH. Identifying the combination of the transcriptional regulatory sequences on prostate specific antigen and human glandular kallikrein genes. *J Urol*. 2004b;172:2029–2034.