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# Biphasic Effect of Androgens on Prostate Cancer Cells and Its Correlation With Androgen Receptor Coactivator Dopa Decarboxylase

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## Abstract

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The aim of this study was to explore the mechanism underlying the dual effect of androgen on prostate cancer cells and further explore its correlation with dopa decarboxylase (DDC), an androgen receptor (AR) coactivator and a traditional neuroendocrine differentiation (NED) marker. Cell proliferation and cycling after treatment with synthetic nonmetabolizable androgen R1881 was determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) method and flow cytometry. Differential gene expression was analyzed by cDNA microarrays. DDC expression during the dual effect of R1881 was further explored with microarray, quantitative reverse transcriptase–polymerase chain reaction (RT-PCR), Western blot, and enzyme activity assays. Proliferation of LNCaP cells was inhibited by 1 nM R1881 but stimulated by 0.1 nM R1881. Compared with the untreated cells, 320 (2.26%; 170 up-regulated, 150 down-regulated) and 4608 (32.65%; 2046 up-regulated, 2562 down-regulated) genes were found to be expressed differentially in the 1 nM and 0.1 nM R1881-treated cells, respectively. The results were partially confirmed by RT-PCR and Western blot. The DDC gene was down-regulated in the 1 nM R1881-treated cells and up-regulated in 0.1 nM R1881- and 30 nM hydroxyflutamide-treated cells. The enzymatic activity of DDC in the latter 2 groups was also strengthened. Meanwhile, the NED markers CgA and synaptophysin were not affected by these AR activators. R1881 had a dose-dependent biphasic effect on LNCaP

cell proliferation. AR coactivator DDC was respectively down- and up-regulated in high and low concentrations of R1881. DDC up-regulation by exogenous AR activators is not accompanied by up-regulation of definitive NED markers.

Key words: Neuroendocrine differentiation, methyltoenolone, cDNA microarray

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Prostate cancer is an important male genital gland malignant carcinoma. In the United States, the mortality rate in man due to prostate cancer ranks second, only lower than that of lung cancer (Freeman et al, 1994). Its incidence increased steadily from 1981 to 1989 and then steeply in the early 1990s. In 1996 alone, there were 317 000 new cases of prostate cancer, and 41 400 patients died of this disease in the United States (Sarma et al, 2002). In some well-developed areas in China, a dramatic increase in the incidence of prostate cancer has been observed that was considered related to the changes in lifestyle and diet pattern (Hsing et al, 1998).

Androgens could act as growth hormone for prostate cells and prostate cancer cells through androgen receptors' (AR) activation. Meanwhile, 5 α-dihydrotestosterone (DHT) at concentrations higher than 0.3 nM was reported to result in progressively lower proliferation of LNCaP cells (a human prostate cancer cell line) (Sonnenschein et al, 1989). It was suggested that the dose response of prostate cancer cells to androgens was biphasic, and a faulty shutoff response is a crucial event for the tumorigenesis of these cells. However, the molecular mechanism of this biphasic effect remains unclear.

Dopa decarboxylase (DDC) has been found to be an AR coactivator (Wafa et al, 2003) in prostate cells and a neuroendocrine differentiation (NED) marker. The DDC contains an LXXLL motif (amino acids 153–157), which plays an essential role in coactivator interaction with steroid receptors. The DDC-enhanced AR transactivation in prostate cancer cells PC3 and LNCaP could be significantly reduced with bicalutamide treatment, suggesting that DDC exerts its effect on AR through a ligand-dependent pathway. Before the AR coactivator role, DDC had been recognized as an NED marker (Wafa et al, 2003), which is usually associated with more aggressive disease in adenocarcinoma of the human prostate.

In this work, we experimented with the synthetic androgen methyltrienolone (17 ß-hydroxy-17 α-methylestra-4,9,11-triene-3-one, R1881) and the androgen-responsive prostate cancer cell line LNCaP, which could be activated by both androgens and some antiandrogens (Olea et al, 1990; Veldscholte et al, 1992). We first confirmed that R1881 has a dose-dependent biphasic effect on LNCaP cell growth. Then we examined, first with microarray technology and then low-throughput methods, differentially expressed genes at growth-promoting and growth-inhibiting R1881 concentrations. The changes of DDC expression were explored to illustrate its roles in androgens' dual effect.

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### *Cells and Materials*

LNCaP cells (kindly provided by Prof Jian-Guang Zhou) were cultured in phenol-free RPMI-1640 medium plus 10% charcoal-stripped fetal bovine serum (Hyclone, South Logan, Utah). The synthetic androgen R1881 (Perkin-Elmer Inc, Waltham, Mass) was dissolved in phosphate-buffered saline (PBS) (pH 7.2) at the concentration of 100 M, stored at – 20° C, and added into the medium at the specified

concentration. For the mock treatment control group, an equal volume of pure ethanol was dissolved in PBS and used as the mock stock solution to be added into the medium.

Indocarbocyanine (Cy3)-deoxycytidine triphosphate (Cy3-dCTP) and indodicarbocyanine (Cy5)-deoxycytidine triphosphate (Cy5-dCTP) were purchased from Amersham Pharmacia Biotech (Piscataway, NJ) and Oligotex mRNA Midi Kit from Qiagen (Valencia, Calif). ScanArray 4000 laser scanner was from GSI Lomonics (Moorpark, Calif). GenePix Pro 3.0 software came from Axon Molecular Device, Sunnyvale, Calif.

## Methods

*Cell Growth Assay*— MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) stains live but not dead cells, and the optical density absorbance at 492 nm wavelength correlates well with the cell number. Cells of different groups were harvested and inoculated into 96-well culture plates (Costar, Cambridge, Mass) at the concentration of $5 \times 10^3$ per well in 200 µL RPMI-1640. At 24 hours after inoculation, the cells were treated respectively with media containing $10^{-7}$ M, $10^{-8}$ M, $10^{-9}$ M, $10^{-10}$ M, $10^{-11}$ M, $10^{-12}$ M, and $10^{-13}$ M R1881. Then the plates were incubated at 37° Cin 5% $CO_2$ humidified incubator. At respectively 1, 3, 5, and 7 days after drug treatment, some plates were taken, media were pipetted off, and 5 mg/mL MTT diluted in PBS was added at 15 µL per well. After 4 hours of incubation in the same incubator, MTT was removed and 150 µL-per-well dimethylsulfoxide was added. The absorbance at 492 nm was read by a BIOHIT BP800 plate reader (Biohit, Helsinki, Finland).

*Analysis of Cell Cycle Changes*— Cells of groups A, B, and C and control group were collected and placed into 6-well plates at a density of $1 \times 10^5$/mL, washed with 0.01 M PBS (pH 7.2), fixed in 70% ethanol for 18 hours, resuspended in PBS, and stained with propidium iodide (PI; 100 µg/mL) for 30 minutes. Flow cytometry was carried out using blue light Argon-Ion laser (excitation wavelength, 488 nm; laser power, 200 mW), and red fluorescence from the PI (which labels DNA) was recorded.

*Analysis of Gene Expression Changes Underlying Biphasic Effect of R1881 by Gene Chip Analysis*— After about 5 days of continuous culturing, cells were inoculated into 6-well plates at the concentration of $10^6$ per well and treated with 1 nM R1881 (group A), 0.1 nM R1881 (group B), or mock treatment medium (control group).

Two sets of gene chip assays were performed: group A vs control and group B vs control. Cell mRNA was extracted by Trizol and purified by Oligotex Midi Kit (QIAGEN). Microarray analysis was performed by the Human Gene Expression chip (version H80s; Biostar, Shanghai, China), which contains 14 114 human genes. Fluorescent probes were synthesized by reverse transcription of 100 µg mRNA with 50 units of avian myeloblastosis virus (AMV) reverse transcriptase (RT) (Takara Shuzo, Kyoto, Japan) in the presence of Cy3- or Cy5-dCTP (Amersham, Arlington Heights, Ill). Then Cy3- and Cy5-labeled probes were prepared and incubated in the cDNA chip at 42° C for 6 hours, washed twice with 2x SSC/0.2% sodium dodecyl sulfate (SDS) at 60° C for 30 minutes, and then washed again with the same buffer for 5 minutes. Finally, the chip was washed with 0.05x standard saline citrate (SSC) at room temperature for 10 minutes, and signals were quantified with the ScanArray 4000 and the Quant Array Software. All the Cy3 fluorescent units were normalized according to the normalized factor, and Cy5 fluorescent intensity was counted as 200 if it were below 200 fluorescent units. The expression changes of genes were considered as up-regulated if the Cy5/Cy3 signal ratio was greater than 2.0 and down-regulated if the ratio was less than 0.5.

*Semiquantitative RT-PCR*— Total RNA was extracted by the QIAGEN RNA isolation kit (Qiagen); 5 mg/mL RNA was used for the first-strand cDNA synthesis (TaKaRa Co, Dalian, China). In each reaction,

100 mL solution containing 3 mmol random hexamers, 25 mM Tris-HCl, 37 mM KCl, 1.5 mM $MgCl_2$, 10 mM dithiothreitol (DTT), 0.25 mM deoxyribonucleoside triphosphate (dNTP), 40 units of RNAsin, an RNase inhibitor, 50 U/mL Super Taq DNA polymerase, and 200 units of RT was used. The annealing mixture was incubated at room temperature for 15 minutes and then incubated in a water bath at 41° C for 60 minutes. The RT enzyme was inactivated by heating the solution to 95° C for 5 minutes. Polymerase chain reaction (PCR) was then carried out using the Perkin-Elmer PCR reaction kit and primers. The PCR was performed for 30 cycles consisting of denaturation at 94° C for 1 minute, annealing at 57° C for 1 minute, and extension at 72° C for 2 minutes. The PCR products were analyzed on 1.5% agarose gel. To calibrate the sample loaded in quantitative PCR, glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA was determined at the same time. Two microliters of PCR product was amplified again using the 2 rat GAPDH primers at 94° C for 30 seconds, 60° C for 30 seconds, and 72° C for 40 seconds for 28 cycles, and 72° C for 5 minutes for 29 cycles, and the optical density of the GAPDH band (337 bp) was measured. The ratio of DDC mRNA/GAPDH mRNA was the relative amount of DDC mRNA. Gene primers were synthesized by Beijing Oake Co (Beijing, China).

*DDC Function Assay*— Cell lysates were dounce-homogenized in ice-cold homogenization buffer (Roche Molecular Biochemicals, Basel, Switzerland). The homogenates were centrifuged at 10 000 x *g* for 10 minutes at 4° C. The supernatant was collected; protein concentrations were determined (Bio-Rad [Hercules, Calif] protein assay kit), and they were employed for immunoblot or enzyme activity assays.

*Sequence analysis of DDC mRNA in R1881 induced inhibition of cell growth.* Total cellular RNA isolated from both control group cells and experimental groups were used for RT-PCR assays. Four sets of primer pairs were used to generate amplicons representing various sets of exonic sequences from the DDC gene: 5'-TTTTCAACATGGATTCCCGTG-3' (forward) and 5'-ATCAGATGTGTAAGCAACCAGCTT-3' (reverse); 5'-TACTGGCTGCTCGGACTAAAGTTA-3' (forward) and 5'-CACCATTCAGAAGATACCGGAATT-3' (reverse); 5'-GGGTCCCATCTGCAACCA-3' (forward) and 5'-AAACCACATTTTCAAAGAGCGAAAT-3' (reverse); and 5'-GCCTTTAATATGGAGCCTGTTTATC-3' (forward) and 5'-GTGGTAGTTATTTTTCTC-3' (reverse).

The amplicons were analyzed by agarose-gel electrophoresis. In addition, a single amplicon containing the open reading frame that encodes the active form of DDC was purified from the gel, and its nucleotide sequence was determined.

*DDC enzymatic activity assay.* DDC activity was assayed using the procedure of Lamprecht and Coyle with modifications. Reaction mixtures (250 μL) contained 10 μg/mL pyridoxal phosphate (Sigma Chemical Co, St Louis, Mo), 100 μg/mL ascorbic acid (Sigma), 600 μM L-DOPA (Sigma), 30 mM sodium phosphate (pH 7.2), and DL-[$^{14}$C]DOPA (American Radiolabeled Chemicals, St Louis, MO; diluted to a final specific activity of 1 μCi/μmol; 0.15 μCi per assay). The reaction mixture was incubated for 15 minutes at 37° C and then terminated by adding 1 mL solution containing 4% perchloric acid and 2 mM 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron; Sigma). [$^{14}$C]$CO_2$ generated during the course of the assay was collected on Whatman filter paper affixed to the top of the reaction vessel after being soaked in a solution of 20% $\alpha$— phenylethylamine (Sigma) and 80% methanol. Radioactivity was measured by scintillation counting. Controls studies established that the assay was linear with respect to time (0— 20 minutes) and input lysate protein concentration (0— 5 μg).

*Western Blot Assay of DDC and NED Markers*— Protein extracts were prepared from cells using a protein lysis buffer containing 50 mM Tris-HCl (pH 7.5), 2.0 mM phenylmethylsulfonyl fluoride, 5.0 mM iodoacetamide, 5.0 mM EDTA, 150 mM NaCl, 0.5% nonylphenoxy polyethoxy ethanol, and 0.5% nonanoyl-N-methylglucamide. Protease inhibitors leupeptin (2 μg/mL) and pepstatin (1 μg/mL) (Roche, Mannheim, Germany) were added just prior to the addition of lysis buffer to the cells. Protein concentrations

in the extracts were quantitated using bicinchoninic acid protein assay (Pierce, Rockford, Ill). From each extract, 10 to 50 µg of total protein was separated on a 12% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) with a 5% stacking gel. After electrophoresis, proteins were transferred to 0.2 µm polyvinylidene diflouride (PVDF) membranes (Bio-Rad) using transfer buffer that contained 25 mM Tris-HCl and 700 mM glycine. Membranes were blocked in 7% powdered milk dissolved in 1x TTBS (14 mM Tris, 154 mM NaCl, and 0.1% Tween-20, pH adjusted to 7.5 with HCl) overnight at 4° C. Primary antibodies were incubated for 2 hours at room temperature in 5% powdered milk dissolved in 1x Tween-20 in TTBS. The murine antibody for DDC was provided by Sigma, and the rabbit antibody for chromogranin A (CgA) was provided by Lab Vision, Fremont. The antigen-antibody complexes were detected using the appropriate secondary antibodies conjugated to horseradish peroxidase (Promega, Madison, Wisc) dissolved in 1x TTBS with 5% powdered milk and incubated with membranes for 1 hour at room temperature. Membranes were developed using ECL+ (Amersham Pharmacia Biotech, Arlington Heights, Ill) and films exposed for appropriate times to detect signal.

### *Statistical Analysis*

The experiments were repeated at least 3 times, and the results were expressed as mean ± SD. Statistical analysis was done using 2-tailed Student's *t* test and *P* values at a level of 95% confidence interval.

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### *Morphologic and Cell Cycle Changes Induced by R1881*

No apparent morphologic changes in LNCaP cells, besides cells becoming more spherical and having higher density, were observed after 5 days of R1881 treatment at any concentration tested. The results of flow cytometry indicated that cells treated with 1 nM R1881 had a higher percentage of cells in $G_1$ phase than the mock-treated cells ($P < .05$), whereas cells treated with 0.1–0.001 nM R1881 had a significantly lower percentage of cells in $G_1$ phase and higher percentage of cells in S phage (Figure 1; Table 1).

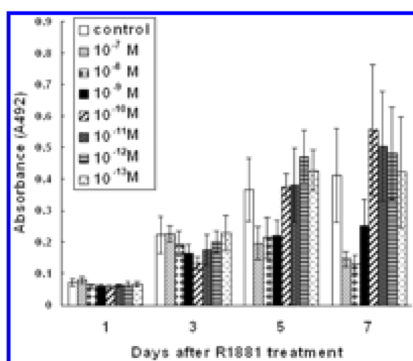


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Figure 1. The growth of LNCaP cells (indicated by absorbance at 492 nM) treated with R1881 of different concentrations. The absorbance was measured with the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) method at 1, 3, 5, and 7 day after treatment. Assays were repeated 3 times, and error bars represent SD.

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Table 1. *Percentage of $G_1$- and S-phase cells of different groups*[*]

## *R1881 Exerts a Biphasic Effect on Prostate Cancer Cell Growth*

The dose-dependent effect of R1881 on prostate cancer cell proliferation is shown in Figure 2. At 1 nM and higher concentrations, R1881 inhibited growth of LNCaP cells, whereas at 0.1 nM and lower concentrations, R1881 stimulated cell growth. The results were the average from triplicate experiments. This and the cell cyle data indicate a biphasic effect of R1881 on LNCaP proliferation (ie, promoting at lower concentrations and inhibiting at high concentrations).

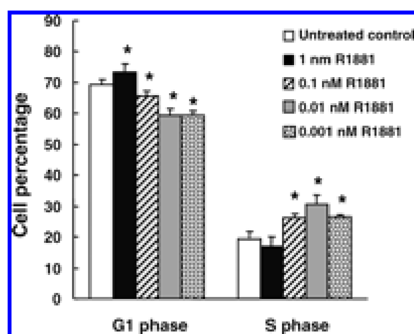


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Figure 2. Percentage of LNCaP cells in $G_0/G_1$ and S phase under treatment of different concentrations of R1881 for 7 days. Cytometry assays were repeated 3 times, and error bars represent SD.

## *Genetic Changes Underlying the Biphasic Effect of R1881*

First, the stability of the RNA isolated from the cells was examined by agarose gel electrophoresis, and the 18S and 28S bands were clear. The absorbance ratio A260/A280 was higher than 2.0, indicating that the extracted mRNA samples were suitable for cDNA microarray assay.

Using the criteria offered by the chip manufacturer (ie, Cy5/Cy3 more than 2.0, up-regulated; Cy5/Cy3 less than 0.5, down-regulated) there were 320 (3.93%) and 4608 (32.65%) genes whose mRNA was expressed differentially, respectively, in the 1 nM R1881 group (A) and the 0.1 nM R1881 group (B). In group A, 150 genes were up-regulated and 170 genes were down-regulated, including DDC. In group B, 2046 genes were up-regulated, including DDC, and 2562 down-regulated. The top 10 up-regulated and top 10 down-regulated genes in group A and B are listed in Tables 2 and 3. In both groups, the expression of AR and KLK3 (encoding prostate-specific antigen [PSA], a regularly monitored marker in prostate cancer) did not change significantly.

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Table 2. *Genes up-regulated and down-regulated in group A (1 nM R1881) chip assay*[*]

| View this table: [in this window] [in a new window] | Table 3. *Genes up-regulated and down-regulated in group B (0.1 nM R1881) chip assay*[*] |
|---|---|

The mRNA expression changes of all the AR coregulator genes observed by microarray were confirmed by RT-PCR. The down-regulation of DDC in the 1 nM R1881 group and its up-regulation in the 0.1 nM group were confirmed (Figure 3). In the 0.1 nM R1881-treated group, FHL2, NCOR1, and PIAS3 were down-regulated, and DDC, TBLR1, and ACTN2 were all up-regulated (Figures 2 and 4; Tables 4 and 5).

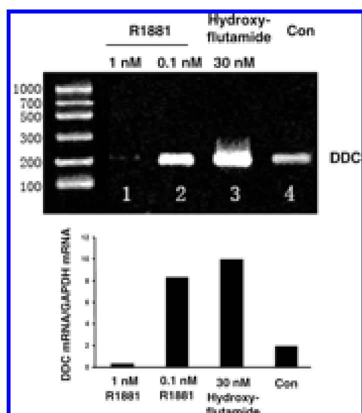


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Figure 3. Semiquantitative reverse transcriptase–polymerase chain reaction (RT-PCR) determination of the relative amount of dopa decarboxylase (DDC) mRNA expression in LNCaP cells treated with 2 concentrations of R1881, 30 nM hydroxyflutamide, or untreated. **(A)** Agarose gel analysis of the RT-PCR products. **(B)** The ratio of DDC mRNA/GAPDH mRNA in different groups.

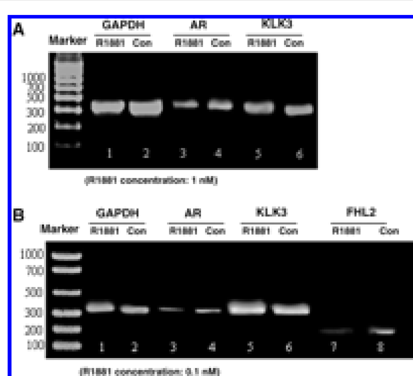


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Figure 4. Semiquantitative reverse transcriptase–polymerase chain reaction determination of the mRNA expression of androgen receptor (AR) and KLK3 genes in the 1 nM R1881-treated group **(A)** and the mRNA expression of AR, KLK3, and FHL2 genes in the 0.1 nM R1881-treated group **(B)**.

| View this table: [in this window] [in a new window] | Table 4. *Androgen receptor coregulators' expression in group A* |
|---|---|

| View this table: [in this window] [in a new window] | Table 5. *Androgen receptor coregulators' expression in group B* |
|---|---|

### *DDC Is Also Up-Regulated After T877a-Mutated AR Activation in LNCaP Cells*

The gene chip experiment of 0.1 nM R1881 (group B) vs control found that DDC was up-regulated, with the Cy5/Cy3 ratio being 4.835. To verify the up-regulation of the DDC gene by R1881, another set of gene chip experiments (30 nM hydroxyfluramide vs control) was performed. Hydroxyflutamide is a traditional AR antagonist but has been reported to activate the T877A-mutated AR in LNCaP cells, thus stimulating LNCaP cells (Olea et al, 1990).

In our experiment, after treatment of 30 nM hydroxyflutamide, 421 (2.97 %) differentially expressed genes were identified by the microarray assay. A total of 157 genes, including DDC, were up-regulated, and 264 genes were down-regulated. All top 10 up-regulated and down-regulated genes in this group are listed in Table 6.

| View this table: [in this window] [in a new window] | Table 6. *Genes up-regulated and down-regulated in group C (30 nM hydroxyflutamide) chip assay** |
|---|---|

Quantitative RT-PCR confirmed that DDC mRNA expression was increased after 0.1 nM R1881 or 30 nM hydroxyflutmide treatment compared with the untreated cells (Figure 3). Alternative splicing of DDC could yield a transcript that encodes its inactive form. However, RT-PCR revealed that DDC produced a single transcript in R1881 or hydroxyflutamide-treated cells. Changes in DDC protein expression were also confirmed by Western blot assay (Figure 5). These overexpressed DDC proteins were demonstrated to be enzymatically active by follow-up radiobiochemical assays, which showed a 6-fold (6.02 ± 0.36 pmol/min/mg protein) and 7-fold (6.64 ± 0.61 pmol/min/mg protein) increase in DDC activity in the 0.1 nM R1881 group and 30 nM hydroxyflutamide group, respectively, compared with the untreated group (0.91 ± 0.178 pmol/min/mg protein) (all in triplicates).

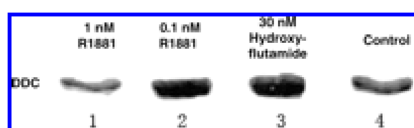


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Figure 5. Western blot analysis of dopa decarboxylase protein in LNCaP cells treated with 2 concentrations of R1881, 30 nM hydroxyflutamide, or untreated.

### DDC Up-Regulation Is Not Accompanied by Overexpression of NED Markers

CgA and synaptophysin are 2 well-recognized markers of NED. CgA has been considered the most sensitive marker and is the most frequently used marker for detecting NE phenotype either at the tissue level or in the general circulation (Mosca et al, 2005). Synaptophysin is an integral membrane glycoprotein with a relative molecular weight of 38 kd, which localizes in the small, clear vesicles present in neuronal cells, tumors, as well as pancreatic islet cells and various NE carcinomas (Huss et al, 2004). In large cell neuroendocrine carcinoma of prostate cases, strongly positive synaptophysin and CgA expression were detected (Evans et al, 2006).

In our experiments, DDC is found to be up-regulated in the 0.1 nM R1881 or 30 nM hydroxyflutamide-treated LNCaP cells. However, in both of these 2 groups, no significant changes in CoA and synaptophysin expression were observed in the Western blot analysis (Figure 6).

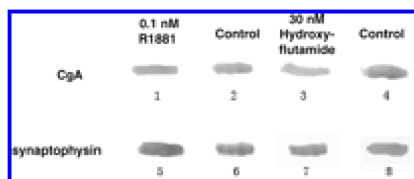


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Figure 6. Western blot analysis of CgA and synaptophysin expression in LNCaP cells treated with 0.1 nM R1881, 30 nM hydroxyflutamide, or untreated.

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The biphasic effect of androgen on prostate cells and in maintaining a steady state of prostate has been well recognized (Prout et al, 1967). Most epithelial cells in male prostate are in a cell cycle— arrested state when plasma androgen levels are high enough. Castration induces cell death, and androgen treatment results in increased cell proliferation until the adult cell number is restored after which no further proliferation occurs even if androgen treatment continues. The effect on androgen-normal prostate cells could also be extended to prostate cancer cells, because androgens of high concentrations could inhibit prostate cancer proliferation (before tolerance develops). Therefore, it is desirable to understand the mechanism by which androgens inhibit cell proliferation, which has remained largely unclear.

In this study, we found that R1881 could inhibit the growth of the prostate cancer cell line LNCaP at a concentration higher than 1 nM, which is accompanied by increased $G_1$/S arrest, whereas concentration of 0.1 nM and below could stimulate cell proliferation. Our study paid special attention to an AR coregulator DDC, which had a notably high expression level in the untreated state; its Cy3 value in the control group was 22017, higher than 96.83% of all genes in the mircoarray (data not all listed). The expression of DDC was down-regulated in the 1 nM R1881-treated group and up-regulated in the 0.1 nM R1881- and 30 nM hydroxyflutamide-treated LNCaP cells.

DDC was found to interact with the ligand-binding domain and the N-terminal domain of AR through its C terminus, which facilitates an increase of the transcriptional activity of the nuclear receptor in

prostate cancer cell lines by modulating the folding of AR and stabilizing the AR dimer through the N-terminal– C-terminal interaction, which is similar to that of NCOA4/ARA70, a well-known AR coactivator. This enhancement was inhibited when the transactivation assays were performed in the presence of an antiandrogen such as bicalutamide, suggesting that DDC exerts its effect on AR through a ligand-dependent pathway. Increased DDC expression and subsequent enhancement of AR activity may cause the prostate cancer cells to have a lower androgen threshold for AR activation, leading to growth-promoting effects under conditions in which androgen levels are limited. In our research, DDC was found to be down-regulated in higher-concentration R1881- (1 nM) treated LNCaP cells but up-regulated in lower-concentration R1881- (0.1 nM) and hydroxyflutamide-treated LNCaP cells, which could support its role in AR activation as a coactivator.

DDC has also been reported to be a tumor marker for NE cell differentiation of prostate cancer, and its enzymatic activity is markedly increased in the prostate of NE cell– transformed transgenic mice (Gazdar et al, 1988). An increase in NE cell content occurs during the progression of prostate cancer and is often an indication of androgen independence or increased aggressiveness of the disease (Nelson et al, 2002). NED of prostate cancer cells was found to be particularly relevant in facilitating prostate cancer progression during the ordinary androgen-suppression therapy (luteinizing hormone-releasing hormone [LHRH] analogs with or without antiandrogens). NE prostate cancer cells produce peptides, hormones, and growth factors that could stimulate proliferation, inhibit apoptosis, and stimulate neoangiogenesis of the neighboring exocrine prostate cancer cells. In hormone refractory disease, NED is a time-dependent phenomenon and is not influenced by conventional antineoplastic treatments. DDC was found to be coexpressed with CgA in 8 of 10 NED specimens, and therefore it was deemed a biomarker of NED. However, in our research, we found DDC was up-regulated in R1881- and hydroxyflutamide-treated LNCaP cells, but this up-regulation of DDC was not accompanied by CgA and synaptophysin up-regulation, which has been proven to be the definitive indicator of NED. Thus, our data suggest that the up-regulation of DDC is a phenomenon in AR activation, but it is not necessarily associated with NED.

In conclusion, we demonstrated that 1) R1881 of 1 nM or higher may inhibit LNCaP cell growth through a $G_1$/S blockage and R1881 of 0.1 nM and below may stimulate LNCaP cell proliferation; 2) DDC and other AR coregulators are implicated in androgen's dual effect—DDC is up-regulated in AR activation and down-regulated in AR inhibition; and 3) DDC is an AR coactivator but not necessarily an NED marker.

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## Footnotes

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|| These authors contributed equally to this article.

## References

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