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Effects of 1,25-Dihydroxyvitamin D_3 on the Distribution of Androgen and Vitamin D Receptors in Human Prostate Neonatal Epithelial Cells

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Abstract Although many studies have examined the mechanisms of 1,25-dihydroxyvitamin D_3 (calcitriol or 1,25 D) action in different prostate cancer cell lines, little is known regarding the influence of this steroid on the normal prostate. The presence of both VDR and AR in normal prostatic tissues raises the distinct possibility of an important role for this hormone in the normal gland. In order to ascertain the possible role of 1,25 D on both AR and VDR in the normal prostate, the effects of calcitriol and dihydrotestosterone (DHT) on the normal human neonatal prostatic epithelial cell line, 267B-1, were examined. These studies were approached by focusing on how 1,25 D in the presence or absence of DHT affects the distribution of AR and VDR in the cytoplasmic and nuclear compartments of the cells in terms of their protein levels and DNA binding activities. Immunoblot analyses show that 1,25 D increases the AR protein level in both the cytoplasmic and nuclear fractions but not the VDR protein level. On the other hand, the gel shift assays demonstrate that 1,25 D increases both the AR– and VDR–DNA binding activities in the nuclear fraction, whereas there is no increase in DNA binding activities in the cytoplasmic fraction. Addition of DHT along with 1,25 D does not affect the DNA binding activities of both AR and VDR. Overall, these studies suggest that 1,25 D actions on the normal prostate cells may be mediated independently through AR and VDR, respectively. J. Cell. Biochem. 88: 609–622, 2003.

Key words: vitamin D; normal prostate; androgen and vitamin D receptors

1,25-dihydroxyvitamin D_3 (1,25 D) or calcitriol is the principally active hormonal form of vitamin D and is well known for its role in regulating calcium homeostasis in the body by actions in the intestine, bone, kidney, and parathyroid glands. Biological responses of target cells to 1,25 D are mediated at least in part by its nuclear receptor, the vitamin D receptor (VDR) [Baker et al., 1988]. The VDR belongs to the steroid/thyroid/retinoid receptor superfamily, which function as ligand-dependent transcription factors [Haussler et al., 1998]. A number of studies have indicated that the VDR controls target gene transcription as homodimers or by forming a heterodimeric complex with the retinoid X receptor (RXR), the receptor for 9-*cis* retinoid acid (RA), and binding to the vitamin D response element (VDRE) present in the promoter region of target genes.

VDRs have been identified in numerous cell types, including breast, kidney, testes, and prostate [Haussler, 1986; Peehl et al., 1994]. The antiproliferative and growth-regulatory effects of 1,25 D and its analogues on various cell types, including normal and neoplastic cells are well established [Colston et al., 1981; Tanaka et al., 1982; Mangelsdorf et al., 1984; McLane et al., 1990; Peehl et al., 1994]. The presence of VDRs in the human prostate cancer cell line LNCaP was first reported by Miller et al. [1992]. VDRs have since been found in other established human prostate cancer cell lines, primary cultures of normal prostate and cancer cells, as well as in normal prostate epithelial and stromal cell lines grown in culture [Peehl et al., 1994]. Studies with many of these cell systems have demonstrated the antiproliferative effects of 1,25 D and its analogues [Skowronski et al., 1993; Miller et al., 1995; Schwartz

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et al., 1995]. Similar antiproliferative and prodifferentiative effects are evident in primary cultures of normal prostate cells in vitro [Peehl et al., 1994]. Moreover, 1,25 D and its analogues significantly inhibit the cellular proliferation of prostate cancer cell line LNCaP, which expresses both the VDR and the androgen receptor (AR).

Investigations by Feldman's laboratory [Zhao et al., 1999, 2000] as well as other groups [Hsieh et al., 1996; Hsieh and Wu, 1997] have demonstrated cross talk between 1,25 D and androgens and that the antiproliferative actions of 1,25 D in LNCaP cells are androgen-dependent. Furthermore, they showed that 1,25 D increased the levels of AR messenger RNA (mRNA) and AR protein in a concentration- and time-dependent manner, and that Casodex (anti-androgen) blocked the inhibitory activity of 1,25 D [Zhao et al., 1999]. Recently, the same group [Zhao et al., 2000] demonstrated that the growth inhibitory action of 1,25 D in the prostate cancer cell lines, MDA PCa 2a and MDA PCa 2b, was androgen independent. They showed that 1,25 D significantly increased the levels of AR mRNA, but Casodex did not block the antiproliferative activity of 1,25 D [Zhao et al., 2000]. Overall, these studies suggest that 1,25 D can inhibit the growth of prostate cancer cells via both androgen dependent and independent manners.

Although studies have examined the mechanism of 1,25 D action in different prostate cancer cell lines, little data is available regarding the influence of this steroid on the normal prostate. In addition to the LNCaP data, studies have now demonstrated a role of 1,25 D in the growth regulation of the normal prostate [Konety et al., 1996, 2000; Krill et al., 1999]. These studies have established that interactions may exist between vitamin D and the actions of androgens in the normal prostate. We previously demonstrated that when adult male rats are castrated and administered a super physiological dose of 1,25 D, the resulting prostates of these animals are more than twice the size of that of the control animals treated with vehicle alone [Konety et al., 1996]. When the rats are castrated and then treated with exogenous testosterone in addition to 1,25 D, they exhibit greater prostatic differentiation without the increase in size seen in the castrated rats. We have also shown that administration of 1,25 D in rats in utero influences the size and differentiation of the prostate throughout the life span of the animal [Konety et al., 1999]. Therefore, 1,25 D has an "imprinting" effect on the prostate similar to what has been seen with androgens and estrogens [Wernert et al., 1988; Makela et al., 1990; Schulze and Claus, 1990].

The presence of both VDR and AR in normal prostatic tissues raises the distinct possibility of an important role for this hormone in the normal gland. In order to ascertain the possible role of 1,25 D on both AR and VDR in the noncancerous prostate, we examined the effects of calcitriol and dihydrotestosterone (DHT) on the normal human neonatal prostatic epithelial cell line, 267B-1. We show that 1,25 D increases the AR protein level in both the nuclear and cytoplasmic fractions but not on VDR protein level. However, 1,25 D increases both the AR– and VDR–DNA binding activities in the nuclear fraction, whereas there was no increase in DNA binding activities in the cytoplasmic fraction. Addition of DHT along with 1,25 D did not affect the DNA binding activities of both AR and VDR. Overall, these results suggest that 1,25 D may influence the steroid receptors in the normal prostatic cells by modulating their DNA binding activities.

MATERIALS AND METHODS

Hormones, Antibodies, and Oligonucleotides

Calcitriol (1,25 D) was purchased from Sigma (St. Louis, MO) and the stock concentration was prepared to 1 mg/ml in ethanol. The stock concentration of DHT (Sigma) was prepared to 10^{-4} M in ethanol. The mouse monoclonal anti-AR and rabbit polyclonal anti-VDR antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The oligonucleotide probes containing consensus binding motif for AR (20 ng/μl) and VDR (20 ng/μl) were also purchased from Santa Cruz Biotechnology.

Cell Culture

The human neonatal prostatic epithelial cell line 267B-1 was obtained from BRFF, Inc. (Ijamsville, MD). The 267B-1 cells were grown in T-75 tissue culture flasks coated with FNC coating mix (BRFF, Inc.) and maintained in serum free P4-8F cultured medium (BRFF, Inc.). The cells were grown at 37°C in a humidified incubator with 5% CO_2.

Cytoplasmic and Nuclear Extracts Preparation

For immunoblot analyses, the cells were grown overnight in T-75 tissue culture flasks. Following this incubation period, the 267B-1 cells were treated with media containing either 5 or 10 μM of 1,25 D (in the presence or absence of 10 nM DHT) or ethanol (control ± 10 nM DHT) for additional 24 h. The cytoplasmic and nuclear extracts were then prepared using NE-PER kit (Pierce, Rockford, IL) according to manufacture's protocol. Protein concentrations were determined by using the Coomassie plus protein assay kit (Pierce) according to the manufacturer's protocol.

Immunoblot Analysis

One hundred micrograms of either cytoplasmic or nuclear extracts were loaded and separated by 7.5% SDS/PAGE along with a separate lane containing 10 μl of Rainbow Markers (Amersham Life Sciences, Arlington Heights, IL). Proteins were then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA) utilizing a semi-dry transfer apparatus (Bio-Rad, Richmond, CA). The membranes were incubated overnight in 5% non-fat dry milk in phosphate buffered saline (PBS) with 0.2% Tween-20. The membranes were washed several times followed by 1-h incubation with either a 1:250 dilution of anti-AR primary antibody or 1:500 dilution of anti-VDR antibody in PBS with 2% non-fat dry milk and 0.2% Tween-20 at room temperature. The membranes were then washed again three times (10 min for each wash) with PBS and 0.2% Tween-20 and incubated for 1 h with appropriate secondary antibody conjugated with horseradish peroxidase at 1:5,000 dilution (Amersham Life Sciences) at room temperature. The membranes were then washed with PBS and 0.2% Tween-20 (three 10-min washes). To assure equal protein loading, all the membranes were stained with 0.5% Ponceau-S stain (Sigma) diluted in 1 × PBS and 0.1% acetic acid. Proteins were detected by a chemiluminescence reaction using the ECL Immunoblot kit (Amersham Life Sciences). To quantitate the bands, both AR and VDR protein bands were then analyzed densitometrically by FX-Phosphoimager Analysis (quantity one). Statistical analyses were performed with two-way ANOVA and the significant values were indicated with P value < 0.05.

Electrophoresis Mobility Shift Assay (EMSA) and Competition Assay

Twenty micrograms of both cytoplasmic and nuclear lysates were incubated in a final volume of 20 μl of 10 mM HEPES (pH 7.9), 80 mM NaCl, 10% glycerol, 1 mM DTT, 1 mM EDTA, and 100 μg/ml poly(deoxyinosinic–deoxycytidylic acid) with either ^{32}P labeled double-stranded AR or VDR consensus binding motif for 30 min at room temperature. For competition analyses, 10, 50, and 100 ng of cold unlabeled probes were added to the cell extracts, respectively. The protein–DNA complexes were resolved on a 4.5% nondenaturing polyacrylamide gel containing 2.5% glycerol in 0.25 × Tris-borate EDTA at room temperature, and the results were autoradiographed with FX Imager (Bio-Rad).

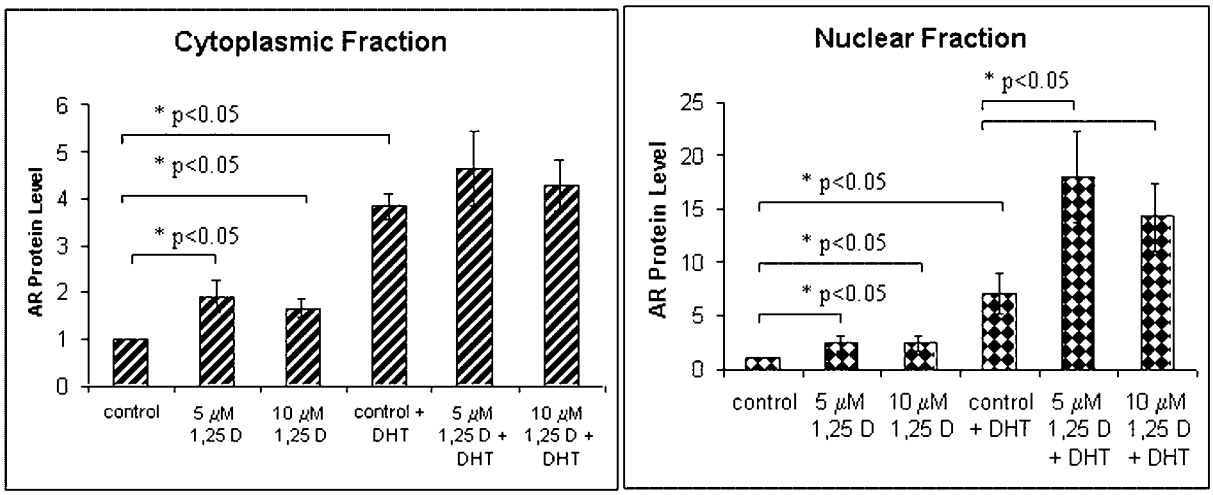
Immunofluorescence

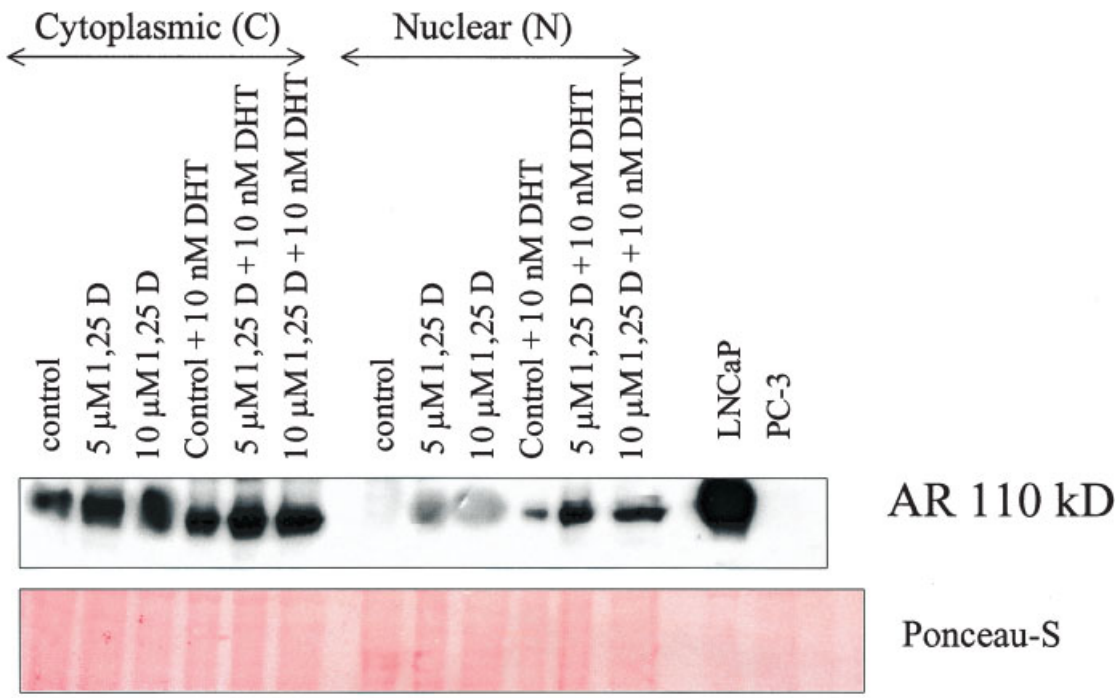
Briefly, the cells were plated into four-well chamber slides (Falcon) at 500 cells/well. The cells were grown overnight and then treated with either 10 mM of 1,25 D (±10 nM DHT) or ethanol (control ± 10 nM DHT) for 24 h. Following the treatment, the cells were fixed with 4% parafolmadehyde (Sigma) and blocked with blocking solution (1 × PBS with 0.2% Tween-20, 5% goat serum, and 2% BSA) for 1 h at room temperature. The cells were then incubated with either anti-AR or anti-VDR antibody (St. Cruz Biotechnology) at a dilution of 1:100 or with 1 × PBS as negative controls for 1 h at room temperature. The cells were then washed with 1 × PBS and 0.2% Tween-20 for four times (5 min each wash), followed by incubation with fluorescein-conjugated secondary antibody (St. Cruz) at a dilution of 1:200 for 1 h at room temperature. The cells were washed again with 1 × PBS and 0.2% Tween-20 for six times (5 min each wash) and then counterstained with Hoechst for nuclei staining. The cells were then observed and photographed with a fluorescence microscope equipped with live camera (Zeiss) at 40 × magnification.

RESULTS

Immunoblot analyses were performed to investigate the distribution of AR and VDR in the cytoplasmic and nuclear extracts in the 267B-1 cells, and if 1,25 D had any effect in influencing the AR and VDR expression levels. Figure 1 shows representation of immunoblot analyses on AR and VDR protein levels as well as

densitometric quantitation on the AR and VDR bands. As shown in Figure 1A, control (ethanol-treated) cells expressed AR with a molecular weight of 110 kDa in the cytoplasmic fraction, but none in the nuclear fraction. When the cells were exposed to 1,25 D at 5 and 10 μM, respectively, for 24 h, the AR protein levels in both the cytoplasmic and nuclear fractions were increased by almost 2- to 2.5-fold ($P < 0.05$). When the cells were further exposed to 10 nM





A

Fig. 1. Representative of immunoblot analysis of: (**A**) androgen receptor (AR) at 110 kDa and (**B**) vitamin D receptor (VDR) at 48–55 kDa from the cytoplasmic and nuclear extracts from the 267B-1 cells. The intensities of the bands (n = 4) were analyzed densitometrically by FX-phosphoimager analysis (quantity one). Ponceau-S staining shows equal loading of the protein and error bars represent standard error mean. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

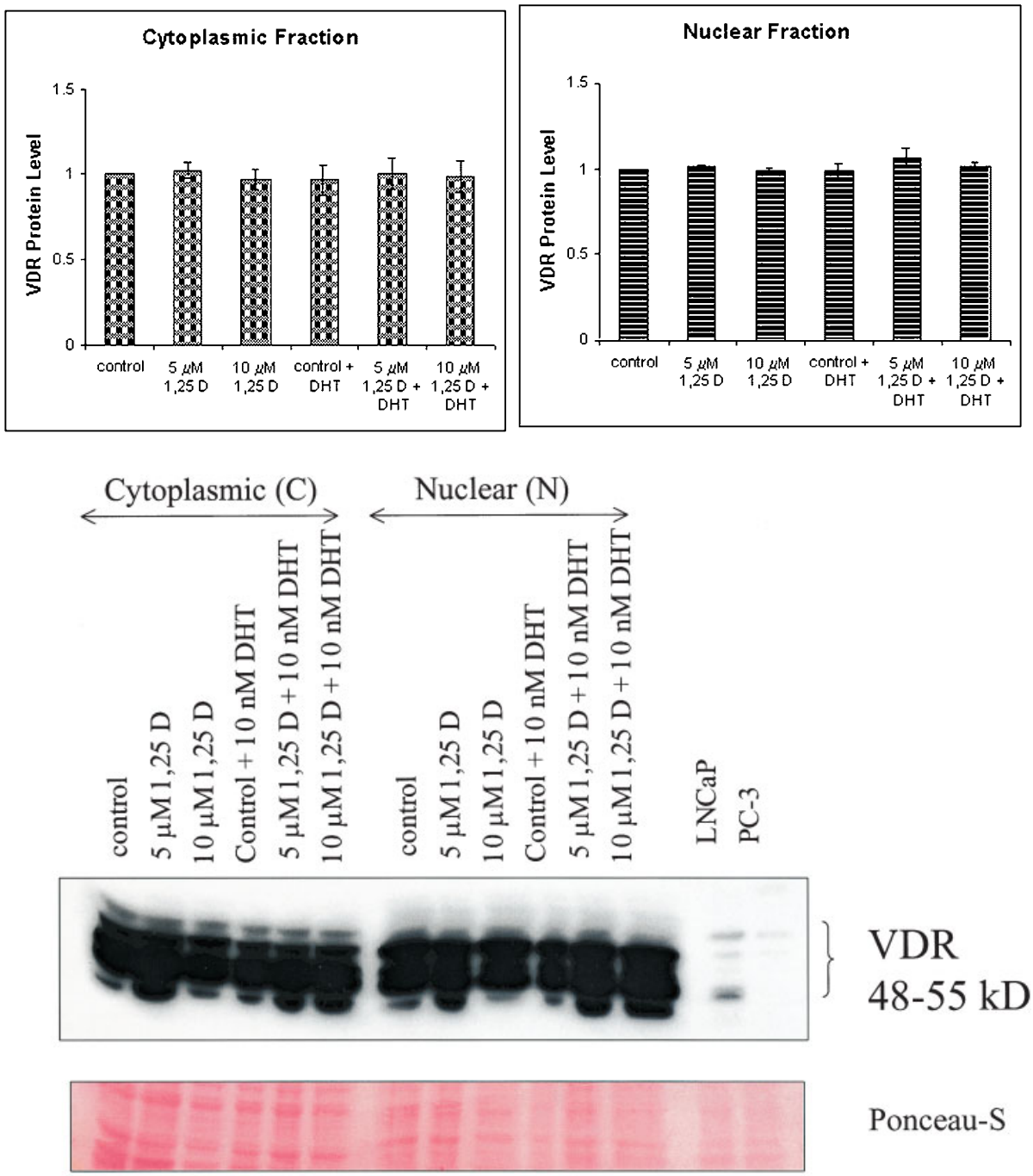


Fig. 1. (*Continued*)

DHT, the expression of AR protein was increased by 4-fold ($P < 0.05$) in the cytoplasmic fraction and by 7-fold ($P < 0.05$) in the nuclear fraction. Interestingly, when 1,25 D was added in the presence of DHT, the AR protein levels in the nuclear fraction were increased by about 14- to 17-fold ($P < 0.05$) compared to the control-ethanol treated group, and by about 2- to 2.5-fold when compared to the cells treated with DHT alone.

Immunoblot analyses of VDR protein showed that 267B-1 cells also express VDR in both cytoplasmic and nuclear compartments at different molecular weights ranging from 48 to 55 kDa (Fig. 1B). The presence of VDR at various molecular weights (48, 50, 52, and 55 kDa) has been reported by a number of investigators [Kivineva et al., 1998; Langub et al., 2000; Kallay et al., 2001]. Thus, these results are in agreement with the reported molecular weights of VDR. As a positive control, whole cell lysates from human prostate cancer cell line LNCaP, which is known to express VDR was also included. As shown in Figure 1B, LNCaP cells express VDRs at different molecular weights (48, 52, and 55 kDa). The presence of VDRs at different molecular weights in LNCaP cells supports the fact that the VDRs observed in 267B-1 cells are indeed VDRs and not background bands. When the 267B-1 cells were exposed to 1,25 D in the presence or absence of DHT, there was no difference in VDR expression in both the cytoplasmic and nuclear compartments.

The effects of 1,25 D and DHT on the distribution of AR and VDR in the cytoplasmic and nuclear fractions in terms of their DNA binding activities were also examined. The electrophoresis mobility shift assays (EMSA) were utilized to assess the binding activities of AR and VDR to the androgen responsive element (ARE) and vitamin D responsive element (VDRE), respectively. As shown in Figure 2, EMSA analysis revealed that the AR and VDR DNA binding activities occurred primarily in the nuclear compartment. When the cells were treated with 1,25 D, there was an increase in the DNA binding activity for both AR and VDR in the nuclear compartment. 1,25 D at 10 μM seemed to have a more pronounced effect in increasing

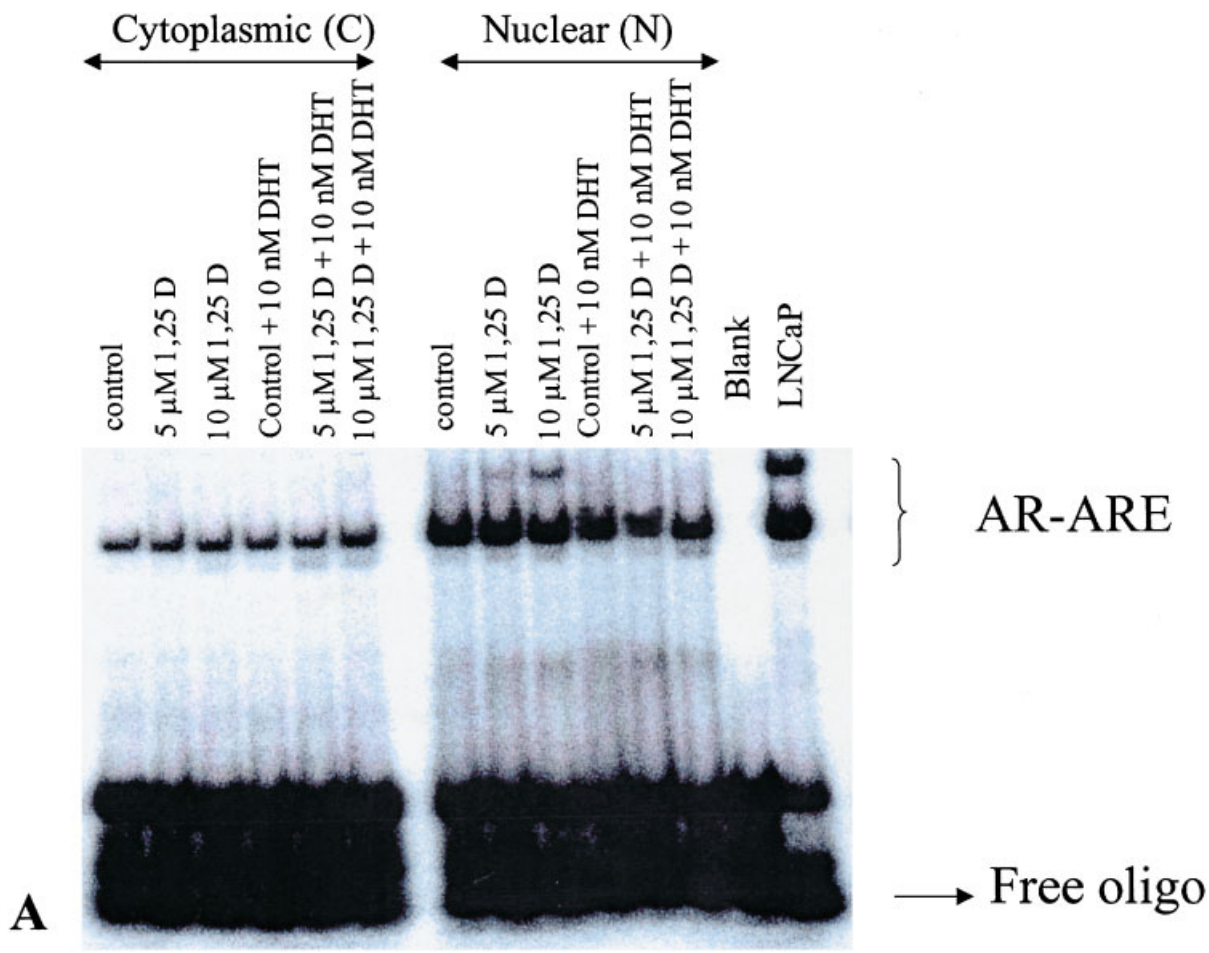


Fig. 2. Electrophoresis mobility shift assay (EMSA) analysis of: (**A**) AR–ARE and (**B**) VDR–VDRE from the cytoplasmic and nuclear extracts from the 267B-1 cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

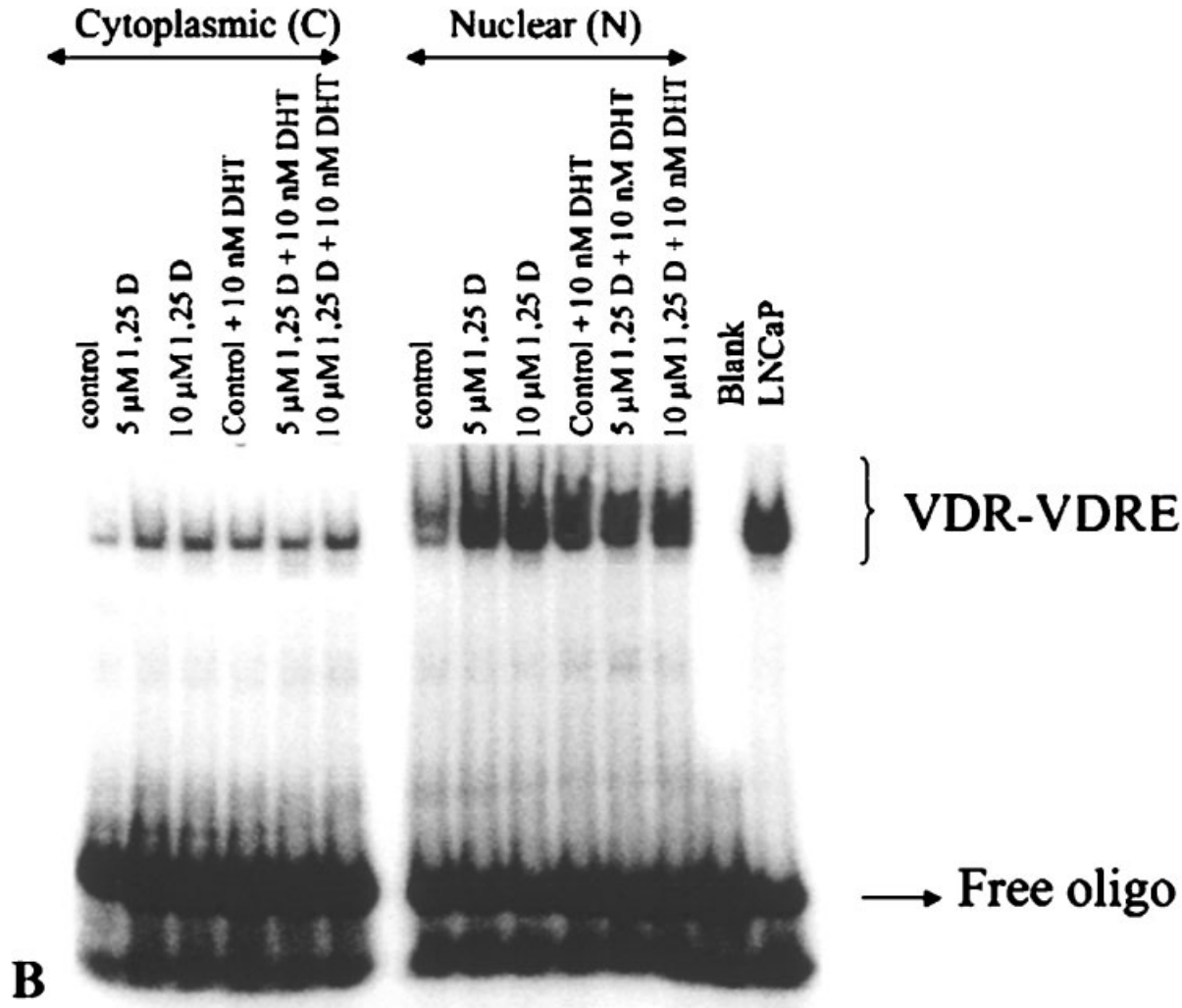


Fig. 2. (*Continued*)

the AR binding activity in the nuclear fraction, whereas 1,25 D at both 5 and 10 μM had the same effect in increasing the VDR binding activity in the nuclear fraction. Interestingly, addition of 10 nM DHT did not change the DNA binding activities of both AR and VDR, regardless of the presence or absence of 1,25 D.

To test the specificity of the binding, competition assays were performed with cold unlabeled AR and VDR consensus oligonucleotides. The competition assays showed that cold AR and VDR oligonucleotides at 10, 50, and 100 ng competed off the binding activity of both AR and VDR (Fig. 3). To further confirm the specificity in the binding activities, mutant consensus ARE and VDRE that contained mutations in their ability to bind the receptors were also tested in the binding assays, and these studies suggested that AR and VDR binding activities were specific (results not shown).

To further investigate how 1,25 D and DHT may influence the AR and VDR distribution, immunofluorescence analyses to examine the localization of these steroid receptors in the 267B-1 cells following exposures to 10 μM 1,25 D in the presence or absence of DHT were performed. These results showed that both AR and VDR (Figs. 4 and 5, respectively) were localized in both cytoplasmic and nuclear compartments of the 267B-1 cells. However, the staining pattern of these receptors showed that both AR and VDR were present at higher levels in the nuclear compartment. The results also demonstrated that addition of 1,25 D in the presence or absence of DHT did not change the levels of VDR protein expression, which is in

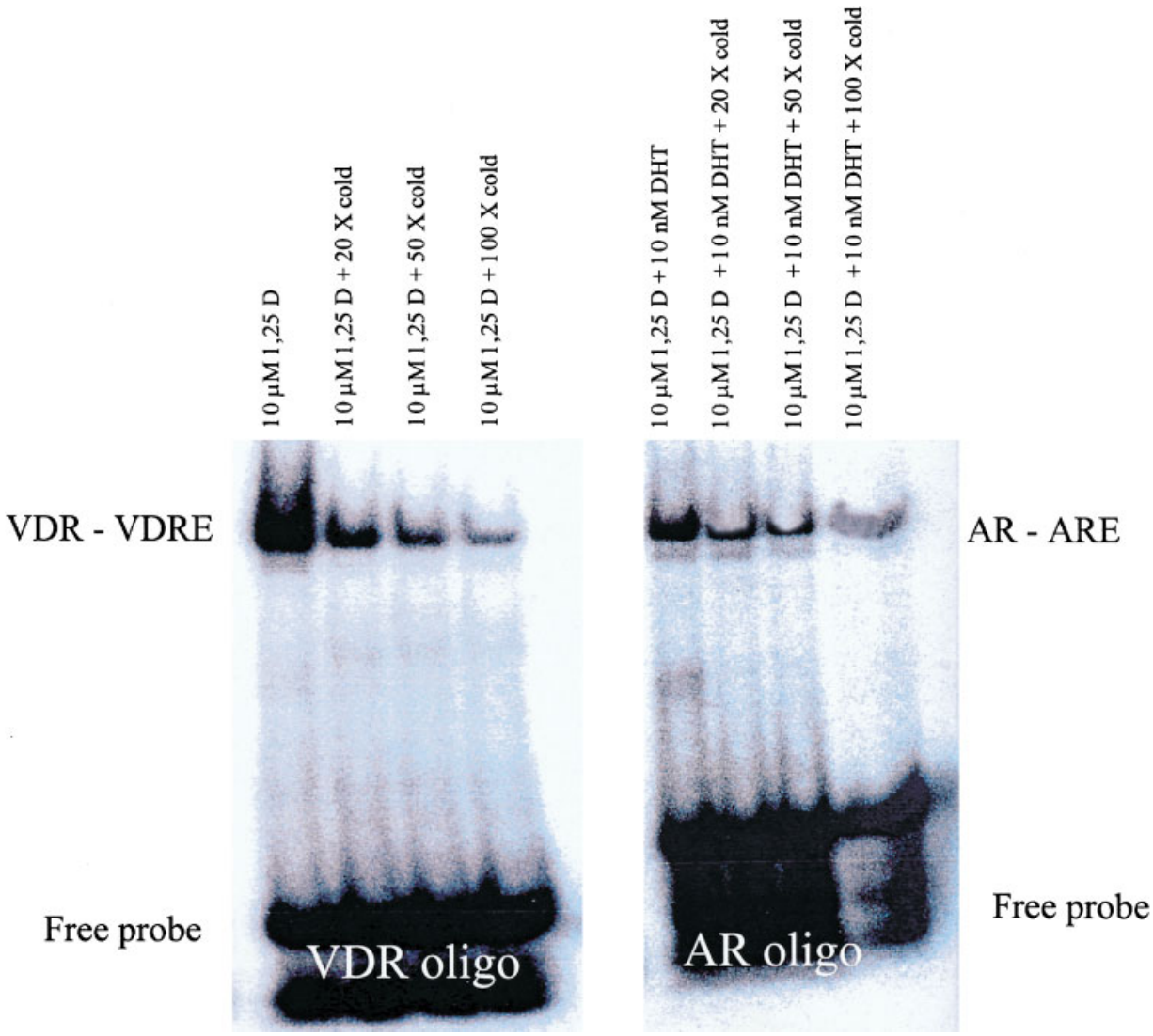


Fig. 3. Competition assay to test for binding specificity using AR and VDR oligonucleotides with the presence of cold, unlabeled oligonucleotides at 10 (20×), 50 (50×), and 100 (100×) ng, respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com]

agreement with our immunoblot results. It is interesting to point out that the AR immunoblot analyses (Fig. 1A) showed that AR was predominantly present in the cytoplasmic fraction of 267B-1 cells treated with either ethanol (control) or 1,25 D, whereas the immunofluorescence analyses showed staining of AR (Fig. 4) in both cytoplasmic and nuclear fractions. The differences in the AR protein level between the control and 1,25 D treated groups were also not detected by the immunofluorescence analyses. This could be due to differences in the sensitivity between immunoblot and immunofluorescence techniques. The AR protein may be detected more readily in the immunoblot analysis rather than in the immunofluorescence analysis.

DISCUSSION

In this study, the effects of 1,25 D and DHT on the regulation of steroid receptors (in particular AR and VDR) in the normal human neonatal prostate epithelial cell line 267B-1 are reported. This study focused on how 1,25 D in the presence or absence of DHT affects the distribution of AR and VDR in the cytoplasmic and nuclear compartments of the cells in terms of their protein levels and DNA binding activities. We have previously studied the effects of

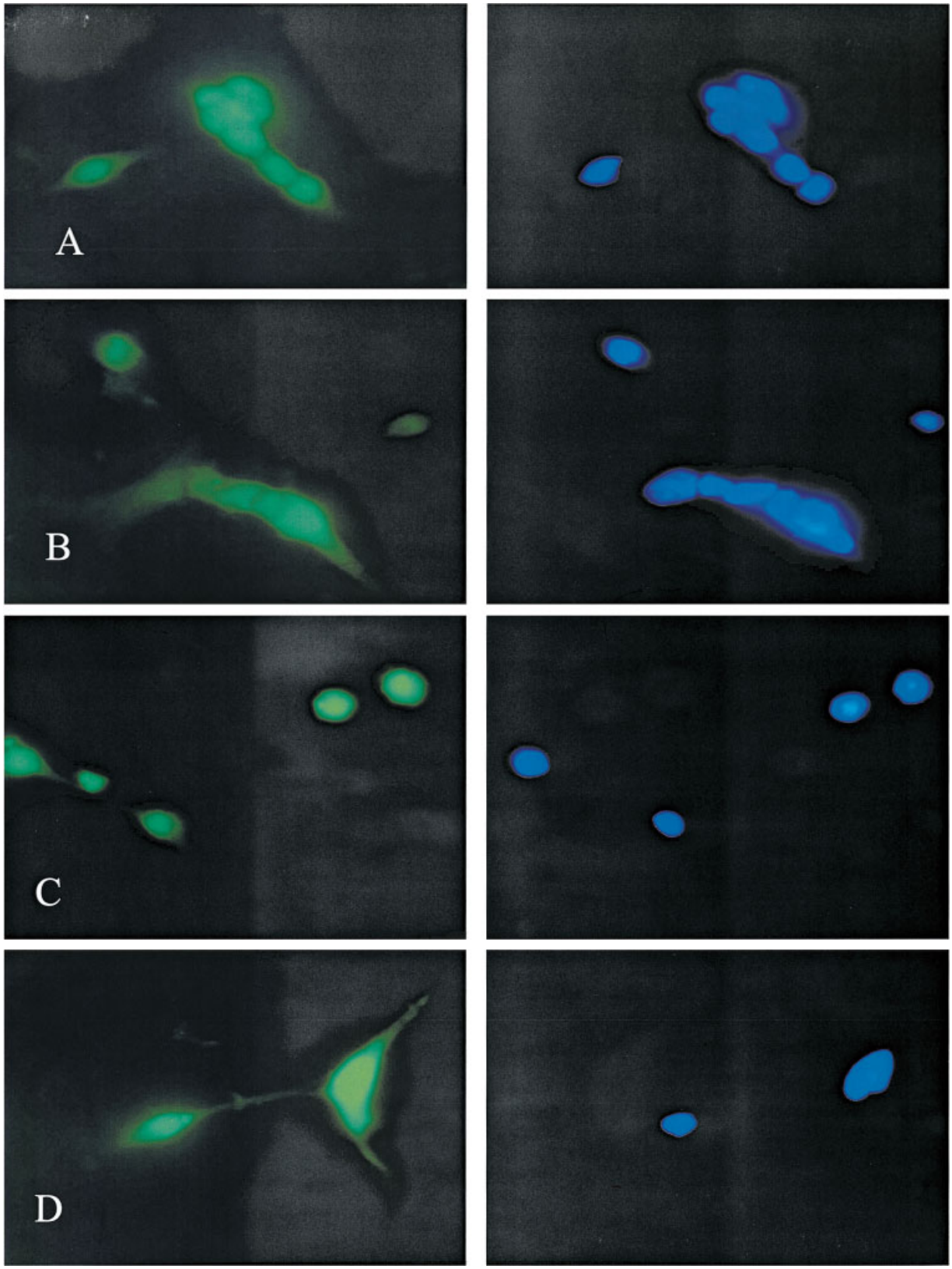


Fig. 4. Immunofluoresence analysis of AR from: (**A**) control, (**B**) 1,25 D, (**C**) control with DHT, and (**D**) 1,25 D with DHT treated groups. Figures on the **left panel** show staining on AR protein with mouse monoclonal anti-AR antibody and fluorescein conjugated goat anti-mouse IgG; figures on the **right panel** show nuclei staining with Hoechst. The cells were grown over night in four-well chamber slides and exposed to either ethanol (control) or 10 μM 1,25 D in the presence or absence of 10 nM DHT for another 24 h.

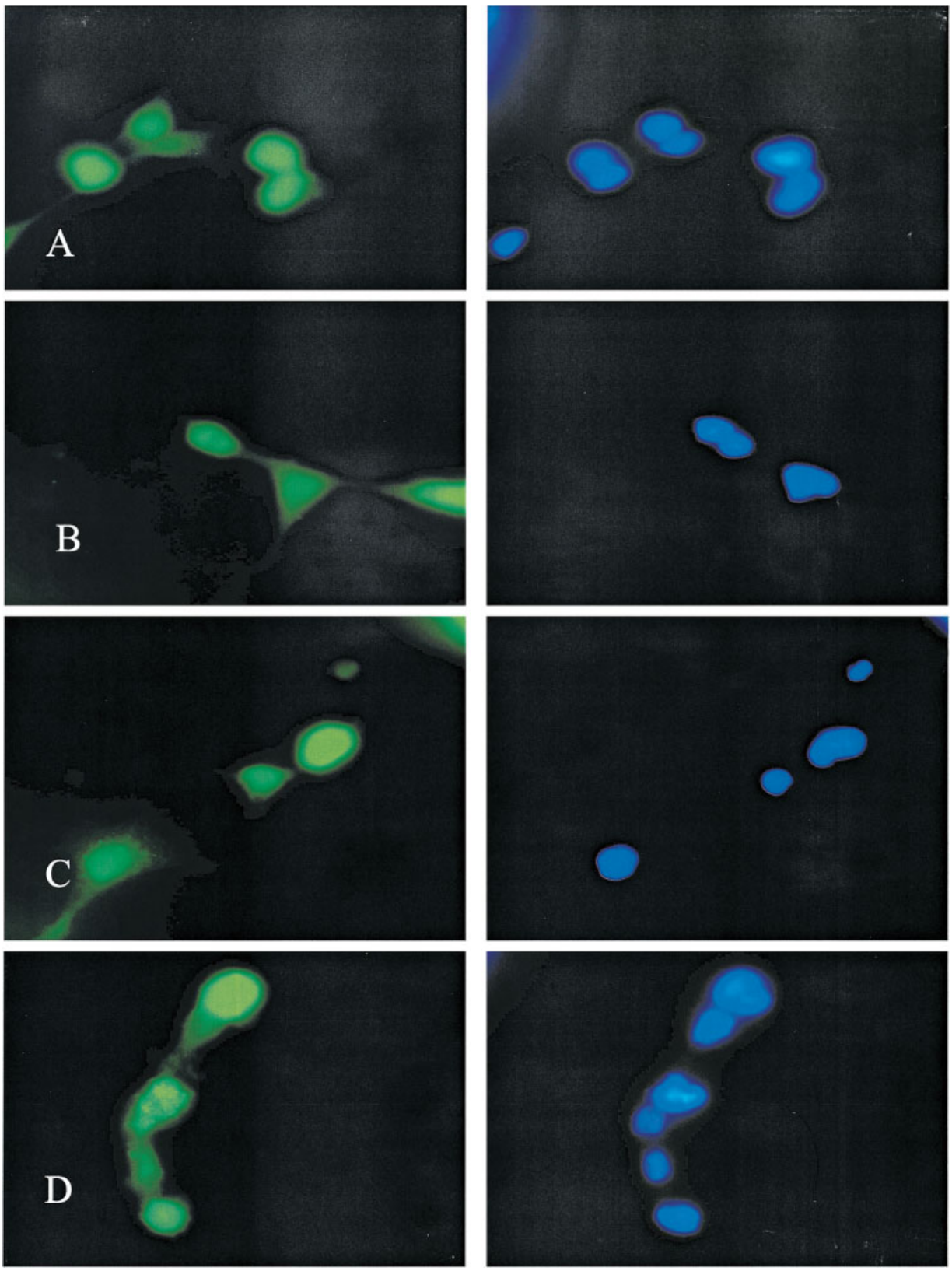


Fig. 5. Immunofluoresence analysis of vitamin D receptor (VDR) from: (**A**) control, (**B**) 1,25 D, (**C**) control with DHT, and (**D**) 1,25 D with DHT treated groups. Figures on the **left panel** show staining on VDR protein with rabbit polyclonal anti-VDR antibody and fluorescein conjugated goat anti-rabbit IgG; figures on the **right panel** show nuclei staining with Hoechst. The cells were grown over night in four-well chamber slides and exposed to either ethanol (control) or 10 μM 1,25 D in the presence or absence of 10 nM DHT for another 24 h.

1,25 D at super-physiological concentrations (0–100 μM) on the 267B-1 cells, and have shown that 1,25 D inhibits the growth of 267B-1 cells with and IC_{50} (concentration for 50% inhibition) of 50 mM when the cells were treated for 24 h [Konety et al., 2000]. Based on these results, the 267B-1 cells were treated with 1,25 D at 5 and 10 μM in the presence or absence of 10 nM DHT. These concentrations of 1,25 D were effective in inhibiting cell growth without generating any cytotoxicity on the cells.

The results demonstrate that 267B-1 cells express AR protein at 110 kDa and when these cells were exposed to 1,25 D at 5 and 10 μM for 24 h, the AR protein level was increased by 2- to 2.5-fold in both the cytoplasmic and nuclear fractions, whereas 1,25 D increased the AR DNA-binding activity only in the nuclear fraction. When 1,25 D was administered along with DHT, the AR protein level was increased by about 14- to 17-fold in the nuclear fraction, whereas the DNA binding activity remained unchanged. Previous studies by Feldman's laboratory [Zhao et al., 1999, 2000] and others [Hsieh et al., 1996; Hsieh and Wu, 1997] have demonstrated that 1,25 D significantly upregulates the AR mRNA levels in human prostate cancer cell lines LNCaP, MDA PCa 2a, and MDA PCa 2b. 1,25 D is also demonstrated by Feldman's laboratory [Zhao et al., 1999] to upregulate the AR protein level in LNCaP cells. Using Casodex as anti-androgen, they also showed that the growth inhibitory actions of 1,25 D are androgen-dependent in LNCaP cells, whereas the growth inhibitory actions of 1,25 D in both MDA PCa 2a and MDA PCa 2b are androgen-independent [Zhao et al., 2000]. Our results support their findings in terms that 1,25 D upregulate AR expression at the protein level in both the cytoplasmic and nuclear fractions of the 267B-1 cells.

1,25 D alone appears sufficient to upregulate the AR protein level in both the cytoplasmic and nuclear compartments. Although the increase in AR DNA binding activity following 1,25 D exposure was observed only in the nuclear fraction, we speculate that there could be a "shuffling" effect of AR protein between the cytoplasmic and nuclear compartments. It is possible that AR protein levels are initially upregulated by 1,25 D in the nuclear compartment. The initial increase in AR protein in the nucleus may recruit other nuclear co-factors to bind to the ARE to start the transcription machinery. Once the binding activity was enhanced by the co-factors, some of the AR protein may be exported out to the cytoplasmic compartment by some unknown chaperone proteins. Thus the increase in the DNA binding activity could be reflective/indicative of the binding of the co-factors along with the active AR to ARE in the nucleus. The fact that exposures of 1,25 D along with DHT increased the AR protein level in the nuclear compartment but not the DNA binding activity suggests that the presence of DHT may somehow maintain the AR protein in the nucleus by forming ligand–receptor complex thus not generating any binding activity. When DHT is absent, the AR may become free and be able to recruit other co-factors to activate binding to the DNA. Further investigations on involvement of nuclear co-factors for AR at transcription level, as well as involvement of chaperone proteins that may be involved in transporting or "shuffling" the AR protein between the cytoplasmic and nuclear compartments must still be established in order to ascertain this concept.

The 267B-1 cells also express VDRs at molecular weights ranging from 48 to 55 kDa. The presence of VDRs in different cell types has been reported by a number of investigators [Kivineva et al., 1998; Langub et al., 2000; Kallay et al., 2001] at molecular weights of 48, 50, 52, and 55 kDa. In this study, LNCaP cells, which have been reported to posses VDR [Miller et al., 1992], also express VDRs at different molecular weights of 48, 52, and 55 kDa. The VDR has previously been identified to interact closely with the nuclear matrix of human and rat genitourinary tissues at varying molecular weights (26, 37, 48, 52, 55, and 57 kDa) [Nangia et al., 1998]. The differences in these molecular weights could be representative of different isoforms or post-translational modification of the VDR. Similarly, the presence of VDRs at varying molecular weight in 267B-1 cells could be indicative of different splice variants or post-translationally modified VDRs.

When 267B-1 cells were exposed to 1,25 D in the presence or absence of DHT for 24 h, there was no difference in VDR protein levels in both the cytoplasmic and nuclear fractions. In contrast, 1,25 D alone was able to increase the VDR DNA binding activity in the nuclear compartment. Addition of DHT together with 1,25 D did not change the binding activity in the nuclear compartment. We speculate that 1,25 D may not

affect the VDR expression directly in terms of the amount/level of VDR protein. However, 1,25 D may affect the activity of the splice variants or the post-translationally modified forms of VDRs to recruit nuclear co-factors to bind to the VDRE and activate transcription. Several co-factors such as TFIIB and DRIP205 have been reported to interact with VDRs in order to activate transcription [Barletta et al., 2002; Jurutka et al., 2002]. These findings suggest that 1,25 D may increase the activity of the post-translationally modified form of VDRs (possibly phosphorylated forms) to recruit nuclear co-factors, which then leads to increase in DNA binding activity. A number of investigators have demonstrated that VDR phosphorylation may influence DNA binding, protein–protein interactions, as well as transactivation function [Hilliard et al., 1994; Desai et al., 1995; Buitrago et al., 2000; Jurutka et al., 2002]. Human VDR has been reported to be phosphorylated by protein kinases A and C, as well as casein kinase II [Jurutka et al., 2002]. In addition, phosphorylation of human VDR has also been reported to enhance transactivation of VDRE-linked reporter gene in COS-7 cells transfected with human VDR [Jurutka et al., 2002]. Using gel mobility shift and Western blot analyses, Desai et al. [1995] showed that binding of the VDR–RXR complex to both the osteocalcin and osteopontin VDREs was inhibited by serine–threonine phosphatase inhibitor. Thus, the results presented here suggest the possibility of VDR phosphorylation that may lead to DNA binding activity. Further investigations are clearly warranted to examine the possible involvement of different VDR splice variants as well as their ability to recruit nuclear co-factors in influencing the DNA binding activity as well as transcription activity.

Taken together, these studies suggest that 1,25 D influences the AR and VDR distribution in 267B-1 cells by two separate pathways: by upregulating AR protein and by upregulating VDR protein activity (proposed model outlined in Fig. 6). 1,25 D may initially upregulate AR protein expression in the nucleus, which then leads to recruitment of nuclear co-factors to increase AR DNA binding activity and possibly transcription. Once the transcription is finished, some of the AR protein may then be transported out to the cytoplasmic compart-

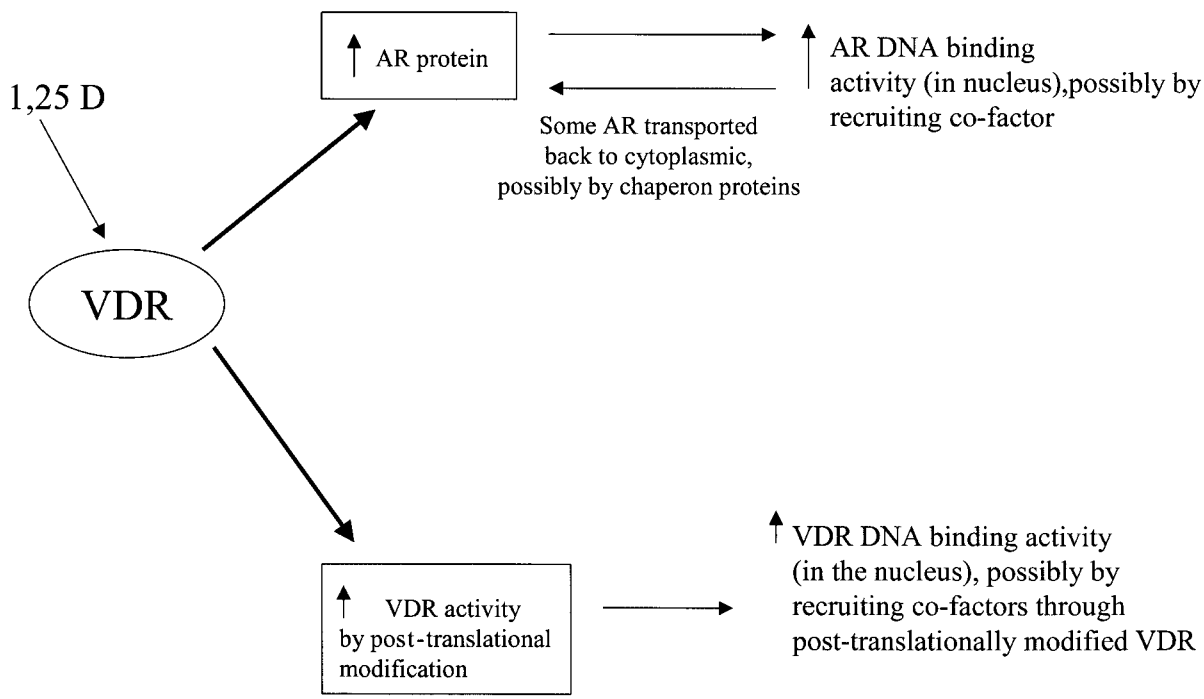


Fig. 6. Proposed model for the role of 1,25 D on the distribution of AR and VDR. 1,25 D may initially upregulate AR protein expression in the nucleus, which then leads to recruitment of nuclear co-factors to increase AR DNA binding activity and possibly transcription. AR would then be transported out to the cytoplasmic, possibly by chaperone proteins. On the other hand, 1,25 D does not upregulate the VDR protein level, but it may increase the DNA binding activities of some VDR splice variants to recruit nuclear co-factors, which would lead to an increase in DNA binding activity and possibly transcription.

ment, possibly via chaperone proteins. Thus, the increase observed in the AR protein level following 1,25 D exposure is reflected in both the cytoplasmic and nuclear fractions. On the other hand, 1,25 D does not upregulate the VDR protein level, but it may increase the DNA binding activities of some VDR splice variants to recruit nuclear co-actors, which leads to an increase in DNA binding activity and possibly transcription.

In summary, the experiments have demonstrated that: (i) 1,25 D regulates the AR and VDR in normal human prostate epithelial cells 267B-1, (ii) 1,25 D upregulates the AR protein level in both the cytoplasmic and nuclear fractions, whereas 1,25 D does not have any effects in the regulation of VDR protein level, and (iii) 1,25 D increases the AR and VDR DNA binding activities in the nuclear fractions, possibly by recruiting nuclear co-factors and influencing the activity of the receptors to bind to the DNA. Overall, it appears that 1,25 D actions on the normal prostate cells may be mediated independently through AR and VDR, respectively.

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