

# Human Prostate Cells Synthesize 1,25-Dihydroxyvitamin D<sub>3</sub> from 25-Hydroxyvitamin D<sub>3</sub><sup>1</sup>

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## Abstract

Epidemiological and laboratory data support a role for vitamin D in the growth and differentiation of human prostatic cells. These findings prompted us to ask whether prostatic cells could convert 25-hydroxyvitamin D<sub>3</sub> (25-OH-D<sub>3</sub>), the major circulating metabolite of vitamin D<sub>3</sub>, to 1,25-dihydroxyvitamin D<sub>3</sub> [1,25(OH)<sub>2</sub>D<sub>3</sub>], the hormonally active metabolite, in a manner similar to cultured human keratinocytes. Therefore, we investigated three well-characterized human prostate cancer cell lines, LNCaP, DU 145, and PC-3; two primary cultures of cells derived from noncancerous human prostates (one normal and one benign prostatic hyperplasia); and primary cultures of normal human keratinocytes for their ability to synthesize 1,25(OH)<sub>2</sub>D<sub>3</sub>. Assays were performed in the presence of 25-OH-D<sub>3</sub> as the enzyme substrate and 1,2-dianilinoethane, an antioxidant and free radical scavenger, and in the presence and absence of clotrimazole, a cytochrome P450 inhibitor.

DU 145 and PC-3 cells produced  $0.31 \pm 0.06$  and  $0.07 \pm 0.01$  pmol of 1,25(OH)<sub>2</sub>D<sub>3</sub>/mg protein/h, respectively. No measurable 1,25(OH)<sub>2</sub>D<sub>3</sub> was detected in LNCaP cells. The normal and benign prostatic hyperplasia primary cultures and keratinocyte cultures produced  $3.08 \pm 1.56$ ,  $1.05 \pm 0.31$ , and  $2.1 \pm 0.1$  pmol of 1,25(OH)<sub>2</sub>D<sub>3</sub>/mg protein/h, respectively, using a calf thymus receptor binding assay to measure 1,25(OH)<sub>2</sub>D<sub>3</sub> in the presence of 1,2-dianilinoethane. The identity of the analyte as 1,25(OH)<sub>2</sub>D<sub>3</sub> was supported by high performance liquid chromatography using [<sup>3</sup>H]25-OH-D<sub>3</sub> as the enzyme substrate and a solvent system that is specific for 1,25(OH)<sub>2</sub>D<sub>3</sub>. The production of 1,25(OH)<sub>2</sub>D<sub>3</sub> in the prostate cancer cell lines and in the primary cultures was completely inhibited in the presence of clotrimazole.

This report demonstrates that two of three human prostate cancer cell lines, as well as primary cultures of noncancerous prostatic cells, possess 1 $\alpha$ -hydroxylase activity and can synthesize 1,25(OH)<sub>2</sub>D<sub>3</sub> from 25-OH-D<sub>3</sub>. Together with recent data indicating that 1,25(OH)<sub>2</sub>D<sub>3</sub> inhibits the invasiveness of human prostate cancer cells (G. G. Schwartz *et al.*, *Cancer Epidemiol. Biomark. Prev.*, 6: 727–732, 1997), these data suggest a potential role for 25-OH-D<sub>3</sub> in the chemoprevention of invasive prostate cancer.

## Introduction

Apart from its role in calcium homeostasis, 1,25(OH)<sub>2</sub>D<sub>3</sub> is now known to play important roles in the regulation of cell growth and differentiation (1). The synthesis of 1,25(OH)<sub>2</sub>D<sub>3</sub> begins with the cutaneous production of vitamin D after exposure to sunlight or after the intestinal absorption of vitamin D<sub>2</sub> or vitamin D<sub>3</sub> obtained from the diet. To become biologically active, vitamin D must undergo two hydroxylation steps. The first hydroxylation occurs in the liver at the 25th carbon position, forming 25-OH-D<sub>3</sub>, the major circulating metabolite of vitamin D. The second hydroxylation occurs in the kidney at the 1 $\alpha$  position, forming 1,25(OH)<sub>2</sub>D<sub>3</sub>, the hormonally active metabolite (2). Although the kidney is the major source of 1,25(OH)<sub>2</sub>D<sub>3</sub>, the enzyme that converts 25-OH-D<sub>3</sub> to 1,25(OH)<sub>2</sub>D<sub>3</sub>, 1 $\alpha$ -hydroxylase, is also present in several types of nonrenal cells, *e.g.*, activated macrophages and keratinocytes (3, 4). The production of 1,25(OH)<sub>2</sub>D<sub>3</sub> in these cells suggests an autocrine/paracrine role for 1,25(OH)<sub>2</sub>D<sub>3</sub> in which it locally modulates cell proliferation and differentiation (5, 6).

Considerable evidence indicates that 1,25(OH)<sub>2</sub>D<sub>3</sub> modulates the growth and differentiation of prostatic cells (7). This evidence includes the ubiquitous presence of receptors for 1,25(OH)<sub>2</sub>D<sub>3</sub> (vitamin D receptors) in human prostatic cells (8–10), and the antiproliferative and prodifferentiating effects of 1,25(OH)<sub>2</sub>D<sub>3</sub> on these cells *in vitro* and *in vivo* (11–14). These findings led us to evaluate whether prostatic cells also possess 1 $\alpha$ -hydroxylase activity. We investigated three well-characterized human prostate cancer cell lines, LNCaP, DU 145, and PC-3, and two primary cultures of cells derived from noncancerous human prostates. We report that two of these cell lines, DU 145 and PC-3, as well as the primary cultures, can synthesize 1,25(OH)<sub>2</sub>D<sub>3</sub> from its precursor, 25-OH-D<sub>3</sub>.

## Materials and Methods

**Cell Lines.** DU 145, PC-3, and LNCaP cell lines were obtained from the American Type Culture Collection (Rockville, MD). All cell lines were tested and found to be free of *Mycoplasma* contamination.

**Culture Conditions.** Prostate cancer cell lines were cultured following several passages *in vitro*. Cells were routinely cultured in complete medium [RPMI 1640 supplemented with

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10% fetal bovine serum (HyClone Labs, Logan, UT) and 10 µg/ml gentamicin (Life Technologies, Inc., Gaithersburg, MD). Cells were seeded in 35-mm culture dishes (Corning-Costar, Cambridge, MA) at  $1 \times 10^5$  cells/dish in complete medium. Medium was changed to a serum-free medium (RPMI 1640 containing 10 µg/ml insulin, 10 µg/ml transferrin, and 1 ng/ml selenous acid) 24 h before adding 25-OH-D<sub>3</sub> (15).

Primary cultures of human prostatic epithelial cells were established and characterized as described by Lokeshwar *et al.* (15). Prostatic epithelial cells cultured in a serum-free defined medium (Mammary Epithelial Growth Medium; Clontech, San Diego, CA) express luminal epithelium-specific cytokeratins (cytokeratins 8 and 18) as detected immunohistochemically using an anti-cytokeratin antibody, CAM 5.2 (Becton Dickinson, Mountain View, CA). The serum-free medium contains MCDF170 supplemented with 25 ng/ml epidermal growth factor, 0.5 µg/ml hydrocortisone,  $1 \times 10^{-4}$  methanolamine, 5 µg/ml insulin, 5 µg/ml transferrin, and 70 µg/ml whole bovine pituitary extract (16).

Prostatic cells used for this study were at their first passage *in vitro* and were cultured in the serum-free RPMI 1640 during incubation with vitamin D metabolites. We investigated two primary cultures: NP96-5, cultured from the histologically normal prostate of a 23-year-old Caucasian organ donor; and BPH96-11, cultured from an open prostatectomy specimen of a 56-year-old Caucasian with BPH.<sup>3</sup> Tissues were obtained according to a human subjects protocol approved by a University Institutional Review Board. Histological examination of adjacent tissue sections taken from specimens used for cultures confirmed their identity as normal or BPH cultures.

**Keratinocyte Culture.** Because 1-hydroxylase activity has been well-established in keratinocytes, we used cultured human keratinocytes for comparison with the prostatic cultures. Keratinocytes were grown in culture following a modification of the method of Rheinwald and Green (17), as described in detail previously (18, 19). Briefly, keratinocytes were obtained from neonatal foreskin after trypsinization at 4°C. Keratinocytes were plated and grown on lethally irradiated 3T3 fibroblast feeder cells in a serum-free basal medium containing 0.15 mM calcium and supplemented with growth factors including bovine pituitary extract (3 µg/ml), epidermal growth factor (25 ng/ml), insulin (5 µg/ml), and prostaglandin E<sub>1</sub> (50 ng/ml). To enhance the plating efficiency, 0.1 µg/ml cholera toxin and 200 ng/ml hydrocortisone were added into the medium during the initial plating of the primary culture and the subsequent subcultures. Cells were fed and maintained without cholera toxin and hydrocortisone and used for enzyme assay.

**25-Hydroxyvitamin D-1α-hydroxylase (1α-Hydroxylase) Assay.** 1α-Hydroxylase activity was determined in monolayer cultures of the cell lines and primary cultures. The assays were performed in the presence of 50 nM 25-OH-D<sub>3</sub> as the enzyme substrate (20) and DPPD (Sigma-Aldrich, Allentown, PA), an antioxidant and a known inhibitor of free radical-generated 1,25(OH)<sub>2</sub>D (21, 22). Assays were also performed in the presence and absence of the cytochrome P450 inhibitor, clotrimazole (20 µM; Sigma Chemical Co., St. Louis, MO; Ref. 23). After 2 h of incubation at 37°C, cultures were placed on ice, and media were removed. Immediately afterward, 1 ml of methanol was added to extract 25-OH-D<sub>3</sub> and 1α,25(OH)<sub>2</sub>D<sub>3</sub>. A 10-µl aliquot of 1α,25-dihydroxy[26,27-methyl-<sup>3</sup>H]vitamin D<sub>3</sub> con-

**Table 1** Synthesis of 1,25(OH)<sub>2</sub>D<sub>3</sub> by three human prostatic cancer cell lines and primary cultures of prostate cells and keratinocytes in the presence and absence of the cytochrome P450 inhibitor, clotrimazole.

Cell type	1,25(OH) <sub>2</sub> D <sub>3</sub> produced (pmol/mg protein/h) <sup>a</sup>	
	P450 inhibitor absent	P450 inhibitor present
DU 145	0.31 ± 0.06 (n = 6)	Undetectable
PC-3	0.07 ± 0.01 (n = 6)	Undetectable
LNCaP	Undetectable	Undetectable
NP96-5	3.08 ± 1.56 (n = 3)	Undetectable
BPH96-11	1.05 ± 0.31 (n = 3)	Undetectable
Human keratinocytes	2.1 ± 0.1 (n = 3)	Undetectable

<sup>a</sup> n, number of replications; errors are SE.

taining 1000 cpm radioactivity in ethanol was also added to each well for calculating the recovery. After extraction at room temperature for 15 min, the methanol extract was transferred to a glass test tube, and the cells were washed with an additional 0.5 ml of methanol. The extract and wash were combined, dried down with a stream of nitrogen, and redissolved in 1 ml of acetonitrile, followed by the addition of 1 ml of 0.4 M K<sub>2</sub>HPO<sub>4</sub> (pH 10.0). The mixture was then applied to a C-18-OH reversed-phase cartridge (24, 25). The fraction containing 1,25(OH)<sub>2</sub>D<sub>3</sub> was then dried down under a stream of nitrogen and reconstituted in 200 µl of ethanol. Two 40-µl aliquots were taken for 1,25(OH)<sub>2</sub>D<sub>3</sub> analysis by thymus receptor binding assay as described by Chen *et al.* (24).

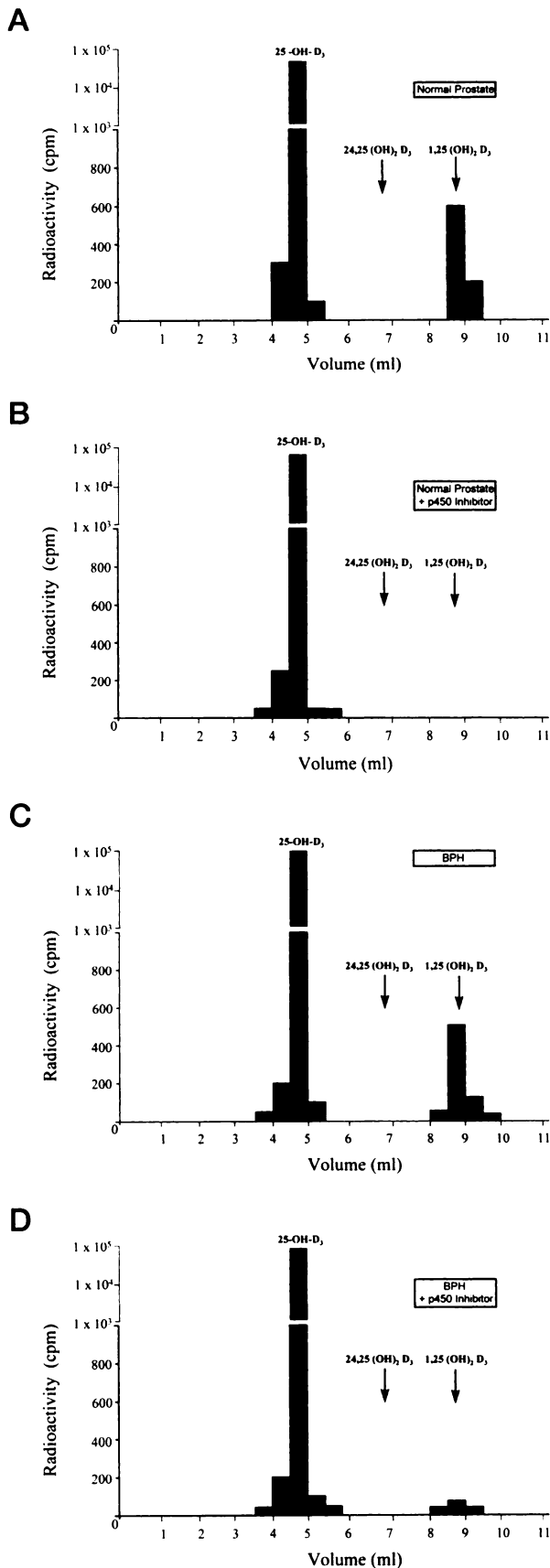
The 1α-hydroxylase activity was also determined in the primary cultures of prostatic cells by using radioactive 25-OH-D<sub>3</sub> (20 ng of nonradioactive 25-OH-D<sub>3</sub> and 0.91 µCi of [<sup>3</sup>H]25-OH-D<sub>3</sub>) instead of only nonradioactive 25-OH-D<sub>3</sub> as a substrate. The incubation medium, time, temperature, extraction procedure, and C-18-OH cartridge chromatography were the same as described for the thymus receptor method, except that the fraction eluted from C-18-OH cartridge with 10% methylene chloride in hexane (25-OH-D<sub>3</sub> fraction) and with 6% isopropanol in *n*-hexane [1,25(OH)<sub>2</sub>D<sub>3</sub> and 24,25(OH)<sub>2</sub>D<sub>3</sub> fraction; Ref. 25] was dried down under nitrogen and redissolved in methylene chloride:isopropanol (19:1) for high performance liquid chromatographic analysis as described below.

**HPLC.** A 30-µl aliquot was mixed with 10 µl each of standard nonradioactive 25-OH-D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> and was applied to an Econosphere silica column (5-µm particle size, 250 × 4.6 mm) with a flow rate of 0.5 ml/min using a methylene chloride:isopropanol (19:1) solvent system as the mobile phase (26, 28). Thirty fractions were collected at 1-minute intervals from each HPLC. Fractions were allowed to evaporate by air to dryness, followed by the addition of scintillation fluid and counting with a beta counter. The retention volume for 25-OH-D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub>, and 1,25(OH)<sub>2</sub>D<sub>3</sub> was calibrated by applying standard 25-OH-D<sub>3</sub>, 24,25(OH)<sub>2</sub>D<sub>3</sub>, and 1,25(OH)<sub>2</sub>D<sub>3</sub> to the HPLC column before, during, and after unknown sample application. The protein concentration in each 35-mm dish was determined as described by Bradford (27). The enzyme activity was expressed as pmol of 1,25(OH)<sub>2</sub>D<sub>3</sub>/mg protein/h.

## Results

Table 1 demonstrates the production of 1,25(OH)<sub>2</sub>D<sub>3</sub> in three cultured human prostate cancer cell lines, two primary cultures of human prostatic cells, and cultured normal human keratinocytes. DU 145 and PC-3 produced 0.31 ± 0.06 and 0.07 ± 0.01

<sup>3</sup> The abbreviations used are: BPH, benign prostatic hyperplasia; DPPD, 1,2-dianilinoethane; HPLC, high-performance liquid chromatography.



pmol of 1,25(OH)<sub>2</sub>D<sub>3</sub>/mg protein/h, respectively. The production of 1,25(OH)<sub>2</sub>D<sub>3</sub> was completely inhibited in the presence of clotrimazole. Conversely, no measurable 1,25(OH)<sub>2</sub>D<sub>3</sub> was detected in LNCaP cells. We also grew primary cultures of prostatic cells from two patients, NP96-5 (normal prostate) and BPH96-11 (BPH), and determined their enzyme activities in the presence of DPPD and in the presence and absence of clotrimazole. The NP96-5 and BPH96-11 cultures produced  $3.08 \pm 1.56$  pmol/mg protein/h and  $1.05 \pm 0.31$  pmol/mg protein/h 1,25(OH)<sub>2</sub>D<sub>3</sub>, respectively, in the presence of DPPD and in the absence of cytochrome P450 inhibitor. The 1 $\alpha$ -hydroxylase activity found in the two primary cultures of prostatic cells was comparable with that found in normal human keratinocytes (Table 1). As in the two cell lines, the production of 1,25(OH)<sub>2</sub>D<sub>3</sub> in the primary cultures of prostatic cells and keratinocytes was completely inhibited in the presence of clotrimazole (Table 1).

The enzyme activity detected by thymus receptor binding assay in the primary cultures of prostatic cells was further supported by HPLC analysis using a solvent system that specifically separates 1,25(OH)<sub>2</sub>D<sub>3</sub> from another metabolite of 25-OH-D<sub>3</sub>, 10-oxo-19-nor-25-OH-D<sub>3</sub>. This metabolite, which is present in significant quantity in kidney homogenates of rats and chicken, is known to comigrate with 1,25(OH)<sub>2</sub>D<sub>3</sub> on normal phase HPLC with the *n*-hexane:isopropanol (9:1) solvent system, the traditional chromatographic system for isolating 1,25(OH)<sub>2</sub>D<sub>3</sub>. Therefore, to ensure that 1,25(OH)<sub>2</sub>D<sub>3</sub> was separated from any 10-oxo-19-nor-25-OH-D<sub>3</sub> present, we used the methylene chloride:isopropanol (19:1) normal phase solvent system. In addition, 1 $\alpha$ -hydroxylase activity was determined by using [<sup>3</sup>H]25-OH-D<sub>3</sub> as substrate.

Fig. 1 demonstrates typical HPLC chromatograms of the two primary prostate cell cultures in the presence and absence of clotrimazole.

## Discussion

After incubation with 25-OH-D<sub>3</sub>, DU 145 and PC-3 prostate cancer cell lines and the two primary cultures produced detectable levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> (Table 1). There are several reasons for believing that the product is authentic 1,25(OH)<sub>2</sub>D<sub>3</sub>: (a) the radioactive metabolite generated from [<sup>3</sup>H]25-OH-D<sub>3</sub> comigrated with authentic standard 1,25(OH)<sub>2</sub>D<sub>3</sub> in an HPLC solvent well-established for the separation and identification of 1,25(OH)<sub>2</sub>D<sub>3</sub> (26, 28). Not only was 1,25(OH)<sub>2</sub>D<sub>3</sub> well-separated from 24,25(OH)<sub>2</sub>D<sub>3</sub> (Fig. 1), but it was also separated from a commonly found metabolite, 10-oxo-19-nor-25-OH-D<sub>3</sub>; (b) the enzyme product was detected by using a specific calf

**Fig. 1.** HPLC elution profile of tritium activity of lipid extracts from cultured normal human prostate primary cultures or BPH cultures incubated with [<sup>3</sup>H]25-OH-D<sub>3</sub>. Second passage of normal human prostate cells (A and B) or cells derived from BPH cultures (C and D) were incubated with nonradioactive 50 nM 25-OH-D<sub>3</sub>, 0.91  $\mu$ Ci/nmol [<sup>3</sup>H]25-OH-D<sub>3</sub>, and 10  $\mu$ M DPPD at 37°C for 2 h in the absence (A and C) or presence of 20  $\mu$ M clotrimazole (B and D). The lipid extract was applied to a C-18-OH cartridge. The fraction eluted from the cartridge with 6% isopropyl alcohol in *n*-hexane was dried down under nitrogen and reconstituted in the normal phase solvent containing methylene chloride: isopropyl alcohol (19:1). Aliquots of 30  $\mu$ l of sample plus 10  $\mu$ l of each 25-OH-D<sub>3</sub> (100 ng) and 1,25(OH)<sub>2</sub>D<sub>3</sub> (100 ng) as standards (as indicated) were run simultaneously on a 5- $\mu$ m particle size Econosphere normal phase silica column using 5% isopropyl alcohol in methylene chloride at a flow rate of 0.5 ml/min. Thirty 1-min fractions were collected, dried down under nitrogen, and counted for radioactivity. The retention volume for 24,25(OH)<sub>2</sub>D<sub>3</sub> was determined by applying standard 24,25(OH)<sub>2</sub>D<sub>3</sub> along with 25-OH-D<sub>3</sub> and 1,25(OH)<sub>2</sub>D<sub>3</sub> into the same HPLC chromatography and using the same mobile phase for elution.



thymus receptor binding assay, which has very poor binding affinity for 24,25(OH)<sub>2</sub>D<sub>3</sub> and 25,26(OH)<sub>2</sub>D<sub>3</sub> (25). In addition, possible contamination by 25-OH-D<sub>3</sub> was eliminated because the fraction used for analysis of 1,25(OH)<sub>2</sub>D<sub>3</sub> contained no 25-OH-D<sub>3</sub> (24, 25); (c) the 100% inhibition of 1,25(OH)<sub>2</sub>D<sub>3</sub> production by the specific cytochrome P450 inhibitor, clotrimazole, further suggests that a cytochrome P450-dependent 1 $\alpha$ -hydroxylase similar to that found in kidney proximal tubular cells is present in these prostatic cells (29); and (d) the identity of the analyte as [<sup>3</sup>H]1,25(OH)<sub>2</sub>D<sub>3</sub> from the radioactive substrate was supported by including DPPD in the presence and absence of clotrimazole during HPLC analysis. The addition of the P450 inhibitor almost totally inhibited the conversion of [<sup>3</sup>H]25-OH-D<sub>3</sub> to [<sup>3</sup>H]1,25(OH)<sub>2</sub>D<sub>3</sub> (Fig. 1, B and D). Thus, DU 145 and PC-3 human prostate cancer cells, as well as two primary cultures derived from noncancerous human prostates, possess 1 $\alpha$ -hydroxylase activity and are capable of converting the major circulating metabolite of vitamin D<sub>3</sub>, 25-OH-D<sub>3</sub>, to the hormonally active vitamin D metabolite, 1,25(OH)<sub>2</sub>D<sub>3</sub>.

No detectable 1,25(OH)<sub>2</sub>D<sub>3</sub> was produced by LNCaP cells. 24-Hydroxylase activity has also been reported to be low or undetectable in LNCaP and highest in DU 145 (8, 9). However, for reasons discussed above, it is unlikely that the present findings for 1 $\alpha$ -hydroxylase activity are contaminated by 24,25(OH)<sub>2</sub>D<sub>3</sub>.

Both primary cultures of noncancerous prostate cells produced 1,25(OH)<sub>2</sub>D<sub>3</sub> at levels 10–40-fold higher than the cell lines. Very high levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> were observed from the culture derived from the 23-year-old organ donor. The quantities of 1,25(OH)<sub>2</sub>D<sub>3</sub> produced by the two primary noncancerous human prostate cells,  $3.08 \pm 1.56$  and  $1.05 \pm 0.31$  pmol/mg protein/h (normal and BPH, respectively), are comparable with those produced in cultured human keratinocytes ( $2.1 \pm 0.1$  pmol/mg protein/h) and to the HEP 62 hepatoma cell line ( $2.3$  pmol/mg protein/h; Ref. 30) and human T-lymphotropic virus-transformed lymphocytes ( $1.6$  pmol/mg protein/h; Ref. 31). These values are at least 10-fold higher than those reported in other extrarenal sites such as human bone cells ( $0.068$  pmol/mg protein/h; Ref. 32).

The enzyme activity found in the primary cultures of prostate cells is comparable with that found in primary cultures of renal proximal tubular cells,  $5.3$ – $5.6$  pmol/h/mg protein (33). Because it is established that renal 1 $\alpha$ -hydroxylase is the major (and probably the sole) source of the enzyme responsible for maintaining the circulating concentration of 1,25(OH)<sub>2</sub>D<sub>3</sub> under normal physiological conditions, we believe that the amount of 1,25(OH)<sub>2</sub>D<sub>3</sub> produced by prostatic cells is likely to be physiologically significant, at least for the microenvironment of prostatic cells. We have shown recently that levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> as low as  $10^{-11}$  M can significantly inhibit the invasiveness of human prostate cancer cells through an artificial basement membrane composed of human amnions (34).

The synthesis of 1,25(OH)<sub>2</sub>D<sub>3</sub> by prostatic cells *in vitro* may clarify an important question concerning the epidemiology of prostate cancer with respect to vitamin D. Hanchette and Schwartz (35) have shown that prostate cancer mortality rates per county in the contiguous United States are inversely correlated with levels of UV radiation. They interpreted these findings to suggest that 1,25(OH)<sub>2</sub>D<sub>3</sub> maintains the differentiated phenotype of prostatic cells and that low levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> may increase the risk for fatal prostate cancer (36). However, although systemic levels of 25-OH-D<sub>3</sub> are known to be dependent on exposure to UV radiation, systemic levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> in normal individuals are very tightly regulated and generally are not correlated with systemic levels

of 25-OH-D<sub>3</sub> (37, 38). Thus, a mechanism by which UV radiation or vitamin D could result in increased exposure of prostatic cells to 1,25(OH)<sub>2</sub>D<sub>3</sub> was unclear. This apparent paradox would be resolved if, like prostatic cells *in vitro*, prostatic cells *in vivo* synthesize 1,25(OH)<sub>2</sub>D<sub>3</sub> locally from 25-OH-D<sub>3</sub>.

These findings also have implications for the design and interpretation of biomarker studies of prostate cancer with respect to vitamin D. For example, if the prostate gland synthesizes 1,25(OH)<sub>2</sub>D<sub>3</sub> *in vivo*, then systemic levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> measured in serum (39, 40) may not reflect levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> at the level of the target cell. Thus, the risk of prostate cancer may be influenced by intraprostatic as well as systemic levels of vitamin D. A dissociation between systemic and intraprostatic hormone levels in men has been demonstrated for another steroid hormone that is activated enzymatically by prostatic cells, dihydrotestosterone (41).

Finally, the synthesis of 1,25(OH)<sub>2</sub>D<sub>3</sub> by prostatic cells may have implications for the use of vitamin D metabolites in prostate cancer chemoprevention. It is now well-established that 1,25(OH)<sub>2</sub>D<sub>3</sub> exerts antiproliferative and pro-differentiating effects on normal and cancerous prostate cells. Moreover, we have shown that *in vitro*, physiological levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> significantly inhibit the invasiveness of DU 145 cells through an artificial basement membrane. This inhibition of invasiveness is correlated with a decrease in the secreted levels of type IV collagenase (matrix metalloproteinases 2 and 9; Ref. 34). We have shown that 1,25(OH)<sub>2</sub>D<sub>3</sub> also exhibits impressive antimetastatic effects on prostate cancer cells *in vivo* (42). These findings support a role for 1,25(OH)<sub>2</sub>D<sub>3</sub> in the chemoprevention of invasive prostate cancer. However, 1,25(OH)<sub>2</sub>D<sub>3</sub> may not be suitable for use as a chemopreventive agent because of the risk of hypercalcemia (43). Our present findings raise the possibility that, by increasing the available substrate, supplementation of men with 25-OH-D<sub>3</sub> could promote the local synthesis of 1,25(OH)<sub>2</sub>D<sub>3</sub> by prostatic cells. However, whether 25-OH-D<sub>3</sub> supplementation of men who are already vitamin D sufficient would promote the synthesis of physiologically significant levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> by prostatic cells *in vivo* remains to be demonstrated.

In summary, we report that DU 145 and PC-3 human prostate cancer cells and noncancerous human prostate cells in primary culture can convert 25-OH-D<sub>3</sub> to the hormonally active metabolite, 1,25(OH)<sub>2</sub>D<sub>3</sub>. Together with recent data indicating that vitamin D receptors are ubiquitous in prostatic cells and that 1,25(OH)<sub>2</sub>D<sub>3</sub> exerts antiproliferative, pro-differentiating, and antimetastatic effects in these cells, our data suggest that 1,25(OH)<sub>2</sub>D<sub>3</sub> may exert an autocrine/paracrine role in the prostate. These findings may provide a mechanism for the observed north-south gradient in prostate cancer mortality and support the potential use of 25-OH-D<sub>3</sub> in the chemoprevention of invasive prostate cancer.

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