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Expression Patterns of Vitamin D Receptor in Human Prostate

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Abstract UV exposure and serum levels of vitamin D have been linked in several studies with prostate cancer risk. At the cellular level, the principal action of vitamin D is mediated though vitamin D receptors (VDR). Since prostate cancer is a disease strongly associated with age, we examined the presence of VDR in normal prostate from donors of various ages to determine if the VDR expression pattern changed with age. We also compared the VDR expression in the peripheral and central zones of the prostate to determine if the expression pattern varied by location. Immunohistochemical studies were performed on paraffin-embedded tissue from cases selected by the following age decades; 10–19, 20–29, 30–39, 40–49, 50–59, and 60–69. Both the central and peripheral zones were examined for VDR expression. The intensity of VDR expression in prostate was compared with expression in different types of human tissues. Mean VDR expression was lowest in the 10–19 years of age group. The intensity of the nuclear VDR was higher though the fifth decade, and then declined in cases of ages 60–70. When multiple sections of the same donor prostate were compared, VDR expression was greater in the peripheral zone compared to the central zone. J. Cell. Biochem. 82: 566–572, 2001. © 2001 Wiley-Liss, Inc.

Key words: human prostate; vitamin D receptor; aging

Prostate cancer is a leading cause of cancer deaths around the world, and the leading risk factor is age [Puntoni et al., 1995; Majeed et al., 2000; Nakata et al., 2000]. The components of risk imposed by advancing age are not well understood, but are thought to be due in part to an environmental exposure. UV light and serum levels of vitamin D have been linked in some studies with prostate cancer risk [Schwartz and Hulka, 1990; Corder et al., 1992; Hanchette and Schwartz, 1992]. Schwartz described a relationship between decreased UV exposure and increased prostate cancer mortality [Schwartz and Hulka, 1990]. Further investigation is required to explore the VDR expression of normal prostate, and if changes occur with age.

VDR modulates the principal cellular response to 1,25-D. VDR is a protein which is highly conserved across species. Among the various isoforms and polymorphisms studied in relation to prostate cancer risk [Taylor et al., 1996 and Ingles et al., 1997], Habuchi et al showed that the *Bsm*I allele might provide a protective effect [Habuchi et al., 2000] against prostate cancer and benign prostatic hyperplasia. An in vitro study showed that cells, without VDR expression, could be transfected with VDR and become sensitive to the growth inhibitory effects of 1,25-D [Miller et al., 1995].

The presence of VDR has been shown to determine the ability of the target cells to respond to 1,25-D during development. The relative abundance of VDR varies during puberty, adolescence, adulthood, and aging adults. Horst et al. [1990] demonstrated that VDR levels in rat intestines and kidney [Koszewski et al., 1990] decreased in aging rats. Liang et al. [1994] corroborated those results by showing a 23% decrease in the mRNA of VDR in aging rats. In human beings, Ebeling et al. [1992] established that VDR decreases with age in duodenal biopsies of 35 females. No prior studies, however, have been performed to systematically examine the relationship between age and VDR

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expression in different zones of normal human prostate.

The peripheral zone is historically considered to be the site of the majority of prostate cancers. In human beings, the peripheral and central zones of the prostate differ substantially in terms of morphology. In normal prostate, the boundary between the two zones is distinct. It is postulated that the anatomical and histological differences reflect biological differences as well. For this reason, we investigated the VDR immunoreactivity occurring in the peripheral area of normal prostate compared to the central zone. Since human beings are one of a few known species to develop prostate cancer, it is important to understand the fundamental regulation of the prostate gland in them.

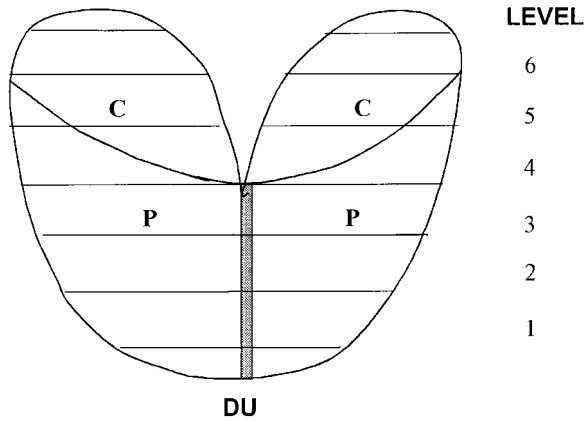

Fig. 1. Sectioned levels of the prostate from the distal urethal margin (DU) to the proximal bladder neck margin. C: Central zone, P: Peripheral zone.

MATERIALS AND METHODS

Case Selection

The prostate donors, for this investigation, were selected from the Western Pennsylvania Tissue Bank, after accession from the regional organ procurement agency CORE (Center for Organ Recovery and Education). Selection was based on the following criteria: (1) age of the donor based on the medical record, (2) documentation of the level of the sample in the pathology report, and (3) verification by a genitourinary pathologist that the tissue was free of adenocarcinoma or suspicious lesions.

Organ Sectioning. At this stage, the proximal bladder neck margin and distal urethal margin were removed, and the specimen was oriented so that the anterior surface faced up. This piece of tissue was, then, serially sectioned perpendicular to its plane, distal to proximal in 3–4 mm thick slices and labeled level 1 to 6 respectively (Fig. 1). The cut surfaces of all slices were examined for tumor, which grossly appears as a slightly raised, firm, yellow, tan, or white area distinct from the surrounding parenchyma. If tumor was observed, the case was not selected for the age or zonal comparison. Two of the cases were disqualified after inspection. The slices, selected, were fixed with 10% neutral/phosphate buffered formalin overnight.

The sample group consisted of 27 cases, and was distributed over the age categories 10–19 (4 cases); 20–29 (6 cases); 30–39 (3 cases); 40–49 (4 cases) 50–59 (6 cases); and 60–69 (4 cases). The scarcity of normal prostates, over the age of 60, prohibited measuring prostate VDR in patients in their seventies and eighties.

Immunohistochemistry

Paraffin-embedded tissues, from selected cases, were cut at 4 microns, deparaffinized in xylene and hydrated though graduated alcohols. The endogenous peroxidase was quenched in a 1:10 ratio of 30% H_2O_2: methanol for 15 min. Antigen retrieval was performed using citric acid (10X) solution at pH 6.0, heated with a microwave pressure cooker, according to Biogenex protocol (15 min at 100% power, followed by 5 min at 40% power). Following antigen retrieval, the slides were allowed to stand at room temperature for 60 min.

The protein blocking solution was normal goat serum diluted 1:10 in PBS. The slides were incubated in a humid chamber for 20 min with normal goat serum as the blocking solution. The primary antibody for vitamin D Receptor (Affinity Bioreagents, Inc., Golden CO, 1:300 dilution in PBS) was incubated with the tissue sections for 2 h at room temperature. After thorough rinsing, secondary antibody, biotinylated anti-rat (1:200) was incubated with tissue sections for 30 min. Avidin-Biotin complex was added according to the manufacturer's instructions (Vector, Burlington, VT). The chromogen, diaminobenzidine (DAB), was applied and then slides were counterstained in Shandon's hematoxylin for 3 min, dehydrated and coverslipped.

The same lot of antibody was utilized throughout the analysis, and the same reference case was included with each run. Negative controls

for 25% of the cases were included with each batch, and were treated identically except primary antibody was omitted from negative controls. Representative sections of skin, liver, and pancreas were selected from paraffin-embedded archives for positive controls. In addition, a checkerboard of normal and tumor tissues from Biogenex was tested with the VDR antibody to compare prostate VDR abundance with other human tissues.

Three investigators were blinded with regard to the age of the patient, during the analysis. The slides were scored on the basis of the intensity and number of nuclei which stained positively on a scale of 1 to 3. Cases received a score of 3, if greater than 75% of the nuclei were positive indicated by a solid appearance. Cases received a score of 2, if 50–75% of the nuclei were positive and the intensity of obtaining resulted in a more granular appearance. A score of 1 was assigned to cases, in which the nuclei were outlined with low intensity staining.

Statistical Analysis

Statistical analysis of the results was performed with the Graphpad Prism (San Diego, CA) version 3.00 for Windows. We detected no seasonal difference in mean VDR expression between tissues collected in the summer compared to winter in the sample group. The comparison of the age decades and the effect of level distribution was performed by one-way analysis of variance (ANOVA). A two-way ANOVA was included in order to examine the interaction between zonal VDR expression and age.

The zonal effect of VDR was analyzed with a Student's *t*-test with Welch's correction [Motulsky, 1999] to compare two group means from the central zone and peripheral zone. The results were confirmed with a paired comparison of samples from the same case but different zones with a Mann-Whitney non-parametric test.

RESULTS

The monoclonal antibody utilized in this study resulted in positive nuclear immunoreactivity in both epithelial and stromal prostate tissues. As shown in Figure 2A, the nuclei of the peripheral epithelium appear small and dark within columnar cells. Figure 2B shows a higher magnification of the boxed area displayed in Figure 2A.

VDR Expression in Human Prostate by Age

Twenty-seven cases met the criteria for age and location of the sample from the prostate in order to be included in this analysis. Epithelial glands from levels 3, 4, and 5 were utilized in the age comparison. These levels were equally represented in each of the age categories. Thus the effect of age was not due to the distribution of levels within age categories. Figure 3 displays the intensity of nuclear VDR staining by age categories of prostate donors from combined locations. The youngest age category was composed of donors from 10–19 years of age. Figure 2D illustrates the central zone of a 10-year-old prostate weakly positive for VDR, scored as 1 because the nuclei were outlined instead of solid in appearance. This group had the lowest mean VDR intensity of 1.9. In comparison, Figure 2C demonstrates strong positive immunoreactivity to VDR in a 20-year-old male donor. There was an increase in VDR expression in the 20–50 years of age categories, culminating in a mean score of 2.6 for the fifth decade. During the sixth decade, VDR declined by an average of 22% from the preceding age category to 2.0. The decline in VDR at the beginning and end of the age spectrum, however, was not statistically significant. The power was limited due to the sample size, thus the possibility of a failing to detect a true difference was increased.

VDR Expression in Human by Prostate Location

We next undertook an examination of VDR by the location of the samples from the prostate. Evaluation of VDR expression was analyzed for samples derived from levels 1,2, or 3 vs. 5 or 6. These levels incorporate either the peripheral zone (1–3) or the central zone (5–6). A decreased level of intensity was noted in samples obtained from the central zone.

Three separate statistical analyses were performed to analyze the difference between VDR expression in the central vs. the peripheral zone. A test for normality was passed, allowing the use of parametric testing. An unpaired *t*-test detected a significant difference between the peripheral zone (2.50 ± 13) and the central zone ($1.95 \pm .05$) with $P < .001$. Welch's correction for unequal variances was used in the analysis.

Next a paired design, in which each donor acted as an age-matched control, was performed

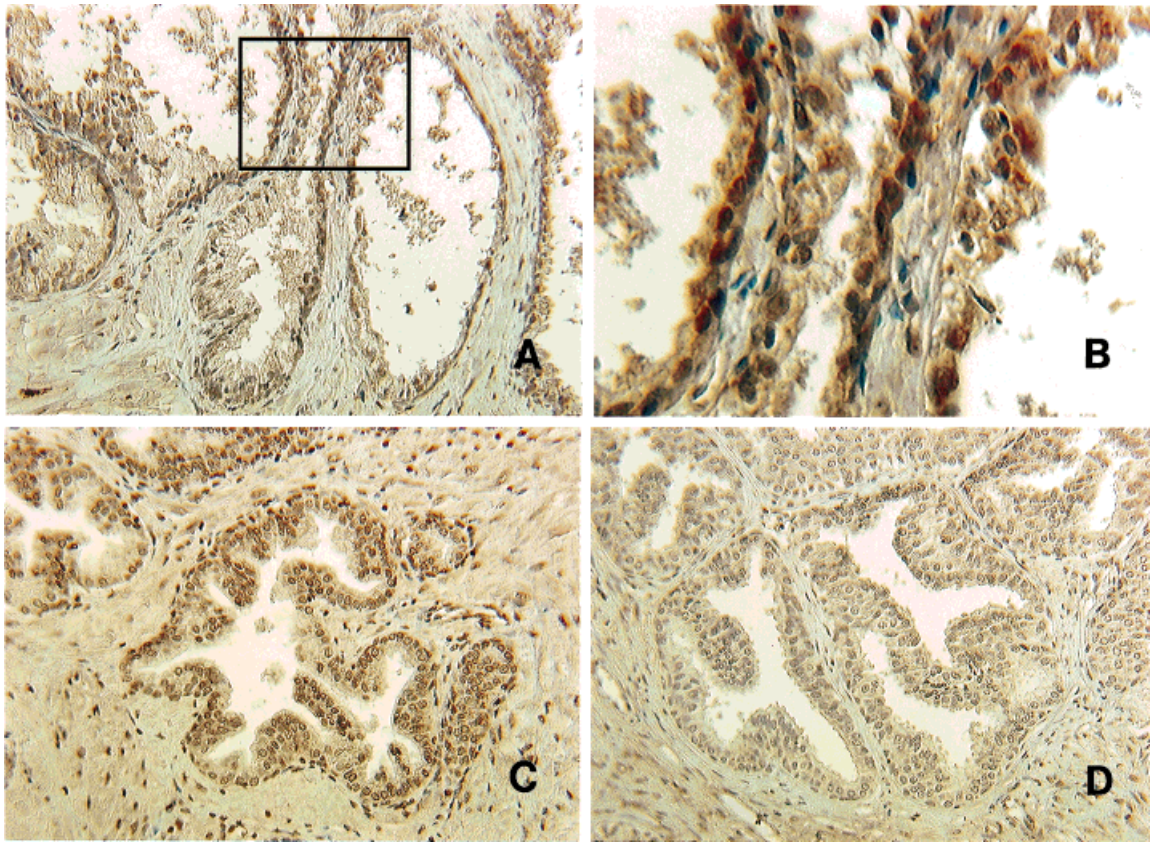

Fig. 2. Representative photographs of the immunohistochemical analysis of prostate tissues. Incubation with a monoclonal antibody against VDR at 1:300 dilution shows positive staining in both epithelial and stromal regions of the tissue. **A:** Positive staining of nuclei in the peripheral zone. Magnification × 100. **B:** Positive staining of nuclei in the boxed area of A at higher magnification × 200. **C:** Strong positive staining (score = 3) of nuclei in the central zone of a 20 year old prostate. Magnification × 100. **D:** Weak positive staining (score = 1) of the nuclei of the central zone of a 10 year old prostate. Magnification × 100.

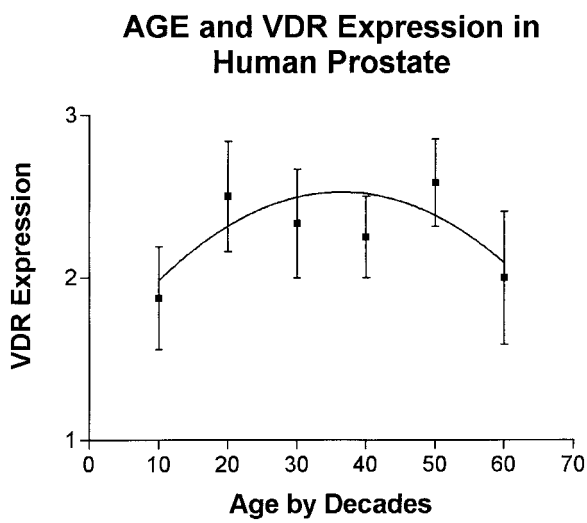

Fig. 3. Analysis of VDR expression by age decades. The mean VDR expression was expressed as an average for the number of samples per age group with the standard error (SE).

on cases where multiple levels from different zones were available from the same case. These results are demonstrated for pairs from four independent donors in Figure 4. The mean score for the central zone was 1.95 compared to a mean score of 2.63 for the peripheral zone. A two-tailed t-test detected a significant difference between the two means ($P < .01$). Because some authors prefer to analyze ordinal data with non-parametric methods, we also performed a Mann-Whitney test on the matched dataset. The median VDR scores of the central and peripheral zones were significantly different ($P < .05$) by this method.

Interaction of Zone and Age Effects

Since the difference in VDR expression between the central and peripheral zone was significant, these results prompted us to test if

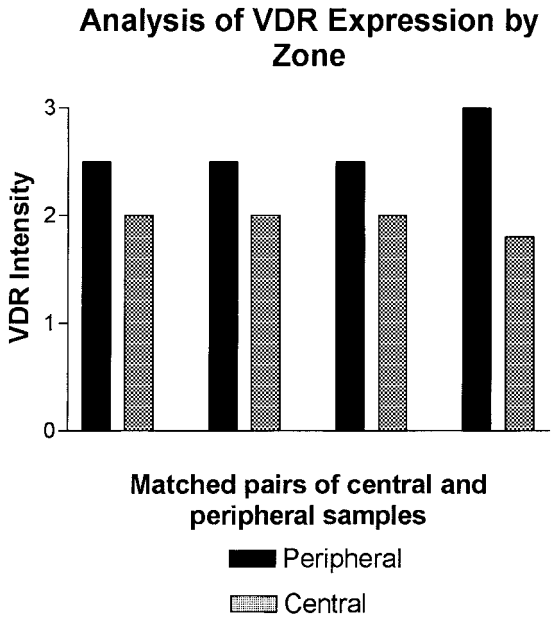

Fig. 4. Analysis of VDR expression by prostate location. Tissue samples from the central and peripheral regions of the same prostate donor were analyzed for VDR expression. Four independent cases demonstrated the peripheral zone expressed a significantly higher level of VDR.

the zonal VDR expression was affected by age. A two-way ANOVA was performed in order to assess the interaction between the age of the donor and the VDR expression by zone of the prostate sample. Examination of mean VDR expression, by peripheral and central zone samples, showed a consistent decrease of VDR in the central zone across age groups that was sampled from 0–59. In the 60–69 years of age group, however, mean VDR expression was equivalent in the central and peripheral zone. A test for significance of the interaction of age and zone was not significant, and therefore, the zone effect was not strongly influenced by age.

VDR Expression in Different Human Tissue Types

Normal tissues, from a variety of human tissues, were compared on the basis of VDR staining. Sections from skin, liver, and pancreas from archival tissue at the University of Pittsburgh were examined immunohistochemically with the same monoclonal antibody. In addition, representative tumor sections were also included. Table I describes the staining pattern for selected tissues from archival and Biogenex sources. All of the tissues demon-

TABLE I. Human Tissues Tested for VDR Immunoreactivity

	Cell Type	VDR Intensity	Location
Normal Tissue Type			
Adrenal	Epithelial	+	Cytoplasmic
Bladder	Epithelial	+	Nuclear
Breast	Epithelial	+++	Nuclear
Brain	Stellate nerve	++	Nuclear
Colon	Epithelial	+++	Nuclear
Heart	Muscle	++	Nuclear, cytoplasm
Kidney	Epithelial	++	Nuclear
Liver	Epithelial	++	Nuclear
Lung	Epithelial	++	Nuclear
Lymph Node	Lymphocytes	+++	Nuclear
Ovary	Stromal	+	Cytoplasmic
Pancreas	Epithelial	++	Nuclear
Parathyroid	Chief cells	+++	Nuclear
Prostate	Epithelial	+	Nuclear
Spleen	Blood cells	+++	Nuclear
Skin	Epithelial	+++	Nuclear
Stomach	Epithelial	+	Nuclear
Thyroid	Epithelial	+++	Nuclear
Tongue	Epithelial	++	Nuclear
Tumor Tissue			
Astrocytoma	Astrocytes	–	Nuclear
Breast	Epithelial	++	Nuclear
Colon	Epithelial	+++	Nuclear
Liver	Hepatocyte	Variable	Cytoplasmic
Lymphoma	Lymphocyte	+++	Nuclear
Melanoma	Epithelial	++	Nuclear
Neuroblastoma	Nerve	+++	Nuclear
Ovary	Epithelial	+++	Nuclear
Pancreas	Epithelial	++	Nuclear
Prostate	Epithelial	+++	Nuclear
Sarcoma	Stromal	++	Nuclear
Thyroid	Epithelial	+++	Nuclear

strated some reactivity with the VDR antibody, but the degree of responses was variable. Skin tissue, which is known to synthesize 1,25-D in response to sunlight, was utilized as a positive control. Skin, lymph node, testis, and parathyroid tissue were strongly positive. Adrenal, ovary, and stomach were weakly positive.

The corresponding tumor tissue (checkerboard-1, Biogenex) showed generally as strong or stronger reactivity compared to its normal counterpart. Normal prostate tissue was moderately positive, whereas prostate tumor was strongly positive.

DISCUSSION

This is the first study that systematically compares a wide range of ages and different prostate zones for VDR expression in human prostate. We found that in normal prostate, VDR was more predominant in the peripheral than the central zone. Since prostate cancer is more often reported in the peripheral zone, it suggests an association between VDR and the regulation of tissue growth in the peripheral zone. The zone effect, however, was not significantly influenced by age.

Our results also indicate that marginally lower VDR expression was observed at the younger and older extremes of the age distribution. Prior studies in rats have shown a decrease in VDR expression with old age. In this study the decline of 22% in VDR expression from ages 50–59 compared to 60–69 was not statistically significant. There were a limited number of normal donors in the older age categories which restricted the sample size, and thus the power of the analysis.

Our survey of VDR expression shows that it is widely distributed in human tissues. These results correlate with the cellular distribution reported in prior immunohistochemical studies in other tissues [Merke et al., 1983; Walters et al., 1986; Kivineva et al., 1998]. As documented in several studies [Eisman et al., 1980; Colston et al., 1982; Berger et al., 1987], tumorigenic cells were also found to express VDR. In this study, we found that the tumorigenic prostate tissues contained VDR to the same or greater extent as the corresponding normal sample. Since many studies have shown that cells with higher VDR content may be more responsive to the effects of 1,25-D, this evidence supports the anti-proliferative and pro-differentiating potential of 1,25-D in cancer therapy.

In summary, this is the first comparative immunohistochemical study of VDR expression by age and location utilizing human donor prostates. Our results demonstrate that (1) VDR is distributed unequally in the human prostate, with a greater level of expression occurring in the peripheral zone; (2) VDR expression is lowest in ages 10–19, increases in ages 20–59, then declines from ages 60–69; (3) there is ubiquitous distribution of VDR across a variety of human tissues, including tumorigenic tissue. The stronger VDR expression, in the peripheral zone, suggests a possible therapeutic advantage in the region where the majority of prostate cancers occur.

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REFERENCES

Berger U, Wilson P, McClelland RA, Colston K, Haussler MR, Pike JW, Coombes RC. 1987. Immunocytochemical detection of 1,25-dihydroxyvitamin D receptors in breast cancer. Canc Res 47:6793–6799.

Colston K, Colston MJ, Fieldsteel AH, Feldman D. 1982. 1,25-dihydroxyvitamin D receptors in human epithelial cancer cell lines. Canc Res 42:856–859.

Corder EH, Guess HA, Hulka BS. 1992. Vitamin D and prostate cancer: A prediagnostic study with stored sera. Cancer Epidemiol Biomarkers Prev 2:467–472.

Ebeling PR, Sandgren ME, DiMagno EP, Lane AW, DeLuca HF, Riggs BL. 1992. Evidence of an age-related decrease in intestinal responsiveness to vitamin D: relationship between serum 1,25-dihydroxyvitamin D_3 and intestinal vitamin D receptor concentrations in normal women. J Clin Endocrinol Metab 75:176–182.

Eisman JA, Martin TJ, MacIntyre I, Frampton RJ, Moseley JM, Whitehead R. 1980. 1,25-dihydroxyvitamin D_3 receptors in a cultured human breast cancer cell line (MCF-7). Biochem Biophys Res Commun 93:9–15.

Habuchi T, Suzuki T, Sasaki R, Wang L, Sato K, Satoh S, Akao T, Tsuchiya N, Shimoda N, Wada Y, Koizumi A, Chihara J, Ogawa O, Kato T. 2000. Association of vitamin D receptor gene polymorphiswith prostate cancer and benign prostatic hyperplasia in a Japanese population. Canc Res 60:305–308.

Hanchette MA, Schwartz G. 1992. Geographic patterns of prostate cancer mortality. Cancer 70:2861–2869.

Horst RL, Goff JP, Reinhardt TA. 1990. Advancing age results in the reduction of intestinal and bone 1,25-dihydroxyvitamin D receptor. Endocrinology 126:1053–1057.

Ingles SA, Ross RK, Yu MC, Irvine RA, LaPeva G, Haile RW, Coetzee GA. 1997. Association of prostate cancer with genetic polymorphisms in vitamin D receptor and androgen receptor. J Natl Cancer Inst 89:166–170.

Kivineva M, Blauer M, Syvala H, Tammela T, Tuohimaa P. 1998. Localization of 1,25-dihydroxyvitamin D_3 receptor (VDR) expression in human prostate. J Steroid Biochem Mol Biol 66(3):121–127.

Koszewski NJ, Reinhardt TA, Beitz DC, Horst RL. 1990. Developmental changes in rat kidney 1,25-dihydroxyvitamin D_3 receptor. Biochem Biophys Res Commun 170:65–72.

Liang CT, Barnes J, Imanaka S, DeLuca HF. 1994. Alterations in mRNA expression of duodenal 1,25-dihydroxyvitamin D_3 receptor and vitamin D-dependent calcium binding protein in aged Wistar rats. Exp Gerontol 29:179–186.

Majeed A, Babb P, Jones J, Quinn M. 2000. Trends in prostate cancer incidence, mortality and survival in England and Wales 1971–1998. BJU Int 85(9):1058–1062.

Merke J, Kreusser W, Bier B, Ritz E. 1983. Demonstration and characterization of a testicular receptor for 1,25-dihydroxyvitamin D_3 inthe rat. Eur J Biochem 130: 303–308.

Miller GJ, Stapleton GE, Hedlund TE, Moffatt KA. 1995. Vitamin D receptor expression, 24-hydroxylase activity, and inhibition of growth by 1α,25-dihydroxyvitamin D_3 in seven human prostatic carcinoma cell lines. Clin Canc Res 1:997–1003.

Motulsky HJ. 1999. Analyzing data with Graphpad Prism. San Diego, CA: Graphpad Software, Inc. www.graphpad.com.

Nakata S, Takahashi H, Ohtake N, Takei T, Yamanaka H. 2000. Trends and characteristics in prostate cancer mortality in Japan. Int J Urol 7(7):254–257.

Puntoni R, Ceppi M, Casella C, Conti E, Crosignani P, De Lisi V, Gafa L, Stanta G, Zanett R, Vercelli M. 1995. Cancer incidence and mortality in the elderly: profile of the Italian problem. Cancer Control 2 (Suppl 1):11–13.

Schwartz GG and Hulka BS. 1990. Is vitamin D deficiency a risk factor for prostate cancer (hypothesis)? Anticancer Res 10:1307–1311.

Taylor JA, Hirvonen A, Watson M, Pittman G, Mohler JL, Bell DA. 1996. Association of prostate cancer with vitamin D receptor gene polymorphism. Cancer Res 56:4108–4110.

Walters MR, Wicker DC, Riggle PC. 1986. 1,25-dihydroxyvitamin D_3 receptor identified in rat heart. J Mol Cell Cardiol 18:67–72.