Vitamin D Receptor Protein Expression in Tumor Tissue and Prostate Cancer Progression

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose

Data suggest that circulating 25-hydroxyvitamin D [25(OH)D] interacts with the vitamin D receptor (VDR) to decrease proliferation and increase apoptosis for some malignancies, although evidence for prostate cancer is less clear. How VDR expression in tumor tissue may influence prostate cancer progression has not been evaluated in large studies.

Patients and Methods

We examined protein expression of VDR in tumor tissue among 841 patients with prostate cancer in relation to risk of lethal prostate cancer within two prospective cohorts, the Physicians' Health Study and Health Professionals Follow-Up Study. We also examined the association of VDR expression with prediagnostic circulating 25(OH)D and 1,25-dihydroxyvitamin D levels and with two VDR single nucleotide polymorphisms, FokI and BsmI.

Results

Men whose tumors had high VDR expression had significantly lower prostate-specific antigen (PSA) at diagnosis (P for trend $< .001$), lower Gleason score (P for trend $< .001$), and less advanced tumor stage (P for trend $< .001$) and were more likely to have tumors harboring the *TMPRSS2:ERG* fusion (P for trend = .009). Compared with the lowest quartile, men whose tumors had the highest VDR expression had significantly reduced risk of lethal prostate cancer (hazard ratio [HR], 0.17; 95% CI, 0.07 to 0.41). This association was only slightly attenuated after adjustment for Gleason score and PSA at diagnosis (HR, 0.33; 95% CI, 0.13 to 0.83) or, additionally, for tumor stage (HR, 0.37; 95% CI, 0.14 to 0.94). Neither prediagnostic plasma vitamin D levels nor VDR polymorphisms were associated with VDR expression.

Conclusion

High VDR expression in prostate tumors is associated with a reduced risk of lethal cancer, suggesting a role of the vitamin D pathway in prostate cancer progression.

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INTRODUCTION

Vitamin D has potential anticancer effects mediated through the **vitamin D receptor (VDR)**, including promotion of cell differentiation and apoptosis and inhibition of cellular proliferation, **angiogenesis**, and tumor cell invasion.⁶⁻⁸ Epidemiologic studies investigating the association of the vitamin D pathway and prostate cancer have focused primarily on circulating 1,25-dihydroxyvitamin D [1,25(OH)₂D], **25-hydroxyvitamin D** [25(OH)D], and VDR polymorphisms. Studies have mostly presented null or nonsignificant associations of serum levels of 25(OH)D and 1,25(OH)₂D with prostate cancer risk.¹⁰⁻¹⁵ Several VDR single nucleotide polymorphisms have been described^{6,16}; although most studies found no associations between these VDR single

nucleotide polymorphisms and prostate cancer risk,¹⁶⁻¹⁹ two studies found that men with low 25(OH)D levels and the BsmI *BB* allele had a lower risk of prostate cancer.^{18,19} Men with low 25(OH)D levels and the less functional FokI *f* allele had an almost two-fold increase in risk for total prostate cancer compared with men with the FokI *FF/Ff* allele and high 25(OH)D levels; the association was even stronger for advanced-stage or high-grade disease.¹⁷ Therefore, VDR polymorphisms could possibly interact with VDR protein expression to modify lethal prostate cancer risk.

In the present study, we investigated VDR protein expression and lethal prostate cancer in the Physicians' Health Study (PHS) and the Health Professionals Follow-Up Study (HPFS). We also investigated associations and interactions between two

VDR polymorphisms and vitamin D metabolites with VDR expression to determine the combined association with lethal disease.

PATIENTS AND METHODS

Study Population

This study was nested among men with prostate cancer who were participants in the PHS and HPFS. The PHS is a randomized primary prevention trial for cardiovascular disease and cancer among 29,067 US male physicians. In PHS I, which began in 1982, men were randomly assigned to aspirin and β -carotene, and in PHS II, which began in 1997, men were randomly assigned to vitamin E, C, and a multivitamin.^{20,21} Blood samples were collected before random assignment in 1982 from 14,916 physicians (68%).¹³ The HPFS is an ongoing prospective cohort study of 51,529 US male dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians, which began in 1986. Between 1993 and 1995, blood samples were collected from 18,018 of the participants.¹²

Clinical and Follow-Up Data

Patients with prostate cancer were initially identified by participant report and confirmed by review of medical records and pathology reports. Information on tumor stage (TNM staging system) and prostate-specific antigen (PSA) levels at diagnosis were abstracted from medical records. Through questionnaires, we collected information on their prostate cancer course, including development of metastases, which was confirmed by treating physicians. Review of death certificates and medical records confirmed date and cause of death. Death from prostate cancer was considered when there was evidence of metastases and disease progression and no other plausible cause of death from medical record review. Follow-up for mortality was more than 99% complete through 2009.

Tissue Microarray Construction

The current study included 841 patients with prostate cancer, including 316 patients in the PHS, diagnosed between January 1983 and December 2004, and 525 patients in the HPFS, diagnosed between March 1986 and December 2002, on whom we collected archival formalin-fixed, paraffin-embedded tumor tissue specimens for tissue microarray (TMA). The majority of tissue (96%) came from prostatectomy specimens, with the remaining coming from transurethral resection of the prostate tissue. All specimens were reviewed by study pathologists (M.F., M.L., and R.F.), who provided uniform evaluation of Gleason score and identified areas of high-density tumor for construction of tumor TMAs. Using a manual arrayer, we sampled at least three tumor cores (0.6 mm) from each specimen and embedded the cores in the recipient TMA block; nine TMAs containing the 841 patient samples were used in this study.

VDR Immunohistochemistry

For immunohistochemistry, 5- μ m sections of each TMA were cut and mounted on slides and subsequently dewaxed and rehydrated with xylene and ethanol. After immersion in 10 mmol/L of citrate buffer, sections underwent microwave pretreatment for 5 minutes for optimal antigen retrieval. Tissue sections were then treated with a peroxidase block (Dako, Glostrup, Denmark). The primary antibody (anti-VDR rabbit polyclonal [C20], sc-1008; Santa Cruz Biotechnology, Santa Cruz, CA) was incubated at a 1:600 dilution for 1 hour at room temperature. A biotin-labeled secondary antibody (Dako) was applied, followed by horseradish peroxidase, the visualizing agent diaminobenzidine (Vector Laboratories, Burlingame, CA) and a hematoxylin counterstain (BioGenex, San Ramon, CA). Because this antibody binds to the C terminus of the VDR protein, VDR protein expression encompasses VDR expression from all alleles of the BsmI and FokI polymorphisms. We have previously shown the specificity of this VDR antibody, which stains VDR in the cytoplasm and membrane.²² VDR staining was analyzed using CRi Vectra, a semiautomated, quantitative image analysis system (CRi, Woburn, MA) that categorizes pixels based on the intensity of the diaminobenzidine spectra, which represents a combination of the percentage of area that is positively

stained, and the intensity of the staining (Fig 1). This final score was averaged across cores for each specimen.

TMPSR2:ERG Fusion Status

For a subset of patients ($n = 184$), we determined in the tumors whether *TMPSR2:ERG*, the common gene fusion identified in prostate cancer, was present. We assessed presence of the fusion directly on the PHS tumor microarrays using a fluorescent in situ hybridization (FISH) assay modified from Tomlins et al.²³ The dual-color, interphase, break-apart FISH assay used two differentially labeled probes that span the telomeric and centromeric regions of *ERG*, which identifies both the *TMPSR2:ERG* fusion through rearrangement and through interstitial deletion.²⁴ In tumors where *TMPSR2:ERG* was not assessable by FISH ($n = 37$), quantitative polymerase chain reaction was used to determine tumor fusion status, as previously described.²⁵ cDNA was synthesized using the Illumina kit (Illumina, San Diego, CA), and *TMPSR2:ERG* was determined using SYBR green assay (Qiagen, Hilden, Germany) with *TMPSR2-ERG_f* and *TMPSR2-ERG_r* primers (GenBank accession code NM_DQ204772).²³ Normalization and proper controls were used.²⁵

VDR Polymorphism and Vitamin D Metabolites

Plasma concentrations of 25(OH)D and 1,25(OH)₂D were assayed as part of nested case-control studies^{10,12,17,18} using the radioimmunosorbent assay as previously described.^{13,26,27} The PHS samples were assayed at two different time points (two batches), and HPFS samples were measured at three time points (three batches) based on date of cancer diagnosis.^{10,12,17,18} The mean intrapair coefficients of variation from blinded quality control samples were less than 10% for 25(OH)D and 1,25(OH)₂D in PHS and HPFS.^{10,12,17}

Data on genetic polymorphisms were available from previous case-control studies. DNA was extracted from baseline blood specimens and amplified using GenomiPhi DNA Amplification Kit (GE Healthcare, Chalfont St Giles, United Kingdom). The FokI and BsmI genotypes were determined using the TaqMan Gene Expression Assay (Applied Biosystems, Foster City, CA) and analyzed at the Harvard Partners Center for Genetics and Genomics (Cambridge, MA).

Statistical Analysis

We divided men into equal quartiles based on the distribution of VDR expression in tumors among men in both cohorts using the proc rank procedure in SAS (SAS Institute, Cary, NC). We compared selected clinical characteristics of the men across quartiles of VDR expression. Because PSA at diagnosis was not normally distributed, we log-transformed this variable. We used analysis of variance (ANOVA) for continuous variables and the Mantel-Haenszel χ^2 test for ordinal variables to evaluate significance for the associations across VDR quartiles.

Hazard ratios and 95% CIs were calculated using Cox proportional hazards regression to assess VDR tumor expression as a predictor of lethal prostate cancer, defined as prostate cancer death or development of bone metastases. Follow-up time was calculated from date of cancer diagnosis to development of lethal disease or censored at death from other causes or at end of follow-up (PHS, March 2009; HPFS, August 2009). Models were adjusted for age at diagnosis and tumor microarray to control for potential batch variation and cohort. We further adjusted for PSA at diagnosis, Gleason score, and tumor stage to investigate the potential for VDR to predict outcome beyond these clinical characteristics.

In a subset of men, we examined the relationship between prediagnostic plasma 25(OH)D levels and tumor VDR protein expression ($n = 220$). We used ANOVA to estimate the difference in mean VDR expression across high and low 25(OH)D levels. We analyzed the association between VDR polymorphisms and VDR protein expression (FokI, $n = 286$; BsmI, $n = 284$). Using ANOVA, we evaluated the difference in mean VDR expression across the FokI and BsmI genotypes. We used the likelihood ratio test to determine the interaction between VDR polymorphisms and high/low VDR expression. Analyses were performed using SAS (version 9.1.3). This study was approved by the Partners Healthcare and Harvard School of Public Health Institutional Review Boards.

Fig 1. Representative images of vitamin D receptor (VDR) protein expression in selected prostate tumor tissue microarray cores from the Physicians' Health Study and Health Professionals Follow-Up Study by Gleason score: (A) Gleason 3 + 3; (B) Gleason 3 + 4; (C) Gleason 4 + 3; and (D) Gleason 4 + 4. Images were taken at $\times 20$ magnification. When staining was present in prostate tumors, it showed cytoplasmic/membranous staining across a large percentage of cells. Nuclear staining of VDR was absent.

RESULTS

The average age at prostate cancer diagnosis was 65.7 years, with an average of 11.8 years of follow-up time after diagnosis (Table 1). Participants were predominantly white (96%). Most men (70%) had locally confined prostate cancer, and the mean PSA level was 11.9 ng/mL. During follow-up, 67 men died of prostate cancer, and 10 men developed bone metastases, for a total of 77 lethal events. For men with baseline plasma vitamin D levels ($n = 220$), the time between blood draw and cancer diagnosis was 6 years (standard deviation, 4 years).

VDR tumor staining was evident in the cytoplasm/membrane and was not nuclear. There was considerable variability in staining of VDR across the prostate cancer tumors, ranging from tumors showing high intensity of staining across most of the prostate tumor nodule to almost no VDR staining in the TMA cores (Fig 1). To examine inpatient variability of VDR expression, we calculated intraclass correlation coefficients (ICCs) across an individual's TMA cores. There

was high concordance of VDR expression across a patient's TMA cores, with an ICC of 77.2 (95% CI, 74.7 to 79.6).

Men with higher VDR tumor expression had lower PSA levels at diagnosis (17.5 ng/mL in the lowest quartile v 8.8 ng/mL in the highest quartile; P for trend $< .001$). Moreover, compared with men with the lowest staining based on quartiles, men with high tumor VDR expression were more likely to have localized disease at prostatectomy (60.4% v 75.4%, respectively; P for trend $< .001$). Protein expression of VDR was highest among men with Gleason 3 + 3 tumors and decreased with increasing pathologic Gleason score (P for trend $< .001$; Table 1 and Fig 1).

For a subset of patients in the PHS, we characterized the tumor specimens for presence of the common *TMPRSS2:ERG* fusion. Men whose tumors had the highest expression of VDR were more likely to have fusion-positive prostate cancer (54%) compared with men with the lowest expression of VDR (29%). Moreover, men with the *TMPRSS2:ERG* fusion that occurred via the deletion mechanism had

Table 1. Demographics and Clinical Characteristics of Patients With Prostate Cancer in the Physicians' Health Study and Health Professionals Follow-Up Study Across Quartiles of VDR Protein Expression in Tumor Tissue (1983 to 2004)

TABLE 2. Summary of VDR Protein Expression in Tumor Nodes (N=841 to 809)												
Demographic or Clinical Characteristic	No. of Patients	VDR Protein Expression										P for Trend
		Overall (N = 841)		Quartile 1 (n = 210)		Quartile 2 (n = 210)		Quartile 3 (n = 211)		Quartile 4 (n = 210)		
		No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	
VDR expression	841											
Mean		30.4		6.0		20.7		37.1		57.9		
SD		20.2		3.9		4.5		4.8		8.9		
Age at diagnosis, years	841											.001*
Mean		65.7		67.3		65.6		64.8		65.3		
SE		0.2		0.4		0.4		0.4		0.4		
BMI at diagnosis, kg/m ²	834											.484*
Mean		25.6		25.9		25.3		25.6		25.5		
SE		0.1		0.3		0.3		0.2		0.2		
PSA at diagnosis, ng/mL	709											< .001*
Mean		11.9		17.5		11.8		9.9		8.8		
SE		1.0		3.7		1.6		0.8		0.5		
Follow-up time, years	841											.182*
Mean		11.8		11.5		12.1		11.4		12.3		
SE		0.2		0.3		0.3		0.3		0.3		
Gleason score	841											
2-6		185	22.0	36	17.1	47	22.4	46	21.8	56	26.7	
7 (3 + 4)		317	37.7	70	33.3	72	34.3	85	40.3	90	42.9	
7 (4 + 3)		193	23.0	56	26.7	51	24.3	41	19.4	45	21.4	
8-10		146	17.3	48	22.9	40	19.0	39	18.5	19	9.0	< .001†
Tumor stage	722											
T1-2		506	70.1	113	60.4	119	68.0	136	76.8	138	75.4	
T3		189	26.2	63	33.7	48	27.4	37	20.9	41	22.4	
T4N1M1		27	3.7	11	5.9	8	4.6	4	2.3	4	2.2	< .001†
<i>TMPPRS2:ERG</i> status	257											
Fusion		114	44.4	19	28.8	31	49.2	27	45.8	37	53.6	
No Fusion		143	55.6	47	71.2	32	50.8	32	54.2	32	46.4	.009†

Abbreviations: VDR, vitamin D receptor; SD, standard deviation; BMI, body mass index; PSA, prostate-specific antigen.

*Analysis of variance *P* value for trend test.†Mantel-Haenszel χ^2 test.

higher VDR expression than men without the *TMPRSS2:ERG* fusion or with the fusion through rearrangement ($P = .01$, data not shown).

The incidence rate of lethal prostate cancer was 7.7 per 1,000 person-years. The incidence rate of lethal disease was lowest among men with the highest tumor protein expression of VDR (3.1 per 1,000 person-years) compared with men in the lowest three quartiles of expression (8 to 10 per 1,000 person-years). In the survival analysis, tumor VDR protein expression was inversely

associated with lethal prostate cancer, especially in the highest quartile (Table 2). Adjustment for Gleason score and PSA at diagnosis, as well as additionally controlling for tumor stage, attenuated the hazard ratios only modestly.

We previously measured circulating levels of 25(OH)D and 1,25(OH)₂D on prediagnostic blood specimens from men in the PHS,¹⁷ and 220 of these men also had tumor VDR protein expression. On average, blood was collected 6 years before cancer diagnosis. We

Table 2. HRs of Lethal Prostate Cancer Among 841 Men With Prostate Cancer in the Physicians' Health Study and Health Professionals Follow-Up Study (1983 to 2004)

(1999 to 2004)									
Factor	VDR Protein Expression								P (quartile 1 v quartile 4)
	Quartile 1		Quartile 2		Quartile 3		Quartile 4		
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	
No. of lethal events	23		22		24		8		
No. of person-years	2,417		2,534		2,400		2,586		
Univariate analysis*	1.00 (Ref)		0.78	0.43 to 1.44	0.74	0.40 to 1.37	0.17	0.07 to 0.41	< .001
Model 1†	1.00 (Ref)		0.94	0.51 to 1.73	0.91	0.49 to 1.70	0.21	0.09 to 0.50	< .001
Model 2‡	1.00 (Ref)		1.13	0.60 to 2.13	1.37	0.72 to 2.59	0.32	0.13 to 0.83	.018
Model 2 + TNM	1.00 (Ref)		1.15	0.61 to 2.16	1.57	0.83 to 2.95	0.37	0.14 to 0.94	.036

Abbreviations: HR, hazard ratio; VDR, vitamin D receptor; Ref, reference.

*Adjusted for tumor microarray.

†Adjusted for tumor microarray and age at diagnosis.

‡Adjusted for tumor microarray, age at diagnosis, Gleason score, and prostate-specific antigen levels at diagnosis.

Table 3. Associations Between VDR Polymorphisms (FokI and BsmI) and VDR Protein Expression in Tumor Tissue in Relation to Lethal Prostate Cancer in Men From the Physicians' Health Study and Health Professionals Follow-Up Study

Genotype	Frequency of Genotype (%)	VDR Expression*		P†	VDR Expression						P for Interaction	
		Mean	95% CI		Low (< median)			High (≥ median)				
					HR*	95% CI	No. of Patients With Lethal Prostate Cancer/Total No. of Person-Years	HR*	95% CI	No. of Patients With Lethal Prostate Cancer/Total No. of Person-Years		
FokI (n = 286)												
FF/Ff	83.9	30.5	27.8 to 33.2		1.23	0.51 to 2.93	12/1,533	1.00 (Ref)			9/1,416	
Ff	16.1	30.4	24.3 to 36.5	.97	2.45	0.75 to 8.02	4/265	1.37	0.29 to 6.33		2/258	.633
BsmI (n = 284)												
bb/Bb	80.6	31.0	28.2 to 33.7		0.85	0.35 to 2.07	9/1,362	1.00 (Ref)			11/1,412	
BB	19.4	29.3	23.7 to 34.9	.68	2.54	0.98 to 6.57	7/392	—			0/274	.007

Abbreviations: VDR, vitamin D receptor; HR, hazard ratio.

*Adjusted for age at diagnosis and cohort.

†P value using analysis of variance.

observed no significant difference ($P = .4$) in mean VDR tumor expression between men who had low (mean VDR, 29.8; 95% CI, 26.0 to 33.6) or high (mean VDR, 27.5; 95% CI, 23.7 to 31.3) circulating levels of 25(OH)D, after adjusting for age at diagnosis, cohort, and time between diagnosis and blood draw. Similarly, VDR expression did not vary ($P = .6$) between men with low (mean VDR, 27.8; 95% CI, 24.0 to 31.7) or high (mean VDR, 29.5; 95% CI, 25.7 to 33.3) circulating levels of the more active metabolite 1,25(OH)₂D.

In Table 3, we compare mean tumor VDR expression according to variation in two function variants of VDR, FokI and BsmI. We found no significant association between the VDR polymorphisms, BsmI or FokI, and VDR tumor protein expression. However, there was a suggestion of an interaction between the genetic variants in VDR and VDR tumor expression to predict lethal prostate cancer. Among men with the BsmI BB genotype and high VDR protein expression, no lethal events occurred. In contrast, men with the BB genotype and low VDR expression had a 2.6-fold increased risk of developing lethal prostate cancer when compared with men with the b allele and low VDR expression. FokI did not significantly interact with VDR expression to modify lethal prostate cancer risk.

DISCUSSION

In this prospective study, men with high VDR protein tumor expression had more favorable clinical characteristics, including lower pathologic Gleason score and more locally confined tumors. Moreover, we found a strong inverse association between tumor VDR protein expression and lethal prostate cancer. After adjusting for PSA at diagnosis, Gleason score, and tumor stage, this inverse association was somewhat attenuated but remained strongly inverse and statistically significant. Our findings could either reflect that VDR is involved in a biologic pathway leading to lethal prostate cancer or that it is indirectly associated with lethal prostate cancer as a marker of other causal mechanisms associated with poorly differentiated or advanced-stage prostate cancer. In either case, VDR expression does seem to be a predictive marker of prostate cancer progression beyond standard clinical features. Furthermore, in vitro and in vivo studies that show VDR inhibits growth of prostate tumor cells and reduces prostate

tumor size in xenografts support a biologic role of VDR expression in aggressive prostate cancer.^{28,29}

The reduction in risk of lethal prostate cancer was restricted to the highest quartile of VDR expression. This result may suggest a threshold of VDR protein expression that tumors must achieve for VDR to influence tumor biology. Alternatively, because the CIs for quartiles 2 and 3 were relatively wide, this appearance of a threshold could be a result of chance variation.

We found that VDR expression varied across a molecular subclass of prostate cancer defined by *TMPRSS2:ERG*, which fuses *TMPRSS2*, a gene regulated by androgens, and *ERG*, a known oncogenic transcription factor.^{23,30} Men whose tumors were *TMPRSS2:ERG* positive through deletion in genomic DNA had significantly higher VDR protein expression in tumor tissue than men who were fusion negative or fusion positive through rearrangement. In prior studies, fusion through deletion has been associated with a more aggressive prostate cancer phenotype and would therefore be expected to be more prevalent in men with low VDR protein expression.^{24,30-32} The relationship we found between VDR expression and fusion status could be explained by the potential effect of VDR and 1,25(OH)₂D on *TMPRSS2:ERG* expression as suggested by Washington and Weigel.³³ Although 1,25(OH)₂D induces *TMPRSS2:ERG* expression, prostate tumor growth is still inhibited in the fusion-positive cell line. Therefore, fusion-positive tumors may be more responsive to vitamin D signaling, and so with high VDR protein expression, growth is still inhibited.

We found no associations between circulating levels of vitamin D metabolites and VDR protein expression. However, we assessed vitamin D metabolites 6 years before diagnosis. Levels more proximate to diagnosis may be more relevant in terms of tumor VDR protein expression. Within HPFS, we previously found a good correlation of circulating vitamin D levels across a 3-year window, with an ICC of 0.70, suggesting that a single measure does provide some reliable estimate of exposure in the short term.¹² Although VDR polymorphisms (BsmI and FokI) were not associated with VDR protein expression, these gene variants could influence VDR function and thus alter prostate cancer progression.³⁴⁻³⁸ Consistent with previous studies comparing VDR polymorphisms and aggressive disease, men with the less functional ff allele had an increased risk of developing lethal

prostate cancer, although our results were not statistically significant.¹⁷ We noted a significant interaction between the BsmI allele (*BB* v *Bb/bb*) and VDR protein expression and lethal prostate cancer; among men with the *BB* allele, those with low VDR expression had a 2.6-fold increased risk of lethal prostate cancer risk, but men with high VDR expression were at lower risk. This difference could reflect the importance of VDR protein expression or simply reflect chance.

The long clinical follow-up permitted use of lethal prostate cancer as the outcome, an important clinical phenotype of such a heterogeneous cancer. Furthermore, we used unbiased quantitative image analyses to score VDR protein expression, and Gleason scores were standardized by study pathologists. Heterogeneity of VDR staining across the tumor specimens may be an issue when using TMAs. However, we sampled at least three TMA cores per prostate cancer specimen and showed a relatively high intraclass correlation of VDR staining.

This study was nested among men with available prostate tumor tissue, primarily men undergoing prostatectomy as curative treatment. We retrieved 73% of eligible tumor specimens, and the clinical characteristics of the prostatectomy patients with and without available tumor tissue are quite similar.

The VDR staining in the prostate tumors was apparent in the cytoplasm and membrane but not in the nucleus. Although nuclear staining of VDR is likely relevant, it is plausible that the VDR antigens may be processed quickly in the nucleus after functioning. Thus, cytoplasmic staining may be a surrogate of VDR actions that are ultimately mediated in the nucleus. At the same time, a significant portion of VDR resides in the cytoplasm and cytoplasmic membrane, where it exerts nongenomic actions outside of the nucleus.^{39,40} The nongenomic actions of VDR include regulation of calcium and chloride channel activity, protein kinase C activation, and phospholipase C activity occurring through cytoplasmic signaling pathways such as protein kinase and mitogen-activated protein kinase.⁴¹⁻⁴⁶ Cytoplasmic VDR may cooperate with important driver mutations, such as a

positive association with PIK3CA mutations in colorectal cancer.²² Cytoplasmic VDR localization in cancer has been well described in various cancers.^{22,47-52}

In conclusion, these results support an important role of the vitamin D pathway on risk of progression to lethal prostate cancer. If these findings are confirmed, VDR protein expression in tumor could help improve prognostic prediction and guide treatment decisions.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Glossary Terms

25-hydroxyvitamin D: 25-hydroxyvitamin D₃, the main circulating form of vitamin D, is produced in the liver when vitamin D₃ (cholecalciferol) from UV irradiation and dietary intake is hydroxylated by mitochondrial and microsomal enzymes in the liver. 25-hydroxyvitamin D₃ is considered the best indicator of vitamin D status, because it reflects not only skin exposure to UV light and total vitamin D intake, but also cholecalciferol production in the skin and hydroxylation of all sources of cholecalciferol in the liver.

Angiogenesis: The process involved in the generation of new blood vessels. While this is a normal process that naturally occurs and is controlled by “on” and “off” switches, blocking tumor angiogenesis (antiangiogenesis) disrupts the blood supply to tumors, thereby preventing tumor growth.

Antigen: A substance that promotes, or is the target of, an immune response.

Cox proportional hazards regression: The Cox proportional hazards regression model is a statistical model for regression analysis of censored survival data. It examines the relationship of censored survival distribution to one or more covariates. It produces a baseline survival curve, covariate coefficient estimates with their standard errors, risk ratios, 95% CIs, and significance levels.

FISH (fluorescent in situ hybridization): In situ hybridization is a sensitive method that is generally used to detect specific gene sequences in tissue sections or cell preparations by hybridizing the complementary strand of a nucleotide probe to the sequence of interest. FISH uses a fluorescent probe to increase the sensitivity of in situ hybridization.

Genetic polymorphisms: A genetic variant seen in at least 1% of the population. Because proteins are gene products, their polymorphisms reflect allelic differences in the gene. The advent of restriction enzymes, which digest DNA to fragments based on sequence specificity, has ushered in an era of restriction fragment length polymorphisms (RFLPs) in which changes in DNA sequence(s) are manifest as restriction fragments of different size(s) when cleaved with a specific restriction enzyme. Polymorphisms are used in tissue typing, in determining disease, in pharmacogenetics, and in assessing genetic diversity.

Immunohistochemistry: The application of antigen-antibody interactions to histochemical techniques. Typically, a tissue section is mounted on a slide and is incubated with antibodies (polyclonal or monoclonal) specific to the antigen (primary reaction). The antigen-antibody signal is then amplified using a second antibody conjugated to a complex of peroxidase-antiperoxidase (PAP), avidin-biotin-peroxidase (ABC) or avidin-biotin alkaline phosphatase. In the presence of substrate and chromogen, the enzyme forms a colored deposit at the sites of antibody-antigen binding. Immunofluorescence is an alternate approach to visualize antigens. In this technique, the primary antigen-antibody signal is amplified using a second antibody conjugated to a fluorochrome. On UV light absorption, the fluorochrome emits its own light at a longer wavelength (fluorescence), thus allowing localization of antibody-antigen complexes.

PSA (prostate-specific antigen): A protein produced by cells of the prostate gland, the blood level of PSA is used as a tumor marker for men who may be suspected of having prostate cancer. Most physicians consider 0 to 4.0 ng/mL as the normal range. Levels of 4 to 10 and 10 to 20 ng/mL are considered slightly and moderately elevated, respectively. PSA levels have to be complemented with other tests to make a firm diagnosis of prostate cancer.

Tissue microarray: Used to analyze the expression of genes of interest simultaneously in multiple tissue samples, tissue microarrays consist of hundreds of individual tissue samples placed on slides ranging from 2 to 3 mm in diameter. Using conventional histochemical and molecular detection techniques, tissue microarrays are powerful tools to evaluate the expression of genes of interest in tissue samples. In cancer research, tissue microarrays are used to analyze the frequency of a molecular alteration in different tumor type, to evaluate prognostic markers, and to test potential diagnostic markers.

Vitamin D receptors (VDR): The vitamin D receptor is a member of the nuclear hormone-receptor superfamily of protein receptors. This intracellular receptor has both hormone-binding and DNA-binding domains. Upon binding by 1,25-dihydroxycholecalciferol, the active metabolite of vitamin D, the receptor complexes with the retinoic acid receptor. This heterodimer subsequently recruits several co-activator molecules and binds to a DNA response element located in the promoter region of target genes to either activate or suppress transcription.