[CANCER RESEARCH 62, 2695–2698, May 1, 2002]

DD3^{PCA3}, a Very Sensitive and Specific Marker to Detect Prostate Tumors

Jacques B. de Kok,[1] Gerald W. Verhaegh, Rian W. Roelofs, Daphne Hessels, Lambertus A. Kiemeney, Tilly W. Aalders, Dorine W. Swinkels, and Jack A. Schalken

Departments of Clinical Chemistry [J. B. d. K., R. W. R., D. W. S.], Experimental Urology [G. W. V., D. H., L. A. K., T. W. A., J. A. S.], and Epidemiology and Biostatistics [L. A. K.], University Medical Centre Nijmegen, 6500 HB Nijmegen, the Netherlands

ABSTRACT

We identified *DD3*^{PCA3} as one of the most prostate cancer-specific genes at present (M. J. Bussemakers *et al*. Cancer Res., *59*: 5975–5979, 1999). Consequently, *DD3*^{PCA3} is an interesting candidate for use as a diagnostic and/or prognostic marker. In this study we developed a method for the accurate quantification of *DD3*^{PCA3} mRNA, using real-time quantitative reverse transcription-PCR. *DD3*^{PCA3} was expressed at low levels in normal prostate but not in 21 selected other normal tissues, blood, or 39 tumor samples other than prostate. The diagnostic and prognostic value of *DD3*^{PCA3} in normal, hyperplastic, and malignant prostate tissues was determined and compared with another promising tumor marker for prostate cancer, telomerase reverse transcriptase (*hTERT* gene), the expression of which is related to telomerase activity. Sensitivity and specificity estimates for both genes were calculated as the area under the receiver-operating characteristics curve (AUC-ROC). *DD3*^{PCA3} (AUC-ROC, 0.98) demonstrated better diagnostic efficacy than *hTERT* (AUC-ROC, 0.88). Moreover, the median increase in mRNA expression in tumor tissues compared with nonmalignant prostate tissues was much higher for *DD3*^{PCA3} (34-fold) than for *hTERT* (6-fold). In tumor tissues, the median expression of *DD3*^{PCA3} was much higher than *hTERT* (5849 *versus* 10 normalized mRNA copies). A significant relationship was observed only between tumor stage and *hTERT* gene expression. We conclude that expression of the *DD3*^{PCA3} gene is a very sensitive and specific marker for the detection of prostate tumor cells in a high background of normal (prostate) cells. Consequently, DD3 measurements may be used for clinical application in prostate needle biopsies or bodily fluids such as blood, ejaculate, urine, or prostate massage fluid.

INTRODUCTION

Prostate cancer is the most frequently diagnosed cancer in the Western male population and the second leading cause of cancer mortality. Patients with organ-confined prostate cancer are generally treated with radical surgery or radiation therapy. Alternatively, if the tumor has spread locally or distantly, androgen ablation is the standard therapy used. Unfortunately, most of the latter patients will develop progressive disease, and there is no other effective treatment available. Therefore, methods are necessary that can detect the tumor early, at a time when the tumor is still locally confined and potentially curable by radical prostatectomy.

A significant contribution to the early detection of prostate cancer has been the discovery of PSA[2] and subsequent development of various immunological assays in serum. Serum PSA is now recognized as the premier marker for detection of prostate cancer and can be used for screening selected populations of patients and for monitoring patients after therapy (1). However, serum PSA levels are regularly elevated in men with BPH, prostatitis, and other nonmalignant disorders, resulting in reduced specificity (1).

Novel cancer-specific markers have been identified that may aid early diagnosis and help to differentiate between tumor and nonmalignant growth. Telomerase activity is one of the most promising markers. High activity has been detected in the majority (90%) of prostate tumors (2, 3), whereas only low or absent activity was observed in normal and BPH tissues (3–5). An alternative for telomerase activity measurement is the quantification of telomerase reverse transcriptase mRNA (*hTERT* gene) by use of real-time quantitative PCR (6). Because *hTERT* expression is the rate-limiting determinant of the telomerase enzyme (7), accurate quantification of *hTERT* mRNA copies may better differentiate between malignant and benign prostate growth than semiquantitative telomerase activity measurements.

Next to *hTERT* gene expression, other genes, such as *PSGR* and *PCGEM1*, have recently been identified that have more prostate-specific expression (8, 9). We identified and characterized *DD3*^{PCA3} (*DD3*), a new prostate-specific gene (10). Northern blot analysis showed that *DD3* mRNA is expressed at low levels in normal prostate and is abundantly present in prostate tumor tissues (10). A more quantitative, reproducible, and sensitive assay is necessary to test *DD3* as a diagnostic or prognostic marker in clinical samples, which often contain only small amounts of mRNA.

MATERIALS AND METHODS

Tissues. Normal tissue samples were obtained from autopsies with a postmortem delay of <4 h. These tissues, together with 39 frozen tissues from nine different types of tumors (lung, esophagus, ileum, colon, pancreas, testis, breast, bladder, and melanoma), were randomly selected from the tissue bank of the Department of Pathology (University Medical Centre Nijmegen). Prostate tissue sections (normal, BPH, and tumor) were carefully selected and removed by a pathologist from fresh prostates of patients after radical prostatectomy at the Canisius-Wilhelmina Hospital (Nijmegen, the Netherlands) and the University Medical Centre Nijmegen. The stages (TNM) of these tumors were determined at the Department of Pathology of both hospitals. Frozen tumor, BPH, and normal prostate tissues were step-sectioned with evaluation by H&E staining at regular intervals. This evaluation included determination of percentage of normal, BPH, and tumor cells, together with Gleason score of the tumor sections.

RNA Isolation and cDNA Synthesis. Total RNA from healthy and tumor tissues was isolated by disruption of 10–25 frozen, 20-μm sections in 1 ml of RNAzolB (Biotex Laboratories Inc, Houston, TX) or TRIzol (Life Technologies, Inc., Breda, the Netherlands) using a sterile pestle. After the manufacturer's protocols were completed, RNA was further purified using the RNeasy kit (Qiagen, Hilden, Germany). RNA was quantified spectrophotometrically.

Purified RNA (0.2–1.0 μg) was added to RNase-free water to a final volume of 10 μl, denatured for 5 min at 90°C, and cooled immediately on ice. Reverse transcription mixture (10 μl) was added, containing first strand buffer (Life Technologies, Inc.), 200 units of Moloney murine leukemia virus (Life Technologies, Inc.), 20 units of RNasin (Promega, Madison Wisconsin), 10 mM DTT, 4.75 μM random hexamers, and 600 μM deoxynucleotides. After annealing of the hexanucleotides for 10 min at 20°C, cDNA synthesis was performed for 45 min at 42°C, followed by an enzyme inactivation step for 5 min at 95°C. cDNA was stored at −20°C until use.

Quantitative Measurement of *PCA3*, *hTERT*, and 18S rRNA. The gene sequence of *DD3* is presented in GenBank accession no. AF103907. The structure of the *DD3* gene with exon-intron boundaries was described previously (10).

Received 9/17/01; accepted 2/26/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, at 564 AKC, UMC Nijmegen, P. O. Box 9101, 6500 HB Nijmegen, the Netherlands. Fax: 31-24-3541743; E-mail: J.dekok@ckcl.azn.nl.

[2] The abbreviations used are: PSA, prostate-specific antigen; BPH, benign prostate hyperplasia; *hTERT*, human telomerase reverse transcriptase; *DD3*, prostate cancer antigen 3 (PCA3, according to HUGO nomenclature); FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine; Ct, threshold cycle; AUC, area under the curve; ROC, receiver-operating characteristic.

Table 1 *DD3 expression in normal tissues*

Tissue	*rRNA*[a] ($\times 10^8$)	*DD3*[a]	Normalized *DD3*[b]
Skin	4.8	0	0
Skeletal muscle	48.0	0	0
Heart	12.0	0	0
Lymph gland	26.0	0	0
Spleen	38.0	0	0
Bone marrow	4.6	0	0
Lung	12.0	0	0
Stomach	20.0	0	0
Ileum	13.0	0	0
Colon	16.0	0	0
Liver	40.0	0	0
Pancreas	24.0	0	0
Testis	28.0	0	0
Bladder	34.0	0	0
Kidney	52.0	390	8
Uterus	44.0	0	0
Thyroid gland	2.8	0	0
Brain	14.0	0	0
Esophagus	7.2	0	0
Ovaries	7.8	0	0
Breast	0.6	0	0
Prostate	4.6	699	152
Leukocytes	34.0	0	0

[a] Ct values were transformed to absolute gene copy numbers by calibration curves (5-log range) of rRNA and DD3 plasmid calibrators. The functions of the calibration curves were: $f(Ct_{rRNA}) = -3.40 \log [rRNA \text{ copies}/20] + 40.80$; and $f(Ct_{DD3}) = -3.84 \log [DD3 \text{ copies}] + 45.32$.

[b] Normalized $DD3 = (DD3/rRNA) \times 10^8$.

Primers and a TaqMan probe for the cDNA-specific real-time quantitative PCR assay were designed: (*a*) DD3-95F (located in exon 1), 5′-GGTGGGAAGGACCTGATGATAC-3′, (*b*) DD3-521R (located in exon 4a), 5′-GGGCGAGGCTCATCGAT-3′, and probe DD3-FAM (located in exon 4a), 5′-FAM-AGAAATGCCCGGCCGCCATC-TAMRA-3′. In the quantitative PCR assay we added 30 pmol of each primer, 20 pmol of TaqMan probe, 25 μl of Universal Master Mix (Applied Biosystems, Foster City, CA), and 1 μl of cDNA in a total reaction volume of 50 μl. After enzyme inactivation for 10 min at 95°C, 40 two-step cycles were performed (30 s at 95°C, 75 s at 60°C). Because 95% of the *DD3* mRNA splice products consist of exons 1, 3, and 4a, the size of the majority of PCR amplicons was 262 bp (10). When no increase in fluorescence was detected after 40 PCR cycles, samples were regarded as negative for *DD3* expression.

For quantitative *hTERT* mRNA measurements, we used 45 pmol of primer LT5 (5′-CGGAAGAGTGTCTGGAGCAA-3′), 15 pmol of primer LT6 (5′-GGATGAAGCGGAGTCTGGA-3′), 10 pmol of probe (5′-FAM-TTGCAAAGCATTGGAATCAGACAGCACT-TAMRA 3′), 25 μl of Universal Master Mix, and 1 μl of cDNA in a 50-μl reaction volume. PCR cycling parameters were 10 min at 95°C, followed by 40 cycles of 30 s at 95°C and 60 s at 60°C.

The level of 18S rRNA (*rRNA*) expression was measured in all samples to normalize *DD3* and *hTERT* expression for sample-to-sample differences in RNA input, RNA quality, and reverse transcription efficiency. For quantification of *rRNA* we used a Pre Developed Assay Reagent and followed the accompanying instructions (Applied Biosystems). To prevent low Ct values from interfering with calculations of the threshold baseline, cDNA samples were diluted 20-fold in water. Amplification parameters were identical to those for *hTERT*, but only 25 PCR cycles were performed.

The principle of the real-time PCR is described by Gibson *et al.* (11). PCR reactions were performed in the ABI Prism 7700 Sequence Detection System (Applied Biosystems), and Ct values for *DD3*, *hTERT*, and 18S rRNA were derived by the computer. To be able to transform the Ct values into absolute mRNA copy numbers, we used a calibration curve (5-log range) that was prepared from a dilution series of linearized plasmid containing the *DD3*, *hTERT*, or *rRNA* amplicon insert.

Statistical Analysis. The distributions of normalized *hTERT* and normalized *DD3* were characterized by their median values and ranges. Differences in these markers between normal and tumor tissue were tested for statistical significance with the nonparametric Mann-Whitney *U* test. The nonparametric Kruskal-Wallis test was used to test differences between prostate cancer patients with different tumor stages and Gleason scores.

To visualize the efficacy of the two markers to discriminate tumor tissue from normal tissue (in the absence of an arbitrary cutoff value), we summarized the data in a ROC curve. This curve plots the sensitivity (true positives) on the *Y* axis against 1 − the specificity (false positives) on the *X* axis, considering each observed value as a possible cutoff value. The AUC was calculated as a single measure for the discriminative efficacy of a marker. When a marker has no discriminative value, the ROC curve will lie close to the diagonal and the AUC is close to 0.5. When a test has strong discriminative value, the ROC curve will move up to the upper left-hand corner and the AUC will be close to 1.0. The Statistical Package for Social Sciences (SPSS) was used for analyses.

RESULTS

Prostate Specificity of *DD3*. Absolute copy numbers of *DD3* mRNA were determined from cDNA from 22 different normal tissues and peripheral blood leukocytes. These absolute copy numbers were normalized to the expression of *rRNA* (Table 1). Normal prostate tissue expressed *DD3* mRNA at a low level. *DD3* was absent in all other tissues, except for kidney, in which *DD3* was expressed at an insignificant level.

In addition, *DD3* mRNA expression was measured in 39 tumor samples representing nine different tumor types (Table 2). *DD3* mRNA could not be detected in all tested tumor samples. The absence of expression of *DD3* in the vast majority of both normal and tumor tissues other than prostate demonstrated the high prostate specificity of this novel tumor marker.

Diagnostic Performance of *hTERT* and *DD3* as Prostate Tumor Markers. *DD3* and *hTERT* mRNA expression was quantitatively measured in 11 normal prostate tissues, 5 BPH samples, and 31 prostate adenocarcinomas (Table 3). No significant differences in *hTERT* or *DD3* mRNA expression were detected between normal and BPH tissues. Therefore, normal and BPH tissues together were defined as one group of nonmalignant tissues.

Median *hTERT* mRNA expression levels between nonmalignant (median, 1.7; range, 0–5.2) and tumor tissues (median, 10.1; range, 0.7–76.1) were significantly different ($P < 0.0001$). Median up-regulation of *hTERT* expression from normal to tumor tissues was 6-fold. A significant relationship was found between *hTERT* expression and tumor stage ($P = 0.02$), but not with Gleason score.

For *DD3*, median normalized expression was also significantly different between nonmalignant (median, 174; range, 0–593) and malignant prostate tissues (median, 5,849; range, 334–39,456; $P < 0.0001$). Median up-regulation of *DD3* from normal to tumor tissues was 34-fold. Even in tissues containing ≤10% tumor cells, normalized *DD3* expression was clearly higher than that in nonmalignant tissues (Table 3, *samples 38–47*). No significant correlation was found between *DD3* expression and tumor stage or Gleason score.

A ROC curve was constructed for both markers (Fig. 1). The AUC-ROC represents the diagnostic efficacy of the continuous test result. The AUC-ROC was 0.98 (95% confidence interval, 0.96–1.00) for *DD3* and 0.88 (95% confidence interval, 0.78–0.97) for *hTERT*, indicating good discrimination power for both tests.

Table 2 *Normalized DD3 expression in pooled tumor tissues*
See descriptions of *rRNA*, *DD3*, and normalized *DD3* in footnotes for Table 1.

Tumor pool	*n*	*rRNA* ($\times 10^8$)	*DD3*	Normalized *DD3*
Lung	5	13.0	0	0
Esophagus	6	7.6	0	0
Ileum	4	15.0	0	0
Colon	5	18.0	0	0
Pancreas	3	92.0	0	0
Testis	4	15.0	0	0
Breast	4	8.0	0	0
Bladder	5	13.0	0	0
Melanoma	3	8.8	0	0

Table 3 *Normalized DD3 and hTERT expression in normal prostate, BPH, and adenocarcinoma tissue samples*

Sample no.	Pathological			Tumor in tissue sample (%)	Expression[b]			Normalized *hTERT*[f]	Normalized *DD3*[g]
	Tissue	Stage[a]	Gleason score[a]		*rRNA*[c] ($\times 10^8$)	*hTERT*[d]	*DD3*[e] ($\times 10^3$)		
1	Normal				5.0	17	0.9	3.4	187
2	Normal				11.0	54	0.0	4.9	0
3	Normal				6.9	7	1.1	1.0	155
4	Normal				6.1	10	1.6	1.6	264
5	Normal				8.6	15	3.1	1.7	364
6	Normal				6.6	12	3.9	1.8	593
7	Normal				5.1	0	0.1	0.0	11
8	Normal				3.5	5	0.6	1.4	181
9	Normal				4.3	0	0.9	0.0	212
10	Normal				4.2	9	0.7	2.1	166
11	Normal				3.9	6	1.5	1.5	372
12	BPH				5.5	18	1.8	3.3	326
13	BPH				12.0	7	0.6	0.6	13
14	BPH				6.1	32	0.1	5.2	10
15	BPH				4.3	21	0.04	4.9	9
16	BPH				11.0	18	0.4	1.6	40
17	Tumor	pT_3	8	70	4.6	39	49.6	8.5	10,783
18	Tumor	pT_3	8	50	5.7	60	30.4	10.5	5,340
19	Tumor	pT_3	6	70	8.4	75	169.0	8.9	20,095
20	Tumor	pT_3	7	80	7.5	278	111.0	37.1	14,865
21	Tumor	pT_3	4	50	11.0	433	255.0	39.4	23,190
22	Tumor	pT_3	6	65	7.0	266	63.0	38.0	8,998
23	Tumor	pT_3	7	20	7.2	120	146.0	16.7	20,292
24	Tumor	pT_2	6	40	7.5	13	34.5	1.7	4,599
25	Tumor	pT_3	6	80	8.8	165	4.7	18.8	531
26	Tumor	pT_3	7	75	4.3	306	16.2	71.2	3,779
27	Tumor	pT_3	5	80	6.1	92	241.0	15.1	39,456
28	Tumor	pT_2	7	70	7.2	202	226.0	28.1	31,457
29	Tumor	pT_3	5	80	7.8	425	39.2	54.5	5,025
30	Tumor	pT_3	5	90	10.0	471	197.0	47.1	19,716
31	Tumor	pT_2	7	20	5.7	4	43.4	0.7	7,620
32	Tumor	pT_3	5	70	5.9	76	41.9	12.9	7,101
33	Tumor	pT_3	7	20	5.0	10	2.5	2.0	492
34	Tumor	pT_2	7	40	4.2	6	1.5	1.4	356
35	Tumor	pT_3	7	30	4.2	54	5.8	12.9	1,380
36	Tumor	pT_2	7	30	4.9	43	1.6	8.8	334
37	Tumor	pT_3	5	80	2.8	62	16.4	22.1	5,849
38	Tumor			5	1.7	12	25.6	7.1	15,059
39	Tumor			10	7.7	51	14.9	6.6	1,941
40	Tumor			5 (+ 70% PIN)[h]	5.6	426	23.1	76.1	4,131
41	Tumor			10 (+ 55% PIN)	4.8	15	56.5	3.1	11,776
42	Tumor			10	4.2	29	8.6	6.9	2,038
43	Tumor			5	5.2	34	38.5	6.5	7,404
44	Tumor			<1	6.4	6	6.3	0.9	988
45	Tumor			10	7.2	35	20.0	4.9	2,778
46	Tumor			5	9.3	94	94.4	10.1	10,172
47	Tumor			<1	10.0	47	9.6	4.7	962

[a] Only adenocarcinomas samples that contained ≥20% tumor cells were used for statistical analysis for a relationship between *DD3* or *hTERT* expression and pathological stage or Gleason score. Gleason score was defined as the sum of the two most common histological grades detected in the tissue sections by pathological review.
[b] Ct values for all samples were transformed to absolute gene copy numbers, using calibration curves (5-log range) of plasmid calibrators.
[c] $f(Ct_{rRNA}) = -3.38 \log [rRNA \text{ copies}/20] + 38.71$ ($r^2 = 1.0$).
[d] $f(Ct_{hTERT}) = -3.39 \log [hTERT \text{ copies}] + 40.43$ ($r^2 = 0.99$).
[e] $f(Ct_{DD3}) = -3.74 \log [DD3 \text{ copies}] + 40.19$ ($r^2 = 0.99$).
[f] Normalized $hTERT = (hTERT/rRNA) \times 10^8$.
[g] Normalized $DD3 = (DD3/rRNA) \times 10^8$.
[h] PIN, prostatic intraepithelial neoplasia.

DISCUSSION

We developed a method for the accurate quantification of *DD3* mRNA expression and showed that *DD3* was exclusively expressed in the prostate. For diagnosis of tumor, *DD3* expression was compared with the expression of *hTERT* mRNA. *hTERT* gene expression is the rate-limiting determinant of telomerase activity (7) and at present the most common tumor marker (12). Sensitivity and specificity estimates for both genes were calculated as the AUC-ROC. Although both *hTERT* and *DD3* could differentiate between malignant and nonmalignant prostate tissues, *DD3* showed much better diagnostic characteristics than *hTERT*, with AUC-ROC values of 0.98 for *DD3* and 0.88 for *hTERT*. The median increase in mRNA expression from nonmalignant to malignant tissues was only 6-fold for *hTERT*, whereas the median *DD3* increase was 34-fold. A large increase is important for the detection of a few malignant cells in a high background of normal prostate cells. This was clearly shown for 10 tissue samples containing ≤10% tumor cells, in which *DD3* showed higher sensitivity than *hTERT*. Moreover, in prostate tumors, the median normalized expression of *DD3* mRNA was ~580-fold higher than *hTERT* mRNA (5849 *versus* 10 normalized copies, respectively). This high expression in tumor cells may also be advantageous for the detection of the few malignant prostate cells shed into blood, urine, prostatic massage fluid, or ejaculate. In addition, *DD3* mRNA expression could not be detected in leukocytes, which are regularly present in these bodily fluids, whereas *hTERT* mRNA is expressed in leukocytes and may cause false positivity (13, 14).

We previously detected a statistically significant relationship between the expression of *hTERT* mRNA and tumor stage and grade in urothelial cell carcinomas (15). Others found a similar relationship for breast tumors and Wilms' tumors (16, 17). In the present study in prostate tumors, this correlation was observed only for stage, but not for Gleason score

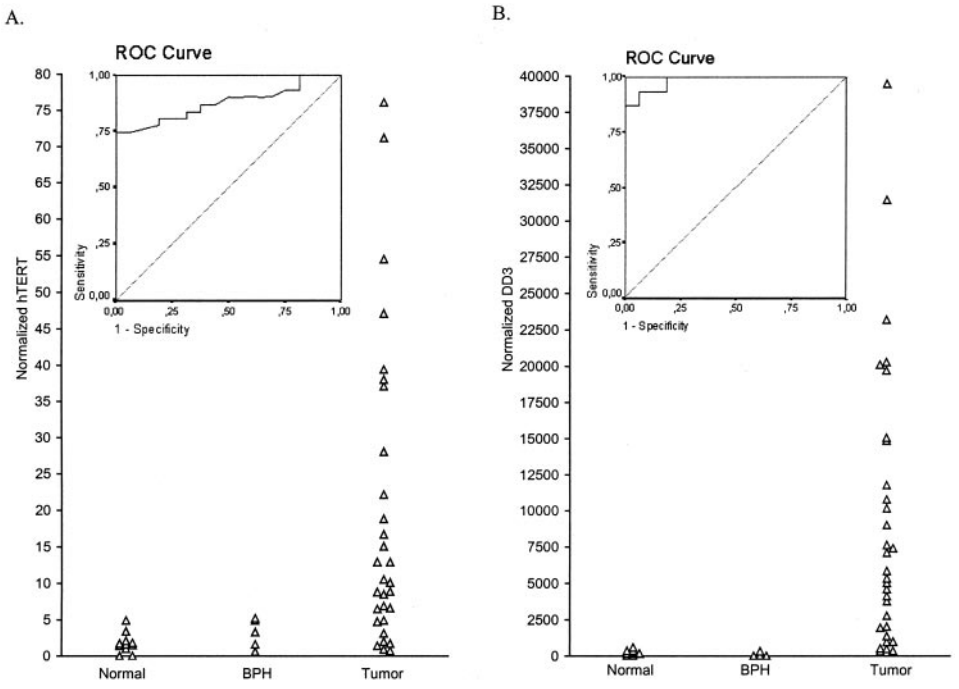

Fig. 1. Normalized expression of *hTERT* mRNA (*A*) and *DD3* mRNA (*B*) in normal prostate, BPH, and tumor tissues. ROC curves (*insets*) are shown to demonstrate diagnostic efficacy of each marker.

(grade). The 21 analyzed prostate tissues (with tumor cells ≥20%) were derived from radical prostatectomies, with only little variation in stage and grade. This low number may have caused the lack of correlation with grade. In contrast, Latil *et al.* (18) did not detect a correlation between *hTERT* expression and pathological parameters for 33 prostate tumor samples with a broader range of stage and Gleason grade.

Typically, both we (present study) and Latil *et al.* (18) detected low *hTERT* expression in the majority of samples from normal prostate tissues and BPH. This contrasts with findings using the telomeric repeat amplification protocol, with which telomerase activity could not be detected in most normal and BPH tissues (3–5). The difference can be explained by the increased sensitivity of the real-time reverse transcription-PCR assay (16, 18). Moreover, low *hTERT* expression in normal prostate tissues corroborates findings that prostatic tissues contain stem cells (19), which generally express telomerase activity.

Recently, *DD3* has been described as one of the most prostate cancer-specific genes (10). Unfortunately, the biological function of *DD3* has not been unraveled, and no homology to any gene present in the computer databases has been found (10). Because of the lack of extensive open reading frames, we suspect that the gene functions as a noncoding RNA (10). Therefore, a mRNA-based method was necessary to quantify the expression of *DD3* in tissues. Our quantitative gene expression data showed that *DD3* is regulated by a unique prostate cancer-specific transcriptional mechanism. However, no correlation between transcriptional activity and tumor stage or grade was detected. For future clinical applications, our real-time, quantitative reverse transcription-PCR test will provide a very sensitive and specific tool to detect prostate tumor cells in tissue biopsies and bodily fluids.

REFERENCES

1. Pannek, J., and Partin, A. W. Prostate-specific antigen: what's new in 1997. Oncology, *11:* 1273–1278, 1997.
2. Zhang, W., Kapusta, L. R., Slingerland, J. M., and Klotz, L. H. Telomerase activity in prostate cancer, prostatic intraepithelial neoplasia, and benign prostatic epithelium. Cancer Res., *58:* 619–621, 1998.
3. Lin, Y., Uemura, H., Fujinami, K., Hosaka, M., Harada, M., and Kubota, Y. Telomerase activity in primary prostate cancer. J. Urol., *157:* 1161–1165, 1997.
4. Sommerfeld, H. J., Meeker, A. K., Piatyszek, M. A., Bova, G. S., Shay, J. W., and Coffey, D. S. Telomerase activity: a prevalent marker of malignant human prostate tissue. Cancer Res., *56:* 218–222, 1996.
5. Caldarera, E., Crooks, N. H., Muir, G. H., Pavone-Macaluso, M., and Carmichael, P. L. An appraisal of telomerase activity in benign prostatic hyperplasia. Prostate, *45:* 267–270, 2000.
6. de Kok, J. B., Zendman, A. J., van de Locht, L. T., Ruers, T. J. M., van Muijen, G. N., Mensink, E., and Swinkels, D. W. Real-time *hTERT* quantification: a promising telomerase-associated tumor marker. Lab. Investig., *79:* 911–912, 1999.
7. Meyerson, M., Counter, C. M., Eaton, E. N., Ellisen, L. W., Steiner, P., Caddle, S. D., Ziaugra, L., Beijersbergen, R. L., Davidoff, M. J., Liu, Q., Bacchetti, S., Haber, D. A., and Weinberg, R. A. *hEST2*, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell, *90:* 785–795, 1997.
8. Xu, L. L., Stackhouse, B. G., Florence, K., Zhang, W., Shanmugam, N., Sesterhenn, I. A., Zou, Z., Srikantan, V., Augustus, M., Roschke, V., Carter, K., McLeod, D. G., Moul, J. W., Soppett, D., and Srivastava, S. *PSGR*, a novel prostate-specific gene with homology to a G protein-coupled receptor, is overexpressed in prostate cancer. Cancer Res., *60:* 6568–6572, 2000.
9. Srikantan, V., Zou, Z., Petrovics, G., Xu, L., Augustus, M., Davis, L., Livezey, J. R., Connell, T., Sesterhenn, I. A., Yoshino, K., Buzard, G. S., Mostofi, F. K., McLeod, D. G., Moul, J. W., and Srivastava, S. *PCGEM1*, a prostate-specific gene, is overexpressed in prostate cancer. Proc. Natl. Acad. Sci. USA, *97:* 12216–12221, 2000.
10. Bussemakers, M. J., van Bokhoven, A., Verhaegh, G. W., Smit, F. P., Karthaus, H. F., Schalken, J. A., Debruyne, F. M., Ru, N., and Isaacs, W. B. *DD3*: a new prostate-specific gene, highly overexpressed in prostate cancer. Cancer Res., *59:* 5975–5979, 1999.
11. Gibson, U. E., Heid, C. A., and Williams, P. M. A novel method for real time quantitative RT-PCR. Genome Res., *6:* 995–1001, 1996.
12. Shay, J. W., and Bacchetti, S. A survey of telomerase activity in human cancer. Eur. J. Cancer, *33:* 787–791, 1997.
13. Broccoli, D., Young, J. W., and de Lange, T. Telomerase activity in normal and malignant hematopoietic cells. Proc. Natl. Acad. Sci. USA, *92:* 9082–9086, 1995.
14. Liu, K., Schoonmaker, M. M., Levine, B. L., June, C. H., Hodes, R. J., and Weng, N. P. Constitutive and regulated expression of telomerase reverse transcriptase (*hTERT*) in human lymphocytes. Proc. Natl. Acad. Sci. USA, *96:* 5147–5152, 1999.
15. de Kok, J. B., Schalken, J. A., Aalders, T. W., Ruers, T. J., Willems, H. L., and Swinkels, D. W. Quantitative measurement of telomerase reverse transcriptase (*hTERT*) mRNA in urothelial cell carcinomas. Int. J. Cancer, *87:* 217–220, 2000.
16. Dome, J. S., Chung, S., Bergemann, T., Umbricht, C. B., Saji, M., Carey, L. A., Grundy, P. E., Perlman, E. J., Breslow, N. E., and Sukumar, S. High telomerase reverse transcriptase (*hTERT*) messenger RNA level correlates with tumor recurrence in patients with favorable histology Wilms' tumor. Cancer Res., *59:* 4301–4307, 1999.
17. Bieche, I., Nogues, C., Paradis, V., Olivi, M., Bedossa, P., Lidereau, R., and Vidaud, M. Quantitation of *hTERT* gene expression in sporadic breast tumors with a real-time reverse transcription-polymerase chain reaction assay. Clin. Cancer Res., *6:* 452–459, 2000.
18. Latil, A., Vidaud, D., Valeri, A., Fournier, G., Vidaud, M., Lidereau, R., Cussenot, O., and Biache, I. *hTERT* expression correlates with MYC over-expression in human prostate cancer. Int. J. Cancer, *89:* 172–176, 2000.
19. Foster, C. S., and Ke, Y. Stem cells in prostatic epithelia. Int. J. Exp. Pathol., *78:* 311–329, 1997.

DD3^{PCA3}, a Very Sensitive and Specific Marker to Detect Prostate Tumors

Jacques B. de Kok, Gerald W. Verhaegh, Rian W. Roelofs, et al.

Cancer Res 2002;62:2695-2698.