Expression of human telomerase reverse transcriptase, Survivin, DD3 and PCGEM1 messenger RNA in archival prostate carcinoma tissue

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Introduction: *The wide spectrum of biological behavior displayed by prostate cancer (PCa) warrants investigation of potential PCa-specific biomarkers that could identify more aggressive tumor types and therefore provide prognostic value. Upregulation of expression of human telomerase reverse transcriptase (hTERT), Survivin, DD3 and PCGEM1 mRNAs in PCa lesions has recently been described. The purpose of this study was to evaluate the clinical value of detection of over-expression of these biomarkers in the diagnosis and prognosis of PCa.*

Material and methods: *Archival formalin-fixed, paraffin-embedded (FFPE) prostatectomy tissue from 26 patients with PCa (Gleason score 3-9, mean 7) and 14 patients with benign prostatic hyperplasia (BPH) were analyzed by reverse transcription polymerase chain reaction (RT-PCR) for semiquantitative transcript levels of hTERT, Survivin, DD3 and PCGEM1. In addition, 25 matched normal (MN) tissue samples were examined. The expression of biomarker mRNA relative to b2-microglobulin mRNA was determined using AlphaImager 2200 data analysis software.*

Results: *The biomarkers had sensitivities ranging from 91% to 100%. Clinical specificities evaluated with the BPH tissue were the following: hTERT mRNA (93%), DD3 mRNA (57%), Survivin (29%) and PCGEM1 (14%). Biomarker expressions were up to 13.5-fold higher in PCa tissue as compared to MN tissue. None of the tumor biomarkers showed a positive correlation with pathological stage and Gleason score.*

Conclusions: *The results of this study indicate potential utility of the hTERT mRNA and DD3 mRNA as diagnostic but not prognostic biomarkers for PCa.*

Key Words: prostate cancer, benign prostatic hyperplasia, hTERT, Survivin, DD3, PCGEM1, RT-PCR

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Introduction

Prostate cancer remains the most common non-cutaneous malignancy and the third leading cause of cancer mortality among men in Canada.[1] Serum prostate specific antigen (PSA) has an important role in early detection but is also frequently elevated in men with benign prostatic hyperplasia, prostatitis and other nonmalignant disorders.[2] A major limitation of the

PSA test is a lack of prostate cancer sensitivity and specificity especially in the intermediate PSA range of 4-10 ng/ml. Limited PSA specificity has resulted in a significant increase in the number of unnecessary prostate biopsy procedures and in the number of equivocal prostate cancer biopsy specimens.[3] Once diagnosed, current prognostic criteria such as pathological stage, Gleason score and PSA levels cannot predict cancer behavior sufficiently in individual patients. The wide spectrum of biological behavior displayed by prostate carcinoma would suggest that no single biological marker would likely provide prognostic information necessary to make a significant impact on patient management. The formulation of a panel of tumor biomarkers reflecting the ability of the neoplasm to proliferate, invade, metastasize and evade apoptosis, might provide more predictive prognostic information from tumor biopsies.

Prostate cancer initiation and progression involves multiple molecular alterations including up-regulated expression of a number of cancer-related genes. Recent gene discovery approaches have led to the identification of several new prostate-specific genes such as DD3[4] and PCGEM1.[5] Both of these genes represent a new class of noncoding RNA riboregulators highly overexpressed at all stages of prostate cancer and in some prostatic intraepithelial neoplastic (PIN) lesions. A novel member of the inhibitor of apoptosis (IAP) family, Survivin, has been re-expressed in all the most common human cancers including prostate cancer but not in normal adult tissues.[6] Survivin is induced by angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and angiopoietin-1 (Ang-1) which may explain its elevated levels in malignant tumors,[7,8] and downregulated by overexpression of effector cell protease receptor 1 (ERP-1).[9] High levels of Survivin expression have been associated with poor clinical outcome in colon and gastric cancers.[10] The human telomerase reverse transcriptase (hTERT) is the catalytic subunit of the telomerase ribonucleoprotein complex known to be required for cellular immortality and malignancy.[11] The hTERT mRNA expression has been considered a surrogate marker for telomerase activity based on its parallel detection in urological malignancies including prostate adenocarcinoma.[12,13] The purpose of this translational research study was to evaluate diagnostic performance of four tumor biomarkers mRNA hTERT, Survivin, DD3 mRNA and PCGEM1 in the detection of prostate carcinoma using a semiquantitative RT-PCR approach, as well as, to delineate the relationship, if any, between any of these biomarkers and cancer pathological stage and grade.

Materials and methods

Patients, specimens and processing

Archival formalin-fixed, paraffin-embedded (FFPE) tissue specimens from 26 patients with primary prostate cancer were analyzed in this retrospective study. This cohort included patients who had undergone radical prostatectomy, without preoperative hormonal therapy or another adjuvant therapy at the St. Joseph's Health Care London between 1997-2001. In addition, 25 matching normal (adjacent) tissue and 14 benign prostatic hyperplasia (BPH) tissue samples were examined. The BPH samples were obtained by transurethral resection. Six serial 10 μm tissue sections were cut from archival FFPE blocks, with four tissue sections placed in sterile tubes for tumor biomarker analysis and two adjacent tissue sections, representing the first and last section, were stained with hematoxylin and eosin (H&E) for histological assessment of the percentage of tumor cells. The tissue sections were cut with disposable blades using a microtome that was cleaned with CitriSolv (Fisher Sci., ON) and 100% ethanol between sections to eliminate possibility of contamination between patient specimens. The 1997 TNM system was used for pathological staging.[14] Grading of the primary cancer was conducted according to the Gleason system.[15] The study was approved by the University of Western Ontario Health Science Research Ethics Board.

RNA extraction

Two FFPE tissue sections (10 μm) were deparaffinized using 1 ml CitriSolv (Fisher Sci., 15 min) and rehydrated through three washes with 100% ethanol (5 min each) and one wash with 80% ethanol (10 min). Total RNA was extracted from the air-dried tissue pellet using 0.5 ml lysis buffer (20 mM Tris-Cl pH7.4, 20 mM EDTA, 1% SDS, 1 mg/ml proteinase K (Roche Diagnostics, Laval QC)). After 16 hour incubation at 55^0C, RNA was purified by two extractions with phenol:chloroform:isoamyl alcohol pH4.3 (Ambion, Austin TX) and precipitated with 1 vol of isopropanol, 0.1 vol of 3M sodium acetate (pH6.0), 10 μg/ml of linear acrylamide (Ambion) for 2 hr at -20^0C. To remove genomic DNA, all samples were treated with DNaseI as recommended by Roche Diagnostics. DNaseI heat inactivation (70^0C, 30 min) step served also for elimination of methylol groups. After two extractions with phenol:chloroform:isoamyl alcohol RNA was precipitated, resuspended in 30 μl of molecular-grade water and stored at -70^0C. RNA concentration was determined using the fluorometric

assay, RiboGreen™ (Molecular Probes, Eugene OR) as previously described.[16]

Reverse transcription-PCR

Complementary DNA (cDNA) was synthesized from 500 ng of total RNA in a 25 µl reaction containing 50 mM Tris-HCl (pH7.3), 75 mM KCl, 3 mM $MgCl_2$, 0.25 mM dNTP (Roche Diagnostics), 5 mM random hexamers, 8 mM dithiothreitol (Invitrogen Corp, Burlington ON), 2 0U of SUPERase-IN RNase inhibitor (Ambion) and 200 U of SuperScript RNase H^- RT (Invitrogen) using incubation conditions recommended by the supplier.

For analysis of expression of hTERT mRNA and Survivin (SURV) mRNA in archival FFPE tissue, optimized multiplex RT-PCR assays (with a housekeeping ß2-microglobulin (ß2 Mgl)) were developed. For analysis of DD3 and PCGEM1 mRNAs, single RT-PCR assays combined with simultaneous testing of ß2 Mgl mRNA (at the same conditions) were used due to difficulties with development of optimized multiplexed formats limited to short target sequences. Briefly, 4 µl aliquots of cDNA were subjected to PCR amplifications using primers listed in Table 1. Amplifications were performed in 50 µl reactions containing 50 mM Tris-HCl (pH8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, and 2.5 U of Platinum™ Taq DNA polymerase with 0.4 µM of DD3, PCGEM1, ß2 Mgl primers in a single format and 0.8 µM hTERT primers (or 0.4 µM of SURV primers) with 0.2 µM of ß2 Mgl primers in a multiplex PCR format. Reactions were subjected to a 5 minute incubation at 94°C, followed by 40 cycles of 94°C for 30 sec, 62°C for 30 sec, 70°C for 30 sec and a final 7 minute extension at 72°C in Perkin-Elmer GeneAmp 2400. For amplification of PCGEM1 cDNA 50°C annealing temperature was used. Each PCR batch included a positive control generated from prostate cancer cell line (LNCaP, DU-145 or PC-3) with known mRNA expression of biomarker gene and a negative sample without cDNA as a reagent control. "No RT" controls were carried out with 300 ng of RNA in all cases using the same RT reaction mix but substituting DEPC-H_2O for reverse transcriptase. PCR products were detected by a 2% agarose gel electrophoresis and ethidium bromide staining using DNA molecular marker VIII (Roche Diagnostics). A sample was considered positive for the presence of biomarker mRNA based on the detection of a specific biomarker amplicon with a biomarker / ß2 Mgl ratio (%) of at least 15 for hTERT and SURV, 20 for PCGEM1, and 45 for DD3. Due to splicing of the DD3 mRNA two amplicons sized 262 bp and 130 bp[17] were observed in the majority of samples. A sample was considered negative based on the absence of a specific biomarker amplicon and the presence of the 130 bp ß2 Mgl amplicon.

Determination of detection sensitivity of RT-PCR assays

The prostate cancer cell line LNCaP obtained from American Type Culture Collection (Manassa, VA) was propagated in RPMI (Invitrogen) with 10% fetal bovine serum and 1% penicillin/streptomycin in 5% CO_2 at 30°C. LNCaP cells were fixed in 10% neutral buffered formaline for 24 hours, washed in PBS and

TABLE 1. **Primers sequences**

Target gene transcript	Designation	Sequence 5'-3'	Sequence accession no.	Amplicon size (bp)	Reference
hTERT (RT A domain)	hTERT-2108F hTERT-2220R	cgc ctg agc tgt act ttg tc gca gta cgt gtt ctg ggg tt	NM001168	113	this study
Survivin (BIR domain)	SURV-97F SURV-211R	acc acc gca tct cta cat tc caa gtc tgg ctc gtt ctc ag	AY007685.2	116	this study
DD3	DD3-95F DD3-521R	ggt ggg aag gac ctg atg ata c ggg cga ggc tca tcg at	AF104907	262, 130	de Kok et al[17]
PCGEM1	PCGEM1-152F PCGEM1-261R	cgt aac ctg tgt ctg caa c gat aag gtc acg ttg agt c	AF223389.1	110	this study
ß2Mgl	ß2Mgl-775F ß2Mgl-904R	ctg aag ctg aca gca ttc gg gct gga tga cgt gag taa acc	AF072097.1	130	this study

The location of amplified cDNA region is indicated by a 5' nucleotide position designation of forward (F) and reverse (R) primers.

$1x10^8$ cells/ml mixed with an equal volume of 1% agarose (ICN Biomedicals, Aurora, OH) were solidified in a plug mold (Biorad Laboratories, Canada). After incubation in 70% ethanol for 16 hours, the cell plugs were embedded in Paraplast Tissue Embedding Medium (Fisher Sci.) according to suppliers' protocol. Total RNA was extracted from FFPE cell plugs containing approximately $4x10^6$ cells per plug and RNA concentration was determined using RiboGreen' assay. The LNCaP RNA cell equivalent was estimated at 0.04 ng/cell. The RNA was serially diluted to 10 cell equivalents and subjected to RT reaction as described above.

Data analysis

The ß2-microglobulin (ß2 Mgl) expression was used to normalize tumor biomarker mRNA expression for sample-to-sample differences in RNA input, RNA quality and RT-PCR assay efficiency. The ß2 Mgl mRNA expression was noted at a similar level in prostate cancer tissue and benign prostatic hyperplastic tissue. The expression of biomarker mRNA relative to ß2 Mgl mRNA was determined by measuring the density of respective DNA bands and quantification of digitized images using AlphaImager 2200 and AlphaEase software. For tumor biomarker analyzed in a single PCR format, ß2 Mgl mRNA determinations conducted in parallel were used for calculation of tumor biomarker mRNA to ß2 Mgl mRNA ratios. The significance of associations between tumor biomarkers and pathological stage or grade were evaluated using Fisher's exact test with P value less than 0.05 considered statistically significant.

Results

Development of RT-PCR based tumor biomarker mRNA assays

The optimized RT-PCR assays were devised in this study for the examination of expression of tumor biomarkers mRNA in formalin-fixed, paraffin-embedded (FFPE) prostate cancer tissue. Initially, an efficient method of total RNA extraction from archival FFPE tissue was developed that produced well amplifable mRNA of housekeeping gene b2 Mgl in a size of up to 260-bp. The b2 Mgl gene transcript was selected as an internal control in our RT-PCR assays based on its moderate copy number and the lack of pseudogenes,[18] which could potentially lead to the amplification of a similar size intronless b2 Mgl sequence.

The overall recovery of total RNA from archival tumor tissue sections varied from 2.4 to 6 mg per section (10 mm) and from 6.8 to 13 ng per mm^2 of paraffin-embedded tissue. The RNA extraction procedure included the removal of methylol groups formed during the interaction of formaldehyde with RNA during tissue fixation[19] thus increasing the quantity of demodified template mRNA without secondary structures, essential for cDNA synthesis. For analysis of hTERT mRNA and Survivin mRNA expression novel multiplex RT-PCR assays were developed. The assays were directed to the detection of hTERT and Survivin critical functional domains (RT A and BIR domains, respectively) based on recent reports of a higher frequency of their detection in the main types of carcinomas.[20-22]

The optimizations of RT-PCR assays (sensitivity) were conducted for the hTERT and Survivin biomarkers with human prostate cancer cell lines LNCaP, DU-145 and PC-3, and for the DD3 and PCGEM1 biomarkers with the LNCaP cell line. The DD3 and PCGEM1 transcripts were not expressed in DU-145 and PC-3 cell lines. The RT-PCR assays had an excellent detection sensitivity evidenced by their ability to detect approximately 5 LNCaP cells (as per RNA cell equivalents) expressing Survivin, DD3, and PCGEM1 mRNA (data not shown). The hTERT/b2 Mgl RT-PCR assay was able to detect approximately 10 LNCaP cells (as per RNA cell equivalents) expressing hTERT transcript (data not shown). All four optimized RT-PCR assays produced specific target biomarker amplicons of expected sequence and size.

Tumor biomarker mRNA expression in prostate carcinoma and matching normal tissue

The patients' age at malignancy diagnosis ranged from 51 to 69 years, with mean age of 60 years. All cancer cases were first reviewed by surgical pathologists at our institution and subsequently reassessed by a dedicated GU pathologist with expertise in prostate pathology. In 12 patients tumors were pathologic stage T2(a,b) and 14 were stage T3(a,b,c). Based on histological assessment of the percentage of tumor cells versus normal epithelial and stromal cells, FFPE tissue specimens from 21 of 26 patients found to contain 50% or more cancer cells were included in this comparative study.

The hTERT mRNA expression was detected in 19 of 21 (91%) archival prostate cancer tissue samples with relative expression levels ranging from 18 to 91 (mean, 42.2). The Survivin mRNA was found in all 21 (100%) analyzed tissue samples with relative expression levels from 30 to 103 (mean, 76.3). The mRNA expression of DD3 and PCGEM1 biomarkers was observed in 20 of 21 (95%) and all

TABLE 2. **Tumor biomarker mRNA expression in archival prostate carcinoma tissue stratified by stage and Gleason score**

Biomarker mRNA	No. of positive patients / Total no. tested (%)				
	Stage		Gleason score		Total
	pT2a-b	pT3a-c	3-6	7-9	
hTERT	6/7 (86)	13/14 (93)	6/7 (86)	13/14 (93)	19/21 (91)
Survivin	7/7 (100)	14/14 (100)	7/7 (100)	14/14 (100)	21/21 (100)
DD3	7/7 (100)	13/14 (93)	7/7 (100)	13/14 (93)	20/21 (95)
PCGEM1	7/7 (100)	14/14 (100)	7/7 (100)	14/14 (100)	21/21 (100)

Gleason score was defined as the sum of two most common histological grades detected in the tissue sections by pathological review. Abbreviations: pT2 stage, tumor confined within the prostate (a, involves half a lobe or less; b, involves more than half a lobe but not both lobes); pT3, tumor extends through prostatic capsule (a, unilateral extracapsular extension; b, bilateral extracapsular extension; c, tumor invades seminal vesicle(s)).

21 (100%) tumor samples, respectively, with relative expression levels from 138 to 343 (mean, 233) for DD3 and 39 to 369 (mean, 241) for PCGEM1. In the 5 of 26 excluded archival prostate cancer tissue samples with 10%-30% content of cancer cells (mean, 16%) tumor biomarkers were detected at a reduced frequency (hTERT 1/5; Survivin 4/5; DD3 4/5; PCGEM1 4/5) and lower expression levels. The expression of tumor biomarker mRNA was also noted in matching histologically normal tissue. The hTERT, Survivin, DD3 and PCGEM1 transcripts were detected in 13/25 (52%), 20/25 (80%), 15/25 (60%) and 19/25 (76%) matching normal tissue samples, respectively. The biomarker expression levels were 1.2 to 13.5-fold higher in prostate cancer tissue versus the corresponding normal tissue. There was no significant difference in the detection rate of any of examined biomarker mRNA and the pathological stage or grade of prostate carcinomas, Table 2. The difference observed between the frequency of hTERT mRNA expression in low Gleason ($\leq$6) tumors (86%) versus high Gleason ($\geq$7) tumors (93%) was also not significant (p=0.521).

Tumor biomarker mRNA expression in benign prostatic hyperplasia tissue

The specificity of the four biomarkers was assessed using archival benign prostatic hyperplasia tissue samples from 14 patients without prostate cancer, Table 3. As the potential effects of thermal artifact on the clarity to utilize the studied biomarkers is unknown, tissue was selected in which no gross visible thermal change was seen. The frequencies of correct identification of nonmalignant prostatic hyperplasia tissue specimens with the use of tumor biomarker RT-PCR assays were the following: hTERT, 13/14 (93%); DD3, 8/14 (57%); Survivin, 4/14 (29%); and PCGEM1, 2/14 (14%). Out of four evaluated tumor biomarkers only hTERT mRNA displayed a high clinical specificity. A similar trend was also observed when matching normal tissue samples were analysed for tumor biomarker mRNA expression. Adjacent normal tissue was correctly identified as nonmalignant by hTERT mRNA with 48% frequency and DD3 mRNA with 40% frequency. PCGEM1 and Survivin were specific in the recognition of benign status of only 20%-24% of matching normal samples, Table 3.

TABLE 3. **Specificity of tumor biomarker RT-PCR assays in the detection of prostate cancer in archival tissue**

Biomarker	No. of negative patients* / Total no. tested (%)	
	Matching normal	Benign prostatic hyperplasia
hTERT mRNA	12/25 (48)	13/14 (93)
DD3 mRNA	10/25 (40)	8/14 (57)
Survivin mRNA	5/25 (20)	4/14 (29)
PCGEM1 mRNA	6/25 (24)	2/14 (14)

* "Negative" patients were defined based on tumor biomarker / ß2Mgl ratio (%) lower than 15 for hTERT, SURV, and lower than 20 and 45 for PCGEM1 and DD3, respectively.

Discussion

This retrospective study was conducted with archival paraffin-embedded prostate tumor tissues recognized as an excellent source of morphologically defined specimens with extensive clinicopathological data. The use of FFPE tumor tissue over fresh/frozen tissue offers an advantage of the accessibility to a broad range of prostate cancer types and grades representing different stages of disease progression, rarely available in a prospective study. In view of limited success reported earlier during the recovery of amplifiable biomarker mRNA from FFPE tissues,[23,24] an efficient procedure for RNA isolation was developed in this study, which permitted for reproducible recovery of the b2 Mgl housekeeping mRNA in a size of up to 270 bases. To assure optimal detection sensitivity, the RT-PCR assays were targeted to the amplification of short regions of biomarker transcripts (≤130 bases). Current procedures used for routine processing of tumor tissue are designed for optimal preservation of tissue architecture and cell morphology without consideration for the integrity of molecular cell components such as RNA. The quality of RNA from archival tissues is known to be modified by various factors such the fixative, the fixation time, and post-mortem time. Although there is little information available concerning the stability and turnover of mRNAs in clinical specimens, it is believed that the delay in tissue fixation could affect representation of mRNA species extracted from paraffin-embedded blocks, since half-lives of human mRNA species vary from a few minutes to 24 hours. Another important factor known to affect the integrity of RNA in archival tissue is formalin fixation. The interaction between formaldehyde and nucleotide monomers leads to covalent RNA modification by the addition of N-methylol groups onto nucleotide base amino groups and subsequent formation of methylene crosslinks between RNA strands.[25] There is evidence that the RNA secondary structures induced by formaldehyde interaction interfere with the synthesis of longer cDNA fragments during reverse transcription reaction.[26] Recently Masuda et al[19] demonstrated that the chemical modification of RNA by formalin is reversible when RNA extracted from paraffin-embedded tissue is heat-treated to 70°C prior to reverse transcription.

This observation was confirmed in our earlier studies and the step of restoration of formalin-modified mRNA template activity was incorporated in our procedure of RNA preparation for biomarker RT-PCR assays. All paraffin-embedded prostatectomy specimens analyzed in this study that were stored for 2 to 6 years had well amplifiable housekeeping b2 Mgl transcript (130 bases) prepared by the above method.

The RT-PCR assays targeted to the detection of hTERT, Survivin, DD3 and PCGEM1 transcripts correctly detected prostate cancer in 91% to 100% patients with confirmed histopathological diagnosis. The clinical specificity evaluated with BPH specimens was the highest for hTERT mRNA (93%), moderate for DD3 mRNA (57%), and the lowest for Survivin mRNA (29%) and PCGEM mRNA (14%). Survivin and hTERT mRNA expression were increased in nearly all examined prostate carcinomas when compared to semiquantitative levels of their expression in histologically benign tissues from the same gland. These findings support the earlier suggestion that inhibition of apoptosis and an extended replicative capacity of cells are involved in the neoplastic transformation.[6] Dysregulation of apoptosis has been implicated in carcinogenesis by abnormally prolonging cell survival and facilitating the accumulation of transforming mutations. Survivin exerts its antiapoptotic activity by binding to and inhibiting the active forms of terminal effectors, caspase-3 and caspase-7.[27] A single BIR domain in Survivin, unusual among IAP family members, is sufficient for caspase interaction and protection against apoptosis.[28] Survivin overexpression was recently reported in several prostate cancer cell lines[29] (the result also observed in this study while testing LNCaP, DU-145 and PC-3) and in primary prostate cancer.[30] The findings of the present study are in agreement with previous reports regarding a high diagnostic sensitivity and specificity of hTERT mRNA in the detection of prostate tumors using fresh/frozen tissue.[17,31]

The two newly described prostate-specific genes, DD3[4] and PCGEM1[5] were overexpressed in this study in all examined primary prostate carcinomas and androgen responsive cell line LNCaP (but not in the androgen-insensitive cell lines PC-3, DU-145). Both of these genes produce non-coding (or non-translatable) mRNA-like transcripts that function, likely, as RNA riboregulators and/or are involved in regulation of expression of linked genes.[4,5,32] Initial examination of DD3 expression in prostatectomy specimens using Northern blot analysis demonstrated 10 to 100-fold overexpression of DD3 transcripts in prostate adenocarcinomas and detectable DD3 expression in normal prostate and BPH tissues when a more sensitive RT-PCR approach was applied.[4] The results of the semiquantitative evaluation of biomarker expression levels in the present study suggest that DD3 mRNA can discriminate between malignant and matching benign prostate. There was

up to 13.5-fold increase (mean, 4-fold) in DD3 mRNA expression in malignant prostate lesions when compared to the corresponding normal prostatic tissue. This data is confirmed by a recent quantitative assessment of DD3 mRNA levels in 31 prostate carcinomas showing 34-fold median increase in DD3 mRNA expression level in tumor tissues compared to nonmalignant tissues.[17] Nevertheless, the detection of similar levels of DD3 mRNA in malignant and normal prostatic tissue in over 10% of examined cases argues against independent use of this biomarker for diagnostic purposes.

Another promising prostate-specific gene, PCGEM1 was recently found by RT-PCR assay to be overexpressed in 13/23 (56%) of fresh/frozen prostate cancer samples, with 13% tumor samples showing reduced expression.[5] PCGEM1 expression was localized to glandular epithelial cells in tumor and normal specimens by *in situ* hybridization with generally lower levels in normal prostatic epithelium.[5] A variable expression of PCGEM1 mRNA was also observed in the current archival prostatectomy tissue study (mean, 241; range, 39 to 369) using RT-PCR assay capable of detecting approximately 5 LNCaP cell RNA equivalents. Inspite of the fact that PCGEM1 gene function appears to be prostate-specific,[5] the high rate of its BPH-associated overexpression does not indicate prostate cancer specificity.

A series of archival BPH tissues were examined in this study for the expression levels of above biomarkers. While only 7% of BPH tissue samples had detectable hTERT mRNA, DD3, Survivin and PCGEM transcripts were expressed in 43% to 86% of benign BPH tissues. It is of interest that consistent co-expression of DD3, Survivin, and PCGEM1 biomarkers at the levels resembling those seen in the majority of prostate carcinomas was observed in 5/14 (36%) of BPH specimens. As an alternative explanation, the high rate of expression of Survivin, DD3 and PCGEM1 in the benign-appearing areas of the prostate gland may be attributed to early onset of cancer-related molecular alterations arising within the transitional zone of the prostate gland. Although prostate adenocarcinomas develop mainly within peripheral zone of the gland (70%), these tumors may also arise in the transitional zone (25%), especially in foci adjacent to BPH.[33]

The hTERT, Survivin, DD3 and PCGEM1 mRNA expression was not observed in this study to be significantly increased in proportion to the aggressiveness of the cancers. Transcriptional activity of none of the examined biomarkers displayed a significant positive correlation with pathological stage or degree of cell differentiation estimated by Gleason score.

Translational studies such as this work are helpful in assessing the clinical value of candidate tumor biomarkers that may be useful in differentiating between tumor and nonmalignant growth and aiding in the prediction of prognosis. Results of the present investigation suggest that semiquantitative levels of hTERT mRNA expression permitted the detection of prostate cancer in archival tissue specimens with 91% sensitivity and distinguished neoplastic lesions from BPH with 93% specificity. The overall diagnostic performance of hTERT mRNA and DD3 mRNA might benefit from combining them into a multimarker panel assay. In spite of excellent sensitivity two other potential biomarkers evaluated in this study, Survivin and PCGEM1 displayed low specificity in prostate cancer diagnosis. A larger cohort of patients representing all stages and grades of prostate cancer requires study to confirm these findings.

Biomarker gene expression is increasingly considered as an objective supplementary approach to the histopathological work-up of precancerous and cancerous lesions of the prostate. Future laser-assisted microdissection of atypical prostate lesions and subsequent evaluation of multiple biomarker genes by quantitative RT-PCR assays or microarrays will likely enhance the pattern-oriented histological grading system that is currently in use. □

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