Telomerase Activity in Prostate Cancer, Prostatic Intraepithelial Neoplasia, and Benign Prostatic Epithelium

Weiben Zhang, Linda R. Kapusta, Joyce M. Slingerland, and Laurence H. Klotz

Departments of Urology [W. Z., L. H. K.] and Pathology [L. R. K.], Division of Cancer Biology Research [W. Z., J. M. S., L. H. K.], Sunnybrook Health Science Center, University of Toronto, Toronto, Ontario M4N 3 M5, Canada

Abstract

Telomerase is a ribonucleoprotein that synthesizes telomeric DNA on chromosomal ends. Telomerase activation has been seen in many immortal cell lines and cancers. Telomerase activity was analyzed in prostate carcinoma; in coexistent prostatic intraepithelial neoplasia (PIN), benign prostatic hyperplasia (BPH), atrophy and normal tissue; and in benign prostate glands. Telomerase activity was detected in 80 of 87 (92%) prostate cancers. Forty-one matched samples (from a total of 32 cases) were available for comparative analysis. The presence of telomerase activity in adjacent PIN, BPH, and normal tissue was correlated with telomerase activity in the malignant epithelium. In these adjacent tissues, telomerase activity was found in 11 of 15 (73%) PINs, 13 of 26 (50%) BPHs, and 1 of 6 (16%) atrophy and 4 of 11 (36%) normal tissues. In contrast to the BPH tissue from cancer-bearing glands, all 16 BPH specimens from patients only diagnosed with BPH were telomerase activity negative. In cancer samples, there was no correlation between telomerase activity and Gleason grade or preoperation prostate-specific antigen level. Our data indicate that telomerase activity is present in most prostate cancers. The high rate of telomerase activity in the benign-appearing areas of these glands may be attributed either to the presence of occult cancer cells or to early molecular alterations of cancer that were histologically apparent.

Introduction

Prostate cancer is characterized by marked biological heterogeneity. Microscopic foci of prostate cancer are present in over 40% of males over 50 years and in a substantial proportion of younger men (1, 2). This is a unique phenomenon among human cancers. These latent malignancies are typically of minimal volume (<0.1 mm³) and have an indolent clinical course. In contrast, larger-volume clinically detected cancers may progress during the patient’s lifetime, posing the threat of distant metastases and death. Differing between the indolent, non-life-threatening lesions and the biologically aggressive cancers is difficult and represents the major challenge in managing this disease (3). A marker that permits the identification of histological cancer that is phenotypically benign would be of substantial utility. The present study was undertaken to ascertain whether telomerase could distinguish these two prostate cancer phenotypes.

Telomerase is a ribonucleoprotein enzyme that synthesizes telomeric repeats onto chromosomal ends using a segment of its RNA component as a template (4). The enzyme contains protein components that include reverse transcriptase activity (5) and a RNA molecule that contains the C-rich strand of telomere repeats in the template region (6). Telomerase activity is directly involved in telomere maintenance, and its activation may play a role in cell immortality. It has been postulated that the immortalization of tumor cells relies on the aberrant reactivation of the telomerase enzyme.

The highly sensitive PCR-based assay TRAP³ permits evaluation of telomerase activity (7–9). Telomerase activity has been identified in most human malignancies and cancer cell lines (7, 10), suggesting that telomerase activation may be a critical step in cell immortalization and oncogenesis.

We have assessed telomerase activity in a cohort of prostate cancer patients treated with radical prostatectomy to determine whether there was a correlation between telomerase activity and other markers of biological phenotype, including serum PSA level and Gleason grade. In addition, we compared telomerase activity in noncancerous regions of cancer-bearing glands, particularly PIN and BPH.

Materials and Methods

Tissue Samples. Specimens of prostate cancer were obtained from 101 patients who underwent radical prostatectomy between 1992 and 1996. Tissue samples had been stored at −80°C in the tissue bank. A slide prepared from the frozen material was stained with H&E. All samples were histologically characterized as tumor, PIN (high grade), BPH, atrophy, or normal. Sixteen BPH samples obtained by cold-cup technique from noncancer patients at the time of transurethral prostatectomy were analyzed for telomerase activity as negative controls.

Cell Culture. Two well-defined, established human prostate cancer cell lines, PC-3 (11) and Du145 (12), were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Life Technologies, Inc., Bethesda, MD).

Telomerase Assay. Telomerase activity was assayed by using the protocol developed by Kim et al. (7). Cores of tissue (1–3 mm) were punched out from histologically predefined areas of the prostate. Extracts were prepared by powdering the tissue under liquid nitrogen in a Bio-pulverizer (Biospec Products, Bartlesville, OK) or homogenized in a Kontes tube with matching pestles (Kontes, Vineland, NJ) rotated at 450 rpm with 2 μl of ice-cold lysis buffer (10 mM Tris-HCl (pH 7.5), 1 mM MgCl₂, 1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 5 mM β-mercaptoethanol, 0.5% Triton X-100, 10% glycerol) per mg of tissue, incubated for 25 min on ice, and centrifuged at 16,000 x g for 25 min at 4°C (13). Extracts from cell lines were prepared by washing with ice-cold washing buffer (10 mM HEPES-KOH (pH 7.5), 1.5 mM MgCl₂, 10 mM KCl, and 1 DTT), centrifuged at 1,500 rpm for 10 min at 4°C, pelleted again, resuspended in 50 μl of ice-cold lysis buffer, and processed as described for tissue extracts. Supernatants were aliquoted, collected, and stored at −80°C. The protein concentration of the extract was measured by the Bradford assay (Bio-Rad). Two μl of cell extract (3 μg protein/μl) were used for each telomerase assay by the TRAP method. The PCR conditions were 32 cycles at 94°C for 40 s, 50°C for 40 s, and 72°C for 50 s (5 min for the final step). Each reaction mixture contained 2 μl of 5 × 10⁻¹⁸ g of an ITAS for the identification of false negative tumor samples that contained Taq polymerase inhibitor (14). One-half of the PCR product was analyzed by electrophoresis in 0.5% Tris-borate EDTA buffer on 12% polyacrylamide nondenaturing gels. To estimate telomere

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2 To whom requests for reprints should be addressed, at Department of Urology, M-G 408, Sunnybrook Health Science Center, 2075 Bayview Avenue, North York, Ontario, M4N 3 M5, Canada. Phone: (416) 480-4788; Fax: (416) 480-6121; E-mail: laurence.klotz@utoronto.ca.

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The abbreviations used are: TRAP, telomeric repeat amplification protocol; BPH, benign prostatic hyperplasia; PIN, prostatic intraepithelial neoplasia; PSA, prostate-specific antigen; ITAS, internal telomerase assay standard.
erase activity in tissue samples, we compared the intensity of the TRAP assay-generated DNA ladder with that of the ITAS signal using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). A cell extract from a sample with known telomerase activity was used as a positive control. As a negative control, lysis buffer was substituted for cell extract (7, 15), or the extract from the positive control was pretreated by 1 μg of RNase (5′-3′; Boehringer Mannheim, Mannheim, Germany) for 20 min at 37°C. Extracts that produced 6-bp ladders with three or more bands were considered telomerase positive. Equal volumes of positive control and negative extracts were mixed and retested for telomerase activity to identify samples containing inhibitors of telomerase or Taq. A kit for telomerase detection (Oncor, Inc., Gaithersburg, MD) was also used to provide an alternative method to verify the telomerase-negative results by TRAP.

Statistical Analysis. The significance of the differences among proportions was evaluated using the χ² test. Correlation between telomerase activity and other clinicopathological characteristics was performed using the t test.

Results

Telomerase-positive extracts produced a characteristic 6-bp ladder shown in Fig. 1. Lanes 3 and Lanes 7–10 represent telomerase-positive extracts. These 6-bp ladders do not appear in Lane 1, in which lysis buffer was substituted for positive cell extract, and in Lanes 2 and 6, in which positive controls were pretreated with RNase. Lanes 4 and 5 demonstrate lack of telomerase activity in the BPH and normal areas adjacent to telomerase-positive cancer (Lane 3) from the same gland (case 1). Lanes 8–10 demonstrate the presence of telomerase activity in BPH, PIN, and normal tissues adjacent to telomerase-positive malignant tissue (Lane 7) coexistent in the same prostate gland (case 2).

Of 87 evaluated prostate cancer tissues, 80 tumors (92%) were positive for telomerase activity. In cores taken from adjacent tissue areas, telomerase activity was found in 11 of 15 (73%) PINs, 13 of 26 (50%) BPHs, and 1 of 6 (16%) atrophy and 4 of 11 (36%) benign epithelium (P = 0.0001; Table 1). Two prostate cancer lines (PC-3 and DU145) also showed telomerase activity.

There were 32 cases in which 1 core from the tumor area and 1 or more additional cores from histologically characterized adjacent areas of PIN, BPH, and atrophy or normal areas were available for comparative analysis. The finding of telomerase activity in adjacent PIN, BPH, and normal tissue was associated in every case with telomerase activity in the malignant epithelium (Table 2). In contrast, in the two telomerase-negative cancer cases, all of the other histological areas sampled were also negative for telomerase activity.

All 16 BPH specimens removed from patients who did not have cancer were negative for telomerase. This contrasted with the tumor-associated BPH, which was frequently positive for telomerase activity (50%).

In six of seven cancers that were devoid of telomerase activity, the average pretreatment PSA level and Gleason score were lower than those in cancers whose telomerase activity was positive, but this difference did not achieve statistical significance (Table 3). Five-year follow-up was available on five of the seven patients; four of five have undetectable PSA levels, and one has early evidence of biochemical failure.

Discussion

Telomerase activation is a characteristic of immortalized tumor cells and is thought to contribute to the mechanism by which these cells abort the normal process of senescence. Telomerase activity has been detected in approximately 80–90% of tumor samples from many types of malignancies and is likely a necessary component of sustained malignant growth.

The present study demonstrated detectable telomerase activity in 80 of 87 (92%) prostate cancers, which is similar to earlier reports in smaller patient cohorts (3, 7). These findings suggest that telomerase activation may be important in the pathogenesis of human prostate cancer. We found no significant correlation between telomerase activity, preoperative PSA levels, or Gleason score.

This result contrasts with the report of Lin et al. (16), who found that the level of telomerase activity in prostate cancer was correlated with the primary Gleason pattern (although not with the total Gleason score) using a semiquantitative dilution technique. The small number of telomerase negative prostate cancers (five) in our series may have resulted in the failure to find such a relationship. Furthermore, the nonlinearity of our TRAP assay makes statements about relative levels of telomerase activity difficult (14). Given the technical limi-

Table 1 Telomerase activity in prostate cancer-bearing glands according to histological type

<table>
<thead>
<tr>
<th>Prostate cancer</th>
<th>Tissues adjacent to cancer</th>
<th>PIN</th>
<th>BPH</th>
<th>Atrophy</th>
<th>Normal</th>
<th>BPH only</th>
</tr>
</thead>
<tbody>
<tr>
<td>87</td>
<td>15</td>
<td>26</td>
<td>6</td>
<td>11</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>No. of samples</td>
<td>TA* positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%)</td>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 (92)</td>
<td>11 (73)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13 (50)</td>
<td>4 (36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (0)</td>
<td>0 (0)</td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

* TA, telomerase activity.
tation of our TRAP assay and the limited number of cases assayed, we were unable to confirm results of Lin et al. (16) with regard to Gleason score and telomerase activity.

PIN is a premalignant lesion. The prevalence of PIN is significantly higher in prostates harboring prostate cancer (33–100%) than it is in prostates that do not contain invasive cancer (4–18%; Ref. 17). PIN can precede carcinoma by 10 years or more (18). Mutations in tumor suppressor genes have been associated with PIN (19). The molecular pathogenesis of this precancerous lesion is still obscure. In 1995, Kim et al. (7) reported telomerase activity in three of five cases of PIN. The present study is the first to confirm this finding. We detected telomerase activity in 11 of 15 cases (73%) of high-grade PIN. In 13 matched samples, 1 core from the tumor and another core from histologically characterized adjacent PIN were studied. In 11 of these 13 cases, both the PIN sample and the invasive cancer proved telomerase positive (Table 2). In the other two glands, telomerase activity was detected in the cancerous area but not in the adjacent PIN.

The presence of telomerase in a high proportion of PIN supports the hypothesis that this lesion is a precursor of prostate cancer and harbors early molecular alterations leading to malignancy.

In benign-appearing tissue from cancer-bearing glands, telomerase activity was detected in 13 of 26 (50%) of BPHs and 5 of 17 (29%) normal tissues (including atrophy; Table 1). In total, 28 matched samples containing elements of BPH, atrophy, or normal tissues coexistent with carcinoma were analyzed. Telomerase activity was identified in the benign elements in 14 of 28 cases. All of these 14 cases were derived from glands bearing cancers that were telomerase positive (Table 2). In this study, telomerase activity was present in a higher proportion of nonmalignant tissues than in other studies, where detectable telomerase activity was identified in only 5–12% of BPH and was not detected in normal epithelium taken from the matched malignant glands (3, 7, 20). This contrasts with the complete absence of telomerase activity in the BPH samples taken from prostate glands devoid of cancer in the present study.

It is possible that occult cancer cells deep to the histologically characterized surface of the benign-appearing cores account for the high frequency of telomerase activity in this tissue. Pathological characterization evaluates the surface of the core only. In a 3-mm-long core, the presence of cancer cells deep to the surface cannot be excluded. The TRAP assay is sensitive enough to detect telomerase activity from 1–10 telomerase-expressing cells (21). This data could also be explained by the provocative hypothesis that early in the development of prostate cancer, telomerase activation may occur before histological alterations. Activation of telomerase in benign-appearing tissue may correlate with continued proliferation beyond the limits of the normal finite prostatic epithelial life span. This would represent an important step in the premalignant progression toward prostate cancers.

This observation also raised the possibility that telomerase detection could provide a more sensitive means than conventional histology for the detection of infiltrating cancer cells in benign-appearing tissue (21). Conversely, the absence of telomerase activity in histological prostate cancer may portend a very favorable biological phenotype and provide a rationale for conservative management. Additional studies will be required to evaluate the clinical significance of telomerase activity in histologically benign tissue and the absence of telomerase in histological cancer.

Acknowledgments

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References


Table 2 Correlation of telomerase activity (TA) in different nonmalignant samples from prostate cancer-bearing gland

<table>
<thead>
<tr>
<th>PIN</th>
<th>BPH</th>
<th>Atrophy</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples from cancer-bearing glands</td>
<td>13</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>No. of TA-positive samples</td>
<td>11</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>% of nonmalignant samples showing TA</td>
<td>85% (11/13)</td>
<td>60% (12/20)</td>
<td>0% (0/2)</td>
</tr>
</tbody>
</table>

* In all of the TA-positive nonmalignant samples, the associated invasive tumor was TA positive. This demonstrates that 100% of TA-positive nonmalignant tissue was derived from TA-positive cancer-bearing glands.

Table 3 Telomerase activity (TA). grade, and PSA

<table>
<thead>
<tr>
<th>TA-positive samples</th>
<th>No.</th>
<th>TA-negative samples</th>
<th>No.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation PSA (µg/l)</td>
<td>10.7 ± 8.6</td>
<td>67</td>
<td>7.9 ± 5.1</td>
<td>6</td>
</tr>
<tr>
<td>Gleason score</td>
<td>6.7 ± 1.1</td>
<td>54</td>
<td>6.0 ± 0.7</td>
<td>5</td>
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</tbody>
</table>
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