Relative Contribution of Normal and Neoplastic Cells Determines Telomerase Activity and Telomere Length in Primary Cancers of the Prostate, Colon, and Sarcoma

Monika Engelhardt, Juan Albanell, Pamela Drullinsky, Wei Han, Jose Guillem, Howard I. Scher, Viktor Reuter, and Malcolm A. S. Moore

James Ewing Laboratory of Developmental Hematopoiesis [M. E., J. A., P. D., W. H., M. A. S. M.], Genitourinary Oncology Service [H. I. S.], and Departments of Surgery [J. G.] and Pathology [V. R.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021; and Department of Medicine, Cornell University Medical College, New York, New York [H. I. S.]

ABSTRACT

Telomerase and telomere length are increasingly investigated as potential diagnostic and prognostic markers in human tumors. Among other factors, telomerase and telomere length may be influenced by the degree of tumor cell content in tumor specimens. We studied telomerase activity and telomere length with concomitant integration of histopathological data to determine whether both were influenced by the amount of tumor cells. We measured telomerase in 153 specimens: in 51 solid tumor blocks; in 51 cryostat sections; and in 51 adjacent normal tissues from patients in 153 specimens: in 51 solid tumor blocks; in 51 cryostat sections; and in 51 adjacent normal tissues from patients with sarcoma (n = 10) and colorectal (n = 11) and prostate cancer (n = 30) using the sensitive and rapid detection telomeric repeat amplification protocol assay. Telomere length was determined by telomere restriction fragment Southern blot analysis. From cryostat sections, tumor cell infiltration was assessed. Telomerase activity was detected in all colorectal tumors and sarcomas, as expected. In primary prostate cancer, however, telomerase activity was less frequently observed (14 of 30, 47%). Moreover, a decreased intensity compared to colon cancer and sarcoma was evident (P < 0.001). The median tumor cell infiltration was significantly higher in sarcoma (65%) and colon (30%) compared to prostate cancer (5%; P < 0.001). There was a positive correlation between tumor cell infiltration and telomerase activity (r = 0.89; P < 0.001). Telomere restriction fragments in tumors were shorter compared to the normal tissues with peak differences in colon, sarcoma, and prostate of 1.8, 2.8, and 1 kilobase pairs, respectively (P < 0.002). Our data suggest the presence of a positive correlation between the degree of tumor cell content in human solid tumors and the level of telomerase activity detected. We demonstrated that the amount of tumor cells also affects telomere restriction fragment analysis. Therefore, with the predominance of normal cells in tumor specimens, telomerase activity measured may not reflect the malignant phenotype, and telomere loss may be underestimated. This phenomenon was most evident in prostate cancer. Our results will have implications for the future when telomerase activity and telomere lengths may be used for early screening, diagnosis, and prognosis determinations and when telomerase inhibitors are applied to clinical practice.

INTRODUCTION

Telomeres are specialized chromosomal end structures composed of TTAGGG repeats in humans. They function to protect chromosomes from degradation, fusion, and recombination events (1). In the absence of compensatory mechanisms, telomeres shorten with each cell division because the termini of linear molecules are not replicated by conventional DNA polymerases (2, 3). To date, telomere shortening has been observed in most dividing somatic cells. This suggests that when a critical telomere length is reached, cell senescence occurs (4, 5). Telomerase has been identified as a ribonucleoprotein enzyme that can compensate for telomere loss by adding TTAGGG nucleotide repeats onto chromosomal ends (1–4). Low levels of telomerase activity have recently been detected in human primitive hematopoietic cells (5–7). However, in most other normal somatic cells, telomerase has not been detected, and consequently telomere shortening occurs after a limited number of population doublings (8–10). In contrast, in germ cell tissue, spontaneously immortalized tumor cell lines, and the majority of tumors, high telomerase activity, stable telomere length, and unlimited proliferative potential have been observed (4, 11–26).

Previous studies have suggested an association of tumor cell infiltration and telomerase activity (18, 21, 27), but to date no study has conclusively correlated the effect of tumor infiltration on telomerase level and telomere length. In this study, we present a detailed analysis of three different types of solid tumors in an attempt to clarify this phenomenon. We selected sarcoma and colon cancer, both considered to be aggressively proliferating tumors, and prostate cancer, which in comparison to the former two is thought to be more indolent. Our results...
demonstrate the value of the careful integration of pathological evaluation when interpreting telomerase activity and telomere length measurements.

MATERIALS AND METHODS

Tissue Samples. Frozen tissues from 51 solid tumors and 51 normal adjacent tissues from patients with sarcoma (n = 10) and colon (n = 11) and prostate cancer (n = 30) were retrieved from our tissue bank. All cases were reviewed by pathologists. Fifteen prostate tumors were staged T2, 12 were T3, and 3 were T4. Twenty-four prostate cancer specimens were moderately differentiated, 4 were poorly differentiated, and 2 were well differentiated. Fourteen prostate cancer patients had tumors with Gleason grade 5/6 compared to 16 with Gleason grade 7/8 (n = 12) and 9/10 (n = 4). In colon, two specimens were staged T1, three were T2, four were T3, and two were T4. Four colon specimens were moderate, five were poor, and two were well differentiated. Nine high-grade sarcomas (three synovial sarcomas, two fibrosarcomas, two leiomyosarcomas, one liposarcoma, and one neurofibrosarcoma) and one low-grade sarcoma (fibrosarcoma) were evaluated. From each tumor sample, one-half was used immediately for protein and DNA preparation (solid tumor block preparation); the other half was embedded for cryostat sections using tissue Tek II OCT (Micos Ink; Tissue Tek Diagnostos, Mishawaka, IN). The embedded tissue was completely frozen in liquid nitrogen, and successive 5-, 60- and 5-mm cryostat sections were obtained in a “sandwich technique.” Top and bottom 5-mm sections were mounted on glass slides and were stained with H&E for light microscopy interpretation of tumor cell infiltration. The quantitation of the tumor cell infiltration was assessed by visual estimation from both top and bottom 5-μm sections by a pathologist in a blind fashion. The middle 60-μm section was assayed for telomerase.

TRAP Assay. Cells from 153 specimens: from 51 solid tumor blocks; from 51 cryostat sections; and from 51 normal adjacent tissues from sarcoma and colorectal and prostate cancer patients were processed as described previously (4, 28). Tissue samples were thinly sliced, homogenized with ice-cold lysis buffer (106 cells/100 μl): 0.5% 3-(3-cholamidopropyl)-dimethylammonio]-l-propane-sulfonate, 10 mM Tris-HCl (pH 7.5), 1 mM MgCl2, 1 mM EGTA, 10% glycerol, 5 mM β-mercaptoethanol, and 1 ng/ml leupeptin; and disposable pestles rotated at 450 rpm by a drill until the tissue was dispersed. Tissues were incubated on ice for 30 min and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected, and the protein concentration was determined by the Bradford assay (Bio-Rad Laboratories, Richmond, CA). Aliquots were diluted to 1 μg protein/μl and stored at −80°C. In brief, the TRAP assay was performed as follows (4, 11, 28). Two μg of protein extract were assayed in a 200-μl reaction mixture [0.75 mM 4-methyl-umbelliferoyl phosphate, 112 mM 2-amino-2-methyl-1-propanol buffer (pH 10.4), 3.75 mM MgSO4, and 1 mg/ml polyvinyl pyrrolidone], were incubated at 37°C for 1 h, and the reaction was stopped by adding 20 μl of 1 M NaOH. The fluorescence of the product was read on a fluorescence plate reader (CytoFluor 2350). Alkaline phosphatase activity was found present in all solid tumor and normal adjacent tissue preparations and 60-μm sections, with no significant difference in tumor and normal tissue control specimens.

TRF Assay. Telomere length was determined by TRF4 Southern blot analysis as described previously (4, 6, 9, 16). Genomic DNA from tumor and normal adjacent tissues was digested with 400 μl of DNA extraction buffer [100 mM NaCl, 40 mM Tris (pH 8.0), 20 mM EDTA (pH 8.0), and 0.5% SDS] and proteinase K (0.1 mg/ml). Extraction was performed using
phenol/chloroform. Five to ten μg of extracted DNA were digested with 10 units of MSPI and RSAI (Boehringer Mannheim, Indianapolis, IN) for 12–24 h at 37°C. Integrity of the DNA before digestion and completeness of digestion were monitored by gel electrophoresis. Electrophoresis of digested genomic DNA was performed in 0.5% agarose gels in 45 mM TBE buffer (pH 8.0) for a total of 660–700 V h. After electrophoresis, gels were depurinated in 0.2 M NaOH/1.5 M NaCl, neutralized in 0.5 M Tris/1.5 M NaCl, and transferred to a nylon membrane (Schleicher & Schuell, Keene, NH) using 20× SSC and dried for 1 h at 70°C. The telomeric probe (TTAGGG)₃ (Genset, San Francisco, CA) was 5'-end labeled with [γ³²P]ATP using T4 polynucleotide kinase (Boehringer Mannheim). Prehybridization and hybridization were performed at 50°C using 5× Denhardt’s solution, 5× SSC/0.1 M Na₂HPO₄/0.01 M Na₄P₂O₇, and 30 μg of salmon sperm DNA per ml/0.1 mM ATP. Membranes were washed in 0.5× SSC/0.1% SDS solution. Telomeric smears were visualized by exposing the membranes to an image plate. The mean lengths of TRFs were analyzed with a phosphorimage analyzer (Molecular Dynamics). Mean TRF lengths were defined as Σ(OD/L) / Σ(OD), where OD is the densitometer output and L is the length of DNA in position (1, 31). Sums were calculated over the range of 2–23 kbp. Telomere peak values were measured by estimating the band size corresponding to the point with the highest absorbance (16). Differences in TRF peak values were defined as a divergence of at least 2 kbp.

**Calculation of Telomere Length Changes by Varying Admixture of Normal Cells.** The accuracy of the mean and peak TRF values to estimate telomere length in tumors can be affected by the ratio of normal versus tumor cells. We created computer-based simulations to assess the effects of tumor heterogeneity on TRF values. Based on these simulations, we estimated the influence on telomere length measurements by an admixture of normal cells in tumor samples. We used the following formula to recalculate TRF length for all 51 tumor specimens: Length of TRF

\[
\text{Length of TRF} = \frac{\Sigma(OD/L)_{\text{tumor}} - (\Sigma(OD/L)_{\text{tumor}} - \Sigma(OD/L)_{\text{normal}})}{\text{admixture of normal cells in %/100}}
\]

**Calculation of Number of Cell Divisions.** An evaluation of the number of PDs in tumor specimens, based on differences in mean and peak TRF length between normal and tumors, was performed. The following formula was used:

\[
\text{Mean or peak TRF (kbp) of normal specimen} = \frac{\text{number of PDs}}{\text{tumor specimen: 0.05 kbp} = \text{number of PDs}}
\]

**Statistics.** Comparisons among groups were made with standard statistical tests. Results are expressed as median and range except when stated otherwise. The relationship between tumor cell infiltration and telomerase activity, and telomere length and telomerase activity, were estimated by linear regression and correlation analysis. Statistical significance of the data obtained was analyzed by the Wilcoxon rank sum test and Student’s t test (Statworks, Cricket Software, Philadelphia, PA). A P less than 0.05 was considered statistically significant.

**RESULTS**

**Telomerase Activity Levels in Solid Tumor Block Specimens.** From solid block specimens, all 11 colon and 10 sarcoma specimens displayed telomerase activity (Table 1; Fig. 1). Forty-seven% of prostate cancer specimens (14 of 30) had telomerase activity with a median activity 20-fold lower than found in colon and sarcoma (P < 0.001; Table 1; Fig. 1A). Dountitrating the protein 10-fold (0.2 μg of protein) and 100-fold (0.02 μg of protein) demonstrated persistent telomerase activity in colon and sarcoma. With the dilution of prostate samples already at limited levels, no telomerase activity in 10- and 100-fold dilutions was detectable. In all normal adjacent tissues, telomerase activity was undetectable, in agreement with the reported absence of telomerase in most somatic tissues (2, 4, 13–15).

**Telomerase Activity Levels in Cryostat Sections.** In 51 tumor specimens, 5-μm cryostat sections were evaluated histopathologically. A high tumor cell content in sarcoma (median, 65%) and in colon (median, 30%) was found compared to significantly lower tumor cells in prostate sections (median, 5%; P < 0.001; Fig. 2). From all 51 tumors embedded for cryostats, not all had tumor cells detectable in the 5-μm section. Tumor cells were visualized in 9 of 11 colon, 8 of 10 sarcoma, and 10 of 30 prostate sections (Table 1). Assays of nine of nine colon (100%) and six of eight sarcoma (75%) sections showed telomerase activity in the 60-μm cryostat section (Table 1). Two sarcoma sections, despite verified tumor cells, failed to reveal telomerase from the 60-μm preparation. These, however, also had weak telomerase activity in the larger solid tumor block preparations. In 2 of 11 colon and 2 of 10 sarcoma sections, only

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**Table 1** Telomerase activity and tumor cell infiltration in solid tumors (median and ranges)

<table>
<thead>
<tr>
<th>Solid tumor preparation</th>
<th>60-μm sections</th>
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<tbody>
<tr>
<td></td>
<td>Histologically verified tumor</td>
</tr>
<tr>
<td></td>
<td>Telomerase +</td>
</tr>
<tr>
<td>Colon 11</td>
<td>11/11</td>
</tr>
<tr>
<td>Sarcoma 10</td>
<td>10/10</td>
</tr>
<tr>
<td>Prostate 30</td>
<td>14/30</td>
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</table>

* Telomerase activity expressed as a percentage of the neuroblastoma cell line (SK-N-SH standard) control.

b P < 0.001 compared to telomerase activity in colon and sarcoma.
normal tissue was detectable and revealed no telomerase activity (Fig. 1B). In prostate, all sections assayed with verified tumor cells (10 of 10) showed telomerase activity in the 60-μm cryostat section (Fig. 1B). In the remaining 20, only normal tissue was detectable (Fig. 1B). Of these 20, in 15 no telomerase activity was present, but in 5 "normal" prostate sections, low telomerase activity was unexpectedly found (Table 1). In these, a histological reevaluation was performed once telomerase was known, which showed tumor cells in one retrospectively (Table 1). The detectability of telomerase in four of five prostate specimens without histological tumor cell verification was most likely due to the lack of tumor cell infiltration in 5-μm sections as opposed to the 60-μm section.

**Correlation Analysis of Tumor Cell Infiltration and Telomerase Activity.** A correlation analysis of tumor cell infiltration and average telomerase activity, calculated from at least three independent TRAP assays, demonstrated a positive correlation \( r = 0.89; P < 0.001 \); Fig. 3). High telomerase activity was observed in specimens that consisted predominantly of tumor cells. The same trend of high telomerase activity in samples with increased tumor cell content was found when each tumor type was analyzed separately. Increased tumor cell content was defined as tumor infiltration over the median infiltration present in each tissue: over 65% in sarcoma, 30% in colon, and 5% in prostate cancer. In sarcoma, median telomerase activity was higher with 31% (range, 20-45.2%) in high tumor infiltration specimens versus 10.3% (range, 9.5-13%) in specimens with low tumor cell infiltration \( P = 0.008 \). In colon, telomerase activity was 31% (range, 20-49%) in high tumor infiltration specimens versus 15.9% (range, 9-16.4%) in low tumor cell infiltration specimens \( P = 0.02 \); and in prostate cancer, 8.5% (range, 5.3-15.6%) versus 0% (range, 0-8.8%);
Fig. 2  Tumor samples were used for cryostat sections of 5, 60, and 5 μm obtained in a “sandwich technique.” Thin top and bottom sections (5-μm) were stained with H&E for light microscopy interpretation of tumor infiltration. A, section of sarcoma in which over 90% of cells are tumor (×200). B, section containing normal colonic mucosa, stroma, and colonic adenocarcinoma (arrow). Approximately 50% of cells are tumor (×200). C, section containing predominantly benign prostatic glands and stroma. A few glands of prostatic adenocarcinoma representing less than 5% of the tissue sampled is also present (arrow; ×200).
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Fig. 3 Histological evaluation of tumor cell infiltration using cryostat sections was performed in 51 tumor specimens to determine tumor versus noncancerous stroma cells. Median tumor infiltration was high in sarcoma (65%) and in colon (30%) compared to significantly lower tumor infiltration in prostate (5%). A correlation analysis of tumor infiltration and telomerase activity given in percentage of the neuroblatoma control showed a positive correlation (r = 0.89; P < 0.001). Increased telomerase activity was found in specimens that consisted predominantly of tumor cells.

TRF Length Measurements and Effect of Tumor Cell Infiltration on Telomere Length Analysis. In colon and sarcoma, all tumors contained shorter telomeres than the adjacent normal tissue. In prostate cancer, shorter and equal TRF lengths compared to normal tissue were observed (Fig. 4). Mean values are shown in Table 2. In 7 of 11 colon samples, 3 of 10 sarcoma samples, and 7 of 30 prostate samples, two distinct peak TRF values of at least ≥2 kbp difference were observed, with the higher peak corresponding to that of the adjacent normal control and the lower peak most likely representing the tumor (see “Materials and Methods”). Utilization of peak TRF values demonstrated shorter telomere lengths in tumor specimens compared to mean TRF values (Table 2). Therefore, peak values seemed to more accurately define tumor telomeres than mean TRF values, and the resultant differences between tumor and normal tissue peak TRFs were 1.8 kbp (P = 0.001), 2.8 kbp (P = 0.001), and 1 kbp (P = 0.002) in colon, sarcoma, and prostate, respectively (Table 2). The accuracy of the mean and peak TRF values to estimate telomere length in tumors can be affected by the ratio of normal versus tumor cells. We created computer-based simulations to assess the effects of tumor heterogeneity on TRF values. Our simulations assumed that tumors are composed of two distinct cell populations, A and B. A is the population of normal cells, and B is the tumor cell population. Arbitrary mean and peak TRF values were assigned to both A and B in the Microsoft Excel program used to calculate TRF values. A and B were artificially mixed in the following manner: 100% A; 90% A, 10% B; 75% A, 25% B; 50% A, 50% B; 25% A, 75% B; 10% A, 90% B; and 100% B. The resulting mean and peak TRF values suggested that:

(a) if the TRF values of A and B are close (i.e., A = 6 kbp, B = 5 kbp) and both populations are mixed, only a single peak TRF will be detectable. This value will be different than the peaks of A and B and closer to the predominant population. Mean TRF values are also closer to the predominant population; and

(b) if the TRF values of A and B are very different (i.e., A = 10 kbp, B = 4 kbp) and both populations are mixed, two peaks will be detectable. These two peaks are similar or identical to the peaks of A and B. This suggests that when two peaks are observed in a tumor specimen, they probably reflect the tumor versus normal cell population. In terms of mean TRF analysis, only one value is generated that will be closer to the predominant cell population. These models suggest that telomere length loss in tumor specimens is underestimated, especially when the degree of tumor cell infiltration is low. Based on these simulations, we estimated the influence on telomere length measurements by admixture of normal cells in tumor samples. We used the formula to recalculate TRF length for all 51 tumor specimens:

\[
\text{TRF}_{\text{admixture}} = \frac{\text{TRF}_{\text{tumor}} \times (\text{normal admixture} \times \text{TRF}_{\text{normal}} + (1 - \text{normal admixture}) \times \text{TRF}_{\text{tumor}})}{100}
\]

where TRF<sub>admixture</sub> is the corrected TRF length, TRF<sub>tumor</sub> is the tumor TRF length, normal admixture is the percentage of normal cells in the tumor specimen, and TRF<sub>normal</sub> is the mean TRF length of normal cells. This formula accurately predicted the TRF values of either A or B from the simulations in which both were mixed. This mathematical model used to calculate possible TRF changes (“corrected” TRF) and applied for tumor cell infiltration of 90, 80, 50, 10, and 5%, demonstrated TRF length changes of 0.1, 0.2, 0.5, 0.9, and 0.95 kbp. According to these calculations, “corrected” telomere lengths in colon, sarcoma, and prostate cancer specimens were 5.8, 5.25, and 5 kbp, respectively (Table 2). Therefore, corrected for their tumor cell content, colon, sarcoma, and prostate cancer displayed similar telomere lengths (Table 2).

Correlation Analysis of Telomere Length versus Telomerase Activity. When evaluating telomerase-positive and -negative samples, we found telomere lengths that were significantly longer in telomerase-positive samples compared to those with no or weak (<10%) telomerase activity (Table 3). Mean TRF lengths were 6.7 kbp, and peaks were 6.1 kbp in all telomerase-positive tumor specimens, compared to mean TRFs of 5.2 kbp and peaks of 4.8 kbp in specimens with no or low telomerase activity (P = 0.001). Telomere lengths in each tumor subgroup displayed the same trend (Table 3).

There was a weak positive correlation between telomere length and telomerase activity. Analyzing TRFs that were uncorrected and corrected for their tumor cell infiltration showed correlation coefficients of r = 0.54 and r = 0.45 (P < 0.05), respectively, implying that elongated telomeres may correlate with higher telomerase activity.
Colon

Sarcoma

Prostate

Fig. 4  Results of a representative Southern blot analysis depicting telomere restriction fragments in colon, sarcoma, and prostate tumor samples (T) and adjacent tissue (N). In colon and sarcoma, all tumors showed shorter telomeres than their normal adjacent tissue. In prostate, shorter and equal TRF lengths compared to normal tissue were observed. Mean TRF differences between tumor and normal in colon, sarcoma, and prostate were 1 kbp ($P = 0.04$), 1.9 kbp ($P = 0.007$), and 0.5 kbp ($P = 0.08$), respectively.

Table 2  Mean and peak TRF values in tumor samples and in normal adjacent tissue

<table>
<thead>
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<th>Mean TRF (kb)</th>
<th>Peak TRF (kb)</th>
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<tr>
<td></td>
<td>Tumor</td>
<td>Normal</td>
</tr>
<tr>
<td>Colon</td>
<td>11</td>
<td>6.5 ± 1.1</td>
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<tr>
<td></td>
<td>“corrected” TRF$^b$</td>
<td>5.8</td>
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<tr>
<td>Sarcoma</td>
<td>10</td>
<td>5.5 ± 1.1</td>
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<tr>
<td></td>
<td>“corrected” TRF$^b$</td>
<td>5.25</td>
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<tr>
<td>Prostate</td>
<td>30</td>
<td>5.9 ± 0.9</td>
</tr>
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<td></td>
<td>“corrected” TRF$^b$</td>
<td>5</td>
</tr>
</tbody>
</table>

$^a$ Corrected for admixture of normal cells in tumor specimens using the formula: $(\Sigma(OD/L)_t)(\Sigma(OD)_t)$ - $(\Sigma(OD/L)_n)(\Sigma(OD)_n)$ normal × (admixture of normal cells in %/100).

$^b$ Corrected for admixture of normal cells in tumor specimens using the formula: $(\Sigma(OD/L)_t)(\Sigma(OD)_t)$ - $(\Sigma(OD/L)_n)(\Sigma(OD)_n)$ normal × (admixture of normal cells in %/100).

DISCUSSION

Telomerase activity has been reported in a wide variety of tumor types (12–26). Telomerase activation is thought to correspond to a late event in the process of multistage tumorigenesis, which results in stabilization of telomeres and subsequent immortalization (2). Recently, high telomerase activity has been correlated with a worse clinical prognosis (12, 14). However, conflicting results have been reported concerning telomerase activity with regard to clinical stage and pathological grade, suggesting that telomerase may be influenced by parameters other than clinicopathological features (12, 14, 32–34). Integration of histopathological analysis in telomerase activity and telomere length measurements in 51 solid tumors and 51 matched normal specimens was performed in this study to clarify these conflicts. Although previous studies have suggested an association of tumor cell infiltration and telomerase activity (18, 21, 27), no study to date has conclusively investigated this phenomenon. Sarcoma and colon cancer were used as representatives of more aggressively growing tumors, and prostate cancer was selected as a representative tumor with a more indolent growth pattern.

We determined that telomerase and telomere lengths have different values in various tumor types and that both are influenced by the degree of tumor cell infiltration. Our data are in agreement with previous reports that showed telomerase to be highly expressed in colon cancer (23). In sarcoma, which has not been well studied, a similarly high telomerase expression was observed. However, in primary prostate cancer, undetectable or substantially lower telomerase activity was found, which contrasts to prior findings detecting telomerase in 85% of specimens tested (25). Histological evaluation of 51 cryostat sections revealed that tumor infiltration in sarcoma and colon was significantly higher compared to prostate cancer. This finding indicates that low telomerase activity in our prostate cancer
specimens reflects a decreased tumor cell burden and the predominance of a normal cell population. Correlation analysis demonstrated that the degree of tumor cell content correlated with telomerase activity. Thus, the quantitatively different presence of tumor cells accounts for our results in prostate cancer and cautions future interpretation of telomerase levels without the concomitant analysis of tumor cell content. Using cryostat sections and solid tumor block preparations for telomerase evaluation illustrated well the value of the highly sensitive TRAP assay as a diagnostic marker for malignancy. Both in small cryostat sections and in specimens with less than 5% tumor cell infiltration, telomerase activity was detectable. This was supported by our histological reevaluation, which revealed tumor cells retrospectively in at least one case. Therefore, telomerase may be used as an adjunct to histopathological evaluation, especially in tumors with low tumor cell infiltration such as prostate cancer, where it may be possible for a pathologist to miss the presence of only a few cancer cells.

In malignant cells, telomere length measurements have been used as a marker for cell proliferation and PDs that occur prior to telomerase activation at a level sufficient to stabilize telomeres (1, 4, 9, 12, 16, 18, 21, 22, 25, 35). There are several theoretical mechanisms accounting for telomere shortening in malignancy. Short telomeres are thought to result from an increased number of PDs in tumors or because telomerase is turned on late in tumorigenesis (6, 8–10, 12). Telomerase may also elonate only some tumor telomeres, thus preventing cell crisis, or there may be a growth advantage for cells with short TRFs (6, 12, 15, 16, 22). Finally, unstable tumor telomeres may lose TTAGGG repeats more readily, accounting for the short telomeres found in tumors (6, 13, 22, 35).

We used a mathematical model that adjusts telomere length changes by varying the admixture of normal cells, which demonstrated that mean TRF values in tumor specimens were increasingly effected by the predominance of normal cells. We also observed two peak values in some tumor specimens: the higher corresponding to that of the adjacent normal control, and the lower peak that of the tumor. This observation was supported by our histopathological evaluations showing a mixture of normal and tumor cells in every tumor specimen. We illustrated that in tumors with scarce tumor cells like in our prostate cancer specimens, tumor telomeres mistakenly appeared to have shortened very little and were almost the same length as their normal controls. Therefore, our calculation model demonstrated that the acquisition of mean TRF values alone may be inaccurate, especially when specimens have very different tumor and normal telomere lengths. For this reason, both mean and peak values were analyzed in this study. To our knowledge, comparative analysis between different types of tumors with respect to the significance of mean and peak TRFs and the effect of tumor cell content have not been reported. We found on average 1–2 kbp shorter mean TRF values in colon and sarcoma compared to their normal adjacent control. In prostate with low tumor infiltration, shorter or equal TRFs were observed, revealing less striking differences between tumor and normal controls. Analysis of peak TRF values more accurately revealed telomere lengths in tumors that were on average 1 kbp shorter compared to mean values and demonstrated more impressive differences between tumor and normal specimens.

In line with previous studies that have reported high telomerase activity frequently associated with altered TRF length (6, 12, 13, 15, 36, 37), we observed 1–2 kbp longer telomeres in strongly telomerase-positive samples compared to specimens with low or undetectable telomerase activity. This indicates that telomerase may successfully stabilize telomeres. We found, however, that the longest telomeres did not always correlate with highest telomerase activity, which suggests that once telomerase is activated, telomeres can be stabilized at any length. Using “uncorrected” TRFs values, calculations of the approximate number of PDs (assuming a base pair loss of 50 bp/cell division) occurring in the “premalignant” phase prior to telomerase up-regulation revealed more than double the number of PDs in sarcoma and colon compared to prostate specimens. However, when telomere length values were used that were corrected for their tumor cell infiltration, PDs of 34, 35, and 27 in colon, sarcoma, and prostate were found, suggesting that telomerase is turned on after approximately 30 PDs in solid tumors, when telomeres have shortened to approximately 5–6 kbp.

In summary, our results demonstrate that telomerase activity and telomeres in solid tumors vary according to the degree of tumor cell infiltration. Therefore, when there is a high predominance of normal cells within a tumor, telomerase may not be a sensitive marker for the malignant phenotype and telomere loss may be underestimated. Moreover, our study demonstrates the importance of analyzing both mean and peak TRF values because mean values reflect the admixture of normal and tumor cells rather than the malignant population alone. Therefore, integration of tumor histology and tumor cell infiltration may be important for future interpretation of telomerase and telomere length investigations. This will have implications for the future when telomerase activity and telomere lengths may be interesting targets for early screening, diagnosis, and prognosis determinations and when telomerase inhibitors are used clinically.

ACKNOWLEDGMENTS

We thank Dr. K. R. Prowse (Geron Corporation, Menlo Park, CA) for expert help in establishing the TRF assay and Dr. N. W. Kim (Geron Corporation) for providing the alkaline phosphatase protocol.
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Clinical Cancer Research 1857
Relative contribution of normal and neoplastic cells determines telomerase activity and telomere length in primary cancers of the prostate, colon, and sarcoma.

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