Clinical Implications of Telomerase Activity Levels in Acute Leukemia

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ABSTRACT

In the present study, we used the telomeric repeat amplification protocol assay, an internal telomerase assay standard, and an automatic DNA sequencer to detect and quantitate telomerase activity in blood samples obtained from normal and acute leukemia patients. Telomerase activity was analyzed in 78 acute leukemia patients and ranged from 0.65 to 147 relative to the internal standard. Compared to the age-matched normal levels of telomerase activity in the peripheral blood cells, we determined that 45 (81.8%) of 55 acute myeloid leukemia (AML) and 16 (69.6%) of 23 acute lymphoid leukemia patients had elevated telomerase activity. There was no relationship between peak telomere length and telomerase activity in both acute lymphoid leukemia and AML patients. In AML, the level of telomerase activity was associated with French-American-British subtypes and cytogenetics, and patients with elevated telomerase activity had high leukocyte counts and more frequent extramedullary involvement during the disease. Among 78 patients, 5 had high levels of telomerase activities similar to immortalized leukemia cell lines; these 5 patients had a very poor prognosis (P < 0.05). The levels of telomerase activity significantly decreased in patients in complete remission. Most of the patients in complete remission showed a normal level of telomerase activity; however, two of them had low to moderate telomerase activity, and they relapsed shortly after entering complete remission. In relapsed patients, there is a general trend for increased telomerase levels, and 2 of the 13 patients retained high telomerase activity, whereas the other 11 had normal to moderate telomerase activity. These results suggest that telomerase activity may be a useful additional method for monitoring the disease condition in acute leukemia patients.

INTRODUCTION

Telomeres, the ends of chromosomes, consist of simple tandem repeats. In humans, 10–15 kb of TTAGGG repeats are found at the ends of all chromosomes (1). Telomeres progressively shorten with age in blood cells and fibroblasts (2, 3). Approximately 50–100 bp of telomeric erosion per each cell division has been demonstrated (4, 5). In hematopoietic cells, however, only 40-bp telomere losses per year have been reported in vivo (6). This apparent difference between the in vitro rate of telomere shortening may be due to the presence of telomerase activity in hematopoietic stem cells in vivo. However, even in vivo the level of telomerase activity is insufficient to completely maintain the telomere length (7) such as is found in the germ line and in most cancer cells.

Telomerase activity is observed in over 85% of primary human malignancies, suggesting that it may be a new marker of cancer and raising the possibility that anti-telomerase therapy may provide a new generation of cancer therapeutics (8–10). Unlike solid tumors, it is somewhat difficult to assess the biological significance of telomerase activity in leukemia, because recent studies have demonstrated low levels of telomerase activity in blood cells obtained from normal individuals (11–14). The development of a PCR-based telomerase assay (the TRAP3 assay) has permitted the analysis of a large number of clinical samples (15, 16). However, the original TRAP assay has a number of limitations, including difficulty in quantitating the enzyme activity and that some samples may contain inhibitors of PCR, yielding false-negative results. We modified the TRAP assay using an internal telomerase assay standard, ITAS (17), and an automatic DNA sequencer, which resulted in increased reliability, linearity, and rapid quantification (18).

To determine whether telomere length and telomerase activity is associated with biological properties in acute leukemia cells and whether telomere length measurements and telomerase activity assays could be a new diagnostic and therapeutic method in managing the care of patients with acute leukemia, in the present study we examined telomere length as well as telomerase activity in acute leukemia cells using these modified methods.

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The abbreviations used are: TRAP, telomeric repeat amplification protocol; ITAS, internal telomerase assay standard; AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; TRF, terminal restriction fragment; APL, acute promyelocytic leukemia; ATRA, all-trans retinoic acid; attg, attograms.
MATERIALS AND METHODS

Patients and Cells. We examined 102 samples from 80 consecutive patients with de novo acute leukemia (1–76 years of age): 57 with AML and 23 patients with ALL. De novo acute leukemia was diagnosed according to the French-American-British criteria (19). All of the ALL patients had precursor B-cell phenotype, and no patient with T-cell ALL was included in this study. Two of the 80 patients were excluded from the study because of the presence of PCR inhibitor. In 72 of 80 ALL and AML patients, either peripheral blood or bone marrow cells to obtain more than 80% blasts were obtained at diagnosis, and samples obtained from the remaining 8 patients contained 31–65% blasts. Among 80 patients, 20 patients were also examined at complete remission state and/or relapse. Peripheral blood mononuclear cells obtained from 70 healthy volunteers (4–90 years of age) and bone marrow cells obtained from 12 healthy volunteers (30–72 years of age) were used as controls of telomerase activity and telomere length. All samples were acquired after informed consent was obtained from patients. All of the samples obtained from peripheral blood or bone marrow cells were separated using Ficoll-Hypaque gradient, and the number of cells were counted; then cell pellets were stored at –80°C immediately. Immortalized human leukemia cell lines, HL60, U937, HAL-01, and OM9;22, were also analyzed as positive controls of telomerase activity (20). Cytogenetics was routinely performed after short term (<48 h) culture (21).

Telomerase Assay. Telomerase activity was assessed according to the method of Kim et al. (15) and Piuttszek et al. (16) with modifications using an ITAS and an automated DNA sequencer (17, 18). Briefly, 50 μl of TRAP reactions, including 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (CHAPS), 10 pmol of fluorescein-labeled TS forward primer (5′-AATCCGCTGCAGACAGTT-3′), 1 μg of T4 gene 32 protein (Boehringer Mannheim, Indianapolis, IN), and 2 units of Taq DNA polymerase (Takara Shuzou, Shiga, Japan) were placed over lyophilized fluorescein labeled 10 pmol of CX reverse primer (5′-CCCTAACCCTTACCTAC- CCTAA-3′) using a wax barrier (Perkin-Elmer, Norwalk, CT). The microtube was incubated for 20 min at room temperature to allow for telomerase-mediated extension of the TS primer. The mixture then was heated at 90°C for 90 s and amplified using PCR (30 rounds at 94°C for 30 s, 50°C for 30 s, and 72°C for 1.5 min).

For standardization of telomerase activity, we used 10 att assay of ITAS, which did not interfere with the TRAP assay (17). Amplification of the ITAS generated a 150-bp product, which could be reamplified using the same TS and CX primers used to amplify the telomerase ladder in the standard TRAP assay (18). Serial 3-fold dilutions of cell extracts were assayed in the absence and in the presence of ITAS, because overamplified PCR products may give unreliable results. RNase pre-treatment of duplicate samples of the extract was also performed as reported previously (20).

The PCR products (1 μl) were subjected to 8% denaturing electrophoresis in an automated laser fluorescence DNA sequencer II (Pharmacia Biotech, Inc., Uppsala, Sweden) and analyzed by the Fragment Manager program (Pharmacia Biotech, Inc.). Each fluorescent peak was quantitated in terms of size, peak height, and peak area (18, 20). To compare the relative amount of telomerase activity between samples, the TRAP assay signals corresponding to telomerase activity were normalized to that of ITAS. Experiments were carried out at least twice to improve the reliability of the results. We calculated mean relative values of telomerase activity in each sample.

Telomeric Repeat Analysis. TRF analysis was done as reported previously (21). Briefly, 10 μg of HindI-digested DNA were size-fractionated by electrophoresis on 0.8% horizontal agarose gels. Following electrophoresis, the DNA samples were denatured, neutralized, transferred to a nylon membrane (Hybond N; Amersham Corp., Buckinghamshire, United Kingdom), and cross-linked with UV light. The filter was hybridized to a 5′-32P-labeled (TTAGGG)6 telomeric probe. The filter then was washed, dried, and exposed to Fuji XR film with an intensifying screen. The smears of the autoradiograms were captured on an Image Master (Pharmacia Biotech, Inc.), and the telomere length was assessed quantitatively (21). We then defined the peak TRF in each sample as the peak intensity of the telomere length, in kilobases (21). We have shown previously that no particular changes were noted in TRF length among peripheral mononuclear blood cells, separated peripheral neutrophils, and nonfractionated bone marrow mononuclear cells (22), in keeping with the previous observation (23). Therefore, we defined age-matched TRFs using peripheral blood mononuclear cells from healthy volunteers. We determined that shortening of telomere repeats in the peripheral mononuclear cells obtained from normal healthy volunteers is related to aging: T = 12.669 – 0.060 x A (where T is TRF in kilobases and A is age in years). Thus, we defined acute leukemia patients with shortened telomeres as those whose TRFs were shorter than expected relative to age (i.e., < mean – 2 × SD).

Statistical Analysis. We used the Kaplan-Meier method to calculate survival curves (24). We also used the χ2 test when appropriate. Hematological data are expressed as means ± SD. Comparisons between groups were analyzed using the Mann-Whitney U test. Values of P < 0.05 were considered significant. The statistical tests were performed using the Statview (Brain Power, Inc., Calabasas, CA) software package for the Macintosh personal computer.

RESULTS

Telomerase Activity in Peripheral Blood and Bone Marrow Mononuclear Cells from Healthy Volunteers and Immortalized Leukemia Cell Lines. Using an extract derived from 1 × 10⁶ cell equivalents per assay, most but not all samples from healthy volunteers had RNA-sensitive, detectable telomerase signals showing 6-bp periodicity. To determine the possible association between levels of telomerase activity and age, the telomerase activity signals in 2 × 10⁸ cells from each individual were normalized to the signal from 10 attg per assay of ITAS, then expressed as a relative value of ITAS. The mean relative value of telomerase activity of the peripheral blood mononuclear cells was generally stronger in children than in older individuals (0 at 90 years of age to 13.4 at 4 years of age; Fig. 1) similar to results reported previously (11). The mean relative value of telomerase activity of the bone marrow cells was 0.6 (72 years of age) to 1.6 (39 years of age). Telomerase
activity is generally less than 1.0 in most of the samples obtained from healthy volunteers over age 40 years. Based on the presence of telomerase activity in peripheral blood and bone marrow cells obtained from healthy volunteers, we defined acute leukemia patients with elevated telomerase activity as those whose telomerase activities were higher than expected relative to age (i.e., > mean ± 2 × SD). Although we only could obtain a few bone marrow samples from healthy volunteers, there was no significant difference in telomerase activity between peripheral blood mononuclear cells and bone marrow samples from healthy volunteers. Thus, we used telomerase activity of peripheral blood mononuclear cells as a control telomerase activity.

Among four established leukemia cell lines tested, HAL-01 showed the highest relative value of telomerase activity (190), followed by U937 (174), OM9;22 (150), and HL60 (72). Therefore, we defined that, if patient samples had a value of above 50, it was considered to have high telomerase activity. Although there is a difference in the basal telomerase activity with age as reported previously (11, 12), we arbitrarily classified acute leukemia patients with elevated telomerase activity as follows: low, above normal and up to 10 relative value; moderate, 10 to 50 relative value; and high, ≥50 relative value.

Telomerase Activity in Acute Leukemia Patients. Among 80 patients with acute leukemia, 2 AML samples obtained from bone marrow were excluded from the statistical analysis because of the presence of inhibitors detected by using ITAS. Both telomerase signals and ITAS were detected after 10-fold dilution in one of these patients, indicating that cell lysates may occasionally contain inhibitors of Taq polymerase (Fig. 2). However, telomerase activity was detected in the remaining 78 patients (Table 1; Figs. 2 and 3). Compared to the normal levels of telomerase activity in the peripheral blood cells, we determined that 45 of 55 AML patients had elevated telomerase activity (18 had high/moderate and 27 had low telomerase levels), which ranged from 2.6 to 65 (Table 1). In ALL, 16 of 23 had elevated telomerase activity (11 had high/moderate and 5 had low telomerase activity), and the levels of telomerase activity ranged from 5.2 to 147 (Table 1). Five patients with childhood ALL were included in this study; 4 of 5 patients had normal level telomerase activity (6.4 relative telomerase activity at 1 year of age, 1.9 at 2 years of age, 2.1 at 3 years of age, and 13.7 at 13 years of age). The remaining patient (25 at 8 years of age) had moderate telomerase activity.

Among the 78 acute leukemia patients with elevated telomerase activity, 5 patients (1 AML and 4 ALL) had a high telomerase activity (Table 2). No significant difference in telomerase activity was noted between AML and ALL (11.5 ± 12.4 versus 24.7 ± 36.3). The incidence of patients with shortened TRFs was significantly higher in AML when compared to ALL (47 of 55 patients in AML versus 8 of 23 in ALL; P < 0.01); however, no correlation was observed between TRF peak lengths and telomerase activity in each patient (Fig. 4).

Telomerase Activity Compared to Other Clinical Features in Acute Leukemia. To determine whether specific clinical features are associated with the level of telomerase

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**Fig. 1** Telomerase activity (2 × 10⁵ cells) of peripheral blood (●; n = 70) and bone marrow (○; n = 12) mononuclear cells obtained from healthy volunteers. The telomerase activity in each subject is expressed as mean relative value compared to an ITAS (vertical axis). The horizontal axis indicates the ages of subjects in years. The mean and SD of age-matched relative value obtained from peripheral blood cells is as follows: ages (years) 0–19 (n = 7), 6.3 ± 3.1; ages 20–39 (n = 32), 1.1 ± 1.7; ages 40–59 (n = 15), 0.7 ± 0.8; ages 60–79 (n = 10), 0.7 ± 0.8; and ages over 80 (n = 7), 0.7 ± 0.6.

**Fig. 2** Representative fluorocurves showing telomerase activity in seven acute leukemia patients (2000-cell equivalent per assay) and immortalized human leukemia cell line U937 (100-cell equivalent per assay). Multiple peaks correspond to telomeric repeats that were synthesized by the presence of telomerase in leukemia cells. To quantitate the telomerase activity, we used the ITAS showing a peak at 150 bp. The results analyzed by the Fragment Manager system and the area of telomerase signals are compared to the area of the ITAS signal. In each subject, telomerase activity is expressed as relative value to ITAS. Top, size markers (100 and 150 bp). The level of telomerase activity is heterogeneous among patients. One patient with AML-M5b had no detectable telomerase or ITAS when 2000 cells were analyzed but did show telomerase signals and ITAS after 10-fold dilution. This suggests the presence of a PCR or Taq inhibitors in this extract.
activity, we compared peak TRF, hematological findings, cytogenetics, and the remission rates between patients with various levels of telomerase activity. AML patients with high/moderate telomerase activity had some distinct clinical and hematological features, including high leukocyte counts (97.1 ± 85.6 x 10^9/liter versus 32.1 ± 36.7 x 10^9/liter; *P* = 0.002), and more frequent extramedullary involvement during the disease (6 of 8 versus 0 of 37; *P* < 0.01). Patterns of telomerase activity were also associated with French-American-British subtypes and cytogenetics (*P* < 0.05; Table 3). Most of the patients (90%) with AML-M3 had normal to low telomerase activity. In contrast to AML, no significant difference in hematological parameters, cytogenetics, and complete remission rate was observed in ALL patients with various levels of telomerase activity.

Because the number of patients of each AML and ALL groups with various levels of telomerase activity was not large enough to obtain statistically significant survival probabilities, we analyzed survival probabilities among all of the acute leukemia patients. Among 78 acute leukemia patients, no statistically significant difference in survival probability was evident among patients with normal range telomerase activity or those with low or moderate levels of telomerase activity (data not shown). However, patients with a high telomerase activity (relative value, >50) had a poor prognosis (*P* < 0.05) compared to those with telomerase activity less than 50 (Fig. 5).

We next compared telomerase levels in two hematological different phases. After entering complete remission (bone marrow blasts were <1.2% in all of the patients analyzed), the telomerase activity decreased in all 15 patients (13 patients with AML and 2 with ALL) compared to that at the time of diagnosis (15.7 ± 16.7 versus 2.5 ± 2.8; *P* < 0.005; Fig. 6, left). Most of the patients in complete remission showed normal levels of telomerase activity: two (one each of AML and ALL) had low to moderate telomerase activity in the remission state and they relapsed within 3 months after entering remission. One of these patients with moderate telomerase activity in the remission state was an AML patient with t(6;9) translocation (patient no. 1 in Table 3). While the patient had detectable DEK/CAN mRNA at the time of diagnosis, DEK/CAN mRNA was not detected in the remission state, although increased telomerase activity was ob-

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**Table 1 Correlation between telomere length, telomerase activity, and clinical hematological findings between AML and ALL**

<table>
<thead>
<tr>
<th></th>
<th>AML (n = 55)</th>
<th>ALL (n = 23)</th>
<th>Significance <em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Telomerase activity*</td>
<td>11.52 ± 12.42</td>
<td>24.66 ± 36.31</td>
<td>NS*</td>
</tr>
<tr>
<td>Median</td>
<td>6.2</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.65–64.0</td>
<td>1.77–147.0</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>27</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>17</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>TRFs (kb)</td>
<td>6.06 ± 3.00</td>
<td>7.27 ± 2.91</td>
<td>0.0521</td>
</tr>
<tr>
<td>Median</td>
<td>5.2</td>
<td>8.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>2.4–19.6</td>
<td>2.4–14.0</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortened TRFs</td>
<td>45c</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>TRFs within normal range</td>
<td>10</td>
<td>15</td>
<td>&lt;0.01c</td>
</tr>
<tr>
<td>Elongated TRFs</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>46 ± 21</td>
<td>38 ± 16</td>
<td>0.097</td>
</tr>
<tr>
<td>Median</td>
<td>45</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>4–76</td>
<td>1–74</td>
<td></td>
</tr>
</tbody>
</table>

* Mean relative value of internal standard. Normal, mean relative value < age-matched mean + 2 × SD; Low, above normal to 10 relative value; Moderate, 10 to 50 relative value; High, >50 relative value.

* NS, not significant.

* Including two patients with the presence of inhibitor.

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</tr>
</thead>
</table>

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**Fig. 3** Telomerase activity expressed as relative value (vertical axis) compared to the stage of AML or ALL diagnosis (horizontal axis). The telomerase activity was calculated by using ITAS (see "Materials and Methods"). Bars, SD.
served (data not shown). We then analyzed 13 patients (8 patients with AML and 5 with ALL) to determine whether telomerase activity is elevated at relapse phase. Two (one each of AML and ALL patient) of the 13 patients showed very high elevations of telomerase activity at the relapse phase (Fig. 6, right), and they had a poor prognosis. However, the relationship between telomerase activity levels at relapse and prognostic outcome was not statistically significant. This indicates that telomerase activity is not always associated with disease progression, but in some acute leukemia patients, further elevation of telomerase activity is linked to disease recurrence.

**DISCUSSION**

Acute leukemia comprises a group of disorders arising from hematopoietic stem cells. The biological property of leukemia cells may depend on cell lineage of leukemia, state of differentiation, and clinical stage. In an initial study by Kim et al. (15), 14 of 16 ALL cases were found to have telomerase activity (15). However, this study was conducted before it was appreciated that normal blood cells had weak but detectable telomerase activity (11–14). Counter et al. (13) reported that normal blood cells had low levels of telomerase activity (2% level of immortalized B cell line) and that 7 AML samples had higher levels of telomerase activity than normal bone marrow cells. The semiquantitative study by Broccoli et al. (14) demonstrated considerable overlap of telomerase activities between normal subjects and leukemia patients. Therefore, unlike solid tumors, it is difficult to determine whether the level of telomerase activity in each hematological neoplasia has clinical implications. For this reason, we examined age-matched telomerase activity in normal peripheral blood mononuclear cells (12), then studied clinical and hematological findings in acute leukemia patients.

In agreement with a previous report (11), we found that there is a reduction of telomerase activity with increasing age (12). Because the basal level of telomerase activity is 10-fold higher in children compared to adults, we arbitrarily classified the level of telomerase activity into subgroup relative to age-related levels of telomerase. Using this classification, it is possible to avoid overlap between normal individuals and adult acute leukemia, especially patients aged over 40. However, because the number of cases in this study is small, it is difficult to assess the level of telomerase activity in childhood leukemia. Because peripheral blood mononuclear cells are mainly composed of lymphocytes, the weak telomerase activity is likely to be derived from T and B lymphocytes as well as hematopoietic stem cells that are circulating in the periph-

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**Table 2** Clinical and hematological features of acute leukemia patients with a high telomerase activity (>50 relative value)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Age/Sex</th>
<th>Leukocytes (X10^9/liter)</th>
<th>Hb (g/liter)</th>
<th>Platelets (X10^9/liter)</th>
<th>Peripheral blasts (%)</th>
<th>Marrow blasts (%)</th>
<th>Telomerase activity</th>
<th>Telomere length (kb)</th>
<th>Survival (mo)</th>
<th>Cytogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML-M1</td>
<td>26/F</td>
<td>243.7</td>
<td>123</td>
<td>50</td>
<td>96</td>
<td>94</td>
<td>64</td>
<td>4.6</td>
<td>4.4</td>
<td>t(6;9)(p21;q34)</td>
</tr>
<tr>
<td>ALL-L2</td>
<td>17/F</td>
<td>81.5</td>
<td>122</td>
<td>76</td>
<td>70</td>
<td>97</td>
<td>105</td>
<td>10.4</td>
<td>2</td>
<td>t(17;19)(p13)</td>
</tr>
<tr>
<td>ALL-L2</td>
<td>62/F</td>
<td>17.8</td>
<td>67</td>
<td>25</td>
<td>75</td>
<td>94</td>
<td>65</td>
<td>11.9</td>
<td>15</td>
<td>Normal</td>
</tr>
<tr>
<td>ALL-L2</td>
<td>19/F</td>
<td>130.5</td>
<td>123</td>
<td>38</td>
<td>92</td>
<td>95</td>
<td>147</td>
<td>8.0</td>
<td>9</td>
<td>t(9;22)(q34;q11)</td>
</tr>
<tr>
<td>ALL-L2</td>
<td>70/F</td>
<td>22.0</td>
<td>62</td>
<td>24</td>
<td>96</td>
<td>98</td>
<td>52</td>
<td>5.1</td>
<td>3</td>
<td>t(9;22)(q34;q11)</td>
</tr>
</tbody>
</table>

**Table 3** Correlation of Telomerase Activity and Cytogenetic Status

<table>
<thead>
<tr>
<th>Cytogenetics</th>
<th>High</th>
<th>Moderate</th>
<th>Low</th>
<th>Normal</th>
<th>Significant P*</th>
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</thead>
<tbody>
<tr>
<td>AML (no. of patients)*</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>Normal karyotypes</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>NS</td>
</tr>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>t(15;17)(q22;q21)</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>inv(16)(p13q22)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>11q23 translocation</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>ALL (no. of patients)*</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Normal karyotypes</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>t(4;11)(q21;q23)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>t(9;22)</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

* Significant value (P < 0.05) determined by χ² test.

* Normal, mean relative value < age-matched mean + 2 × SD; Low, above normal to 10 relative value; Moderate, 10 to 50 relative value; High, >50 relative value. NS, not significant.
eral blood. Because it is difficult to get bone marrow samples from healthy volunteers, we alternatively used peripheral blood cells as age-matched controls. We used mononuclear cells obtained from acute leukemia patients at diagnosis that were composed of more than 80% immature leukemia cells; therefore, the level of telomerase activity largely reflects the biological properties of leukemia cells that were derived from leukemic stem cells. Although we did not compare each fraction of cells, the levels of telomerase activities were elevated in 45 of 55 AML and 16 of 23 ALL patients, suggesting that fraction of proliferating cells are increased in such cases. Hiyama et al. (11) described that the level of telomerase activity increases in hematopoietic progenitor cells upon their proliferation and that the committed stem cell rather than the most primitive stem cell had increased level of telomerase activity. Because Holt et al. (25) have reported recently that cells with telomerase activity down-regulate telomerase activity levels with quiescence, these data suggest that most primitive hematopoietic stem cells have low levels of telomerase activity because they are likely to be quiescent most of the time. However, upon stimulation to proliferate, telomerase activity appears to be up-regulated in their immediate progeny, which may help to slow down the rate of telomere erosion as part of the rapid turnover of such cells. The more mature cells then become quiescent again and down-regulate telomerase activity (26, 27).

These findings suggest the following explanations to begin to understand the biological significance of telomerase activity in acute leukemia cells. Because the level of telomerase activity may be linked to the fraction of cells with a high proliferative capability in each acute leukemia patient, telomerase would not be elevated if the leukemic stem cells were not proliferating rapidly. In addition, the level of telomerase activity may be down-regulated when leukemia cells differentiate. Based on these concepts, we studied the clinical and hematological findings in acute leukemia patients to determine if any parameters correlated with the level of telomerase activity.

In the present study, elevated telomerase activity in AML is associated with high leukocyte counts and extramedullary infiltration, e.g., central nervous system involvement and myeloblastoma. This may reflect a fraction of cells with high proliferative capability in such patients. Holt et al. (25) reported that telomerase activity is present in G1, S, and G2-M phases of the cell cycle. Taken together with our findings, acute leukemia cells with elevated telomerase activity may be affected by cell cycle-dependent anti-leukemia drugs as well as future anti-telomerase cancer therapies. Although patients with a very high telomerase activity are not common in acute leukemia, we found that 5 of 78 fresh leukemia patients and 2 of 13 relapsed patients had a very high telomerase activity and had a poor prognosis, similar to those results reported in neuroblastoma (28), gastric cancer (29), and more recently, AML (26). Although these preliminary studies indicate that telomerase activity levels may be a new marker in predicting the outcome of patients with neoplasms, additional studies comparing proliferation capability (e.g., proliferating cell nuclear antigen and Ki-67) as well as DNA or RNA synthesis studies will be required using multivariate analysis to determine if telomerase is an independent prognostic marker.

Interestingly all but one acute leukemia patient had specific chromosomal translocations such as t(6;9)(p21;q34), t(17;19)(q21;p13), and t(9;22)(q34;q11), which cause a chimeric protein such as DEK/CAN, E2A/HLF, and BCR/ABL. It is likely that such translocations occur as an early genetic event, then reactivation or up-regulation of telomerase may occur due to rapid cell division during disease progression. Therefore, high telomerase activity may be linked to disease severity in acute leukemia patients, but additional studies with a larger sample size will be required to confirm this.

Approximately 20% of patients with acute leukemia did not show elevated telomerase levels. Zhang et al. (26) reported that telomerase activity is inhibited by a differentiation-inducing agent in HL60 cells (26). We observed an association between APL.

**Fig. 5** The survival of the acute leukemia patients separated by telomerase activity levels; high, ≥50 telomerase activity (---); moderate, 10–50 telomerase activity (---); low, normal range–10 telomerase activity (---); and normal, normal-range telomerase activity (---). A significant difference is observed between patients with high telomerase activity and those with low/moderate telomerase activity or those with normal telomerase activity (P < 0.05 as determined by the Kaplan-Meier test).

**Fig. 6** Changes of telomerase activity at two different hematological phases. Left, all but two acute leukemia patients normalized their telomerase activity after entering complete remission. Right, at the time of relapse, the telomerase activity was elevated in two patients. Dx, at the time of diagnosis; CR, complete remission; Re, relapse. The vertical axis shows telomerase activity expressed in relative value. Arrowheads, ALL patients.
These findings indicate that telomerase activity may be repressed in such patients. The reverse transcription-PCR assay to therapy may be an indication of differentiation responsiveness in ATRA-treated APL cells. More importantly, detection of high telomerase activity in patients on ATRA therapy may be an indication of chemotheraphy failure.

We found that the levels of telomerase activity in acute leukemia significantly decreased in the complete remission state. The reduced level of telomerase activity may reflect normal peripheral blood cells. Interestingly, the remission durations of two patients whose telomerase activity did not normalize were extremely short, indicating minimal residual disease in such patients. The reverse transcription-PCR assay to detect chimeric mRNA is widely used for monitoring leukemia patients with specific translocation; however, a patient with t(6;9) showed moderate telomerase activity, even when DEK/CAN mRNA was not detectable by reverse transcription-PCR (data not shown). The present study suggests that sequential and quantitative measurement of telomerase activity may be useful in monitoring acute leukemia patients.

Although some caution is urged about the interpretation in the present studies, because the number of cases studied was small, we believe analysis of telomerase activity may contribute to our understanding of the developmental stages not only in leukemic stem cells but also in normal hematopoietic stem cells. In conclusion, it is anticipated that future studies will clarify the biological significance of telomerase activity in acute leukemia and eventually provide additional information for the treatment of acute leukemia patients.

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Clinical implications of telomerase activity levels in acute leukemia.

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