Telomerase Activity and Telomere Length in Acute and Chronic Leukemia, Pre- and Post-ex Vivo Culture


ABSTRACT

We studied telomerase regulation and telomere length in hematopoietic progenitor cells from peripheral blood and bone marrow from patients with acute and chronic leukemia and myeloproliferative diseases. CD34+ cells from a total of 93 patients with either acute myeloid leukemia (AML; n = 25), chronic myeloid leukemia (CML; n = 21), chronic lymphocytic leukemia (CLL; n = 18), polycythemia vera (PV; n = 16), or myelodysplastic syndromes (MDS; n = 13) were analyzed before and in 19 patients after ex vivo expansion in the presence of multiple cytokines (kit ligand, interleukin-3, interleukin-6, and granulocyte colony-stimulating factor plus erythropoietin). Compared with hematopoietic progenitor cells from normal donors (n = 108), telomerase activity (TA) was increased 2- to 5-fold in chronic phase (CP)-CML, CLL, PV, and MDS. In AML, accelerated phase (AP) and blastic phase (BP)-CML, basal TA was 10- to 50-fold higher than normal. TA of CP-CML CD34+ cells was up-regulated within 72 h of ex vivo culture, peaked after 1 week, and decreased below detection after 2 weeks. In contrast, TA in AP/BP-CML and AML CD34+ cells was down-regulated after 1 week of culture and decreased further thereafter. The expansion potential of CD34+ cells from patients with leukemia was considerably decreased compared with CD34+ cells from normal donors. The average expansion of cells from leukemic individuals was 6.5-, 2.3-, 0.6-, and 0.2-fold in weeks 1, 2, 3, and 4, respectively, whereas expansion of normal cells was 5- to 15-fold higher. In serial expansion culture, a median telomeric loss of 0.7 kbp was observed during 3–4 weeks of expansion. Our results demonstrate that up-regulation of telomerase is similar in CD34+ cells from CP-CML, CLL, PV, and MDS patients and in normal hematopoietic cells during the first week of culture, whereas in AML and AP/BP-CML, telomerase is high at baseline and down-regulated during expansion culture. High levels of telomerase in leukemic progenitors at baseline may be a feature of both the malignant phenotype and rapid cycling. Telomerase down-regulation during culture of leukemic cells may be due to the decreased expansion potential or repression of normal hematopoiesis, or in AML it may be due to the partial differentiation of AML cells. Shown previously to be associated with loss of TA, telomere shortening during ex vivo expansion correlated with low levels of TA, particularly in chronic leukemia and MDS progenitors where telomerase was insufficient to protect against telomere loss due to intense proliferation.

INTRODUCTION

Human telomeres are specialized chromosomal end structures composed of G-rich simple repeat sequences that are important for the function and genome integrity (1). Because conventional DNA polymerases cannot fully replicate the extreme ends of linear chromosomes, each cell division results in DNA loss. Telomere shortening has been observed in dividing somatic cells, eventually leading to cell senescence when telomeres become critically short (1–4). Telomerase is a ribonucleoprotein which adds telomeric repeats, using an RNA subunit as a template. Telomerase expression thereby prevents telomeric shortening during cell division and allows cells to bypass replicative senescence (5). Although high TA is typically found in tumor cells of various origin (6–12), borderline TA has also been detected in human primitive hematopoietic cells and in unstimulated lymphocytes where TA increases significantly with cytokine-induced expansion, cell proliferation, and cell cycle activation (13, 14). Although previous studies have demonstrated expression of TA associated with most human solid tumors and hematological malignancies (5–12), no study to date has investigated telomerase regulation and telomere changes in acute and chronic leukemia before and after ex vivo culture in response to cytokine stimulation. Normal and neoplastic hematopoietic cells can be expanded ex vivo and differentiated into specific lineages with the use of exogenous growth factors, drugs, and differentiating agents (15–18). This has allowed the elucidation of important growth factors and adhesion molecules that are differentially regulated during normal and leukemic cell growth, the identification of normal versus leukemic primitive progenitor cells, the partial detection of defects at the leukemic stem cell level, and the selective expansion of normal versus leukemic hematopoietic cells in ex vivo culture (15–21). In contrast to normal progenitor cells, clonal progeny of leukemic cells may have a limited ability to proliferate and differentiate, abnormalities in apoptotic pathways that enable persistence of the leukemic clone and may express TA, which promotes longevity (16–25). In this study, we analyzed whether TA and TRF in BM and PB samples from adult patients with acute and chronic leukemia and MDS differ from healthy donor specimens and whether TA and TRF measurements performed on leukemic samples show significant changes before and after ex vivo culture. The hypothesis of the study was that telomeres would be stable under ex vivo expansion conditions in AML (because telomerase would be high, whereas in CML and MDS, telomeres should progressively shorten because telomerase levels are not high enough to stabilize telomeres) and to use CLL CD34+ cells as controls for normal stem cells.

MATERIALS AND METHODS

Patients and Cell Specimens. Specimens were collected from PB and BM of 93 leukemia patients with either AML (n = 25), CML (n = 21), CLL (n = 18), PV (n = 16), or MDS (n = 13) at the time of diagnosis. For ex vivo culture studies, 19 patient samples (8 CML, 5 AML, 4 PV, and 2 CLL) were used. According to the French-American-British classification, 3 of 25 AML were classified as M0, 5 as M1, 8 as M2, 1 as M3, 5 as M5, 1 as M7, and 2 had transformed from MDS to AML. In CML, of 21 patients, 13 were in CP and 8 were in AP or BP. In CLL, of 18 patients, 14 had stable disease, whereas...
4 had transformed to high-grade non-Hodgkin’s lymphoma. In MDS, three patients had RA, five had RA with sideroblasts, and five had RAEB. In each of four AML and four MDS patients, serial specimens were obtained throughout their disease course. All PV patients had chronic phase disease. Patients from which specimens were obtained at diagnosis and during the course of their disease had received no prior therapy, whereas those samples from patients obtained during the course of their disease had received ≥6 chemotherapy cycles in 34 cases and ≥6 chemotherapy cycles in 13 cases.

RESULTS

Patient Characteristics. Table 1A summarizes the clinical patient characteristics. The numbers of male and female patients were comparable in groups as well as in subgroups of patients. PB and BM specimens were collected at diagnosis and during the course of the disease (Table 1B). A total of 54 patients were analyzed at diagnosis, 47 during the course of their disease, and 19 before and after ex vivo culture. Patients from which specimens were obtained at diagnosis had received no prior therapy, whereas those samples from patients obtained during the course of their disease had received ≥6 chemotherapy cycles in 34 cases and ≥6 chemotherapy cycles in 13 cases.

TA and Telomere Length in Healthy Donors. Table 2 summarizes the results obtained from healthy donors (n = 108). Median TA was higher in CD34+ cells than in MNC and higher in PB and BM compared with CB cells (Table 2). TA in MNC and CD34+ cells using PB and BM was low and decreased in intensity with age (TA_{MNC} = 4.21 - 0.0482 × A; and TA_{CD34+} = 8.2 - 0.065 × A; where TA is telomerase activity in TPG and A is age in years), as previously reported (25). Because Southern blot TRF analysis is resolved as a characteristic smear of cellular DNA due to interchromosomal and intercellular heterogeneity, both mean and peak TRF values were analyzed. Mean and peak TRFs in CD34+ cells were similar with 7.8 and 8.1 kbp, respectively (Table 2). Telomere lengths of CB CD34+ cells were longer than from PB and BM, in accordance with their higher TA values.

Table 1A Patient demographics

| Cell Source and Separation of CD34+ Cells and Subsets. BM and PB specimens from healthy donors and patients were obtained after gaining informed written consent. CB, which was otherwise to be discarded, was informed of the consent process according to the policy of the Institutional Review Board of Research Associates of the New York University Medical Center. Heparinized MNC from healthy donors and patients were separated by Ficoll-Paque (Pharmacia, Uppsala, Sweden). CD34+ cells were selected with immunomagnetic beads (Dynal, Oslo, Norway) (13) and used for suspension culture assay, progenitor assay (colony assay), TRAP, and TRF analysis.

Suspension Culture Assay (Delta Culture). CD34+ cells were cultured at 4 × 10^6 cells per ml in Iscove’s modified Dulbecco’s medium plus 20% FCS supplemented with gentamicin and mononothioglycerol in the presence of a five-factor cytokine cocktail of kit ligand (20 ng/ml), interleukin-3 (50 ng/ml), interleukin-6 (100 units/ml), erythropoietin (6 units/ml), and granulocyte colony-stimulating factor (1000 units/ml; K36EG), as previously reported (13). The CD34+ cell recovery after 1 week of culture was similar for patient and healthy donor specimens with a median of 2.21%. However, the highest CD34+ cell yield (5.6%) was obtained from AML samples; next were CML, CLL, and PV samples.

Weekly passages and colony assays were performed for 3–4 weeks until no further cellular expansion was observed.

Colonies, CD34+ cells (1 × 10^3/ml) and ex vivo expanded CD34+ cells (2 × 10^3–4 × 10^7/ml) were cultured in triplicate in Iscove’s modified Dulbecco’s medium containing 0.36% agarose (FMC Bioproducts, Rockland, ME), 20% FCS, and cytokines (K36EG) (13). After 14 days of incubation, CFU-granulocyte-macrophage was scored using an inverted microscope.

TRAP Assay. The assay which incorporates an internal PCR control (designated telomerase substrate-nontelomerase) was performed as described previously (5, 26–28). In brief, two μg of protein extract were assayed in reaction tubes containing 50 μl of the TRAP reaction mixture. For each assay, a negative control and 0.1 amol of the quantification standard olinucleotide R8 were used. The amount of TA for each reaction was calculated as published (9, 10, 25–28), expressing the final quantitation as TPG. One unit of TPG was defined as 0.001 amol (or 600 molecules) of telomerase substrate primers extended by telomerase present in the extract with at least three telomeric repeats. The assay was in the linear range from 0.001 amol (1 TPG) to 1 amol (1000 TPG) of R8. This range extended over three logs of our target protein concentration (data not shown). All results were determined from at least three to six independent TRAP assays.

TRF Assay. DNA isolation and TRF analyses were performed as described (3, 4, 13, 25). In brief, genomic DNA was digested with MSP I and RSA I (Boehringer Mannheim, Indianapolis, IN); electrophoresis was performed in 0.5% agarose gels; and gels were depurinated, denatured, neutralized, transferred to a nylon membrane using 20× SSC, dried for 1 h, and hybridized with a 5'-end labeled telomeric probe (TTAGGG)\_n (Genset, La Jolla, CA). Telomeric smears were visualized by exposing the membranes to imaging plates with mean and peak TRF lengths analyzed as reported (13, 25, 29, 30).

Statistics. Comparisons among groups were made with standard statistical tests. Results are expressed as median values except when stated otherwise. Statistical significance of the data obtained was analyzed with the Wilcoxon rank sum test and the Student t test.

Table 1A Patient demographics

<table>
<thead>
<tr>
<th>Median patient age</th>
<th>No.</th>
<th>Male</th>
<th>Female</th>
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</thead>
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<td>[years (range)]</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>93</td>
<td>48</td>
</tr>
<tr>
<td>AML</td>
<td>49</td>
<td>25</td>
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</tr>
<tr>
<td>CML</td>
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<td>13</td>
</tr>
<tr>
<td>CLL</td>
<td>66</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>PV</td>
<td>60</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>MDS</td>
<td>55</td>
<td>13</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 1B Analyses performed based on disease type, number of patients, and disease status

<table>
<thead>
<tr>
<th>Total</th>
<th>No. at diagnosis</th>
<th>No. during course of disease</th>
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</thead>
<tbody>
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<tr>
<td>Total</td>
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<tr>
<td>AML</td>
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</tr>
<tr>
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</tr>
<tr>
<td>MDS</td>
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</table>

* n = 8 patients with serial specimens obtained at diagnosis and during the course of their disease.

Table 2 TA and TRF in healthy donors [median (range)]

<table>
<thead>
<tr>
<th>All donors</th>
<th>Cord blood</th>
<th>Peripheral blood</th>
<th>Bone marrow</th>
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<tbody>
<tr>
<td>Number</td>
<td>108</td>
<td>21</td>
<td>78</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35 (0–92)</td>
<td>0</td>
<td>46.5 (17–92)</td>
</tr>
<tr>
<td>TA (TPG) in MNC</td>
<td>1 (0–6)</td>
<td>0</td>
<td>2 (0–6)</td>
</tr>
<tr>
<td>TA (TPG) in CD34+</td>
<td>3 (0–15)</td>
<td>2 (0–5)</td>
<td>6 (0–15)</td>
</tr>
<tr>
<td>Mean TRF (kbp)</td>
<td>7.8 (5.1–13.6)</td>
<td>10.8 (5.1–13.6)</td>
<td>7.3 (5.1–9.9)</td>
</tr>
<tr>
<td>Peak TRF (kbp)</td>
<td>8.1 (5.5–13)</td>
<td>10.3 (9–13)</td>
<td>7.5 (5.5–9)</td>
</tr>
<tr>
<td>TRF loss in culture (kbp)</td>
<td>1.1 (0.4–2.9)</td>
<td>1.0 (0.4–2.9)</td>
<td>1.0 (0.4–2.9)</td>
</tr>
</tbody>
</table>
with previous studies (2, 13). Telomere length in MNC cells declined with age (TL = 10.052 – 0.054 × A [r = −0.6; p < 0.05], where TL is the TRF in kbp and A is age in years) (Fig. 1A). CD34⁺ cells were a median of 0.2 kbp longer than MNC with 8.7 versus 8.5 kbp TRFs, respectively (n = 11; n = 6 CB, n = 5 PB); however, no significant telomere length difference in CD34⁺ cells and MNC was observed (Fig. 1B). In ex vivo culture, CB (n = 12), PB (n = 20), and BM (n = 7) CD34⁺ cells were found to lose 1–2 kbp over a 4-week period, with a comparable TRF loss in all three cell sources (Table 2).

**TA and Telomere Length in Leukemia Patients.** Fig. 2 summarizes TA and telomere length in leukemia and MPD patients as well as in healthy donors. Increased TA and short telomeres were defined as such when TA in patients was higher than normal [>(mean − 2 × SD)] and when their TRF lengths were shorter than normal relative to their age-matched healthy donors [i.e., <(mean − 2 × SD)]. Median TA in all patients was increased 7.7-fold, and median telomeres were 1.2 kbp shorter compared with age-matched healthy donors (p < 0.0001). Median TA in CD34⁺ cells from PV and CLL was similar to normal CD34⁺ cells, moderately increased in CML and MDS (p < 0.05), and increased 18-fold in AML specimens (Fig. 2A). Telomere length, particularly in AML and CML, was significantly shorter (p < 0.01), decreased to a lesser extend in PV and MDS (p < 0.05), and was not significantly different for CLL patients who had similar telomere lengths as age-matched healthy donors (Fig. 2B). Again, we found no significant telomere length difference in CD34⁺ and MNC from CML patients; however, telomeres of CD34⁺ cells were a median of 0.4 kbp longer than those of MNC with 4.8 versus 4.4 kbp, respectively (n = 8) (Fig. 2C).

**TA and Telomere Length at Diagnosis and during the Course of Disease.** TA in all patients was significantly increased in diagnostic specimens with 15 TPG (range: 0–55.3) compared with 2.4 TPG (range: 0–14.3) in follow-up specimens (p < 0.0001). This was true for all subgroups of patients (PV, 2.8 versus 2 TPG; CLL, 5 versus 2.6 TPG; CML, 15.1 versus 1 TPG; AML, 32 versus 2.16 TPG) except for MDS (7 versus 7.3 TPG) (Fig. 3A). In serial samples from AML patients taken at diagnosis and after courses of induction chemotherapy (n = 4), telomerase decreased 14.8-fold after chemotherapy, correlating with the reduction of leukemic cells and the attainment of normal cells in BM and PB. In serial specimens of four MDS patients, TA increased with disease progression. Particularly in one patient who progressed from RA to RAEB, TA increased 11-fold, and in progression from RAEB to overt AML, TA increased 18-fold (Fig. 3B). No significant difference in telomere lengths could be demonstrated in patient specimens at diagnosis and follow-up, with 5.7 (range: 3.2–7.7) versus 5.6 kbp (range: 3.4–8.2), respectively (p = 0.833). A trend toward telomere shortening was observed in subgroups of patients with PV, CML, and MCL, in which telomeres tended to shorten as the disease continued, with 5.9 versus 5.7, 6.6 versus 6.1, and 5.1 versus 4.7 kbp, respectively. In contrast, longer telomeres were found in AML and MDS patients after induction chemotherapy (6.6 and 6.1 kbp) compared with those found in diagnostic specimens (5 and 5.9 kbp). Most likely this was due to the loss of the leukemic clone (with
shorter telomeres) and the emergence of normal hematopoietic cells (with longer telomeres) after induction therapy (Fig. 3C).

**Telomeres in Patients with <6 and >6 Chemotherapy Cycles.** Patients who received moderate pretreatment (<6 chemotherapy cycles) had significantly longer telomeres than patients who received extensive pretreatment (>6 chemotherapy cycles), with a median of 6.4 (range: 4.3–8.6) versus 5.0 kbp (range: 3.2–7.6), respectively (p < 0.0001). Patients with no pretreatment had telomeres of 6.5 kbp, suggesting that the initial chemotherapy cycles both eradicated the leukemic clone and shortened the telomeres of normal hematopoietic cells (with the latter phenomenon predominating with successive chemotherapy). However, it cannot be ruled out that telomere length differences were also due to differences in the number of leukemic cells in patient specimens, e.g., that patients who required more therapy had more leukemic cells and, therefore, that this added to the effects of the chemotherapy on the results. Nevertheless, it was of interest that CD34+ cells from CLL patients progressively shortened from 6.6 to 6.1 and 5.9 kbp, whereas CD34+ cells from CML, PV, MDS, and AML patients initially increased from 6.1 to 6.7 kbp and then decreased to 5 kbp with 0, <6, or >6 chemotherapy cycles, respectively.

**TA and Telomere Length in Patients with Stable and Accelerated Disease Course.** Patients with stable versus accelerated disease where defined as a group with CP versus AP and BP disease and were defined in MDS/AML as a patient group with RA/RAS (RA with sideroblasts) versus RAEB/AML disease. In CLL, patients with stage RAI I–III disease and patients with transformed aggressive lymphocytic lymphoma (Richter’s syndrome) were subdivided. TA was significantly lower in all patients with stable compared with accelerated disease, with 2.8 TPG (range: 0–14.3) compared with 21 TPG (range: 2–41.5), respectively (p < 0.0001). This was also true for subgroups of CLL, CML, and MDS/AML, with 1.9 versus 31.5, 2.3 versus 5, and 4.95 versus 15 TPG, respectively. Telomeres in patients with stable disease were 5.9 kbp (range: 4.2–8.6) compared with 5 kbp (range: 3.2–7.6) in patients with progressive disease (p = 0.091). 6.5 versus 6.4 kbp in CLL, 5.6 versus 4.6 kbp in CML, and 5.9 versus 5.1 kbp in AML/MDS.

**TA and Telomeres in PB Compared with BM AML and MDS Specimens.** BM specimens obtained from AML and MDS patients at diagnosis had slightly elevated TA (1.6-fold higher) and shorter telomeres (4.9 kbp) compared with PB specimens (5.2 kbp) from the same patient. TA was identical in BM and PB specimens from patients in complete remission, displaying a low or undetectable TA with a median of 1.4 TPG. Telomeres in remission patients (with a median age of 46 years) were 6.94 kbp in BM and 6.9 in PB, which was 0.6 kbp shorter compared with age-matched healthy donor controls.

**TA, Telomere Dynamics, and Cell Expansion under ex Vivo Culture Conditions.** Like TA kinetics observed in healthy donors (Fig. 4A), telomerase was up-regulated within 72 h of ex vivo culture of CD34+ cells from CP-CML, PV, and CLL patients (Fig. 4B). TA in these cultured cells peaked after 1 week and decreased below detection after 2 weeks of culture (Fig. 4A). In contrast, in AP/BP-CML and AML patients, telomerase in CD34+ cells was down-regulated after 1 week of expansion and decreased further thereafter (Figs. 4, A and B, and 5A). Telomerase up-regulation in CP-CML, PV, and CLL patients was lower than that observed in normal hematopoietic cells, whereas in AP/BP-CML and AML, telomerase was elevated at baseline and highly exceeded levels found in healthy donors (Fig. 5A). The expansion potential of CD34+ cells from patient samples was considerably decreased compared with normal hematopoiesis (Fig. 5B). The cell expansion in healthy donors in weeks 1 to 4 was 36-, 37-, 3.5-, and 1.2-fold, respectively, whereas in patients, the expansion potential was significantly reduced with 6.5-, 2.3-, 0.6-, and 0.2-fold, respectively. The expansion potential was also reduced in each patient subpopulation, as shown in Fig. 5B. The cell expansion potential of CML samples was higher compared with patients with AML, PV, or CML (Fig. 5B). Lower amounts of TA in weeks 2 to 4 of culture were associated with telomere erosion during ex vivo culture. During 3 to 4 weeks of culture, a median telomeric loss of 0.7 kbp was detected in patient samples compared with 1.1 kbp in healthy donors (Fig. 5C). Higher TA in AML and AP/BP-CML specimens correlated with a reduced telomere loss compared with lower TA and a greater TRF loss in CLL and PV patients (r = −0.61) (Fig. 5D).
Fig. 4. A, telomerase kinetics during ex vivo culture. Lanes 1–5: TA in healthy donors with up-regulation after 1 week of culture. Lanes 6–8: TA in AP/BP-CML with a substantial decrease during ex vivo culture. Lanes 9–12: same phenomenon but in AML. Lanes 13–16: TA in CLL CD34+ with similar telomerase up-regulation as observed in healthy donors. B, TA in CD34+ cells from CP-CML displayed a similar up-regulation within 72 h of ex vivo expansion as in healthy donors.

DISCUSSION

Previous studies have shown that telomeres within hematopoietic cells and other somatic tissues progressively shorten with age in in vivo and ex vivo culture (1, 2, 4, 13) and that telomeric DNA loss may correlate with genetic instability, the pathogenesis of de novo MDS or leukemia, and disease progression (22–25, 31). However, no study to date has conclusively addressed TA and telomere length changes before and after ex vivo culture in acute and chronic myeloid leukemias, myelodysplastic syndromes, CLL, and PV. Therefore, we sought to determine whether TA and TRF measurements in adult leukemia patients differ from healthy donors and whether both show significant changes before and after ex vivo culture. We found that TA in MNC and CD34+ cells from healthy donors was low and that TA in CD34+ cells was increased compared with MNC. Therefore, TA appears to be required for maintenance and proliferation potential of CD34+ cells. Very low levels of telomerase were found in CB cells (most likely due to their quiescent nature) where only <2% of cells are in cell cycle, whereas higher TA was observed in more actively cycling PB and BM cells. In leukemia (especially in diagnostic specimens analyzed in this study) telomerase was highly increased in contrast to normal hematopoietic cells. TA rose with the acuteness and aggressiveness of the leukemic disease: (a) was low in PV and CLL; (b) increased moderately in CML and MDS; and (c) increased significantly in AML patients. Although other investigators have reported less consistent telomerase expression in leukemia with no correlation to biological and clinical parameters (32), our results are in line with previous reports (11, 12, 22–25) and suggest that telomerase plays an important role in the process of multistage leukemogenesis, correlates with disease progression, and decreases to borderline activity with the attainment of complete remission in leukemic patients.

Telomeres had progressively shortened, particularly in AML and CML, to a lesser extent in PV and MDS, and least in CLL CD34+ cells. Short telomeres in leukemia and MPD support the concept that there is progressive telomere erosion in patients with ongoing or active disease and that telomerase was turned on after extensive

Fig. 5. A, similar to TA kinetics observed in healthy donors, telomerase was up-regulated during ex vivo expansion of CD34+ cells from CP-CML, PV, and CLL patients, peaked after 1 week, and decreased below detection thereafter. In contrast, in AML, telomerase was down-regulated after 1 week and decreased even further. B, expansion potential of CD34+ cells in leukemia was considerably decreased compared with normal hematopoiesis. The cell expansion potential in CML patients was higher compared with AML, PV, or CLL and correlated with the amount of TA. C, in serial culture within 3–4 weeks of expansion, a median telomeric loss of 0.7 kbp was detected, with a reduced loss in AML and CML correlating with higher TA compared with a greater loss in PV and CLL when TA was low (D).
proliferation (25, 31). Assuming a bp loss of 50/cell division, average population doublings in CLL CD34⁺ cells (used as controls for normal stem cells) were low but increased 20-fold in PV and MDS and 45- to 50-fold in CML and AML. Telomere reduction was previously demonstrated in acute and chronic leukemia (22, 25, 31), in MDS (33, 34), and in others such as aplastic anemia (35), IDDM (insulin-dependent diabetes mellitus) (36), and scleroderma (37). Telomere shortening in disease states has been linked to chromosome abnormalities and disease progression, leading to increased proliferation, cell apoptosis, and/or immune destruction (12, 22–25, 30, 33, 34). Comparison of diagnostic and follow-up samples from leukemic patients revealed significantly increased TA in diagnostic specimens compared with those of patients exposed to >6 chemotherapy cycles, accounting for a telomere length difference of 1.4 kbp (or 28 years of premature aging). Telomes in patients in complete remission were a median of 0.6 kbp shorter compared with age-matched healthy donors, accounting for 12 years of premature aging. Telomere shortening in hematopoietic cells has been observed after standard chemotherapy (25), after intensified treatment protocols such as high-dose chemotherapy in autologous (38) and allogeneic transplantation (39, 40), in cells treated with cisplatin (41), and after irradiation (42). Telomere reduction associated with transplantation has been demonstrated to correlate inversely with the number of nucleated cells infused (40), suggesting that the proliferative pressure is less intense with large progenitor cell support. Telomere erosion after high-dose chemotherapy protocols seems likely to occur as a consequence of strong proliferative stress, possibly accounting for hematopoietic abnormalities such as reduced hematopoietic and stromal cell compartments, impaired CFU and long-term culture-initiating cell (LTC-IC) capacity, poor response of progenitors to growth factors in vivo and ex vivo.

Fig. 6. A, the colony number in each week of culture as well as the total colony numbers (B) were significantly lower in CD34⁺ cells from AML compared with CL, PV, and CML patients. Compared with healthy donors, cell counts (C) and colony counts (D) were 1–5 log decreased in MPD and leukemia in expansion culture. Cell counts in healthy donors reached the maximum cell expansion in weeks 1 and 2 and decreased thereafter compared with significantly lower numbers in weeks 1–4 in leukemia patients (C). In line with the decreased cell expansion potential, total colony numbers (D), the fold cell increase (E), and the fold progenitor expansion (F) were also significantly diminished in leukemia compared with normal donors.
and the high incidence of MDS that has been observed after autologous and allogeneic transplantation (43, 44).

We and others have previously demonstrated TA in normal hematopoietic cells and that TA further increases in ex vivo culture which thereby prevents progressive telomere shortening despite high cell turnover (12, 13). We found that the kinetics of TA in CD34+ cells from CP-CML, PV, and CLL patients were similar to normal hematopoiesis, whereas high TA in AP/BL-CML and AML specimens was down-regulated. The amount of telomerase correlated with the cell expansion potential, in which elevated TA was associated with greater proliferative potential, whereas a decline in proliferation and increased cell apoptosis was observed with subsequent telomere erosion in weeks 2–4 of expansion when TA was low. C. Eaves and M. Dexter have convincingly demonstrated that leukemic cell lines or acute and chronic leukemia samples can be grown in suspension culture, colony-forming assay, LTC-IC assay, or severe combined immune-deficient (SCID) mice. In these systems, growth and maturation into specific lineages is observed and can be modulated by use of exogenous growth factors and differentiating agents (19–21). Ex vivo culture of leukemic cells, as documented in AML and CML patients, previously allowed the identification and isolation of primitive normal stem cells, partial reconstitution of normal hematopoiesis, and loss of the malignant cell clone (19–21, 45, 46). Telomerase down-regulation in our culture system seemed to demonstrate the phenomenon whereby, in chronic and acute leukemia, malignant and normal hematopoietic coexist, and in many cases primitive hematopoietic cells proliferate normally, whereas the malignant clones die out ex vivo, presumably via terminal differentiation or apoptotic cell death. Leukemic cells may exhibit aberrant differentiation, altered susceptibility to apoptosis, and modified enzyme activity, as observed for telomerase. Apoptotic death and/or terminal differentiation of leukemic cells can be induced, with appropriate cytokines and/or use of retinoic acid in the case of promyelocytic leukemia (11, 17, 47). During culture we observed that telomere loss inversely correlated with levels of telomerase: high TA in AML prevented excessive telomere shortening, was too low in chronic leukemia and MDS to completely prevent telomere loss, and was comparable with normal hematopoiesis in CLL CD34+ cells. Why the expansion potential of CLL progenitors was reduced even though TA and telomere loss were comparable with normal hematopoiesis seems only insufficiently explained by their advanced age and pretreatment rather than by the impaired biological features responsible for the perturbed ex vivo culture kinetics. Nevertheless, loss of TA and progressive telomere erosion in acute and chronic leukemia upon expansion may have demonstrated (a) the loss of the malignant phenotype (in favor of normal hematopoietic progenitors), (b) induction of terminal differentiation of leukemic cells, and/or (c) senescence and apoptosis of leukemic cells with critically short telomeres after further shortening during suspension culture. That telomere loss plays a crucial role for replicative cell senescence has been shown in telomerase knock-out mice, in which telomere loss led to depletion of male germ cells, diminished hematopoietic colony formation, impaired mitogen-induced proliferation of primary spleenocytes, an increase in apoptosis, and (with critically short telomeres) cessation of proliferation (48, 49).

On the basis of these investigations, we conclude that TA (a) is up-regulated in freshly isolated acute and chronic leukemia cells, (b) correlates with acute versus chronic forms of leukemic disease and disease progression and correlates inversely with response to therapy, and (c) is down-regulated in acute leukemia during expansion, the latter phenomenon due to either partial differentiation of leukemic cells or leukemic cell apoptosis in culture. Telomere shortening in culture most likely occurs because telomerase levels are insufficient, particularly in chronic leukemic or MDS progenitors, to protect against telomere bp loss upon proliferation. Alternatively, telomere shortening may reflect the emergence of nonleukemic populations with low TA which is unable to prevent telomere shortening upon extensive proliferation (13). Although telomerase may not be the only mechanism for maintaining chromosome ends and alternative lengthening of telomeres also functions to elongate telomeres (50), TA seems vital for cell survival and organ homeostasis in most proliferating cells through maintenance of telomere structures during cell division. Novel telomerase inhibitors, as recently shown using a genetic approach (51), should be tested in hematopoietic culture systems with use of normal and malignant hematopoietic cells because telomerase dynamics, cell proliferation, and telomere length changes can be determined.

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Telomerase Activity and Telomere Length in Acute and Chronic Leukemia, Pre- and Post- ex Vivo Culture


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