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Telomere deregulations possess cytogenetic, phenotype, and prognostic specificities in acute leukemias

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Objective. **Telomeres are protected by tightly regulated factors and elongated by telomerase. Short and/or deprotected chromosomes are recombinogenic and thereby cancer prone.**

Materials and Methods. **Together with the quantification of telomerase activity (TA), measuring telomere length (TL) and expression of the genes that govern telomere protection and elongation are useful for assessing telomere homeostasis.**

Results. **By these means we demonstrate that TL, *hTERT*, and TA are in the order acute myelogenous leukemia (AML) > T-cell acute lymphoblastic leukemia (T-ALL) > B-cell acute lymphoblastic leukemia (B-ALL) > T-ALL > AML, and B-ALL > AML > T-ALL. AML0 and AML3 display the lowest amounts of *hTERT* transcripts, and ALL and AML cells with cytogenetic abnormalities possess the shortest telomeres. *hTERT* expression includes phenotype-specific RNA maturation and correlates with TA but not with TL. A wide ratio of TA to *hTERT* expression between leukemia subtypes suggests phenotype-specific *hTERT* post-transcriptional deregulations. B- and T-ALL overexpress *Ku70* and *Pinx1,* T-ALL *PTOP* and *RAP1*, and B-ALL *TRF2*, the expression of which is significantly higher in cases with abnormal karyotype. *hTERT* transcription and TL correlate with response to intensive chemotherapy, and *hTERT* and *RAD50* are independent prognostic factors for survival.**

Conclusions. **Each leukemia subtype possesses specific telomere dysregulations that rely on phenotype, karyotype, response to treatment, and survival.** © **2011 ISEH - Society for Hematology and Stem Cells. Published by Elsevier Inc.**

Chromosome and gene rearrangements are the hallmark of leukemia. Abnormal chromosome extremities favor such rearrangements and are thereby involved in leukemogenesis. Telomeres, the extremities of chromosomes, consist of repetitive DNA sequences and proteins, creating a specialized structure called the telosome [1] that protects chromosome ends from fusion and degradation [2]. In the absence of special telomere maintenance mechanisms, linear chromosomes shorten progressively with every round of DNA replication, eventually leading to cellular senescence or apoptosis. A cellular reverse transcriptase called *telomerase* counteracts telomere shortening. It is composed of a catalytic protein subunit, telomerase reverse transcriptase (hTERT) and an RNA template (hTR). Telomere protection relies on specific factors, the shelterin complex [3] and several additional proteins involved in DNA damage response and replication [2].

Chromosomal ends with critically shortened or deprotected telomeres are highly recombinogenic and, in certain cellular and genetic backgrounds, telomere dysfunctions may promote chromosome rearrangements and thereby

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tumor development [4]. Hence the majority of human tumor cells possess shorter telomeres than their normal counterparts, suggesting that abnormal telomere shortening is frequently involved in cancer. Malignant cells regularly bypass replicative senescence and paradoxically combine high telomerase activity (TA) with short telomere length [4]. Therefore, telomere dysfunctions are connected with tumor development and persistence. In consequence, unraveling specific telomerase and telosome changes in cancer or leukemia should provide new knowledge about oncogenesis, useful clinical markers and specific therapeutic targets.

In acute myelogenous leukemia (AML), TA correlates with hTERT but not with hTR expression, and is higher at time of diagnosis or progression than at time of remission [5]. The shortest telomeres characterize AML patients with multiple cytogenetic abnormalities [6,7]. In children, the highest hTERT messenger RNA (mRNA) values are observed in B-cell acute lymphoblastic leukemia (B-ALL) followed by T-cell acute lymphoblastic leukemia (T-ALL) and AML [8]. However, the positive correlation between hTERT overexpression, increased TA, and leukemogenesis does not seem to be mandatory as hTERT underexpression has recently been shown at some stage of chronic myeloid leukemia [9,10], adult T-cell leukemia/lymphoma [11,12], and chronic lymphocytic leukemia [13]. In addition to the quantitative modulation of hTERT expression, dysregulation of hTERT mRNA maturation has been evidenced in chronic myeloid leukemia [9,10]. Regarding telomerase access to telomeres in hematologic malignancies, chronic lymphocytic leukemia is known to display numerous and important changes in the expression of telosome genes [13]. Little is known about the expression of these factors in acute leukemias. In vitro, the expressions of TRF1, PINX1, TANK1, and TANK2 are differentially modulated upon differentiation of acute promylocytic cell lines [14].

Here we investigated TA, telomere length, hTERT, and telomere genes expression in adult acute leukemias. Significant differences were evidenced for TA, hTERT, and telomere genes expression between ALL and AML cells. These define a specific pattern of telomere deregulation with respect to the leukemic phenotype. This pattern was found to possess prognostic implications and might generate useful diagnostic or prognostic markers and therapeutic targets.

Materials and methods

Samples studied

The medical ethics committee of the Hospices Civils de Lyon approved the study. Informed consent was obtained from patients and healthy volunteers in accordance with the Declaration of Helsinki and with institutional guidelines. Bone marrow leukemic cells were obtained before treatment from 57 acute leukemia patients with $>85\%$ bone marrow blasts, including 37 patients with AML, 13 with B-ALL, and 7 with T-ALL. Diagnosis of leukemia was based on routine morphologic evaluation, immunophenotyping, cytochemical smears, and karyotyping. Age-matched control cells derived from donors corresponded to bone marrow mononuclear cells (BMMNCs, 25 samples) and purified $CD34^+$ bone marrow cells ($n = 16$).

Isolation of $CD34^+$ normal bone marrow cells

Mononuclear cell fractions from bone marrow donors were separated using a Ficoll-Hypaque gradient. $CD34^+$ cells were then isolated by immunomagnetic microbead selection using a Dynal CD34 progenitor cell selection system (Dynal Biotechnologies) according to manufacturer's instructions. The kits contained Dynabeads and DETACHaBEAD for isolation and release of pure $CD34^+$ cells with an intact antigen profile. Purity was controlled by fluorescence-activated cell sorting analysis with $CD34^+$–fluorescein isothiocyanate antibody (Dako).

Telomere restriction fragment length analysis

The average telomere length in mononuclear cells was measured in all samples using the TeloTAGGG Telomere length Assay (Roche Diagnostics, Mannheim, Germany). Briefly, purified genomic DNA (6–8 μg) was extracted by phenol chloroform and digested by specific restriction enzymes. The DNA fragments were separated by gel electrophoresis and transferred to a nylon membrane by Southern blotting. The blotted DNA fragments were hybridized to a digoxigenin-labeled probe specific to telomere repeats and incubated with a digoxigenin-specific antibody coupled to alkaline phosphate. Finally, the immobilized probe was visualized by a sensitive chemiluminescence substrate and the average telomere restriction fragment (TRF) length was assessed by comparing the signals relative to a molecular weight standard.

Quantification of telomerase activity

Quantitative determination of telomerase activity was performed using the telomeric repeat amplification protocol with teloTAGGG telomerase PCR ELISAPLUS kit (Roche Diagnostics, Mannheim, Germany), as described previously [11]. Relative TA was expressed as the absorbance of the sample compared to the absorbance of the control template.

Real-time quantitative reverse transcriptase–polymerase chain reaction (PCR).

Total cellular RNA from patient and control samples was isolated using TriZol reagent according to the manufacturer's instructions (Invitrogen, Life Technologies, Carlsbad, CA, USA). Before reverse transcription, RNA was treated by DNase (Amersham) to prevent DNA contamination. First-strand complementary DNA (cDNA) was synthesized from 0.5 μg RNA using random primers (Promega) and Superscript II reverse transcriptase (Invitrogen). RNA concentration and purity were determined by ultraviolet spectrophotometry. Sequences of primers are described in Supplementary Table E1 (online only, available at www.exphem.org). Primer sets used to quantify gene expression were first tested in PCR with a control cDNA to ensure specific amplification, as evidenced by the presence of a unique specific signal after agarose gel electrophoresis. PCR assays were performed on an ABI Prism 7000 sequence detection system (Applied Biosystems) using 5 μL cDNA, 6 μL SYBR Green Master Mix, 0.25 μL Rox (Invitrogen) and 0.75 μL primers at 10 μM. Thermal cycling consisted of a first cycle at 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds