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Impact of telomere length on survival in classic and variant hairy cell leukemia

Evgeny Arons[a], Hong Zhou[a], Daniel C. Edelman[b], Allison Gomez[b], Seth M. Steinberg[c], David Petersen[b], Yonghong Wang[b], Paul S. Meltzer[b], Robert J. Kreitman[a,*]

[a] Laboratory of Molecular Biology, National Cancer Institute (NCI), NIH, United States
[b] Cancer Genetics Branch, NCI, NIH, United States
[c] Biostatistics and Data Management Section, Office of the Clinical Director, Center for Cancer Research, National Cancer Institute, NIH, 9000 Rockville Pike, 37/5124b, Bethesda, MD 20892-4255, United States

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ABSTRACT

Telomeres, which protect the ends of chromosomes, are shortened in several hematologic malignancies, often with adverse prognostic implications, but their effect on prognosis of classic and variant hairy cell leukemia (HCL and HCLv) has not been reported. HCL/HCLv genomic DNA from 46 patients was studied by PCR to determine the ratio of telomere to single copy gene number (T/S). T/S was unrelated to diagnosis of HCL or HCLv ($p = 0.27$), but shorter T/S was associated with unmutated immunoglobulin rearrangements ($p = 0.033$) and age above the median at diagnosis ($p = 0.017$). Low T/S was associated with shorter overall survival from diagnosis (OS), particularly T/S <0.655 ($p = 0.0064$, adjusted $p = 0.019$). Shorter OS was also associated with presence of unmutated ($p < 0.0001$) or IGHV4-34+ ($p < 0.0001$) rearrangements, or increasing age ($p = 0.0002$). Multivariable analysis with Cox modeling showed that short T/S along with either unmutated or IGHV4-34+ rearrangements remained associated with reduced OS ($p = 0.0071$, $p = 0.0024$, respectively) after age adjustment. While T/S is relatively long in HCL and the disease usually indolent with excellent survival, shortened telomeres in HCL/HCLv are associated with decreased survival. Shortened T/S could represent a risk factor needing further investigation/intervention to determine if non-chemotherapy treatment options, in addition to or instead of chemotherapy, might be particularly useful.

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1. Introduction

Classic hairy cell leukemia (HCL) is a B-cell malignancy with distinctive immunophenotype, typically expressing BRAF V600E mutation, CD20, CD22, CD25, CD11c, CD103, CD123, annexin A1 (Anxa1), and tartrate-resistant acid phosphatase (TRAP) [1–4]. Purine analog therapy is highly effective, with most patients achieving durable complete remissions (CR) [5,6]. HCL variant (HCLv) was first identified by Cawley et al. [7] and recently recognized by the World Health Organization as a separate disorder [4]. HCLv lacks CD25, annexin A1, TRAP, and BRAF V600E, and patients respond poorly to purine analogs, with only partial response in less than 50%, CR in less than 10% and relatively poor overall survival (OS) from diagnosis [3,8–11]. We recently reported that HCL expressing the immunoglobulin heavy-chain variable (IGHV) rearrangement type IGHV4-34 expresses wild-type BRAF and has a poor prognosis like HCLv, whether immunophenotypically consistent with HCLv or classic HCL [10,12]. Mutations other than BRAF V600E have been found in HCLv and IGHV4-34+ HCL, including several within the MAPK pathway [13].

Telomerase activity (TA) prevents the further shortening of telomeres, composed of arrays of TTAGGG DNA repeats up to 25 kb, which associate with specific nucleoproteins to protect the 3' ends of chromosomes from translocations, double-strand breaks and recombinations [14]. Multiple cell divisions can result in successively shorter telomere length (TL), leading to age-related senescence including apoptosis, but high TA can be associated with immortalization and development of leukemias and lymphomas [15]. Although naive normal B-cells lack TA, germinal center (GC) B-cells, which undergo at least 20 cell divisions, have high TA, thus preventing TL shortening [16].

In the B-cell malignancies, including chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), lymphoplasmacytic

* Corresponding author. Fax: +301 451 5765.
E-mail address: kreitmar@mail.nih.gov (R.J. Kreitman).

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lymphoma (LPL), and Waldenström's macroglobulinemia (WM), TA and TL have been observed to vary with aggressiveness and disease type [15,17–19]. In CLL, TL is highly variable and short telomeres have been associated with unmutated IGHV status, high-risk genomic aberrations, and poor outcome [15,20,21]. In contrast to CLL, TL in MCL and DLBCL was not associated with prognosis or disease outcome [17,18]. One of these studies contained 12 cases of HCL [18], and showed that TL was longer in HCL and LPL than in FL or non-GC-like DLBCL, suggesting a stronger relationship of HCL to the GC-B-cell. TL data have not been reported for HCLv. To study telomeres in both HCL and HCLv, we determined relative TL (RTL) in patients with these B-cell disorders.

2. Materials and methods

2.1. Patients and blood samples

Blood was obtained from 46 patients with either HCL or HCLv as part of Institutional Review Board (IRB)-approved sample collection protocols at NCI. DNA was obtained from blood in sodium heparin tubes by the Qiagen AllPrep kit (Qiagen, Valencia, CA). HCL/HCLv cells were purified by Ficoll centrifugation followed by negative isolation of B-cells to remove T-cells, NK cells, monocytes, and other non-B-cells using the Dynabeads Untouched Human B Cells Kit (Thermo Fisher, Grand Island, NY). Samples finally had >90% HCL/HCLv cells provided that the leukemic cells prior to purification comprised >90% of the B-cells, as determined by flow cytometry. IGHV sequencing to determine closest germline sequence and% homology to germline was performed as previously described [10,12].

2.2. Telomere length analysis

The monochrome multiplex quantitative PCR (MMQPCR) method [22,23] was performed using the Rotor-Gene Corbett 3000 (Qiagen). This method has been used for many years and is reliable with low intra-individual variability [24–26]. Samples chosen for DNA preparation contained >90% HCL cells, to insure that telomere data would pertain to the malignant cells and not the normal cells in the sample. The telomere and single copy gene (SCG) albumin primers were used at a final reaction concentration of 900 nM each: telg, ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT; telc, TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA; albu, CGGCGGCGGGCGGCGCGGGCTGGGCGGaatgctgcacagaatccttg; albd, GCCCGGCCCGCCGCGCCCGTCCCGCCGgaaaagcatggtcgcctgtt. Normal female DNA dilutions for the standard curve contained a final amount of 200 ng, 50 ng, 12.5 ng, 3.125 ng in their respective tubes at a final volume of 15 μL. Samples to be measured for TL and the positive control each contained a final amount of 20ng DNA in a total volume of 15 μL. The standard curve was performed in duplicate for each run and samples were processed in triplicate. Syto 82 Orange Fluorescent Nucleic Acid Stain (Molecular Probes) and Quantitect Multiplex NoRox PCR Master Mix (Qiagen) were used at a final reaction concentration of 5 μM and 1X, respectively. The following were the thermal conditions: initial holding stage 1 (95 °C, 15 min), cycling stage 2 of two repeats (94 °C, 15 s; 49 °C, 15 s), cycling stage 3 of 28 repeats (94 °C, 15s; 62 °C, 10 s; 74 °C, acquired reading, 15s; 84 °C, 10 s; 88 °C, acquired reading, 15s), and melt curve (ramp from 75 °C to 95 °C, rising by 0.2° each step, waiting for 60s on the first step and 15s for each step afterwards). The Ct values acquired at 74 °C and 88 °C of cycling stage 3 correspond to the telomere and albumin signals, respectively. The MMQPCR data was analyzed using the Rotor-Gene 6 software version 6.1. Two standard curves were generated for telomere and SCG albumin using the auto-find threshold option. Using each sample's telomere Ct and albumin Ct, telomere ng and albumin ng were interpolated from their respective standard curve. Telomere ng/Albumin ng generated T/S ratios for each reaction. The average T/S ratio from samples run in triplicate and standard deviation values were used in our analysis.

2.3. Statistical methods

Dichotomous patient characteristics were compared between two groups using a Fisher's exact test, and an exact form of the Wilcoxon rank sum test was used for comparing continuous parameters between two groups. For these analyses, the p-values are reported without adjustment for multiple comparisons since they are considered exploratory and descriptive.

The correlation between continuous parameters was determined using Spearman non-parametric correlation analysis. For these analyses, $|r| > 0.70$ would be considered a strong correlation; $0.50 < |r| < 0.70$ would be considered moderately strong; $0.30 < |r| < 0.50$ would be considered weak to moderately strong, and if $|r| < 0.30$, the correlation is weak. Since the p-value for a correlation coefficient is a test of whether $r = 0$, this is less important in interpreting the result than the strength of the correlation itself.

Survival was determined from date of diagnosis until date of death or date last reported to be alive. Survival probabilities as a function of time were determined by the Kaplan–Meier method, with the significance of the difference among curves compared using the log-rank test. The T/S ratio was divided into quartiles and the patients with values in these 4 categories were initially compared with respect to their association with survival. Subsequently, a single division was made to separate patients into two groups on the basis of the best association with survival. In this case, the p-value was adjusted by multiplying the unadjusted p-value by 3 to account for the implicit number of tests needed to arrive at the ultimate division. Age was also explored in a similar fashion and divided into three categories based on the survival curves. To further assess the importance of the multiple factors considered jointly for their association with survival, a Cox proportional hazards model analysis was performed.

All p-values are two-tailed, and except as noted above, are presented without any adjustment for multiple comparisons.

3. Results

3.1. Patients tested, clinical factors

Of the 46 patients tested, 27 had classic HCL and 19 had the variant HCLv. As shown in Table 1, median age at diagnosis was lower for HCL than for HCLv (46.6 vs 55.3 years, $p = 0.028$). Males outnumbered females 37 to 9, with no difference in ratio between HCL and HCLv ($p = 0.46$). The median number of prior courses of purine analog, including not only cladribine (CdA) and pentostatin (DCF), but also bendamustine and fludarabine, was 3 (range: 0–8) for HCL and one (range: 0–6) for HCLv ($p = 0.016$). The higher number of purine analogs for HCL compared to HCLv was expected since the more rapid progression in the latter results in patients presenting earlier for clinical trials. Median white blood cell (WBC) count at the time of T/S measurement was 28.4 (range: 0.8–141) per nL in HCL vs 25.3 (range: 4.99–304) in HCLv ($p = 0.51$). All patients had active disease with median 7.14 HCL cells/nL (range: 0.00092–134) vs 19.2 (range: 0.475–286) HCLv cells/nL in the blood ($p = 0.71$), as determined by flow cytometry. Seventeen of 27 (63%) with HCL had prior splenectomy, vs 7 of 19 (37%) with HCLv ($p = 0.13$). While WBC and HCL counts were not significantly different between HCL and HCLv, they were different as expected in patients with vs without prior splenectomy (Table 1, $p = 0.020$, $p = 0.031$).