EBioMedicine 2 (2015) 591–596

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Original Article

Blood Telomere Length Attrition and Cancer Development in the Normative Aging Study Cohort

Lifang Hou [a,b,*], Brian Thomas Joyce [a,c], Tao Gao [a], Lei Liu [a,b], Yinan Zheng [d], Frank J. Penedo [b,e], Siran Liu [f], Wei Zhang [a], Raymond Bergan [b,g], Qi Dai [h], Pantel Vokonas [i,j], Mirjam Hoxha [k], Joel Schwartz [l], Andrea Baccarelli [l]

[a] Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, 680 N. Lake Shore Drive, Suite 1400, Chicago, IL 60611, USA
[b] Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, 303 E. Chicago Ave., Olson Pavilion 8350, Chicago, IL 60611, USA
[c] Division of Epidemiology and Biostatistics, School of Public Health, University of Illinois—Chicago, 1603 W. Taylor St., Chicago, IL 60613, USA
[d] Institute for Public Health and Medicine, Northwestern University Feinberg School of Medicine, 633 N. St. Clair St., Chicago, IL 60611, USA
[e] Department of Medical Social Sciences, Northwestern University Feinberg School of Medicine, 633 N. St. Clair St., Chicago, IL 60611, USA
[f] Department of Biomedical Engineering, Northwestern University, 2145 Sheridan Road, Rm E310, Evanston, IL 60208, USA
[g] Department of Medicine, Division of Hematology and Oncology, Northwestern University Feinberg School of Medicine, 251 E. Huron St., Suite 3-150, Chicago, IL 60611, USA
[h] Vanderbilt University Medical Center, 2525 West End Ave, Suite 319, Nashville, TN 37203, USA
[i] VA Normative Aging Study, VA Boston Healthcare System, 150 South Huntington Ave, Boston, MA 02130, USA
[j] Department of Medicine, Boston University School of Medicine, Boston, MA 02118, USA
[k] Molecular Epidemiology and Environmental Epigenetics Laboratory, Department of Clinical Sciences and Community Health, Università degli Studi di Milano, San Barnaba 8, 20122 Milan, Italy
[l] Department of Environmental Health, Harvard School of Public Health, 655 Huntington Ave, Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 12 March 2015
Received in revised form 9 April 2015
Accepted 10 April 2015
Available online 13 April 2015

Keywords:
Telomere
Longitudinal study
Cancer incidence

ABSTRACT

Background: Accelerated telomere shortening may cause cancer via chromosomal instability, making it a potentially useful biomarker. However, publications on blood telomere length (BTL) and cancer are inconsistent. We prospectively examined BTL measures over time and cancer incidence.
Methods: We included 792 Normative Aging Study participants with 1–4 BTL measurements from 1999 to 2012. We used linear mixed-effects models to examine BTL attrition by cancer status (relative to increasing age and decreasing years pre-diagnosis), Cox models for time-dependent associations, and logistic regression for cancer incidence stratified by years between BTL measurement and diagnosis.
Findings: Age-related BTL attrition was faster in cancer cases pre-diagnosis than in cancer-free participants ($p_{difference} = 0.017$); all participants had similar age-adjusted BTL 8–14 years pre-diagnosis, followed by decelerated attrition in cancer cases resulting in longer BTL three ($p = 0.003$) and four ($p = 0.012$) years pre-diagnosis. Longer time-dependent BTL was associated with prostate cancer (HR = 1.79, $p = 0.03$), and longer BTL measured ≤4 years pre-diagnosis with any (OR = 3.27, $p < 0.001$) and prostate cancers (OR = 6.87, $p < 0.001$).
Interpretation: Age-related BTL attrition was faster in cancer cases but their age-adjusted BTL attrition began decelerating as diagnosis approached. This may explain prior inconsistencies and help develop BTL as a cancer detection biomarker.

1. Introduction

Telomeres are tandem repeats of TTAGGG nucleotides at the ends of eukaryotic chromosomes that, along with telomere binding proteins, help maintain genomic stability (Ma et al., 2011). Studies show that blood telomere length (BTL) decreases with age and that environmental exposures causing oxidative stress and chronic inflammation accelerate this process (Jennings et al., 2000; von Zglinicki, 2002). Shortened telomeres are often involved in cellular senescence or apoptosis. However, if their shortening becomes critical, such biological responses can be inhibited, resulting in genomic instability (Kong et al., 2013; Frias et al., 2012) including chromosomal rearrangements, and both gains and losses of chromosomal segments (Lundblad and Szostak, 1989), all essential steps in carcinogenesis. For these reasons, telomeres have long been an object of study for potential early involvement in cancer development (Londono-Vallejo, 2008; DePinho, 2000). One major weakness to tissue-specific telomere length in tumors is that it is only measurable after disease development, and thus can be affected by both cancer and treatment.

Blood leukocytes play an important role in carcinogenesis via inflammatory response and pro-apoptotic processes. Leukocyte infiltration is critical early in carcinogenesis and has been linked to many

* Corresponding author at: Department of Preventive Medicine, Northwestern University, 680 N. Lake Shore Drive, Ste. 1400, USA.
E-mail address: l-hou@northwestern.edu (L. Hou).

http://dx.doi.org/10.1016/j.ebiom.2015.04.008

cancers including pancreatic (Schnekenburger et al., 2008) and colorectal (Ichikawa et al., 2011). Thus, studying BTL in DNA collected before cancer development can provide important information on its role in cancer etiology and serve a valuable predictive purpose. However, BTL has been extensively studied in relation to cancer risk with inconsistent results (Hou et al., 2012a; Willeit et al., 2010). One possible explanation is that most studies reporting shorter BTL in cancer patients relative to controls are retrospective studies in which BTL was measured post-diagnosis, a finding which could be a consequence of cancer development or treatment, not a cause (Hou et al., 2012a). For example, Unryn et al. showed that patients with neck and head tumors who went through eight weeks of chemotherapy had a mean telomere loss of 660 base pairs (Unryn et al., 2006). Results have also been inconsistent in prospective studies where BTL was measured pre-diagnostically, some reporting increased cancer risk in participants with shorter BTL, and others with longer (Hou et al., 2012a). Most studies examined BTL at a single time point only, and none to our knowledge measured BTL more than once before cancer diagnosis, making it difficult to examine the causal relationship between BTL attrition and cancer risk. Longitudinal studies of BTL with multiple pre-diagnostic measurements may be more informative about how BTL contributes to cancer risk, and provide critical information on the relationship between BTL and cancer development and diagnosis. Our objective is to examine BTL attrition over time in relation to risk of developing cancer, specifically: 1) How BTL changes with time affect, and are affected by, cancer development and 2) whether BTL measured prior to clinical diagnosis is associated with risk of developing cancer.

2. Methods

2.1. Study Design and Participants

The Normative Aging Study (NAS) was established by the US Department of Veteran Affairs (VA) in 1963 with an initial cohort of 2280 healthy men. Initial eligibility criteria at enrollment included veteran status, residence in the Boston area, ages 21–80, and no history of hypertension, heart disease, cancer, diabetes, or other chronic health conditions. From 1963 to 1999, 981 participants died and 470 were lost to follow up. Statistical comparisons between the remaining 829 participants and those lost to follow up revealed no significant differences in subject characteristics (age, BMI, etc.). Participants were recalled for clinical examinations every 3–5 years. Starting in 1999, these included 7-ml blood samples for DNA analysis. Between January 1st, 1999 and December 31st, 2012, 802/829 (96.7%) of active participants agreed to donate blood. Our study population included participants who had 1–4 clinical visits during which blood was collected, and non-missing data for BTL from at least one of those visits, resulting in a total population of 792. Of these, 227 (28.7%) participants had data from one visit, 202 (25.5%) from two, 229 (28.9%) from three, and 134 (16.9%) from four. This study was approved by the Institutional Review Boards of all participating institutions, and all participants provided written consent.

2.2. Identification of Cancer Cases

Information on cancer diagnosis was obtained from questionnaires and confirmed via review of medical records. Among the 792 participants, 213 were diagnosed with cancer (75 prostate, 97 skin, 41 other) before their first blood draw (baseline). After examining associations between BTL and prevalent cancers, these participants were excluded, and subsequent analyses only examined pre-diagnostic BTL measurements. Among the remaining 579 participants free of cancer at baseline, 135 new cancer cases occurred (53 prostate, 42 skin, 10 lung, nine leukemia, five bladder, four colon, two stomach, two liver, two pancreas, and six unspecified) during median 10.6 year follow-up. Participants' mean age at diagnosis was 75.9 ± 6.6 years. Participants who were cancer-free for the full duration of the study were censored after their last recorded visit.

2.3. Telomere Measurement

BTL was measured using quantitative real-time polymerase chain reaction (qPCR) (Cawthon, 2002). Relative BTL was measured by the ratio of the telomere (T) repeat copy number to single-copy gene (S) copy number (T:S ratio) in a given sample and reported as relative units expressing the ratio between test DNA BTL and reference pooled DNA BTL. The latter was created using DNA from 475 participants randomly selected (400 ng per sample) and used to generate a fresh standard curve from 0.25 to 20 ng/μL in every T and S PCR run. qPCR primer sets for T and human beta-globin, taken as the reference S, as well as qPCR mix composition were previously described (Hou et al., 2009). We ran all samples in triplicate, and the average of the three T measurements was divided by the average of the three S measurements to calculate the average T:S ratio. The intra-assay coefficient of variation for the T/S ratio was 8.1%. The average coefficient of variation for the T reaction was 8%, and for the S reaction was 5.6%. When the coefficient of variation for T or S reactions was higher than 15%, the measurement was repeated.

2.4. Statistical Analysis

After our initial descriptive analysis of BTL and subject characteristics by visit, we performed a second descriptive analysis using a repeated measures study to examine associations between participant characteristics at baseline and cumulative mean BTL (BTL averaged across all visits) among cancer-free participants only. Next, we used linear mixed-effects models to compare rates of BTL attrition over time by cancer status (those who developed cancer at some point during follow-up, and those who did not). BTL attrition rate was examined relative to increasing age, and age-adjusted BTL attrition was also examined relative to decreasing years pre-diagnosis (pre-censoring in the case of cancer-free subjects). Next, we used Cox proportional hazards regression models to estimate time-dependent associations between BTL and time to diagnosis/censoring. Finally, based on our above analysis, we performed logistic regression of BTL and cancer stratified by time between BTL measurement and diagnosis/censoring (≤4, 4–8 and >8 years).

All multivariable models adjusted for age at baseline, race, education, BMI, smoking status, pack-years of smoking, and alcohol consumption. For ease of tabular presentation, continuous variables were categorized into tertiles but retained in continuous form for all analyses. We examined the effect of adjusting for white blood cell count and proportion neutrophils, but including these variables did not appreciably affect our results, prompting their exclusion. We also excluded participants missing any data for outcome, BTL, or covariates. Figures were generated using R v3.0.2 and all other analyses used SAS (version 9.3, SAS Institute). We used two-sided tests to compare means and BTL attrition rates, and set a statistical significance threshold of $p = 0.05$.

2.5. Funding Source

This study was funded by the Epidemiology Research and Information Center, U.S. Department of Veterans Affairs; NIEHS R01-ES015172. Additional funding support was provided by the Northwestern University Robert H. Lurie Comprehensive Cancer Center Rosenberg Research Fund. The funding institutions had no role in the study design, data collection or analysis, interpretation of findings, manuscript preparation, or decision to pursue peer-review publication.