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Shorter telomeres associate with a reduced risk of melanoma development

Hongmei Nan^{1,*}, Mengmeng Du^{1,2,*}, Immaculata De Vivo^{1,2}, JoAnn E. Manson^{1,2,3}, Simin Liu, MD⁵, Anne McTiernan⁶, J. David Curb⁷, Lawrence S. Lessin⁸, Matthew R. Bonner⁹, Qun Guo¹, Abrar A. Qureshi^{1,4}, David J. Hunter^{1,2}, and Jiali Han^{1,2,4,†}

¹Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

²Department of Epidemiology, Harvard School of Public Health, Boston, MA

³Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

⁴Clinical Research Program, Department of Dermatology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

⁵Program on Genomics and Nutrition, Department of Epidemiology, University of California, Los Angeles, School of Public Health, Los Angeles, California

⁶Fred Hutchinson Cancer Research Center, Division of Public Health Sciences, Seattle, WA

⁷Pacific Health Research Institute, Honolulu, Hawaii

⁸Medstar Research Institute and Howard University, Washington, DC

⁹Department of Social and Preventive Medicine, School of Public Health and Health Professions, University of Buffalo, Buffalo, NY

Abstract

Epidemiological studies have linked shortened telomeres with the development of many cancers. However, recent studies have suggested that longer telomeres may lead to prolonged senescence in melanocytes, providing increased opportunity for malignant transformation. We therefore examined whether shorter pre-diagnostically measured relative telomere length in peripheral blood

[†]Correspondence and requests for reprints to: Jiali Han, Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115, U.S.A.; Telephone: 617-525-2098; Fax: 617-525-2008; jiali.han@channing.harvard.edu.

*Hongmei Nan and Mengmeng Du contributed equally to this work.

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AUTHORS' CONTRIBUTIONS

Hongmei Nan: data analysis, literature search, data interpretation and manuscript writing

Mengmeng Du: data collection, literature search, data interpretation and manuscript writing

Immaculata De Vivo: data collection and manuscript revision

JoAnn E. Manson: study design and manuscript writing

Simin Liu: study design and manuscript revision

Anne McTiernan: study design and manuscript revision

J. David Curb: study design and manuscript revision

Lawrence Lessin: study design and manuscript revision

Matthew R. Bonner: study design and manuscript revision

Qun Guo: data analysis and manuscript revision

Abrar A. Qureshi: data interpretation and manuscript revision

David J. Hunter: data interpretation and manuscript revision

Jiali Han: literature search, tables, study design, data collection, data analysis, data interpretation, and manuscript writing

leukocytes (PBLs) was associated with a decreased risk of cutaneous melanoma. Telomere length in prospectively collected PBLs was measured in incident melanoma cases and age-matched controls selected from participants in three large prospective cohorts: the Women's Health Initiative Observational Study (WHI-OS), the Health Professionals Follow-up Study (HPFS), and the Nurses' Health Study (NHS). Shorter telomere lengths were associated with decreased risk of melanoma in each cohort. The p for trend across quartiles was 0.03 in the WHI-OS and 0.008 in the HPFS. When combining these two datasets with published data in the NHS (p for trend, 0.09), compared with individuals in the fourth quartile (the longest telomere lengths), those in the first quartile had an odds ratio of 0.43 (95% CI, 0.28–0.68) (p for trend, 0.0003). Unlike findings for other tumors, shorter telomeres were significantly associated with a decreased risk of melanoma in this study, suggesting a unique role of telomeres in melanoma development.

Keywords

Telomere length; peripheral blood leukocytes; melanoma; skin cancer; prospective study

INTRODUCTION

Telomeres are long hexameric (TTAGGG)_{*n*} repeats capping both ends of linear eukaryotic chromosomes. They play a critical role in maintaining genomic stability by preventing fusion of chromosomal ends, nucleolytic decay, end-to-end fusion, and atypical recombination(1). Human telomeres shorten by 30–200 base pairs after each cycle of mitotic division and have been likened to a “molecular clock” reflecting the number of divisions a cell has undergone(2). Epidemiological studies have observed links between shortened telomere length in peripheral blood leukocytes (PBLs) and the development of many cancers, such as bladder, gastric, and renal cancers(3–6). However, telomere shortening has been implicated in several aspects of tumorigenesis, including senescence, apoptosis, and genomic instability(7–9). Hence, depending on the distinct proliferative features of different cell types, telomere shortening may play roles in both suppressing and facilitating carcinogenesis(10).

Cutaneous melanoma is the most serious form of skin cancer and accounts for 74% of all deaths from skin cancer(11). Melanoma arises from the malignant transformation of melanocytes, which reside in the basal layer of the epidermis(12). Melanocytes are characterized by low levels of proliferation and have a limited capacity to undergo apoptosis(13). Instead, melanocytes are more likely to senesce in response to oncogenic stress, which allows them to remain functional while preventing propagation of the oncogenic mutation(13). Longer telomeres in melanocytes that already have oncogenic mutations (activating BRAF and silenced/deleted p16) may delay senescence, allowing these cells to acquire additional mutations and increasing the probability of malignant transformation. Prolonged senescence leads to the increased formation of nevi(14–15), which are strongly associated with increased risk of melanoma(16–17).

To evaluate whether shorter telomeres are associated with a decreased risk of melanoma, we conducted a prospective study nested within three large cohorts: the Women's Health Initiative Observational Study (WHI-OS), the Health Professionals Follow-up Study (HPFS), and the Nurses' Health Study (NHS), to examine the relation between pre-diagnostically measured telomere lengths in PBLs and risk of incident melanoma.

MATERIALS AND METHODS

Study Population

The study population consisted of 557 melanoma cases and 579 controls selected from individuals who had previously provided a blood sample in three large prospective cohorts: the WHI-OS (233 female cases and 237 female controls), the HPFS (120 male cases and 120 male controls), and the NHS (204 female cases and 222 female controls, published previously (18)). Detailed descriptions of these three cohorts, blood collection protocols, and selection of cases and controls are presented in Supplementary Information. Briefly, we used blood collection time as the baseline for follow-up. Incident melanoma cases and matched controls (on age, ± 1 year) were selected from each cohort for up to 15 years of follow-up in WHI-OS, 13 years in HPFS, and 11 years in NHS. Only pathologically confirmed melanoma cases diagnosed after blood collection were included in this study. All cases and controls were free of previously diagnosed cancer and were of self-reported European ancestry. The study protocol was approved by the Committee on Use of Human Subjects of Brigham and Women's Hospital, Boston, MA in accordance with an assurance filed and approved by the Department of Health and Human Services, and written informed consent was obtained from all participants.

Telomere Length Measurement

Details of the measurement method for relative telomere length are described in Supplementary Information. Briefly, we assessed relative telomere length of PBL DNA using a modified quantitative polymerase chain reaction (PCR) assay, which determines the Telomere repeat copy number to Single-copy gene (*36B4*) copy number (T/S) ratio, a value proportional to the average telomere length(19). All samples for both the telomere and single-copy gene (*36B4*) reactions were performed in triplicate on different plates. In addition to the samples, each 384-well plate contained a 6-point standard curve from 1.25ng to 20ng using pooled buffy coat-derived genomic DNA. The purpose of the standard curve was to assess and compensate for inter-plate variations in PCR efficiency. The slope of the standard curve for both the telomere and *36B4* reactions was -3.65 ± 0.11 , and acceptable linear correlation coefficient (r^2) values for both reactions was 0.99. The T/S ratio ($-dCt$) for each sample was calculated by subtracting the average *36B4* Ct value from the average telomere Ct value. The relative T/S ratio ($-ddCt$) was determined by subtracting the T/S ratio of the 5 ng standard curve point from the T/S ratio of each unknown sample. Relative telomere lengths were reported as the exponentiated relative T/S ratio.

Blinded quality control (QC) samples were interspersed throughout the dataset to assess variability of threshold cycle (Ct) values. These quality controls were duplicated samples. From 11 pairs of duplicate samples, the intraclass correlation coefficient (ICC) for the telomere assay was 0.83 (95% CI, 0.65–0.93), and that for the *36B4* single-gene assay was 0.79 (95% CI, 0.59–0.91).

Statistical Analyses

We examined separately the association between telomere length and melanoma risk in the WHI-OS and the HPFS, followed by combined analyses of the WHI-OS, HPFS, and NHS to increase statistical power. We categorized the participants into quartiles based on the relative telomere length distribution in each cohort-specific control population; the fourth quartile (i.e., those with the longest telomere lengths), served as the reference group. We used conditional logistic regression to calculate odds ratios (OR) and 95% confidence intervals (95% CI). Tests for trend were conducted by assigning the cohort-specific median value of each quartile of telomere length among controls to both cases and controls in each category and modeling it as a continuous variable. Before combining the data, we assessed for

heterogeneity between the results across the three cohorts by conducting a likelihood ratio test comparing nested models with and without interaction terms between cohort (modeled categorically) and telomere length (modeled continuously). For the combined analysis, data were pooled and individuals (cases and controls) in the same quartile of telomere length in each cohort were grouped together. To perform tests for trend in the combined analysis, the weighted median value for each quartile was calculated by weighting cohort-specific medians by the proportion of observations in each cohort, among controls only.

To determine if the association between telomere length and melanoma risk differed by tanning ability, hair color, and mole count, we conducted likelihood ratio tests comparing nested models with and without interaction terms between telomere length and these variables. We modeled tanning ability, hair color and mole count as ordinal variables and telomere length continuously. The *p* values were two-sided; *p* values <0.05 were considered statistically significant. We used SAS Version 9.1 software (SAS Institute Inc, Cary, NC).

RESULTS

Descriptive Characteristics of the Study Population

Basic characteristics of cases and controls in the three cohorts are presented in Table 1. The mean ages at diagnosis of incident melanoma cases in the WHI-OS, HPFS, and NHS were 68.2, 65.3, and 63.6 years, respectively. This was later than the age of diagnosis in the general US population (60 years)(20), and was likely because participants included in this study were near 60 years and free of diagnosed melanoma at the start of follow-up. The childhood tanning ability of cases was less than that of controls across the three cohorts. In the HPFS and NHS, melanoma cases were more likely to have red or blonde hair compared to controls. In addition, melanoma cases tended to have a higher mole count on the arms than controls.

Telomere Length and Risk of Incident Melanoma

The results for the association between telomere length and the risk of melanoma are presented in Table 2. In the separate analyses, shorter telomere lengths were associated with a decreased risk of melanoma in both the WHI-OS (*p* for trend, 0.03) and the HPFS (*p* for trend, 0.008). These results were consistent with the non-significant association previously reported among 204 melanoma cases and 222 controls in the NHS conducted by our group, as shown in Table 2 for the purpose of comparison (*p* for trend, 0.09)(18). The association between telomere length and melanoma risk did not statistically differ across the three cohorts (*p* for interaction, 0.75). In the combined analysis of all three cohorts, compared with individuals in the fourth quartile, those in the first quartile had an OR of 0.43 (95% CI, 0.28–0.68) (*p* for trend, 0.0003). This result remained essentially the same after adjusting for melanoma risk factors (OR=0.43; 95% CI, 0.27–0.70 for first vs. fourth quartile; *p* for trend, 0.0009). To rule out the influence of preclinical disease from cases diagnosed soon after blood collection, we conducted a sensitivity analysis excluding cases diagnosed within the first two years of blood collection, and the results remained similar in 486 cases and 467 controls [OR (95% CI), 0.44 (0.27–0.72) for first vs. fourth quartile]. We did not observe effect modification of the association between telomere length and melanoma risk by tanning ability (*p* for interaction, 0.10), mole count (*p* for interaction, 0.07), or hair color (*p* for interaction, 0.46). In addition, there was no appreciable difference in the results by age at diagnosis (first vs. fourth quartile; OR=0.51 (95% CI, 0.26–0.99) for ≤ 65 years; OR=0.38 (95% CI, 0.20–0.72) for >65 years).

DISCUSSION

The observed positive association between relative telomere lengths and melanoma risk contrasts with the inverse associations previously reported for basal cell carcinoma of the skin and other tumors(3–6, 18). Recently, a germline genetic variant rs401681[C] near telomere reverse transcriptase (*TERT*), a gene responsible for telomere maintenance, was associated with decreased risk of melanoma, but with increased risk of basal cell carcinoma of the skin and other tumors at various sites (lung, bladder, prostate, and cervical cancer) (21–22). Consistent with this finding, our data suggest that the role of telomere biology in melanoma pathogenesis differ from that in other tumors.

One potential mechanism underlying this difference is that shorter telomere lengths protect against the malignant transformation of cells within melanocytic nevi by limiting proliferative capacity and triggering the entry to senescence stage. Recent studies have demonstrated that senescence provides a unique barrier to the progression of melanoma(23). Senescence is tightly controlled, induced by telomere shortening, and appears to be regulated in melanocytes in a manner that differs at least partially from other studied cell types(15, 24).

Melanocytic nevi (moles) are considered a potential precursor of melanoma, and a relatively high number of nevi on the body is a well-known risk factor(17, 25). Supporting the above mechanism and our melanoma association results, a previous study reported a positive correlation between PBL telomere lengths and total body nevus counts in 1,897 Caucasian women aged 18 to 79 years (age-adjusted *p*-value, 0.0001)(26). Consistent with those findings, in our previous study, shorter telomeres in PBLs were associated with fewer moles on the arms in 870 controls (age-adjusted *p*-value, 0.003)(18). Our results on telomere length and melanoma risk remained essentially unchanged after adjusting for mole count, as well as other risk factors, suggesting that telomere shortening may help prevent melanoma development by reducing the malignant transformation of cells within melanocytic nevi, rather than by decreasing nevus count.

Similar to our findings for melanoma, unlike other chronic diseases(3, 27–30), shorter telomeres have been associated with decreased risk of Parkinson's disease. A nested case-control study of 96 cases and 172 age-matched controls within the HPFS suggested that men with shorter telomeres had a lower risk of Parkinson's disease (multivariate adjusted RR for the lowest vs. the highest quartile 0.33; 95% CI: 0.12–0.90)(31). Emerging evidence suggests a potential link between Parkinson's disease and melanoma. Studies have observed a decreased risk of cancer among Parkinson's disease patients, with the important exception of melanoma(32–33). Melanoma and Parkinson's disease share similar host susceptibility risk factors, such as red hair color and family history of melanoma(34–35). Unlike other chronic diseases, cigarette smoking and rotating nightshift work are both associated with a lower risk of both diseases(36–39). Melanocytes are derived from a single group of neural crest cells, and we speculate that dopamine-containing nerve cells and melanocytes may share unique molecular mechanisms that defend against cellular damage and stress.

A potential limitation of this study is that relative telomere lengths were not directly measured in DNA extracted from skin tissue. However, telomere length in PBLs has been significantly correlated with that in skin tissues (r^2 , 0.71; *p*-value, 0.018), indicating that telomere length in PBLs could serve as a surrogate marker(40). As telomere length may uniquely influence progression towards malignancy in different cell types, a similar distribution of telomere lengths may not necessarily reflect similar telomere biology in melanocytes compared with other tissues.

In the current study, PBL relative telomere length was measured using quantitative PCR, a widely used method in epidemiological telomere research. Although this technique does not measure the absolute telomere length, as demonstrated in previous studies, the T/S ratio obtained using quantitative PCR assay is highly reproducible and shows strong correlation with mean telomere restriction fragments as measured by traditional Southern blot, suggesting the reliability and validity of the quantitative PCR-based approach(19, 30, 41).

We identified a significant association between shorter relative telomere length and decreased risk of incident melanoma. Both men and women were included in this study, and this relationship should be generalizable to Caucasian populations. This finding provides novel insights supporting the notion that telomere-induced senescence may uniquely influence melanoma development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

PBLs	Peripheral blood leukocytes
HPFS	Health Professionals Follow-up Study
WHI	Women's Health Initiative
NHS	Nurses' Health Study

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WHI study appendix

SHORT LIST OF WHI INVESTIGATORS

Program Office: (National Heart, Lung, and Blood Institute, Bethesda, Maryland) Jacques Rossouw, Shari Ludlam, Joan McGowan, Leslie Ford, and Nancy Geller.

Clinical Coordinating Center: (Fred Hutchinson Cancer Research Center, Seattle, WA) Ross Prentice, Garnet Anderson, Andrea LaCroix, Charles L. Kooperberg; (Medical Research Labs, Highland Heights, KY) Evan Stein; (University of California at San Francisco, San Francisco, CA) Steven Cummings.

Clinical Centers: (Albert Einstein College of Medicine, Bronx, NY) Sylvia Wassertheil-Smoller; (Baylor College of Medicine, Houston, TX) Haleh Sangi-Haghpeykar; (Brigham and Women's Hospital, Harvard Medical School, Boston, MA) JoAnn E. Manson; (Brown University, Providence, RI) Charles B. Eaton; (Emory University, Atlanta, GA) Lawrence S. Phillips; (Fred Hutchinson Cancer Research Center, Seattle, WA) Shirley Beresford; (George Washington University Medical Center, Washington, DC) Lisa Martin; (Los

Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA) Rowan Chlebowski; (Kaiser Permanente Center for Health Research, Portland, OR) Erin LeBlanc; (Kaiser Permanente Division of Research, Oakland, CA) Bette Caan; (Medical College of Wisconsin, Milwaukee, WI) Jane Morley Kotchen; (MedStar Research Institute/Howard University, Washington, DC) Barbara V. Howard; (Northwestern University, Chicago/Evanston, IL) Linda Van Horn; (Rush Medical Center, Chicago, IL) Henry Black; (Stanford Prevention Research Center, Stanford, CA) Marcia L. Stefanick; (State University of New York at Stony Brook, Stony Brook, NY) Dorothy Lane; (The Ohio State University, Columbus, OH) Rebecca Jackson; (University of Alabama at Birmingham, Birmingham, AL) Cora E. Lewis; (University of Arizona, Tucson/Phoenix, AZ) Cynthia A. Thomson; (University at Buffalo, Buffalo, NY) Jean Wactawski-Wende; (University of California at Davis, Sacramento, CA) John Robbins; (University of California at Irvine, CA) F. Allan Hubbell; (University of California at Los Angeles, Los Angeles, CA) Lauren Nathan; (University of California at San Diego, LaJolla/Chula Vista, CA) Robert D. Langer; (University of Cincinnati, Cincinnati, OH) Margery Gass; (University of Florida, Gainesville/Jacksonville, FL) Marian Limacher; (University of Hawaii, Honolulu, HI) J. David Curb; (University of Iowa, Iowa City/Davenport, IA) Robert Wallace; (University of Massachusetts/Fallon Clinic, Worcester, MA) Judith Ockene; (University of Medicine and Dentistry of New Jersey, Newark, NJ) Norman Lasser; (University of Miami, Miami, FL) Mary Jo O'Sullivan; (University of Minnesota, Minneapolis, MN) Karen Margolis; (University of Nevada, Reno, NV) Robert Brunner; (University of North Carolina, Chapel Hill, NC) Gerardo Heiss; (University of Pittsburgh, Pittsburgh, PA) Lewis Kuller; (University of Tennessee Health Science Center, Memphis, TN) Karen C. Johnson; (University of Texas Health Science Center, San Antonio, TX) Robert Brzyski; (University of Wisconsin, Madison, WI) Gloria E. Sarto; (Wake Forest University School of Medicine, Winston-Salem, NC) Mara Vitolins; (Wayne State University School of Medicine/Hutzel Hospital, Detroit, MI) Michael S. Simon.

Women's Health Initiative Memory Study: (Wake Forest University School of Medicine, Winston-Salem, NC) Sally Shumaker.

Table 1

Characteristics of melanoma cases and controls in each cohort

Characteristic	WHI-OS (females)		HPFS (males)		NHS (females)	
	Cases (n=233)	Controls (n=237)	Cases (n=120)	Controls (n=120)	Cases (n=204)	Controls (n=222)
Age at diagnosis, mean (SD) ^a	68.2 (7.3)	NA	65.3 (9.5)	NA	63.6 (7.5)	NA
Age at blood draw, mean (SD)	63.8 (6.8)	63.9 (7.0)	59.9 (9.1)	59.9 (9.0)	57.5 (7.1)	57.8 (7.0)
Red or blonde hair color (%) ^b	NA	NA	21.3	12.2	23	11.2
Tanning ability, tan without burn (%)	17.8	26.4	14.9	30.2	17.4	24.8
Mole count on the arms, 3+ (%) ^b	NA	NA	27.7	9.4	23.9	8.8

^aStandard deviation

^bThe information on hair color and mole count was not available in the WHI-OS.

NA: not applicable (age at diagnosis) or not available (hair color and mole count)

Table 2
Odds ratio (OR) and 95% confidence interval (CI) of incident melanoma by relative telomere length

	4 th quartile ^d	3 rd quartile	2 nd quartile	1 st quartile	<i>p</i> for trend
WHI-OS (females)					
Cases (%)	58 (24.9)	76 (32.6)	60 (25.8)	39 (16.7)	
Controls (%)	59 (25.0)	59 (25.0)	60 (25.0)	59 (25.0)	
OR (95% CI)	1.00	1.14 (0.62–2.11)	0.77 (0.39–1.53)	0.40 (0.18–0.88)	0.03
HPFS (males)					
Cases (%)	41 (34.2)	31 (25.8)	28 (23.3)	20 (16.7)	
Controls (%)	28 (23.3)	33 (27.5)	27 (22.5)	32 (26.7)	
OR (95% CI)	1.00	0.49 (0.21–1.12)	0.47 (0.20–1.12)	0.18 (0.06–0.56)	0.008
NHS^c (females)					
Cases (%)	63 (30.9)	49 (24.0)	44 (21.6)	48 (23.5)	
Controls (%)	55 (24.8)	54 (24.3)	58 (26.1)	55 (24.8)	
OR (95% CI)	1.00	0.76 (0.43–1.34)	0.54 (0.29–1.01)	0.59 (0.31–1.13)	0.09
Combined study of WHI-OS, HPFS, and NHS					
Cases (%)	162 (29.1)	156 (28.0)	132 (23.7)	107 (19.2)	
Controls (%)	142 (24.5)	146 (25.2)	145 (25.0)	146 (25.2)	
OR (95% CI)	1.00	0.84 (0.58–1.21)	0.62 (0.41–0.93)	0.43 (0.28–0.68)	0.0003
Multivariate OR (95% CI) ^b	1.00	0.84 (0.57–1.24)	0.60 (0.39–0.94)	0.43 (0.27–0.70)	0.0009

^d Longest relative telomere length group

^b Conditional logistic regression adjusting for hair color, tanning ability, and the number of moles on arms

^c NHS results were published previously (18).