Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

Research Article

Cancer Prevention Research

DNA Methylation of Telomere-Related Genes and Cancer Risk

Brian T. Joyce[1], Yinan Zheng[1], Drew Nannini[1], Zhou Zhang[1], Lei Liu[2], Tao Gao[1], Masha Kocherginsky[3], Robert Murphy[4], Hushan Yang[5], Chad J. Achenbach[6], Lewis R. Roberts[7], Mirjam Hoxha[8], Jincheng Shen[9], Pantel Vokonas[10,11], Joel Schwartz[12], Andrea Baccarelli[13], and Lifang Hou[1]

Abstract

Researchers hypothesized that telomere shortening facilitates carcinogenesis. Previous studies found inconsistent associations between blood leukocyte telomere length (LTL) and cancer. Epigenetic reprogramming of telomere maintenance mechanisms may help explain this inconsistency. We examined associations between DNA methylation in telomere-related genes (TRG) and cancer. We analyzed 475 participants providing 889 samples 1 to 3 times (median follow-up, 10.1 years) from 1999 to 2013 in the Normative Aging Study. All participants were cancer-free at each visit and blood leukocytes profiled using the Illumina 450K array. Of 121 participants who developed cancer, 34 had prostate cancer, 10 melanoma, 34 unknown skin malignancies, and 43 another cancer. We examined 2,651 CpGs from 80 TRGs and applied a combination of Cox and mixed models to identify CpGs prospectively associated with cancer (at FDR < 0.05). We also explored trajectories of DNA methylation, logistic regression stratified by time to diagnosis/censoring, and cross-sectional models of LTL at first blood draw. We identified 30 CpGs on 23 TRGs whose methylation was positively associated with cancer incidence ($\beta = 1.0–6.93$) and one protective CpG in *MAD1L1* ($\beta = -0.65$), of which 87% were located in TRG promoters. Methylation trajectories of 21 CpGs increased in cancer cases relative to controls; at 4 to 8 years prediagnosis/censoring, 17 CpGs were positively associated with cancer. Three CpGs were cross-sectionally associated with LTL. TRG methylation may be a mechanism through which LTL dynamics reflect cancer risk. Future research should confirm these findings and explore potential mechanisms underlying these findings, including telomere maintenance and DNA repair dysfunction. *Cancer Prev Res; 11(8); 511–22. ©2018 AACR.*

Introduction

Telomeres are tandem TTAGGG nucleotide repeats that "cap" the ends of eukaryotic chromosomes and serve to maintain genomic stability and limit cellular proliferation (1). Blood leukocyte telomere length (LTL) shortens with age, and this process can be accelerated by exposure to environmental risk factors (in particular those known to cause oxidative stress and/or chronic inflammation, two major carcinogenic pathways; ref. 2). Prior studies demonstrated that LTL shortening may reflect *in situ* changes in telomere length among precancerous and cancerous cells (2) and that cellular senescence induced by critical telomere shortening and the Hayflick limit is generally thought to be a tumor-suppressive process, which cancer cells must overcome early in carcinogenesis (3). However, the exact role of LTL in cancer development remains uncertain. There are numerous studies reporting associations between LTL and cancer risk (2), with largely inconsistent results. These inconsistencies may be due to

[1]Center for Population Epigenetics, Robert H. Lurie Comprehensive Cancer Center and Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois. [2]Division of Biostatistics, Washington University in St. Louis, St. Louis, Missouri. [3]Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois. [4]Center for Global Health, Feinberg School of Medicine, Northwestern University, Chicago, Illinois. [5]Division of Population Science, Department of Medical Oncology, Sidney Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania. [6]Department of Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois. [7]Division of Gastroenterology and Hepatology, Department of Medicine, Mayo Clinic, Rochester, Minnesota. [8]Molecular Epidemiology and Environmental Epigenetics Laboratory, Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy. [9]Department of Population Health Sciences, University of Utah School of Medicine, Salt Lake City, Utah. [10]VA Normative Aging Study, VA Boston Healthcare System, Boston, Massachusetts. [11]Department of Medicine, Boston University School of Medicine, Boston, Massachusetts. [12]Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts. [13]Department of Environmental Health Science, Mailman School of Public Health, Columbia University, New York, New York.

Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

Corresponding Author: Brian T. Joyce, Northwestern University, 680 N. Lake Shore Drive, Suite 1400, Chicago, IL 60611. Phone: 312-503-5407; Fax: 312-908-9588; E-mail: b-joyce@northwestern.edu

doi: 10.1158/1940-6207.CAPR-17-0413

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

differences in study design (e.g., variations in time between LTL measurement and cancer diagnosis) and relatively sparse data from prospective observational studies. Our recent prospective study found that incident cancer cases experienced accelerated LTL shortening until around 4 years prior to diagnosis, at which point their LTL stabilized relative to controls (4), suggesting a dynamic relationship between LTL and cancer development.

The underlying regulatory mechanisms responsible for the telomere shortening-lengthening balance and its related cancer risk are only partially understood at present. A previous study of genetic mutations in telomere-related genes (TRG) found limited associations with LTL (5). This may be because of the low genetic variability of these genes in human populations (6). Conversely, a genome-wide meta-analysis identified loci at TRGs associated with both LTL and cancer (7). One possible alternative to a genetic mechanism is epigenetic control of TRGs. In human studies, LTL has been associated with DNA methylation in subtelomeric regions and selected loci within TRGs (8) and repetitive elements Alu and LINE-1 (surrogates for global methylation; ref. 9). The rate of telomere shortening over time was also associated with LINE-1 methylation, suggesting a time-dependent association between DNA methylation and telomere length (9).

However, to our knowledge no prior population-based studies have examined DNA methylation of TRGs in relation to LTL dynamics and cancer risk, particularly in a prospective, longitudinal setting. In light of our prior finding of the shift from accelerated telomere shortening to telomere stabilization prior to cancer diagnosis (4), a prospective examination of epigenetic changes in TRGs may shed light on the involvement of LTL dynamics in carcinogenesis. Thus, our primary objective is to assess whether blood DNA methylation in TRGs is prospectively associated with cancer risk. Our secondary objective is to explore whether DNA methylation of cancer-associated CpG sites on TRGs is associated with LTL.

Materials and Methods

Study population

The Normative Aging Study (NAS) was established in 1963 by the U.S. Department of Veterans Affairs to assess the determinants of healthy aging in an initial cohort of 2,280 men. Eligibility criteria included being between the ages of 21 and 80, veteran status, living in the Boston area, and having no history of chronic health conditions (cardiovascular disease, cancer, etc.). Participants returned for clinical examinations every 3 to 5 years, and starting in 1999, these examinations included a 7-mL blood draw for genetic and epigenetic analysis. From enrollment to 1999, 981 participants died and 470 were lost to follow-up (primarily by moving away from the Boston area); descriptive analysis previously found no differences in characteristics between either of these subgroups and the 829 participants remaining as of 1999 (4).

Between January 1, 1999, and December 31, 2013, 802 of 829 (96.7%) active participants consented to blood donation (median follow-up time, 10.1 years). Of these, 686 were randomly selected for whole-epigenome profiling using the Illumina Infinium HumanMethylation450 BeadChip array, and 491 were cancer-free at the time of their first methylation measurement. To minimize confounding due to genetic ancestry, we excluded 16 participants of non-white race, leaving 889 observations of 475 participants for analysis. In total, 157 (33%) participants had data from one blood draw, 222 (47%) participants from two blood draws, and 96 (20%) subjects from three blood draws. Among this final set, 121 cases developed cancer (34 prostate, 34 unspecified skin malignancies, 10 melanomas, 8 lung, 5 bladder, 4 colorectal, 26 others) and 354 participants remained cancer-free for our entire follow-up. Information on medical history obtained from questionnaires was confirmed via blinded medical record review and included cancer diagnoses and comorbidities.

We identified TRGs using a PubMed literature search for genes linked to telomere maintenance, elongation, and repair (5–7, 10–29). This resulted in 80 TRGs (Table 1) containing 2,651 CpG sites available in our dataset, which we list with accompanying annotation information (and mean/SD methylation at the first blood draw) in

Table 1. Number of CpGs in each gene of interest by pathway

Helicase		Repair		Other	
BLM	17	*ATM*	59	*ACYP2*	26
DDX1	10	*BTBD12*	28	*BHMT*	15
DDX11	19	*DCLRE1C*	20	*BICD1*	29
PIF1	15	*DDB1*	17	*C17orf68*	21
RECQL	14	*FEN1*	25	*CLPTM1L*	53
RECQL4	19	*HMBOX1*	24	*CXCR4*	26
RECQL5	54	*MRE11A*	21	*DCAF4*	24
WRN	41	*MSH2*	14	*DCLRE1B*	16
Shelterin		*NBN*	10	*EHMT2*	177
ACD	31	*PARP3*	19	*MAD1L1*	731
POT1	15	*PCNA*	26	*MCM4*	14
RAP1A	17	*PML*	31	*MEN1*	24
TERF1	12	*RAD50*	14	*MPHOSPH6*	15
TERF2	15	*RAD51*	18	*MTR*	22
TERF2IP	17	*RAD51AP1*	16	*MTRR*	20
TINF2	15	*RAD51C*	15	*MYC*	37
Telomerase		*RAD51L1*	78	*NAF1*	18
DKC1	22	*RAD51L3*	15	*OBFC1*	18
GAR1	14	*RAD54L*	13	*PARP1*	19
NHP2	19	*SIRT1*	17	*PARP2*	9
NOP10	11	*SIRT6*	17	*PIK3C3*	11
TEP1	13	*SMC5*	9	*PINX1*	30
TERC	9	*SMC6*	16	*PRKDC*	37
TERT	100	*TP53BP1*	30	*PRMT8*	35
WRAP53	34	*XRCC6*	20	*PXK*	21
				RTEL1	33
				SIP1	9
				TNKS	28
				TNKS2	16
				UCP2	13
				ZNF208	9
				ZNF676	1

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

Supplementary Table S1. For ease of presentation, we also classified genes [based on Mirabello and colleagues' work (5) or literature review and GeneCard search] into one of five telomere-related pathways: Helicase, Shelterin, Telomerase, Repair, or Other.

Telomere measurement

Laboratory methods for measuring LTL in the NAS have been described previously (4). In brief, LTL was measured using quantitative qPCR. Relative LTL was measured by taking 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 LTL and reference pooled DNA LTL. The latter was created using DNA from 475 participants (400 ng/sample) and used to generate a fresh standard curve from 0.25 to 20 ng/μL in every T and S qPCR run. All samples were run 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 5.6%. When the coefficient of variation for the T or S reactions was higher than 15%, the measurement was repeated.

DNA methylation measurement

Buffy coat DNA was isolated from each sample via the QIAamp DNA Blood Kit (QIAGEN) and a 0.5 μg aliquot was bisulfite converted with the EZ-96 DNA Methylation Kit (Zymo Research). In the NAS, this was done on blood collected between 1999 and 2007. DNA methylation was subsequently detected by the Infinium HumanMethylation450 BeadChip platform (Northwestern University, Feinberg School of Medicine, Center for Genetic Medicine, Chicago, IL). Technical effects due to the plate/chip were minimized by utilizing a two-stage age-stratified algorithm to randomize the samples, thereby ensuring comparable age distribution across plates/chips.

Quality control samples consisted of replicate pairs and a single sample that was run within and between plates/chips to help detect batch effects. Analytic plates were run consecutively, by the same technician, and processed and read on the same scanner. Quality control approaches also included the detection and removal of 15 DNA samples and 949 probes via the pfilter command in the Bioconductor wateRmelon package, which excluded DNA samples containing >1% of probes with detection *P* values >0.05 and probes having >1% of samples with detection *P* value >0.05 (after omitting samples excluded above). Furthermore, we also excluded probes with specific design and/or annotation, namely 65 with genotyping function, 3,091 used for detecting CpH methylation, and 3,688 containing an SNP in the last 10 bases with a minor allele frequency greater than 0.01 in the CEU reference set. A number of these probes were already excluded by the pfilter command, so after these steps, we finally obtained 477,927 probes (i.e., ∼98.4% out of 485,512), which were used to obtain DNA methylation. Finally, we applied a 3-part, preprocessing pipeline to our data: (i) background correction via the out-of-band (noob) method by Triche and colleagues (30); (ii) dye-bias adjustment by the Bioconductor methylumi package (31); and (iii) probe-type correction with BMIQ according to Teschendorff and colleagues (2013; ref. 32), as provided by wateRmelon (33).

Statistical analysis

For descriptive analyses, we performed χ^2 or Kruskal–Wallis tests to assess differences in participant characteristics at the first methylation measurement by cancer status (patients who would later develop cancer during the study period vs. those who remained cancer-free throughout). We next used a joint model under the shared random effects model framework (reduced method by Liu and Hang; ref. 34) to combine our repeated methylation measures (linear mixed model) and time to cancer diagnosis data (Cox proportional hazards model) and to examine associations between cancer incidence and DNA methylation of all 2,651 CpG sites of interest.

This method was designed as an extension of the shared random effects model and uses a Gaussian quadrature technique with a piecewise constant baseline hazard to approximate the baseline hazard in a Cox model, while incorporating repeated measures as with a mixed model. A traditional approach to evaluating longitudinal biomarkers with time to event data is to use observed values as a time-varying covariate in a Cox proportional hazards model. However, this requires a complete set of repeated measures in a time-continuous process, whereas in reality, our biomarkers of interest are measured only at discrete time points, generally not including the time of event occurrence (35). Although the value of the biomarker at event time can be obtained by, for example, last observation carried forward (LOCF), this practice could be crude and lead to inappropriate inferences, especially when the time interval between biomarker measurement and disease outcome is long (35). Furthermore patient survival to event occurrence might depend on the "underlying true" (or expected) values of biomarkers, rather than the observed values with measurement errors; in this situation, a traditional model would be biased toward the null (35).

Thus, we used a joint model of longitudinal biomarkers and survival. Our model accounts for selection bias by the random effects shared between the mixed model of methylation markers and survival model for time to event. Rather than LOCF, the missing methylation measures at the event time can be imputed by empirical Bayes estimate (posterior expected value of random effects conditional on the observed data) from the mixed model, based on the observed history of individuals who did not have an event up to that time. Also, the "underlying true" (expected) biomarkers, rather than the observed values accompanying

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

measurement errors, are incorporated in the survival model, which address the "biased toward the null" concern. This model is designed to maximize statistical power and minimize bias in the analysis of correlated repeated measures (e.g., DNA methylation data) with time to an event as the outcome, without making assumptions regarding the data structure or missingness. The model failed to converge for 37 of the 2,651 CpG sites (1.4%), which were excluded from analysis. We used the Benjamini–Hochberg FDR to correct for all of the remaining 2,614 tests and report CpG sites with FDR <0.05 as significant. We conducted a sensitivity analysis of all significant CpG sites with the 34 unspecified skin malignancies excluded, and examined unadjusted Pearson correlations between all significant CpG sites.

For subsequent analysis of these cancer-associated loci, we used linear mixed-effects models to compare age-adjusted DNA methylation trajectory by cancer status to examine the DNA methylation at the significant loci over time. We tested significance in these analyses via an interaction term between years to diagnosis/censoring and cancer status (0 = cancer-free for the entire follow-up, 1 = cases diagnosed during follow-up) for each CpG site, and also report differences by each year to diagnosis/censoring. Next, we compared our prior results showing that LTL was associated with cancer incidence when measured up to 4 years prediagnosis (4). To do this, we performed logistic regressions of cancer status on methylation at each CpG site by each interval between blood sample collection and diagnosis/censoring (0–4 years, 4–8 years, and >8 years). All methylation values were standardized to have a standard deviation equal to 1 for this analysis. For participants with multiple observations within the same stratum, we used the first observation from each subject only. We also explored cross-sectional associations between methylation at each of these significant CpG sites and LTL, both measured at the first blood draw only and restricted to subjects who were cancer-free for the entire follow-up to minimize potential confounding by age- and cancer-related factors. All of the above analyses were conducted using SAS v. 9.4 (SAS Institute) and adjusted for age, BMI, education, smoking status and pack-years, alcohol consumption, blood cell type abundances (CD8, CD4, natural killer, B cells, and monocytes; ref. 36), and five principal components (previously calculated to represent 95% of DNA processing batch effects), all based on our prior work studying DNA methylation in this cohort (37).

Bioinformatic analysis

Finally, we performed a regulatory enrichment analysis of the 31 cancer-associated CpG sites using R v. 3.4.0. We used DNase I hypersensitivity sites (DNase), transcription factor–binding sites (TFBS), and annotations of histone modification ChIP peaks pooled across cell lines (data available in the ENCODE Analysis Hub at the European Bioinformatics Institute). For each regulatory element, we then calculated the number of overlapping CpGs among the 31 significant CpGs (observed) and 10,000 sets of randomly selected CpGs across the genome (expected). We calculated the ratio of observed to mean expected as the enrichment fold and obtained an empirical *P* value from the distribution of the expected in the background.

Results

Table 2 shows the characteristics of all participants at the first blood draw by cancer status. Briefly, participants who were cancer-free for the full follow-up were slightly older than those who later developed cancer. Our descriptive analysis identified no other significant differences in participant characteristics across cancer status. Table 3 shows the results of the joint model, with 31 CpG sites on 23 TRGs associated with cancer incidence at FDR <0.05. These included CpGs on two genes in each of the Helicase (*PIF1*, *RECQL4*), Shelterin (*ACD*, *TINF2*), and Telomerase (*DKC1*, *WRAP53*) pathways; 10 DNA repair genes linked to telomeres (*BTBD12*, *DCLRE1C*, *DDB1*, *FEN1*, *HMBOX1*, *MSH2*, *PARP3*, *RAD51L3*, *RAD54L*, and *SIRT6*); and seven other TRGs (*BICD1*, *CLPTM1L*, *MAD1L1*, *MTRR*, *MYC*, *RTEL1*, and *SIP1*). Four TRGs had multiple significant hits: *DDB1* (five CpGs), *DCLRE1C* (three CpGs), *PARP3* (two CpGs), and *MAD1L1* (two CpGs). Of the 31 significant CpG sites, 27 (87%) were located in gene promoter regions, three (10%) were located in the 5′UTR, and one (3%) in the gene body. Similarly, 27 of 31 (87%) significant CpG sites were located in CpG islands, two (7%) were located in open sea regions, and one each in north (3%) and south (3%) shores. Supplementary Table S2 shows the results of our sensitivity analysis with unspecified skin malignancies excluded. Supplementary Figure S1 plots the beta coefficients by genomic location for all CpGs on a given gene, for six genes of interest (*TINF2*, *PIF1*, *DDB1*, *DKC1*, *PARP3*, and *MYC*; figures for other genes

Table 2. Characteristics of study participants at first blood draw by cancer status

	Cancer-free Mean ± SD/*n* (%)	Incident cancer Mean ± SD/*n* (%)	*P*
N	354	121	
Age, years	72.2 ± 6.9	70.6 ± 6.4	0.02[a]
BMI, kg/m^2	28.3±4.2	28.2 ± 4.0	0.85
Smoking status			
Never	94 (26.6%)	37 (30.6%)	0.68
Current	17 (4.8%)	6 (5.0%)	
Former	243 (68.6%)	78 (64.5%)	
Pack-years of smoking	20.5 ± 24.2	21.7 ± 24.3	0.76
Average alcohol consumption			
0–1 drinks/day	288 (81.4%)	102 (84.3%)	0.47
2+ drinks/day	66 (18.6%)	19 (15.7%)	
Education, years			
High School grad or less	105 (29.1%)	24 (19.8%)	0.22
Some college/college grad	168 (47.5%)	66 (54.6%)	
Any professional/graduate school	83 (23.5%)	31 (25.6%)	
Leukocyte telomere length	1.29 ± 0.49	1.32 ± 0.51	0.71

[a]Statistically significant at $P < 0.05$.

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

Table 3. Cancer-associated CpGs in TRGs by pathway at FDR < 0.05

Pathway	Gene	CpG	Region	Island	β[a]	95% CI	FDR
Helicase	*PIF1*	cg11013726	5′UTR	Island	1.00	0.57–1.43	0.02
	RECQL4	cg17368874	TSS200	Island	6.67	3.13–10.20	0.04
Shelterin	*ACD*	cg04265926	TSS1500	Island	6.67	3.09–10.25	0.04
	TINF2	cg02271180	1stExon	Island	1.99	0.88–3.10	0.05
Telomerase	*DKC1*	cg19944582	TSS200	Island	5.80	2.74–8.85	0.04
	WRAP53	cg25053252	TSS1500	Island	5.47	2.82–8.12	0.03
Repair	*BTBD12*	cg04157159	TSS200	Island	4.25	1.92–6.58	0.04
	DCLRE1C	cg14369264	TSS1500	Island	1.11	0.53–1.69	0.04
	DCLRE1C	cg24866702	TSS200	Island	6.53	3.12–9.95	0.04
	DCLRE1C	cg04785461	TSS200	Island	5.38	2.37–8.40	0.05
	DDB1	cg23053918	1stExon	Island	5.45	2.75–8.15	0.03
	DDB1	cg20772347	TSS200	Island	5.68	2.65–8.72	0.04
	DDB1	cg24840365	TSS200	Island	5.49	2.55–8.43	0.04
	DDB1	cg25530631	1stExon	Island	6.63	3.04–10.22	0.04
	DDB1	cg08724919	1stExon	Island	1.45	0.64–2.26	0.05
	FEN1	cg25628257	TSS200	Island	3.95	2.03–5.87	0.03
	HMBOX1	cg14143435	TSS200	N_Shore	1.41	0.73–2.10	0.03
	MSH2	cg00547758	5′UTR	Island	6.23	2.97–9.48	0.04
	PARP3	cg14974841	TSS1500	Island	5.22	2.49–7.95	0.04
	PARP3	cg14262432	TSS200	Island	6.93	3.00–10.86	0.05
	RAD51L3	cg19223675	TSS200	S_Shore	5.16	2.27–8.05	0.05
	RAD54L	cg24955114	TSS1500	OpenSea	6.16	2.67–9.66	0.05
	SIRT6	cg15034464	5′UTR	Island	5.11	2.78–7.44	0.03
Other	*BICD1*	cg21587861	TSS200	Island	6.93	3.12–10.75	0.04
	CLPTM1L	cg19739264	1stExon	Island	4.89	2.37–7.41	0.04
	MAD1L1	cg09776772	Body	OpenSea	-0.65	−0.97 to −0.33	0.03
	MAD1L1	cg13247668	TSS200	Island	5.00	2.30–7.71	0.04
	MTRR	cg26627933	1stExon	Island	5.45	2.73–8.16	0.03
	MYC	cg07871324	TSS1500	Island	5.81	2.91–8.71	0.03
	RTEL1	cg27236539	TSS200	Island	1.25	0.56–1.94	0.04
	SIP1	cg15533434	TSS200	Island	5.28	2.48–8.09	0.04

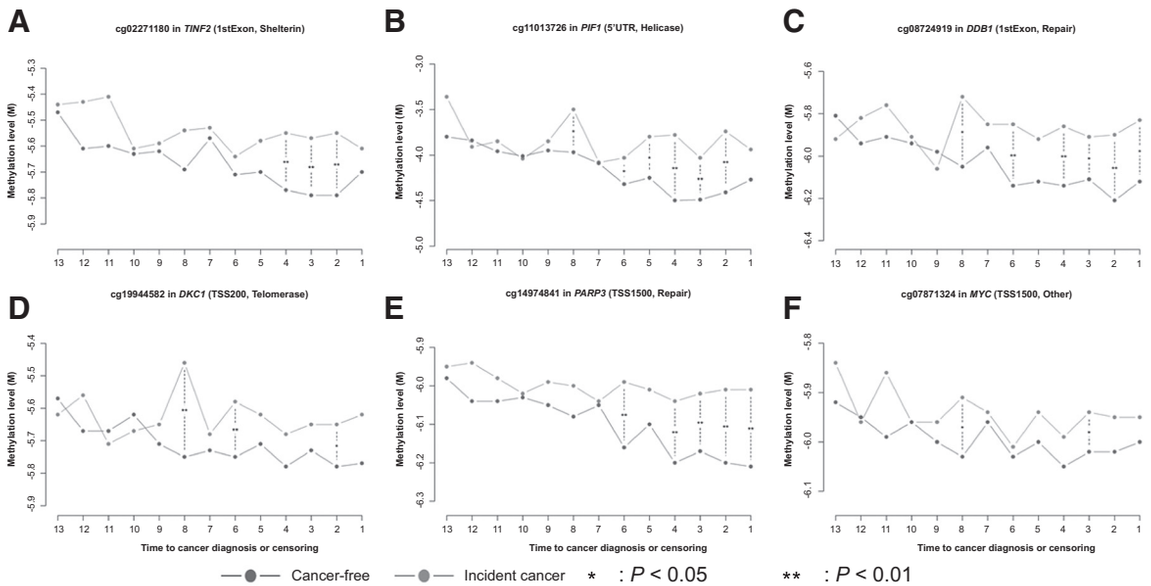
[a]Beta coefficients represent the average difference in methylation (M-value) between cases and controls.

available upon request). Generally speaking, CpGs on the same gene as our primary findings tended to be associated with cancer incidence in the same direction as the primary finding, albeit not to the same degree of statistical significance. In the unadjusted correlation analysis, methylation of significant CpG sites tended to be significantly correlated with one another (0.3–0.6 for almost all CpG sites; data available upon request) despite their disparate locations in the genome.

For the trajectory analyses, overall we found significant differences in methylation over time by cancer status in 21 of 31 cancer-associated CpG sites including both of the CpGs in each of the Helicase, Shelterin, and Telomerase pathways (Supplementary Table S3). Figure 1 plots the trajectory analyses of DNA methylation over time at select noteworthy CpG sites by cancer status. CpGs on *TINF2* (an important telomere-regulating gene) as well as *PIF1* (in the Helicase pathway) and the DNA repair genes *DDB1* and *PARP3* (Fig. 1A–C and E, respectively) showed strong trends with higher methylation in subjects developing cancer, generally beginning around 4 to 6 years prediagnosis/censoring. Conversely, CpGs on *DKC1* in the Telomerase pathway as well as *MYC* (Fig. 1D and F, respectively) showed few differences between cancer cases and cancer-free subjects, and no clear temporal trend. For many CpG sites, methylation trajectories between cancer cases and cancer-free participants began to diverge as early as 6 to 8 years prior to diagnosis/censoring, with clear trends visible for most cancer-CpG sites beginning 4 years prediagnosis (see Supplementary Fig. S2 for corresponding figures with 95% CIs added; Supplementary Fig. S3 contains figures for the remaining 25 CpG sites).

Table 4 shows the results of the logistic regression analysis of DNA methylation and later cancer status at 0 to 4 and 4 to 8 years prediagnosis/censoring. In the stratum of 0 to 4 years prediagnosis/censoring, we found 11 CpG sites associated with cancer incidence: one CpG in each of the Shelterin (*TINF2*) and Telomerase (*WRAP53*) pathways, one CpG on each of three TRGs in the DNA Repair pathway (*PARP3*, *FEN1*, and *SIRT6*) and four CpGs on a fourth (*DDB1*), and one CpG on each of *CLPTM1L* and *MAD1L1*. In the stratum of 4 to 8 years prediagnosis/censoring, we found 17 CpG sites associated with cancer incidence: one CpG in each of the Helicase (*RECQL4*), Shelterin (*ACD*), and Telomerase (*DKC1*) pathways; eight CpGs on six TRGs in the DNA repair pathway (*DCLRE1C*, *DDB1*, *MSH2*, *PARP3*, *RAD51L3*, and *SIRT6*); and one CpG on each of *BICD1*, *CLPTM1L*, *MAD1L1*, *MTRR*, *RTEL1*, and *SIP1*. Methylation at four CpG sites (on *CLPTM1L*, *DDB1*, *PARP3*, and *SIRT6*) was associated with incident cancer in both time strata. Supplementary Table S4 shows the logistic regression results in samples collected more than 8 years prediagnosis/censoring; one CpG on *PARP3* was associated with cancer incidence.

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413



Note: Supplementary Table S2 shows corresponding tabular results; Supplementary fig. S2 shows results with 95% confidence intervals.

Figure 1.
DNA methylation by years to cancer diagnosis/censoring and cancer status for select CpG sites.

Table 4. Logistic regression results stratified by time interval between blood draw and diagnosis/censoring

			0–<4 years		4–<8 years	
Number of observations			208		171	
CpG	Gene	Pathway	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*
cg11013726	*PIF1*	Helicase	1.21 (0.73–2.02)	0.46	1.14 (0.72–1.78)	0.58
cg17368874	*RECQL4*	Helicase	1.38 (0.84–2.24)	0.20	1.67 (1.04–2.69)	0.03[b]
cg04265926	*ACD*	Shelterin	1.13 (0.70–1.81)	0.62	2.18 (1.40–3.41)	<0.01[b]
cg02271180	*TINF2*	Shelterin	1.82 (1.09–3.02)	0.02[b]	1.32 (0.84–2.06)	0.22
cg19944582	*DKC1*	Telomerase	1.35 (0.87–2.11)	0.18	2.43 (1.44–4.09)	<0.01[b]
cg25053252	*WRAP53*	Telomerase	2.04 (1.27–3.28)	<0.01[b]	1.56 (0.96–2.54)	0.07
cg04157159	*BTBD12*	Repair	1.03 (0.62–1.70)	0.91	1.45 (0.95–2.20)	0.08
cg04785461	*DCLRE1C*	Repair	0.94 (0.52–1.69)	0.84	1.76 (1.15–2.69)	0.01[b]
cg14369264	DCLRE1C	Repair	1.38 (0.82–2.33)	0.23	2.06 (1.33–3.20)	<0.01[b]
cg24866702	*DCLRE1C*	Repair	1.60 (0.94–2.72)	0.08	2.03 (1.28–3.21)	<0.01[b]
cg08724919	*DDB1*	Repair	1.78 (1.09–2.88)	0.02[b]	1.61 (1.05–2.46)	0.03[b]
cg20772347	*DDB1*	Repair	1.87 (1.12–3.12)	0.02[b]	1.66 (1.00–2.75)	0.05
cg23053918	*DDB1*	Repair	2.23 (1.29–3.86)	<0.01[b]	1.32 (0.88–2.00)	0.18
cg24840365	*DDB1*	Repair	2.16 (1.20–3.86)	0.01[b]	1.10 (0.71–1.71)	0.66
cg25530631	*DDB1*	Repair	1.18 (0.78–1.78)	0.43	1.31 (0.85–1.99)	0.22
cg25628257	*FEN1*	Repair	2.30 (1.31–4.02)	<0.01[b]	1.54 (0.97–2.45)	0.07
cg14143435	*HMBOX1*	Repair	1.47 (0.92–2.33)	0.10	1.45 (0.93–2.25)	0.10
cg00547758	*MSH2*	Repair	1.39 (0.84–2.29)	0.20	1.77 (1.12–2.81)	0.01[b]
cg14262432	*PARP3*	Repair	1.08 (0.70–1.66)	0.73	1.37 (0.82–2.27)	0.23
cg14974841	*PARP3*	Repair	1.61 (1.03–2.53)	0.04[b]	1.60 (1.00–2.56)	0.05[b]
cg19223675	*RAD51L3*	Repair	1.50 (0.94–2.40)	0.09	1.90 (1.20–3.00)	0.01[b]
cg24955114	*RAD54L*	Repair	1.44 (0.95–2.18)	0.09	1.38 (0.80–2.40)	0.25
cg15034464	*SIRT6*	Repair	1.54 (0.92–2.57)	0.10	1.50 (1.01–2.24)	0.05[b]
cg21587861	*BICD1*	Other	1.09 (0.70–1.68)	0.71	2.03 (1.20–3.42)	0.01[b]
cg19739264	*CLPTM1L*	Other	2.09 (1.24–3.53)	0.01[b]	1.67 (1.10–2.54)	0.02[b]
cg09776772	*MAD1L1*	Other	0.54 (0.33–0.87)	0.01[b]	1.01 (0.66–1.54)	0.96
cg13247668	*MAD1L1*	Other	1.66 (0.99–2.77)	0.05	1.98 (1.22–3.21)	0.01[b]
cg26627933	*MTRR*	Other	1.47 (0.97–2.24)	0.07	2.01 (1.23–3.27)	0.01[b]
cg07871324	*MYC*	Other	1.40 (0.90–2.17)	0.14	1.20 (0.78–1.84)	0.40
cg27236539	*RTEL1*	Other	1.38 (0.80–2.38)	0.25	1.61 (1.06–2.45)	0.02[b]
cg15533434	*SIP1*	Other	2.64 (1.48–4.69)	<0.01[b]	2.32 (1.34–4.04)	<0.01[b]

[a]ORs represent the increase in odds of later being diagnosed with cancer for each 1-SD increase in methylation at that CpG site (see Supplementary Table S1 for SD values).
[b]Significant at $P < 0.05$; DNA methylation was standardized to SD = 1 prior to modeling.

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

Table 5. Associations between cancer-associated CpG sites and telomere length at first blood draw ($N = 346$)

CpG	Gene	Pathway	β	95% CI	*P*
cg11013726	*PIF1*	Helicase	0.03	−0.07–0.12	0.56
cg17368874	*RECQL4*	Helicase	0.06	−0.02–0.14	0.14
cg04265926	*ACD*	Shelterin	0.05	−0.10–0.19	0.52
cg02271180	*TINF2*	Shelterin	−0.02	−0.20–0.17	0.87
cg19944582	*DKC1*	Telomerase	−0.13	−0.33–0.08	0.23
cg25053252	*WRAP53*	Telomerase	0.07	−0.20–0.34	0.60
cg04157159	*BTBD12*	Repair	0.02	−0.13–0.18	0.76
cg04785461	*DCLRE1C*	Repair	−0.01	−0.12–0.10	0.88
cg14369264	*DCLRE1C*	Repair	0.04	−0.04–0.13	0.31
cg24866702	*DCLRE1C*	Repair	0.25	0.08–0.41	<0.01[a]
cg08724919	*DDB1*	Repair	0.07	−0.06–0.21	0.29
cg20772347	*DDB1*	Repair	0.14	−0.09–0.37	0.24
cg23053918	*DDB1*	Repair	0.21	−0.11–0.53	0.20
cg24840365	*DDB1*	Repair	0.12	−0.18–0.43	0.43
cg25530631	*DDB1*	Repair	0.23	0.05–0.42	0.01[a]
cg25628257	*FEN1*	Repair	0.06	−0.21–0.34	0.65
cg14143435	*HMBOX1*	Repair	−0.01	−0.22–0.19	0.91
cg00547758	*MSH2*	Repair	0.12	0.01–0.23	0.03[a]
cg14262432	*PARP3*	Repair	0.05	−0.09–0.18	0.49
cg14974841	*PARP3*	Repair	0.12	−0.16–0.40	0.40
cg19223675	*RAD51L3*	Repair	−0.05	−0.28–0.19	0.69
cg24955114	*RAD54L*	Repair	0.10	−0.22–0.42	0.54
cg15034464	*SIRT6*	Repair	0.09	−0.09–0.28	0.33
cg21587861	*BICD1*	Other	−0.12	−0.27–0.03	0.13
cg19739264	*CLPTM1L*	Other	0.01	−0.18–0.21	0.90
cg09776772	*MAD1L1*	Other	0.00	−0.08–0.09	0.94
cg13247668	*MAD1L1*	Other	0.08	−0.17–0.32	0.54
cg26627933	*MTRR*	Other	0.10	−0.23–0.42	0.56
cg07871324	*MYC*	Other	0.06	−0.24–0.35	0.71
cg27236539	*RTEL1*	Other	−0.01	−0.22–0.20	0.96
cg15533434	*SIP1*	Other	0.11	−0.07–0.29	0.23

[a]Significant at $P < 0.05$.

Table 5 shows the results of our cross-sectional models of LTL on DNA methylation. DNA methylation of three CpG sites, all of them on DNA repair genes, was positively associated with LTL at the first blood draw: cg24866702 on *DCLRE1C*, cg00547758 on *MSH2*, and cg25530631 on *DDB1*. We found no other significant associations between DNA methylation of TRGs and LTL. Finally, Supplementary Fig. S4 shows the results of our regulatory element enrichment analysis. Five histone modifications (notably H3K27ac, H3K4me2, H3K4me3, H3K79me2, and H3K9ac) were significantly enriched at the CpG sites significantly associated with cancer (all $P < 0.001$). Supplementary Table S5 contains more detailed tabular findings.

Discussion

To our knowledge, this is the first study to identify DNA methylation changes in TRGs that are prospectively associated with cancer. In this cohort, we identified positive associations between cancer incidence and methylation at 30 CpG sites (and one negative association), most in gene promoter regions, on 23 genes related to telomere maintenance and regulation. Over time, methylation of 21 CpG sites began to diverge by later cancer status several years prior to diagnosis/censoring. In general, cancer cases experienced increased static methylation and cancer-free participants experienced decreased methylation. Furthermore, our logistic regression identified 11 and 17 CpG sites where methylation at 0 to 4 years and 4 to 8 years prediagnosis/censoring, respectively, was associated with cancer incidence (including four CpGs in both strata). Finally, in participants who remained cancer free, at the first blood draw, DNA methylation at three CpG sites was associated with telomere length. Few studies have examined these genes as potential blood-based cancer biomarkers; thus, our findings should be validated in other populations. Nonetheless, these findings suggest mechanisms through which cancer cells may be able to alter telomere homeostasis, possibly as a precursor to clinical disease, thus indicating DNA methylation of TRGs as a potentially useful biomarker of cancer.

We identified methylation of CpG sites (cg19944582 and cg25053252) in the promoters of two genes (*DKC1* and *WRAP53*) involved with telomerase, a well-characterized telomere maintenance pathway, as positively associated with cancer. The two genes involved in the telomerase pathway, *DKC1* and *WRAP53*, jointly promote telomerase expression and telomere maintenance. Mutations of *DKC1* were identified in cancer cells (38), as was promoter hypermethylation of *DKC1* (39). Further evidence suggests that reductions in *DKC1* expression may increase cancer susceptibility through nontelomere mechanisms, such as

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

reduced *p53* expression (40), which may partially explain the lack of association with telomere length in our cross-sectional analysis. *DKC1* downregulation has also been associated with exposure to arsenic, a known carcinogen (41). Similarly, reduced *WRAP53* expression was associated with cancer prognosis (42). Methylation of these genes may thus be involved with cancer risk and/or progression independent of telomere length.

Our study also identified methylation of two CpGs (cg04265926 and cg02271180) in the promoters of two genes (*ACD* and *TINF2*) in the shelterin pathway, another well-characterized telomere maintenance pathway, as positively associated with cancer. Changes in shelterin complex expression have been implicated in a variety of cancer types, including germline mutations in both *ACD* (43) and *TINF2* (44). However, limited evidence exists to support this hypothesis as previous DNA methylation studies of telomerase-associated genes tended to focus on *TERT*. However, studies of *TERT* methylation in blood leukocytes found no associations with cancer (45), concordant with our findings. One possible explanation is that the normally strict regulatory control of *TERT* may be preserved in blood leukocytes even in participants experiencing carcinogenesis, suggesting that future studies of blood DNA methylation should focus on other shelterin complex genes.

Among other DNA repair genes, we identified methylation at multiple loci within the promoters of three genes (*PARP3*, *DCLRE1C*, and *DDB1*) as positively associated with cancer. A prior study of cancer samples found downregulation of both *PARP3* and *DCLRE1C* in cancer cells and was additionally associated with telomerase reactivation (13). Downregulation of *DCLRE1C* has also been associated with chronic exposure to ionizing radiation (46). Furthermore, methylation at one of the significant loci on each of *DCLRE1C* and *DDB1* was also positively associated with telomere length at the first blood draw. Thus, epigenetic repression of these DNA repair genes may be one mechanism through which cancer cells can activate telomere maintenance mechanisms.

In our prior examination of LTL and cancer incidence in this same cohort, we identified cancer-associated accelerated LTL shortening that stabilized starting approximately 4 years prior to diagnosis (4). We observed that higher DNA methylation at CpG sites on 15 TRGs of interest in this study was associated with cancer status 4 to 8 years prior to diagnosis (Table 4). In addition, we observed significantly different methylation trajectories between cancer cases and cancer-free participants in 21 sites on 17 genes. Examples of this divergence can be seen with four CpGs (cg02271180 in *TINF2*, cg11013726 in *PIF1*, cg08724919 in *DDB1*, and cg14974841 in *PARP3*) in Fig. 1. In all of these cases, DNA methylation began to significantly differ between cases and controls beginning at least 4 years prediagnosis/censoring. Finally, three CpGs (cg24866702 on *DCLRE1C*, cg25530631 on *DDB1*, and cg00547758 on *MSH2*) were positively associated with telomere length in our cross-sectional analysis. These findings all occurred prior to (or at the same time as) the shift in LTL change that our previous study observed. Our trajectory analyses suggest that increased DNA methylation at these and other sites may be an early event in the development of cancer, either reflecting constitutive exposures that also increase cancer risk or correlating with DNA methylation changes occurring in cancer cells, that remains detectable for years. Also of note, methylation of both cg24866702 and cg00547758 was associated with both telomere length at the first blood draw and with cancer risk 4 to 8 years prior to diagnosis (but not 0–4 years prior). This suggests that these methylation changes occur prior to our previously observed change in telomere length and may be involved in driving this change via a DNA repair-related mechanism. As studies of the relationship between LTL measured at a single time point and cancer risk remain unclear (47), alterations in DNA methylation of these TRGs may help explain the between-study differences (e.g., differences in the timing of LTL measurement relative to cancer diagnosis). Together, our results suggest that studying DNA methylation in blood leukocytes is promising for future research into the role of dynamic changes in telomere length during cancer development, and that incorporating epigenetic data may help improve the utility of telomere length in blood leukocytes as a cancer biomarker.

Finally, although we lacked gene expression data, we were able to identify enrichment of numerous important regulatory elements in the set of CpGs associated with cancer. These include H2A.Z, TFBS, and DNase, which may all point toward a role of methylation of these CpGs in *cis*-regulatory changes and potential transcriptional activation (consistent with most of the CpGs being located in gene promoter regions). We also identified five activating histones and one repressive histone in association with our set of CpGs at $P < 0.001$. The repressive histone, H3K9me1, has been previously found to have altered levels in some cancers (48). Similarly, the activating histone marker H3K27ac has been found to be dysregulated in cancer (49). Expression levels of some of these histones have previously been associated with DNA methylation (50). Taken together, these findings bolster our conclusion that the identified CpG sites in these important TRGs may affect gene expression.

This study is subject to limitations. Although the longitudinal nature of our study design allowed us to explore aspects of the temporal associations between DNA methylation of TRGs, LTL, and current cancer risk, it remains challenging to accommodate a formal mediation analysis of longitudinal mechanisms. Our conclusions regarding the interplay of DNA methylation and LTL in carcinogenesis over time thus require confirmation in additional prospective studies. In addition, the study population of the NAS is not representative, and thus, more diverse

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

populations should be studied to validate our findings, although there is little reason to believe these mechanisms would substantially vary by gender or by race. Furthermore, the sample sizes of most specific cancer types in the NAS were too small to permit a statistically rigorous exploration of these associations for individual cancers. Although we hypothesized that dysregulation of telomere maintenance mechanisms are a general mechanism affecting many different cancer types, this should also be tested in larger studies. Similarly, the relatively small number of cases coupled with our time stratification limited the sample size for each time stratum analyzed. This may have resulted in false negatives, which may explain some associations that were significant 4 to 8 years prediagnosis but not 0 to 4 years. Alternatively, this discrepancy may reflect a subset of cancer-free subjects who developed cancer after the end of our study period (and were thus effectively misclassified). Validation with a longer follow-up and/or larger study population would be necessary to test these possible explanations. The dearth of significant associations between DNA methylation and LTL in our cross-sectional model may also be a consequence of reduced sample size; the dynamic natures of LTL and methylation (and their potential relationships with one another and with cancer) limited us to a cross-sectional model. Thus, our findings may represent a lack of a biological effect or a lack of statistical power and should be interpreted with caution until they can be validated. Similarly, the NAS dataset lacked gene expression data to provide functional verification of our findings posited above. Future research should verify the relationship between DNA methylation and expression of these specific genes.

Nonetheless, this study provides an important, potentially mechanistic explanation for the dynamic relationship between LTL and cancer that we previously observed. Future studies should confirm and explore these CpG sites and genes as potential early detection biomarkers and therapeutic targets; the strong correlations between most CpG sites in our analysis (despite their disparate locations on the genome) further bolster the possibility of these CpGs collectively making a biomarker in the future. Future research in larger, more diverse populations should focus on examining changes in the DNA methylation of these TRGs in terms of gene expression, LTL, and cancer to further elucidate the temporal sequence of these events and their potential role in mechanisms of carcinogenesis. DNA methylation of TRGs could be an important early event in carcinogenesis and, with appropriate confirmation, could have extremely valuable clinical applications for cancer. These DNA methylation changes in blood leukocytes may have been induced by environmental exposures (pollutants, nutrients, etc.) acting constitutionally; thus, our findings may provide important information on one possible mechanism of action for previously identified carcinogenic exposures. Future research should explore this possibility by examining potential exposure–methylation relationships in the genes we identified. Furthermore, if epigenetic changes in these genes do influence the length of cancer cells' telomeres, therapeutically targeting these changes could theoretically induce cellular senescence in cancer cells and thus provide a new effective, safe, and targeted therapy for cancer. However, for this to happen, future studies will need to validate the epigenetic changes we have identified in blood both in cancer and in normal healthy tissue. Nonetheless, these findings provide important information for future cancer early detection, prevention, and treatment. This may be particularly true in populations with underlying immune dysfunction or chronic inflammation (e.g., chronic HIV infection, autoimmune disorders). Exploring these pathways may also facilitate the use of cancer immunotherapies to correct immune dysfunction and cancer-specific immune responses.

Disclosure of Potential Conflicts of Interest

M. Kocherginsky has provided expert testimony for The University of Chicago. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: B.T. Joyce, Y. Zheng, J. Schwartz, L. Hou
Development of methodology: B.T. Joyce, L. Liu, M. Hoxha, L. Hou
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M. Hoxha, P. Vokonas, J. Schwartz, A. Baccarelli
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B.T. Joyce, Y. Zheng, Z. Zhang, L. Liu, M. Kocherginsky, R. Murphy, J. Shen
Writing, review, and/or revision of the manuscript: B.T. Joyce, Y. Zheng, D. Nannini, Z. Zhang, L. Liu, T. Gao, M. Kocherginsky, R. Murphy, H. Yang, C.J. Achenbach, L.R. Roberts, J. Shen, J. Schwartz, L. Hou
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B.T. Joyce, R. Murphy, J. Shen, P. Vokonas
Study supervision: B.T. Joyce, L. Liu, P. Vokonas, L. Hou

Acknowledgments

The Normative Aging Study is supported by the Epidemiology Research and Information Center of the U.S. Department of Veterans Affairs (NIEHS R01- ES015172) and is a research component of the Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC). L. Hou received additional support from the Northwestern University Robert H. Lurie Comprehensive Cancer Center Rosenberg Research Fund. L. Hou, R. Murphy, and L.R. Roberts also received support from the NCI: 1U54CA221205-01 and D43 TW009575. A. Baccarelli and J. Schwartz received additional support from the National Institute of Environmental Health Sciences; NIEHS R01-ES021733, NIEHS R01-ES015172, NIEHS-R01ES025225, NIEHS P30-ES009089, and NIEHS P30-ES00002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 15, 2017; revised April 3, 2018; accepted May 22, 2018; published first June 12, 2018.

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

References

1. Frias C, Pampalona J, Genesca A, Tusell L. Telomere dysfunction and genome instability. Front Biosci (Landmark Ed) 2012;17: 2181–96.
2. Hou L, Zhang X, Gawron AJ, Liu J. Surrogate tissue telomere length and cancer risk: shorter or longer? Cancer Lett 2012;319: 130–5.
3. Giaimo S, d'Adda di Fagagna F. Is cellular senescence an example of antagonistic pleiotropy? Aging Cell 2012;11:378–83.
4. Hou L, Joyce BT, Gao T, Liu L, Zheng Y, Penedo FJ, et al. Blood telomere length attrition and cancer development in the normative aging study cohort. EBioMedicine 2015;2:591–6.
5. Mirabello L, Yu K, Kraft P, De Vivo I, Hunter DJ, Prescott J, et al. The association of telomere length and genetic variation in telomere biology genes. Hum Mutat 2010;31:1050–8.
6. Mirabello L, Yeager M, Chowdhury S, Qi L, Deng X, Wang Z, et al. Worldwide genetic structure in 37 genes important in telomere biology. Heredity 2012;108:124–33.
7. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, et al. Identification of seven loci affecting mean telomere length and their association with disease. Nat Genet 2013;45:422–7.
8. Buxton JL, Suderman M, Pappas JJ, Borghol N, McArdle W, Blakemore AI, et al. Human leukocyte telomere length is associated with DNA methylation levels in multiple subtelomeric and imprinted loci. Sci Rep 2014;4:4954.
9. Wong JY, De Vivo I, Lin X, Grashow R, Cavallari J, Christiani DC. The association between global DNA methylation and telomere length in a longitudinal study of boilermakers. Genet Epidemiol 2014;38:254–64.
10. Dilley RL, Verma P, Cho NW, Winters HD, Wondisford AR, Greenberg RA. Break-induced telomere synthesis underlies alternative telomere maintenance. Nature 2016;539:54–8.
11. Dimitrova N, Chen YC, Spector DL, de Lange T. 53BP1 promotes non-homologous end joining of telomeres by increasing chromatin mobility. Nature 2008;456:524–8.
12. Feng X, Luo Z, Jiang S, Li F, Han X, Hu Y, et al. The telomere-associated homeobox-containing protein TAH1/HMBOX1 participates in telomere maintenance in ALT cells. J Cell Sci 2013;126 (Pt 17):3982–9.
13. Frias C, Garcia-Aranda C, De Juan C, Moran A, Ortega P, Gomez A, et al. Telomere shortening is associated with poor prognosis and telomerase activity correlates with DNA repair impairment in non-small cell lung cancer. Lung Cancer 2008;60:416–25.
14. Jia G, Su L, Singhal S, Liu X. Emerging roles of SIRT6 on telomere maintenance, DNA repair, metabolism and mammalian aging. Mol Cell Biochem 2012;364:345–50.
15. Karami S, Han Y, Pande M, Cheng I, Rudd J, Pierce BL, et al. Telomere structure and maintenance gene variants and risk of five cancer types. Int J Cancer 2016;139:2655–70.
16. Kim S, Bi X, Czarny-Ratajczak M, Dai J, Welsh DA, Myers L, et al. Telomere maintenance genes SIRT1 and XRCC6 impact age-related decline in telomere length but only SIRT1 is associated with human longevity. Biogerontology 2012;13:119–31.
17. Kim S, Parks CG, Xu Z, Carswell G, DeRoo LA, Sandler DP, et al. Association between genetic variants in DNA and histone methylation and telomere length. PLoS One 2012;7:e40504.
18. Lee JH, Cheng R, Honig LS, Feitosa M, Kammerer CM, Kang MS, et al. Genome wide association and linkage analyses identified three loci-4q25, 17q23.2, and 10q11.21-associated with variation in leukocyte telomere length: the Long Life Family Study. Front Genet 2013;4:310.
19. Levy D, Neuhausen SL, Hunt SC, Kimura M, Hwang SJ, Chen W, et al. Genome-wide association identifies OBFC1 as a locus involved in human leukocyte telomere biology. Proc Natl Acad Sci U S A 2010;107:9293–8.
20. Mangino M, Christiansen L, Stone R, Hunt SC, Horvath K, Eisenberg DT, et al. DCAF4, a novel gene associated with leucocyte telomere length. J Med Genet 2015;52:157–62.
21. Marchesini M, Matocci R, Tasselli L, Cambiaghi V, Orleth A, Furia L, et al. PML is required for telomere stability in non-neoplastic human cells. Oncogene 2016;35:1811–21.
22. Mendez-Bermudez A, Royle NJ. Deficiency in DNA mismatch repair increases the rate of telomere shortening in normal human cells. Hum Mutat 2011;32:939–46.
23. Mirabello L, Richards EG, Duong LM, Yu K, Wang Z, Cawthon R, et al. Telomere length and variation in telomere biology genes in individuals with osteosarcoma. Int J Mol Epidemiol Genet 2011;2:19–29.
24. Pooley KA, Bojesen SE, Weischer M, Nielsen SF, Thompson D, Amin Al Olama A, et al. A genome-wide association scan (GWAS) for mean telomere length within the COGS project: identified loci show little association with hormone-related cancer risk. Hum Mol Genet 2013;22:5056–64.
25. Potts PR, Yu H. The SMC5/6 complex maintains telomere length in ALT cancer cells through SUMOylation of telomere-binding proteins. Nat Struct Mol Biol 2007;14:581–90.
26. Saharia A, Teasley DC, Duxin JP, Dao B, Chiappinelli KB, Stewart SA. FEN1 ensures telomere stability by facilitating replication fork re-initiation. J Biol Chem 2010;285:27057–66.
27. Stern JL, Zyner KG, Pickett HA, Cohen SB, Bryan TM. Telomerase recruitment requires both TCAB1 and Cajal bodies independently. Mol Cell Biol 2012;32:2384–95.
28. Wilson JS, Tejera AM, Castor D, Toth R, Blasco MA, Rouse J. Localization-dependent and -independent roles of SLX4 in regulating telomeres. Cell Rep 2013;4:853–60.
29. Zee RY, Ridker PM, Chasman DI. Genetic variants in eleven telomere-associated genes and the risk of incident cardio/cerebrovascular disease: The Women's Genome Health Study. Clin Chim Acta 2011;412:199–202.
30. Triche TJ Jr, Weisenberger DJ, Van Den Berg D, Laird PW, Siegmund KD. Low-level processing of Illumina Infinium DNA Methylation BeadArrays. Nucleic Acids Res 2013;41:e90.
31. Davis S, Du P, Bilke S, Triche T Jr, Bootwalla M. methylumi: Handle Illumina methylation data. 2015. Available from: https://rdrr.io/bioc/methylumi/.
32. Teschendorff AE, Marabita F, Lechner M, Bartlett T, Tegner J, Gomez-Cabrero D, et al. A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. Bioinformatics 2013; 29:189–96.
33. Pidsley R, CC YW, Volta M, Lunnon K, Mill J, Schalkwyk LC. A data-driven approach to preprocessing Illumina 450K methylation array data. BMC Genomics 2013;14:293.
34. Liu L, Huang X. Joint analysis of correlated repeated measures and recurrent events processes in the presence of death, with application to a study on acquired immune deficiency syndrome. J R Stat Soc Ser C Appl Stat 2009;58:65–81.
35. Liu Y, Liu L. Joint models for longitudinal data and time-to-event occurrence. Routledge International Handbook of Advanced Quantitative Methods in Nursing Research. Taylor and Francis Inc., New York, NY; 2015.
36. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics 2012; 13:86.

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

37. Zheng Y, Joyce BT, Colicino E, Liu L, Zhang W, Dai Q, et al. Blood epigenetic age may predict cancer incidence and mortality. EBioMedicine 2016;5:68–73.
38. Bellodi C, Krasnykh O, Haynes N, Theodoropoulou M, Peng G, Montanaro L, et al. Loss of function of the tumor suppressor DKC1 perturbs p27 translation control and contributes to pituitary tumorigenesis. Cancer Res 2010;70:6026–35.
39. Smith IM, Mithani SK, Mydlarz WK, Chang SS, Califano JA. Inactivation of the tumor suppressor genes causing the hereditary syndromes predisposing to head and neck cancer via promoter hypermethylation in sporadic head and neck cancers. ORL J Otorhinolaryngol Relat Spec 2010;72:44–50.
40. Montanaro L. Dyskerin and cancer: more than telomerase. The defect in mRNA translation helps in explaining how a proliferative defect leads to cancer. J Pathol 2010;222:345–9.
41. Gao J, Roy S, Tong L, Argos M, Jasmine F, Rahaman R, et al. Arsenic exposure, telomere length, and expression of telomere-related genes among Bangladeshi individuals. Environ Res 2015;136:462–9.
42. Zhang H, Wang DW, Adell G, Sun XF. WRAP53 is an independent prognostic factor in rectal cancer- a study of Swedish clinical trial of preoperative radiotherapy in rectal cancer patients. BMC Cancer 2012;12:294.
43. Aoude LG, Pritchard AL, Robles-Espinoza CD, Wadt K, Harland M, Choi J, et al. Nonsense mutations in the shelterin complex genes ACD and TERF2IP in familial melanoma. J Natl Cancer Inst 2015;107:pii:dju408.
44. Hartmann K, Illing A, Leithauser F, Baisantry A, Quintanilla-Martinez L, Rudolph KL. Gene dosage reductions of Trf1 and/or Tin2 induce telomere DNA damage and lymphoma formation in aging mice. Leukemia 2016;30:749–53.
45. Hoxha M, Fabris S, Agnelli L, Bollati V, Cutrona G, Matis S, et al. Relevance of telomere/telomerase system impairment in early stage chronic lymphocytic leukemia. Genes Chromosomes Cancer 2014;53:612–21.
46. Fachin AL, Mello SS, Sandrin-Garcia P, Junta CM, Ghilardi-Netto T, Donadi EA, et al. Gene expression profiles in radiation workers occupationally exposed to ionizing radiation. J Radiat Res 2009;50:61–71.
47. Zhang X, Zhao Q, Zhu W, Liu T, Xie SH, Zhong LX, et al. The association of telomere length in peripheral blood cells with cancer risk: a systematic review and meta-analysis of prospective studies. Cancer Epidemiol Biomarkers Prev 2017;26:1381–90.
48. Ellinger J, Bachmann A, Goke F, Behbahani TE, Baumann C, Heukamp LC, et al. Alterations of global histone H3K9 and H3K27 methylation levels in bladder cancer. Urol Int 2014;93:113–8.
49. Song Y, Li ZX, Liu X, Wang R, Li LW, Zhang Q. The Wnt/beta-catenin and PI3K/Akt signaling pathways promote EMT in gastric cancer by epigenetic regulation via H3 lysine 27 acetylation. Tumour Biol 2017;39:1010428317712617.
50. Okitsu CY, Hsieh CL. DNA methylation dictates histone H3K4 methylation. Mol Cell Biol 2007;27:2746–57.

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

Published OnlineFirst June 12, 2018; DOI: 10.1158/1940-6207.CAPR-17-0413

Cancer Prevention Research

DNA Methylation of Telomere-Related Genes and Cancer Risk

Brian T. Joyce, Yinan Zheng, Drew Nannini, et al.

Cancer Prev Res 2018;11:511-522. Published OnlineFirst June 12, 2018.