Research Article

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Telomere Length and Lung Cancer Mortality among Heavy Smokers

Jennifer A. Doherty[1,2], Laurie Grieshober[1], John R. Houck[2], Matthew J. Barnett[2], Jean De Dieu Tapsoba[2], Mark Thornquist[2], Ching-Yun Wang[2], Gary E. Goodman[2], and Chu Chen[2,3,4]

Abstract

Background: Accumulating evidence suggests that short telomere length is associated with increased overall mortality, but the relationship with cancer mortality is less clear. We examined whether telomere length (global, and chromosome arm 5p- and 13q-specific) is associated with lung cancer mortality among cases from the β-Carotene and Retinol Efficacy Trial of heavy smokers.

Methods: Telomere length was measured on average 6 years before diagnosis for 788 lung cancer cases. Adjusted Cox proportional hazards models of all-cause and lung cancer–specific mortality were assessed for lung cancer overall and by histotype.

Results: Short telomere length was associated with increased mortality for small cell lung cancer (SCLC), particularly stage III/IV SCLC [HR and 95% confidence interval for shortest vs. longest telomere length tertile: 3.32 (1.78–6.21)]. Associations were strongest for those randomized to the active intervention and when telomere length was measured ≤5 years before diagnosis. All-cause mortality patterns were similar. Short chromosome 5p telomere length was suggestively associated with lung cancer mortality, but there was no association with chromosome 13q telomere length.

Conclusions: Our large prospective study suggests that among heavy smokers who developed lung cancer, short prediagnosis telomere length is associated with increased risk of death from SCLC.

Impact: This is the first study to examine telomere length and mortality in lung cancer cases by histotype. If the association between short telomere length and SCLC mortality is replicated, elucidation of mechanisms through which telomere length influences survival for this highly aggressive cancer may inform more effective use of telomere-targeted therapeutics. *Cancer Epidemiol Biomarkers Prev; 27(7); 829–37. ©2018 AACR.*

Introduction

Lung cancer is the leading cause of cancer-related death worldwide, with over one million deaths annually (1). More Americans die from lung cancer than prostate, breast, and colorectal cancers combined (2). Molecular markers of prognosis and treatment efficacy could help to improve outcomes for lung cancer, for which 5-year survival is only 18% (3).

Telomeres are chromatin structures that cap chromosome ends, protecting them from erroneous recognition as double-strand DNA breaks, inappropriate enzymatic degradation, and end-to-end fusions (4). They shorten with each cell division, and when they reach a critically short length, they trigger apoptosis or cellular senescence (5). Telomere length provides a measure of the cumulative effects of both intrinsic and extrinsic processes on telomere homeostasis (6). Different chromosomes and chromosome arms of the same chromosome have varying telomere length (7–9). The closest gene to the telomere of chromosome arm 5p is *TERT*, which encodes the catalytic subunit of the enzyme telomerase that maintains telomeres. It is hypothesized that *TERT* may autoregulate its influence on telomere length by interacting with the chromosome 5p telomere (10). Chromosome 13q is of interest because it contains the cell-cycle checkpoint gene *RB1*. Abrogated cell-cycle checkpoint genes like *RB1* may allow damaged cells to escape from senescence, which may result in increased cellular proliferation when combined with telomere maintenance (11).

Short peripheral blood telomere length is reported to be associated with increased all-cause mortality in many (12–24) but not all (25–31) studies—including null associations observed in the very old (32–37). The association with overall cancer mortality is less clear. Although some studies have observed that short peripheral blood telomere length is associated with cancer mortality (17, 38, 39), others have not (13–15, 25–27, 34, 36); however, many of these studies were not designed to evaluate cancer mortality as a primary endpoint. A recent meta-analysis of 13 studies of nonhematologic malignancies observed that short peripheral blood telomere length (measured in some studies before diagnosis and after in others) is associated with increased mortality (40). Only three studies have examined peripheral blood telomere length and survival after lung cancer diagnosis

[1]Department of Population Health Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah. [2]Program in Epidemiology, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington. [3]Department of Epidemiology, School of Public Health, University of Washington, Seattle, Washington. [4]Department of Otolaryngology: Head and Neck Surgery, School of Medicine, University of Washington, Seattle, Washington.

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Corresponding Author: Jennifer A. Doherty, Huntsman Cancer Institute, 2000 Circle of Hope, Room 4721, Salt Lake City, UT 84112-5550. Phone: 801-213-5681; Fax: 603-653-9093; E-mail: jen.doherty@hci.utah.edu

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(39, 41, 42). Weischer and colleagues observed that shorter telomere length measured prior to diagnosis is associated with increased mortality in lung cancer cases from their population-based Danish cohort (39). Similar suggestive associations were observed by Lee and colleagues in their cohort of smokers with chronic obstructive pulmonary disease (COPD; ref. 41). The study by Kim and colleagues reported that long telomere length, measured at diagnosis in early-stage non–small cell lung cancer (NSCLC) cases, is associated with increased risk of recurrence after curative resection (42). In this study, we examine associations between telomere length (global, and chromosome arm 5p- and 13q-specific), measured on average 6 years prior to lung cancer diagnosis, and all-cause and lung cancer–specific mortality in a cohort of heavy smokers who developed lung cancer.

Materials and Methods

Study population

This study includes lung cancer cases from a nested case–control study conducted within the multicenter β-Carotene and Retinol Efficacy Trial (CARET; ref. 43), a randomized, double-blinded, placebo-controlled chemoprevention trial of daily supplementation with β-carotene and retinyl palmitate among very heavy smokers (44–46). Current or former smokers (i.e., quit within 6 years) with a $\geq$20 pack year history, ages 50 to 69 years, were eligible for the trial (n = 14,254). Men with substantial occupational asbestos exposure, ages 45 to 69 years, and current or former heavy smokers (i.e., quit within fifteen years), were also eligible (n = 4,060). Participants completed annual questionnaires with information about smoking history and other risk factors, and blood samples were collected between 1994 and 1997. The intervention was stopped in 1996 after observing higher lung cancer incidence and overall mortality in the intervention compared with the placebo arm. Active participant follow-up for lung cancer and other outcomes continued until 2005, with cancer and death reports confirmed by thorough review of clinical records, pathology reports, and death certificates. Passive follow-up from linkages with two state cancer registries (Washington State Cancer Registry and Connecticut Tumor Registry) and the National Death Index extended follow-up for endpoints through 2013. The original nested case–control study identified cases using endpoint information collected during active participant follow-up (between 1985 and 2005; ref. 43). Cases were eligible for that study if they were lung cancer–free at blood draw (n = 793). Of those eligible, five cases who had been incorrectly diagnosed with lung cancer and one case with discordant sex information after genotyping were excluded, resulting in 787 cases. Another 38 cases did not have enough DNA for telomere length assays, leaving 749 cases. Among the 1,441 controls with sufficient DNA from the original case–control study, selected based on follow-up through 2005, 89 subsequently developed lung cancer. We included these cases in this study for a total of 838 lung cancer cases. Each of the participating CARET institution's institutional review boards approved all study protocols, and written informed consent was provided by all participants.

Laboratory methods

QIAamp DNA Blood Midi kits (Qiagen) were used to extract DNA from blood samples according to manufacturer's instructions. We measured global relative telomere length using two independent singleplex quantitative PCR (qPCR) assays, one for telomere repeats and one for a single copy of hemoglobin subunit beta (HBB; the control gene), in a method modified from Aviv and colleagues (47) and Cawthon and colleagues (48). Telomere to single-copy control gene ratio (T/S) were determined using the approach of McGrath and colleagues (49) and normalized per Aviv and colleagues (47). All samples were measured in duplicate on two separate runs. If there was >7% difference in normalized T/S ratios for a sample, it was assayed a third time and the average of the two closest values was used. Average coefficients of variation for the positive controls was 8.8% over 37 assay runs.

We designed primers to assay 5p and 13q chromosome arm-specific telomere length using a modified STELA protocol (50). We created target sequence-specific primers using a genomic alignment tool (http://www.genome.ucsc.edu) and Repeatmasker (http://www.repeatmasker.org) due to high homology in the subtelomere region, adjacent to the telomere. Specificity was confirmed by sequencing the fragment. Primers were designed for a two-step process that first uses a long PCR to amplify the specific chromosome arm from the subtelomere to the telomere end, followed by qPCR to target regions unique to the subtelomere and the telomere repeat. The Ct value of subtelomere, used as the single copy (S), and the Ct value of the targeted region were used to calculate the T/S ratio. Cq values of samples run in duplicate were evaluated; samples were retested if the Cq standard deviation was >0.3. If the standard deviation remained >0.3 in the repeated run, the values were averaged. Positive controls included on every plate were used to adjust final Cq data for the telomere and telomere adjacent runs.

Samples with low DNA concentration (n = 41) and outliers (n = 9) were excluded, leaving 788 cases in analyses of global telomere length. For the chromosome 5p and 13q assays, 82 and 73 cases did not pass quality control, leaving 756 and 765 cases for analyses, respectively. Additional methods details are available in Doherty and colleagues (51).

Statistical analyses

We evaluated Spearman correlations between continuous global, 5p, and 13q telomere length and age (years), pack years, cigarettes per day, and body mass index (kg/m^2). Log_2-transformed global, 5p, and 13q relative telomere length were evaluated as categorical variables (split at tertiles, quintiles, and deciles based on all lung cancers). The log-rank test was used to evaluate whether survival differed by tertile of telomere length, and the Kruskal–Wallis test was used to evaluate pairwise differences in the median survival times between the telomere length tertiles. Cox proportional hazards models were used to calculate HRs and 95% confidence intervals (CIs) for increasing quantiles of telomere length and lung cancer–specific and all-cause mortality overall, and separately by adenocarcinoma, squamous cell carcinoma, and SCLC histotypes. The "All lung cancer cases" category includes cases with adenocarcinoma, squamous cell carcinoma, and SCLC, as well as 328 cases for whom histotype was specified only as: NSCLC, NOS; other NSCLC; unknown, or missing. HRs and 95% CIs were adjusted for age, sex, race/ethnicity, smoking status, pack years, asbestos exposure, enrollment year, and intervention arm. Stage was only available for a subset of 505 lung cancer cases, 147 adenocarcinoma, 125 squamous cell, and 91 SCLC, so we performed

separate analyses additionally adjusting for stage. Linear trend across telomere length quantiles was evaluated by including an ordinal term (treated as continuous) in the model. The proportional hazards assumption was evaluated using Schoenfeld global test. Subgroup analyses of telomere length and mortality based on age, smoking status, sex, study arm, stage, pack years, and time between blood draw and lung cancer diagnosis were performed. Tests for statistical significance were two-sided, and a P value cutoff with a Bonferroni correction of 7 (the number of subgroups examined) was used ($0.05/7 = 0.007$). All analyses were performed in SAS (version 9.4; SAS).

Results

Characteristics of lung cancer cases at the time of blood draw are presented in Table 1. Briefly, cases were aged 64.2 years on average and the majority were white (95%), male (65%), and current smokers (66%). Participants were followed on average 7.9 years from blood draw through lung cancer diagnosis and ultimately until death or the end of the study period. The time between blood draw and any lung cancer diagnosis was 5.9 years, on average. History of asbestos exposure was highest for squamous cell carcinoma (21%) and lowest for SCLC (13%). Age at blood draw was inversely associated with global, but not chromosome 5p or 13q, telomere length (Spearman correlation $= -0.11$; $P = 0.002$). An inverse association with body mass index (BMI) was observed for chromosome 13q telomere length only (Spearman correlation 0.09, $P = 0.02$). There were no statistically significant correlations between telomere length (global, 5p, or 13q) and pack years or cigarettes per day.

A total of 788 individuals with lung cancer were successfully assayed for telomere length. Of 751 deaths, 635 were attributed to lung cancer. Separately by histotype, 93%, 95%, and 98% of the adenocarcinoma, squamous cell, and small cell cases had died, respectively. There was no deviation from the proportional hazards assumption in the survival analyses of global, 5p, and 13q telomere length (all P-values >0.27). We observed suggestive trends of increasing SCLC mortality associated with decreasing telomere length. For the shortest versus the longest tertile, quintile, and decile of telomere length and SCLC-specific mortality, HRs and 95% CIs were: 1.74 (1.05–2.90) (Table 2), 2.17 (1.10–4.26), and 5.19 (1.69–15.99), respectively, all with $P_{trend} < 0.04$ (Supplementary Table S1). Among SCLC cases with known stage, 86 were stages III or

Table 2. Telomere length and lung cancer–specific mortality by histotype among lung cancer cases[a]

TL tertile[b]	Lung cancer deaths	Total lung cancer cases	HR (95% CI)
All lung cancer cases[c]			
1 (shortest)	212	261	1.08 (0.89–1.32)
2	214	267	1.04 (0.86–1.27)
3 (longest)	209	260	1.00 (Ref.)
		P_{trend}	0.42
Adenocarcinoma			
1 (shortest)	49	59	1.35 (0.90–2.02)
2	43	55	1.17 (0.78–1.78)
3 (longest)	58	75	1.00 (Ref.)
		P_{trend}	0.15
Squamous cell			
1 (shortest)	42	55	0.86 (0.52–1.43)
2	38	53	0.76 (0.46–1.25)
3 (longest)	34	42	1.00 (Ref.)
		P_{trend}	0.63
Small cell			
1 (shortest)	45	47	1.74 (1.05–2.90)
2	37	38	1.66 (0.98–2.83)
3 (longest)	30	36	1.00 (Ref.)
		P_{trend}	0.04

Abbreviation: TL, telomere length.

[a]Cox proportional hazards models adjusted for age at blood draw, sex, race, smoking status at blood draw, asbestos exposure, enrollment year, intervention arm, and pack years at blood draw.

[b]Telomere tertile cutoffs were determined among all lung cancer cases, and are defined by the following nontransformed relative telomere length ranges: 0.23 to <0.84, 0.84 to <1.15, and 1.15 to <2.45.

[c]"All lung cancer cases" includes adenocarcinoma, squamous cell, and small cell, as well as 328 cases for whom histotype was NSCLC, NOS; other NSCLC; unknown or missing.

Table 1. Characteristics of lung cancer cases at blood draw

	All lung cancer cases[a] ($N = 788$)	Adenocarcinoma ($N = 189$)	Squamous cell ($N = 150$)	Small cell ($N = 121$)
Age, years; mean (SD)	64.2 (5.6)	64.1 (5.6)	65.0 (5.6)	64.4 (5.7)
45–54; *N* (%)	52 (7)	14 (7)	9 (6)	8 (7)
55–59; *N* (%)	127 (16)	29 (15)	21 (14)	20 (17)
60–64; *N* (%)	239 (30)	64 (34)	36 (24)	33 (27)
65–69; *N* (%)	233 (30)	49 (26)	53 (35)	41 (34)
70–74; *N* (%)	137 (17)	33 (18)	31 (21)	19 (16)
Race; *N* (%)				
White	747 (95)	182 (96)	141 (94)	116 (96)
Black	22 (3)	1 (1)	5 (3)	0 (0)
Other	19 (2)	6 (3)	4 (3)	5 (4)
Sex, female; *N* (%)	273 (35)	77 (41)	37 (25)	51 (42)
Current smoker; *N* (%)	523 (66)	109 (58)	107 (71)	78 (65)
Pack years; mean (SD)	57.4 (21.5)	56.1 (20.1)	62.4 (26.1)	57.6 (20.3)
Years since quit smoking; mean (SD)	2.2 (4.5)	2.7 (5.1)	2.0 (4.0)	2.1 (4.4)
Intervention arm (assigned to active); *N* (%)	430 (55)	102 (54)	77 (51)	67 (55)
Asbestos exposure; *N* (%)	125 (16)	30 (16)	31 (21)	16 (13)
Years followed[b]; mean (SD)	7.9 (4.9)	7.7 (5.2)	7.8 (5.0)	6.2 (3.8)
Years between blood draw and diagnosis; mean (SD)	5.9 (3.9)	4.9 (3.4)	4.9 (3.2)	4.8 (3.2)

[a]"All lung cancer cases" includes adenocarcinoma, squamous cell, and small cell, as well as 328 cases for whom histotype was NSCLC, NOS; other NSCLC; unknown or missing.

[b]Years followed includes time from blood draw to death or end of the study period.

Table 3. Telomere length and lung cancer–specific mortality among stage III/IIV SCLC cases, stratified by age, smoking status, sex, intervention arm, pack years of smoking, and time between blood draw and diagnosis[a]

Subgroup	TL tertiles	SCLC deaths	Total SCLC cases	HR (95% CI)	P_{trend}
Lung cancer stage					
Stage III/IV	1 (shortest)	29	29	3.32 (1.78–6.21)	0.0002
	2	28	29	1.94 (1.05–3.58)	
	3 (longest)	24	28	1.00 (Ref.)	
Age at blood draw					
≤65 years	1 (shortest)	15	15	4.06 (1.55–10.64)	0.003
	2	15	15	2.71 (1.15–6.37)	
	3 (longest)	13	15	1.00 (Ref.)	
>65 years	1 (shortest)	14	14	6.33 (1.86–21.52)	0.003
	2	13	14	2.12 (0.67–6.71)	
	3 (longest)	11	13	1.00 (Ref.)	
Smoking status at blood draw					
Current smoker	1 (shortest)	18	18	4.77 (1.92–11.84)	0.001
	2	20	21	1.52 (0.68–3.40)	
	3 (longest)	14	16	1.00 (Ref.)	
Former smoker	1 (shortest)	11	11	5.11 (1.24–20.97)	0.006
	2	8	8	15.60 (3.22–75.82)	
	3 (longest)	10	12	1.00 (Ref.)	
Sex					
Men	1 (shortest)	17	17	2.80 (1.25–6.27)	0.01
	2	19	19	1.64 (0.70–3.84)	
	3 (longest)	17	18	1.00 (Ref.)	
Women	1 (shortest)	12	12	5.06 (1.60–16.06)	0.006
	2	9	10	1.97 (0.54–7.18)	
	3 (longest)	7	10	1.00 (Ref.)	
Intervention arm					
Active	1 (shortest)	21	21	8.95 (2.66–30.10)	0.0003
	2	15	15	2.38 (0.84–6.69)	
	3 (longest)	8	11	1.00 (Ref.)	
Placebo	1 (shortest)	8	8	2.01 (0.68–5.94)	0.06
	2	13	14	2.92 (1.12–7.57)	
	3 (longest)	16	17	1.00 (Ref.)	
Pack years at blood draw (median = 52 years)					
≤52 years	1 (shortest)	13	13	3.79 (1.44–9.94)	0.006
	2	18	19	2.41 (0.97–5.99)	
	3 (longest)	11	14	1.00 (Ref.)	
>52 years	1 (shortest)	16	16	3.71 (1.36–10.07)	0.01
	2	10	10	1.27 (0.46–3.46)	
	3 (longest)	13	14	1.00 (Ref.)	
Time between blood draw and lung cancer diagnosis (mean = 5 years)					
0–5 years	1 (shortest)	22	22	6.38 (2.59–15.74)	0.0001
	2	20	21	4.09 (1.68–9.93)	
	3 (longest)	15	17	1.00 (Ref.)	
>5 years	1 (shortest)	7	7	2.58 (0.66–10.11)	0.18
	2	8	8	1.50 (0.38–5.86)	
	3 (longest)	9	11	1.00 (Ref.)	

Abbreviation: TL, telomere length.

[a]Cox proportional hazards models adjusted for age at blood draw, sex, race, asbestos exposure, enrollment year, smoking status at blood draw, intervention arm, and pack years at blood draw (any of these adjustment variables was not included when the analysis was stratified on that variable).

IV, and only five were stages I or II, so we restricted further analyses to late stage (stages III/IV) SCLC. HR and 95% CI for the tertile, quintile, and decile associations between telomere length and late stage SCLC-specific mortality were: 3.32 (1.78–6.21; Table 3), 3.33 (1.54–7.21), and 5.86 (1.64–20.91), respectively, all with $P_{trend} < 0.0007$ (Supplementary Table S2). Associations for late stage SCLC all-cause mortality were generally similar (tertiles presented in Supplementary Table S3). Results adjusted for stage (III vs. IV) were essentially the same. Late-stage SCLC-specific 5-year survival differed by tertile of telomere length (log-rank $P = 0.005$; Supplementary Fig. S1). Median survival for the shortest tertile was only 6 months, compared with 10.8 months ($P = 0.008$) in the longest tertile.

Statistically significant associations (i.e., with a multiple testing-corrected $P_{trend} < 0.007$) between short telomere length and late-stage SCLC-specific (Table 3) and all-cause mortality (Supplementary Table S3) were observed in almost all strata defined by age, smoking status, sex, and pack years. Comparing the shortest to the longest tertile of telomere length, late-stage SCLC-specific mortality associations were suggestively stronger for aged >65 years (HR 6.33; 95% CI, 1.86–21.52; $P_{trend} = 0.003$) than aged ≤65 years (HR 4.06; 95% CI, 1.55–10.64; $P_{trend} = 0.003$), and for women (HR 5.06; 95% CI, 1.60–16.06; $P_{trend} = 0.006$) than men (HR 2.80; 95% CI, 1.25–6.27; $P_{trend} = 0.01$; Table 3). Associations were strongest for those randomized to the active intervention (HR 8.95; 95% CI, 2.66–30.10; $P_{trend} = 0.0003$) versus placebo (HR 2.01; 95% CI, 0.68–5.94; $P_{trend} = 0.06$), and those with telomere length measured 0 to 5 years prior to diagnosis (HR 6.38; 95% CI, 2.59–15.74; $P_{trend} = 0.0001$) versus >5 years prior to diagnosis (HR 2.58; 95% CI, 0.66–10.11;

$P_{trend} = 0.18$; Table 3). Associations for all-cause mortality were similar to SCLC-specific mortality, although generally slightly smaller in magnitude (Supplementary Table S3).

No clear pattern between telomere length and lung cancer–specific mortality was observed for adenocarcinoma or squamous cell carcinoma, before or after adjustment for stage (Table 2; Supplementary Tables S1 and S2). Results were similar for all-cause mortality. For all lung cancer cases combined, short telomere length was suggestively associated with mortality but only for the analysis of deciles. Comparing the shortest to the longest decile of telomere length, the HR and 95% CI for lung cancer–specific mortality was 1.39 (0.98–1.98; Supplementary Table S1), and the corresponding HR and 95% CI for all-cause mortality was 1.43 (1.03–1.99), but we did not observe a trend between decreasing telomere length and mortality (Supplementary Table S1).

Short chromosome 5p telomere length was not associated with mortality for any of the histotypes individually, but it was suggestively associated with increased lung cancer–specific (HR 1.24; 95% CI, 1.02–1.52; $P_{trend} = 0.03$) mortality for all lung cancers, particularly among those $\leq$65 years (HR 1.56; 95% CI, 1.18–2.05; $P_{trend} = 0.003$; Table 4). Similar results were observed for all-cause mortality in all lung cancers (Supplementary Table S4). Associations were generally similar after adjustment for stage. Chromosome 13q telomere length was not associated with mortality before (Supplementary Table S5) or after adjustment for stage.

Discussion

To our knowledge, this is the largest prospective study to date of telomere length and lung cancer mortality, and the first to evaluate associations by histotype. We observed that short telomere length measured prior to diagnosis was associated with increased all-cause and lung cancer–specific mortality for SCLC, but not the other histotypes, among lung cancer cases with an average smoking history of 57 pack years. We also observed that short chromosome 5p telomere length was suggestively modestly associated with increased mortality in lung cancer cases, but not within individual histotypes.

The association with global telomere length was particularly strong for late-stage SCLC, and when telomere length was measured closer to diagnosis. It is possible that telomere length closer to diagnosis reflects a physiologic state that is more relevant to survival outcomes, but it may also reflect preclinical changes associated with the onset of disease. The CARET trial reported higher mortality among individuals randomized to the intervention arm (44); within this group, the association between short telomere length and SCLC mortality was especially strong, suggesting an interaction between pharmacologically high-dose β-carotene/retinyl palmitate and telomere length on survival outcomes. This is plausible given the association between oxidative stress and short telomeres (52–56), and the suspected prooxidant effects of high-dose vitamins (57).

SCLC makes up 16% of all lung cancers, and it is a more aggressive disease than NSCLC, with 5-year relative survival of only 6% compared with 20% for adenocarcinoma and 17% for squamous cell carcinoma (58). For each histotype, associations were generally similar for all-cause and lung cancer–specific mortality, reflecting the short survival time experienced by individuals with lung cancer. The majority of SCLC (76%) are diagnosed at stages III/IV (58). Even though late-stage SCLC survival is particularly poor, we observed that late-stage SCLC cases with telomere length in the shortest tertile had worse median survival than those in the longest tertile (6 months vs. 10.8 months, respectively; $P = 0.008$).

Our results are generally consistent with the limited telomere length and lung cancer mortality literature to date. In their population-based Danish cohort study with up to 20 years of follow-up, Weischer and colleagues reported a 27% increased hazard of death per kilobase pair decrease in telomere length among 522 lung cancer cases (468 deaths; HR 1.27; 95% CI, 1.13–1.43), but histotype-specific results were not reported (39). In a cohort of 4,271 individuals with COPD and on average a 40 pack year smoking history, Lee and colleagues reported that short telomere length is suggestively associated with increased lung cancer mortality ($n = 127$; shortest vs. longest quartile 1.40, 0.94–2.16), but results were not presented by histotype (41). We also observed a suggestive association between short telomere length and increased all-cause (and lung cancer–specific) mortality for all lung cancer cases, but in our study, these associations are driven by the strong associations we observed among SCLC. Our study of individuals who smoked on average 57 pack years and the study by Lee and colleagues demonstrate that even among smokers, short telomere length may be associated with worse survival (41). The study by Kim and colleagues, which assessed telomere length at diagnosis and risk of recurrence for early-stage NSCLC cases ($n = 473$), is quite different from our work because we measured telomere length prior to diagnosis (not at diagnosis) and evaluated mortality (not recurrence). They observed that long telomere length is associated with early-stage adenocarcinoma recurrence (HR 2.19; 95% CI, 1.05–4.55; ref. 42). We did not observe an association between long telomere length and early-stage adenocarcinoma mortality (shortest vs. longest telomere length tertile HR 1.18; 95% CI, 0.44–3.20). Finally, associations between mortality and telomere length in lung tumor tissue compared with paired-normal tissue (59–63) or tumor tissue only (64, 65) have been conflicting. Because telomere dynamics differ for somatic versus germline tissues, results from these studies are not directly comparable to our work.

Although the literature on telomere length and lung cancer mortality is very limited, there is a growing body of evidence linking long telomere length with risk of lung cancer. This association is likely restricted to the adenocarcinoma histotype, supported by prospective studies (66), including ours in the CARET study (51), and studies of genetic risk scores for telomere length (67, 68). It is not implausible that short telomere length could be associated with increased mortality from SCLC, whereas long telomere length could be associated with risk of adenocarcinoma, as both long and short telomeres likely represent telomere dysfunction (69), and the histotypes are biologically distinct (70). For example, there are strikingly different patterns of genetic susceptibility by histotype (71), and adenocarcinomas have a much higher frequency of actionable mutations than do the other histotypes (70). Although smoking is associated with all lung cancers, it is most strongly related to SCLC risk (72). As telomere length is influenced by genetic and nongenetic factors such as age (73–75), exposure to cigarette smoking (74, 75), and oxidative stress and inflammation (52–56, 76), it is possible that short telomere length

Table 4. Chromosome 5p telomere length and lung cancer–specific mortality among all lung cancer cases[a], stratified by age, smoking status, sex, intervention arm, stage, pack years of smoking, and time between blood draw and diagnosis[b]

Subgroup	TL tertiles	Lung cancer deaths	Total lung cancer cases	HR (95% CI)	P_{trend}
TL tertile	1 (shortest)	211	250	1.24 (1.02–1.52)	0.03
	2	202	257	1.02 (0.83–1.24)	
	3 (longest)	197	249	1.00 (Ref.)	
Lung cancer stage[c]					
Stage I/II	1 (shortest)	22	41	1.30 (0.69–2.45)	0.42
	2	24	50	1.12 (0.62–2.04)	
	3 (longest)	23	41	1.00 (Ref.)	
Stage III/IV	1 (shortest)	116	122	1.26 (0.96–1.65)	0.10
	2	114	119	1.03 (0.78–1.36)	
	3 (longest)	100	109	1.00 (Ref.)	
Age at blood draw					
≤65 years	1 (shortest)	106	119	1.56 (1.18–2.05)	0.003
	2	113	143	1.04 (0.80–1.37)	
	3 (longest)	104	133	1.00 (Ref.)	
>65 years	1 (shortest)	105	131	1.07 (0.80–1.43)	0.64
	2	89	114	1.06 (0.78–1.43)	
	3 (longest)	93	116	1.00 (Ref.)	
Smoking status at blood draw					
Current smoker	1 (shortest)	143	165	1.36 (1.07–1.74)	0.01
	2	138	175	0.96 (0.75–1.23)	
	3 (longest)	131	162	1.00 (Ref.)	
Former smoker	1 (shortest)	68	85	1.05 (0.74–1.49)	0.79
	2	64	82	1.14 (0.80–1.61)	
	3 (longest)	66	87	1.00 (Ref.)	
Sex					
Men	1 (shortest)	145	173	1.29 (1.01–1.66)	0.04
	2	130	164	1.03 (0.81–1.32)	
	3 (longest)	125	157	1.00 (Ref.)	
Women	1 (shortest)	66	77	1.16 (0.82–1.63)	0.42
	2	72	93	0.96 (0.69–1.34)	
	3 (longest)	72	92	1.00 (Ref.)	
Intervention arm					
Active	1 (shortest)	119	140	1.31 (1.01–1.72)	0.05
	2	107	131	0.96 (0.73–1.26)	
	3 (longest)	112	138	1.00 (Ref.)	
Placebo	1 (shortest)	92	110	1.19 (0.88–1.62)	0.26
	2	95	126	1.06 (0.79–1.42)	
	3 (longest)	85	111	1.00 (Ref.)	
Pack years at blood draw (median = 52 years)					
≤52 years	1 (shortest)	117	131	1.34 (1.01–1.77)	0.04
	2	101	126	1.04 (0.78–1.39)	
	3 (longest)	96	122	1.00 (Ref.)	
>52 years	1 (shortest)	94	119	1.12 (0.84–1.50)	0.45
	2	101	131	0.95 (0.71–1.26)	
	3 (longest)	101	127	1.00 (Ref.)	
Time between blood draw and lung cancer diagnosis (mean = 5 years)					
0–5 years	1 (shortest)	106	122	1.15 (0.86–1.55)	0.34
	2	99	111	0.99 (0.74–1.33)	
	3 (longest)	94	108	1.00 (Ref.)	
>5 years	1 (shortest)	105	128	1.31 (0.99–1.73)	0.06
	2	103	146	1.03 (0.78–1.36)	
	3 (longest)	103	141	1.00 (Ref.)	

Abbreviation: TL, telomere length.

[a]All lung cancer cases includes adenocarcinoma, squamous cell carcinoma, and SCLC cases, as well as 313 cases for whom histotype was NSCLC, NOS; other NSCLC; unknown or missing.

[b]Cox proportional hazards models adjusted for age at blood draw, sex, race, asbestos exposure, enrollment year, smoking status at blood draw, intervention arm, and pack years at blood draw (any of these adjustment variables was not included when the analysis stratified on that variable).

[c]274 cases are missing stage data.

reflects higher cumulative exposure to factors that may be associated with poorer survival. Also, because telomere length measured in peripheral blood is a weighted average of the telomere lengths of circulating immune cells (77), it could reflect varying immune profiles.

Regarding telomere length and mortality for cancer types other than lung cancer, the recent meta-analysis that combined 13 studies of various solid cancer types reported that short peripheral blood telomere length is associated with increased mortality (40), but results for individual cancer types are inconsistent. Weischer and colleagues observed that short telomere length measured prior to diagnosis is associated not only with mortality from lung cancer, but also melanoma and glioma, and with favorable survival for esophageal cancer

(39). Conflicting results have been reported for cancers of the breast (39, 78–80), colon and/or rectum (39, 81, 82), esophagus (39, 83), kidney (39, 84, 85), liver (39, 86–88), stomach (39, 89), urinary tract (39, 90, 91), and ovary (39, 92, 93), although the two studies of glioma to date both reported associations between short telomere length and increased mortality (39, 94).

Our study has several strengths: all cases were heavy smokers, reducing the possibility of confounding by smoking status; we measured telomere length prior to diagnosis so it is ostensibly not affected by diagnosed lung cancer or its treatment; and we were able to evaluate chromosome 5p and 13q telomere length. Although this is the largest study to date, data on histotype and stage were missing for some of the study participants, which reduced the sample sizes for histotype-specific and stage-adjusted analyses. Still, we were able to evaluate associations after controlling for stage in approximately 80% of the cases. Finally, DNA was extracted from whole blood using QIAamp kits, which have been reported to yield shorter telomere length measurements (95–97). If the distribution of telomere length was compressed, this may have attenuated the HRs and therefore may have limited our ability to detect differences in survival.

Our findings—that short global telomere length is associated with increased mortality for late-stage SCLC, and that short chromosome 5p telomere length is suggestively associated with increased mortality for all lung cancers—are novel, and require evaluation in other populations. Given that we observed a stronger association with SCLC when telomere length was measured closer to diagnosis, it may be of interest to determine whether telomere length measured at diagnosis but before treatment is more strongly associated with survival. If replicated, studies elucidating mechanisms through which peripheral blood telomere length influences survival for this highly aggressive cancer are warranted, and may inform more effective use of telomere-targeted therapeutics (6, 11).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J.A. Doherty, G.E. Goodman, C. Chen
Development of methodology: J.A. Doherty, L. Grieshober, J.R. Houck
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.R. Houck, M. Thornquist, G.E. Goodman
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.A. Doherty, L. Grieshober, J.R. Houck, J.D.D. Tapsoba, M. Thornquist, C.-Y. Wang, G.E. Goodman
Writing, review, and/or revision of the manuscript: J.A. Doherty, L. Grieshober, J.R. Houck, M.J. Barnett, J.D.D. Tapsoba, C.-Y. Wang, G.E. Goodman, C. Chen
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): L. Grieshober, J.R. Houck, M.J. Barnett, M. Thornquist, G.E. Goodman, C. Chen
Study supervision: J.A. Doherty, C. Chen

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