Review

Cancer Epidemiology, Biomarkers & Prevention

Systematic Review of Genetic Variation in Chromosome 5p15.33 and Telomere Length as Predictive and Prognostic Biomarkers for Lung Cancer

Linda Kachuri[1,2,3], Lidija Latifovic[3], Geoffrey Liu[2,4,5], and Rayjean J. Hung[1,2]

Abstract

Lung cancer remains the leading cause of cancer mortality worldwide. Known histomolecular characteristics and genomic profiles provide limited insight into factors influencing patient outcomes. Telomere length (TL) is important for genomic integrity and has been a growing area of interest as agents targeting telomerase are being evaluated. Chromosome 5p15.33, an established cancer susceptibility locus, contains a telomerase-regulatory gene, *TERT*, and *CLPTM1L*, a gene associated with cisplatin-induced apoptosis. This review offers a summary of the clinical utility of 5p15.33 polymorphisms and TL. A total of 621 abstracts were screened, and 14 studies (7 for 5p15.33, 7 for TL) were reviewed. Endpoints included overall survival (OS), progression-free survival (PFS), therapy response, and toxicity. Of the 23 genetic variants identified, significant associations with OS and/or PFS were reported for rs401681 (*CLPTM1L*), rs4975616 (*TERT-CLPTM1L*), and rs2736109 (*TERT*). Both shorter and longer TL, in tumor and blood, was linked to OS and PFS. Overall, consistent evidence across multiple studies of 5p15.33 polymorphisms and TL was lacking. Despite the potential to become useful prognostic biomarkers in lung cancer, the limited number of reports and their methodologic limitations highlight the need for larger, carefully designed studies with clinically defined subpopulations and higher resolution genetic analyses. *Cancer Epidemiol Biomarkers Prev; 25(12); 1537–49. ©2016 AACR.*

Introduction

Lung cancer is the most commonly diagnosed cancer and the leading cause of cancer death worldwide (1). Despite recent advances in its treatment, the general prognosis for lung cancer remains poor, with low 5-year survival rates of under 15% (2). For non–small cell lung cancer (NSCLC), treatment with platinum-based chemotherapy provides modest improvements in the survival of stage IIIB/IV NSCLC patients and the absolute cure rates in stage II/IIIA. However, our current understanding of the factors influencing interindividual variability in outcomes is limited, as clinic–demographic factors (age, sex, smoking, and disease stage) and histomolecular factors, including tumor histology, somatic molecular changes, such as *EGFR* mutations or anaplastic lymphoma kinase (*ALK*) rearrangements, only provide a partial picture (3). This underscores the need to identify noninvasive, reliable molecular markers of disease progression and treatment efficacy.

Telomeres are structures that cap the ends of linear chromosomes and are composed of short tandem repeats of the TTAGGG sequence (4, 5). Telomeres shorten by 20 to 200 base pairs with each round of cell division, due to incomplete DNA replication (6, 7). This occurs in all normal human cells with the exception of adult stem cells and activated lymphocytes (8, 9). Once telomeres become critically short, activation of cell-cycle arrest leads to replicative senescence, followed by apoptosis (10). This mechanism represents a fundamental barrier to cancer initiation by limiting proliferation and maintaining genome stability. Therefore, maintenance of telomere length (TL) is a key step in tumorigenesis and a universal characteristic of immortalized cancer cells (10, 11). The most important telomere lengthening mechanism requires telomerase, an enzyme that is overexpressed in approximately 90% of tumors, but inactive in normal cells (12). Telomerase-independent, alternative lengthening of telomeres (ALT) pathways are also relevant but only occur in a small subset of lung carcinomas originating from neuroendocrine cells (10, 11, 13).

One of the genes critical for maintaining the functionality of telomerase is *TERT*, which is located in chromosome 5p15.33 and encodes the catalytic subunit of telomerase. 5p15.33 (*TERT*) is one of the several genetic loci involved in TL regulation, along with 3q26 (*TERC*), 4q32.2 (*NAF1*), 10q24.33 (*OBFC1*), and 20q13.3 (*RTEL1*; refs. 14–16). Importantly, genome-wide association studies (GWAS) have established 5p15.33 as an important cancer susceptibility locus (17, 18), but few studies have explored the prognostic [i.e., overall survival (OS), progression-free survival (PFS), or disease-free survival] or predictive (i.e., treatment response) value of these risk variants.

[1]Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, Ontario, Canada. [2]Dalla Lana School of Public Health, University of Toronto, Toronto, Ontario, Canada. [3]Prevention & Cancer Control, Cancer Care Ontario, Toronto, Ontario, Canada. [4]Ontario Cancer Institute, Princess Margaret Cancer Center, Toronto, Ontario, Canada. [5]Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada.

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Rayjean J. Hung, Lunenfeld-Tanenbaum Research Institute, Sinai Health System 60 Murray St., Toronto, Ontario, M5T 3L9, Canada. Phone: 416-586-4750; Fax: 416-586-8404; E-mail: rayjean.hung@lunenfeld.ca

doi: 10.1158/1055-9965.EPI-16-0200

Functional studies have also suggested that *TERT* may act as a direct transcriptional regulator of key oncogenic signaling pathways, affecting the induction of target genes critical for cell survival and cancer progression, such as Wnt, Myc NF-κB, and β-catenin (19–21). Activation of *EGFR* signaling has also been suggested as a mechanism through which *TERT* stimulates proliferation (11, 22). In addition to *TERT*, the 5p15.33 region contains another cancer susceptibility gene, *CLPTM1L*, which encodes the cleft lip and palate-associated transmembrane 1 like protein and was shown to have a role in cisplatin-induced apoptosis (23).

Another compelling observation is the inhibition of telomerase and telomere shortening in response to platinum-based and radiotherapy, suggesting that TL and *TERT* may modulate treatment efficacy (24–27). In addition to genetic variation and treatment, TL may also be affected by lifestyle and environmental factors, such as cigarette smoking, alcohol consumption, and air pollution, as well as socioeconomic factors and psychologic stress (28–34). These findings demonstrate that TL is a complex and integrative biomarker that captures the influence of a wide range of potentially relevant factors.

The motivation for the current review is to provide a comprehensive discussion of the relationship between TL, inherited genetic variation in 5p15.33, and the clinical outcomes in lung cancer. The prognostic value of TL in solid tumors was previously reviewed by Bisoffi and colleagues (35) and Svenson and colleagues (36); however, both articles did not focus on lung cancer. A recent meta-analysis of TL and cancer prognosis by Zhang and colleagues (37) did not provide a summary estimate for lung cancer and only included two studies. As the evidence for the clinical utility of TL and genetic variants within the TERT/CLPTM1L locus continues to accumulate, we conducted a systematic review of both TL and 5p15.33 genetic variants to integrate current knowledge related to lung cancer prognosis.

Materials and Methods

Search strategy and eligibility criteria

PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses; ref. 38) methodologic guidelines were followed while incorporating a modified protocol for systematic review of the association between chronic stress and telomere length (PROSPERO registration number: CRD42014009274; ref. 39). Telomerase activity is not a surrogate for TL in a blood or tissue sample, which is the culmination of multiple processes affecting telomere homeostasis, such as replication rate, regulatory proteins, telomerase expression, as well as inherited genetic variation and environmental exposures. For this reason, we limited our review to the prognostic effects of TL and excluded the evaluation of telomerase activity.

A detailed search strategy was developed (Supplementary Materials and Methods), using specific medical subject headings (MeSH) and words from "all fields" to identify studies in MEDLINE (PubMed interface), including the following keywords: "5p15," "TERT," "CLPTM1L," "telomere length," "polymorphism," "single nucleotide polymorphism," "genetic variant," "lung cancer," "outcome," "survival," "prognosis," "response," "chemotherapy," and "radiotherapy." Searches were also performed using EMBASE (OVID interface; 1947–January 2016) and Google Scholar. Literature searches were carried out independently by two reviewers (L. Kachuri and L. Latifovic) and were limited to human studies and English language. Only full reports published on or before January 10, 2016, were included. To ensure sensitivity of the search strategy, citation lists of retrieved articles were checked.

Abstracts of articles that considered any of the following major outcomes were reviewed: OS rate, median survival time, disease-free survival rate, PFS, cancer-specific survival, time to progression, and therapy response, including toxicity. Search results were reviewed to exclude studies of cancer risk (incidence) and descriptive reports detailing the correlation between TL and clinic–pathologic characteristics.

Interrater agreement in abstract screening was assessed using Cohen kappa, and the two reviewers showed a high level of agreement (0.83, 0.66–0.99) for the identification of eligible studies. Discrepancies in eligibility were resolved through discussion between the two reviewers. The final list of eligible studies was confirmed by a senior epidemiologist (R.J. Hung).

Data extraction

Screening of full-text manuscripts and data abstraction were completed independently by two reviewers (L. Kachuri and L. Latifovic) using a standardized data collection form, a modified version of the template by Quinlan and colleagues (39), with explicit inclusion and exclusion criteria (Supplementary Materials and Methods). This approach was adopted to ensure homogeneity of data collection and to minimize subjectivity in the data gathering and entry process. The following data were extracted from eligible studies: authors' names, year of publication, number of case subjects and events, ethnicity, cancer type and histology, methods used for statistical analysis, summary effect estimates, such as ORs, HRs, and their corresponding 95% confidence intervals (CI), and estimates of survival time, such as 5-year survival rates or median survival. Covariate data, such as inclusion/exclusion criteria of lung cancer patients in each study, and stratification or adjustment variables used in the study analyses were recorded.

For studies of 5p15.33 variants, the names and genomic position of each SNP, the minor allele, and genetic model tested were also collected. For studies of TL, we collected information on the DNA source and method of extraction, as well as the method of TL measurement and analysis. Complete concordance (100%) was reached for all variables assessed.

Given the variable outcomes of interest, differences in methodology for TL assessment, and diverse clinical populations, a meta-analysis was not pursued.

Scientific quality assessment

Study quality for observational studies was assessed using the Strengthening the Reporting of Genetic Association Studies (40) and Strengthening the Reporting of Observational Studies in Epidemiology: Molecular Epidemiology (41) tools. Experimental designs were assessed using the Consolidated Standards of Reporting Trials checklist (42). The purpose of this assessment was to identify studies that should be considered for exclusion on the basis of low quality, or failing to report key information. All studies were found to be of acceptable quality and none were excluded using these criteria.

Results

A total of 620 relevant titles and abstracts (385 for TL; 235 for 5p15.33) were identified and screened, and 26 (9 for TL; 15 for

5p15.33) were selected for detailed full-text review (Supplementary Fig. S1). Relatively few publications were selected for full review because the majority of the articles were focused on functional characterizations of the 5p15.33 locus, TERT/CLPTM1L expression, or telomerase activity. Among epidemiologic studies, only articles relevant for lung cancer prognosis were included. Among the 15 articles selected for full-text review, 8 GWASs of lung cancer survival were excluded because they did not report associations for the 5p15.33 region, either in the main text or in the Supplementary Materials and Methods. After these exclusions, 7 studies of genetic variants in 5p15.33 (Supplementary Table S1) were included. After reviewing 9 studies of TL, one report was excluded because it only presented descriptive analyses of TL alterations in lung tumors (43), and another study had an English abstract, but the full text was only available in Japanese (44). A total of 7 studies of TL (Supplementary Table S2) were included in this review.

A range of outcomes was investigated across the 7 studies of 5p15.33 and 7 studies of TL: All-cause mortality was the most common, with OS as the primary outcome, followed by PFS or disease-free survival or risk of disease recurrence, and therapy response. Studies of OS reported median follow-up times of less than 5 years and relied on electronic health care and cancer registry records, as well as active follow-up, to improve the completeness of outcome ascertainment. Censoring was typically defined as the date of the last physician visit. All of the studies were relatively comparable in terms of the methods used for statistical analysis. Cox proportional hazards regression were used in studies of OS and PFS, while logistic regression was used in the three studies investigating therapy response. Of the studies examining OS, only one study treated deaths from non-lung cancer causes as competing events and estimated sub-hazard ratios (SHR) using the Fine and Gray method (45).

Survival rates and median survival times or time to disease progression or recurrence was estimated using the Kaplan–Meier method. Only one study compared Kaplan–Meier curves using the log-rank test as the only method of testing associations with OS and PFS (46). Of the 10 studies that used Cox regression, only two (47, 48) reported verifying the important proportionality assumption.

Chromosome 5p15.33 genetic variants

Study description and methods. A total of 23 genetic variants were investigated in the seven studies of 5p15.33 (Supplementary Table S1). Of these, four (57%) were conducted in NSCLC patients (46, 49–51), one study focused on small-cell lung cancer (SCLC; ref. 52), and two studies included multiple histologic types (47, 53). Xun and colleagues (47) investigated a mixed case series of NSCLC (including large cell), SCLC, and unspecified histology but presented results stratified by histologic subtype. Liang and colleagues (53) evaluated 309 patients: 37.5% squamous carcinoma, 35.6% adenocarcinoma, 21.3% small cell, 0.6% large cell, and 5% unspecified histology. Of these, 115 cases contributed to the therapy response analysis, but the histology distribution of this subset was not specified.

Among the four NSCLC-specific studies, Azad and colleagues (50), de Mello and colleagues (46), and Zhao and colleagues (51) focused exclusively on stage III/IV disease, while a case series by Catarino and colleagues was composed of 89% stage III/IV patients (49, 51). Azad and colleagues (50) included an ethnically mixed (74% Caucasian) sample, and the remaining two studies were in European populations. In addition, Azad and colleagues also restricted analyses to Caucasian patients to minimize confounding by population stratification and verify the consistency of the observed associations (50). All NSCLC studies included several histologic subtypes. The cases in Azad and colleagues (50) and Zhao and colleagues (51) were similar in their respective histology distributions: adenocarcinoma (61% and 63%, respectively), squamous (24% and 22%) and other NSCLC (15% and 13%). Similarly, adenocarcinoma was most common histology (48.3%) in Catarino and colleagues (49), followed by squamous carcinoma (37.5%) and other NSCLC (14.2%). The patients investigated by de Mello and colleagues (46) were divided into squamous (22.9%) and nonsquamous (77.1%) groups. Studies with multiple histologies were conducted in European (46, 47, 49) and Han Chinese populations (53). The single SCLC study was conducted in Han Chinese patients (52).

Overall survival. Of the seven studies investigating the *TERT* and *CLPTM1L* polymorphisms in 5p15.33, five (71%) reported associations for OS (Table 1). However, only two variants in *CLPTM1L*, rs401681 and rs402710, were examined in multiple studies for this outcome.

Inconsistent results were observed across studies for rs401681. The two NSCLC studies found either no association with OS (50) or a longer median survival among 144 Portuguese NSCLC patients in patients carrying the T allele (46). In contrast, in 874 Han Chinese SCLC patients, carriers of the T allele had a higher risk of death compared with those with the CC genotype (HR = 1.29; 95% CI, 1.08–1.55; ref. 52). Statistically significant associations were observed in males (HR = 1.15; 95% CI, 1.01–1.31) and smokers (HR = 1.17; 95% CI, 1.02–1.34; ref. 52). Differences in tumor subtype and ethnic groups might account for differences in results. Of concern, the positive findings of de Mello and colleagues (46) were based on unadjusted comparisons of Kaplan–Meier survival curves and, therefore, do not account for known differences in prognostic factors.

No statistically significant OS associations were observed for rs402710-T in North American (50) and European (46) NSCLC populations. Although the overall OS association was also absent in stratified analyses of data from the EPIC (European Prospective Investigation into Cancer and Nutrition) cohort, Xun and colleagues showed that rs402710-T was associated with lung cancer–specific mortality in current smokers (SHR = 1.21; 95% CI, 1.02–1.43), SCLC patients (SHR = 1.57; 95% CI, 1.09–2.25), and patients with unspecified lung cancer histology (HR = 1.31; 95% CI, 1.02–1.68; ref. 47).

Three studies investigated additional genetic variants in this chromosomal region. First, the Canadian study by Azad and colleagues investigated 8 genetic variants in 5p15.33 that were previously associated with lung cancer risk (50). In their predominantly Caucasian sample of 564 stage III/IV NSCLC patients, only rs4975616-A (*TERT-CLPTM1L*) was statistically significantly associated with OS (per allele HR = 0.75; 95% CI, 0.69–0.91), after adjusting for multiple comparisons and relevant covariates (50). Second, Catarino and colleagues (49) investigated one *TERT* polymorphism (rs2735940) in 226 Portuguese NSCLC patients and observed a statistically significant improvement in OS in carriers of the T allele (HR = 0.52; 95% CI, 0.35–0.77). In analyses stratified by histology, this association remained statistically

Table 1. Associations between 5p15.33 genetic variants and overall survival (OS) in lung cancer patients

SNP (gene)	Study (Ref)	Minor allele	Sample size (events)	Genetic model	Analysis	Effect estimate (95% CI)		*P*	Results
rs2735940 (*TERT*)	Catarino et al. (2010; ref. 49)	T	174 cases (58)	Recessive[a]	Cox regression	HR	0.52 (0.35–0.77)	0.001	Improved OS
rs2736098 (*TERT*)	Azad et al. (2014; ref. 50)	T	564 cases	Log additive	Cox regression	HR	1.14 (0.97–1.28)	0.14	Not associated
rs2736100 (*TERT*)	Li et al. (2014; ref. 52)	C	874 cases (521)	Log additive	Cox regression	HR	0.94 (0.83–1.07)	0.376	Not associated
rs2853677 (*TERT*)	Li et al. (2014; ref. 52)	G	874 cases (521)	Log additive	Cox regression	HR	0.92 (0.81–1.04)	0.184	Not associated
rs31489 (*CLPTM1L*)	De Mello et al. (2013; ref. 46)	A	144 cases	Genotypic	Log-rank test	—	—	0.029	Improved OS
				Dominant	Log-rank test	—	—	0.008	Improved OS
rs401681 (*CLPTM1L*)	Azad et al. (2014; ref. 50)	T	564 cases	Log additive	Cox regression	HR	0.92 (0.79–1.03)	0.09	Not associated
	Li et al. (2014; ref. 52)	T	874 cases (521)	Log additive	Cox regression	HR	1.14 (1.01–1.28)	0.036	Poorer OS
			CC: 209 cases (170)	Genotypic	Cox regression	HR	1.00		
			CT: 224 cases (136)				1.35 (1.11–1.63)	0.022	
			TT: 74 cases (41)				1.20 (0.92–1.57)	0.192	
			CC: 209 cases (170)	Dominant	Cox regression	HR	1.00		
			CT/TT: 298 cases (177)				1.29 (1.08–1.55)	0.005	
	De Mello et al. (2013; ref. 46)	T	144 cases	Genotypic	Log-rank test	—	—	0.046	Improved OS
				Dominant	Log-rank test	—	—	0.021	
rs402710 (*CLPTM1L*)	Azad et al. (2014; ref. 50)	T	564 cases	Log additive	Cox regression	HR	0.99 (0.87–1.11)	0.71	Not associated
	Xun et al. (2011; ref. 47)	T	1094 cases (874)	Log additive	Cox regression	HR	1.07 (0.97–1.19)	0.18	Not associated
			763 cases (537)	Log additive	Cox regression	HR	1.12 (0.98–1.28)	0.11	
			763 cases (537, 58)	Log additive	Cox regression	SHR	1.10 (0.97–1.26)	0.14	
	De Mello et al. (2013; ref. 46)	T	144 cases	Genotypic	Log-rank test	—	—	0.309	Not associated
				Dominant[b]	Log-rank test	—	—	0.958	
rs4635969 (*TERT-CLPTM1L*)	De Mello et al. (2013; ref. 46)	T	144 cases	Genotypic	Log-rank test	—	—	0.337	Not associated
				Dominant	Log-rank test	—	—	0.417	
rs465498 (*CLPTM1L*)	Li et al. (2014; ref. 52)	G	874 cases (521)	Log additive	Cox regression	HR	1.08 (0.90–1.28)	0.4148	Not associated
rs4975616 (*TERT-CLPTM1L*)	Azad et al. (2014; ref. 50)	A	564 cases	Log additive	Cox regression	HR	0.75 (0.69–0.91)	0.002	Improved OS

[a]rs2735940 (hTERT -1327 C/T): minor allele is C.

[b]In this study population, the minor allele was C; however, effects were estimated with respect to the T allele, with rs402710-CC as the reference category.

significant only among patients with nonsquamous (adenocarcinoma and other) tumors (HR = 0.46; 95% CI, 0.27–0.78; ref. 49). Third, De Mello and colleagues reported a significant (unadjusted) association with OS for rs31489 (46). For patients with nonsquamous NSCLC, the rs31489 AA and CA genotypes had longer survival than the rs31489 CC genotype: 13 months (range, 6.98–19.01), and 13 months (range, 6.33–19.66), compared with 6 months (range, 1.10–10.90), respectively (P = 0.029; ref. 46). None of these polymorphisms were evaluated in more than one study.

Disease recurrence and progression. Three studies investigated 5p15.33 polymorphisms with respect to PFS in stage III/IV patients (Table 2). There was some variation in the assessment of PFS among these studies. Specifically, the relevant time period started at diagnosis in the study by Azad and colleagues (50), while Zhao and colleagues (51) considered time only after the start of chemotherapy. This also reflects differences in the eligibility criteria, as Zhao and colleagues (51) only included patients who have undergone chemotherapy.

Both studies found rs401681 to be significantly associated with PFS. Azad and colleagues reported a decreased likelihood of cancer progression for carriers of the T allele (HR = 0.86; 95% CI, 0.76–0.99), and de Mello and colleagues observed significantly improved PFS (log-rank P = 0.021) in patients with the rs401681-TT genotype (7 months; range, 0.001–14.04), compared with CC (2 months; range, 0.95–3.05) and CT (5 months; range, 3.22–6.77) genotypes (46, 50). In addition, Azad and colleagues observed a significant association with PFS for rs4975616-A (HR = 0.74; 95% CI, 0.62–0.89; ref. 50).

A third study by Zhao and colleagues (51) reported inconsistent results for rs2736109, in which longer progression-free time was observed for GA genotypes (P = 0.023) compared with GG, but increased progression likelihood was observed for rs2736109-AA (HR = 1.32; 1.03–1.68). This pattern was more pronounced for individuals aged >58 (log-rank P = 0.007; HR = 0.65; 95% CI, 0.50–0.85; ref. 51).

Treatment response. Three studies investigated response to chemotherapy. Although treatment response was assessed using the RECIST guidelines (54), all studies operationalized their outcome variables differently (Table 3). Liang and colleagues (53) investigated 9 variants in 5p15.33, of which two *CLPTM1L* SNPs (rs401681-T, rs402710-T) were also investigated by De Mello and colleagues (46). However, the two populations were very different and were thus treated differently, albeit both using platinum-based multiagent chemotherapeutic regimens. De Mello and colleagues evaluated Portuguese stage III/IV NSCLC patients, including a subset positive for *EGFR* mutations treated with gefitinib, whereas Liang and colleagues examined extensive stage SCLC patients of Han Chinese descent. Neither study observed statistically significant associations with treatment response.

Zhao and colleagues reported associations with treatment response and grade 3 or 4 toxicity for rs4975605 and rs2736109 in stage III/IV NSCLC Han Chinese patients (51). Overall, rs4975605-CA genotype was associated with a higher likelihood of nonresponse (OR = 1.51; 95% CI, 1.01–2.25; P = 0.046), especially among females ($P = 1.57 \times 10^{-4}$), never-smokers ($P = 1.94 \times 10^{-4}$), never-smoking females ($P = 1.40 \times 10^{-4}$), and stage IV patients (P = 0.003). An increased, but not statistically

Table 2. Associations between 5p15.33 genetic variants and progression-free survival (PFS) or disease recurrence in lung cancer patients

SNP (gene)	Study (Ref)	Minor allele	Sample size (events)	Genetic model	Analysis	Effect estimate (95% CI)		P	Results
rs2736098 (*TERT*)	Azad et al. (2014; ref. 50)	T	564 cases	Log additive	Cox regression	HR	1.18 (0.98–1.34)	0.08	Not associated
rs2736109 (*TERT*)	Zhao et al. (2015; ref. 51)	A	896 cases (558 events)	Heterozygotic	Cox regression	HR	0.88 (0.74–1.06)	0.171	Not associated
					Log-rank test	–	–	0.023	Improved PFS[a]
				Homozygotic	Cox regression	HR	1.23 (0.95–1.60)	0.122	Not associated
					Log-rank test	–	–	0.013	Poorer PFS[b]
				Best fitting[c]	Cox regression	HR	1.32 (1.03–1.68)	0.026	Poorer PFS
rs31489 (*CLPTM1L*)	De Mello et al. (2013; ref. 46)	A	144 cases	Genotypic	Log-rank test	–	–	0.588	Not associated
				Dominant		–	–	0.449	
rs401681 (*CLPTM1L*)	Azad et al. (2014; ref. 50)	T	564 cases	Log additive	Cox regression	HR	0.86 (0.76–0.99)	0.04	Improved PFS
	De Mello et al. (2013; ref. 46)	T	144 cases	Genotypic	Log-rank test	–	–	0.021	Improved PFS[d]
				Dominant		–	–	0.332	Not associated
rs402710 (*CLPTM1L*)	Azad et al. (2014; ref. 50)	T	564 cases	Log additive	Cox regression	HR	0.92 (0.78–1.05)	0.12	Not associated
	De Mello et al. (2013; ref. 46)	T	144 cases	Genotypic	Log-rank test	–	–	0.269	Not associated
				Dominant[e]		–	–	0.654	
rs4635969 (*TERT-CLPTM1L*)	De Mello et al. (2013; ref. 46)	T	144 cases	Genotypic	Log-rank test	–	–	0.665	Not associated
				Dominant		–	–	0.445	
rs4975616 (*TERT-CLPTM1L*)	Azad et al. (2014; ref. 50)	A	564 cases	Log additive	Cox regression	HR	0.74 (0.62–0.89)	0.001	Improved PFS

[a]Longest median disease-free survival time was observed for rs2736109-GA individuals.
[b]Shortest median disease-free survival time was observed for rs2736109-AA individuals.
[c]Stratification analyses by patient characteristic subgroups were performed, and the most significant genetic model was assumed as the best-fitting model.
[d]Longest median disease-free survival time observed for rs401681-TT individuals.
[e]rs402710: In this study population, the minor allele was C; however, effects were estimated with respect to the T allele, with rs402710-CC as the reference category.

Table 3. Associations between 5p15.33 genetic variants and outcomes for first-line chemotherapy in lung cancer patients, including treatment response rate or clinical benefit based on RECIST guidelines, and severe toxicity[a] based on NCI Common Toxicity Criteria

SNP (gene)	Study (Ref)	Patient therapy	Outcome	Minor allele	Sample size (events)	Genetic model	Analysis	Effect estimate (95% CI)		*P*	Results
rs10073340 (*CLPTM1L*)	Liang et al. (2014; ref. 53)	Stage III/IV lung, cisplatin	Response rate: SD/PD vs. CR/PR	T	112 cases (35) CC: 100 cases (33) TC: 12 cases (2)	Log additive Genotypic	Logistic regression	OR OR	1.91 (0.39–9.36) 1 2.00 (0.39–10.3)	0.349 0.335	Not associated
rs2736109 (*TERT*)	Zhao et al. (2015; ref. 51)	Stage IIIA/IV NSCLC: cisplatin-Navelbine, cisplatin-gemcitabine, cisplatin-paclitaxel	Severe toxicity: gastrointestinal	A	GG: 388 cases (26) GA: 454 cases (45) AA: 122 cases (9)	Genotypic	Logistic regression	OR	1 1.68 (0.99–2.83) 1.27 (0.57–2.86)	 0.054 0.560	Not associated
			Severe toxicity: hematologic	A	GG: 388 cases (86) GA: 459 cases (124) AA: 122 cases (22)	Genotypic	Logistic regression	OR	1 1.29 (0.94–1.78) 0.78 (0.46–1.32)	 0.120 0.348	Not associated
			Severe toxicity: neutropenia	A	GG: 371 cases (45) GA: 446 cases (60) AA: 118 cases (10)	Genotypic	Logistic regression	OR	1 1.11 (0.73–1.68) 0.70 (0.34–1.46)	 0.641 0.344	Not associated
			Severe toxicity: anemia	A	GG: 376 cases (7) GA: 448 cases (18) AA: 120 cases (4)	Genotypic	Logistic regression	OR	1 1.90 (0.77–4.71) 2.05 (0.58–7.32)	 0.163 0.267	Not associated
			Severe toxicity: thrombocytopenia	A	GG: 377 cases (13) GA: 452 cases (16) AA: 121 cases (5)	Genotypic	Logistic regression	OR	1 1.05 (0.49–2.23) 1.12 (0.38–3.27)	 0.908 0.838	Not associated
rs31484 (*CLPTM1L*)	Liang et al. (2014; ref. 53)	Stage III/IV lung, cisplatin	Response rate: SD/PD vs. CR/PR	T	112 cases (35) AA: 88 cases (29) TA: 25 cases (6)	Log additive Genotypic	Logistic regression	OR OR	1.26 (0.46–3.44) 1 1.31 (0.45–3.81)	0.424 0.393	Not associated
rs31489 (*CLPTM1L*)	De Mello et al. (2013; ref. 46)	Advanced NSCLC: gefitinib (*EGFR*$^+$), platinum based (*EGFR*$^-$)	Response rate: undefined	A	CC: 48 cases CA: 71 cases AA: 25 cases CA+AA: 96 cases	Genotypic Dominant	Logistic regression	OR OR	1 0.41 (0.12–1.36) 0.63 (0.21–1.88) 0.57 (0.23–1.42)	— 	Not associated
rs380286 (*CLPTM1L*)	Liang et al. (2014; ref. 53)	Stage III/IV lung, cisplatin	Response rate: SD/PD vs. CR/PR	A	113 cases (15) GG: 90 cases (30) GA: 23 cases (5)	Log additive Genotypic	Logistic regression	OR OR	1.56 (0.53–4.57) 1 1.65 (0.53–5.13)	0.312 0.283	Not associated
rs401681 (*CLPTM1L*)	De Mello et al. (2013; ref. 46)	Advanced NSCLC: gefitinib (*EGFR*$^+$), platinum based (*EGFR*$^-$)	Response rate: undefined	T	CC: 40 cases CT: 77 cases TT: 27 cases CT+TT: 104 cases	Genotypic Dominant	Logistic regression	OR OR	1 1.49 (0.54–4.12) 2.15 (0.62–7.46) 1.64 (0.62–4.36)	— 	Not associated
	Liang et al. (2014; ref. 53)	Stage III/IV lung, cisplatin	Response rate: SD/PD vs. CR/PR	T	113 cases (35) CC: 57 cases (19) TC: 50 cases (15) TT: 6 cases (1) TC+TT: 56 cases (16)	Log additive Genotypic Dominant	Logistic regression	OR OR OR	1.22 (0.61–2.42) 1 1.11 (0.46–2.68) 2.39 (0.24–23.50) 1.19 (0.50–2.82)	0.477 0.666 0.584	Not associated
rs402710 (*CLPTM1L*)	De Mello et al. (2013; ref. 46)	Advanced NSCLC: gefitinib (*EGFR*$^+$), platinum based (*EGFR*$^-$)	Response rate: undefined	T	CC: 20 cases CT: 63 cases TT: 61 cases CT+TT: 104 cases	Genotypic Dominant[b]	Logistic regression	OR OR	1 1.06 (0.30–3.72) 0.59 (0.16–2.15) 0.81 (0.25–2.65)	— 	Not associated
	Liang et al. (2014; ref. 53)	Stage III/IV lung, cisplatin	Response rate: SD/PD vs. CR/PR	T	113 cases (15) CC: 58 cases (19) TC: 49 cases (15) TT: 6 cases (1) TC+TT: 56 cases (16)	Log additive Genotypic Dominant	Logistic regression	OR OR OR	1.19 (0.60–2.36) 1 1.05 (0.44–2.55) 2.33 (0.24–22.90) 1.13 (0.48–2.69)	0.539 0.693 0.673	Not associated
rs421629 (*CLPTM1L*)	Liang et al. (2014; ref. 53)	Stage III/IV lung, cisplatin	Response rate: SD/PD vs. CR/PR	T	113 cases (35) CC: 87 cases (29) TC: 26 cases (6)	Log additive Genotypic	Logistic regression	OR OR	1.46 (0.54–3.98) 1 1.55 (0.53–4.50)	0.355 0.321	Not associated

(Continued on the following page)

Table 3. Associations between 5p15.33 genetic variants and outcomes for first-line chemotherapy in lung cancer patients, including treatment response rate or clinical benefit based on RECIST guidelines, and severe toxicity[a] based on NCI Common Toxicity Criteria (Cont'd)

SNP (gene)	Study (Ref)	Patient therapy	Outcome	Minor allele	Sample size (events)	Genetic model	Analysis	Effect estimate (95% CI)		P	Results
rs451360 (*CLPTM1L*)	Liang et al. (2014; ref. 53)	Stage III/IV lung, cisplatin	Response rate: SD/PD vs. CR/PR	T	113 cases (35)	Log additive	Logistic regression	OR	1.11 (0.31–3.97)	0.987	Not associated
					GG: 100 cases (31)	Genotypic		OR	1	0.986	
					GT: 13 cases (4)				1.12 (0.30–4.19)		
rs4635969 (*TERT-CLPTM1L*)	De Mello et al. (2013; ref. 46)	Advanced NSCLC: gefitinib (*EGFR*$^{+}$), platinum based (*EGFR*$^{-}$)	Response rate: undefined	T	CC: 94 cases	Genotypic	Logistic regression	OR	1	—	Not associated
					CT: 44 cases				1.04 (0.43–2.52)		
					TT: 6 cases				1.92 (0.29–12.67)		
					CT+TT: 50 cases	Dominant		OR	1.13 (0.49–2.62)		
rs467095 (*CLPTM1L*)	Liang et al. (2014; ref. 53)	Stage III/IV lung, cisplatin	Response rate: SD/PD vs. CR/PR	G	109 cases (35)	Log additive	Logistic regression	OR	0.83 (0.34–2.04)	0.884	Not associated
					AA: 87 cases (29)	Genotypic		OR	1	0.173	
					AG: 17 cases (3)				1.95 (0.49–7.75)		
					GG: 5 cases (3)				0.33 (0.05–2.23)		
					AG+GG: 22 cases (6)	Dominant		OR	1.14 (0.38–3.42)	0.586	
rs4975605 (*TERT*)	Zhao et al. (2015; ref. 51)	Stage IIIA/IV NSCLC: cisplatin-Navelbine, cisplatin-gemcitabine, cisplatin-paclitaxel	Clinical benefit: PD vs. CR/PR/SD	A	CC: 795 cases (651)	Genotypic	Logistic regression	OR	1		
					CA: 168 cases (125)				1.51 (1.01–2.25)	0.046	↓ Response
					AA: 12 cases (11)				0.41 (0.05–3.26)	0.402	Not associated
					CA+AA: 180 cases (136)	Dominant		OR	1.42 (0.95–2.11)	0.085	
			Severe toxicity: gastrointestinal	A	CC: 784 cases (66)	Genotypic	Logistic regression	OR	1		Not associated
					CA: 168 cases (13)				0.85 (0.45–1.61)	0.615	
					AA: 12 cases (1)				0.86 (0.11–7.06)	0.892	
			Severe toxicity: hematologic		CC: 789 cases (195)	Genotypic	Logistic regression	OR	1		Not associated
					CA: 168 cases (32)				0.74 (0.48–1.13)	0.165	
					AA: 12 cases (5)				2.50 (0.77–8.12)	0.127	
			Severe toxicity: neutropenia		CC: 762 cases (101)	Genotypic	Logistic regression	OR	1		Not associated
					CA: 162 cases (13)				0.58 (0.32–1.07)	0.081	
					AA: 11 cases (1)				0.93 (0.12–7.57)	0.947	
			Severe toxicity: anemia		CC: 772 cases (23)	Genotypic	Logistic regression	OR	1		Not associated
					CA: 161 cases (5)				0.75 (0.25–2.23)	0.601	
					AA: 11 cases (1)				2.85 (0.32–25.84)	0.351	
			Severe toxicity: thrombocytopenia		CC: 777 cases (30)	Genotypic	Logistic regression	OR	1		Not associated
					CA: 162 cases (3)				0.38 (0.11–1.29)	0.122	
					AA: 11 cases (1)				1.30 (0.15–11.21)	0.814	
rs4975616 (*TERT*)	Liang et al. (2014; ref. 53)	Stage III/IV lung, cisplatin	RR: SD/PD vs. CR/PR	G	113 cases (35)	Log additive	Logistic regression	OR	1.46 (0.54–3.98)	0.355	Not associated
					AA: 87 cases (29)	Genotypic		OR	1	0.321	
					AG: 26 cases (6)				1.55 (0.53–4.50)		

Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

[a]Severe treatment toxicity defined as grade 3 or 4 toxicity based on NCI Common Toxicity Criteria v. 3.0.

[b]rs402710: In this study population, the minor allele was C; however, effects were estimated with respect to the T allele, with rs402710-CC as the reference category.

significant, risk of gastrointestinal toxicity was observed for rs2736109-GA (OR = 1.68; 95% CI, 0.99–2.83).

Telomere length

Study description and methods. Seven studies examined the prognostic role of TL in lung cancer, six of which were restricted to NSCLC (Supplementary Table S2). One cohort study did not report the histology distribution (48). Studies were varied in terms of their geographical locations and participant ethnicity. There were three studies conducted in Asian patients from Korea (55), Taiwan (56), and Japan (57), two North American studies with predominantly (>80%) Caucasian patients, and two European studies carried out in Denmark (48) and Spain (58). Two studies focused exclusively on PFS, three only assessed OS, and two studies examined both outcomes. All but one were observational studies. The exception was an open-label randomized phase II clinical trial of imetelstat, a telomerase inhibitor (59). The primary endpoint of this study was PFS among patients with nonprogressive, advanced NSCLC, after platinum-based doublet chemotherapy (59). In the context of this trial, TL was investigated as a predictive biomarker for imetelstat activity. The efficacy of therapy with imetelstat was examined in stratified analyses among patients grouped into the shortest 1/2, shortest 1/3, and shortest 1/4 of tumor TL (59).

Two of the six observational studies were cohorts investigating factors related to cancer risk and progression, where TL was measured in peripheral blood leukocytes (PBL). Weischer and colleagues evaluated lung cancer patients identified from a 20-year prospective follow-up of a population-based sample of 47,102 individuals in Denmark (48). Kim and colleagues collected information from 467 lung cancer patients enrolled at MD Anderson Cancer Center in Houston, Texas (60). PBLs are the most common tissue of choice for measuring TL in studies that involve cancer-free individuals and are considered to be an acceptable surrogate for TL in other tissues or organs of interest. In contrast, tumor TL can be more heterogeneous and reflect the cumulative impact of many tumor-associated factors that influence telomere homeostasis. Both studies using PBL collected samples prior to treatment, and Weischer and colleagues obtained blood prior to cancer diagnosis (48, 60). Therefore, the findings of these studies are unlikely to be confounded by treatment.

The remaining observational studies and clinical trial are best characterized as case series selected using hospital-based sampling, such as patients undergoing resection, who were subsequently followed up for disease recurrence and mortality. These five studies evaluated tumor specimens.

The method of DNA extraction was not reported in two studies. QIAamp was used in three studies (including both cohorts), and two studies used organic extraction with phenol/chloroform. Direct comparison of these methods (61) found that DNA isolated by organic extraction produced consistently similar TL results, whereas QIAamp produced shorter estimates for relative telomere length (RTL) and a restricted range of TL variance, suggesting that studies using QIAamp may be vulnerable to type II error.

There was variability in the methods used to measure TL, which represents an important source of heterogeneity between studies (62, 63). All approaches have their own limitations. Methods that start with genomic DNA include the Southern blot telomeric restriction fragment (TRF) analysis and qPCR. Another set of methods relies on FISH to detect telomere repeats in individual cells or chromosomes.

TRF was used in three studies. It analyzes TL either as a ratio of length in tumor samples to paired normal tissue or in absolute lengths. The most significant drawback of TRF is the requirement for substantial amounts of DNA (1.5–10 μg, minimum 105 cells), which often precludes its use in archival biopsy tissues, where DNA can be degraded or scarce (63, 64). TRF may slightly overestimate TL by including reads from the subtelomeric regions and has a lower sensitivity for very short telomeres, as there is a threshold below which a hybridization signal will not be produced (62, 63). However, despite these limitations, the measurement error of the TRF assay is low, making it a method of choice in many studies.

In studies where hundreds or thousands of blood samples require testing, q-PCR methods are the only high-throughput strategy available (63, 64). Although not as precise as TRF, a recent extension of the traditional qPCR, the monochrome multiplex q-PCR (MM-qPCR), has improved accuracy and allows absolute TL to be estimated (65). Four studies used q-PCR methods and two employed the MM-qPCR assay.

FISH methods, used in the imetelstat trial to validate qPCR techniques (59), can also provide highly accurate TL measurements within single cells; however, these assays require large amounts of viable DNA, requiring that samples be processed promptly after collection and making these methods sensitive to proper DNA collection and storage (63, 64). Similarly to TRF, because FISH methods use hybridization, there will be a threshold below which TL measurement will not be possible. This method was only feasible in the context of a prospective study or trial.

Overall survival. Associations differed across the three studies that investigated the role of TL in OS (Table 4). Two studies linked shorter TL to poor survival outcomes. A large population-based, prospective cohort from Denmark found that among 522 lung cancer patients, those experiencing increased telomere attrition had a higher risk of death (HR per 1,000 bp decrease in TL: 1.27; 95% CI, 1.13–1.43). This study did not examine associations by lung cancer histology or any other relevant subgroups. Similar results were observed by Jeon and colleagues in a study of tumor samples from 164 Korean NSCLC patients (55). Significantly higher mortality was observed for patients in the shortest quartile of RTL compared with all others (HR = 2.67; 95% CI, 1.50–4.75). This association was observed for both adenocarcinoma and squamous cell carcinoma (55). The association between shorter TL and poor survival outcomes was more pronounced for stage I (HR = 5.41; 95% CI, 2.40–12.2), compared with stage II–IIIA disease (HR = 1.51; 95% CI, 0.58–3.92), as well as among current smokers (HR = 3.28; 95% CI, 1.64–6.56).

In contrast, a study by Hsu and colleagues, which examined TL in paired tumor and normal tissues (T/N ratio) in 79 stage I–IV NSCLC patients, observed improved OS in individuals with shorter TL and higher mortality in those with longer or "maintained" TL, defined as T/N >0.75 (56). This study observed improved 4-year cumulative survival among patients with T/N $\leq$0.75 (69.2%), compared with T/N >0.75 (41.3%; $P = 0.0227$). Using T/N $\leq$0.75 as the reference category, they observed higher mortality among patients with TL maintenance (HR for T/N >0.75: 2.54; 95% CI, 1.21–5.32; ref. 56).

The study by Hirashima and colleagues adopted a hypothesis that extreme increases or decreases in TL in tumors when

Table 4. Association between TL and overall survival (OS) in lung cancer patients

Study [Ref]	Sample size (events)	Variable	Analysis	Effect estimate (95% CI)			*P*	Results
Weischer et al. (2013; ref. 48)	522 cases (468)	ATL – continuous (T/S)	Cox regression	HR	Per 1,000 bp decrease	1.27 (1.13–1.43)	—	Shorter TL → poorer OS
Jeon et al. (2012; ref. 55)	164 cases (58)	RTL – quartiles (T/S)	Cox regression	HR	4th:	1.00		Shorter TL → poorer OS
					3rd:	0.69 (0.31–1.53)	0.37	
					2nd:	0.73 (0.32–1.62)	0.43	
					1st:	2.14 (1.06–4.35)	0.03	
				HR	Others:	1.00		
					1st:	2.67 (1.50–4.75)	0.001	
Hsu et al. (2004; ref. 56)	79 cases	RTL – dichotomous (T/N)	Cox regression	RR	T/N≤0.75:	1.00	0.014	Maintained TL → poorer OS
					T/N>0.75:	2.54 (1.21–5.32)		
Hirashima et al. (2000; ref. 57)	72 cases	ATL – dichotomous (outside normal range)	Cox regression	HR	Normal	1.00	0.0033	Abnormal TL → poorer OS
					Altered	3.046 (1.46–6.36)		
Chiappori et al. (2014; ref. 59)	19 cases (15)	RTL – dichotomous (T/S) Short: 33rd percentile	Cox regression	HR	Control	1.00	—	Not associated
					Treatment	0.41 (0.11–1.46)		
	38 cases (31)	RTL – dichotomous (T/S) Long: 66th percentile		HR	Control	1.00	—	
					Treatment	0.51 (0.20–1.28)		
	59 cases	ATL – dichotomous Short: 33rd percentile		HR	Control	1.00	—	Not associated
					Treatment	0.44 (0.11–1.87)		
		ATL – dichotomous Long: 66th percentile		HR	Control	1.00	—	
					Treatment	0.58 (0.25–1.36)		

Abbreviations: RTL, relative telomere length; T/N, ratio of TL in tumor samples vs. paired normal tissue; T/S, ratio of telomere copy number vs. single-gene copy number.

compared with normal tissues are associated with poorer OS. This study used TRF to compare TL in tumor and paired normal tissues of 72 stage I–III NSCLC patients (57). In normal tissues, TL expressed as mean ± standard deviation was 6.2 ± 1.1 kb (57). The authors defined bidirectional TL alterations as TL outside of the mean ± 2 SD range of values, based on the distribution of TL observed in normal tissues (8.4 kb to 4.0 kb). Significantly shorter survival durations were observed in the 25 patients (34.7%) with TL alterations, confirmed on multivariate analysis, demonstrating poorer OS among patients with altered TL (HR = 3.05; 95% CI, 1.46–6.36; ref. 57).

The telomerase inhibitor trial examined PFS in patients stratified by TL (59). Although these differences did not reach statistical significance, the effect of imetelstat on preventing cancer recurrence was largest among patients in the 1/3 shortest TL group (HR = 0.43; 95% CI, 0.14–1.3). The median PFS in the short TL group treated with imetelstat increased to 1.9 months, compared with 1.5 months in the control arm (59). Patients with short TL who were treated with imetelstat were the only group where the cumulative survival rate remained above 50%.

Disease recurrence and progression. Definitions of PFS were very similar among the three observational studies of TL, although only Kim and colleagues (60) specified which sites were included in the recurrence definition. All three studies reported statistically significant associations between TL and cancer recurrence; however, the HRs were in opposing directions (Table 5). A prospective follow-up of 473 NSCLC patients found that longer leukocyte TL was associated with a higher likelihood of recurrence (HR = 1.75; 95% CI, 0.96–3.22; ref. 60). Although the main effects analysis did not reach statistical significance, the HR for longer TL was statistically significant for females with adenocarcinoma (HR = 2.67; 95% CI, 1.19–6.03; ref. 60). In contrast, studies using tumor DNA found telomere shortening to be associated with cancer recurrence (43, 46). Frias and colleagues measured TL using TRF in 77 Spanish NSCLC patients and observed a higher likelihood of recurrence among patients with shorter TL (HR = 1.89; 95% CI, 1.15–3.10; ref. 58). Jeon and colleagues observed poorer PFS among patients in the lowest quartile of tumor TL (HR = 1.92; 95% CI, 1.17–3.14; ref. 55). Similar to the findings for OS, the likelihood of cancer recurrence was greater among stage I (HR = 2.71; 95% CI, 1.39–5.29) compared with stage II–IIIA (HR = 1.31; 95% CI, 0.60–2.84) patients and smokers (HR = 2.30; 95% CI, 1.27–4.15; ref. 55).

The imetelstat trial did not observe any statistically significant differences in PFS between short and long TL strata (59). However, similar to findings for OS, there appeared to be a PFS trend toward a numerically larger treatment benefit among patients with short TL (HR = 0.43; 95% CI, 0.14–1.30), compared with those with medium/long TL (HR = 0.86; 95% CI, 0.39–1.88; ref. 59).

Discussion

Lung cancer contributes to nearly 20% of all cancer deaths (1, 2). Advances in molecular biology and epidemiology can identify accurate and reliable biomarkers and improve stratification of the patients into more precise groups to assist in selection of optimal treatment modalities, as have been seen in the case of *EGFR* mutations and *ALK* rearrangements. Targets of interest currently include *KRAS*, *PIK3CA*, *ROS1*, and *BRAF* mutations (66, 67). TL has been an area of growing interest, as new agents are being evaluated targeting telomerase (59, 68, 69), while the 5p15.33 region, known to be important in lung cancer development, contains an important telomerase-regulatory gene, *TERT*. In addition, *CLPTM1L*, located in the same genetic region, has been associated with cisplatin-induced apoptosis (23). Cisplatin and platinum agents have been the cornerstone of chemotherapeutic management of lung cancers, even though only a third of patients have tumor shrinkage with its use (70). Thus, the prognostic and predictive role of TL and 5p15.33 region polymorphisms on survival and response to platinum-based therapy is of great interest. Our synthesis of current evidence for lung cancer

Table 5. Association between TL and progression-free survival (PFS) or disease recurrence in lung cancer patients

Study (Ref)	Sample size (Events)	Variable	Analysis		Effect estimate (95% CI)		*P*	Results
Kim et al. (2014; ref. 60)	473 cases (151)	RTL – continuous (T/S)	Cox regression	HR	Per 1 unit increase	1.75 (0.96–3.22)	0.07	Longer TL → poorer PFS
Jeon et al. (2012; ref. 55)	164 cases (81)	RTL – quartiles (T/S)	Cox regression	HR	4th:	1.00		Shorter TL → poorer PFS
					3rd:	1.06 (0.55–2.08)	0.85	
					2nd:	1.14 (0.59–2.21)	0.69	
					1st:	1.97 (1.04–3.74)	0.03	
				HR	Others:	1.00		
					1st:	1.92 (1.17–3.14)	0.01	
Frias et al. (2007; ref. 58)	77 cases	RTL - dichotomous (T/N)	Cox regression	RR	T/N≥1:	1.00	0.012	Shorter TL → poorer PFS
					T/N<1:	1.887 (1.147–3.102)		
Chiappori et al. (2014; ref. 59)	19 cases (10)	RTL – dichotomous (T/S) Short: 33rd percentile	Cox regression	HR	Control	1.00	—	Not associated
					Treatment	0.43 (0.14–1.30)		
	38 cases (21)	RTL – dichotomous (T/S) Long: 66th percentile		HR	Control	1.00	—	
					Treatment	0.86 (0.39–1.88)		
	59 cases total	ATL – dichotomous Short: 33rd percentile		HR	Control	1.00	—	Not associated
					Treatment	0.45 (0.14–1.48)		
		ATL – dichotomous Long: 66th percentile		HR	Not reported	—	-	

Abbreviations: ATL, absolute telomere length)); T/N, ratio of TL in tumor samples vs. paired normal tissue; T/S, ratio of telomere copy number vs. single-gene copy number.

uncovers some promising leads and demonstrates potential clinical applications of telomere length and 5p15.33 genetic profiles.

Of the studies focusing on genetic variants in the 5p15.33 region, significant associations with both OS and PFS were reported for rs4975616-A (*TERT-CLPTM1L*) and rs401681-T (*CLPTM1L*). However, although rs4975616-A was only investigated in one study (50), the evidence across three studies of rs401681-T was conflicting. One study reported poorer OS among SCLC patients (52), another observed improved OS for NSCLC (46), while a third study of rs401681-T in NSCLC patients reported no association (50). Variants that were predictive of OS in at least one study included rs2735940-T (*TERT*) and rs402710-T (*CLPTM1L*). In addition, rs2736109-A (*TERT*) was associated with PFS. Although only two SNPs, rs401681 and rs402710-T, were analyzed in more than one study, most of the variants discussed in this review are located in a 62-kb linkage disequilibrium (LD) block, including the 5′-end of *TERT*, its promoter, and the entirety of *CLPTM1L*. Both rs402710-C and rs4975616-A are in strong LD with rs401681-C in European ($R^2 = 1.0$ and $R^2 = 0.87$) and Chinese and Japanese ($R^2 = 0.88$ and $R^2 = 0.42$) populations.

Although the findings for 5p15.33 variants are sparse and conflicting, partly due to a limited number of studies with modest sample sizes, the biological significance of TERT/CLPTM1L and the genetic architecture of this region suggest that 5p15.33 may harbor other variants that could play a role in OS and PFS, as this region appears to be under strong evolutionary constraint and shows relatively little common genetic variation (71). Some functional evidence exists for rs2736109 and rs2735940, both in the *TERT* promoter region. rs2736109 is localized in GATA-2 transcription factor–binding site, and rs2735940 is associated with higher transcriptional and telomerase activity and longer TL (51, 72). rs401681 has also been associated with TL, but not with *TERT* activity (73). These observations point to *TERT* activity and TL regulation as potentially relevant mechanisms in mediating the associations with clinical outcomes in lung cancer.

Our review suggests that the relationship between TL and prognosis is complex and nonlinear. First, differences in the underlying biological processes that are reflected in tumor and blood TL make it challenging to synthesize the associations across these studies. It remains unclear whether the TL abnormalities that are observed in blood are reflective of underlying genetic susceptibility or arise as a consequence of the carcinogenic process, or possibly both. The type of telomere dysfunction that is a more robust predictor of clinical outcomes may also vary by tumor histology and tissue under investigation. Studies measuring leukocyte TL using qPCR offered somewhat conflicting messages, but inconsistencies were also observed among the studies investigating tumor TL measured using the Southern blot TRF assay. Thus, contrasting findings may not be easily explained by differences in tissue or method of TL measurement.

Second, contrasting results highlight potential different mechanisms acting under difference circumstances. Several studies hypothesize that telomeric shortening promotes genomic instability. With the concurrent inactivation of key tumor suppressor genes, such as *p53* and/or *p16/Rb*, a cellular environment may be created that facilitates the acquisition of necessary properties for metastasis and recurrent disease. Conclusions from the PBL study of Weischer and colleagues (48) and tumor studies by Jeon and colleagues (55) and Frias and colleagues (58) are consistent with shorter TL being associated with shorter OS and PFS.

In contrast, longer TL may reflect telomerase reactivation, which promotes tumor growth and immortalization. Therefore, longer TL may be a marker for the acquired enhanced proliferative and survival capacity of malignant cells, which would in turn translate to more aggressive disease. Longer TL may allow for more actively reproducing cells, leading to an accumulation of mutations affecting apoptosis and senescence pathways, and increase the number of viable tumor cells (60, 74). In addition, the sustained replication of unstable tumor cells with longer TL may allow for somatic evolution, such as the acquisition of mutations that contribute to the emergence of clones with enhanced capacity for invasion and metastasis (43, 75, 76). This theory fits with the results reported by the Caucasian-predominant study of Kim and colleagues (60), particularly in females with adenocarcinoma, and in the study of Chinese patients in Hsu and colleagues (56), which also observed adverse survival outcomes in patients with a higher T/N ratio (>0.75).

To complicate matters, *EGFR* mutations and *ALK* rearrangements are found in higher proportions in never-smoking patients, while *EGFR* mutations are associated with being female, of East Asian descent, and having adenocarcinoma (67, 77, 78). Could mechanisms driven by different mutations, such as *EGFR*, be responsible for differences in the opposing relationships between TL and OS? This hypothesis is supported by the observation that EGF activates telomerase through upregulation of *TERT* transcription (79); however, there are no studies directly investigating TL and *TERT* in specific subgroups of patients by mutational status. The bidirectional findings in Japanese patients by Hirashima and colleagues (57) and decreased OS and PFS in Korean smokers by Jeon and colleagues (55) are consistent with this theory of alternative mechanisms; however, these studies did not report *EGFR* or *KRAS* mutation status. In fact, of all the publications reviewed here, only de Mello and colleagues (46) and Chiappori and colleagues (59) reported the number of patients positive for EGFR or KRAS mutations; however, associations with TL were not investigated in these subgroups.

Therefore, it is possible that different aspects of telomere dysfunction may be driven by somatic molecular alterations. Telomere elongation may be an indicator of the immortality of tumor cells (a finding intriguing as it was found in subsets of adenocarcinomas, females, and populations of East Asian descent, where *EGFR* mutations are common), whereas extremely shortened TL in malignant cells may be a marker for aggressive tumors marked by increased genomic instability, where genomic instability is a hallmark of smoking-related lung cancer (43, 80). Data from The Cancer Genome Atlas and other sources support the contention that smoking-related lung cancers have many-fold increases in their mutational landscape, when compared with those with *EGFR* mutations (67, 77).

Also intriguing are the observations from the telomerase inhibitor trial (59). Although this small sample analysis should be considered a negative result given a lack of statistical significance, the results are suggestive of a greater clinical benefit from telomerase inhibition in patients with tumors possessing shorter TL, in a sample with unknown smoking prevalence but a low prevalence (10%) of *EGFR*-positive patients. Furthermore, as telomerase preferentially elongates shorter telomeres (81), it is plausible that blocking its activity confers a larger benefit in the short TL subgroup.

However, despite promising leads offered by the studies in this review, there are several important methodologic caveats that should be acknowledged. First, low statistical power is one of the most significant limitations shared by the studies in this review. With the exception of three reports (47, 48, 52), most lacked sufficient power to detect modest associations with OS and PFS that would be expected for common genetic variants and TL. Second, although studies of candidate polymorphisms had the benefit of being hypothesis driven and limited the number of comparisons, this approach may miss associations for rare and low-frequency variants, and those that were not chosen based on previous GWAS of lung cancer risk. Third, strong and consistent evidence across multiple studies, and validation in different patient subpopulations with respect to stage, histology, and ethnicity, smoking status, and mutational status, is still lacking in the literature linking genetic variants to clinical outcomes in lung cancer. Fourth, functional studies are also needed to help characterize the specific biological pathways through which these SNPs may impact lung cancer recurrence and survival.

For studies of TL, additional methodologic concerns require discussion. First, no attempts were made to refine the associations by cell type, despite the fact that leukocyte TL is an average across all immune cell subpopulations present in blood and may obscure important differences in their TL dynamics. Second, there is no accepted range of normal TL in healthy tissues; therefore, tumor studies that use the T/N ratio and define TL alterations based on departure from values observed in one group may not be applicable to other patient populations. Third, using the T/N ratio to predict survival outcomes may not take into account molecular features of the tumor that could be effect modifiers of the relationship between TL and outcome (55). Fourth, low power in small case series may be exacerbated by methods of TL measurement that have low sensitivity and are prone to measurement error. Finally, the impact of accelerated telomere attrition resulting from the psychologic impact of a cancer diagnosis should be considered, as TL measured after diagnosis may also reflect an increased emotional and physical burden (80, 82).

Future studies can be improved with the use of repeated measurements to better ascertain temporal changes in TL that are associated with clinical outcomes. The presence of intratumor heterogeneity resulting from diverse evolution processes (83, 84) also suggests that examining TL in multiple tissue samples may be informative. Other methodologic concerns include inconsistent reporting of basic characteristics, such as smoking status, ethnicity, as well clinically relevant markers and mutation profiles. None of the genetic association studies carried out an analytic adjustment for the underlying population structure and instead relied on self-reported ethnicity or inferred ethnicity based on patient residence. Although confounding by population stratification is more important for genetic association studies, this should also be considered for studies of TL, as it may vary between ethnic groups (85).

Taken together, the current body of literature offers some encouraging, yet inconsistent findings that continue to support interest in 5p15.33 genetic variants and telomere length as biomarkers with potential clinical utility, not only for prognostication, but also as potential lung cancer treatment targets. However, the limited number of studies and their methodologic limitations leave more unanswered questions than firm conclusions and highlight the need for larger, carefully designed studies with clinically defined subpopulations of lung cancer patients and refined, high-resolution genetic analyses. Leveraging information on relevant biological pathways and telomere function to disentangle complex associations will enhance our ability to uncover findings that may be translated into improved prognostic assessment and treatment approaches. By first studying the associations across specific tumor sites, such as lung cancer, one can further understand the potential interactions between these biomarkers with clinical, demographic, and molecular factors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

L. Kachuri is a trainee in the Canadian Institutes of Health Research (CIHR) Strategic Training for Advanced Genetic Epidemiology (STAGE) Program and is supported by the CIHR Doctoral Research Award: Frederick Banting and Charles Best Canada Graduate Scholarships (GSD-137441).

Received March 10, 2016; revised July 20, 2016; accepted August 17, 2016; published OnlineFirst August 26, 2016.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69–90.
2. Canadian Cancer Society's Advisory Committee on Cancer Statistics. Canadian Cancer Statistics 2015. Toronto, Canada: Canadian Cancer Society; 2015.
3. Horgan AM, Yang B, Azad AK, Amir E, John T, Cescon DW, et al. Pharmacogenetic and germline prognostic markers of lung cancer. J Thorac Oncol 2011;6:296–304.
4. Blackburn EH. The molecular structure of centromeres and telomeres. Annu Rev Biochem 1984;53:163–94.
5. Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, et al. A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. Proc Natl Acad Sci USA 1988;85: 6622–6.
6. Levy MZ, Allsopp RC, Futcher AB, Greider CW, Harley CB. Telomere end-replication problem and cell aging. J Mol Biol 1992;225:951–60.
7. Hayflick L. The limited *in vitro* lifetime of human diploid cell strains. Exp Cell Res 1965;37:614–36.
8. Hiyama K, Hirai Y, Kyoizumi S, Akiyama M, Hiyama E, Piatyszek MA, et al. Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. J Immunol 1995;155:3711–5.
9. Liu K, Schoonmaker MM, Levine BL, June CH, Hodes RJ, Weng NP. Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes. Proc Natl Acad Sci USA 1999;96: 5147–52.
10. Xu L, Li S, Stohr BA. The role of telomere biology in cancer. Annu Rev Pathol 2013;8:49–78.
11. Low KC, Tergaonkar V. Telomerase: central regulator of all of the hallmarks of cancer. Trends Biochem Sci 2013;38:426–34.
12. Newbold RF. The significance of telomerase activation and cellular immortalization in human cancer. Mutagenesis 2002;17:539–50.
13. Heaphy CM, Subhawong AP, Hong SM, Goggins MG, Montgomery EA, Gabrielson E, et al. Prevalence of the alternative lengthening of telomeres telomere maintenance mechanism in human cancer subtypes. Am J Pathol 2011;179:1608–15.
14. 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.
15. Prescott J, Kraft P, Chasman DI, Savage SA, Mirabello L, Berndt SI, et al. Genome-wide association study of relative telomere length. PLoS One 2011;6:e19635.
16. 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 USA 2010;107:9293–8.
17. McKay JD, Hung RJ, Gaborieau V, Boffetta P, Chabrier A, Byrnes G, et al. Lung cancer susceptibility locus at 5p15.33. Nat Genet 2008;40:1404–6.
18. Landi MT, Chatterjee N, Yu K, Goldin LR, Goldstein AM, Rotunno M, et al. A genome-wide association study of lung cancer identifies a region of chromosome 5p15 associated with risk for adenocarcinoma. Am J Hum Genet 2009;85:679–91.
19. Choi J, Southworth LK, Sarin KY, Venteicher AS, Ma W, Chang W, et al. TERT promotes epithelial proliferation through transcriptional control of a Myc- and Wnt-related developmental program. PLoS Genet 2008;4:e10.
20. Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. Cell 2012;149:1192–205.
21. Ghosh A, Saginc G, Leow SC, Khattar E, Shin EM, Yan TD, et al. Telomerase directly regulates NF-kappaB-dependent transcription. Nat Cell Biol 2012;14:1270–81.
22. Smith LL, Coller HA, Roberts JM. Telomerase modulates expression of growth-controlling genes and enhances cell proliferation. Nat Cell Biol 2003;5:474–9.
23. Yamamoto K, Okamoto A, Isonishi S, Ochiai K, Ohtake Y. A novel gene, CRR9, which was up-regulated in CDDP-resistant ovarian tumor cell line, was associated with apoptosis. Biochem Biophys Res Commun 2001;280:1148–54.
24. Colangelo D, Osella D. Telomerase inhibition and cancer: might platinum based drugs have a future as anti-telomerase pharmacological approach? Curr Med Chem 2005;12:3091–102.
25. Mese H, Ueyama Y, Suzuki A, Nakayama S, Sasaki A, Hamakawa H, et al. Inhibition of telomerase activity as a measure of tumor cell killing by cisplatin in squamous cell carcinoma cell line. Chemotherapy 2001;47: 136–42.
26. Furuta M, Nozawa K, Takemura M, Izuta S, Murate T, Tsuchiya M, et al. A novel platinum compound inhibits telomerase activity in vitro and reduces telomere length in a human hepatoma cell line. Int J Cancer 2003;104: 709–15.
27. Mirjolet C, Boidot R, Saliques S, Ghiringhelli F, Maingon P, Crehange G. The role of telomeres in predicting individual radiosensitivity of patients with cancer in the era of personalized radiotherapy. Cancer Treat Rev 2015;41:354–60.
28. Rode L, Nordestgaard BG, Bojesen SE. Peripheral blood leukocyte telomere length and mortality among 64,637 individuals from the general population. J Natl Cancer Inst 2015;107:djv074.
29. Babizhayev MA, Savel'yeva EL, Moskvina SN, Yegorov YE. Telomere length is a biomarker of cumulative oxidative stress, biologic age, and an independent predictor of survival and therapeutic treatment requirement associated with smoking behavior. Am J Ther 2011;18:e209–26.
30. Strandberg TE, Strandberg AY, Saijonmaa O, Tilvis RS, Pitkala KH, Fyhrquist F. Association between alcohol consumption in healthy midlife and telomere length in older men. The Helsinki Businessmen Study. Eur J Epidemiol 2012;27:815–22.
31. Zhang X, Lin S, Funk WE, Hou L. Environmental and occupational exposure to chemicals and telomere length in human studies. Occup Environ Med 2013;70:743–9.
32. Needham BL, Adler N, Gregorich S, Rehkopf D, Lin J, Blackburn EH, et al. Socioeconomic status, health behavior, and leukocyte telomere length in the National Health and Nutrition Examination Survey, 1999–2002. Soc Sci Med 2013;85:1–8.
33. Needham BL, Mezuk B, Bareis N, Lin J, Blackburn EH, Epel ES. Depression, anxiety and telomere length in young adults: evidence from the National Health and Nutrition Examination Survey. Mol Psychiatry 2015;20:520–8.
34. Latifovic L, Peacock SD, Massey TE, King WD. The influence of alcohol consumption, cigarette smoking, and physical activity on leukocyte telomere length. Cancer Epidemiol Biomarkers Prev 2016;25:374–80.
35. Bisoffi M, Heaphy CM, Griffith JK. Telomeres: prognostic markers for solid tumors. Int J Cancer 2006;119:2255–60.
36. Svenson U, Roos G. Telomere length as a biological marker in malignancy. Biochim Biophys Acta 2009;1792:317–23.
37. Zhang C, Chen X, Li L, Zhou Y, Wang C, Hou S. The association between telomere length and cancer prognosis: evidence from a meta-analysis. PLoS One 2015;10:e0133174.
38. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. J Clin Epidemiol 2009;62:1006–12.
39. Quinlan J, Tu MT, Langlois EV, Kapoor M, Ziegler D, Fahmi H, et al. Protocol for a systematic review of the association between chronic stress during the life course and telomere length. Syst Rev 2014;3:40.
40. Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von Elm E, et al. Strengthening the reporting of genetic association studies (STREGA)–an extension of the STROBE statement. Genet Epidemiol 2009;33:581–98.
41. Gallo V, Egger M, McCormack V, Farmer PB, Ioannidis JP, Kirsch-Volders M, et al. STrengthening the Reporting of OBservational studies in Epidemiology–Molecular Epidemiology STROBE-ME: an extension of the STROBE statement. J Clin Epidemiol 2011;64:1350–63.
42. Schulz KF, Altman DG, Moher D; CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. BMJ 2010;340:c332.
43. Shirotani Y, Hiyama K, Ishioka S, Inyaku K, Awaya Y, Yonehara S, et al. Alteration in length of telomeric repeats in lung cancer. Lung Cancer 1994;11:29–41.
44. Hirashima T, Komiya T, Nitta T, Kobayashi M, Kawase I. [Prognostic significance of alterations in telomeric repeat length in patients with pathological stage I-II non-small cell lung cancer]. Nihon Rinsho 1998;56:1264–71.
45. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94:496–509.
46. de Mello RA, Ferreira M, Soares-Pires F, Costa S, Cunha J, Oliveira P, et al. The impact of polymorphic variations in the 5p15, 6p12, 6p21 and 15q25 Loci on the risk and prognosis of Portuguese patients with non-small cell lung cancer. PLoS One 2013;8:e72373.

47. Xun WW, Brennan P, Tjonneland A, Vogel U, Overvad K, Kaaks R, et al. Single-nucleotide polymorphisms (5p15.33, 15q25.1, 6p22.1, 6q27 and 7p15.3) and lung cancer survival in the European prospective investigation into cancer and nutrition (EPIC). Mutagenesis 2011; 26:657–66.
48. Weischer M, Nordestgaard BG, Cawthon RM, Freiberg JJ, Tybjaerg-Hansen A, Bojesen SE. Short telomere length, cancer survival, and cancer risk in 47102 individuals. J Natl Cancer Inst 2013;105:459–68.
49. Catarino R, Araujo A, Coelho A, Gomes M, Nogueira A, Lopes C, et al. Prognostic significance of telomerase polymorphism in non-small cell lung cancer. Clin Cancer Res 2010;16:3706–12.
50. Azad AK, Qiu X, Boyd K, Kuang Q, Emami M, Perera N, et al. A genetic sequence variant (GSV) at susceptibility loci of 5p15.33 (TERT-CLPTM1L) is associated with survival outcome in locally advanced and metastatic non-small-cell lung cancer (NSCLC). Lung Cancer 2014;84:289–94.
51. Zhao X, Wang S, Wu J, Li X, Wang X, Gao Z, et al. Association of TERT polymorphisms with clinical outcome of non-small cell lung cancer patients. PLoS One 2015;10:e0129232.
52. Li D, Wei L, Xu B, Yu D, Chang J, Yuan P, et al. Association of GWAS-Identified lung cancer susceptibility loci with survival length in patients with small-cell lung cancer treated with platinum-based chemotherapy. PLoS One 2014;9:e113574.
53. Liang Y, Thakur A, Gao L, Wang T, Zhang S, Ren H, et al. Correlation of CLPTM1L polymorphisms with lung cancer susceptibility and response to cisplatin-based chemotherapy in a Chinese Han population. Tumour Biol 2014;35:12075–82.
54. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
55. Jeon HS, Choi YY, Choi JE, Lee WK, Lee E, Yoo SS, et al. Telomere length of tumor tissues and survival in patients with early stage non-small cell lung cancer. Mol Carcinog 2014;53:272–9.
56. Hsu CP, Miaw J, Shai SE, Chen CY. Correlation between telomerase expression and terminal restriction fragment length ratio in non-small cell lung cancer–an adjusted measurement and its clinical significance. Eur J Cardiothorac Surg 2004;26:425–31.
57. Hirashima T, Komiya T, Nitta T, Takada Y, Kobayashi M, Masuda N, et al. Prognostic significance of telomeric repeat length alterations in pathological stage I-IIIA non-small cell lung cancer. Anticancer Res 2000;20:2181–7.
58. 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.
59. Chiappori AA, Kolevska T, Spigel DR, Hager S, Rarick M, Gadgeel S, et al. A randomized phase II study of the telomerase inhibitor imetelstat as maintenance therapy for advanced non-small cell lung cancer. Ann Oncol 2015;26:354–62.
60. Kim ES, Ye Y, Vaporciyan AA, Xing J, Huang M, Gu J, et al. Telomere length and recurrence risk after curative resection in patients with early-stage non-small cell lung cancer: a prospective cohort study. J Thorac Oncol 2015;10:302–8.
61. Cunningham JM, Johnson RA, Litzelman K, Skinner HG, Seo S, Engelman CD, et al. Telomere length varies by DNA extraction method: implications for epidemiologic research. Cancer Epidemiol Biomarkers Prev 2013;22: 2047–54.
62. Baird DM. New developments in telomere length analysis. Exp Gerontol 2005;40:363–8.
63. Aubert G, Hills M, Lansdorp PM. Telomere length measurement-caveats and a critical assessment of the available technologies and tools. Mutat Res 2012;730:59–67.
64. Kimura M, Stone RC, Hunt SC, Skurnick J, Lu X, Cao X, et al. Measurement of telomere length by the Southern blot analysis of terminal restriction fragment lengths. Nat Protoc 2010;5:1596–607.
65. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res 2009;37:e21.
66. Johnson DH, Schiller JH, Bunn PAJrRecent clinical advances in lung cancer management. J Clin Oncol 2014;32:973–82.
67. The Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. Nature 2014;511:543–50.
68. Williams SC. No end in sight for telomerase-targeted cancer drugs. Nat Med 2013;19:6.
69. Ruden M, Puri N. Novel anticancer therapeutics targeting telomerase. Cancer Treat Rev 2013;39:444–56.
70. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, et al. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002;346:92–8.
71. Savage SA, Stewart BJ, Eckert A, Kiley M, Liao JS, Chanock SJ. Genetic variation, nucleotide diversity, and linkage disequilibrium in seven telomere stability genes suggest that these genes may be under constraint. Hum Mut 2005;26:343–50.
72. Matsubara Y, Murata M, Yoshida T, Watanabe K, Saito I, Miyaki K, et al. Telomere length of normal leukocytes is affected by a functional polymorphism of hTERT. Biochem Biophys Res Commun 2006;341:128–31.
73. Rafnar T, Sulem P, Stacey SN, Geller F, Gudmundsson J, Sigurdsson A, et al. Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. Nat Genet 2009;41:221–7.
74. Hackett JA, Greider CW. Balancing instability: dual roles for telomerase and telomere dysfunction in tumorigenesis. Oncogene 2002;21:619–26.
75. Albertson DG, Collins C, McCormick F, Gray JW. Chromosome aberrations in solid tumors. Nat Genet 2003;34:369–76.
76. Chin L, Artandi SE, Shen Q, Tam A, Lee SL, Gottlieb GJ, et al. p53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. Cell 1999;97:527–38.
77. Govindan R, Ding L, Griffith M, Subramanian J, Dees ND, Kanchi KL, et al. Genomic landscape of non-small cell lung cancer in smokers and never-smokers. Cell 2012;150:1121–34.
78. Xu J, He J, Yang H, Luo X, Liang Z, Chen J, et al. Somatic mutation analysis of EGFR, KRAS, BRAF and PIK3CA in 861 patients with non-small cell lung cancer. Cancer Biomark 2011;10:63–9.
79. Maida Y, Kyo S, Kanaya T, Wang Z, Yatabe N, Tanaka M, et al. Direct activation of telomerase by EGF through Ets-mediated transactivation of TERT via MAP kinase signaling pathway. Oncogene 2002;21:4071–9.
80. Prescott J, Wentzensen IM, Savage SA, De Vivo I. Epidemiologic evidence for a role of telomere dysfunction in cancer etiology. Mutat Res 2012;730:75–84.
81. Britt-Compton B, Capper R, Rowson J, Baird DM. Short telomeres are preferentially elongated by telomerase in human cells. FEBS Lett 2009;583:3076–80.
82. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, et al. Accelerated telomere shortening in response to life stress. Proc Natl Acad Sci USA 2004;101:17312–5.
83. de Bruin EC, McGranahan N, Mitter R, Salm M, Wedge DC, Yates L, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. Science 2014;346:251–6.
84. Zhang J, Fujimoto J, Zhang J, Wedge DC, Song X, Zhang J, et al. Intratumor heterogeneity in localized lung adenocarcinomas delineated by multi-region sequencing. Science 2014;346:256–9.
85. Hunt SC, Chen W, Gardner JP, Kimura M, Srinivasan SR, Eckfeldt JH, et al. Leukocyte telomeres are longer in African Americans than in whites: the National Heart, Lung, and Blood Institute Family Heart Study and the Bogalusa Heart Study. Aging Cell 2008;7:451–8.