Association between leukocyte telomere length and the risk of pancreatic cancer: Findings from a prospective study

Hung N. Luu¹,2*, Joyce Y. Huang¹, Renwei Wang¹, Jennifer Adams-Haduch¹, Aizhen Jin³, Woon-Puay Koh³,4, Jian-Min Yuan¹,2

¹ Division of Cancer Control and Population Sciences, UPMC Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA, United States of America, ² Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh PA, United States of America, ³ Health Services and Systems Research, Duke-NUS Medical School Singapore, Singapore, ⁴ Saw Swee Hock School of Public Health, National University of Singapore, Singapore

* luuh@upmc.edu, hnl11@pitt.edu

Abstract

Introduction

Telomeres and telomerase play important role in maintaining chromosome integrity and genomic stability. Recent epidemiologic data showed inconsistent findings which suggested that both short and long leukocyte telomeres could be associated with increased risk of pancreatic cancer. We prospectively examined the association between telomere length and pancreatic cancer risk in a population-based cohort study.

Methods

The Singapore Chinese Health Study recruited 63,257 Chinese aged 45 to 74 years from 1993 to 1998 in Singapore. Relative telomere length in peripheral blood leukocytes was quantified using a validated monochrome multiplex quantitative polymerase chain reaction method in 26,540 participants, including 116 participants who later developed pancreatic cancer after an average of 13 years of follow-up. Cox proportional hazard regression method was used to calculate hazard ratio (HR) and its 95% confidence interval (CI) of pancreatic cancer risk associated with telomere length, with adjustment for confounding factors.

Results

Longer telomeres were significantly associated with higher risk of pancreatic cancer ($P_{trend} = 0.02$). Compared with lowest quartile, subjects with highest quartile of telomere length had an HR of 2.18 (95% CI: 1.25–3.80) for developing pancreatic cancer. In stratified analysis, this association remained among pancreatic adenocarcinoma patients but not among pancreatic non-adenocarcinoma patients. In continuous scale, the HRs and 95% CIs were 3.08 (1.17–8.11) for adenocarcinoma patients and 1.47 (0.43–5.06) for non-adenocarcinoma patients. The HRs and 95% CIs of the highest quartile of telomere length, compared with the lowest quartile, for adenocarcinoma and non-adenocarcinoma were 2.50 (1.22–5.13) and 1.64 (0.70–3.82), respectively.

Data Availability Statement: De-identified data relevant to the report can be shared and is available upon request through the University of Pittsburgh for researchers who meet the criteria for access to confidential data. Data are available from the University of Pittsburgh Institutional Data Access / Ethics Committee with the following contact information: 3500 Fifth Avenue, Hieber Building Main Office, Suite 106 Pittsburgh, PA 15213. Main Phone: (412) 383-1480. Main Fax: (412) 383-1508. Email: askirb@pitt.edu.
The length of follow-up from the collection of blood for the measurement of telomere length to the diagnosis of cancer (median = 8.0, range: from 5.0 months to 16.2 years) had no significant impact on the association between telomere length and pancreatic cancer risk.

Conclusions
The present study demonstrates that longer telomeres are associated with increased risk of overall pancreatic cancer.

Introduction
Although pancreatic cancer may be uncommon, it is a highly lethal malignancy. Worldwide, pancreatic cancer ranks 7th in cancer mortality with approximately 432,250 deaths out of 459,000 new cases a year [1]. In the U.S., it ranks 4th in cancer mortality in both sexes [2]. Compared to other more common cancers, pancreatic cancer has relatively short survival time and the low 5-year survival rate after cancer diagnosis [2] as most cases are diagnosed at an advanced stage. Moreover, while incidence and mortality rates of other cancers have been declining in the past four decades, the incidence rate of pancreatic cancer has been increasing by 1.5% per year [2,3], and the survival rate of pancreatic cancer patients remained constant over the same period [2,4]. Established risk factors for pancreatic cancer include chronic pancreatitis, obesity, type 2 diabetes, and tobacco smoking [5]. Collectively, these risk factors are attributable to less than half of pancreatic cancer burden in the U.S [5]. Hence, there is an urgent need to identify the underlying risk factors of pancreatic cancer to understand the process of carcinogenesis for better prevention and control of pancreatic cancer.

Telomeres, which are tandem DNA repeats at the ends of chromosomes, and telomerase, an enzyme that maintains telomere length, play important role in genomic stability as they protect chromosome ends from degradation, fusion and irregular recombination [6,7]. Human telomeres are approximately 10–15 kb and shorten approximately 30–200 bp after each cycle of cell division [8]. The rate of telomere shortening across individuals depends on both genetic and environmental factors (i.e., smoking, diabetes, obesity or physical activity) [9,10]. In normal circumstances, inactivity of telomerase and the incomplete replication of linear DNA molecules at the end of each chromosome result in telomere shortening, leading to cell senescence and triggering the programmed cell death (apoptosis) [6]. Cell senescence and apoptosis can prevent malignant transformation of damaged cells, thus reducing the likelihood of cancer development. On the other hand, under abnormal circumstances, cycles of cell division do not cause telomere shortening, may be through the activation of telomerase or other unknown mechanisms, resulting in evading cell senescence and apoptosis, considering a hallmark of cancer [11]. Consequently, individuals with longer telomeres relative to their counterparts at the same chronological age may have a higher chance of developing cancer.

Previous epidemiologic studies showed mixed findings that both short [12–14] and long leukocyte [12,14–16] telomere lengths were associated with increased risk of pancreatic cancer. Possible underlying factors accounting for these inconsistent results might be variations in study design, characteristics of study populations or follow-up duration, time between blood collection and the diagnosis date of pancreatic cancer, methods used to measure telomere length in different laboratories and other confounding factors that may impact on telomere length [17]. The Mendelian randomization approach, which usually avoids the potential...
confounding effect of environmental exposure on the relation between phenotypically measured telomere length and risk of pancreatic cancer, has shown that genetically predicted telomere length is not associated with pancreatic cancer risk [18]. They, however, observed that one short telomere-related allele (rs10936599, T) was associated with decreased risk of pancreatic cancer but the other short telomere-related allele (rs2736100, A) was associated with increased risk of pancreatic cancer.

These conflicting results from previous epidemiologic studies prompted us to perform the present analysis to examine the association between leukocyte telomere length and risk of pancreatic cancer in a prospective cohort study in Singapore.

**Methods**

**Study population**

The current analysis was based on the data from the Singapore Chinese Health Study, a population-based prospective cohort study established between April 1993 and December 1998 by the recruitment of middle-age and elderly Chinese living in government-built housing estates, where 86% of the Singapore population resided in during the period of recruitment. The participants belonged to either the Hokkien dialect group who originated from the Fujian province, or the Cantonese dialect group who originated from the Guangdong province in southern China. Detailed information on designs and methods has been described elsewhere [19]. Briefly, at baseline, participants were interviewed at their homes by trained interviewers, using a structured questionnaire to collect information on demographics, body weight and height, lifetime use of tobacco, current physical activity, menstrual/reproductive history (women only), occupational exposure, medical history, and family history of cancer. Body mass index (BMI) was calculated as the weight in kilograms divided by height in meters squared. All study participants provided written informed consent. The present study was approved by the Institutional Review Boards of the National University of Singapore and the University of Pittsburgh.

Blood and urine samples were initially collected from a 3% random sample of cohort participants in 1994–1999. Between July 1999 and December 2003, all surviving cohort subjects were re-contacted for a telephone interview to update information on alcohol use, tobacco use, medical history, current physical activity, and body weight. At the telephone interviews, participants were asked to donate blood. We began blood and urine collection from all consented surviving cohort participants at the beginning of year 2000. For those declining our request for blood donation, mouthwash samples were collected instead.

Of all the subjects that we re-contacted successfully, 28,346 subjects (approximately 57%) consented to donating blood for research. The study participants who provided blood samples were younger than those who did not (mean ± standard deviation-SD: 60.9 ± 7.7 versus 62.4 ± 8.2 years of age), more educated (33.6% versus 25.1% having secondary or higher education), more likely to be men (45.5% versus 39.1%), and had slightly higher prevalence of smoking (32% versus 30.2% ever smokers) and regular alcohol consumption (18.3% versus 14.8% weekly or daily consumers of alcohol).

**Dietary assessment**

Dietary assessment in SCHS used semi-quantitative food frequency questionnaires (FFQs) that was validated against a series of 24-hour dietary recall (24-HDR) interviews [20] and selected biomarker studies on random subsets of cohort participants [21,22]. The SCHS FFQ contained 165 food items and food groups commonly consumed in Singapore. Study participants were asked how frequently (in 8 categories: ranging from “never or hardly ever” to “two
or more times a day”) they consumed the food or food group and followed by a question on the amount of food consumed, using photographs to choose from three portion sizes (small, medium, large). Average daily intake of approximately 100 nutrients and non-nutrient compounds was computed for each study participant using the Singapore Food Composition Database [20]. The reproducibility and validity of the SCHS FFQ were evaluated in 1,022 individuals with two 24-hour recalls, one weekday and one weekend during 12 months. The correlation coefficients for the majority of calorie-adjusted nutrients ranged from 0.50 to 0.75 [20].

For each of the 4 types of alcoholic beverages (beer, wine, western hard liquor, Chinese hard liquor), participants were asked to choose from eight frequency categories: never or hardly, once a month, 2–3 times a month, once a week, 2–3 times a week, 4–6 times a week, once a day, and 2 or more times a day. Consumers were then asked to choose from four defined portion sizes. For beer, the portion sizes were 1 small bottle (375 ml) or less, 2 small bottles or 1 large bottle (750 ml), 2 large bottles, and 3 large bottles or more. For wine, the portion sizes were 1 glass (118 ml) or less, 2, 3, and 4 glass or more. For Chinese or western hard liquor, the portion sizes were 1 shot (30 ml) or less, 2, 3, and 4 shots or more. One drink was defined as 375 ml of beer (13.6 g of ethanol), 118 ml of wine (11.7 g of ethanol), or 30 ml of western or Chinese hard liquor (10.9 g of ethanol).

Assessment of pancreatic cancer cases

Incident pancreatic cancer cases and all deaths within the cohort was identified, using the International Classification of Diseases-Oncology, 2nd Edition code C25 via linkage of all cohort participants with the database of the nationwide Singapore Cancer Registry and the Birth and Death Registry that have complete records of incident cancer and death cases in Singapore, respectively [23]. All histologic types of pancreatic cancer were included in the current analysis, including neoplasm malignant (n = 5), carcinoma (n = 4), pleomorphic carcinoma (n = 1), adenocarcinoma (n = 49), mucinous adenocarcinoma (n = 3), infiltrating duct adenocarcinoma (n = 13), carcinoid tumor malignant (n = 1), neuroendocrine carcinoma (n = 1), intraductal papillary-mucinous carcinoma (n = 2), as well as 38 pancreatic cancer cases with unknown histology. Of the total 116 cases, 65 were adenocarcinoma and the remaining 51 were other/unknown histology of pancreatic cancer. The ascertainment of cancer incidence and deaths among all study participants were virtually complete as to the time of this study, only 56 (<0.1%) of the entire cohort participants were known to be lost to follow-up due to migration out of Singapore or for other reasons.

Measurements of leukocyte telomere length

Genomic DNA was extracted from peripheral blood using QIAamp 96 DNA Blood kits (Qia-gen, Valencia, CA) according to the manufacturer’s protocol. Leukocyte telomere length was measured using a validated monochrome multiplex qPCR method, as described elsewhere [24]. Briefly, this method measures the relative average telomere length in genomic DNA by determining the ratio of telomere repeat copy number (T) to single (albumin) gene copy number (S) in experimental samples relative to a reference sample (the T/S ratio). The DNA sample for the standard curve was composed of an equimolar pool of 77 samples selected from participants of the Singapore Chinese Health Study who were identified in a prior study; the telomere length values of all the 77 samples were within 10% of the population mean. This pooled DNA sample was run on all qPCR plates: 8 replicates for each of four concentrations (4, 0.8, 0.16, and 0.032 ng/µl). Thermal cycling was carried out on an Applied Biosystem 7900 HT instrument, using PCR cycling conditions as described previously [24]. Real-time PCR cycle
thresholds, determined independently for the albumin gene (ALB) and telomere (TEL) amplification traces for all wells (experimental and standard DNA samples), were used to calculate telomere length [24] with the 384-well plate-based normalization of telomere length, which was more robust than the overall standard-curve based normalization. All experimental DNA samples were assayed in duplicate, and the average value of the two replicates was used for final analysis for each subject. The mean intra-assay coefficient of variation, as a measure of reproducibility for telomere length, was 3.5% over all technical sample duplicates.

Statistical analysis
The relative average telomere length (i.e., the T/S ratio) for each individual was included in the final statistical analysis. After excluding 1,585 participants with a history of cancer at the time of blood collection and an additional 221 subjects with unavailable telomere length measurement due to assay problems, the current analysis included 26,540 subjects. As of December 31, 2016, with an average follow-up of 12.8 years after their donation of blood sample, 116 among them had developed pancreatic cancer. For each study participant, person-years at risk were counted from the date of blood draw to the date of pancreatic cancer diagnosis, death, migration out of Singapore, or December 31, 2016, whichever occurred first.

Means and standard deviations (SDs) were calculated for continuous variables while counts and proportions were calculated for categorical variables. We used the \( t \)-test to compare differences for continuous variables and the \( \chi^2 \) test to compare differences for categorical variables between pancreatic cancer cases and the rest of the cohort participants, as well as across quartiles of telomere length.

Cox proportional hazard regression method was performed to calculate hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs) for developing pancreatic cancer associated with higher quartiles compared with the lowest quartile. We tested for linear trend by treating the telomere length quartiles as an ordinal variable in the Cox proportional hazard model. We did not find violation of the proportional hazards assumption in our dataset when we examined this assumption using time-varying covariates (i.e., an interaction between leukocyte telomere length and the event time in log) in the Cox models \( (P = 0.876) \).

All Cox proportional hazard regression models included measures of smoking history—number of cigarettes smoked per day (never smokers, 1–12, 13–22, or 23+), number of years of smoking (never smokers, 1–19, 20–39, or 40+), and number of years since last smoked for quitters (current smokers, <1, 1–4, 5–19, 20+ years since last smoked, or never smokers). Other potential confounders included in the multivariate Cox proportional hazards models were age, sex, dialect group (Hokkien or Cantonese), level of education (no formal education, primary school, secondary or higher education), body mass index \((<20, 20 to <24, 24 to <28, \text{ or } \geq 28 \text{ kg/m}^2)\), and alcohol consumption (non-drinkers, 1 to <7, or \( \geq 7 \) drinks per week), history of diabetes (no or yes), and physical activity (no or yes). The weekly physical activity was defined as any moderate or vigorous activity, or strenuous sports lasting at least 30 minutes. In the current analysis, all information of participant characteristics, except education, were from follow-up 1 interview, which were closer to the sample collection date. The BMI categories was grouped based on the recommendation for Asians by the World Health Organization (WHO) [25]. These covariates were selected based on findings from our prior study [26] and others [5,27]. Specifically, in the previous study [26], we found that shorter leukocyte telomeres were associated with older age, male gender, lower level of education, ever smoking, daily drinkers of alcoholic beverages, and less physical activity. These factors were also associated with higher risk of pancreatic cancer. Therefore, these risk factors were included as covariates in the multivariable Cox regression models.
We also performed stratified analysis and sensitivity analyses by examining the robustness of the association between leukocyte telomere length and pancreatic cancer risk. Major risk factors for sub-group analyses were: smoking status (ever vs. never smoked), sex (male vs. females), BMI status (<25 vs. ≥25), and alcohol consumption (non-drinkers vs. drinkers). For sensitivity analysis, we performed a stratified analysis using median duration from blood draw to the date of diagnosis of pancreatic cancer (<8 years vs. ≥8 years). We conducted additional analysis after excluding cases and person-years observed within the first 3 years after blood draw. All statistical analyses were conducted using SAS, version 9.4 (SAS Institute Inc., Cary, NC). All P values presented are two-sided, and P < 0.05 was considered statistically significant.

Results
The mean (±SD) age at the cancer diagnosis of the 116 pancreatic cancer cases identified in this cohort was 74.1 (±8.3) years. The median time interval between blood collection and pancreatic cancer diagnosis was 8.0 years (from 5.0 months to 16.2 years).

Table 1 compares the baseline characteristics of pancreatic cancer patients with the remaining participants of the cohort. Cases were older than non-cases. The differences in distributions of other demographic and lifestyles between cases and non-cases were not statistically significant (all P's >0.05).

Table 2 shows selected characteristics of participants by relative average telomere length (i.e., T/S ratio). Compared to those in the lowest quartile for telomere length, those in the highest quartile were expectantly about 5 years younger in average at the time of blood sampling. They also had slightly higher BMI and were more likely to be women or engage in weekly physical exercise while were less likely to smoke, drink alcohol daily or report a history of diabetes (P<0.02).

Telomere length was positively associated with pancreatic cancer risk after adjustment for potential confounders (Table 3). Compared with the lowest quartile, participants with the highest quartile of telomere length were more than two times more likely to develop pancreatic cancer (HR = 2.18, 95% CI: 1.25–3.80, P<0.02). In the analysis of pancreatic adenocarcinoma only (n = 65), the results were comparable to the results that included all pancreatic cancer cases. The HRs (95% CIs) were 3.08 (1.17–8.11) for adenocarcinoma patients as compared with 2.29 (1.07–4.92) for all cases (Table 3). Although the association between telomere length and risk of non-adenocarcinoma or non-histologically confirmed pancreatic cancer was not linear and not statistically significant, the significantly elevated HR was observed for second quartile of telomere length (HR = 2.3, 95% CI: 1.06–4.96). The small sample size and heterogeneity of pancreatic cancer histology may contribute to the observed variation of risk estimates in this subgroup analysis. The difference in the telomere length-risk associations between the two histological subgroups was not statistically significant (P<0.05).

Thus, all subsequent subgroup analyses were performed all pancreatic cancer cases.

In stratified analysis (Table 4), statistically significant association between longer telomeres and higher risk of pancreatic cancer was present in never smokers (HR = 3.04, 95% CI: 1.43–6.47 comparing the highest with the lowest quartile, P<0.007) but not in ever smokers, in men (HR = 2.91, 95% CI: 1.32–6.41, P<0.03) but not in women, in individuals without a history of diabetes (HR = 2.50, 95% CI: 1.33–4.70, P<0.01) but not in those with diabetes, or in those with <25 kg/m² of BMI (HR = 2.44, 95% CI: 1.25–4.76 P<0.02) but not in their heavier counterparts. However, the heterogeneity in the telomere length-pancreatic cancer risk associations between these contrasting groups was not statistically significant (all P<0.05) (Table 4). When data were analyzed by the duration from blood collection to
diagnosis, the association between telomere length and risk of pancreatic cancer was slightly stronger for shorter duration (<8 years) than that for longer duration (≥8 years); the corresponding HRs (95% CIs) of pancreatic cancer for the highest related to the lowest quartile were 3.11 (95% CI: 1.34–7.21) and 1.63 (95% CI: 0.78–3.41), respectively (S1 Table). However, their difference was not statistically significant ($P_{\text{heterogeneity}} = 0.730$). We repeated analysis after excluding first 3 years of data (i.e., both cases and person-years), the results remained the same (Data not shown).

### Table 1. Distributions of characteristics among study participants. The Singapore Chinese Healthy Study, 1993–2016.

| Characteristics                                      | Cases (n = 116) n, % | Non-cases (n = 26,422) n, % | P-value |
|------------------------------------------------------|----------------------|-----------------------------|---------|
| Mean age (±SD), years $^b$                           | 66.24±7.86           | 62.80±7.64                  | $<$0.0001 |
| Gender ($^a$)                                        |                      |                             |         |
| Male                                                 | 60 (51.72)           | 12,173 (46.07)              | 0.22    |
| Female                                               | 56 (48.28)           | 14,249 (53.93)              |         |
| Dialect $^a$                                         |                      |                             |         |
| Cantonese                                            | 61 (52.59)           | 13,405 (50.73)              | 0.69    |
| Hokkien                                              | 55 (47.41)           | 13,017 (49.27)              |         |
| Highest level of education ($^a$)                    |                      |                             |         |
| No formal education                                  | 24 (20.69)           | 5,501 (20.82)               | 0.65    |
| Primary school                                       | 57 (49.14)           | 11,974 (45.32)              |         |
| Secondary school or higher                           | 35 (30.17)           | 8,947 (33.86)               |         |
| Mean body mass index (±SD), Kg/m$^2$                 | 23.20±3.78           | 23.25±3.50                  | 0.95    |
| Smoking status ($^b$)                                |                      |                             |         |
| Never smoker                                         | 72 (62.07)           | 17,949 (67.93)              | 0.23    |
| Former smoker                                        | 25 (21.55)           | 4,181 (15.83)               |         |
| Current smoker                                       | 19 (16.38)           | 4,292 (16.24)               |         |
| Alcohol consumption ($^b$)                           |                      |                             |         |
| Non-drinkers                                         | 92 (79.31)           | 21,484 (81.31)              | 0.85    |
| 1 to <7 drinks/week                                  | 18 (15.52)           | 3,753 (14.20)               |         |
| ≥7 drinks/week                                       | 6 (5.17)             | 1,185 (4.49)                |         |
| Diabetes ($^b$)                                      |                      |                             |         |
| No                                                   | 95 (81.90)           | 22,694 (85.90)              | 0.22    |
| Yes                                                  | 21 (18.10)           | 3,728 (14.10)               |         |
| Any weekly physical activity ($^{**}$) ($^b$)        |                      |                             |         |
| No                                                   | 76 (65.52)           | 16,985 (64.27)              | 0.78    |
| Yes                                                  | 40 (34.48)           | 9,437 (35.73)               |         |
| Mean time from blood collection to cancer diagnosis (±SD) | 8.88±4.23           | -                           |         |

$^a$The weekly physical activity was defined as any moderate or vigorous activity, or strenuous sports lasting at least 30 minutes

$^b$Variables reported at baseline

$^{**}$Variables reported at the follow-up 1.

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### Discussion

In the present analysis of the population-based prospective cohort study of 26,540 individuals with an average follow-up of 13 years, we found that longer telomeres in peripheral blood leukocytes were associated with significant increase in the risk of pancreatic cancer. The duration of follow-up did not have significant impact on the association between telomere length and risk of pancreatic cancer.
Previous epidemiological studies [12–16] reported notably inconsistent results that both short [12–14] and long leukocyte [12,14–16] telomere lengths were associated with increased risk of pancreatic cancer. Two studies [12,14] reported a U-shaped association between telomere length and pancreatic cancer risk, i.e., both shorter and longer telomeres were associated with higher risk of pancreatic cancer. Accordingly, in a retrospective case-control study of 499 pancreatic cancer cases and 963 controls matched with frequencies of age group, sex and residence in Mayo Clinic, Skinner et al. [12] found a U-shaped association between leukocyte telomere length and risk of pancreatic cancer. The other study was conducted by Zhang et al. [14], a nested case-control study of 900 pancreatic cancer cases and 900 matched controls within
the Prevention Study of Adolescent Population in Liaoning Province, China, which found a similar U-shaped association. In addition, three nested case-control studies of pancreatic cancer in Western populations also reported mixed results. One study in the U.S [13] of 386 pancreatic cancer cases and 896 controls from 5 prospective cohort studies and matched by year of birth, prospective cohort, smoking status, fasting status at blood collection, found that longer telomeres were associated with significantly lower risk of pancreatic cancer. The other two studies, both in Europe [i.e., a case-control study of 193 pancreatic cancer cases versus 660 controls from the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Study [15] and a case-control study of 331 matched case-control pairs from the European Prospective Investigation into Cancer and Nutrition (EPIC) Cohort [16]], found that longer telomeres were associated with statistically significant higher risk of pancreatic cancer. The results of these European studies [15,16] were comparable with the finding of our current study.

The inconsistency of the association between leukocyte telomere length and pancreatic cancer risk in different studies might be due to differences in study design (retrospective vs. prospective studies), study populations (Asian vs. European descendants), variation in the length of follow-up, and different prevalence rates of established risk factors (i.e., smoking, diabetes, physical activity or obesity), time between blood collection and the diagnosis date of pancreatic cancer, methods used to measure telomere length in different laboratories, as well as other

| Relative average telomere length by pancreatic histology | Person-years | Number of cases | HR (95% CI)* |
|-----------------------------------------------------------|--------------|----------------|--------------|
| **Overall cases**                                          |              |                |              |
| Continuous Variable                                       | 348,979      | 116            | 2.29 (1.07–4.92) |
| Quartile Variable                                         |              |                |              |
| Q1 (shortest)                                             | 83,015       | 22             | Ref.         |
| Q2                                                        | 86,507       | 35             | 1.81 (1.06–3.10) |
| Q3                                                        | 88,240       | 25             | 1.45 (0.81–2.58) |
| Q4 (longest)                                              | 91,217       | 34             | 2.18 (1.25–3.80) |
| **P_trend**                                                |              |                | 0.02         |
| **Adenocarcinoma**                                        |              |                |              |
| Continuous Variable                                       | 348,549      | 65             | 3.08 (1.17–8.11) |
| Quartile Variable                                         |              |                |              |
| Q1 (shortest)                                             | 82,942       | 12             | 1.00         |
| Q2                                                        | 86,340       | 16             | 1.45 (0.68–3.07) |
| Q3                                                        | 88,146       | 13             | 1.26 (0.57–2.80) |
| Q4 (longest)                                              | 91,121       | 24             | 2.50 (1.22–5.13) |
| **P_trend**                                                |              |                | 0.02         |
| **Non-Adenocarcinoma/unknown Histology**                   |              |                |              |
| Continuous Variable                                       | 348,424      | 51             | 1.47 (0.43–5.06) |
| Quartile Variable                                         |              |                |              |
| Q1 (shortest)                                             | 82,887       | 10             | 1.00         |
| Q2                                                        | 86,383       | 19             | 2.30 (1.06–4.96) |
| Q3                                                        | 88,129       | 12             | 1.69 (0.72–3.96) |
| Q4 (longest)                                              | 91,025       | 10             | 1.63 (0.66–4.03) |
| **P_trend**                                                |              |                | 0.23         |

*Adjusted for age, sex, education, dialect group, smoking status, alcohol drinking, BMI, diabetes history, and weekly physical activity.

*Including 38 pancreatic cancer cases with unknown histology.
Table 4. Association between relative average telomere length and pancreatic cancer risk among participants stratified by selected characteristics in the Singapore Chinese Health Study, 1993–2016.

| Relative telomere length | Person-years | Number of cases | HR (95% CI)* |
|--------------------------|--------------|-----------------|--------------|
| **By Smoking Status**    |              |                 |              |
| Never Smokers            |              |                 |              |
| Q1 (shortest)            | 52,980       | 10              | Ref.         |
| Q2                       | 59,579       | 20              | 2.13 (0.99–4.56) |
| Q3                       | 64,130       | 17              | 1.91 (0.87–4.21) |
| Q4 (longest)             | 69,061       | 25              | 3.04 (1.43–6.47) |
| \( P_{\text{trend}} \)  |              |                 | 0.007        |
| Ever Smokers             |              |                 |              |
| Q1 (shortest)            | 30,035       | 12              | Ref.         |
| Q2                       | 26,928       | 15              | 1.54 (0.72–3.32) |
| Q3                       | 24,110       | 8               | 1.02 (0.41–2.52) |
| Q4 (longest)             | 22,155       | 9               | 1.35 (0.56–3.28) |
| \( P_{\text{trend}} \)  |              |                 | 0.71         |
| \( P_{\text{interaction}} \) |          |                 | 0.23         |
| **By Gender**            |              |                 |              |
| Male                     |              |                 |              |
| Q1 (shortest)            | 43,313       | 11              | Ref.         |
| Q2                       | 40,081       | 22              | 2.58 (1.25–5.34) |
| Q3                       | 36,365       | 11              | 1.59 (0.68–3.70) |
| Q4 (longest)             | 33,296       | 16              | 2.91 (1.32–6.41) |
| \( P_{\text{trend}} \)  |              |                 | 0.03         |
| \( P_{\text{interaction}} \) |          |                 | 0.64         |
| Female                   |              |                 |              |
| Q1 (shortest)            | 39,702       | 11              | Ref.         |
| Q2                       | 46,426       | 13              | 1.15 (0.51–2.57) |
| Q3                       | 51,875       | 14              | 1.24 (0.56–2.76) |
| Q4 (longest)             | 57,920       | 18              | 1.57 (0.72–3.40) |
| \( P_{\text{trend}} \)  |              |                 | 0.24         |
| \( P_{\text{interaction}} \) |          |                 | 0.64         |
| **By Diabetes History**  |              |                 |              |
| No Diabetes History      |              |                 |              |
| Q1 (shortest)            | 71,672       | 16              | Ref.         |
| Q2                       | 75,455       | 29              | 2.05 (1.11–3.78) |
| Q3                       | 77,296       | 21              | 1.64 (0.85–3.18) |
| Q4 (longest)             | 79,890       | 29              | 2.50 (1.33–4.70) |
| \( P_{\text{trend}} \)  |              |                 | 0.01         |
| Diabetes History         |              |                 |              |
| Q1 (shortest)            | 11,343       | 6               | Ref.         |
| Q2                       | 11,052       | 6               | 1.17 (0.38–3.65) |
| Q3                       | 10,944       | 4               | 0.92 (0.26–3.30) |
| Q4 (longest)             | 11,327       | 5               | 1.35 (0.40–4.58) |
| \( P_{\text{trend}} \)  |              |                 | 0.74         |
| \( P_{\text{interaction}} \) |          |                 | 0.28         |
| **By BMI Status**        |              |                 |              |
| BMI < 25                 |              |                 |              |
| Q1 (shortest)            | 60,735       | 15              | Ref.         |
| Q2                       | 62,344       | 24              | 1.89 (0.99–3.62) |

(Continued)
unmeasured potential confounders[17]. For example, smoking [28,29], diabetes [30] and obesity [31] or physical activity [32,33] are known to be associated with telomere length as well in our study [26]. Lynch et al. [15] reported a positive association between telomere length and pancreatic cancer risk in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study that comprised male smokers only while in both Skinner et al. [12] and Zhang et al. [14] studies, the prevalence of current smokers at baseline for cases and controls were 11% vs. 32% and 31% vs. 20%, respectively; which were different from ours (i.e., 17.1% in cases vs. 16.2% in controls). Additionally, current participants of the Singapore Chinese Health Study were leaner (mean BMI = 23.3 in cases and 23.2 in non-cases) compared to study participants of all other five studies [12–16] where mean BMI was more than 25.0. The history of diabetes in our study (20% in cases and 14.1% in controls) was much lower than that in the studies by Skinner et al. [12] (74.5% in cases and 48.3% in controls) and Zhang et al. [14] (43.0% in cases and 39.3% in controls). Consequently, the differences in age and BMI, and proportions of male subjects, smokers and diabetics among various study populations could yield differential association between telomere length and pancreatic cancer risk.

In addition, the difference in time interval between dates of blood collection and diagnosis of pancreatic cancer might also contribute to the inconsistency of results from different studies. In the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Study, Lynch et al. [15] found that the positive association between telomere length and pancreatic cancer risk was observed in subjects diagnosed within the first five years of blood draw, but not those diagnosed greater than five years after blood draw. Conversely, in another study of five US prospective cohorts, Bao et al. [13] reported that shorter pre-diagnostic leucocyte telomere length was associated with increased risk of pancreatic cancer. In our study, we examined but did not find the duration of follow-up had significant impact on the risk of pancreatic cancer associated with telomere length. The different results by time intervals between dates of blood collection and diagnosis of pancreatic cancer (<8 years vs. ≥8 years) might be reflective of tumor adaptive mechanism for escaping cellular senescence and apoptosis. Telomere elongation at the initial stages of tumorigenesis may be due to activation of telomerase to ensure that tumor cells escape apoptosis and proceed to divide and proliferate indefinitely, suggesting a possible reverse causality [17]. Our observed association that was statistically significant only within the first 8 years supports this speculation.
Studies using genetic variants as proxy measures for telomere length could avoid the potential biases due to disease progression and treatment, and other attributes to telomere shortening (i.e., aging and oxidative stress), reverse causation issue or inter-laboratory variability in the measurement of telomere length. Recently, Antwi et al. [18] constructed a genetic risk score of eight polymorphisms that were identified to be associated with leukocyte telomere length in genome-wide association study [34] and found no association between this genetic risk score and pancreatic cancer risk. Noted that this study only involved 1,500 cases and 1,499 non-cancer controls, a modest sample size for such type of effort. They, however, found that one short telomere-related allele (rs10936599, T) was associated with decreased risk of pancreatic cancer whereas another short telomere-related allele (rs2736100, A) was associated with increased risk of pancreatic cancer [18]. However, a recent study by Haycock et al. [35], using Mendelian randomization approach of GWAS data in a larger sample size (n = 5,105 pancreatic cancer cases versus 8,739 controls) found that the risk estimate for pancreatic cancer associated genotypes related to longer telomeres was not statistically significant (OR = 0.86, 95% CI: 0.57–1.32). Another approach might be to evaluate whether the association between leukocyte telomere length and pancreatic cancer risk is modulated by individual variation in telomere maintenance genes, such as TERT, TRC, TRF1, TRF2, POT1 or Rap1. Indeed, in a nested case-control study of five cohorts, Bao et al. [13] found that three SNPs (rs401681, rs2736100, rs2736098) at TERT were associated with pancreatic cancer risk, of which the minor allele for rs401681 was associated with shorter telomeres.

Several experimental studies have indicated that telomere length in peripheral blood leukocyte was positively correlated with telomeres in buccal cells [36], fibroblasts [36], skin [37,38], and synovial membrane [38]. A study by Daniali et al. [39] found that telomere length in peripheral blood leukocytes were highly correlated with those in the muscle cells ($r^2 = 0.71$), fat ($r^2 = 0.69$), and skin ($r^2 = 0.69$) (all $P$s <0.0001). Furthermore, Gardner et al. [40] showed that telomere length in both skin and skeletal muscle cells was highly correlated with that in the pancreas tissue after controlling for chronological age. These data strongly suggest that peripheral blood leukocytes can be used as non-invasive surrogates for pancreas and other tissue types in the measurement of telomere length.

The biological mechanism linking longer telomere to pancreatic cancer is unclear. It is well known that in normal condition shorter telomeres may act as tumor suppressors [41] that can protect against carcinogenesis by triggering programmed cell death in the presence of functional cell cycle checkpoints and intact apoptotic pathways [42]. In contrast, cells with longer telomeres have higher proliferative capacity and more cell division. Each round of genome replication has the potential to introduce genetic mutations and chromosomal alterations, which may promote malignant transformation [43]. In addition, the mobilization of younger immune response cells, such as T cells is associated with longer telomeres [44,45], which might be involved in the promotion of carcinogenesis [46]. Furthermore, peripheral blood leukocyte telomere length may serve as an indicator of other factors for pancreatic cancer risk. Accordingly, smoking or oxidative stress might lead to telomere shortening, which then triggers cell mechanism such as increase in telomerase activity or activation of the telomerase independently, resulting in telomere lengthening [47]. Further studies are thus needed to elucidate the biological mechanisms for long telomeres in the development of pancreatic cancer.

Our study has several strengths. The prospective design minimized the potential impact of progression and treatment of pancreatic cancer on telomere length, since telomere length was determined on an average of 8.5 years before pancreatic cancer diagnosis. The long-term and complete follow-up further reduced potential bias due to the impact of undiagnosed pancreatic cancer on telomere length. A comprehensive adjustment for smoking, alcohol use, physical...
activity, BMI and history of diabetes minimized their potential confounding effect on the telomere length-pancreatic cancer risk association.

Our study also has some limitations. First, telomere length was measured in leukocyte rather than in target tissue. It is noted, however, that prior study shown high correlation of telomere length measures between the two tissue types [39,40]. Second, telomere length was measured at one-time point, which may not representative for true telomere length over time. This one-time point measurement would preclude our ability to evaluate the attrition rate of telomere length and the risk of pancreatic cancer development overtime. Third, relatively small number of cases hampered sub-group analyses, resulting in wide confidence intervals of risk estimates. Forth, as with any observational studies, residual confounding from measured (e.g., BMI, smoking and physical inactivity) or unmeasured factors could not be completely ruled out.

In summary, our study shows a dose-dependent association for peripheral blood telomere length with increased risk of pancreatic cancer. These findings, together with results from prior studies support a potential etiological role of longer telomeres for the development of pancreatic cancer. Future research efforts are warranted to elucidate the biological mechanism for longer telomeres in the development of pancreatic cancer.

Supporting information

S1 Table. Association between relative average telomere length and pancreatic cancer risk among participants stratified by median duration from blood collection to diagnosis in the Singapore Chinese Health Study, 1993–2016.

(DOCX)

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Author Contributions

Conceptualization: Hung N. Luu, Joyce Y. Huang, Woon-Puay Koh, Jian-Min Yuan.

Data curation: Hung N. Luu, Joyce Y. Huang, Renwei Wang, Aizhen Jin, Woon-Puay Koh, Jian-Min Yuan.

Formal analysis: Hung N. Luu, Joyce Y. Huang, Renwei Wang, Jennifer Adams-Haduch, Aizhen Jin, Woon-Puay Koh, Jian-Min Yuan.

Funding acquisition: Woon-Puay Koh, Jian-Min Yuan.

Investigation: Hung N. Luu, Joyce Y. Huang, Renwei Wang, Jennifer Adams-Haduch, Aizhen Jin, Woon-Puay Koh, Jian-Min Yuan.

Methodology: Hung N. Luu, Renwei Wang, Jennifer Adams-Haduch, Aizhen Jin, Woon-Puay Koh, Jian-Min Yuan.

Project administration: Aizhen Jin, Jian-Min Yuan.

Resources: Hung N. Luu, Jennifer Adams-Haduch, Woon-Puay Koh, Jian-Min Yuan.
Software: Renwei Wang, Aizhen Jin.

Supervision: Hung N. Luu, Woon-Puay Koh, Jian-Min Yuan.

Validation: Hung N. Luu, Woon-Puay Koh.

Writing – original draft: Hung N. Luu, Joyce Y. Huang, Renwei Wang, Jennifer Adams-Haduch, Aizhen Jin, Woon-Puay Koh, Jian-Min Yuan.

Writing – review & editing: Hung N. Luu, Joyce Y. Huang, Renwei Wang, Aizhen Jin, Woon-Puay Koh, Jian-Min Yuan.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018; 68: 394–424. https://doi.org/10.3322/caac.21492 PMID: 30207593

2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018; 68: 7–30. https://doi.org/10.3322/caac.21442 PMID: 29313949

3. Brune KA, Lau B, Palmisano E, Canto M, Goggins MG, Huban RH, et al. Importance of age of onset in pancreatic cancer kindreds. J Natl Cancer Inst. 2010; 102: 119–126. https://doi.org/10.1093/jnci/djp466 PMID: 20068195

4. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerassimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010; 7: 248–249. https://doi.org/10.1038/nmeth0410-248 PMID: 20354512

5. Maisonneuve P, Lowenfels AB. Risk factors for pancreatic cancer: a summary review of meta-analytical studies. Int J Epidemiol. 2015; 44: 186–198. https://doi.org/10.1093/ije/dyu240 PMID: 25502106

6. Stewart SA, Weinberg RA. Telomeres: cancer to human aging. Annu Rev Cell Dev Biol. 2006; 22: 531–557. https://doi.org/10.1146/annurev.cellbio.22.010305.104518 PMID: 16824017

7. Blackburn EH. Telomeres and telomerase: the means to the end (Nobel lecture). Angew Chem Int Ed Engl. 2010; 49: 7405–7421. https://doi.org/10.1002/anie.201002387 PMID: 20821774

8. Harley CB. Human ageing and telomeres. Ciba Found Symp. 1997; 211: 129–139; discussion 139–144. PMID: 9524755

9. Slagboom PE, Droog S, Boomsma DI. Genetic determination of telomere size in humans: a twin study of three age groups. Am J Hum Genet. 1994; 55: 876–882. PMID: 7977549

10. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, et al. Obesity, cigarette smoking, and telomere length in women. Lancet. 2005; 366: 662–664. https://doi.org/10.1016/S0140-6736(05) 66639-5 PMID: 16112303

11. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144: 646–674. https://doi.org/10.1016/j.cell.2011.02.013 PMID: 21376230

12. Skinner HG, Gangnon RE, Litzelman K, Johnson RA, Chari ST, Petersen GM, et al. Telomere length and pancreatic cancer: a case-control study. Cancer Epidemiol Biomark Prev. 2012; 21: 2095–2100. https://doi.org/10.1158/1055-9965.EPI-12-0671 PMID: 23093543

13. Bao Y, Prescott J, Yuan C, Zhang M, Kraft P, Babic A, et al. Leukocyte telomere length, genetic variants at theTERTgene region and risk of pancreatic cancer. Gut. 2017; 66: 1116–1122. https://doi.org/10.1136/gutjnl-2016-312510 PMID: 27797938

14. Zhang R, Zhao J, Xu J, Liu F. Association of peripheral leukocyte telomere length and its variation with pancreatic cancer and colorectal cancer risk in Chinese population. Oncotarget. 2016; 7: 38579–38585. https://doi.org/10.18632/oncotarget.9536 PMID: 27503261

15. Lynch SM, Major JM, Cawthon R, Weinstein SJ, Virtamo J, Lan Q, et al. A prospective analysis of telomere length and pancreatic cancer in the alpha-tocopherol beta-carotene cancer (ATBC) prevention study. Int J Cancer. 2013; 133: 2672–2680. https://doi.org/10.1002/ijc.28272 PMID: 23674344

16. Campa D, Marguten B, De Vivo I, Bourton-Ruault M-C, Racine A, Severi G, et al. Leukocyte telomere length in relation to pancreatic cancer risk: a prospective study. Cancer Epidemiol Biomark Prev. 2014; 23: 2447–2454. https://doi.org/10.1158/1055-9965.EPI-14-0247 PMID: 25103821

17. Antwi SO, Petersen GM. Leukocyte Telomere Length and Pancreatic Cancer Risk: Updated Epidemiologic Review. Pancreas. 2018; 47: 265–271. https://doi.org/10.1097/MPA.0000000000000995 PMID: 29424808
18. Antwi SO, Bamlet WR, Broderick BT, Chaffee KG, Oberg A, Jatoi A, et al. Genetically Predicted Telomere Length is not Associated with Pancreatic Cancer Risk. Cancer Epidemiol Biomark Prev. 2017; 26: 971–974. https://doi.org/10.1158/1055-9965.EPI-17-0100 PMID: 28264873

19. Yuan J-M, Stram DO, Arakawa K, Lee H-P, Yu MC. Dietary cryptoxanthin and reduced risk of lung cancer: the Singapore Chinese Health Study. Cancer Epidemiol Biomark Prev. 2003; 12: 890–898.

20. Hankin JH, Stram DO, Arakawa K, Park S, Low SH, Lee HP, et al. Singapore Chinese Health Study: development, validation, and calibration of the quantitative food frequency questionnaire. Nutr Cancer. 2001; 39: 187–195. https://doi.org/10.1207/S15327914nc392_5 PMID: 11759279

21. Seow A, Shi CY, Franke AA, Hankin JH, Lee HP, Yu MC. Isoflavonoid levels in spot urine are associated with frequency of dietary soy intake in a population-based sample of middle-aged and older Chinese in Singapore. Cancer Epidemiol Biomark Prev. 1998; 7: 135–140.

22. Seow A, Shi CY, Chung FL, Jiao D, Hankin JH, Lee HP, et al. Urinary total isothiocyanate (ITC) in a population-based sample of middle-aged and older Chinese in Singapore: relationship with dietary total ITC and glutathione S-transferase M1/T1/P1 genotypes. Cancer Epidemiol Biomark Prev. 1998; 7: 775–781.

23. Parkin DM, Whelan S, Ferlay J, Thomas D, editors. Cancer incidence in five continents. Vol. 8: Cancer incidence in five continents; Vol. 8. Lyon: IARC Press; 2002.

24. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res. 2009; 37: e21. https://doi.org/10.1093/nar/gkn1027 PMID: 19129229

25. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet Lond Engl. 2004; 363: 157–163. https://doi.org/10.1016/S0140-6736(03)15268-3

26. Yuan J-M, Beckman KB, Wang R, Bull C, Adams-Haduch J, Huang JY, et al. Leukocyte telomere length in relation to risk of lung adenocarcinoma incidence: Findings from the Singapore Chinese Health Study. Int J Cancer. 2018; 142: 2234–2243. https://doi.org/10.1002/ijc.31251 PMID: 29318605

27. Huang JY, Butler LM, Wang R, Jin A, Koh W-P, Yuan J-M. Dietary Intake of One-Carbon Metabolism-Related Nutrients and Pancreatic Cancer Risk: The Singapore Chinese Health Study. Cancer Epidemiol Biomark Prev. 2016; 25: 417–424. https://doi.org/10.1158/1055-9965.EPI-15-0594 PMID: 26711329

28. Huzen J, Veldhuisen DJ, Samani NJ, Zwirnerman AH, Codd V, et al. Telomere length loss due to smoking and metabolic traits. J Intern Med. 2014; 275: 155–163. https://doi.org/10.1111/jiom.12149 PMID: 24118582

29. Astuti Y, Wardhana A, Watkins J, Wulaningsih W, PILAR Research Network. Cigarette smoking and telomere length: A systematic review of 84 studies and meta-analysis. Environ Res. 2017; 158: 480–489. https://doi.org/10.1016/j.envres.2017.06.038 PMID: 28704792

30. Antwi SO, Oberg AL, Shivappa N, Bamlet WR, Chaffee KG, Steck SE, et al. Pancreatic cancer: associations of inflammatory potential of diet, cigarette smoking and long-standing diabetes. Carcinogenesis. 2016; 37: 481–490. https://doi.org/10.1093/carcin/bgw022 PMID: 26905587

31. Rode L, Nordestgaard BG, Weischer M, Bojesen SE. Increased body mass index, elevated C-reactive protein, and short telomere length. J Clin Endocrinol Metab. 2014; 99: E1671–1675. https://doi.org/10.1210/jc.2014-1161 PMID: 24762112

32. Latifovic L, Peacock SD, Massey TE, King WD. The Influence of Alcohol Consumption, Cigarette Smoking, and Physical Activity on Leukocyte Telomere Length. Cancer Epidemiol Biomark Prev. 2016; 25: 374–380. https://doi.org/10.1158/1055-9965.EPI-14-1364 PMID: 26656293

33. Du M, Prescott J, Kraft P, Han J, Giovannucci E, Hankinson SE, et al. Physical activity, sedentary behavior, and leukocyte telomere length in women. Am J Epidemiol. 2012; 175: 414–422. https://doi.org/10.1093/aje/kwr330 PMID: 22302075

34. Codd V, Nelson CP, Allbright 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: 422–427, 427e1-2. https://doi.org/10.1038/ng.2528 PMID: 23535734

35. Telomeres Mendelian Randomization Collaboration, Haycock PC, Burgess S, Nounu A, Zheng J, Okoli GN, et al. Association Between Telomere Length and Risk of Cancer and Non-Neoplastic Diseases: A Mendelian Randomization Study. JAMA Oncol. 2017; 3: 636–651. https://doi.org/10.1001/jamaoncol.2016.5945 PMID: 28241208

36. Gadalla SM, Cawthon R, Giri N, Alter BP, Savage SA. Telomere length in blood, buccal cells, and fibroblasts from patients with inherited bone marrow failure syndromes. Aging. 2010; 2: 867–874. https://doi.org/10.18632/aging.100239 PMID: 21113082

37. Granick M, Kimura M, Kim S, Danial L, Cao X, Herbig U, et al. Telomere dynamics in keloids. Eplasty. 2011; 11: e15. PMID: 21436892
38. Friedrich U, Griese E, Schwab M, Fritz P, Thon K, Klotz U. Telomere length in different tissues of elderly patients. Mech Ageing Dev. 2000; 119: 89–99. PMID: 11080530
39. Daniali L, Benetos A, Susser E, Kark JD, Labat C, Kimura M, et al. Telomeres shorten at equivalent rates in somatic tissues of adults. Nat Commun. 2013; 4: 1597. https://doi.org/10.1038/ncomms2602
40. Gardner JP, Kimura M, Chai W, Durrani JF, Tchakmakjian L, Cao X, et al. Telomere dynamics in macaques and humans. J Gerontol A Biol Sci Med Sci. 2007; 62: 367–374. https://doi.org/10.1093/gerona/62.4.367 PMID: 17452729
41. Maciejowski J, de Lange T. Telomeres in cancer: tumour suppression and genome instability. Nat Rev Mol Cell Biol. 2017; 18: 175–186. https://doi.org/10.1038/nrm.2016.171 PMID: 28096526
42. d’Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, et al. A DNA damage checkpoint response in telomere-initiated senescence. Nature. 2003; 426: 194–198. https://doi.org/10.1038/nature02118 PMID: 14608368
43. Tomasetti C, Vogelstein B. Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science. 2015; 347: 78–81. https://doi.org/10.1126/science.1260825 PMID: 25554788
44. Kaszubowska L. Telomere shortening and ageing of the immune system. J Physiol Pharmacol. 2008; 59 Suppl 9: 169–186.
45. Aladdin H, Katzenstein T, Dreves A-M, Ryder L, Gerstoft J, Skinhøj P, et al. T-cell receptor excisional circles, telomere length, proliferation and apoptosis in peripheral blood mononuclear cells of human immunodeficiency virus-infected individuals after 18 months of treatment induced viral suppression. Scand J Immunol. 2003; 57: 485–492. https://doi.org/10.1046/j.1365-3083.2003.01258.x PMID: 12753506
46. Zamarron BF, Chen W. Dual roles of immune cells and their factors in cancer development and progression. Int J Biol Sci. 2011; 7: 651–658. https://doi.org/10.7150/ijbs.7.651 PMID: 21647333
47. Stewart SA, Bertuch AA. The role of telomeres and telomerase in cancer research. Cancer Res. 2010; 70: 7365–7371. https://doi.org/10.1158/0008-5472.CAN-10-1373 PMID: 20841475