



Mini-review

Surrogate tissue telomere length and cancer risk: Shorter or Longer?

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ABSTRACT

Telomeres play a critical role in chromosome stability. Telomere length (TL) shortening is a risk factor for cancers. Measuring TL in surrogate tissues that can be easily collected may provide a potential tool for early detection of cancers. A number of studies on surrogate tissue TL and cancer risks have been conducted and results are inconsistent, including positive, negative, or null associations. In this article, we reviewed the published data on surrogate tissue TL in relation to cancer risks, discussed the possible reasons for the differences in the results and future directions and challenges for this line of research.

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1. Introduction

Telomeres are complexes of tandem repeats of DNA (10–15 kb, 5′-TTAGGG-3′) and associated proteins that cap eukaryotic chromosomes, which protect chromosome stability from nucleolytic degradation, chromosome ends infusion, and chromosome aberrant recombination [1]. Telomeres shorten with the increase in age. Inflammation and oxidative stress have been shown to result in accelerated telomere length (TL) shortening [2–7]. Telomerase plays a key role in stabilizing telomeres by adding hexameric (TTAGGG) repeats to the telomeric ends of the chromosomes, thus compensating for the continued erosion of telomeres [1]. Telomerase activity can be detected in most of malignant tumors and some somatic tissues, including blood leukocytes [8–10]. Therefore, telomeric DNA is dynamic, and TL is considered the result of the balance between processes that shorten telomeres (i.e., the end-replication problem) and processes that lengthen telomeres involving telomerase in somatic cells that need to divide regularly, such as blood leukocytes [11,12].

Telomere shortening in genomic DNA appears to reflect lifetime cumulative oxidative stress and chronic inflammation [2–6], two major pathways of carcinogenesis. Short telomeres are associated with cellular senescence and decreased tissue renewal capacity

[13,14]. It has been postulated that shortened telomeres could play a causal role in carcinogenesis by instigating chromosomal instability, thus promoting neoplastic transformation [15] (Fig. 1). Investigating TL in cancer patients and their counterparts may provide mechanistic insight for cancer etiology and help identify high-risk populations for cancer development.

2. Telomere length and cancer risks

Investigations on TL and cancer have been extensively conducted on tumor tissues [16,17]. However, measurement of TL in tumor tissue may have limited risk predictive value because tumor tissues can only be obtained invasively and expensively after disease development and/or even during or after treatment, which may affect TL in the tissue. In addition, compensating for the telomere erosive process with telomerase expression in cancer cells may also affect TL in tumors. Thus, measurement of TL in tumor tissue has limited value as a marker for early cancer detection or for etiological research. Non-tumor surrogate tissues, such as blood leukocytes, sputum, and buccal cells, can be easily and inexpensively collected and TL in these tissues may represent both a valuable resource to evaluate TL-related risks in research investigations and a potential tool to screen individuals for their future risk of cancer. As listed in Table 1, to date, a number of studies have examined TL in surrogate tissues in relation to cancer risks. However, the results have been inconsistent, showing positive, inverse, or null associations between TL and cancer risks, with the majority of studies reporting short TL associated with cancer risks in retrospective studies. Two recent meta-analyses have shown that the

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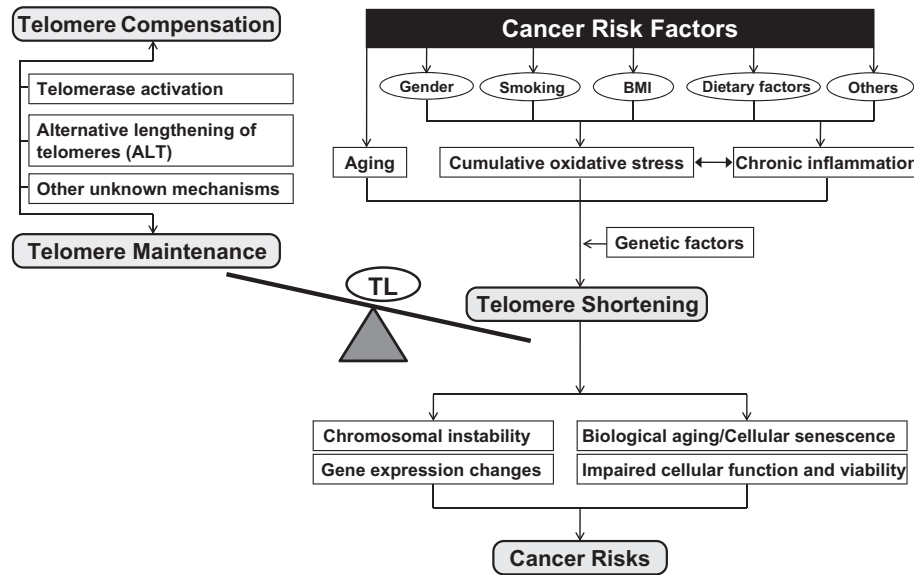


Fig. 1. Hypothesized conceptual model for telomere length shortening and cancer risk. Telomere length is the result of the balance between telomere shortening and maintenance processes. Age-related telomere length shortening can be accelerated by factors that increase oxidative stress and/or cause chronic inflammation. Shortened telomere length may cause chromosomal instability and impaired cellular function, which may contribute to increased cancer risks.

Table 1
Studies evaluating association of TL in surrogate tissues with cancer risk

Cancer site & Ref.	Study design	Tissue types	Sample Size	Methods	TL in cancer patients relative to controls
Head/neck [3]	Retrospective	Blood leukocyte	92 cases, 92 controls	Southern Blot	Short
Lung [3]	Retrospective	Blood leukocyte	54 cases, 54 controls	Southern Blot; FISH	Short
Lung [20]	Retrospective	Blood leukocyte	243 cases, 243 controls	Q-PCR	Short
Lung [21]	Retrospective	Sputum	120 cases, 110 controls	Q-PCR	Short
Kidney [3]	Retrospective	Blood leukocyte	32 cases, 32 controls	Southern Blot; FISH	Short
Kidney [22]	Retrospective	Blood leukocyte	65 cases, 65 controls	FISH	Short
Bladder [3]	Retrospective	Blood leukocyte	135 cases, 135 controls	Southern Blot	Short
Bladder [23]	Retrospective	Buccal	63 cases, 93 controls	Q-PCR	Short
Bladder [24]	Retrospective	Blood leukocyte	184 cases, 192 controls	Q-PCR	Short
Ovarian [25]	Retrospective	Blood leukocyte	99 cases, 100 controls	Q-PCR	Short
Breast [26]	Prospective & Retrospective	Blood leukocyte	2243 cases, 2181 controls	Q-PCR	None (prospective)
Short (retrospective)					
Breast [27]	Retrospective	Blood leukocyte	1067 cases, 1100 controls	Q-PCR	Short
Breast [28]	Retrospective	Blood leukocyte	287 cases, 350 controls	Q-PCR	Short
Gastric [29]	Retrospective	Blood leukocyte	300 cases, 416 controls	Q-PCR	Short
Gastric [30]	Retrospective	Blood leukocyte	396 cases, 378 controls	Q-PCR	Short
Skin (BCC) [31]	Retrospective	Blood leukocyte	300 cases, 870 controls	Q-PCR	Short
Colorectal [26]	Prospective & Retrospective	Blood leukocyte	2,249 cases, 2161 controls	Q-PCR	None (prospective)
Short (retrospective)					
Osteosarcoma [32]	Retrospective	Blood leukocyte	98 cases, 69 controls	Q-PCR	Short
Esophagus [33]	Prospective	Blood leukocyte	300 cases	Q-PCR	Short
Overall risks [34]	Prospective	Blood leukocyte	92 cases	Q-PCR	Short
NHL [35]	Age matched pair	Blood leukocyte	40 cases, 40 controls	FISH	Short
Skin (melanoma) [31]	Retrospective	Blood leukocyte	218 cases, 870 controls	Q-PCR	Long
Breast [43]	Retrospective	Blood leukocyte	365 cases, 446 controls	Q-PCR	Long
Breast [44]	Retrospective	Blood leukocyte	102 cases, 50 controls	Q-PCR	Long
Lung [45]	Retrospective	Blood leukocyte	229 cases, 229 controls	Q-PCR	Long
HCC [46]	Retrospective	Blood leukocyte	240 cases, 240 controls	Q-PCR	Long
NHL [47]	Prospective	Blood leukocyte	107 cases, 107 controls	Q-PCR	Long
Kidney [48]	Retrospective	Blood leukocyte	105 cases	Q-PCR	Long
Breast [59]	Retrospective	Blood leukocyte	153 cases, 159 controls	Q-PCR, FISH	None
Breast [60]	Retrospective	Blood leukocyte	140 cases, 108 controls	Southern Blot	None
Breast [61]	Case-cohort	Blood leukocyte	342 cases, 735 subcohort	Q-PCR	None
Prostate [62]	Retrospective	Blood leukocyte	612 cases, 1049 controls	Q-PCR	None
Skin (SCC) [31]	Retrospective	Blood leukocyte	285cases, 870 controls	Q-PCR	None
Skin (SCC) [63]	Retrospective	Blood leukocyte	241 cases, 241 controls	Q-PCR	None
Endometrial [64]	Retrospective	Blood leukocyte	551 cases, 1320 controls	Q-PCR	None
Breast [65]	Prospective	Blood leukocyte	1122 cases, 1147 controls	Q-PCR	None
Colorectal [66]	Prospective	Blood leukocyte	357 cases, 134 controls	Q-PCR	None
Colorectal [67]	Prospective	Blood leukocyte	191 cases, 306 controls	Q-PCR	None

BCC, basal cell carcinoma; SCC, squamous-cell carcinoma; HCC, hepatocellular carcinoma.

associations of short TL with risk for various cancers were stronger in retrospective studies compared with prospective studies [18,19]. In this review, we summarize the previous observations on blood TL and cancer risk by shorter or longer TL, or null

association, provide possible explanations, and discuss the potential challenges and future directions in TL-cancer research using surrogate tissues.

2.1. Shorter TL and cancer risks

Most studies have reported that shorter TL in surrogate tissues is associated with increased cancer risks. The majority of these studies are retrospective case-controls studies, including the studies of head and neck [3], lung [3,20,21], renal cell [3,22], bladder [3,23,24], ovarian [25], breast [26–28], gastric [29,30], basal cell carcinoma (BCC) [31], colorectal cancer (CRC) [26], and osteosarcoma [32]. For instance, we recently reported that gastric cancer risk was doubled (OR = 2.04, 95% CI = 1.33–3.13) among subjects in the lowest compared to the highest quartile of TL [29]. Gastric cancer cases had significantly shorter TL (mean TL \pm SD = 1.25 \pm 0.34) than controls (1.34 \pm 0.35) (p = 0.0008). In addition, there are two prospective studies on esophageal cancer [33] and overall cancer risk [34], and an age-matched pair study of Non-Hodgkin's lymphoma (NHL) [35].

The shorter TL in cancer patients compared to controls without cancer is biologically plausible. Telomeres at the ends of chromosomes play important roles in maintaining genomic integrity and stability [36–38]. When telomeres become critically shortened, cells undergo either replicative senescence or apoptosis [39]. If such processes are bypassed, cells continue to proliferate through activation of telomerase, leading to genomic instability. The accumulated mutations, genetic lesions, and inactivated tumor suppressor checkpoints may ultimately result in cancer [15,40].

Evidence suggests that aging-related telomere attrition can be accelerated by cancer risk factors such as obesity, smoking and dietary factors [2,10,23,24,33,41,42]. Our investigation of TL in gastric cancer confirmed that factors increasing oxidative stress or inflammation, such as cigarette smoking, decreased fruit and vegetable intake, and chronic *H. Pylori* infection are associated with shorter TL in blood DNA [29]. Therefore, it is biologically plausible that environmental factors may cause cancer via, at least partially, TL shortening mechanism. However, the majority of current studies reporting short TL associated with cancer risks were retrospective studies, in which samples were collected after the cancer diagnosis. Shorter TL in cancer patients in these studies could be caused by the reverse-causation problem, a limitation in retrospective case-control design.

2.2. Longer TL and cancer risks

Although most studies observed shorter blood leukocyte TL in cancer patients, seven recent studies have reported an association between longer TL in blood leukocyte DNA and cancer risk, including case-control studies of melanoma [31], breast [43,44], lung cancer [45] and hepatocellular carcinoma (HCC) [46], a prospective studies of NHL [47] and a retrospective study of renal cell carcinoma (RCC) [48].

These authors posited biologically reasonable explanations for their findings. As breast cancer is a hormone-related cancer, the prolonged estrogen exposure in breast cancer patients could be a possible reason for the longer TL observed in Svenson's and Gramatges's studies [43,44]. This explanation is supported by reports of longer TL in women than men caused by estrogen exposure [49–52], and longer TL in postmenopausal women with hormone replacement therapy than those without hormone replacement therapy [53]. Moreover, the anti-oxidative capacity of estrogen may be contributing to TL maintenance [54]. Similarly, Svenson et al. found that RCC patients with longer TL had a significantly worse prognosis compared with patients with shorter TL [48]. In RCC patients, down-regulation of the immune response

could theoretically lead to less telomere attrition [48]. It is also possible that patients with longer TL had elevated levels of various telomerase-stimulating factors [48]. In response to oncogenic stress, cutaneous melanocytes are most likely to senesce due to their low level of proliferation and limited capacity to undergo apoptosis [55]. However, mutated cells with longer TL might experience a delay in senescence as a result of the great replication potential. The observed longer TL in melanoma patients might also stem from this mechanism [31]. Longer TL was found in lung cancer patients [45]. In this study, lung cancer patients smoked more than controls thus may have a higher chance to have inflammation [56]. Therefore, the composition of blood leukocytes may be different between patients and controls. TL can be different by blood leukocyte sub-types [57,58], which might have led to the observed longer TL in lung cancer patients. Longer TL was also found in NHL patients in a prospective study by Lan et al. [47]. A recent study examined 240 HCC patients, 240 healthy controls, and 120 noncancer controls with chronic liver disease (CLD) has reported that HCC cases exhibited a significantly longer TL than CLD controls and healthy controls. They also showed that individuals with long TL had a significantly increased risk of HCC relative to either healthy or CLD controls [46]. As Lan [47] and Liu [46] have discussed, cells with longer telomeres may favor a delayed cell senescence, therefore, these cells could have more opportunities to develop chromosomal instability and acquire genetic abnormalities, thus being at higher risk of carcinogenic transformation [55]. However, because the systemic immune system is very dynamic, using peripheral blood leukocytes might be not the perfect surrogate tissue for estimating overall TL, a biomarker of biological aging.

2.3. Null association between TL and cancer risk

Null associations were also demonstrated in seven retrospective case-control studies of breast [59–61], prostate [62], squamous-cell carcinoma (SCC) [31,63] and endometrial cancer [64], and five prospective studies of breast [26,65] and colorectal cancer [26,66,67]. It is well documented that in breast cancers, chromosomal abnormalities are not random and concentrate on certain chromosome arms [68–70]. Thus, the null association of overall TL with various cancers might be caused by telomeres on “irrelevant” chromosomes instead of telomeres on specific chromosomes that play pathological roles. Pooley et al. evaluated the association between TL with CRC in a prospective and a retrospective case-control study. The null association of TL with CRC in the prospective study [26] agrees with the other two prospective studies of colorectal cancer [66,67], while the shorter TL observed in the retrospective study suggests that majority of telomere attrition occurs after CRC diagnosis rather than before or during cancer development.

2.4. Possible reasons for the inconsistent results and limitations of study surrogate tissue TL

Differences in study design, biological sample collection and processing, specific cancer site, limited statistical power, variability in confounding factors, and differences in laboratory measurement of TL, may be contributing factors to inconsistent results in telomere association studies. TL has been measured using various methods, including terminal restriction fragment (TRF), quantitative fluorescence in situ hybridization (QFISH), flow FISH and real-time PCR. TRF, a traditional method of measuring TL, requires large amounts of DNA and time [71], and results are dependent on the particular restriction enzymes used. Currently, real-time PCR is the most economical and versatile high-throughput method and TL measurements are highly consistent with the Southern blot assay [72].

In case-control and some cohort studies, biological samples were collected after the disease diagnosis and/or treatment initiation. Studying prospectively collected pre-diagnostic surrogate samples can avoid the potential impact caused by cancer development and treatment. However, malignant changes may occur prior to the diagnosis [73,74]. Therefore, it remains unclear whether the timing of sample collection plays a role in the variability of findings across studies with pre-diagnostic surrogate tissues. Our recent investigation on buccal cell TL and prostate cancer risk in subjects with pre- or post-diagnostic samples has shown that shorter TL in pre-diagnostic samples collected four or more years prior to prostate cancer diagnosis was associated with prostate cancer risk but not in post-diagnostic samples or pre-diagnostic samples collected three or fewer years prior to the diagnosis (data not shown). The finding of no association between TL in pre-diagnostic buccal cell DNA collected three or fewer years prior to prostate cancer diagnosis is consistent with the null association of blood leukocyte TL with prostate cancer risk reported in a nested case-control study in the US Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial [62,75], which included only cases who had provided blood samples three or fewer years prior to the diagnosis.

TL is the result of the balance between telomere shortening and lengthening processes. The age-related TL shortening can be accelerated by increased oxidative stress and telomere lengthening occurs when TL integrity maintenance mechanisms are activated by certain conditions, such as cancer development. Although the exact mechanisms for reversal of age-related TL shortening in normal somatic cells are largely unknown, these observations suggest that the timing interval between sample collection and cancer diagnosis may be a contributing factor for the differences in the published data on the association between TL and cancer risk. It is possible that peripheral blood leukocyte telomere lengthening could be an overall activation of the immune system during first a few years of tumor formation, i.e., some years before disease diagnosis. An alternative explanation could be when surrogate tissue TL becomes critically shortened due to exposure to risk factors, it may in turn trigger compensatory mechanisms, such as increased telomerase activity and an alternative non-telomerase-based mechanism lengthening telomeres that maintain TL integrity [9]. Thus, the suitability of peripheral blood leukocytes as surrogate tissue for somatic TL measurements may be limited due to several reasons. TL in samples collected close to cancer diagnosis may be lengthened by such processes, which might negate the association of shortened TL with cancer risk. However, the compensatory mechanism of TL in surrogate tissues in cancer patients is unclear and likely to be similar to the ones in tumor tissues. For example, a recent study has demonstrated that comprehensive lifestyle improvement, including increased physical activity and healthier diet, are significantly associated with increases in telomerase activity in blood leukocytes in prostate cancer patients, which may potentially lengthen shortened TL [10]. In addition, blood leukocyte TL can also be modified by cancer treatment. A recent study has shown TL shortening in breast cancer patients after completion of chemotherapy [76]. The differential TL between subtypes of blood leukocyte populations, and the fact that in most studies the DNA was extracted from mixed blood leukocytes also limit the interpretation of TL in blood leukocytes. In addition, TL in blood leukocyte DNA may be different from TL in targeted tissues. For examples, a study has shown that, although leukocyte TL in aplastic anemia (AA), a human hematopoietic disease with proclivity to hematologic cancers, patients was comparable to age-matched controls, bone marrow cells from these AA patients had shorter telomeres than controls [77]. AA patients with shorter bone marrow cell TL had a higher proportion of aneuploidy cells and a higher frequency of structural chromosomal aberrations compared with patients with longer telomeres and controls [77]. Thus, more studies exam-

ining TL in blood and other surrogate tissues in comparison with TL in targeted tissues are needed.

3. Future directions in TL-cancer research using surrogate tissues

Our understanding of telomeres and telomere biology has advanced greatly since the discovery of telomerase and telomere maintenance [78]. However, a variety of issues remain to be addressed in telomere-cancer research using non-tumor surrogate tissues.

Some sub-populations of blood leukocytes may have shorter telomeres than others. For example, it has been shown that TLs are different in memory vs. naive and CD41 vs. CD81 T-cells [79]. These subsets of cells may play specific roles in cancer, and thus, assessing telomere dynamics in leukocyte subsets may be essential to answering specific questions in telomere cancer research.

It is still unclear whether telomeres on all chromosomes or only telomeres on specific chromosomes contribute to tumorigenesis. It has been suggested that loss of telomere function occurs preferentially on chromosomes with critically short telomeres. It was observed by Leach et al. [80] that human chromosomes with shorter telomeres have a higher frequency of acquired somatic cell aneuploidy in cultured lymphocytes. A recent study provided the first epidemiologic evidence that short telomere length of specific chromosomes, such as 17p and 12q, is associated with an increased risk of esophageal cancer [81]. Another study observed shorter telomeres on chromosome 9p-short were strongly associated with an increased risk of breast cancer [82]. Therefore, more studies measuring TL on specific chromosomes of different cancer types are needed.

TL at birth, and telomere attrition and maintenance afterwards are genetically determined. The T allele of the -1381 promoter SNP in the telomerase gene (*TERT*, T > C, rs2735940) was associated with significantly longer telomeres and increased telomerase activity than in individuals with the CC genotype [83]. Relative to other genes, the extent of genetic variation is limited in genes important in telomere biology [84], suggesting that genetic variation in these genes may not be tolerated because of the important role that these genes play in maintaining the telomere integrity and thus in chromosomal stability. Consequently, these genetic variants may be more likely to be significant risk factors for diseases in which chromosomal instability is implicated, such as cancer. A study by Savage et al. has shown suggestive associations between SNPs in *TERT* and the risk of breast cancer among individuals with a family history of the malignancy [85]. Therefore, it is important to consider the effect of gene-environmental interaction in TL-cancer studies.

Either excessively short or long TL may both contribute to cancer development if the balance is broken. Further investigations, taking into account the above-mentioned and other factors, are needed to improve our understanding of surrogate tissue TL as a biomarker for cancer risk and progression. Because of its dynamic nature, large longitudinal studies are required to study telomere dynamics in surrogate tissue in relation to cancer initiation and development. Ultimately, study of TL in easily accessible surrogate tissues could lead to the development of telomere and/or telomerase-related measures for cancer prevention and therapy.

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