Telomere in Cancer Development

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Abstract

Telomeres are long hexameric (TTAGGG)n repeats at the ends of the p and q arms of linear eukaryotic chromosomes. Telomeres play a critical role in maintaining the structural integrity of chromosomes by preventing fusion of chromosomal ends, nucleolytic decay, end-to-end fusion, and atypical recombination. Telomere length in peripheral blood lymphocytes (PBLs) has emerged as a potential biomarker of aging and risk of age-related diseases such as cancers. Telomere length is a complex trait that is shaped by genetic and environmental determinants. In this review, we summarize the previous studies on the association of telomere length and telomere-related genetic variants with the risk of various cancers. *[N A J Med Sci. 2010;3(2):48-52.]*

Key Words: *Telomere length, cancer*

Telomere Shortening and Cancer

Telomeres are distinctive structures capping both ends of linear eukaryotic chromosomes. Human telomeres consist of long hexameric (TTAGGG)n repeats, which are associated with a number of telomere-related proteins. The repeating sequences of telomeres comprise double-strand DNA with a G-rich single-strand 3' overhang end.[1] Studies in mice and yeast have demonstrated the critical role of telomeres in maintaining the structural integrity of chromosomes by preventing fusion of chromosomal ends, nucleolytic decay,end-to-end fusion, and atypical recombination.[2] However, at each cell division, DNA polymerases fail to completely replicate telomeres, resulting in cumulative erosion of the telomeres.[3,4] This phenomenon is called the "end-replication problem". Telomeric repeats shorten by 30-200 bp after each cycle of mitotic division and have been likened to a "molecular clock" reflecting the number of divisions a cell has undergone.[5] Telomere length is maintained by telomerase, a ribonucleoprotein enzyme. Telomerase appends chromosome ends with hexameric repeats to restore telomere length. Telomerase activity is generally low in replicative somatic tissues but high in germ cells, in which chromosomal integrity is critical for fecundity.[6-8] Most adult somatic tissues do not express telomerase, resulting in the progressive loss of telomeric DNA with age.

Telomere shortening plays conflicting roles in cancer development. On one hand, the progressive loss of telomeric repeats with each cell division limits the total number of times a cell can divide. When telomeres shorten to a critical length, a cell cycle checkpoint is triggered, proliferation is blocked, and the cell enters replicative senescence. Senescent cells express β-galactosidase, develop a characteristic flat and vacuole-rich cytoplasmic morphology, and undergo a permanent and irreversible cell cycle arrest in G1 phase, but remain metabolically active.[9] This constraint on proliferation prevents the accumulation of oncogenic mutations and subsequent malignant transformation. However, if the Rb and/or p53 signalling pathways have been inactivated, cell division continues, resulting in further telomere shortening with a concurrent increase in genomic instability.[10,11] Eventually, the dividing cell reaches crisis, a second proliferation block characterized by gross chromosomal aberrations. The vast majority of these cells undergo apoptosis. Once a threshold length of 12.8 repeats (77 base pairs) is reached, chromosome ends become fusogenic, resulting in chromosome instability,[12] which may lead to malignant transformation. Rarely a cell may trigger the reactivation of telomerase, which at this stage is thought to facilitate tumor initiation and progression. Reactivation of telomerase is detected in more than 80% of human tumors,[13] making it one of the most common abnormalities in cancer cells.

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Telomere Length in Peripheral Blood Leukocytes (Pbls) and Cancer Risk

Correlation between telomere length in PBL and target tissue

A correlation between telomere length in PBLs and in some tissues has been demonstrated. Friedrich et al[14] measured telomere length in PBLs and that in skin and synovial tissues of nine elderly patients. Although the mean telomere length was shorter in PBLs than in skin and synovium, the lengths in PBLs were significantly correlated with those in skin and synovium. These authors concluded that "the measurement of telomere length in easily accessible tissues such as blood could serve as a surrogate parameter for the relative telomere length in other tissues."

Association between PBL telomere length and cancer risk

Telomere length in PBLs has emerged as a potential biomarker of aging and risk of age-related diseases such as cancers. A few epidemiologic studies have examined the relationship between PBL telomere length and the risk of various cancers (**Table 1**). In a subset of participants in Wu et al. [15], shorter PBL telomere length was significantly correlated with baseline DNA damage as measured by the Comet assay and with mutagen sensitivity in lymphocytes after gamma-irradiation or exposure to benzo[*a*]pyrene diol epoxide, suggesting that telomere length is a marker of DNA damage and of susceptibility to such damage.

Table 1. Telomere length and the risk of various cancers from previous studies.

	Cases/Controls	OR (95% CI)	Overall telomere length	*p* for trend
Combined cancer sites*				
Wu et al. 2003 [15]	221/164	4.4 (2.1-9.3)	shortest vs. longest quartile	0.001
Head and neck cancer				
Wu et al. 2003 [15]	92/92	5.1 (1.9-13.8)	shortest vs. longest quartile	-
Bladder cancer				
Broberg et al. 2005 [42]	63/93	4.0 (1.5-11.0)	shortest vs. longest quartile	-
Breast cancer				
Shen et al. 2007 [43]	287/350	1.6 (0.9-2.7)	shortest vs. longest quartile	0.14
Shen et al. 2009 [44]	1,067/1,110	1.1 (0.9-1.4)	shortest vs. longest quartile	0.69
	Premenopausal women	1.6 (1.1-2.5)	shortest vs. longest quartile	0.01
De Vivo et al. 2007 [45]	1122/1147	1.3 (0.8-1.9)	shortest vs. longest quartile	0.2
Bladder cancer				
McGrath et al. 2007 [46]	184/192	1.9 (1.1-3.4)	shortest vs. longest quartile	0.006
Lung cancer				
Jang et al. 2008 [47]	243/243	8.7 (4.1-18.7)	shortest vs. longest quartile	<0.0001
Hosgood et al. 2009 [37]	120/110	1.6 (0.8-3.2)	shortest vs. longest tertile	0.28
Shen et al. 2009 [48]	229/229	0.6 (0.4-1.1)	shortest vs. longest quartile	0.04
Prostate cancer				
Mirabello et al. 2009 [49]	612/1049	0.8 (0.6-1.0)	shortest vs. longest quartile	0.34
Non-Hodgkin lymphoma				
Lan et al. 2009 [50]	107/107	0.3 (0.1-0.7)	shortest vs. longest quartile	0.003

* Combined from the cases of bladder, lung, and renal cell cancers and matched controls.

Association between PBL chromosome-specific telomere length and cancer risk

Several studies suggest that the regulation of telomere length in mammalian cells may be chromosome-specific.[16,17] It has been suggested that there is a chromosome-specific pattern of telomere lengths.[18] Moreover, Graakjaer et al.[17] showed that this pattern is partly inherited in humans and maintained throughout life. Modified real-time PCR-based single telomere length analysis (STELA) in PBLs has been developed to measure telomere lengths from several individual chromosomes (17p, 2p, 11q, 12q, and XpYp).[16,19,20] The telomeres on chromosome 17p exhibit the shortest length in senescent cells.[16,21]

Telomerase-expressing cancer cells revealed both allelic variation and chromosome-specific telomere length, with 17p displaying the shortest allelic telomere length.[16] These observations provide evidence of chromosome-specific factors regulating the individual chromosome telomeres and raise the possibility that the relatively short telomeres on chromosome 17p contribute to the frequent loss of 17p alleles in human cancers. The p53 tumor suppressor gene is located on 17p, and loss of heterozygosity (LOH) at this chromosome arm has been found in early-stage neoplasia.[22]

It is conceivable that the telomere at this chromosome end has evolved to elicit a more stringent response to a critical telomere erosion to protect against LOH.

Martens et al[21] showed that telomere lengths on specific chromosome arms are very similar in different tissues donated by the same individual. The first published epidemiologic study evaluating chromosome-specific telomere length as a potential biomarker for cancer risk revealed that shorter telomeres on 17p were significantly associated with an increased risk of esophageal cancer (p=0.003).[20]

Telomere-related Genetic Variants and Cancer Risk

Telomere length has been shown to have a substantial heritable component in twin studies.[23-26] Genome-wide microsatellite studies have identified specific loci that are linked to telomere length, but specific genes have not been established.[23,27] These studies strongly suggest that between-person genetic variation plays a major role in the rate of telomere shortening in humans.

Telomere length maintenance genes

Given the role of telomerase in maintaining telomere length in germ and neoplastic tissue, genetic factors such as single nucleotide polymorphisms (SNPs), which can regulate telomerase expression, may affect susceptibility to cancer. Recent studies by Matsubara et al[28] have identified a potential functional polymorphism (rs2735940) in the hTERT promoter that affects telomere length in leukocyte-derived genomic DNA. Using reporter-based assays, Matsubara et al[28] demonstrated that hTERT promoter activity was significantly higher in the -1327T allele than in the -1327C allele (p = 0.0004). The -1327T allele carriers also had significantly longer telomeres compared to those with the homozygous -1327C allele (p = 0.0007). A follow-up case-control study consisting of 104 coronary artery disease (CAD) patients and 115 matched controls showed a higher frequency of the -1327C/C genotype in CAD patients (51.9% vs. 36.5%, p = 0.02).[29] Among the 104 CAD patients, leukocyte telomere length in individuals with the -1327C/C genotype (7.62+/-2.19 kb, mean+/-SD) was shorter than in individuals with the -1327T/C and -1327T/T genotypes (8.74+/-2.92, p = 0.03).[29] It is evident that the -1327T/C polymorphism in the hTERT promoter may be an important factor in modulating telomerase activity and telomere length. Therefore, further discovery and characterization of SNPs that play a role in regulating telomerase activity will become a critical avenue of research in assessing susceptibility to chronic diseases such as cancer.

Table 2. Genes involved in telomere stability and maintenance.

Gene symbol	Gene name	Location
TERT	Telomerase reverse transcriptase	5p15.33
POT1	Protection of telomeres 1	7q31.33
TRF1	Telomeric repeat binding factor 1	8q13
TRF2	Telomeric repeat binding factor 2	16q22.1
TNKS	Tankyrase	8p23.1
TINF2	TRF1 interacting nuclear factor 2	14q11.2
TRF2IP	TRF2 interacting protein	16q23.1

Although numerous factors are involved in the telomere maintenance complex, seven prominent candidates are chosen for their enzymatic activity or DNA binding properties: TERT, POT1, TNKS, TRF1, TRF2, TINF2, and TRF2IP (**Table 2**). The *TERT* gene encodes the reverse transcriptase subunit of telomerase, and its significance is mentioned in previous sections. In recent years, the Protection of Telomeres (*POT1*) gene has emerged as a critical component of the telosome complex. *POT1* encodes a protein that binds to the single-stranded G-overhang at the distal ends of telomeres. The primary function of POT1 is to regulate the length of the G-overhang and prevent telomeric

ends from being recognized as strand breaks by the DNA repair machinery, thus circumventing non-homologous end joining of telomere ends.[30] Telomere Repeat Factors (TRF1 and TRF2) are proteins of the telosome complex that bind to double-stranded TTAGGG telomeric repeats. Both TRF factors act as DNA 'anchors' and complex with other proteins of the telosome such as Rap1, Tin2, and Tpp1. The telosome complex creates a closed state in the telomere called the T-loop that is stabilized when the POT1-bound single-stranded G-overhang is brought close to upstream double-stranded telomeric repeats bound to TRF1 and TRF2.[31] The *TRF1* gene is a negative regulator of telomerase activity.[32] The tankyrase *(TNKS)* gene increases telomere length by inhibiting *TRF1*.[33] The *TRF2* gene maintains the telomeric structure that protects chromosomal ends.[34] The TRF7-interacting nuclear factor 2 *(TINF2)* gene interacts with both *TRF1* and *TRF2*. The TRF2 interacting protein (TRF2IP, also known as Rap1) is a negative regulator of telomere length.[35]

Association between telomere-related genetic variants and cancer risk

Most recently, through a GWAS on basal cell carcinoma (BCC), Rafnar et al identified a locus (rs401681[C]) harboring the TERT gene with an increased risk of BCC.[36] They tested this SNP for association with 16 additional cancer types, and found that the SNP rs401681[C] was associated with an increased risk of lung cancer, bladder cancer, and prostate and cervix cancer, but a reduced risk of melanoma.[36] Hosgood et al evaluated the association between 17 SNPs in *POT1*, *TERT*, and *TERF2* genes and lung cancer risk in 120 Chinese lung cancer cases and 110 controls, and they observed significant associations of POT1 rs10244817, TERT rs2075786, and TERF2 rs251796 with lung cancer risk.[37] Choi et al observed that the TERT rs2735940 and rs2736098, and TNKS1 rs6985140 are associated with an increased risk of lung cancer.[38] For breast cancer, three SNPs in the TERT gene (rs2736109, rs3816659, and rs2853669), one SNP in the POT1 gene (rs33964002), one SNP in the TERF2 gene (rs3785074), and one SNP in the TNKS2 gene (rs10509637) have been reported to be associated with an increased risk of breast cancer.[39,40]

Summary

Telomere length in peripheral blood lymphocytes (PBLs) has emerged as a potential biomarker of aging and risk of age-related diseases such as cancers. Most previous epidemiologic studies reported that shorter telomeres are associated with an altered risk of various cancers. Telomere shortening is implicated in several aspects of tumorigenesis, including senescence, apoptosis, and genomic instability. Because of the distinct proliferative features of different cells, telomere length may play roles in both suppressing and facilitating carcinogenesis.[41] Overall, these findings highlight the need for improved understanding of the role of telomere in carcinogenesis and the necessity of prospective evaluation of telomere length and cancer risk.

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