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Telomeric DNA length in cerebral gray and white matter is associated with longevity in individuals aged 70 years or older

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Abstract

Many studies have demonstrated the association between telomere length in mitotic cells and carcinogenesis and mortality, but little attention has been focused on post-mitotic cells and human life expectancy. We assessed the relationship between telomere length in cerebral gray and white matter and longevity in 72 autopsied Japanese patients aged 0–100 years using Southern blot hybridization. The mean telomere lengths in the gray and white matter were 12.3 ± 2.5 kilobase pairs and 11.4 ± 2.1 kilobase pairs, respectively. The mean telomere lengths in 60–69 year decadal group were less than those of neonates, and declined further in the 70–79-year age group, but those in groups of further advanced age were longer than in the 70–79 year group (70–79 < 80–89 < 90–100 years of age). Thus, the 90–100-year age group possessed significantly longer telomeres than the 70s ($p = 0.029$). Autopsy protocols showed a decrease in the rate of cancer death in individuals in their 80s ($p = 0.041$) and 90s ($p = 0.017$) versus those in their 60s, and in their 80s the mean telomere length in the gray matter from cancer death patients was significantly shorter than that of patients who died of other diseases ($p = 0.04$). These data suggest that innate telomere lengths are maintained very well in the cerebrum, and are associated with longevity. Our study lends indispensable support to the hypothesis that longer telomeres protect the genome from instability (a major cause of carcinogenesis) and are beneficial for longevity.
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Keywords: Telomere length; Aging; Cerebral gray matter; Cerebral white matter; Southern blotting; Longevity; Life expectancy; Cancer death; Carcinogenesis

1. Introduction

Human telomeric DNA is considered to protect chromosomes against degeneration, recombination, fusion, and loss (Blackburn, 1991). Human somatic cells are known to have a limited proliferative life span when serially cultured in vitro. As they approach this limit, they cease to replicate and enter a state of senescence (Hayflick and Moorhead, 1961). This replication arrest in vitro is due at least in part to telomere shortening because it can be bypassed by transfection with the telomerase catalytic subunit gene (Bodnar et al., 1998). The telomere hypothesis of cellular aging suggests that when telomere shortening on a particular chromosome reaches a critical level, a DNA damage checkpoint mechanism may be initiated and the cells stop dividing (Harley et al., 1990; Allsopp et al., 1992). Telomere lengths in vivo have also been measured

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in various human tissues. The previous data suggested that the dynamics of telomere synthesis and loss are unique to each individual, resulting in a wide range of individual specific telomere lengths (Vaziri et al., 1993). Furthermore, data on specific attrition rates of telomeres in replicating somatic cells have been accumulated through the use of several different techniques (Harley et al., 1990; Takubo et al., 1999; Lansdorp et al., 1996; Meeker et al., 2002; Mayer et al., 2006; Cawthon, 2002; Baird et al., 2003). On the other hand, very little is known about telomere attrition in post-mitotic cells, such as neurons and myocardial cells, in which telomere shortening may not occur in the absence of mitosis (Allsopp et al., 1995; Butler et al., 1998; Takubo et al., 2002). The cerebral cortex contains neurons and glial cells, while the white matter has a predominance of oligodendrocytes. Since neurons in adults do not show mitosis, and glial cells are known to be replaced very slowly under normal conditions, there has been a prevailing notion that telomeres in the adult brain are in a stable condition. In the present study, to address the issue of telomere metabolism in the human brain, we measured telomere lengths in the gray and white matters separately.

2. Materials and methods

2.1. Patients

We studied unrelated Japanese individuals who had died and were autopsied at the Department of Pathology, Tokyo Metropolitan Geriatric Hospital, Department of Pathology, Saitama Children's Medical Center, and the Japanese Red Cross Medical Center during February 2000 and June 2001. We prepared brain samples from 72 consecutive autopsied individuals (40 men and 32 women) between 0 and 100 years old with a mean age of 73.9 ± 26.0 years (81.3 ± 10.0 years except for neonates). All of the autopsy diagnoses were made by authorized pathologists (Supplementary Table 1). Family members of all those autopsied provided written informed consent for the collection of organs and tissues for educational and scientific use including DNA analysis. The Tokyo Metropolitan Institute of Gerontology Ethics Committee approved the study protocol.

2.2. Procedures

We dissected paired samples of gray and white matter from the occipital lobe in the autopsy room, and stored them at −80 °C until use. Histological examination to check for autolytic change in the tissues adjacent to the sampled areas was performed by K.T., M.S., T.A., H.K. or M.F., and any tissues found to be autolytic were not used. Furthermore, to verify the quality of samples, we performed pulse-field gel electrophoresis as described elsewhere (Schwartz and Cantor, 1984). Only DNA of more than 100 kbp was selected for this study.

Genomic DNA was extracted using the standard method with proteinase K and sodium dodecyl sulfate (SDS). Southern blot hybridization was performed using the standard protocol and described elsewhere (Takubo et al., 1999). Briefly, 5 μg DNA was digested with the restriction enzyme HinfI (Roche, Germany), and the digested DNA was resolved on 0.8% agarose gels at 20 V for 18–19 h in 0.5× TBE buffer. Fractionated DNA fragments were transferred to nylon membranes (Hybond-N+, Amersham, UK) by an alkaline transfer technique using capillary blotting, followed by hybridization for 12 h at 50 °C in 6× SSPE with 1% (w/v) SDS. A $(TTAGGG)^4$ probe was labeled with $[\gamma\text{-}^{32}P]$ATP (Amersham) at the 5′ end with T4 polynucleotide kinase (Toyobo, Japan). The membranes were washed in 2× standard saline citrate buffer (SSC) at room temperature, and then in 6× SSC, 0.1% SDS, at 50 °C for 15 min, then dried with filter papers, and exposed to Fuji Imaging Plates (Fuji Photo Film Co. Ltd., Japan) for 30 min at room temperature. We performed data processing with a BAS-2500 Mac image analyzer (Fuji Photo Film) using the programs Image Reader (version 1.1, Fuji Photo Film) and Mac Bas (version 2.4, Fuji Photo Film). Telomere lengths (modal values) were determined by comparing the position of the maximum radioactivity in each lane with those of molecular size markers (Supplementary Fig. 1). We measured telomere lengths twice for all samples except neonatal cases, and determined their mean value (Supplementary Table 1).

2.3. Statistical analysis

The statistical analyses were done with StatView software version 5.0 (SAS Institute Inc). The differences in mean values were assessed for significance with Student's *t* test, and for correlation significance with Fisher's test.

3. Results

Whole-sample scatter plot analysis revealed only a low rate of decrease of telomere length with aging in both the gray and white matter (Fig. 1). Although the regression lines of telomere length in the gray and white matter descended according to age, a statistically significant relationship between telomere length reduction and age was observed only in the white matter: $y = 12.5 - 0.02x$ ($p = 0.0459$), where y is telomere length in the white matter in kbp, and x is age in years. Importantly, in most individuals, telomeres in the gray matter were longer than those in the white matter. Consequently, the mean telomere length in the gray matter [12.3 ± 2.5 kbp (SD)] was slightly but significantly greater than that in the white matter [11.4 ± 2.1 kbp (SD)] ($p < 0.0001$) (Fig. 1). We also found a marked correlation between the telomere lengths in the gray and white matter in each individual ($r = 0.897$, $p < 0.0001$) (Fig. 2). When we divided the samples into five age groups (0, 60–69, 70–79, 80–89, and 90–100 years),

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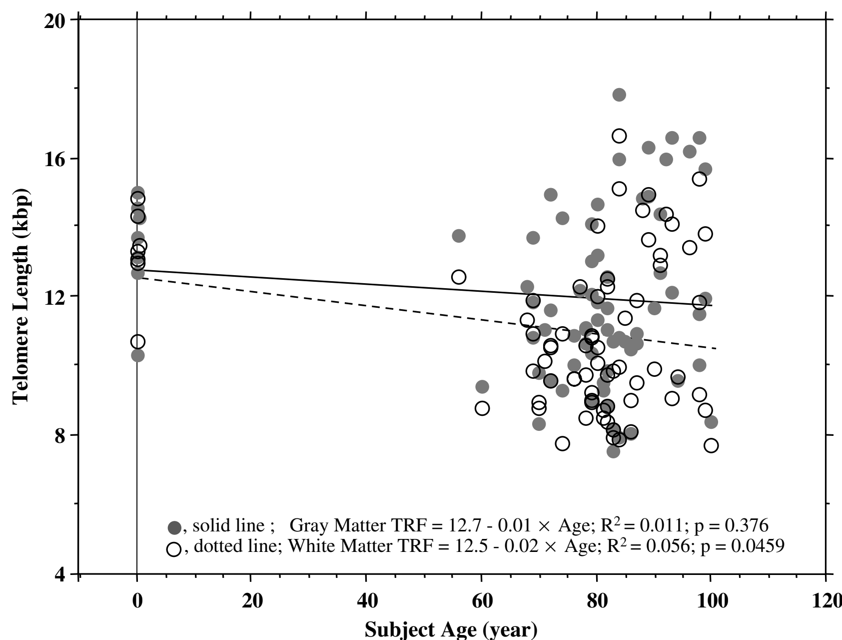

Fig. 1. Regression analysis between telomere reduction and aging in the gray and white matter of the brain, showing scatter plots for samples taken from individuals. There is significant reduction of telomere length only in the white matter. Mean lengths (±SD) of telomeric DNA in the gray and white matter in all cases were 12.3 ± 2.5 kbp and 11.4 ± 2.1 kbp, respectively. Mean telomere length in the gray matter was significantly greater than that in the white matter, with differences of 0.05–0.88 kbp (mean 0.45 kbp) ($p < 0.0001$) in elderly individuals, but the difference was not significant in neonates ($p = 0.534$).

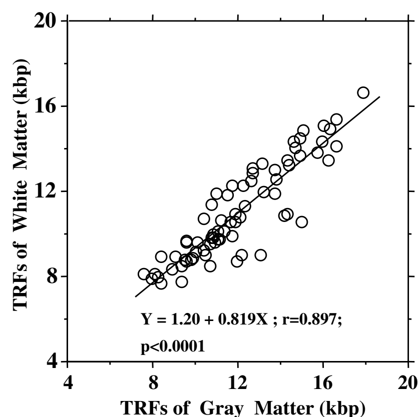

Fig. 2. Robust correlation of telomere lengths between paired samples of gray and white matter from 72 individuals. (1st experiment: $r = 0.897$, $p < 0.0001$, 2nd experiment: $r = 0.762$, $p < 0.0001$: data not shown).

again we found that the telomeres in the gray matter were slightly but significantly longer than those in the white matter in the 4 older age groups (means ± SD); 60s ($n = 5$): 11.6 ± 1.6 kbp > 10.6 ± 1.2 kbp ($p = 0.0064$), 70s ($n = 19$): 11.3 ± 1.8 kbp > 9.8 ± 1.1 kbp ($p = 0.0004$), 80s ($n = 26$): 11.5 ± 2.8 kbp > 11.0 ± 2.6 kbp ($p = 0.0002$), 90–100 ($n = 14$): 13.1 ± 2.8 kbp > 11.7 ± 2.5 kbp ($p = 0.0006$) (Fig. 3).

In neonates, however, no significant difference in telomere length was evident between the gray and white matter, 13.4 ± 1.6 kbp and 13.3 ± 1.3 kbp, respectively ($p = 0.534$) (Fig. 3). Comparison of neonates with individuals in their 60s showed that while telomere shortening in the white matter was statistically significant ($p = 0.0050$), that in the gray matter was not ($p = 0.086$). On the other hand, telomere shortening of the white or gray matter did not differ significantly between individuals in their 60s and 70s (white matter: $p = 0.20$, gray matter: $p = 0.68$).

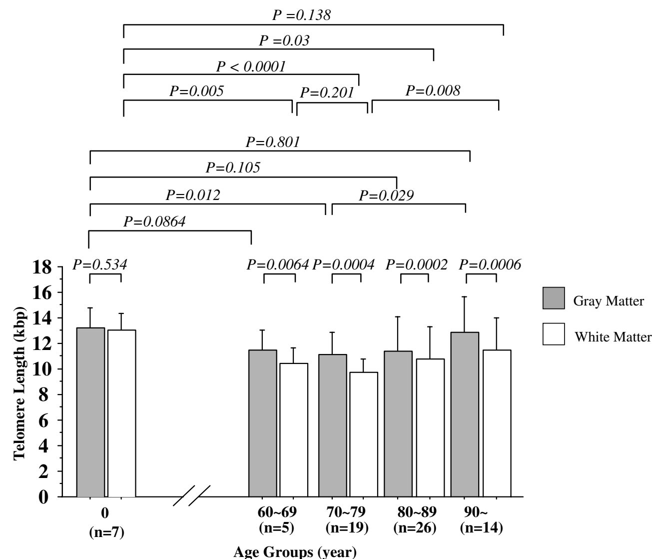

Fig. 3. Mean telomere lengths of the gray and white matter in the five age groups. The telomeres of individuals aged 70–79 years were shorter than those of individuals aged 80–89 years and 90–100 years ($p = 0.029$). The mean telomere lengths (±SD) of the gray and white matter in the five age groups were 13.4 ± 1.6 kbp and 13.3 ± 1.3 kbp (0 year; 7 subjects), 11.6 ± 1.6 kbp and 10.6 ± 1.2 kbp (60–69 years old; 5 subjects), 11.3 ± 1.8 kbp and 9.8 ± 1.1 kbp (70–79 years old; 19 subjects), 11.5 ± 2.8 kbp and 11.0 ± 2.6 kbp (80–89 years old; 26 subjects), 13.2 ± 3.0 kbp and 12.1 ± 2.4 kbp (90–100 years old; 14 subjects).

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Taken together, it is strongly suggested that telomeres in the gray and white matter at birth would be similar in size in specific individuals, and then would become gradually eroded before adulthood in the white matter but mostly preserved in the gray matter during aging, with the consequence that the initial size of telomeric DNA would be well preserved even in aged people, especially in the gray matter.

We found an unexpected and intriguing phenomenon related to telomere status in later life: while the mean telomere lengths of individuals in their 60s and 70s decreased with age, those in groups of further advanced age were longer than in the 70s group (70–79 < 80–89 < 90–100 years of age) (Fig. 3). Consequently the mean telomere lengths in the gray and white matter of 90–100-year-olds were significantly greater than those of individuals in their 70s (gray matter: $p = 0.029$; white matter: $p = 0.008$). Considering the good preservation of telomere length in the brain, these data strongly suggest that individuals born with long telomeres could surpass the mean life expectancy. To address the gender bias of our sample population, we calculated the mean length and SD of the telomere restriction fragment (TRF) from each age group of females and males separately. This revealed that that (1) generally, females had a slightly longer mean TRF than males at each age group, except in their 60s (none of the female subjects) and gray matter TRF in their 70s, although the differences for any of the age groups were not statistically significant (Supplementary Table 2). Furthermore (2), the age group-dependent TRF length tendency observed for the population as a whole was reproduced in both the female and male populations, TRF being longest in newborns, shortest in individuals in their 70s, longer in individuals in their 80s than in individuals in their 70s, and longer in individuals in their 90s than in individuals in their 80s (Supplementary Figure 2). When men and women were analyzed separately, the TRF lengths of the gray and white matter showed statistically significant differences in most of the age groups, but the differences between age groups were not significant. This result might have been due to the reduction in the number of samples.

Fig. 4 shows another intriguing feature of the population analyzed in this study. The patients were divided into groups according to the major cause(s) of death diagnosed at autopsy. The solid circles represent patients who died as a result of carcinomas and hematopoietic malignancies, and the clear circles patients who died of other diseases. The latter category included curatively treated cancer patients and patients with latent cancers who died of other diseases. The cancer death rates for individuals in their 60s, 70s, 80s, and 90–100 were 80% (4 cancer deaths among 5 cases), 32% (6/19), 27% (7/26) and 13% (2/14), respectively. The incidence of the malignancies that were considered to have been the direct cause of death was as follows: lung cancer (6), lymphoma (4), gastric cancer (3), colon cancer (2), hepatoma (1), gallbladder cancer (1), urinary bladder cancer (1), leukaemia (1) and myeloma (1). Although only

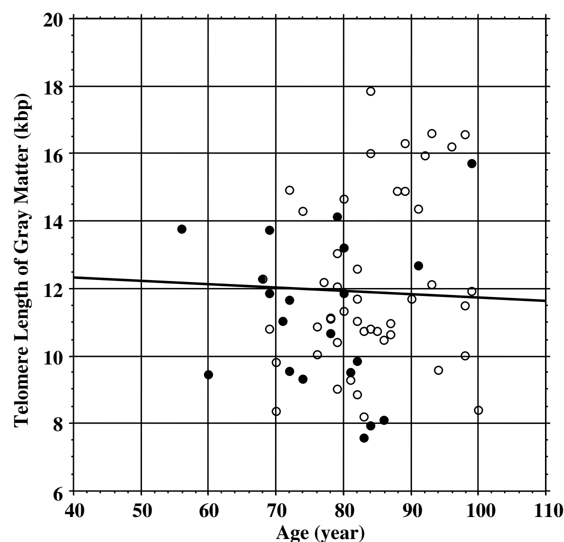

Fig. 4. Cancer death and telomere length in aged Japanese individuals. Solid circles, individuals who died due to carcinomas and hematopoietic malignancies. Clear circles, individuals who died of diseases other than malignancy, including curatively treated cancer patients and patients harboring latent cancer who died of other diseases. Solid line, the linear regression line obtained from whole individual values of telomere length in the gray matter.

a limited number of middle-aged patients were autopsied in our hospitals, the proportion of those who died of cancer was reasonably consistent with that for the Japanese population as a whole (Statistics and Information Department, Ministry's Secretariat, Ministry of Health, Labour and Welfare Japan, 2006). For patients overall, the mean gray matter telomere length in patients who died of cancer ($n = 20$: 11.2 ± 2.3 kbp) was slightly but not significantly less than that in patients who died of other diseases ($n = 45$: 12.1 ± 2.6 kbp) ($p = 0.17$). Our statistical analysis revealed a significantly lower rate of cancer-related death in individuals in their 80s ($p = 0.041$) and 90s ($p = 0.017$) versus those in their 60s. In the 80s age group, the mean telomere length in the gray matter from patients who died of cancer ($n = 7$: 9.7 ± 2.1 kbp) was significantly shorter than that of patients who died of other diseases ($n = 19$: 12.2 ± 2.7 kbp) ($p = 0.04$).

4. Discussion

To date, telomere length in vivo has been measured in various human tissues employing several different techniques, including Southern blotting (Harley et al., 1990; Takubo et al., 2002), PCR amplification (Cawthon, 2002; Baird et al., 2003), and quantitative fluorescence in situ hybridization (QFISH) (Lansdorp et al., 1996; Meeker et al., 2002; Mayer et al., 2006; O'Sullivan et al., 2006). These analyses, including systematic studies by our group, have also revealed specific rates of telomere attrition in replicating somatic cells (Takubo et al., 2002; Ishii et al., 2006). On the other hand, very little is known about telomere attrition in post-mitotic cells, such as neurons and

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myocardial cells, in which telomere shortening may not occur in the absence of mitosis (Allsopp et al., 1995; Butler et al., 1998; Takubo et al., 2002). The cerebral cortex contains nuclei of neurons and glial cells, while the white matter contains no neuronal nuclei, but abundant oligodendrocytes. It is well known that the ratio of glial cells to neurons in the gray matter is approximately 4:1 (Treff, 1967). Since adult neurons do not show mitosis and glial cells are known to be resting or replaced very slowly under normal conditions, the notion that telomeres in the adult cerebral cortex are in a stable condition has prevailed. On the other hand, in the white matter, active myelination continues from the late embryonic stage to adolescence, a period in which oligodendrocytes, which play a central role in myelination, are considered to be proliferating (Gilles et al., 1983; Marcus, 1991). Telomere shortening in the white matter could occur preferentially during adolescence, and our data support this. Since previous studies failed to detect telomerase activity in cerebral tissue samples by the TRAP assay (Kim et al., 1994; Allsopp et al., 1995), telomere elongation in the human brain could be negligible after birth. Consequently, in view of the fact that older individuals have longer telomeres in cerebral tissues, it can be hypothesized that they constitutively possess longer telomeres from early in life, and that de novo telomere elongation due to telomerase activity is unlikely.

In this study we analyzed telomeric DNA in the cerebrum using an authentic Southern blotting method. Although technical innovation has improved telomere measurement, Southern blotting is still the most reliable method and sets a standard for other methods. Indeed, our data were in good agreement with previous analyses of human brain samples ranging from the fetal stage to 76 years of age (Allsopp et al., 1995; Butler et al., 1998). A major difference between our study and previous ones was the targeted population; our study population included much older individuals who had lived beyond the average life expectancy. Inclusion of these much older individuals shed light on some of the fundamental aspects of telomere biology.

In our previous study we compared the telomere lengths of five organs (myocardium, cerebral cortex, liver, renal cortex, spleen) derived from a large number of autopsy cases, and found a robust correlation of telomere length among these organs in any given individual. This strongly suggested that each individual possesses a specific telomere length (Takubo et al., 2002). In the present study we calculated the coefficient of correlation between telomere lengths in the gray and white matters, and obtained a high value (Fig. 2). Although accumulated data from various laboratories on telomere length obtained using different methods have already strongly suggested that telomere length in each individual at birth has a wide range (e.g., Baird et al., 2006), the biological significance of individual telomere length, and the regulatory mechanisms that govern telomere length in vivo are still unclear. Our data also demonstrate that there is considerable inter-individual variation in telomere length at birth and throughout life. The genetic factors responsible for determining telomere length have been investigated in many organisms, particularly the yeast *Saccharomyces cerevisiae*. From the discovery of the essential telomerase gene EST1 (Lundblad and Szostak, 1989) to recent comprehensive studies, numerous genes have been shown to participate in telomere maintenance, either directly or indirectly (Askree et al., 2004). The molecular basis of human telomere metabolism remains largely unexplored. Many genes related to telomere maintenance in yeast may have similar roles in humans. Since the genetic background of humans is considerably larger and more heterogeneous than that of laboratory organisms, not only molecular genetic approaches but also well controlled population studies are essential for clarification of human telomere biology (Epel et al., 2004; Nordfjäll et al., 2005). On the other hand, telomere attrition is thought to be accelerated (or decelerated) by various environmental or epigenetic factors, e.g. oxidative stress (von Zglinicki et al., 1995) or life stress (Epel et al., 2004), and telomere shortening in turn may trigger replicative senescence or chromosomal instability, leading to age-related degeneration or tumorigenesis (Hastie et al., 1990; Nakamura et al., 2000; Vaziri et al., 1994; Rando, 2006). Therefore, it may be possible to say that individuals with longer telomeres are more likely to have better tolerance to the degenerative effects of aging. Indeed, Cawthon et al. have reported a phenomenon that seems to represent a "mirror image" of our present findings. They have suggested that humans with shorter telomeres in peripheral blood have higher mortality, possibly due to age-related diseases such as infections (Cawthon et al., 2003).

Recent life tables have indicated that the average life expectancy of the Japanese population in 2000 was 77.7 years for males and 84.6 years for females. Similar to other developed countries, the major cause of disease death in Japan is cancer throughout most of adulthood, but not in individuals over 80 years old. Even among individuals in their late 70s, half die of cancer. In individuals in their 80s, cancer is still the leading cause of death, accounting for more than one third of all deaths. It is not until individuals have reached their 90s that cancer is replaced as the leading cause of death by cardiovascular and cerebrovascular diseases (Statistics and Information Department, Ministry's Secretariat, Ministry of Health, Labour and Welfare Japan, 2006). To date, numerous studies have demonstrated a reduction of telomere length in carcinomas (Hastie et al., 1990; Nakamura et al., 2000) suggesting that telomere erosion causes genomic instability, thus triggering carcinogenesis. Recently, O'Sullivan et al. have reported that the telomere length in the colon epithelia was positively associated with age in the elderly aged 60 years or older. This positive association was observed exclusively in the epithelial cells, and not in the stromal fibroblasts or the lymphocytes. They have suggested that the association is a consequence of selective survival of the elderly with long telomeres in the colon (O'Sullivan et al., 2006).

These data are partially relevant to our findings. In animal models, however, a critical difference in the tumor spectrum between humans and laboratory mice has received attention (DePinho, 2000), and this may be attributable to the fact that laboratory mice have telomeres ten times longer than those of humans (de Lange, 1994). Indeed, telomerase knockout mice require several generations of backcrossing to achieve critical telomere shortening. The tumorigenesis observed in their epithelial tissues seems to be relevant to the carcinogenesis spectrum of aged humans (Artandi et al., 2000). Recently, it has been shown that double knockout mice lacking the telomerase RNA component (Terc) and Wrn (the murine homologue of human adult-onset premature aging syndrome) genes develop a phenotype resembling human Werner syndrome only in late generations (G3-5) (Chang et al., 2004). Although these painstaking studies serve to reinforce our understanding of telomere biology, phenomena specific to humans can be revealed only through meticulous observation and studies of human populations. The present study lends further fundamental support to the hypothesis that longer telomeres protect the genome from genetic instability and are beneficial for longevity (Rando, 2006). Further studies of aged individuals with long telomeres who have lived beyond the peak age range for cancer might provide some useful clues to the factors underlying human death and longevity.

Contributors

K. Nakamura was responsible for the basic concept and study design, measured telomere lengths, carried out data analysis, and contributed to discussion of the findings and writing of the report. K. Takubo also contributed to the basic study concept, organized the total project, and contributed to discussion and writing of the report. N. Izumiyama-Shimomura and A. Ishii contributed to preparation of tissue samples and discussion. M. Sawabe, T. Arai, H. Kishimoto and M. Fujiwara contributed to preparation of tissue samples and autopsy diagnosis. M. Kato and M. Oshimura contributed to the study design and discussion. N. Ishikawa contributed to data analysis, discussion of the findings and writing the report.

Competing interests statement

The authors declare that they have no competing financial interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.exger.2007.05.003.

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