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Reduced telomere length variation in healthy oldest old

Julius Halaschek-Wiener [a], Irma Vulto [b], Dan Fornika [a], Jennifer Collins [a], Joseph M. Connors [d], Nhu D. Le [c], Peter M. Lansdorp [b,e], Angela Brooks-Wilson [a,e,*]

[a] Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada
[b] Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada
[c] Cancer Control Research, BC Cancer Agency, Vancouver, BC, Canada
[d] Centre for Lymphoid Cancer, BC Cancer Agency, Vancouver, BC, Canada
[e] Department of Medical Genetics, University of British Columbia; Vancouver, BC, Canada

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ABSTRACT

Telomeres protect against DNA degradation at the ends of linear chromosomes. The number of telomere repeats is reduced over time in human aging. Using flow FISH we have assessed telomere length in 134 exceptionally healthy seniors aged 85 or older who have never been diagnosed with cancer, cardiovascular disease, major pulmonary disease, diabetes or Alzheimer disease (the 'Super-seniors') and 47 randomly-ascertained mid-life individuals aged 40–50 years. We compared their telomere lengths to a reference interval based on 400 individuals aged 1–100 years and show that Super-seniors do not have exceptionally long telomeres for their age. Consistent with the known trend of telomere shortening over time; however, they have shorter telomeres than the younger control group.

Furthermore, we show that variability in telomere length was lower in the Super-seniors than in the mid-life controls or the reference data. Reduced telomere length variation was observed for lymphocytes, CD45RA-positive T-cells and memory T-cells. These results suggest that individuals, some types of their somatic cells, or both, may be selected for an optimal rather than extreme telomere length. Selection of individuals and/or cells that have an optimal telomere repeat length could contribute to disease resistance and promote healthy aging.

1. Introduction

Telomeres are structural entities that extend beyond the sequences at the end of chromosomes by several thousand base pairs. They are a composite of DNA and numerous telomere binding proteins, and serve as caps to protect linear DNA molecules from being recognized as double strand breaks, as well as to buffer end replication-related DNA loss (Olovnikov, 1973). Short telomeres can cause chromosomal instability resulting in breakage-fusion cycles and cancer, while cells with extremely long telomeres can escape replicative senescence that would otherwise restrict cell division after a pre-determined number of cell divisions (Hayflick and Moorhead, 1961). Growth, proliferation and tissue regeneration and maintenance all come at the expense of loss of telomere repeats.

Telomere function and the consequences of altered telomere length have been well established at a cellular level (de Lange, 2005; von Zglinicki and Martin-Ruiz, 2005). Less is known about the contribution of telomeres to health, disease or aging at the level of the whole organism. Telomere length is established in the germline and possibly also early in embryonic development by transient activation of the telomerase ribonucleoprotein complex. Only telomerase and alternative lengthening of telomere (ALT) mechanisms are able to elongate telomeres and therefore counteract telomere decay over time. Most human somatic cells lack active telomerase and lose telomere repeats with proliferation (Collins and Mitchell, 2002).

Several studies have shown that telomere length in peripheral blood mononuclear cells is representative of that of many tissues; intra-individual correlation between telomere lengths in different tissues is high (Saretzki and von Zglinicki, 2002; von Zglinicki et al., 2000). Short telomeres in peripheral blood lymphocytes are associated with increased risk for development of head and neck, kidney, bladder and lung carcinomas (Wu et al., 2003). Shorter telomere length has also been associated with mortality from heart disease and infectious disease in people aged 60 years and older (Cawthon et al., 2003). Short telomeres have also been associated with diabetes and heart disease, both age-related

* Corresponding author at: BC Cancer Research Centre, 675 West 10th Avenue, Vancouver, BC, Canada, V5Z 1L3. Tel.: +1 604 675 8156; fax: +1 604 675 8178.
E-mail address: abrooks-wilson@bcgsc.ca (A. Brooks-Wilson).

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diseases (Tentolouris et al., 2007; van der Harst et al., 2007; Wu et al., 2003). Smoking and obesity both accelerate telomere loss and aging in women (Valdes et al., 2005). Telomere length was not associated with either morbidity or mortality; however, in the Leiden 85-plus study, or with survival in a Danish seniors cohort (Bischoff et al., 2006; Martin-Ruiz et al., 2005).

Eighty-five years and older is the group considered by gerontologists to be the 'Oldest Old' (Suzman and Riley, 1985). A minority of individuals survive to 85, and those that do are often afflicted with chronic diseases that impact their quality of life. We hypothesized that exceptionally healthy oldest old would have long telomeres for their age. To test this hypothesis, we analyzed telomere length in leukocytes of healthy oldest old ('Super-seniors') and population-based controls, and compared them to an established reference set of individuals aged 1–100.

2. Materials and methods

2.1. Study subjects

This study was approved by the joint Clinical Research Ethics Board of the University of British Columbia and the BC Cancer Agency. All subjects gave written informed consent. Population-based lists from the BC Medical Services Plan were used to contact potential study subjects 85 years or older, or between 40 and 50 years of age from Metro Vancouver, British Columbia, Canada. Eligibility criteria for the healthy oldest old included being 85 or older, self-reporting as never having been diagnosed with cancer (except non-melanoma skin cancer), cardiovascular or pulmonary disease (except asthma), diabetes or Alzheimer disease; or taking a medication prescribed specifically for one of these diseases. The mid-life comparison group was recruited randomly without regard to health or disease status. Samples used for telomere analyses were from 134 unrelated healthy oldest old aged 85–97 (mean age: 88 years; female:male ratio = 67%:33%). The 47 control individuals were 40–50 years old (mean age: 44 years; female:male ratio = 64%:36%).

2.2. Telomere analysis using flow FISH

Cells were isolated within 48 h of blood draw. Cell preparation and flow FISH analysis were performed as described previously (Baerlocher et al., 2006). 1 ml of whole blood was used for leukocyte isolation. First, red blood cells were lysed in 0.8% NH_4Cl/0.1mM EDTA (Stem Cells Technologies, BC) and the remaining cells (granulocytes, monocytes, lymphocytes, and platelets) were resuspended in 200 μl of hybridization buffer (5% dextrose/10mM Hepes/0.1% BSA) and 200 μl of 80% FCS/20% DMSO and stored in liquid nitrogen. Cells were used for flow FISH analysis within 3 years of preparation.

Automated 96-well multicolor flow FISH is a very sensitive method to measure the average or median telomere length in granulocytes, lymphocytes, CD45RA-positive T-cells, memory T-cells, and B-cells from human blood. An internal standard (bovine thymocyte control cells) of known telomere length is analyzed within the same tube for every sample. The method is based on the quantitative hybridization of a directly labeled fluorescent peptide nucleic acid (PNA) probes to telomere repeats. Comparison of the fluorescent signal of the internal standard, which has known telomere length, to the signal of the test cells allows accurate determination of the telomere length of the latter. Samples were analyzed in quadruplicate using a FACSCalibur flow cytometer (BD Biosciences, CA, USA); unstained cells and cells hybridized with the telomere specific PNA probe were analyzed in duplicates. Cells were also stained with CD45RA–Cy5 (prepared in house) and CD20 (L26-PE, Beckman Coulter, CA, USA) antibodies, to obtain independent telomere length information for lymphocytes, granulocytes, memory T-cells, CD45RA-positive T-cells, and B-cells. We acquired 10,000 events for each sample. Cellquest Pro software (BD Biosciences, CA, USA) was used to quantify the flow cytometry results; median telomere lengths were calculated using an automated Microsoft Excel calculator.

2.3. Statistical analyses

Telomere lengths were compared using the *t*-test, assuming unequal variances (Larsen and Marx, 2005). The variations of the telomere lengths were assessed by comparing the corresponding variances using the *F*-test (Larsen and Marx, 2005). Differences with p-values < 0.05 were considered statistically significant.

3. Results

The flow FISH technique (Baerlocher et al., 2006) was used to measure the telomere lengths of 134 healthy oldest old who had never been diagnosed with cancer (except non-melanoma skin cancer), cardiovascular or pulmonary disease (except asthma), diabetes or Alzheimer disease; and 47 population-based controls aged 40–50 years. Published flow FISH data from identically processed samples from 400 reference individuals without hematological or malignant disease aged 1–100 years was used to generate percentile curves for telomere lengths across the age spectrum (Armanios et al., 2007; Yamaguchi et al., 2005). We considered telomere length values between the 10th and 90th percentiles, for a particular age, to be normal.

Flow FISH uses telomere repeat-specific hybridization probes to derive multi-parameter information on the length of telomere repeats in thousands of individual cells (Baerlocher et al., 2006). Leucocytes were also stained with CD45RA and CD20 antibodies to allow capture of telomere length information for lymphocytes, granulocytes, memory and naïve T-cells and B-cells. Telomere length values for specific leukocyte cell types are shown in Table 1.

Comparison of telomere length between the Super-seniors and mid-life controls was consistent with the known trend of telomere shortening over time. All cell types examined showed shorter telomere lengths in the older age group; these results were highly statistically significant ($p < 0.00002$) for all cell types (Table 1).

The telomere lengths of healthy oldest old and mid-life controls are shown superimposed on reference intervals generated from published data (Armanios et al., 2007; Yamaguchi et al., 2005). Visual inspection of this comparison shows that both the healthy oldest old and the control data are consistent with the pattern of telomere lengths established over time by the reference data. This indicates good reproducibility of the flow FISH protocol. Telomeres of healthy oldest old center mainly between the 10th and 90th percentiles (in the normal range) of the reference set (Fig. 1a–c). In contrast to our expectation based on studies that showed an association of short telomeres with a variety of age-related diseases (cancer, diabetes and heart disease) (Tentolouris et al., 2007; van der Harst et al., 2007; Wu et al., 2003), we found that

Table 1
Telomere length in healthy oldest old and mid-life controls

	Mean telomere length in kb (S.D.)		Telomeres are shorter in HOO than controls	Difference in telomere length variation in HOO versus controls[a]	HOO in the normal range (10–90%) of the reference set
	HOO ($n = 134$)	Controls ($n = 47$)	95% confidence intervals (p-value)	p-Value	% normal (p-value)
Lymphocytes	4.56 (0.75)	6.96 (1.01)	−2.71 to −2.02 ($<1.0E^{-6}$)	0.011	88 (0.005)
Granulocytes	6.40 (0.82)	7.03 (0.95)	−0.95 to −0.33 ($2.0E^{-5}$)	0.18	81 (0.32)
CD45RA-positive cells[b]	4.61 (0.85)	7.63 (1.17)	−3.37 to −2.66 ($<1.0E^{-6}$)	0.006	79 (0.57)
Memory T-cells	4.33 (0.67)	5.90 (0.79)	−1.82 to −1.31 ($<1.0E^{-5}$)	0.15	88 (0.005)
B-cells	6.71 (0.90)	8.09 (0.95)	−1.69 to −1.07 ($<1.0E^{-6}$)	0.66	83 (0.18)

p-Values < 0.05 are considered statistically significant; HOO = healthy oldest old, kb = kilo base pairs; S.D. = standard deviation.
[a] Standard deviations of telomere length values were compared between healthy oldest old and controls.
[b] CD45RA is predominantly expressed on naïve T-cells, but is also represented on some memory/effector T-cell subpopulations.

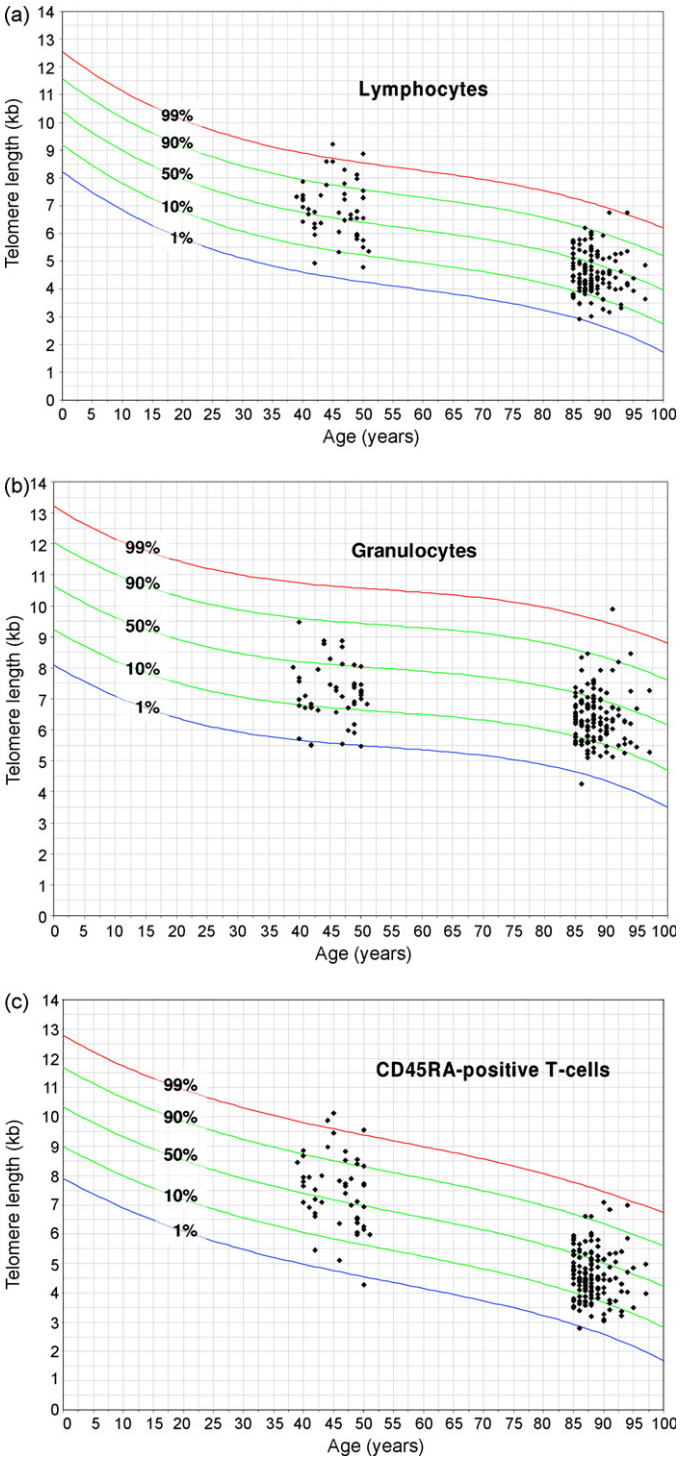

Fig. 1. Telomere lengths in lymphocytes, granulocytes, and CD45RA-positive T-cells of healthy oldest old and mid-life controls. Telomere lengths of 134 healthy oldest old and 47 mid-life controls are plotted against percentile curves previously established using 400 randomly selected reference individuals of various ages (Armanios et al., 2007; Yamaguchi et al., 2005). Telomere lengths are shown for (a) lymphocytes, (b) granulocytes, and (c) CD45RA-positive T-cells.

healthy oldest old, who are free of these diseases, do not have unusually long telomeres.

Fig. 1a–c show the telomere lengths of the healthy oldest old and mid-life controls for lymphocytes, granulocytes and CD45RA-positive T-cells, respectively; relative to percentile curves defined by the reference data. Supplemental online Figure 2a and b show data for memory T-cells and B-cells. Inspection of these data revealed that the telomere lengths of the healthy oldest old appeared to be more tightly clustered than those of the mid-life comparison group. Statistically significant differences in variances were observed for lymphocytes and CD45RA-positive T-cells (Table 1). In addition, we tested whether significantly more Super-seniors are clustered in the normal range, versus the null hypothesis that 80% of individuals fall between the 10th and 90th percentiles. All cell types were close to or above the expected 80% mark; comparisons of lymphocytes and memory T-cells were statistically significant ($p = 0.005$, Table 1). Reduced telomere length variation was observed for lymphocytes, CD45RA-positive T-cells and memory T-cells in one or both of these comparisons.

4. Discussion

Several studies have shown an association between short telomeres and increased mortality from age-related diseases (Cawthon et al., 2003). Mutations in genes that encode either the RNA or the reverse transcriptase component of telomerase, which maintains telomere repeats, are risk factors for and contribute to the pathogenesis of severe human diseases such as aplastic anemia and idiopathic pulmonary fibrosis (Armanios et al., 2007; Yamaguchi et al., 2005). Telomere length was associated with increased myocardial function in seniors over 85 years (Collerton et al., 2007) and inversely associated with age-related illness such as cardiovascular diseases (Benetos et al., 2004; Brouilette et al., 2003) and dementia (Panossian et al., 2003; von Zglinicki et al., 2000). Analysis of telomeres in four smoking-related cancers showed that short telomeres were associated with an increased risk for these malignancies (Wu et al., 2003). Other studies show an association between health-determining parameters such as physical activity level, smoking, body weight, and even socioeconomic status and telomere length in blood cells (Cherkas et al., 2006; Valdes et al., 2005), apparently linking telomere length with health status and potential longevity. Other aging-related studies; however, did not find associations between telomere length and survival (Bischoff et al., 2006) or mortality in the oldest old (Martin-Ruiz et al., 2005).

We hypothesized that individuals who not only lived well into their 9th decade, but also did so without developing cancer, cardiovascular or pulmonary disease, diabetes or Alzheimer disease, would have unusually long telomeres. Our results show; however, that the telomeres of healthy oldest old do not appear different from a reference sample of similar aged individuals. As expected, they are shorter than those of younger individuals.

We did find, however, that telomere length is less variable in healthy oldest old than in mid-life individuals; this difference is observed in three cell types: lymphocytes, CD45RA-positive T-cells and memory T-cells. One possible explanation for this would be that smaller values generally show less variance than larger values, and the healthy oldest old do have shorter telomeres than the younger controls.

Although expression of CD45RA predominates on naïve T-cells, CD45RA is also expressed, at varying levels, on CD8-positive effector T-cells. The proportion of this subset has been shown to increase with aging, in contrast to the naïve T-cells that decline in the elderly (Hong et al., 2004). If naïve T-cells and effector T-cells differ in telomere length, then differences in the proportion of these CD45RA-positive cell types between mid-life controls and the elderly may, to some extent, contribute to telomere length differences between age groups. Other differences in abundance of hematological cell types with age could also potentially influence these results. For simplicity, we have not characterized changes in cell type abundance or telomere length differences between

$CD45RA^+$ cell types but have carried out this work at the level of resolution of flow FISH. Later studies will be necessary to address whether differences in the ratio of naïve to effector T-cells affects telomere length comparisons.

We have also noted that the lymphocytes and memory T-cells of Super-seniors showed a statistically significant enrichment of individuals in the normal telomere length range. This is the first evidence that individuals, or at least some types of their cells, are potentially selected for an optimal telomere length rather than long length. This observation is consistent with our understanding of the cell biology of aging, in which cell proliferation and senescence are delicately balanced. Long telomeres may favor inappropriate escape from senescence leading to cancer; short telomeres can trigger genomic instability also leading to cancer. Support for this hypothesis comes from a very recent breast cancer study by Svenson et al. (2008), who conclude that short telomeres in peripheral blood cells are associated with a better survival outcome and that long telomeres were a significant negative prognostic factor for advanced disease. Telomere shortening in somatic cells may have evolved in long-lived species (and not in mice) as a tumor suppressor mechanism. Cells with long telomeres are at higher risk of transformation, in that aspiring tumor cells have ample time and cell divisions to acquire multiple genetic abnormalities before their telomere length-related tumor suppressor mechanisms are activated. Therefore, both short and long telomeres represent risk factors for tumor development, whereas the average/optimal telomeres in Super-seniors could be protective.

Generally, the inter-individual variance of biological parameters will increase over time due to stochastic events that act to increase heterogeneity. In gerontological studies it has been observed that the elderly are more heterogeneous than other age groups with regard to many individual characteristics of social–psychological and biological nature (Dannefer, 1987; Fraga et al., 2005; Herndon et al., 2002; Nelson and Dannefer, 1992; Rea et al., 2005). Members of an aging cohort become more unlike each other with the passage of time. In contrast, we observed greater telomere length homogeneity in healthy oldest old, which we propose is biologically rooted in the important balance between cell proliferation and senescence.

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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.mad.2008.07.004.

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