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Synchrony of Telomere Length among Hematopoietic Cells

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Abstract

Objective—Little is known about the relations of telomere lengths among leukocyte subsets and between leukocytes and cells up the hematopoietic hierarchy. This information is relevant, since telomere dynamics in granulocytes were postulated to mirror those of hematopoietic stem cells (HSCs).

Subjects and Methods—We examined in newborns' cord blood (UCB) the relationships of telomere length in hematopoietic progenitor cells (HPCs) ($CD34^{+}CD45^{-}$) with that in T lymphocytes and in granulocytes. In addition, we correlated telomere length in granulocytes with that in whole leukocyte samples of individuals with age range between birth and 100 years.

Results—In the UCB, we found strong correlations of telomere length in HPCs with telomere lengths in T lymphocytes (r ranging from 0.882–0.935, p ranging from 0.0038–0.0007) and in granulocytes (r=0.930, p=0.0072). At birth, strong correlations were also observed between telomere length in granulocytes and that in all leukocytes (r=0.979, p=0.0003). Throughout the human lifespan, the relationship between telomere length in granulocytes and that in all leukocytes was $r>0.980$, $p<0.0001$.

Conclusions—Robust synchrony exists among leukocyte subsets throughout the human lifespan; individuals with relatively long (or short) telomeres in one leukocyte subset have long (or short) telomeres in other leukocyte subsets. Moreover, telomere length in leukocytes reflects its length in cells up the hematopoietic hierarchy, i.e., HPCs and, by inference, HSCs. Strong links have been found by many studies between leukocyte telomere length and a host of aging-related diseases. Our findings suggest, therefore, that that these links might be traced to telomere dynamics in HSCs.

Keywords

telomeres; aging; T cell; life span; human

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Conflict of Interest Disclosure

There are no financial conflicts of interest for any of the authors.

Introduction

With a view to gain insight into the role of telomere dynamics (birth telomere length and its age-dependent shortening afterwards) in the biology of human aging, most epidemiological studies have examined the relationships of leukocyte telomere length (LTL) with a host of aging-related diseases [1;2] and survival [3;4]. However, as telomere length varies among subsets of leukocytes [5], concerns have been raised about whether the mean length of telomeres in all leukocytes, i.e. LTL, might capture less accurately or miss altogether relevant associations of telomere length in leukocyte subsets with features of interest [6]. For instance, lymphocyte subsets display variation in telomere length [7;8] and lymphocytes display a faster rate of age-dependent telomere shortening than granulocytes [5]. Whereas several studies have found that telomere length in hematopoietic precursor cells is longer than in more mature myeloid cells [9;10], a question remains about whether telomere length in any leukocyte subset provides information over and above that of LTL about aging-related diseases.

No doubt, leukocyte subsets have central and diverse roles in health and disease, but seeking to understand telomere dynamics in one type of cell vs. another is insightful only in the context of the specific hypothesis that is being tested. For example, if the hypothesis one tests is whether telomere dynamics in memory $CD8^{+}CD28^{-}$ T lymphocytes play a role in the demise of the elderly, then it is only reasonable to examine the relationship between telomere length in $CD8^{+}CD28^{-}$ T cells and death in old individuals. Similarly, if one would like to know whether telomere dynamics in monocytes is somehow involved in the pathogenesis of atherosclerosis, then it makes sense to measure telomere length in monocytes in individuals with or without clinical/subclinical manifestations of the disease.

In what way then can LTL serve to gain insight into the biology of human aging and aging-related diseases? The answer depends on whether the following model is proven valid: Over the life course of the individual, LTL dynamics mirror telomere dynamics in the hematopoietic stem cells (HSCs) [11;12]. If this premise is defensible, then the relationships of LTL with human aging and aging-related diseases suggest a role, whether passive or active, of telomere dynamics and telomere regulating genes in HSCs in the biology of human aging. However, since HSCs are poorly characterized [13] and their estimated numbers in the bone marrow is exceedingly small [14], it is but impossible at present to verify the premise through direct empirical observations. These would have required bone marrow aspirations in large cohorts and the development of methods that precisely characterize HSCs and sort them in sufficient quantities for the measurement of telomere length. For this reason, telomere dynamics in granulocytes, which are post-mitotic cells with a short biological life, have been used in both humans [15] and non-human primates [16] to figure out telomere dynamics in HSCs. Recently, based on extensive simulations, we concluded that LTL dynamics provide information that closely corresponds to that of granulocyte telomere dynamics about the kinetics HSC replication [11]. Empirical data in both human and in the non-human primate support of this concept for early development [5;17].

The dual question that follows is this: To what extent does inter-individual variation in the length of telomeres in circulating leukocytes mirror those in HSCs and how proximate is LTL to telomere length in granulocytes? While HSCs are rare and poorly characterized, hematopoietic progenitor cells (HPCs) are more abundant, better characterized and in the newborn they can be isolated from the umbilical cord blood (UCB) in sufficient numbers for the measurement of their telomere length. Accordingly, we proceeded in two phases: First, we examined in UCB a) the relationships of telomere length in HPCs with that in leukocyte subsets (T lymphocytes and granulocytes), and b) the relationship of telomere length within

leukocyte subsets. Second, we examined the relationship between telomere length in granulocytes and that in all leukocytes in two cohorts, one comprising young adults and the other comprising individuals throughout most of the human lifespan.

Subjects and Methods

First phase

Subjects—UCB was obtained from the placenta of 8 full-term normal newborns (6 males, 2 females); one African American; 6 whites of Hispanic extraction; and 1 of mixed extraction.

Umbilical cord blood processing—UCB in Citric acid/dextrose was obtained from The Southwest Blood and Tissue Center, San Antonio, TX. One ml of blood was spun down for 10 min. at 1500 × g in a cooled centrifuge and the buffy coat layer was collected and saved at −80°C.

Ficoll separation of mononuclear cells—For Ficoll separation, blood was diluted 1:1 in RPMI 1640 medium containing 10% fetal calf serum (GIBCO) and 25 ml were loaded onto a cushion of 25 ml of Ficoll (Sigma) in a 50 ml tube and centrifuged using a standard Ficoll separation protocol. The interface containing the mononuclear fraction was collected, washed counted and stained for further analysis and cell sorting.

UCB processing for immunofluorescence staining—The FACSAria (Becton Dickinson, Mt. View, CA) cell sorter was used for all cell sorting. For determination of percent $CD34^+CD45^-$ cells, samples of 5 million cells were stained for $CD34^+CD45^-$ cells in a 2-color immunofluorescence staining assay against their matched control immunoglobulins as described before [18].

Cells were sorted at 5,000 –10,000 events/sec. A minimum of 100,000 $CD34^+CD45^-$ cells (referred to as $CD34^+$ cells, with purity of 85–95%), were collected. Sorted cells were pooled and frozen and kept at −80°C.

Based on a 4-color immunofluorescence assay, memory and naive $CD4^+$ and $CD8^+$ cells were characterized as follows: $CD4^+CD45RO^+RA^-$ (referred to as $CD4^+$RO cells or $CD4^+$ memory cells), $CD4^+CD45RO^-RA^+$ (referred to as $CD4^+$RA or $CD4^+$ naïve cells), $CD8^+CD45RO^+RA^-$ (referred to as $CD8^+$RO cells or $CD8^+$ memory cells) and $CD8^+CD45RO^-RA^+$(referred to as $CD8^+$RA or $CD8^+$ naïve cells). Antibodies used were anti-CD4-APC; anti CD8-PE-cy7; antiCD45RO-PE and anti CD45RA-FITC. As a control we used isotype and fluorescence matched immunoglobulins as described above. Sorting was performed in a 4-way sorting at a speed of 10,000 events/sec. A minimum of 100,000 cells with a purity range of 80–95% were collected. Sorted cells were pooled and frozen and kept at −80°C.

Sorting of granulocytes was performed by light scatter using the forward scatter and side scatter as parameters for the large granular cells. Technical reasons precluded telomere length measurements in two granulocyte samples.

Second phase

Subjects—There were two cohorts in this phase. The first cohort whose telomere length was measured by Southern blots of the terminal restriction fragments (TRFs), consisted of 24 healthy males (13 African Americans, 11 whites) of age range 22–34 years. The second

cohort, whose telomere length was measured by Flow-FISH, consisted of 400 healthy whites of both sexes with age ranging from birth to 100 years [19].

Telomere length measurements

For the first phase and the smaller cohort of the second phase, the mean length of the TRFs was measured by Southern blots as previously described [20]. For the larger cohort of the second phase, the median length of telomeres was measured by Flow-FISH, as previously described [21].

Informed consent

For the first phase, all UCB units were obtained with all identifiers removed according to IRB approved exempt status protocol. For the second phase, all subjects (or parents/guardians) signed IRB-approved informed consents at the different institutions.

Results

First phase

Figure 1A displays the relationships of telomere lengths in memory and naïve $CD4^+$ and $CD8^+$ T cells with $CD34^+$ cells, which are primarily HPCs. There were strong correlations of telomere lengths in all T cell subsets with telomere length in $CD34^+$ cells. Using paired t test, telomere length was 190 base pairs (bp) shorter in $CD4^+$ memory cells than in $CD4^+$ naïve cells (Figure 2A) and 150 bp shorter in $CD8^+$ memory than $CD8^+$ naïve cells (Figure 2B). In addition, there were strong correlations of telomere length between granulocytes and $CD34^+$ cells (Figure 1B) and between telomere length in granulocytes and LTL (mean telomere length measured in buffy coats) (Figure 1C). While telomere length was respectively longer in naïve $CD4^+$ and $CD8^+$ by 1,017 bp and 860 bp than in $CD34^+$ cells (Figure 3A,B), telomere length in granulocytes was significantly shorter by 270 bp than that in $CD34^+$ cells (Figure 3C).

Second phase

There were robust relationships between telomere length in granulocytes and LTL when telomere length was measured by Southern blot analysis (Figure 4A) and between the median length of telomeres in granulocytes and telomere length of all leukocytes (total Cells), when telomere length was measured by Flow-FISH (Figure 4B). The wider range in the Flow-FISH sample than in the Southern blot sample reflects the considerably wider age range of the Flow-FISH sample and the much greater number of subjects whose telomere length was measured by Flow-FISH.

It is noteworthy that the Flow-FISH results treat each leukocyte as a unit, so that the median accounts for the distributions of telomere lengths in the leukocyte populations. The Southern blot analysis, however, lumps DNA from all leukocytes in the sample into one parameter and determines the distribution of this entity, which reflects the distributions of telomere lengths of the leukocyte populations and within each leukocyte (i.e., telomere lengths in different p and q arms of the 46 chromosomes).

Discussion

The main findings of the study are as follows: (i) at birth, telomere lengths in T cells and granulocytes are strongly correlated with telomere length in HPCs; (ii) in both newborns and adults, telomere length in granulocytes is highly correlated with LTL. These findings extend the concept of synchrony (equivalence) observed within tissues and organs of the newborn [20] and the fetus [22] to HPCs and leukocyte subsets in the newborn. They also show that

adults with long (or short) LTL display long (or short) telomeres in their granulocytes. The synchrony within tissues, organs and hematopoietic cells of the fetus, the newborn and the adult is not absolute, as shown in this work and previously [20;22;23], but clearly, the magnitude of the inter-individual variation in telomere length far exceeds that of the variation in telomere length among cell types within the individual. In this context, the inter-individual variation in leukocyte telomere length among newborns of this study is considerably smaller than those in previous reports [20;24]. This is expected, considering the small number of newborns in the present study, as the larger the sample, the larger the variation.

Of interest are the findings that in newborns, telomere lengths in both memory and naïve $CD4^+$ and $CD8^+$ T cells were considerably longer than in $CD34^+$ cells, perhaps due to telomeres lengthening during maturation of T cells in the thymus, which has robust telomerase activity in macaques [23] and in humans [7]. As the thymus progressively involutes after birth in all vertebrates, including humans [25], the longer length of telomeres in T lymphocytes than in $CD34^+$ cells might be a phenomenon that is characteristic of early development.

It is noteworthy that even in the newborn, as shown in adults [26], memory T cells displayed shorter telomere length than naïve ones, although the difference in telomere length between the two subsets was small compared to that observed in later life. The wider gap in telomere length between memory and naïve T cells in adults than in newborns probably stems from the duration of immunological memory, which is maintained by clonal expansion to sustain the memory T cell pool [5;27].

The robust associations of telomere length of granulocytes with that in $CD34^+$ cells in newborns and with LTL in both newborns and adults support the premise that LTL closely, albeit not perfectly, mirrors telomere length in HSCs [11;15]. Viewed through the prism of HSC dynamics, inter-individual variation in LTL and its age-dependent shortening reflect inter-individual variation in HSC telomere dynamics, a phenomenon observed in the first year of life in the non-human primate [17].

None of this means that deciphering the factors that determine telomere dynamics in subsets of leukocytes is not important for understanding a host of human diseases. That said, given that LTL is highly heritable [5;28–31], the premise that LTL dynamics mirror HSC telomere dynamics leads to the following prediction: Telomere regulating genes might explain inter-individual variation in LTL in the general population, since such variation far surpasses variation among leukocyte subsets within the individual. Recent genome-wide association analyses of LTL have borne out this prediction [32;33], providing a powerful conceptual proof that LTL, and by inference HSC telomere length, is an index of telomere length in all leukocytes, in spite of some variation in telomere length among leukocyte subsets.

Acknowledgments

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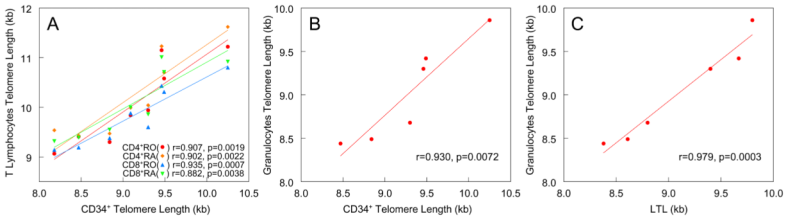

Figure 1. Relationships of telomere lengths among hematopoietic cells in newborns

A. Telomere length of $CD4^+$ and $CD8^+$ memory and naïve T lymphocytes vs. telomere length in $CD34^+$ cells; B. Telomere length in granulocytes vs. telomere length in $CD34^+$ cells; C. Telomere length in granulocytes vs. that in all leukocytes, i.e., leukocyte telomere length (LTL).

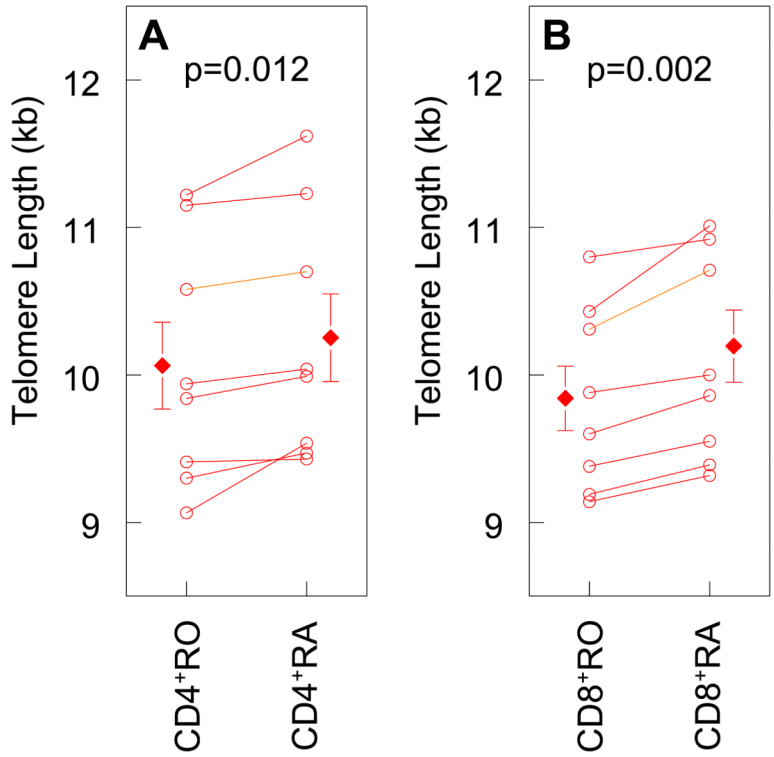

Figure 2. Relationships of telomere lengths between memory and naïve T lymphocytes in newborns

A. $CD4^+$ lymphocytes ($CD4^+$RO-memory, $CD4^+$RA-naïve); B. $CD8^+$ lymphocytes ($CD8^+$RO-memory, $CD8^+$RA-naïve).

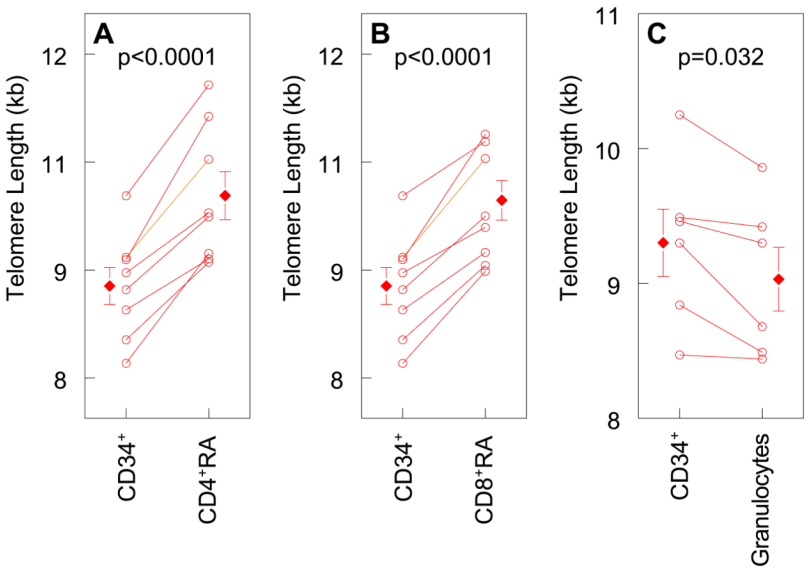

Figure 3. Relationships of CD34$^+$cells with T lymphocytes and granulocytes in newborns

A. CD34$^+$ and CD4$^+$RA-naïve; B. CD34$^+$ and CD8$^+$RA-naïve; C. CD34$^+$ and granulocytes.

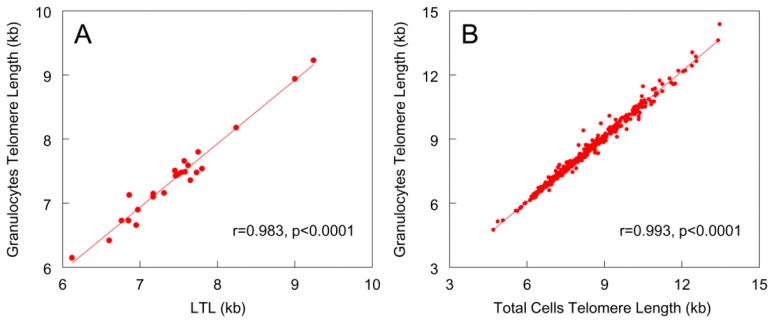

Figure 4. Relationships between telomere length in granulocytes and telomere length in all leukocytes

A. Telomere length, measured by Southern blot analysis of the terminal restriction fragments, in young adults aged 22-34 years (LTL-leukocyte telomere length); B. Telomere length, measured by Flow-FISH, in individuals aged birth-100 years.