Blood Reviews 25 (2011) 261–269

Contents lists available at SciVerse ScienceDirect

Blood Reviews

journal homepage: www.elsevier.com/locate/blre

REVIEW

Telomere biology in hematopoiesis and stem cell transplantation

Shahinaz M. Gadalla [a,b,1], Sharon A. Savage [a,*]

[a] Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA
[b] Cancer Prevention Fellowship Program, National Cancer Institute, Bethesda, MD, USA

ARTICLE INFO

Keywords:
Telomeres
Bone marrow failure disorders
Hematopoietic stem cell transplant
Dyskeratosis congenita
Myelodysplastic syndrome
Pluripotent stem cells

ABSTRACT

Telomeres are long $(TTAGGG)_n$ nucleotide repeats and an associated protein complex located at the end of the chromosomes. They shorten with every cell division and, thus are markers for cellular aging, senescence, and replicative capacity. Telomere dysfunction is linked to several bone marrow disorders, including dyskeratosis congenita, aplastic anemia, myelodysplastic syndrome, and hematopoietic malignancies. Hematopoietic stem cell transplantation (HSCT) provides an opportunity in which to study telomere dynamics in a high cell proliferative environment. Rapid telomere shortening of donor cells occurs in the recipient shortly after HSCT; the degree of telomere attrition does not appear to differ by graft source. As expected, telomeres are longer in recipients of grafts with longer telomeres (*e.g.*, cord blood). Telomere attrition may play a role in, or be a marker of, long term outcome after HSCT, but these data are limited. In this review, we discuss telomere biology in normal and abnormal hematopoiesis, including HSCT.

Published by Elsevier Ltd.

1. Introduction

The replicative capacity of human cells is limited by the inability of DNA polymerase to fully replicate the linear ends of the DNA,[1] a phenomenon called the "end-replication problem". The physiologic solution to the end-replication problem was provided with the discovery of telomeres, the molecular structures at chromosome ends, and their maintenance enzyme, telomerase.[2] Studies of telomere biology now range from a detailed understanding of telomeric structure, function, and regulatory mechanisms, to evaluating their role in aging, and human disease susceptibility, such as aplastic anemia, pulmonary fibrosis, heart disease, chronic inflammatory conditions, and cancer.

Telomeres shorten with successive cell division and, when a critical length is reached, cellular senescence is triggered. During the first weeks after hematopoietic stem cell transplantation (HSCT), the transplanted stem cells undergo extensive cell division to reconstitute the recipients' new hematopoietic cells; this process has the potential to cause significant telomere attrition. In this review, we evaluate the 1) relationship between normal telomere biology and cell growth and maturation in the hematopoietic system, 2) bone marrow disorders associated with telomere biology abnormalities, and 3) telomere dynamics after HSCT, and their potential role in transplant outcomes. Finally, we discuss the clinical implications and directions for research on the role of telomere biology in HSCT.

2. Telomere biology in hematopoietic cells

2.1. Human telomeres

Telomeres consist of long $(TTAGGG)_n$ nucleotide repeats and an associated protein complex located at the end of the chromosomes. They are essential for maintaining chromosomal integrity by preventing chromosome end-to-end fusion.[3] Telomeres shorten with every cell division,[4] and therefore their length is a marker for cell aging, senescence and, replicative capacity. Telomere erosion in human cells represents a unique form of DNA damage, in which repair mechanisms are very limited.[5] When telomeres become critically short, cells become senescent and ultimately die. Alternatively, critically short telomeres may bypass senescence and continue to divide despite the presence of genetic instability. This is often associated with alterations in the p53 or Rb pathways, as observed in cancer cells.[6] Human studies show that telomeres shorten with age in almost all tissues, with the exception of the brain and heart (reviewed by Takubo and colleagues[7]). Longitudinal studies suggest a positive relationship between the rate of telomere attrition and the individual's baseline telomere length[8,9]; individuals with the longest telomeres at baseline appear to have the highest attrition rate overtime. However, the mechanism of this differential telomere length regulation is unknown.

Abbreviations: HSCT, hematopoietic stem cell transplantation; GvHD, graft *versus* host disease.

* Corresponding author at: Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, 6120 Executive Blvd., EPS/7018, Rockville, MD 20852, USA. Tel.: +1 301 496 5785; fax: +1 301 496 1854.
E-mail addresses: gadallas@mail.nih.gov (S.M. Gadalla), savagesh@mail.nih.gov (S.A. Savage).
[1] Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, 6120 Executive Blvd., EPS/7019, Rockville, MD 20852, USA. Tel.: +1 301 435 4721; fax: +1 301 496 1854.

0268-960X/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.blre.2011.06.004

Telomeres are maintained mainly by the telomerase ribonucleoprotein complex and the shelterin protein complex. The catalytic unit of telomerase consists of an RNA template (*TERC*), a reverse transcriptase protein (*TERT*), and the dyskerin protein complex. Telomerase is expressed in embryonic and adult stem cells, and in highly-proliferative cells such as germline cells, skin, intestine, and bone marrow,[10,11] but not in most other somatic cells.[12] Approximately 80 to 90% of cancer cells maintain telomere length by overexpressing telomerase.[13] However, a subset of cancers elongates telomeres through an alternative lengthening mechanism (reviewed by Cesare and Reddel[14]). The shelterin complex consists of six protein subunits: telomeric repeat-binding factors 1, and 2 (gene names *TERF1* and *TERF2*), TRF1-interacting nuclear factor 2 (*TINF2*), TERF2-interacting protein 1 (*TERF2IP*), TIN2-interacting protein 1 (*ACD*), and protection of telomeres (*POT1*).[15] Shelterin is essential for normal telomere maintenance and function by inhibiting telomeric DNA damage response, regulating telomerase, and inhibiting telomeric homologous recombination.[16]

Human telomere length varies significantly between individuals. In human somatic cells, telomere length ranges between 2 and 15 kilobases (Kb),[17] and is often longer in females than males.[18] In addition, intra-individual tissue differences in telomere length have been observed.[19,20] However, within an individual, telomere length appears to be highly-correlated (*i.e.*, individuals with longer telomeres in one tissue tend to have longer telomeres in other tissues).[21–25]

Several methods are available to measure telomere length (reviewed by Baird[26] and Lin and Yan[27]). Terminal restriction fragment (TRF) measurement, based on Southern blots, is considered the gold standard. Other methods include: flow-FISH, which measures telomeres in leukocyte subsets; quantitative PCR (Q-PCR), which measures relative telomere length in extracted DNA as a ratio of telomere repeat copy number (T) to single gene copy number (S); and single telomere length analysis (STELA), a PCR method that measures chromosome-specific telomere length.

2.2. Telomere dynamics in hematopoietic cells

Proper telomere maintenance in hematopoietic stem cells (HSCs) is important because of the need for continuous replication of blood cells throughout life. Telomeres shorten rapidly after birth and during childhood. Early studies demonstrated the presence of longer telomeres in umbilical cord HSCs ($CD34^{+}CD38^{-}$) than in the same cell population in adult bone marrow[28] or in peripheral blood.[29] Telomere shortening in peripheral blood mononuclear cells (PBMCs) during the first 3 years of life was found to be more than fourfold higher than that observed in adults during a period of 3 years.[30] The profound rate of telomere attrition in the first years of life might reflect the expected rapid replication and expansion of hematopoietic stem cells early in life, followed by a decline in the rate of their replication thereafter.[31]

The presence of longer telomeres in umbilical cord blood (UCB) was not limited to stem cells, but was also observed in mature hematopoietic cells, including T-cells, B-cells, NK-cells, and granulocytes, compared with their adult counterparts.[29] Additional studies suggest that lymphocytes have longer telomeres than granulocytes at birth, followed by a high attrition rate in the first year for both cell types. More prominent telomere loss was observed in lymphocytes after the first year of life, resulting in shorter lymphocyte telomeres (as measured by flow-FISH) in old age (>60 years) when compared with that of granulocytes.[31] Telomere length differences between lymphocyte subsets have also been reported: adult peripheral blood B-lymphocytes were shown to have longer telomeres than T-lymphocytes.[32] This was thought to be due to differences in the rate of cell division between B and T cells.[33] In addition, CD8+ cells appear to have longer telomeres than CD4+ cells.[34]

In support of the replicative senescence theory, longer telomeres were found in hematopoietic progenitor cells (CD34+) than in subsets of mature cells (naïve and memory CD4+ and CD8+, and granulocytes). Both CD4+ and CD8+ naïve T-cells have longer telomeres than corresponding memory cells,[35] or effector cells.[34] The longer telomeres in naïve cells might be the result of their higher replicative needs. However, one study found longer telomeres in memory B-cells than naïve cells,[32] a finding that still requires explanation. Overall, telomere length appears to be related to the replicative history of hematopoietic cells and their progenitors but potentially important deviations from this pattern have yet to be explained. Future studies geared toward understanding telomere biology in HSCs subsets will be important in understanding HSC development and their lifespan.

3. Telomere biology and bone marrow related disorders

3.1. Dyskeratosis congenita

The discovery that mutations in dyskerin (*DKC1*) caused X-linked recessive dyskeratosis congenita (DC),[36] and that these mutations resulted in abnormally short telomeres,[37]was the springboard for studies of telomere biology in inherited disorders. DC is a complex disorder, characterized by the diagnostic triad of nail dystrophy, lacy reticular pigmentation of the neck and upper chest, and oral leukoplakia.[38] Patients are at very high risk of bone marrow failure (BMF), myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). By age 30 years, 80–90% of DC patients develop severe aplastic anemia that does not respond to immunosuppressive medications;[39] this is the main cause of death in those patients.[40] Patients with DC are also at high risk of pulmonary fibrosis,[41] and cancer (11-fold higher than the general population).[42]

DC is genetically heterogeneous, including X-linked recessive, autosomal dominant, and autosomal recessive forms. Approximately 25% of patients with DC have the X-linked form with mutations in *DKC1*. DC is also caused by inherited mutations in other critical components of the telomere maintenance pathway, including *TERC* (autosomal dominant), *TERT* (autosomal dominant and recessive), *TINF2* (autosomal dominant), *NOP10* and *NHP2* (both autosomal recessive) and, the most recently discovered autosomal recessive gene, *TCAB1*.[43] A mutation in one of these seven genes is identified in about 60% of patients with classic DC. Although all the DC genes have not yet been discovered, it is important to note that all patients with DC have very short telomeres for their age. Telomere length measurement in leukocyte subsets by flow cytometry with fluorescent *in situ* hybridization (flow-FISH) is sensitive and specific in distinguishing patients with DC from unaffected family members, and from patients with other inherited BMF syndromes.[44] In another study using a different flow-FISH technique which determined relative telomere length in PBMC compared with a control cell line, all patients with DC had very short telomeres, but approximately 30% of patients with other BMF disorders also had telomere length ≤1st percentile.[45] Clinical testing of telomere length by flow-FISH is now recommended for all patients with BMF, if testing for Fanconi anemia is negative.[44,46]

3.2. Other bone marrow failure disorders

Several studies have evaluated telomeres in patients with other inherited BMF syndromes *e.g.*, Fanconi anemia, Diamond-Blackfan anemia, Shwachman-Diamond syndrome, and paroxysmal nocturnal hemoglobinuria.[47] These studies suggest that some of these patients have shorter than average telomeres, but they are not as short as the very short telomeres which characterize DC. TL abnormalities in BMF syndromes other than DC have been observed primarily in granulocyte telomeres,[44] an observation that might reflect the rapid turnover