

Telomeres in health and disease

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Abstract

Telomeres are repetitive ribonucleoprotein complexes present at ends of chromosomes. To synthesize this manuscript, a thorough literature search was done using PubMed, MEDLINE and Cochrane review for English-language literature and data available from the period of 2005–2016 were analyzed for manuscript writing. Telomeres help in maintaining the cellular health, inbuilt cellular mechanisms, metabolism and normal cell cycle. Telomerase is a specialized enzyme that possesses catalytic subunits - reverse transcriptase, Terc and dyskerin. Mutations affecting telomere or any component of telomerase enzyme result in disorders such as dyskeratosis congenita, aplastic anemia, myelodysplastic syndromes and leukemias. Thus, it is important to understand the telomere biology so as to deal with normal physiologic processes such as apoptosis, aging and senescence and tumor development.

Keywords: Apoptosis, dyskerin, senescence, telomerase, telomeres, tumorigenesis

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INTRODUCTION

The word “telomere” has originated from Greek word “telos” meaning “end” and “meros” meaning “part.” The existence of telomeric ends was first suggested by Muller (1930).^[1] “Hayflick limit” describes “the limited numbers of cell divisions that a particular cell undergoes.” This phenomenon was first described by Oeseburg (2010).^[1] Telomere sequences were first described by “Elizabeth Blackburn” and “Joseph Gall” (1978).^[1] Robert Meyazis along with their colleagues identified human telomeric sequence consisting of TTAGG repeats.^[1] Carol Greider discovered the reverse transcriptase “telomerase” in 1985.^[1]

Telomeres are composed of ribonucleoprotein complexes present at the chromosomal ends. These consist of tandem repeats of DNA sequences rich in G bases (TTAGG), which

are bound by six-protein complex termed as “shelterin.” The shelterin complex includes “protection of telomere β 1 (Pot-1) tripeptidyl peptides 1 (TPP1) heterodimer telomere-binding proteins,” “TTAGG repeat-binding factor 1 (TRF1),” “TRF2” along with interacting factors, repressor-activator protein 1 (Rap1) and TRF1 interacting nuclear factor-2 (Tin2). The telomeric chromatin also contains negative regulators of telomeric lengths and telomeric recombination. Shortening of telomeres below a threshold length and/or alterations in functioning of telomere-binding proteins result in loss of telomeric protection, which leads to “end-to-end chromosomal fusion,” “arrest of cell cycle,” and “apoptosis.” Other functions include transcriptional silencing of genes located in vicinity of the telomeres (“subtelomeric silencing”) and correct chromosome segregation during mitosis. Telomeric shortening is associated with cell division owing

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to inability of DNA polymerases to replicate ends of linear chromosomes. This is called “end replication problem.” The enzyme “telomerase” causes addition of TTAGG repeats at the chromosomal ends. This enzyme consists of a catalytic subunit possessing reverse transcriptase telomerase reverse transcriptase (Tert), Terc (an RNA component) that acts as template for DNA synthesis and dyskerin, a protein which binds and stabilizes Terc. Strong telomerase expression is detectable in stem cells and in early stages of embryogenesis. Mutations involving various telomerase components such as Tert, tERC, Dkc1 and shelterin (Tin2) are linked to human genetic disorders such as dyskeratosis congenita and aplastic anemia. These diseases are associated with short or dysfunctional telomeres. Telomeric function is highly dependent on normal functional shelterin. Suppression of shelterin protein results in the development of degenerative pathologies.^[2,3]

TELOMERE REGULATION

Telomeres are composed of repetitive genetic sequences maintained by an enzyme, “telomerase.” Telomerase as a complex contains a reverse transcriptase, hTERT, template RNA (hTERC) and accessory factors (Est1 and dyskerin). Telomerase regulation occurs at the telomere terminus which contains single-stranded DNA (ssDNA)-binding protein (Pot-1). The “end replication problem” originates from utilization of short RNAs to prime DNA synthesis. Deletion of these RNA primers does not impede circular genome as these gaps are not closed by extension of a preceding Okazaki fragment. The telomeric nucleoprotein complex helps cells in distinguishing naturally occurring chromosomal ends from DNA breaks. In the absence of telomeric protection, these chromosomal ends activate response pathways that deal with DNA damage. These pathways signal cell cycle arrest, senescence, or apoptosis. Telomeres prevent inappropriate DNA repair reactions such as exonucleolytic degradation and ligation. Impairment of telomeric function results in unprotected chromosomal end fusion which results in generation of dicentric chromosomes. Alterations in telomere structure and function occur during cancer development as well as in aging process. Telomere erosion limits the proliferation of transformed cells, thus acting also as a “tumor suppressor system.” Telomerase is a two-component ribonucleoprotein enzyme containing a highly conserved reverse transcriptase, TERT and an associated RNA template, TERC. TERT is closely related to the nonlong terminal repeat retroviral reverse transcriptase and Group II introns. It extends the 3' DNA end rather than an RNA primer. The chromosome terminus acts as a primer positioned on an alignment site in TERC like the 3' telomeric end. Extension of this telomere

terminus results in the addition of a telomeric repeat. The repeated steps endow the chromosome ends with repeat arrays representing telomeres.^[2-4]

The human genome contains at least three EST1 orthologs, of which EST1A and B have been shown to encode telomerase-associated proteins. The mammalian EST3 ortholog has not been identified to date. The human telomerase interacts with an RNA-binding protein, “dyskerin,” a pseudouridine synthase which plays a role in ribosomal processing as it binds to numerous small-sized nucleolar RNAs. Functional significance of dyskerin binding to hTERC is observed in dyskeratosis congenita, wherein the X-linked disease is due to genetic mutation in dyskerin while the autosomal dominant form is attributed to hTERC gene mutation.^[2-4]

TELOMERASE LENGTH REGULATION BY TELOMERE-INDEPENDENT MECHANISMS

In humans, telomere lengths are alternatively maintained by alternative lengthening of telomeres mechanisms, exonucleolytic attacks and deletions at a rapid rate (“telomere rapid deletion”). The maintenance of telomerase-independent telomere length results in variable chromosomal ends. However, telomerase involvement maintains a stable size.^[5]

Negative feedback control of telomeric length is mediated by the TRF1 complex. The human telomeric length control is mainly exerted by the “TRF1,” which is a small-sized dimeric protein with a specific sequence TTAGGGTTAG. TRF1 binds to duplex telomeric TTAGGG repeat array. The total numbers of TRF1 molecules per chromosome end are correlated with telomeric length. A long telomere engages large numbers of TRF1 molecules that block the telomerase from addition of more repeats. TRF1 binding to telomeres is inhibited by enzymes, tankyrase 1 and 2. These are poly(ADP-ribose) polymerase identical in amino acid sequence and functions. TIN2, a small protein with unknown domain, forms a ternary protein complex with both TRF1 and tankyrase, thus stabilizing the TRF1-tankyrase interactions. PINX1, a “TRF1-interacting protein,” affects telomeric length control by altering telomerase activity within nucleus.^[4-6]

Telomere becomes shorter in length with each cell division as DNA polymerase I enzyme cannot copy extreme DNA strand ends. On attaining critically short length, cell cycle arrest or cell death takes place. Thus, it can be concluded that telomere length shortens with age. There are mutations associated with three genes on dyskeratosis congenita - “DKC1,” “TERT,” and “TERC.” All patients

suffering from dyskeratosis congenita possess extremely short telomeres. Dyskeratosis congenita due to mutations in TERT and TERC manifests in younger age groups.^[2]

The mammalian “CST” (CTC1-STN1-TEN1) is associated with telomeres. Lack of reduction in “CTC1” or “STN1” results in telomeric defects causes defective DNA replication. CTC1 or STN1 knockdown increases anaphase bridges and multi-telomeric signals. Numerous extra proteins are required along with the standard replication machinery to replicate the telomeric duplex which includes TRF1, Bloom syndrome, flap endonuclease 1, ROTUNDIFOLIA4-LIKE (RTFL), BReast Cancer2 (BRCA2) and RecQ protein ligand4 (RECQL4). Depletion of these proteins results in the formation of fragile telomeres and may appear under replication stem and fork stalling. Fork stalling is inducible by various factors such as repetitive or complex DNA sequences, nucleotide depletion and DNA damage. Once the “replication fork” is stalled, it is required to rapidly restart the maintenance of genomic stability. If restart does not occur, the collapse of “replication fork” leads to ssDNA formation, DNA double-strand breaks and unwanted recombination events.^[3]

Telomerase causes addition of short telomeric repeats to “DNA substrates” using a repeat addition type of processivity. This is partly regulated by a TERT-dependent anchor site.^[4]

Telomeres form G-quartet structures, wherein the four GS bonds together by the “Hoogsteen base pairing.” When these quartets are stretched closely, the overall structure is referred to as the “G-quadruplex.”^[4,6]

Telomere length maintenance and stabilization are complicated procedures requiring telomerase and shelterin complex after replication. This complex facilitates T-loop structure function at chromosomal end preventing NHET/homologous recombination events involving telomeres. “Lamin A Δ exon 9” mutant proteins hinder telomerase accessibility, replication and/or repair which can lead to terminal deletion.^[6]

The telomeric ssDNA-binding protein Pot-1 protects the telomeres from rapid degradation and its regulation. Human Pot-1 interacts with TRF1 duplex telomeric DNA-binding complex. The DNA-binding “Pot-1” is required for inhibition of telomerase activity.^[5]

QUANTIFICATION OF TELOMERE LENGTH

Most common techniques used to measure telomeric length include polymerase chain reaction, Southern blot

and *in situ* hybridization. Southern blotting or telomere “restriction fragment” analyses are considered the gold standard methods. Herein, telomeres are represented as smears and average telomere length is ascertained by the weightage of the smear.^[1]

CLINICAL PARADIGMS IN TELOMERE BIOLOGY

Shelterin together with telomere-DNA acts as a dynamic unit, which prevents the chromosomal ends from being recognized as damaged DNA, thereby preventing their degradation. Telomerase synthesizes new telomere repeats which offset shortening with each cell division. The telomeric length helps in predicting the onset of replicative senescence, which is a permanent state of cell cycle arrest arising after finite numbers of cell divisions on reaching a critically shortened length; the telomeres become dysfunctional and activate DNA damage response identical to double DNA breaks. These “senescent” cells secrete a set of cytokines, chemokines and proteases, which are collectively termed as “senescence-associated secretory phenotype.”

The telomeric dysfunction is associated with decreased cellular metabolism, also. The mutated telomere and telomerase cause telomeric shortening which is manifested in age-related phenotypes. Telomere-mediated disorders exhibit two hallmarks of age-related disease, i.e. degenerative organ failure and cancer predisposition. In children and young adults, telomere-mediated disorders cause bone marrow failure, while in adults, it additionally manifests as “idiopathic pulmonary fibrosis” and “liver cirrhosis.” Adult-onset telomeric disease manifests as familial or sporadic myelodysplastic disease or acute myeloid leukemia. Telomeric defects involving hematopoietic tissues are due to stem cell failure which limits both stem cell numbers and function. This condition is commonly seen in pediatric population which has predominantly high turn-over tissues. In adults, the tissues possess a slow-turnover. Telomeric defects cause disease due to multiple “hits” which accumulate over a period to cause organ failure. This stem cell exhaustion state contributes to tumorigenesis, which explains the tumor-prone nature of telomeric syndromes. Telomeric shortening is a tumor-suppressive mechanism mediated by the p53-mediated apoptosis and cellular senescence.^[7]

Leukocytic telomeric length and telomeric shortening rates are considered the biomarkers of aging process.^[8,9] Cells in culture cease dividing after a certain number of passages and enter into “replicative senescence.” This phenotypic change is characterized by alterations in cell morphology,

genetic and protein expressions. Beta-galactosidase staining is used for the identification of these senescent phenotypes.^[1]

Telomere shortening in healthy aging process is observed in CD4+ T-helper cells, CD8+ “cytotoxic” T- and B-cells at an annual rate of 19–35 base pairs. Chronic stress accelerates telomere shortening. Hence, short telomeric length has been found in patients with mood disorders, in pessimistic women and social deprivation.^[10]

INFLUENCE OF TELOMERIC LENGTH

The telomeric DNA due to its high guanine content is highly susceptible to stress-induced accumulation of 8-oxo-guanine which is inefficiently repaired. Furthermore, the random accumulation of single-stranded breaks arising from hydroxyl radical-mediated attacks on DNA backbone along telomeric and subtelomeric regions. Telomeric stability is influenced by environmental and occupational exposures, psychological conditions, inflammatory state and chronic diseases. The intake of proper diet along with exercise prevents genomic instability.^[9,11]

Telomere is composed of noncoding double-stranded tandem guanine-rich DNA sequences (TTAGGG). These telomeres are extended up to 9–15 kb in humans which end in a 50–300 nucleotide 3' single guanine strand overhang that folds back and enters the double-stranded telomeric helix which forms a large “T-loop.” This T-loop causes formation of a high-order structure which mediates the end-capping. The T-loop stability is closely dependent on the integrity of associated telomere-specific proteins, the “shelterin complex.” This shelterin complex includes proteins such as telomeric repeat-binding factor (TRFS)-1 and TRFS-2. The TRF proteins bind to the “double-stranded” telomeric DNA and directly bind to the single-stranded telomeric DNA and directly interact with TPP1. Rap1 binds to TRF2. TIN2 forms the central component of complex which interacts with TRF1, TRF2 and TPP1. This complex plays a significant role in protecting the chromosomes from being recognized by DNA-damage/repair system as breaks which can activate the p53 or p16^{INK4a} pathway which leads to apoptosis or cellular senescence. The 5' end of TERC contains the template while the 3' end contains “two” binding sites for telomerase-associated proteins. The loss of “TERT” does not alter short-term telomere integrity; however, it affects chromatin configuration and impairs DNA damage response. The telomerase enzyme directly modulates the Wnt/ β -catenin signaling by acting as a cofactor.^[12]

The 3' ends of all chromosomes are single-stranded (lacking a complementary strand) and measure approximately 200 nucleotides in length. Both the 3' single-stranded ends of chromosome loop back on themselves and anneal to the double-stranded part and form a T-loop. After the T-loop formation, the ssDNA forms hydrogen bonds on repetitive complementary sequence on 5' end, thus forming a “D” or “displacement loop.” The 3' end of human telomere is guanine-rich and forms secondary structures known as “G-quadruplex” structure. This structure must be unfolded before initiation of telomerase activity.^[13]

Cells with long telomeric lengths are at higher transformation risk as the tumor cells get extra time and undergo multiple mitotic divisions, thus acquiring genetic abnormalities.^[14]

Exhaustion of stem cell pool is a significant risk factor involved in aging process. Stem cells possess the capacity to divide beyond the “Hayflick limit” due to telomerase activity. In the normal stem cells, telomerase expression is highly regulated. The telomeric length is maintained in cancer cells due to continuous telomerase expression.^[12]

Stem cells and malignant cells possess property of immortality which is dependent on telomere maintenance mechanisms. Telomerase activation mechanism elongates telomere sequences using RNA template. Sarcomatous cells use alternative lengthening mechanisms to maintain telomeric lengths.^[15] Telomeric length is inherited, short, follows polygenic mode of inheritance and possesses high variability with regard to replication of somatic cells and is inversely related to age.^[16,17] Mean leukocytic telomeric length is indicator of biological age and somatic fitness. Shortening of telomeres with repeated cell divisions leads to genetic instability and carcinogenesis.^[16]

CONCLUSION

Telomeres are essential for maintenance of cell cycle, apoptosis and their maintenance. Failure of telomerase enzymatic activity results in tumorigenesis, aging and senescence. Telomere biology is an important area in understanding the cell functions and their metabolic regulation, especially in tumor development.

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Conflicts of interest

There are no conflicts of interest.

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