Roles of Telomeres and Telomerase in Aging

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ABSTRACT

Telomeres are DNA structure that capped the end of linear eukaryotic chromosomes to protect the stability and integrity of the chromosomes. Attrition of telomeres has been known as one of the hallmark of aging. This review leads to huge interest in the biology of telomeres and the related enzyme in the aging and related fields. We attempt to review briefly the biology and functions of telomeres, factors, and mechanisms that control its length and its maintenance. Genetic and epigenetic related dysfunction of telomeres and telomerase will also be discussed. Finally, a list of disorders of telomeres and telomerase (telomeropathies) will be mentioned with potential therapeutic interventions for these disorders, and the potential for delaying cell senescence and health-span extension.

Keywords: Aging, DNA, telomeres

ABSTRAK

Telomeres adalah bagian dari struktur DNA di ujung linier *helix eukaryotic chromosome* sebagai penutup/*cap* untuk menghindari dan melindungi bagian ujung kromosom dari kerusakan dan untuk menjaga kestabilannya. *Telomeres* akan mengalami erosi atau pemendekan yang merupakan tanda penuaan/*aging*. Proses ini mendapat perhatian besar sehubungan dengan peristiwa penuaan. Ulasan singkat ini tentang sifat-sifat biologi dan fungsi *telomeres*. Akan dibahas pula akibat pergeseran/disfungsi genetik dan epigenetik yang menyebabkan perubahan *telomeres d*an telomerase. Di akhir pembahasan, dicantumkan daftar *telomeres* dan telomerase (*telomeropathies)* yang dapat diintervensi dan dapat memperlambat proses penuaan, dengan kata lain memperpanjang usia. **Khing S. Ong, Zack S.T. Liem, Boenjamin Setiawan. Peran *Telomeres* dan Telomerase pada Proses Penuaan.**

Kata kunci: DNA, penuaan, telomeres

INTRODUCTION

A brief history on the discovery of telomere

The importance of telomeres biology, are represented on the Nobel prizes won in this field. Hermann Muller won the 1946 Nobel prize; he is the first who coined the term telomere (Greek: telos for end and meros for part). Barbara McClintock won the 1983 Nobel prize for her works on cytological studies on maize chromosomes end structure and proposed a breakage-fusion-bridge hypothesis.[2] Thomas Cech won 1989 Nobel prize in Chemistry for discovery of RNA catalytic property, that led to the discovery of components of telomere.[3] Last, in 2009 Elizabeth Blackburn and Carol Greider along with Jack Szostak won the Nobel prize for their works on telomeres of a single cell organism tetrahymena and the discovery of telomerase.[2]

TELOMERE STRUCTURE AND FUNCTION

Telomere is a cap at the ends of each linear eukaryotic chromosomes in our cells, consists of several hundreds of non-coding neucleotides AGGGTT (in human) at tandem repeat sequence in the 5'direction, with 3' single stranded DNA overhang.[4] To strengthen the protection of chromosome, telomeres bind to a protein shelterin to stabilized and prevent chromosome from fusion.[4,5] A T-loop structure form at the ends of chromosomes by looping 3' overhang back to be imbedded in the double stranded portion of the chromosome. This process is mediated by telomeric repeat proteins TRF1 and TRF2.[4,5] which are included in the shelterin proteim complex function to protect the end of chromosome and maintenance of telomeres. T-loop results in a triple stranded structure at the point of insertion and form a small loop called D-loop. TRF2 is classified as double stranded telomere binding protein located at the D-loop junction, whereas TRF1 is located on the double stranded portion of the telomere. Over expression of a dominant negative TRF2 cell line would effect the single stranded portion of telomere, and lead to activation of the p53 apoptosis pathway.[4]

With each replication of cell, the length of telomere shortened, because the somatic cell polymerase is incapable of replicate the 3' overhang DNA, as a result the chromosome are less protected with each replication until it come to a replicative senescence state, where it reach the Hayflick limit; each cell type inherently only capable to replicate in a limited cycles (30-80 cycles depending on cell types). Cells from older people and people with premature aging syndromes replicates with much fewer cycles. Maintaining the length of telomere is therefore important

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in replenishment of new cells to replace the worn one, and understanding of how the length of telomere is maintained is essential in the field of aging research.

The function of telomere besides protecting the chromosomes ends is to prevent the activation of DNA damage response pathway which can lead to cell's death. Study by Robin, et al,[6] showed that telomere can also affects genes expression such as silencing by heterochromatin spreading, a process called telomere position effects or TPE. It can also effect gene expression over larger distances (TPE-OLD) by chromatin looping.

These genes expression changes reprogram the cell as telomere shorten and contribute to the dysfunctional changes associated with aging.[6,7] TPE change of gene expression is different from changes of gene expression through epigenetic silencing. However, both process are controlled by gene(s), both appear to result in stem cell dysfunction and are linked to disease risk factors.[8] Cells enter into senescence state, because the gene expression has altered so much that the cell ceases to function normally.[8]

THE LENGTH OF TELOMERE

Changes in telomeric length (TL) with advancing ages varied among individual people.[9] However, in general, the trend is that TL decreases with increasing age, and act as a "molecular clock" in people. A recent study however, showed that TL is only slightly better than 'tossing a coin', as compared to simple measures such as actual age, ability to climb stair, or walk a distance.[10] TL from blood leukocytes is significantly shorter than those from skin cells or synovial cells of the same person,[9] it might also be different in other types of tissues.

Telomere Length and Longevity

As we know, longevity is affected by multiple factors including genetic, epigenetic and environmental factors. TL may play a role in the genetic of longevity. Decreasing TL with advancing age is less steep in women than in men, but some studies showed the differences are not significant.[9]

Due to a gene mutation, Amish families or population generally live longer than other populations in the same area. A study on large Amish families,[11] TL was negatively correlated with age, and no significant differences between genders. But there is a positive correlation and association between daughter's TL and paternal life-span, but not with the maternal life-span, these results suggest a link between TL, aging, and life-span indicating a strong genetic influence, that the authors suggest possibly by an imprinting mechanism; genome imprinting is a differential expression of a gene that depends on the sex of the parent that transmit the gene. The expression of gene inherited from father is different from when it is inherited from mother.

A study on small numbers of centenarian had shown a significantly longer telomeric length in healthy centenarian as compared to unhealthy centenarian. This results suggesting that the length of telomeres is associated with health condition.[12]

Factors Affecting Telomeric Length

1. Sex hormone regulating telomerase gene[13]

Telomeric length is generally longer in women than men, due to estrogen regulation of telomerase, causing lower rate of age-dependent attrition of telomeric length. Danazol, a synthetic androgen also increases the telomeric length in patients with telomere diseases.[14]

2. Meditation, stress relief and positive attitudes have been shown to increase telomeric length.[15]
3. Healthy life-style and exercise also associated with increased telomeric length.[16]

Maintenance of Telomeric Length

With each cycle of cell replication, the loss portion of the telomere is re-build by an enzyme telomerase or telomerase reverse transcriptase (TERT). TERT is responsible for the lengthening of telomere or reversing the attrition of telomere. Using mouse 'engineered' with telomerase deficiency, the mice reveal an age-associated phenotype, including DNA damage and shorter telomeres. Reconstitutions of telomerase activity reverse these aging phenotypes.[4]

Recently another enzyme - ATM-kinase (ataxia telangiectasia mutated protein kinase) - was discovered at John Hopkins by Carol Greider team.

In normal mouse cells inhibition of enzyme PARP1 (poly [ADP-ribose] polymerase 1) would activate ATM-kinase and lengthen the telomeres.[17] Another protein called telomeric zinc finger-associated protein (TZAP) has been discovered[18] that prevent telomere from become too long. TZAP bind preferentially to long telomere and trigger a telomere trimming process.

In vitro study has shown that delivery of modified mRNA encoding TERT would rapidly extend the telomeres in human fibroblasts and myoblasts.[19]

Wu, et al,[9] proposed an alternate strategy for telomere maintenance (ALT) in telomere deficient cells, however it induced substantial telomere length variability. Later, Royle, et al,[10] expanded the ALT by including process that are activated in the absence of telomerase activity such as increase telomere chromatid exchange, blocking the T-loops and activation recombination-based process that restore replication capability.

Although tumors has shorter telomeres compare to normal cells, about 80% of the tumors has increase activity of telomerase to ensure their indefinite cells proliferation. In addition, evidence has been accumulated suggesting that telomerase has extra-telomeric function in tumor cells survival and proliferation.[20]

All the evidence so far suggests that factors governing telomere length is of paramount important in both aging and cancer. Hence, there is a 'Goldilocks effect' for telomere, not too short and not too long, a well balanced optimal length seems to be what is required.

TELOMERE/TELOMERASE AND AGING

Aging is a multifactorial process, and is extremely complex. Of the tentative nine hallmarks proposed for mammalian aging,[21] only genomic instability can be clearly correlated to telomeres attrition.[21]

Can activate the telomerase or TERT rejuvenate cells and tissues? Most studies addressing to answer the question are carried out in mouse model. To provide a 'proof-of-principle', Spain National Cancer Research Center led by Maria Blasco tested the effects of TERT gene therapy in mouse model with

AAV vector of wide tropism expressing mouse TERT, with the results of increased life span of 24% in 1 year-old mice and 13% in 2 year-old mice, and of importance, telomerase treated mice did not develop more cancer than the control untreated mice.[22]

Another study also used mice engineered to be lack of telomerase activity, but can be re-activated by a compound called 4-hydroxytamoxifen (4-OHT). Once telomerase is re-activated by 4-OHT, a wide range of tissue damage are reversed.[23]

With mounting evidence (at least in mice model) of beneficial effect of TERT in delaying or even reversing aging, it is unavoidable that the current trend is to develop therapeutic intervention against aging with controllable amount of TERT activation.

GENETIC AND EPIGENETIC OF TELOMERES

The gene TERT at chromosome 5 provides instruction for making components of telomerase. The TERT component is produced by TERT gene, the other component is produced from TERC (telomerase RNA component) gene.[24] At least 18 mutations in TERT gene have been identified in patients with congenital dyskeratosis, and at least 23 mutations on TERT for idiopathic pulmonary fibrosis. Mutations in TERT gene have also been associated with increase risk for various cancers.[24]

Epigenetic signaling contributes to aging, human diseases and tumorigenesis through methylation of DNA and histone acetylation without altering the DNA sequence. Epigenetic (DNA methylation) age is a biomarker of chronological age that predicts life expectancy.[25] Study had shown that telomere shortening can effect DNA methylation. In human cells culture, it has been shown that TERT would reverse the hypomethylation associated with aging.

A recent study[26] analyzed nearly 10,000 people, and found gene variant mapping to 5 loci with intrinsic epigenetic age acceleration (IEAA) and gene variants in 3 loci associated with extrinsic epigenetic age accelerating (EEAA). It turn out the variants in TERT that are associated with IEAA, paradoxically were also associated with longer instead of shorter telomeric length because of accelerated aging, suggesting an important role of TERT in regulating epigenetic age.

TELOMERE DISEASES, TELOMEROPATHIES

Mutations in telomere genes are associated with diseases known as telomere diseases. Classical examples such as congenital dyskeratosis, idiopathic pulmonary fibrosis, aplastic anemia, and liver fibrosis all have shortened telomere length.[27] Recently a term telomeropathies was given for a "spectrum" of related syndromes.[28]

THERAPY OF TELOMERE SYNDROMES

Currently most interventions for these syndromes are organ transplantation eg. bone marrow, lung and liver which only treating the physical condition. To address the molecular causes of these syndromes, telomerase activating therapy should be considered.

TA-65 OR ASTRAGALUS ROOTS

Astragalus roots has been used for more than 2,000 years in China as a herbal medicine to boost the vitality and strengthen "Qi". TA-65 was commercialized as a telomerase activator. A couple of blog-sites have people testified its effectiveness, with improvement of skin seems to turn more "youthful" appearances, 6 months after taking TA-65. Another potential is gene therapy of TERT with harmless adenovirus as vector, or with CRISPR Ca9 technology.

CONCLUSION

1. Most recent GWAS or genome wide association study from UCLA, Boston University, Stanford University and Institute for aging research at Hebrew Senior Life with nearly 10,000 subjects shown a critical role of TERT in epigenetic clock (age). However it is not clear that this is associated with life expectancy. Unlike study in mouse model from the team led by Maria Blasco, AAV carried mouse TERT treatment clearly enhance the life span of younger and older mice.

2. Since the length of telomeres is different in each person, and or differs in the same person at different time, the interpretation of study results is more difficult. Many studies are conducted in mouse model. However, mouse telomeres are much longer compared to human's, but the lifespan is much shorter than human. Does this mean mouse telomere carried much more deleterious gene expression than human telomere?

3. Therapeutic intervention in delaying aging or cell senescence, might be focused on controllable activation of hTERT either by small molecule, other enzyme or by gene therapy using AAV vector or CRISPR Ca9 technology. As to TA-65 or Astragalus roots, a randomized double blind control study need to be performed.

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