REVIEW ARTICLE

Telomere in Aging and Age-Related Diseases

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Abstract

BACKGROUND: The number of elderly population in the world keep increasing. In their advanced ages, many elderly face years of disability because of multiple chronic diseases, frailty, making them lost their independence. Consequently, this could have impacts on social and economic stability. A huge challenge has been sent for biomedical researchers to compress or at least eliminate this period of disability and increase the health span.

CONTENT: Over the past decades, many studies of telomere biology have demonstrated that telomeres and telomere-associated proteins are implicated in human diseases. Accelerated telomere erosion was clearly correlated with a pack of metabolic and inflammatory diseases. Critically short telomeres or the unprotected end, are likely to form telomeric fusion, generating genomic instability, the cornerstone for carcinogenesis. Enlightening how telomeres involved in the mechanisms underlying the diseases' pathogenesis was expected to uncover new molecular targets for any important diagnosis or therapeutic implications.

SUMMARY: Telomere shortening was foreseen as an imporant mechanism to supress tumor by limiting cellular proliferative capacity by regulating senescence check point activation. Many human diseases and carcinogenesis are causally related to defective telomeres, asserting the importance of telomeres sustainment. Thus, telomere length assessment might serve as an important tool for clinical prognostic, diagnostic, monitoring and management.

KEYWORDS: telomerase, cellular senescence, aging, cancer

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Introduction

Genomic instability was carried along as the cornerstone in cancer development, by accelerating pile of genetic mutation that responsible for cancer cell evolution.(1,2) Genomic instability can occur through a variety of mechanisms, including a defective response to DNA damage, a defect in DNA replication, or a defect in chromosome segregation. The importance of some of these mechanisms has been demonstrated through the study of human genetic diseases that demonstrate both increased chromosome instability and cancer.(3,4)

In every cell cycle, there is always a probability of mistaken for damaged or broken DNA on the ends of eukaryotic chromosomes. This will cause cause cell permanently arrest in damage cellular pathways. Any attempts to repair would address risks for genome integrity. Telomeres, a protein-DNA complex, work out this problem and prevent the chromosome end from initiating a DNA damage response.(5) Telomeres are composed of repeated DNA sequences bound by a series of specialized protein. It can be ideated as the chromosomes protective cap.(6) Any defect in this cap structure could lead to cell cycle arrest or DNA repair activities which promote an end-to-end fusion of chromosomes via non-homologous

end joining (NHEJ).(7) Telomeres are directly affected by the inability of DNA polymerase to completely replicate the 5′ end of a linear chromosome, a situation which known as the end replication problem.(8,9) In each replication, the chromosome terminus loss some DNA and commonly human somatic cells don't have enough capability to compensate. Thus, progressive rounds of replication lead to gradual telomere shortening, until telomeres become critically short and considered as DNA damage.(10) Sensed as damage, a signal will be sent to stop the cell for further divisions and head to senescent pathways. This ability to limit damaged cell proliferation might be one of tumor suppressor mechanism.(11,12)

Contrary, telomere shortening correlates with cellular aging. Therefore, either abnormal telomere shortening or elongation, could detrimental for human health. Extreme telomere shortening due to telomerase deficiency in highly proliferative tissue, for example, can lead to diseases such as dyskeratosis congenita (DKC) or pulmonary fibrosis. (12-15) Conversely, telomerase upregulation leads to the cellular immortalization that is fundamental to cancer cell growth.(16) Telomerase reverse transcriptase (TERT) known to has an essential role in telomere maintenance and in cancer biology.(17) The majority cancer cells depend on the activation of telomerase to gain proliferative immortality. Stem and progenitor cells also express low levels of telomerase.(18) This showed the essential of telomere length regulation for both cellular and organismal well being. Telomere length regulation affect by the structure and composition of the telomere, the availability of telomerase and the interplay between telomere proteins, telomerase and the DNA replication machinery.(19)

Telomeres and Telomerase Biology

Recent comprehensive insight about the mechanisms of age-related diseases, concluded the importance of overall telomere attrition in predicting mortality and those diseases.(20) Telomere roles as a cap to protect the genomic DNA through various mechanisms. One of it is by preventing the recognition of the linear chromosomal DNA end as a broken end, because once it was recognize as a broken end, automatically DNA end-joining, DNA recombination, or DNA repair mechanisms will be processed, leading to unstable chromosomes. Unfortunately, common chromosomal DNA replication machinery cannot completely copy the DNA until the extreme ends of the linear chromosomes, this leads to attrition of chromosome ends after many course of cell divisions.(20)

The structure and function of mammalian telomeres are highly conserved, built on long tandem arrays of duplex TTAGGG repeats ends in a 50- to 400-nt 30 protrusion of the G- rich strand, forming the binding sites for the abundant telomere-specific protein complex, called shelterin (Figure 1). The presence of duplex telomeric repeats, a telomere-specific protein complex, and a 30 protrusion are general themes for all eukaryotic telomeres but the nature of the repeats and proteins vary widely.(21)

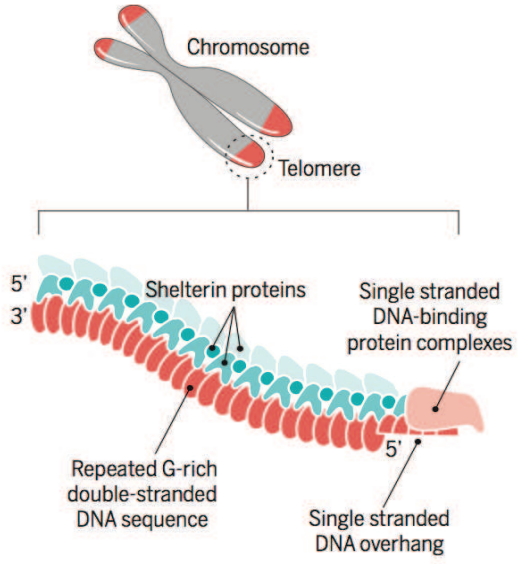

Figure 1. Telomere Structure.(20) (Adapted with permission from The American Association for the Advancement of Science).

Based on recent understanding of the molecular pathways that recognize and repair double-strand breaks in mammalian cells, the end-protection problem can be recast in more precise terms (Figure 2).

There are two independent signaling pathways in mammalian cells that are activated by double-strand breaks. Firts is the ataxia telangiectasia mutated (ATM) kinase pathway, directly activated directly by DNA ends, and then the ataxia telangiectasia and Rad3-related (ATR) kinase pathway, activated by the single-stranded DNA formed when the 5′ end of a double-strand break gets trimmed back, or resected.(5)

To avoid premature cellular senescence and the acceleration age-related diseases, the telomere must have ample reserve length, but also telomere shortening is needed to suppress tumor formation. Then, telomere length homeostasis should be achieved.(22) Mammalian telomeres solve the end-protection problem through the agency of a six-subunit protein complex called shelterin.(23) Shelterin is enriched with specificity for telomeres, through the binding complex of several DNA protein to several DNA sequence.

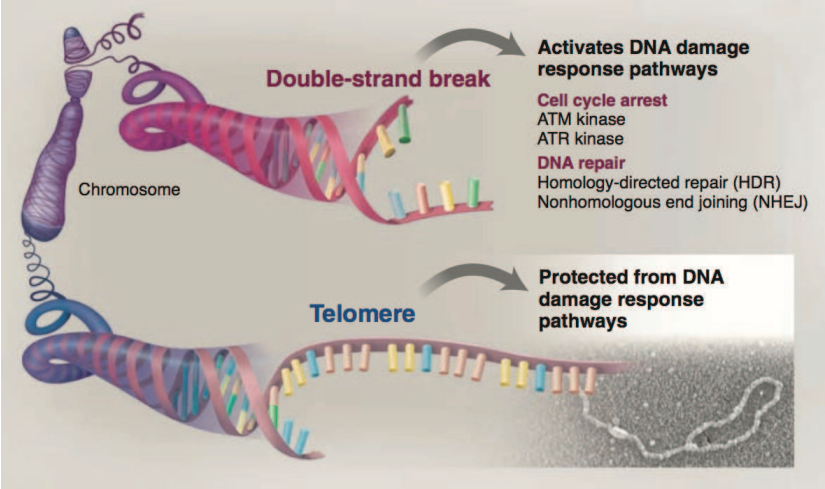

Figure 2. The end-protection problem.(5) (Adapted with permission from The American Association for the Advancement of Science).

Two shelterin subunits, telomeric repeat factor (TRF)1 and TRF2, bind to the TTAGGG sequences in double-stranded DNA, and one subunit, protection of telomeres protein (POT)1, binds to these sequences in single-stranded form. These three proteins are held together by TRF1-interacting nuclear protein (TIN)2 and tripeptidyl peptidse 1 (TPP1), making the selectivity of shelterin for telomeric DNA is admirable.(5) Meanwhile represor/activator protein 1 (Rap1) is a stabilizing protein associated with TRF2. Recent studies found that shelterin is not just a static structural component, but it was emerging as a telomere protecting protein complex which have a DNA remodeling activity to change the structure of the telomeric DNA, acts together with several associated DNA repair factors.(23,24) The sixsubunits of Shelterin on telomeric DNA are shown in Figure 3.

Telomere shortening can also be caused by include nuclease action, chemical (such as oxidative) damage, and DNA replication stress. Telomerase, as well as recombination between telomeric repeats, can counteract these damage-causing process to restore telomere length.(25) Telomere length is balanced on an equilibrium set point. It shorten during replication and lengthened by telomerase. Any imbalance of this equilibrium leads to disease.(26) Telomerase is an RNA-containing reverse transcriptase that adds telomeric repeat DNA to chromosome ends. (27) This prevent telomeres to be shortened in the end replication problem, which is the failure of the DNA replication machinery to duplicate the very end of each chromosome.(28) As stated before, once telomeres shrink to a critical length, signal will be sent for the cell senescent, or alternatively undergo programmed cell death. This is the major tumor-suppressive mechanism, to prevent any replication of damaged DNA. Continuously dividing cells such as germ cells, stem cells, and, importantly, most cancer cells then require telomerase activity for survival.(29,30)

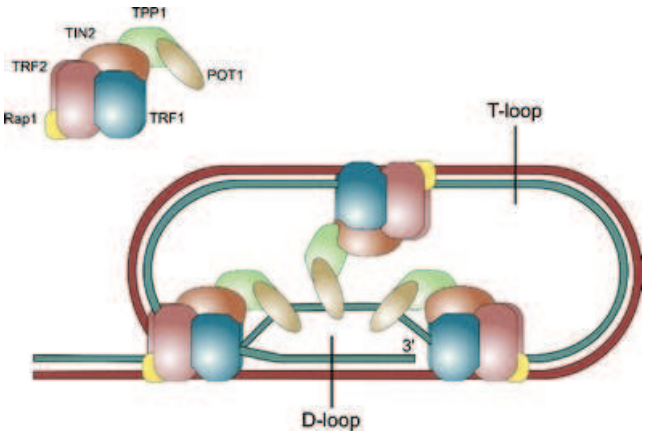

Figure 3. The six known subunits of Shelterin on telomeric DNA.(24) (Adapted with permission from PubMed Central).

Telomerase functions as a ribonucleoprotein enzyme. An integral telomerase RNA (TR) component was required, in addition to the catalytic TERT. Extensive studies have identified some structural and functional features within the TR and TERT essential for activity.(31) In eucaryotes, telomerase catalyzes the extension of telomeric DNA. Human telomerase complex involving Cajal bodies in its intracellular trafficking and its recruitment to telomeres. Once recruited, a separate step activated incuding increase in its repeat addition processivity.(32)

Telomerase is unique among reverse transcriptase (RT) by functioning as a ribonucleoprotein.(33-35) Telomerase's catalytic core is minimally built of the TERT and the integral TR. TERT protein consists of the catalytic site for DNA synthesis, and assembles with the TR to provides the template (Figure 4). When telomerase activity was not needed, many accessory proteins in the holoenzyme take part in crucial roles for telomerase biogenesis, localization, and regulation.(36-40)

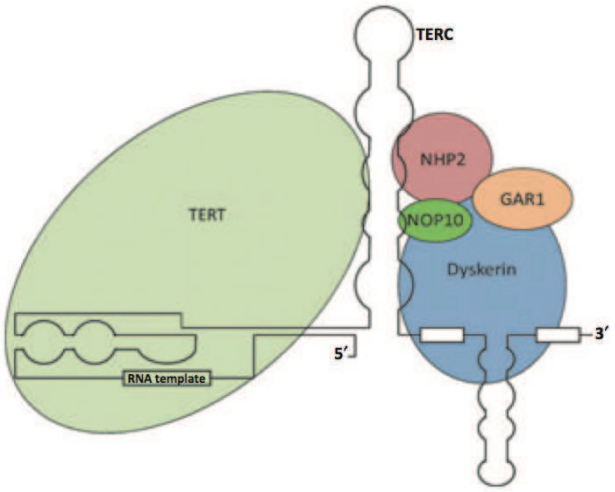

Figure 4. Telomere Structure.(40) (Adapted with permission from John Wiley & Sons, Inc). TERC: telomerase RNA component; NHP2: non-histone protein 2; NOP10: nucleolar protein 10; GAR1: glycine arginine rich.

TERTs usually consist of four domains: the telomerase essential N-terminal (TEN) domain, the telomerase RNA-binding domain (TRBD), the RT domain, and the C-terminal extension (CTE). The TEN domain interacts with telomerase reverse (TER) and traps single-stranded telomeric DNA to promote processive repeat synthesis.(41-43) This processes undergo by capturing the substrate and maintain the association with those single-stranded products.(44) The TRBD confers the specificity of interaction between TERT and TER.(41,45) Motifs preserved in the evolutionarily related retrotransposon RTs forms the active site in RT domain. Here, aspartic acid residues coordinate the magnesium ions needed for catalysis of deoxynucleotide (dNTP) addition.(46) In TERTs, this domain also positions the template and aligns the substrate 3' end.(47,48) RT domain function could be enhanced by the CTE and/or the nucleic acids.(49,50) So far, mutations in at least six telomerase components have been linked to human telomere-mediated disorders such as DKC, aplastic anemia (AA), and idiopathic pulmonary fibrosis (IPF).(16,17,51) Most cancer cells have their telomerase up-regulated so then they keep growing.(52)

Telomerases important role in oncogenesis is highlight due to recurrent mutations recently identified in the promoter of the gene for the hTERT (human TERT) telomerase protein component (53,54), the most frequent mutation in some cancer types (55). These promoter mutations are associated with increased hTERT expression, telomerase activity, and telomere length.(56) Otherwise, telomerase activity, its maturation, or the recruitment to telomeres deficiencies lead to human diseases such as aplastic anemia and DKC.(57)

Cellular Senescense

All the time our cells experience stress and damage continuously either exogenous or endogenously. The responses range from complete recovery to cell death. Proliferating cells can commence a further response by adopting a state of permanently cell growth arrest, termed cellular senescence.(58,59)

Cellular senescence was first defined by Hayflick as the ultimate and irreversible loss of replicative capacity occurring in primary somatic cell culture.(60) His study found that cell growth was reproducibly blocked after a fairly well-defined number (under constant culture conditions) of population doublings (PD), and this suggested the idea of a biological clock or, more specifically, a replication counter (61) that counts biological time in numbers of cell divisions, and after a reproducible number of divisions triggers signaling pathways that block cellular division. Several different processes have been suggested as possible clocking mechanisms (62), but telomere uncapping (63) is by far the best established and most extensively investigated of these besides the epigenetic derepression of the INK4a/ARF locus, and DNA damage.(58) Cellular senescence protects against the development of cancer, while it also may be involved in aging.(59,64) The consequences of cellular senescence is the radically phenotype altering which thought to impair tissue function and predispose tissues. Accumulation of these known as "replicative senescence" will progress to diseases.(65,66) Oxidative stress and activated oncogenes such as Ras have also been shown to trigger cellular senescence.(67,68).

Degree of telomere shortening is quickened by oxidative damage, then telomere shortening could reflect the accumulation of oxidative damage.(69) Aging was known to be correlated with oxidative accumulation and the gradual of senescent cells accumulation induce aging mechanism of mitotic tissues. Senescent display a radically altered phenotype, genetic, morphology, and behavior clearly from its growth-competent counterparts. Supposed that the neighbor cells, extracellular matrix and other structural components affecting the process of aged tissues, increased the risk of cancer.(70-73) Together with senescent cells accumulation, senescence also limiting the regenerative potential of stem cells pools or loss of stem cell function. These two combination probably contribute to simultaneously aging process (Figure 5).(59)

Senescence was mediated by cell's two main tumor suppressor pathways, the ARF/p53 and the INK4a/RB

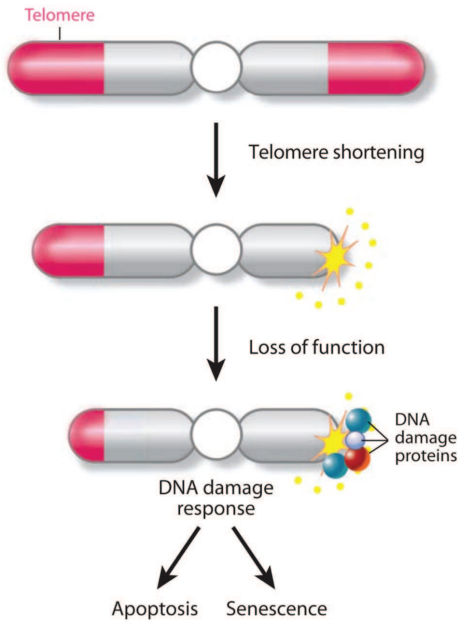

Figure 5. Short telomeres activate a DNA-damage response that leads to apoptosis and senescence.(15) (Adapted with permission from Annual Reviews)

pathway.(74,75) Ectopic expression of oncogenic Ras in primary cells demonstrated a senescent-like arrest mediated by p53 and $p16^{INK4a}$, proved that oncogene-induced senescence could be a mechanism of potentially dangerous cells growth reatrain.(76) Thus, deprivation of these tumor suppressors mechanisms essentially achieve the oncogenic transformation of human cells in vitro (77), and indeed these pathways are frequently disrupted in human cancer cells (74,75).

The senescence growth arrest is not simply a halt to cell proliferation, akin to the reversible growth arrest of quiescence. Rather, senescent cells distinct from quiescence or terminal differentiation.(78) Among the prominent senescence-associated changes in gene expression, numerous cytokines, chemokines, growth factors and proteases of expression and secretion vigorously increased (79-84). This termed as the senescence-associated secretory phenotype (SASP).(85) SASP of senescent cells can cause normal cells to lose their optimal function, leading to tissue degeneration, and cause premalignant cells to proliferate and adopt more malignant phenotypes, promote to full-blown cancer.(85)

Senescence beta-galactosidase (SA-β-GAL) is a frequently used senescence biomarker.(86,87) Encoded by galactosidase beta-1 (GLB1) gene from lysosomal β-D-galactosidase, SA-β-GAL activity increased in senescent cells. The eznymatic activity of SA-β-GAL has to be preserved for detection, so the tissues should be snap-frozen (87). Also, non-senescent cells display β-galactosidase activity in the lysosomes that functions most optimally at pH 4.(88) Therefore, senescent cells underwent a lysosomal compartment expansion, giving rise to an increase in β-galactosidase activity and can be measured at suboptimal pH 6 (hence, SA-β-GAL).(88-91)

Deeper understanding of the senescent phenotype of all mitotic cell-type will provide better assess for the potential consequences of their appearance, therefore we can combat the problem into three strategies: 1) prevention, 2) removal and 3) replacement.(65)

Telomere and Aging

Aging can be defined as the progressive functional decline of tissue function yet results in mortality. The weakening can result from diminished or loss function of post-mitotic cells or due to functional decline in stem cell ability to replace cells, sustain replicarions and cell divisions. Aging should not be understood as disease but a context of evolution, such as The Disposable Soma model, proposed by Thomas Kirkwood in 1977, presumes that our body must budget the amount of energy available to it, and the compromise in energy allocation to the repair function will cause the body gradually to deteriorate with age.(92)

As the world population ages, it has become increasingly important to understand the physiologic consequences of aging and quickly identify those changes that are likely to result in progression to frailty. Frailty, to distinguish it from normal aging, usually implies a state of heightened vulnerability to acute and chronic stressors as consequences of significant reduction in physiologic reserve. It is usually associated with decline in function across multiple systems that in composite contribute to geriatric syndromes, including falls, osteoporotic fractures, incontinence, cognitive decline, anemia, malnutrition, and muscle wasting.(93)

Aging manifest in overall decline in various organs function capacity in maintaining baseline tissue homeostasis and adequate physiological responses under stress (94,95). This process usually gradual, means modest in middle years aged tissues but late in life will accelerate rapidly and likely put organism into serious challenge of regenerative response. At the anatomical and physiological levels, deficient regenerative response and decreased tissue cellularity seems to be closely related to many of classic age medical syndrome, such as muscle atrophy, anemia, feeble immune responses and impaired wound healing.(96)

In term of cell fate and aging, telomeres play important roles by accustom the cellular response to stress and growth stimulation due to any DNA damage and previous cell divisions. To desist from DNA repair pathway activation, hundreds of nucleotides of telomere repeats must cap each chromosome end. Critically short or uncapped telomeres will be repaired by telomerase or recombination, but it was limited in most somatic cells. When too many uncapped telomeres accumulated, then cellular senescence is triggered. Germline cells usually express high levels of telomerase, so the telomere length is maintained. In somatic cells, the length is high diverse but commonly decline with age, as a barrier to tumor growth but as results the cells lose with age.(97)

Throughout a life time, our body supposed to possess a notable ability for continuous extensive and sustained tissue renewal, due to reservoirs of somatic tissue stem cells (98,99), but aging and regenerative researches show parallels blunted proliferative responses and misdirected differentiation of resident tissue stem cells parallel with age. In the other side, these long-lived renewable reservoirs can also affect the health of aged individuals negatively by providing a preferred cellular compartment for malignancy.(99)

Many human studies in genetic disorders has confirmed the relevancy of DNA damage signaling and metabolic regulation to drive the ageing process. Specifically, functional decline of tissue stem cells can primarily instrigated with age-associated telomere damage, alleviation of telomere capping function and associated p53 activation. Together with mitochondrial dysfunction, these will affect in tissues renewal and bioenergetic support. A hypothetical model connecting telomere damage, p53 activation, stem cell, and mitochondrial dysfunction offers a unifying explanation about how telomeres impact aging organism.(96)

The genotoxic stress model of aging, the core telomere-p53 axis integrates well with almost all genetic elements proven to be important in the aging process. First, it accounts for the premature aging phenotypes common to both telomere-dysfunctional mice and those with germline p53 hyperactivation.(100,101) Second, it describes how premature aging happened in mice lacking of SIRT1 or SIRT6, proteins that disable p53 activity.(102) Third, it explains the link between mitochondria and key aging factors: Peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α, PGC-1β, forkhead box O (FOXO) proteins and B lymphoma Mo-MLV insertion region 1 (BMI1); mice with lack of these genes experience accelerated tissue degeneration and mitochondrial dysfunction.(96)

Revealing these networks by constructing a model of interaction between telomeres, stem cells and mitochondria will provide us advance biomarkers for aging and the strategies for therapy, *i.e.,* telomeres stabilization either through brief telomerase reactivation, p53 modulation, mitochondrial function and biogenesis improvement, and mechanistic target of rapamycin (mTOR) and phosphoinositide 3-kinase (PI3K) pathways modulation, to rejuvenate both proliferating and quiescent the aged tissues.(96)

Telomeres and Cancer

Today, withdrawal from the cell cycle after a certain number of cellular divisions (replicative senescence) is known to be triggered by shortened telomeres(103) Recent studies made us learned that cancer cells have evolved their ability to overcome senescence (52,104) because they can maintain the telomere lengths (such as expressing telomerase), then cancer cells are capable to divide indefinitely (104), a biomarker of almost all advanced human cancers.(30)

To limit tumors' clonal proliferation, dominance and ensures a polyclonal composition of (stem) cells in large, long-lived multicellular organisms, human somatic (stem) cells loss its telomeric DNA progressively. Regrettably, this induce somatic cells to ignore or bypass the telomere"checkpoint (105), *e.g.,* because their DNA damage responses are defective. Loss of telomere function like this can results in chromosome fusions, broken chromosomes, break-fusion bridge cycles, translocations, and aneuploidy, creating a genetic instability that grows further genetic alterations rapidly.(106,107) This way, telomere loss could also promote tumor growth by driving selection of cells with defective DNA damage responses (*e.g.,* loss of p5).(29,108) DNA damage responses involving normal and dysfunctional telomeres with intracellular signaling pathways, while DNA repair involving proteins such as ATM, ATR, and p53.(109) Together these demonstrate telomeres as determinant dynamic elements required for genome stability, regulating the cell response under stress and growth stimulation.(97)

Crisis is a period where cell growth and death are in balance. In chromosome end fusions, chromosome breakage-fusion-bridge (BFB) happened leads to genomic instability, chromosome rearrangements, and eventually telomerase engagement. However, telomerase encountered

in approximately 90% of all malignant tumors (52), may predict poor or favorable outcome (110,111), thus making telomerase both a highly attractive biomarker and target for the development of mechanism-based cancer diagnostics, prognostics, and therapeutics.(30)

Genome instability caused by telomere crisis was found to induce chromosome gains and loses (aneuploidy), translocations, gene loss (manifested as loss of heterozygosity (LOH)) and regional amplification through BFB cycles (Figure 6).(108,112,113) The genomic alterations included whole genome reduplication, chromothripsis and kataegis.(114-116) Telomere fusions can occur between different chromosomes or between sister chromatids after DNA replication, thus leading to different outcomes.(4) BFB cycles generally can lead to three outcomes related to cancer: LOH, nonreciprocal translocations and gene amplification. LOH frequently found in cancer-relevant loci, could arise if when a dicentric chromosome breaks and one of the daughter cells inherits a chromosome with a terminal deletion. Nonreciprocal translocations could arise when the DNA end of a broken chromosome invades another chromosome and copies part of this chromosome through a process called break-induced replication.(117,118) Nonreciprocal translocations occur during tumorigenesis in mice with shortening telomeres and are a frequent class of rearrangements in cancer.(119) Sequence analysis of more than 1,000 telomere fusion events has shown that a chromosome end lacking telomere protection can recombine with diverse chromosomeinternal loci (120).

Malignant tumors collectivelly characterized by telomerase expression, to service the unlimited cell proliferation, otherwise most benign and premalignant tumor characterized by the absence of telomerase.(121) Somatic mutations in the proximal promoter of the human TERT now become the most familiar noncoding mutation in cancer.(122)

Therefrom, telomerase become a very attractive target for any immortal cells including cancer stem cells. Telomerase expression, telomere length and cell kinetics between normal and tumor tissues are very different, thus make it more delicate for telomerase to be a relative safe target for many developing therapy such as vaccines, and specific telomerase inhibitor, imetelstat sodium (GRN163L).(123) The key advantages of targeting telomerase in comparison with most other cancer targets are its relative universality, criticality and specificity for cancer cells, including the putative cancer stem cell. Telomerase is expressed in the majority of tumours from all cancer types

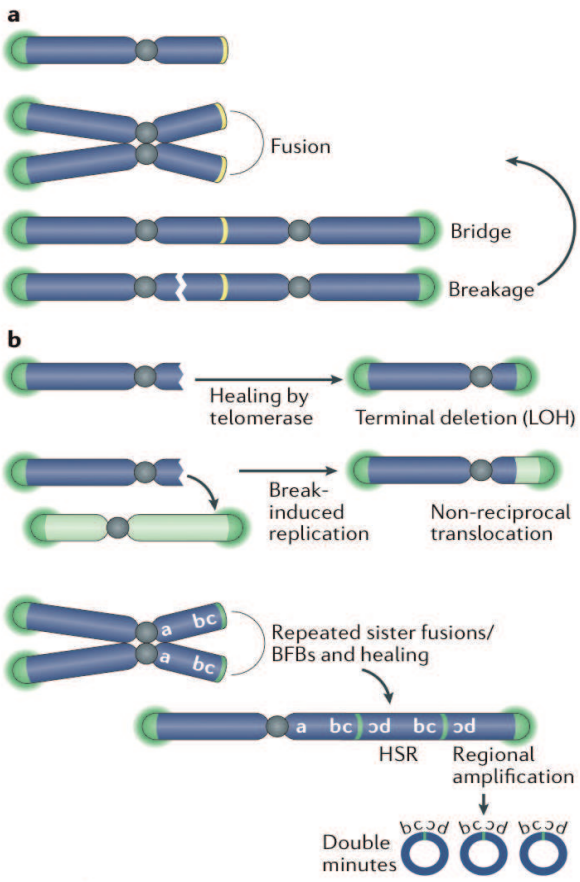

Figure 6. BFB cycles and chromosomal rearrangements during telomere crisis.(110) (Adapted with permission from Nature Publishing Group).

(52,121,124) and some recent studies have suggested that cancer stem or stem-like cells are also telomerase-positive (125-128).

Telomeres and Diseases

Recent findings suggests, as the most risk factors for chronic disease, aging is the feasible modifiable one.(129) not only the apparent signs such as gray hair, wrinkle and spotting skin, muscle wasting, altered adiposity, but aging increase the susceptibility to diseases as people enter the last decades of life, including sufficient immune function, cardiovascular diseases (CVD), cancers, type 2 diabetes mellitus (T2DM), depression, and especially cognitive decline, although they are also could happened as comorbid disease in younger people.(20) Both aging and disease result in the same outcome: the impairment of normal biological function. It would not, therefore, be a surprise if tissue dysfunction resulting from an aging mechanism eventually manifested itself as a disease. Therefore, we expect new development

in prevention and therapeutic methods by understanding the process of aging.(65)

Cellular senescence, as one basic process that play most contribution to age-related dysfunction and chronic sterile inflammation, refers to the essentially irreversible growth arrest that occurs when cells experience potentially oncogenic insults (58,130-134), and now believe that it was the potent anticancer mechanism (135-138). In contrast, despite its name, its discovery over 50 years ago, and increasing data associating senescent cells with aging phenotypes and age-related pathology (59,139-145), while eliminating senescent cells could delay age-related dysfunction (146), at least in a progeroid mouse model.

Cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) are clearly related to age and can reduced life span.(147) in fact, people with same age do not experience the same cardiometabolic outcome, suggest that chronological age is not a precise measure for health status.(148) and we need a better biomarker to identify the cardiometabolic health so we not only can predict but also prevent the disease. Leukocyte telomere length (LTL) may be one such biomarker.(149)

Normally, LTL is reduced in normal aging with considerable inter-individual variation, supposed this reflects the biological age of the cells and organism, which could be different with chronological age (59,150), influenced by own genetic factors, lifestyle and disease. As example, regular exercise slow leukocyte telomere erosion (151,152), while obesity is associated with reductions in LTL (153,154). Current studies reported an association between LTL and chronic diseases including CVD and T2DM (154,155). As we know that those chronic disease, arthritis, together with aging normally involve increased oxidative stress and inflammation. Reduced LTL suggested to be responsible for those stressors.(156)

The 5′-TTAGGG-3′ repeats in the telomere sequence are prone to oxidative damage (8-oxodG). ROS-induced DNA breaks, so oxidative stress could promote telomere shortening in leukocytes and other cells in parallel with aging and chronic disease state.(157,158) Markers of oxidative stress are also elevated in association with shorter LTL in patients with rheumatoid arthritis (159) and T2DM (160). DNA damage in telomeres is very stable and not easily repaired, that's why telomere shortening caused by reactive oxygen species (ROS) will be accelerated.(161) In addition to oxidative DNA damage, impaired calcium homeostasis in Alzheimer disease (AD) patient lymphocytes can also induce telomere erosion.(162,163) Oxidative stress can release calcium from mitochondria then triggers a viscous cycle of telomere shortening, promote more mitochondrial dysfunction that elevating ROS and leads to DNA damage which worsen the telomere shortening.

LTL associated with increasing stress hormones level such as norepinephrine, epinephrine, cortisol and insulin-like growth factor (IGF)-1.(164-166) Exaggerated activation of HPA cause decreased of growth hormone (GH) and affecting the telomere maintenance.(167) Stress responses also affect in decreased dehydroepiandrosterone (DHEA) and increased bulk lymphocyte proliferation and markers of oxidative damage, in total resulting in stress-induced leukocyte telomere erosion.(156,165)

Although not immediately, telomere shortening in leukocytes and microglia can affect neuronal health by compromising the normal functions of these immune cells within the brain.(168,169) The roles of the immune system in the initiation and progression of Newcastle disease (ND) are being actively investigated.(170) Telomere shortening in immune cells, astrocytes and neurons could amplify oxidative stress-dependent senescence and secretion of pro-inflammatory mediators (senescence-associated secretory phenotype), results in diseases progression.(59,168,171). The shortest telomere within a cell showed to pronounce cell senescence the most as well (172,173).

Chronic psychologic stress has been associated with shorter telomeres during childhood and adulthood, although not consistently. Children and adults with adverse and disadvantaged early life experiences (174-178), women who provide care for a family member with a chronic health condition (179-181), those who report high perceived stress (180-182) and women exposed to domestic violence (183) have shorter telomeres in leukocytes and varying subtypes of immune cells compared with those who have not experienced such stressors. Severity and chronicity of depression are also related to shorter telomeres.(184-186) Women with high consistency of healthy behavior appeared to be more protected although exposed to same level of stress, showed that telomere length can be expected to be maintained.(187)

Telomeropathies, a disorder caused by defects in the telomere maintenance machinery, just recently discovered shared a constellation of overlapping syndromes.(188,189) DKC was the first disorder associated to telomerophaty, manifest the diagnostic triad of oral leukoplakia, skin hyperpigmentation, nail dystrophy (190-192), most prominent display organ failure, usually in the bone marrow and a seri of symptoms that less frequently appear such as aplastic anemia or specific lymphopenias. (193,194)

In adulthood, idiopathic pulmonary fibrosis (IPF) is the most common symptom of a telomeropathy.(57) IPF is characterized by progressive failure of the lung coincident with fibrosis and inflammation.(195) Around 8-20% of familial cases of IPF were in responsible of TERC and TERT inherited mutation (196), while 37% of familial cases and 25% of sporadic cases were correlated with shorter telomeres compared to the 10th percentile of the general population, besides as-yet undiscovered genetic or environmental causes (197). Other adult-onset manifestations of impaired telomere maintenance include familial liver cirrhosis (198), aplastic anemia in adulthood (199), and sporadic acute myelogenous leukemia (AML), in which both somatic and germline mutations have been found (200).

Biomarkers of Aging and Diseases

Early 1980s, scientists tried to define aging and its better predicting biomarkers objectively, separate from diseases but not universally accepted while biomarkers for diseases is conceptually more straight forward.(201-203) Valid aging biomarkers, which is describe the rate of aging than chronological age were expected to allow the evaluation of any better prevention and interventions.(204) A simpler set of aging biomarker criteria was proposed by Miller in Butler, *et al.,* are: 1) Biomarkers which cover multiple physiological and behavioral domains, in association with age, so it can predict the outcome of a broad spectrum of age-sensitive tests better than chronological age; 2) It should intertwine biomarkers of aging with biomarkers of disease and suggests that biomarkers of aging will be measuring degenerative changes; 3) The measurement will not alter another age-sensitive tests results or life expectancy of subjects.(205)

The major argument for the development of biomarkers of age-related disease could be summed up as follows: 1) Many age-related diseases develop over long periods and are not observable until they are well established; 2) Successful treatment of disease often requires early diagnosis and treatment; 3) Early biomarkers would permit such treatment when it has a better chance of producing a positive result than treatment begun late in the disease process; and 4) Biomarkers would provide measures to assess the effects of treatment in less than the lifespan of the organism.(204)

Telomeres play quite roles in brain biology and are found shortened in patients with neurodegenerative diseases such as dementia or AD.(206-209) In cellular immunology, telomerase activators were proven to boost immune system of human and mice (210-212), suggested that was associated with stem cells pools mobilizations by telomerase, particularly in this case, the hematopoietic stem cell niches. Telomere shortening also correlates with cardiovascular diseases. Thus, telomeres as indicators of biological aging and diseases. Many studies proven the association between LTL to stroke, myocardial infarction and T2DM. 1 SD in LTL could significantly raise stroke incident (OR 1.21, CI 1.06–1.37; I2=61%), myocardial infarction (OR 1.24; 95% CI 1.04–1.47; I2=68%), and T2DM (OR, 1.37; 95% CI 1.10–1.72; I2=91%). Shortened leukocyte telomere length demonstrates a significant association with stroke, myocardial infarction, and type 2 diabetes mellitus.(149)

β-galactosidase (β-Gal) expressed only in senescent cells, not in pre-senescent or quiescent fibroblasts or keratinocytes. At pH 6.0 using immunohistochemistry, β-Gal can be used as one of the best, reliable and simple methods to measure senescence *in vitro* and *in vivo*.(86,213-219) SA-β-GAL demonstrated a positive correlation with increasing age using human skin samples.(86) So, SA-β-GAL can be used as a marker for senescence in senescent protocol or through senescence-induced methods involving DNA damage agents, oncogenic signals, or over-expression of tumor suppressors such p16 and ARF.(220)

A crucial marker of senescent cell is senescence-associated heterochromatin foci (SAHF). Employing the concept of irreversibility senescent cells, they present a characteristic heterochromatin condensation structure involving the formation of heterochromatic foci (221) which was visible under microscopy, and defined by condensed regions of DNA/chromatin. SAHF are known to silence and repress several E2F-regulated genes such as MCM3, PCNA, or Cyclin A (221-223) and are known to be triggered by several pathways involving p16 or p53 activation.(223)

Conclusively, the ideal biomarker for senescence are cancer and aging marker, therefore ideally can be used for degenerative diseases and cancer studies *in vivo*.(224) Telomere shortening represents the accumulation of DNA damage and its intrinsic mechanism during cell aging, which finally result in senescent.(10,225) The lifestyle factors (such as exercise, smoking, body mass) influence on the aging associated to the expression of serum markers of DNA damage (Cathelicidin-related antimicrobacterial peptide (CRAMP), elongation factor (EF)-1α, Stathmin, n-acetyl-glucosaminidase, and chitinase) in comparison to other described markers of cellular aging ($p16I^{NK4a}$ upregulation and telomere shortening) in human peripheral blood. The study showed that lifestyle factors can affect age-

independent level of DNA damage biomarkers. Smoking and increased BMI significantly correlated with increased the level of biomarkers expression, apart from subjects' chronological age. In contrast, exercise was associated with an age-independent reduction in the expression of biomarkers of DNA damage in human blood.(152)

Telomerase As Theurapeutic Target

We can now understand the limitation on cell division as a mechanism for tumor suppression. Indeed, in mice, cancer growth was hindered due to shorten telomere. On the other hand, when telomeres become very short it also could provoke tumor growth by genome instability. TERT can preserve the telomere length. The human telomerase consists of two subunits: a RNA templates, TERC, and the catalytic subunit, hTERT, which synthesizes the new telomeric DNA from the RNA template.(226) Cells who need a high replicative capacity, such as stem cells and progenitor cells have a higher activity of telomerase.(227) Telomerase elongates telomere, therefore stabilize chromosomes, and rejubenate gene expression pattern. The hTERT, out of the established role, could promote proliferation of resting stem cells via a non-canonical pathway (227) and perform a direct effect on cell transcription and signaling, *e.g.,* as a cofactor in a β-catenin transcriptional complex (228), which plays a role in embryogenesis and development (229). Telomerase alone is not an oncogene, but permissive for carcinogenesis, so uncontrolled induction of telomerase would have a pitfall. ~90% tumor cells express more telomerase, makes telomeres as an overlap target for both anticancer and cell rejuvenation at different cellular and functional levels.(230)

Recent knowledges lead more idea to reversenormal cellular aging process to treat aging symptoms. Many studies tried to develop telomerase activators thay may induce hTERT and/or hTR expression, enhance enzyme activity and/or influence cellular location, and came up single molecule such as cycloastragenol, derived from Astragalus membranaceus root (commercially available as TA-65), proved to transiently activate telomerase in T lymphocytes in the retardation of telomere shortening, increased proliferative potential, and enhanced functional response.(210,231) TA-65 also shown to improve the accelerated immunosenescence in HIV patients and increased the number of senescent memory CD8 T cells. (210,231) Since 2013, TA-65 sold as supplement and give benefit for activating telomerase in immune cells, neonatal keratinocytes, and fibroblasts.(210,232) TA-65 acts via extracellular-signal-regulated kinase (ERK)-pathway activation and subsequent enhancement of telomerase expression without increasing the cancer incidence (233).

Other phytochemicals have been shown to activate telomerase. Resveratrol activates telomerase in mammary epithelial (234) and endothelial progenitor cells (235), most likely due to the upregulation of sirtuin (SIRT)1 although the long-term effects study is not incomplete yet (236). N-acetylcarnosine has been proposed as telomerase activator for cataracts treatment, because reduced telomere length is known intimately involved in opacification, making the lens opaque or cloudy.(237) Another compound that has proven to have a neuroprotective effects in mice and showed delayed progression of amyotrophic lateral sclerosis and increased survival in SOD1 transgenic mice is AGS-499.(238)

Some antioxidants such as N-acetylcysteine may also have indirect effect to upregulate telomerase activity by blocking the nuclear export of telomerase into the cytosol.(239) The α-tocopherol, shown to retain telomerase activity in brain microvascular endothelial cells.(240) ROS damage telomeres directly through vulnerable GGG triplet of the repetitive telomere sequence, and indirectly via telomerase activity modulating and cellular location.(241) So, hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors could also have telomere lengthening effects (242), by interfering with the redox balance of cells (239) and by increasing expression of the telomere stabilizing protein TRF2 (243). Finally, Ginkgo biloba was shown to activate telomerase by inducing PI3K/Akt signaling.(244)

The successful strategies in activating telomerase and rejuvenate cells applied in many tissue engineering and reconstructive surgery for extending cell lifespan. However this may accumulate genetic and epigenetic aberrations that can contribute to malignant transformation.(230) Several approaches for a telomerase-based gene therapy in the treatment of cancer then be developed, due to higher telomerase activity in cancer cells, compared to most other cells.(52) Accordingly, approaches to block TERT have been pursued. One prominent example is Geron specfically modified oligonucleotide chemistry GRN163L which is complementary to TERC and, thus, able to bind to the catalytic center of TERT thereby inhibiting its function.(245) In contrast to direct TERT blockade, immunotherapeutic approaches use TERT-derived peptides to develop vaccines that would activate the immune system to specifically target cancer cells with high TERT expression. GV1001 is such a peptide vaccine that is currently under investigation in a substantial number of clinical trials.(230)

Some difficulties in using telomerase as cancer therapeutic targets includes: First, anti-proliferative effects of telomerase inhibition only induced in cells with short telomeres so the drug need quite a time to be effective, while the tumor is growing. Second, the inhibition of telomerase cannot yet be specific for certain cells, so any highly proliferative cells which need telomerase for survival, namely, stem cells, etc will regard this treatment negatively.(239) Thus, a narrow telomere length window therapy could be the answer for now.(246)

Conclusion

Telomeres physically could define as the edge of chromosomes, to protect them from nucleolytic degradation and DNA repair activities. Traditionally, lack of enzymes limit the ability of DNA replication to fully replicate telomere ends. Tigether with nucleolytic activities, telomere will be eroted in ieach replication, while telomere length homeostasis is essential for cell survival. Shortened telomere generate DNA damage, induce cellular senescence and apoptosis, and cause short telomere syndromes and associated age – related disease. Telomerase act de novo to counterbalance this shortened telomere by adding telomere sequences. On the other hands, elongated telomeres were found in cancer cells. They escape senescence to allow immortal growth. Telomere biology is best viewed in context: It was raising as a powerful inter-active factor for precision medicine in health monitoring and assesing disease, so we need further studies about the genetic and non-genetic determinants interaction in this telomere length maintenance, on different diseases.

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