Telomeres as Therapeutic Targets in Heart Disease

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HIGHLIGHTS

- Age-associated CVDs impose a great burden on current health systems. Despite the fact that current strong evidence supports the links among aging, telomere attrition, and CVDs, there is no clear direction for the development of telomere therapeutics against CVDs.
- This review focuses on immune modulation, CHIP, pharmaceutical interventions, and gene therapy for their therapeutic roles in age-associated CVDs.
- The future goal of telomere cardiovascular therapy in young subjects is to prevent senescence and diseases, whereas in older adult subjects, the goal is restoration of cardiovascular functions. Further studies on the telomere-CHIP-atherosclerosis axis may shed insights on how to achieve these 2 different therapeutic targets.

SUMMARY

Telomeres are double-stranded repeats of G-rich tandem DNA sequences that gradually shorten with each cell division. Aging, inflammation, and oxidative stress accelerate the process of telomere shortening. Telomerase counteracts this process by maintaining and elongating the telomere length. Patients with atherosclerotic diseases and cardiovascular risk factors (e.g., smoking, obesity, sedentary lifestyle, and hypertension) have shorter leukocyte telomere length. Following myocardial infarction, telomerase expression and activity in cardiomyocytes and endothelial cells increase significantly, implying that telomerase plays a role in regulating tissue repairs in heart diseases. Although previous studies have focused on the changes of telomeres in heart diseases and the telomere length as a marker for aging cardiovascular systems, recent studies have explored the potential of telomeres and telomerase in the treatment of cardiovascular diseases. This review discusses the significant advancements of telomere therapeutics in gene therapy, atherosclerosis, anti-inflammation, and immune modulation in patients with cardiovascular diseases.

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Telomeres invariably shorten with age and cell division (3). Although there could be asynchrony of telomere length among different tissues (4), peripheral leukocyte DNA has been most commonly used in clinical studies to measure leukocyte telomere length (LTL) (5). Several methods have been used to measure LTL, including terminal restriction fragment analysis by hybridization with telomere sequence probes, single telomere amplification and blotting, flow cytometry of cells following hybridization with fluorescent peptide nucleic acid probes, quantitative fluorescence in situ hybridization with fluorescent telomere peptide nucleic acid probes, and quantitative polymerase chain reaction assays (6). Due to the different methods used in clinical trials and large interbatch coefficients of variations, there is no current gold standard of telomere length measurement, and therefore, comparison of telomere lengths between different clinical trials could be misleading (7).

Traditional risk factors for cardiovascular diseases (CVD), such as smoking, diabetes mellitus, dyslipidemia, hypertension, obesity, and shift work, have been associated with short LTL (8). In the prospective WOSCOPS (West of Scotland Primary Prevention Study) trial, subjects in the lowest tertile of LTL had a 44% increased risk of 5-year major cardiovascular events compared with subjects in the highest tertile of LTL (9). Another meta-analysis study with 43,725 participants and 8,400 patients revealed that short LTL had a pooled relative risk for coronary heart disease of 1.54 (95% confidence interval: 1.30 to 1.83). In addition, short LTL was associated with coronary artery disease risk independent of traditional vascular risk factors. The association of short LTL with cerebrovascular disease is less significant than that with coronary artery disease (10). Short LTL also affects the prognosis of coronary artery disease. In a prospective WHI (Women’s Health Initiative) study with 1,525 post-menopausal women, shorter LTL was associated with higher risks of mortality (11). Further analysis showed that patients with myocardial infarction had shorter LTL, which was equivalent to that observed in individuals without myocardial infarction but who were 8 to 12 years older in biological age (12). Shorter LTL was also associated with increased proinflammatory activity in high-risk unstable plaque on virtual histology intravascular ultrasound (13) and delayed re-endothelialization after drug-eluting stent implantation (14) in acute coronary syndrome. When patients developed chronic heart failure, they were also observed to have shorter LTL (15). Moreover, short LTL was also associated with congestive heart failure severity and clinical outcomes (16).

Despite the fact that current robust epidemiological and animal study evidence supports the telomere attrition links between age and CVDs, there is no clear route that leads to the development of telomere therapeutics against CVDs in the future due to the following limitations. First, in adult somatic cells, manipulation of the telomere system bears an oncogenic risk (17,18). Thus, therapeutic techniques based on the overexpression of telomerase and other telomere-related signals should be applied after considering cell-type and tissue interactions. Second, there is still no clear mechanistic insight into the link between telomere and/or telomerase and atherosclerosis development (19). Third, the telomere system is complex and regulated by various feedback mechanisms, including circadian rhythm oscillations (20), and a direct interruption of 1 target in the telomere pathway can lead to various side effects.

TELOMERES AND TELOMERASE

Because DNA polymerase is unable to replicate the 3’ ends of chromosomes fully, the so-called “end-replication problem,” telomeres shorten during each cell replicated cycle (21). When telomeres reach a critically short length, genomic instability activates the DNA repair system and induces replicative arrest, senescence, and cell death (22).

In primary human cells, each time a cell divides, 50 to 100 bases are lost from the telomeres on each chromosome. This loss is much larger than the estimation from end-replication mechanisms, indicating that there are other contributing factors for telomere attrition in human cells (23). Oxidative stress and tissue inflammation have been observed to accelerate telomere shortening and reduced replicative lifespans (24). Telomere shortening is considered a biological molecular clock and is the underlying mechanism proposed to explain the limited lifespan of cells in culture, known as the Hayflick limit (25). During the progressive accumulation of senescent cells in aging, there is a marked increase in the secretion of proinflammatory cytokines, adhesion molecules, growth factors, and proteases from senescent cells (26,27). This inflammatory signaling initiates a vicious cycle that enhances telomere dysfunction, triggers replicative senescence, and promotes aging and development of age-associated diseases (28). Telomerase is a crucial component in telomere maintenance and regulation. It consists of an RNA
Telomeres as Therapeutic Targets

Robust epidemiological and genetic evidence linking telomere length and CVD risk support the therapeutic hypothesis that genetic manipulations of the telomere system can be a potential treatment target for CVDs. Mice with genetic knockout of TERC or TERT had progressively shorter telomeres over generations and showed features such as severe developmental defects, aging pathologies, and premature death (38). Reconstitution of TERC or TERT expression in the TERC- or TERT-deficient mice with critically short telomeres resulted in elongation of telomeres, less DNA damage, decreases in aging biomarkers, and delay in age-related pathologies (39). In these mice models, short telomeres and associated pathologies were treated and halted by telomerase re-expression. These findings provided the concept for therapeutic strategies to delay age-associated pathologies by transiently increasing telomerase expression.

Telomerase gene therapy was first achieved by delivering mouse TERT with an adeno-associated virus (AAV) into young and old mice. This nonintegrative gene therapy resulted in elongated telomeres, extended lifespans, and delayed age-associated pathologies, such as insulin sensitivity, osteoporosis, and neuromuscular coordination, in both age groups (40). Importantly, telomerase-treated mice did not develop cancer at a higher rate than the corresponding control group (41). With the nonintegrative and replication incompetent properties of AAVs, this strategy restricted TERT expression to a few cell divisions and provided a relatively genome-safe TERT activation. Thus, these studies in mice supported the feasibility of telomerase activation treatment to overcome the adverse consequences of critically short telomeres. Applications of AAV-TERT gene therapy in specific telomere syndromes also showed expected therapeutic effects in preclinical mouse models, such as aplastic anemia and pulmonary fibrosis (42,43). A report for age-associated diseases, such as CVDs, demonstrated improved ventricular function and limited infarct scars after acute myocardial infarction with TERT gene therapy in a preclinical mouse model (44). TERT gene therapy is a promising candidate that deserves further research efforts for clinical implementation for the treatment of age-associated diseases.

Apart from direct TERT delivery by nonintegrative AAV vectors, new gene therapy methods using modified mRNA for in vitro encoding of TERT in human fibroblasts can transiently increase telomerase activity, rapidly extend telomeres, and increase proliferative capacity without the risks of insertional mutagenesis and off-target effects (45). In addition to proof-of-concept experimental data in mice, the development of safe strategies for transient and controllable telomerase activation in humans can be a subject of future studies.
PHARMACEUTICAL INTERVENTIONS FOR TELOMERES AND TELOMERASE ACTIVITY

Because of the pertinence of telomerase in antiaging gene therapy in mice models, several studies focused on the therapeutic interventions for telomerase modulations in humans. Several cardiovascular medications, which have been used for decades and have been shown to have significant survival benefits in patients, possess the effects of telomere length maintenance and senescence prevention.

Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) exert various pleiotropic effects to prevent the development of atherosclerotic plaque (46). A cross-sectional analysis of 3,496 subjects from the U.S. National Health and Nutrition Examination Survey showed that telomere length appeared to be longer with a longer duration of statin usage (47). Statin therapy was associated with higher telomerase activity independently of multiple covariates, such as age, sex, smoking, lipid profile, and inflammation (48). Statins can enhance telomerase activity and protect telomeres through upregulation of the telomere repeat-binding factor 1 (TRF1), telomere repeat binding factor 2 (TRF2), repressor/activator protein 1 (RAP1), protection of telomere 1 (POT1), TRF1- and TRF2-interacting nuclear protein 2 (TIN2), and ACD shelterin complex subunit and telomerase recruitment factor (TPP1). A more specific analysis of human T-lymphocytes showed that atorvastatin in pharmacologically relevant doses led to a transient increase in telomerase activity in T-cells. This effect, which could be blocked by inhibitors of Akt and phosphatidylinositol-4,5-bisphosphate 3 (PI3)-kinase, was more pronounced in the CD4+ than in the CD8+ T-cell subsets (50). In addition, it also prevented telomere shortening by accelerating DNA repair through Nijmegen breakage syndrome-1 protein stabilization and telomere maintenance in vascular smooth muscle cells (51).
The crosstalk between angiotensin II and telomere systems are noteworthy. Overexpression of TERT in vivo modified the angiotensin II–induced microvascular endothelial dysfunction (52). Angiotensin II induces oxidative stress and senescence in vascular smooth muscle cells with telomerase-independent oxidative stress-induced senescence and telomerase-dependent replicative senescence (53). Acute exposure of vascular smooth muscle cells to angiotensin II results in vascular smooth muscle senescence, which is not associated with telomerase activity changes and cannot be reversed by TERT overexpression. However, long-term exposure of vascular smooth muscle cells to angiotensin II induced reduction in proliferation and replicative senescence with telomere shortening (54). Angiotensin II receptor blockers (e.g., losartan) and angiotensin-converting enzyme inhibitors (e.g., captopril) were both shown to protect endothelial progenitor cells from senescence and dysfunction through telomerase cross-talk (55,56). However, some studies showed that captopril and losartan had no effect on telomere attrition caused by cardiac hypertrophy after abdominal aortic constriction in rats (57). Therefore, the clinical use of angiotensin-converting enzyme inhibitors or angiotensin II inhibitors for the modification of telomere systems requires further clinical studies.

Peroxisome proliferator-activated receptor agonists (e.g., pioglitazone) can increase the activity of telomerase and expression of TRF-2 in mice aorta and in mononuclear cells. Pioglitazone-treated mice were shown to possess the reduced senescence markers, p16, cell-cycle checkpoint kinase 2, and p53 (58). Acute exposure of vascular smooth muscle cells to angiotensin II significantly reversed by pioglitazone through telomerase activity enhancement (59). Moreover, pioglitazone was able to increase the TERT and TRF-2 expression in the hearts of diabetic rats (60).

The low potency telomerase activator TA-65, a bioactive molecule extracted from Astragalus membranaceus, has been historically used in Chinese traditional medicine as an antiaging drug and has been shown to have effects on telomere lengthening in mice. TA-65 treatment induces telomerase-dependent elongation of short telomeres and reverses DNA damages in fibroblasts (61) and human T cells (62). Randomized, double-blind, and placebo-controlled clinical trials showed that TA-65 treatment increased high-density lipoprotein cholesterol and reduced C-reactive protein in patients with metabolic syndrome (63) and was also found to elongate telomeres (64).

In addition, sex hormones were also reported to activate TERT transcription. For decades, androgen therapy was considered as the first treatment choice for aplastic anemia, without a clear understanding of the underlying mechanism. A recent study showed that the upregulated telomerase activity is responsible for the effectiveness of the androgen treatment effect in aplastic anemia. In mice with aplastic anemia induced by short telomeres, testosterone therapy halted telomere attrition and prevented subsequent death, by enhancing telomerase expression and lengthening telomeres (65). Moreover, a synthetic androgen, danazol, which was used in the treatment of human telomeropathies, was shown to elongate telomeres in circulating leukocytes and improve hematological parameters (66).

Although specific telomere-lengthening effects of telomerase activation affect cardiovascular health and aging, noncanonical, extracellular, and nontelomere-lengthening functions of telomerase were recently described (67). The off-target effects of these telomerase activating or telomere-lengthening compounds, including those in mitogen signaling and oncogenesis, should be considered before clinical usage.

**INFLAMMATION, ATHEROSCLEROSIS, AND CLONAL HEMATOPOIESIS OF INDETERMINATE POTENTIAL**

Atherosclerosis is the dominant pathology of CVD, including myocardial infarction, heart failure, stroke, and peripheral artery diseases (68). The prevalence of these diseases progressively increases with age. In addition, the risk factors of atherosclerotic diseases, such as aging, smoking, obesity, sedentary lifestyle, and unhealthy diet, have been reported to be associated with telomere shortening based on observational epidemiological studies (69). As observed in 1 of these studies, each kilobase pair shortening of telomeres in peripheral blood cells was estimated to result in 2.8- to 3.2-fold higher risk of myocardial infarction and stroke (70).

Telomeres in coronary endothelial cells are shorter in patients with atherosclerosis than in healthy individuals (71). Telomere shortening of endothelial cells might play a role in atherogenesis by increasing proinflammatory reactions and promoting high-risk unstable atherosclerotic plaques (72). In human abdominal aorta analysis, shorter telomere and higher attrition were observed in aged vessels with increased shear wall stress (73). Significant telomere attrition, shorter telomeres, and DNA damages were demonstrated in biopsied tissue from a failing heart;
these effects were only specific to cardiomyocytes, regardless of the age of the patients (74). Moreover, asynchronous shortening of the telomere length between cardiac atrial tissue and leukocytes served as a better biomarker than leukocyte length alone for post-cardiovascular surgery events (75).

Atherosclerosis is an inflammatory disease that involves vascular endothelium, smooth muscle, and blood cells (76). With aging, telomeres shorten, and blood cells start to accumulate somatic genetic mutations. As these blood cells gain a competitive expansion advantage, they give rise to some expanded clones of leukocytes that circulate in the peripheral blood, which is termed clonal hematopoiesis of indeterminate potential (CHIP) (77). More than 10% of septuagenarians exhibit CHIP, and its prevalence increases with age (78). Individuals with CHIP have increased cardiovascular mortalities independent of traditional risk factors (e.g., diabetes, hypertension, and dyslipidemia) (79). Current evidence shows that de novo mutations in DNMT3A, TET2, and ASXL1 facilitate the clonal expansion of leukocytes (80,81). Macrophages from TET2-knockout mice resulted in the abnormal activation of the NLRP3 (Nucleotide-Binding Domain, Leucine-Rich-Containing Family, Pyrin Domain-Containing-3)-mediated inflammasome and contribute to enhanced atherosclerosis (82). Mice with CHIP mutations in hematopoietic cells also exhibited aggravated the development of heart failure (83).

Both telomere attrition and CHIP increase with age. Accordingly, progressive leukocyte telomere attrition can lead to genomic instability, which later results in CHIP (84). Therefore, it is assumed that the manipulation of the telomere system would be a possible treatment target of CHIP-related CVDs. Although current clinical evidence supports this concept, further research needs to be conducted. In a whole-genome sequencing study, the strongest association of CHIP was found to be an 8-bp deletion in intron 3 of the TERT gene (85). In the same study, telomere lengths were observed to be significantly shortened in individuals with CHIP. Dyskeratosis congenita is a rare progressive congenital disease with skin pigmentation, nail dystrophy, and leukoplakia of the oral mucosa. Dyskeratosis congenita is characterized by short telomeres with poor telomere maintenance, mainly caused by some abnormal mutations in ribosome and telomerase RNA components (86). Clonal expansion of hematopoietic cells bearing non-synonymous coding somatic mutations is a common feature that occurs in one-half of patients with dyskeratosis congenita (87). The telomerase complex controls hematopoietic cell differentiation and senescence in the induced pluripotent stem cell model (88). The telomere-CHIP-atherosclerosis (TCA) axis may provide several possible therapeutic targets, including modulating telomerase activity, rescuing senescent or mutated clonal cells, and inhibiting the inflammation from CHIP (Central Illustration). However, future investigations are required to understand the TCA axis before comprehensive clinical trials can be undertaken in the future.

Furthermore, several aspects of the TCA axis remain to be understood. First, telomere attrition and CHIP are a progressive, long-term processes, like atherosclerosis, and thus require better cellular or animal models to simulate these 2 chronic processes (e.g., low-density lipoprotein receptor knockout mice for atherosclerosis). Second, the evidence for the connection between telomere attrition and CHIP is based on a clinical association study. Therefore, a future study is required to understand their underlying mechanisms. Third, the telomere length variations between individuals and different attrition rates between tissues are not directly linked to CHIP occurrence and atherosclerosis. The connections could be affected by other factors, such as different responses to critical short telomeres or inflammation. Even among patients with dyskeratosis congenita, >10% of them do not develop CHIP (87). Lastly, there is still no evidence showing that CHIP can be prevented or reversed via telomere modulation.

**IMMUNE MODULATION**

Inflammation is a protective response to injury of a process that delivers leukocytes to sites of infection or tissue damage. Acute inflammation usually lasts for hours and has many positive (e.g., interleukin-6/tumor necrosis factor-α) and negative (e.g., interleukin-10) regulators. If infection and tissue damage persists, if the healing process is somehow disturbed, or if 1 of the negative control mechanisms fails, inflammation may progress to a chronic state that can last for weeks, months, or maybe years (89). In many common chronic diseases, such as atherosclerosis, the chronic inflammatory process does not follow a manifestation of an acute reaction but begins as a low grade and smoldering response (90).

Aging, DNA damage, and stem cell failure are closely associated with low levels of chronic inflammation (91). Chronic low-grade inflammation increases oxidative stress and enhances telomere dysfunction (92). The links between telomere dysfunction and chronic inflammation are bidirectional and can result in complex vicious cycles. Telomerase is active in the human coronary artery.
and its activity is increased during atherosclerosis formation (93). Different proinflammatory mediators increased TERT mRNA and telomerase activity in macrophages in atherosclerosis through nuclear factor-κB signaling (93). Premature telomere erosion in peripheral blood mononuclear cells is a common phenomenon in obesity, myocardial infarction, and atherosclerosis (8). In telomerase-deficient mice, marked increases in proinflammatory cytokines interleukin-6, CXCL16 (Chemokine (C-X-C motif) ligand 16), and tumor necrosis factor-α were observed in pulmonary tissues (94). Chronic inflammation aggravates telomere dysfunction and cellular senescence through oxidative stress activation and cyclooxygenase-2–dependent reactive oxygen species production (95). From this evidence, the telomere system and chronic inflammation are suspected to be linked closely.

The aging process in humans is associated with changes in circadian rhythm patterns (96). Circadian rhythm controls telomeres and telomerase activity through circadian locomotor output cycles kaput
gene–aryl hydrocarbon receptor nuclear translocator-like protein 1 heterodimers (97). Mice and humans with circadian rhythm abnormalities not only have increased vascular senescence (98), impaired endothelial progenitor cell function, enhanced atherosclerosis (99), and obesity (100) but are also prone to chronic inflammation and sepsis (101). The intersection of circadian mechanics into the linkage between telomere and chronic inflammation provides more opportunities in combating atherosclerosis. For example, experimental evidence in mice indicate that melatonin regulates the transactivation of telomerase and the expression of core clock and clock-related genes (102). Melatonin inhibits smooth muscle cell inflammation and atherosclerosis in mice (103,104). For example, physicians in emergency departments are known to lose their telomerase oscillation, and have low telomerase activities and circadian misalignments that increases CVD risk factors (105). Further research on the connections between telomerase and circadian rhythm will shed more light upon this area.

However, the connections between telomere and atherosclerosis are not definitive. Although mice with double deficiency in ApoE and TERC have extensive telomere attritions, a substantial reduction of atherosclerosis was observed in them compared to mice with normal telomerases (106). Short telomeres result in immunosenescence and lead to protection from atherosclerosis (107). Moreover, not all clinical studies have found significant associations between telomere lengths in white blood cells and morbidity or mortalities (108).

AGING CARDIOVASCULAR PATIENTS AND TELOMERE THERAPY

According to World Health Organization data, by 2020, all countries across the world will face significant challenges to their health and social systems due to the aging demographic shift (109). The number of people aged 60 years and older will outnumber children younger than 5 years. The pace of the aging population will be faster in future decades. There are 3 significant differences in telomere therapy between the young and older adult population. First, in older adult individuals, the evidence supporting the telomere length and remaining lifespan is controversial (110). The contradictory results of these studies suggest that in septuagenarians and octogenarians, the role of telomere in survival becomes less important (111). A recent study indicated that healthy lifestyle habits such as not smoking and not being obese at the age of 71 were the most significant associated factors with survival at the age of 85 years or older in men (112). The efficacy of manipulation of the telomere system with younger subjects versus older subjects will need exploratory studies in the future. Second, the goals of telomere cardiovascular therapy in young subjects and older adults subjects are different. In young subjects, the goal of telomere therapy is to prevent cardiovascular senescence and diseases, whereas in older adult subjects, the goal of telomere therapy is to restoration of cardiovascular functions. Third, the prevention of CHIP and atherosclerosis with telomere targets in young subjects requires long-term treatments. Moreover, the efficacy and effectiveness of the treatment involving CHIP detection, prevention, and correction in octogenarians are questionable. Further clinical studies are required to overcome these limitations.

TELOMERE THERAPY FROM THE BENCH TO THE BEDSIDE

Telomere biology could be potentially involved in the development of age-associated CVDs including atherosclerosis, hypertension, myocardial infarction, and heart failure. Critically shortened telomeres activate a series of downstream changes that induce cardiomyocyte cell cycle arrest and cellular senescence (38). The reduced proliferative potential of cardiovascular systems limits the regenerative capacity of aged and injured myocardium and vasculature (113). Thus, therapeutic strategies to restore the proliferative potential of adult cardiovascular systems are considered as a promising alternative treatment for CVDs. In mouse models, telomerase gene transfer therapy provides an attractive way for cardiovascular restoration and deserves future investigations. Although we are still far from applying the current knowledge in daily therapeutic protocols, many studies seem to agree with the fact that a combination of exercise, healthy diet, low everyday stress, and anti-inflammatory agents intake may prove to be beneficial in promoting human longevity by modulating the telomere system and in slowing down the effects of many chronic disorders. The present knowledge in this regard still requires input from different studies, and further investigations are needed to uncover the true molecular relationships involved in the previously described phenomena.

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