

REVIEW ARTICLE

Telomeres and Telomerase in The Aging Heart

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Abstract

BACKGROUND: Aging *per se* is a risk factor for reduced cardiac function and heart diseases, even when adjusted for aging-associated cardiovascular risk factors. Accordingly, aging-related biochemical and cell-biological changes lead to pathophysiological conditions, especially reduced heart function and heart disease.

CONTENT: Telomere dysfunction induces a profound p53-dependent repression of the master regulators of mitochondrial biogenesis and function, peroxisome proliferator-activated receptor gamma coactivator (PGC)-1 α and PGC-1 β in the heart, which leads to bioenergetic compromise due to impaired oxidative phosphorylation and ATP generation. This telomere-p53-PGC mitochondrial/metabolic axis integrates many factors linked to heart aging including increased DNA damage, p53 activation, mitochondrial, and metabolic dysfunction and provides

a molecular basis of how dysfunctional telomeres can compromise cardiomyocytes and stem cell compartments in the heart to precipitate cardiac aging.

SUMMARY: The aging myocardium with telomere shortening and accumulation of senescent cells restricts the tissue regenerative ability, which contributes to systolic or diastolic heart failure. Moreover, patients with ion-channel defects might have genetic imbalance caused by oxidative stress-related accelerated telomere shortening, which may subsequently cause sudden cardiac death. Telomere length can serve as a marker for the biological status of previous cell divisions and DNA damage with inflammation and oxidative stress. It can be integrated into current risk prediction and stratification models for cardiovascular diseases and can be used in precise personalized treatments.

KEYWORDS: aging, telomere, telomerase, aging heart, mitochondria, cardiac stem cell

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Introduction

Besides resulting in continues functional and structural decrease of multiple organs, aging is also inventing some profound effects on heart and the arterial system. Age-related cardiac and vascular changes include impaired endothelial function and intimal proliferation (1), increased arterial stiffness (2-7), left ventricular (LV) diastolic dysfunction (8-10), LV pathological hypertrophy (11), diminished LV systolic reverse capacity (9,10), decreased heart rate variability (12-14), and a reduction in maximal heart rate (15). Furthermore, as a consequence of aging, the

interaction between the heart and arterial system is altered to preserve ventricle-arterial homeostasis.(16)

As people grow older, the prevalence of cardiovascular disease (CVD) is increasing dramatically. More than 80% of cases of coronary artery disease (CAD) and 75% of cases of congestive heart failure (CHF) are found in geriatric patients. The incidence of CVD cases, which includes CAD, CHF, and also stroke, are found in 4 out of 1000 person/years in adult aged 45-54 years old. The number of this incidence raise to 10 cases out of 1000 person/years in adult aged 65-75 years old. And it just grow worse as someone aged ≥ 85 years old, as the number of incidence found to be are 75 cases out of 1000 person/years.(17)

Aging is a big risk factor for CVDs, which are a major cause of chronic disability and the leading cause of death worldwide. The morbidity and mortality rates which are associated with CVDs remain high and cause a dreadful burden on the healthcare system, even after the advanced prevention and treatment of CVDs over the last two decades. As mentioned before, the prevalence of CVDs is increasing in the older population. This shows that CVDs in older population are a major healthcare challenge that should be focused on. A good understanding of the complex interaction between the aging process and CVDs is needed to develop a novel therapeutic target for older patients.(18)

The aging heart is indicated by a faulty responsiveness to stress and by a reduced efficiency of endogenous protective mechanisms (*e.g.*, ischemic pre-conditioning and post-conditioning), resulting in increased vulnerability to injury.(19) As for now, the detail mechanism which is involved in the cardiac senescence still has not been fully known. But, the progressive accrual of macromolecular oxidative harm over the lifetime is invoked as a major factor.(20) Reactive oxygen species (ROS) are steadily created within cells by several enzymatic reactions, including those catalyzed by cyclooxygenases, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and xanthine oxidase; however, the bulk of ROS production occurs as a byproduct of mitochondrial oxidative phosphorylation (OXPHOS).(21)

Several epidemiologic surveys have reported an association of short telomere length (TL) with CVD (22-25) and cardiovascular mortality (26-27). The Cardiovascular Health Study showed that each shortened kb pair of TL correlated to a threefold increased risk of myocardial infarction (MI) and stroke.(22) It was reported in one of the latest systemic review and meta-analysis that there is a stable positive association of decreased leukocyte TL (LTL) with cardiometabolic outcomes, where one standard deviation (SD) decrease in LTL was significantly related to a 21%, 24%, and 37% increased risk of stroke, myocardial infarction (MI), and type 2 diabetes mellitus (T2DM), respectively (5).

Telomeres and Telomerase

Telomeres are special chromatin structures located at the ends of eukaryotic chromosomes that prevent the recognition of chromosomal ends as double-stranded DNA breaks, thereby protecting these regions from recombination and degradation and avoiding a DNA damage cellular response. The

telomeric DNA is composed of noncoding double-stranded repeats of G-rich tandem DNA sequences (TTAGGG in vertebrates) that are extended several thousand base pairs (10 to 15 kb in humans and 25 to 40 kb in mice) and end in a 150 to 300 nucleotide 3' single-stranded overhang (G-strand overhang) (Figure 1).(28,29,30) Several specific proteins are related to telomeric DNA, some of those are telomerase and the telomeric repeat binding factors (TRF)1 and TRF2 which directly bind to the TTAGGG repeat and interact with other factors composing large protein complexes regulating TL and structure.(31)

TL is greatly variable among individuals with the same age. Already at birth, noticeable differences in TL can be found. Several studies have suggested that TL can be predicted by the TL of the parents. Heritability of TL has been estimated to be as high as 82%.(32) Several environmental factors are also associated with telomere length and possible telomere attrition rate. Most important are oxidative stress (33) and factors related to oxidative stress such as smoking (34) and UV radiation (35).

The human telomerase is responsible for maintaining and elongating TL and consists of the telomerase RNA component (TERC) and telomerase reverse transcriptase (TERT), the catalytic component. To maintain TL, TERT uses the TERC as a template to synthesize new telomeric DNA repeats at a single-stranded overhang. Several cells such as germ cells, stem cells, hematopoietic progenitor cells, activated lymphocytes, and most cancer cells, possess a high level of telomerase activity to overcome telomere shortening and control limitless cell division. However, somatic cells generally have a low or undetectable level of telomerase activity with limited longevity. The TL and integrity are regulated through the interplay between the telomerase and shelterin proteins.(36) Telomerase activity decreases with age but increases markedly in response to injury.(37) The telomerase expression in the mammalian's heart is small, yet functionally significant. Telomerase has a role in regulating tissue repair and regenerative, this is shown by a substantial increase in telomerase expression which was found in cardiomyocytes, endothelial cells and fibroblasts of cryoinjured adult mice hearts.(38)

During the shortening process of telomere to its crucial length, the cell enters cellular senescence, starting a series of changes in the gene expression of replicative cell-cycle inhibitors and inhibits proliferation, then eventually into apoptosis (39) as known as replicative senescence. It is known that senescent cells change their morphology and secretory phenotype in autocrine and paracrine patterns. The pattern of this active altered secretion has been named

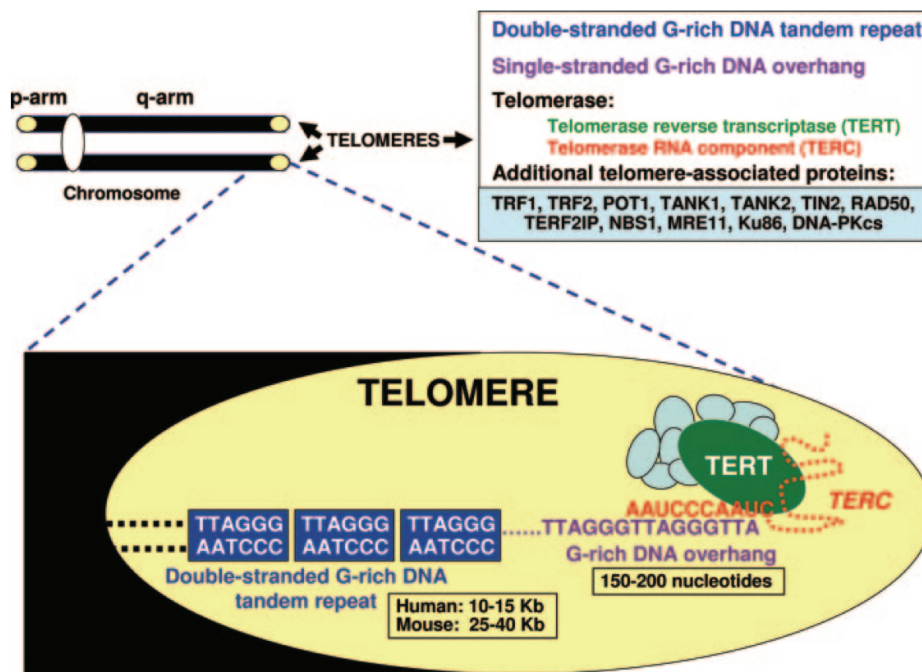


Figure 1. Telomere organization.
 (30) (Adapted with permission from American Heart Association).

as the senescence-associated secretory phenotype (SASP). They secrete interleukin (IL)-6 and IL-8, intercellular adhesion molecule 1 (ICAM-1), metalloproteases, monocyte attractants, plasminogen activator inhibitor 1 (PAI-1) and vascular endothelial growth factor (VEGF).(40,41) Senescent cells are partly responsible for inflammation and also promoting apoptosis, tissue remodeling, and repair through their SASP. Thus, chronic inflammation initiates a vicious cycle which enhances telomere dysfunction and the accumulation of senescent cells. Cell senescence provokes chronic inflammation and accelerates aging and the development of aging-associated diseases.(41)

After each cell division, the length of the telomere shortens, and when a critical shortening is reached, the cell enters senescence or apoptosis.(42,43) Thus, TL is considered a marker of cell senescence and replicative capacity.(42,44) LTL represents the average TL across a heterogeneous population of leukocytes including monocytes, granulocytes and lymphocytes, and can serve as a biological marker of aging.(45)

Aging is Functional Decline of Telomeres, Mitochondria and Stem Cells

Mitochondria play important roles in a myriad of cellular processes including ATP production via oxidative phosphorylation, biosynthetic pathways, cellular redox homeostasis, ion homeostasis, oxygen sensing, signaling,

and regulation of programmed cell death. Mitochondrial dysfunction is central to theories of aging, because age-related changes of mitochondria are likely to impair a host of cellular physiological functions in parallel and contribute to the development of all common age-related diseases.(46)

The original formulation of the mitochondrial theory of aging postulates that raised production of mitochondria-derived ROS could effects in a several macromolecular oxidative modifications, which are a primary causal factor in the aging process and also in the development of age-related diseases.(47,48) Mitochondrial retrograde signaling is a pathway of communication from mitochondria to nucleus, which involves multiple factors that sense and transmit mitochondrial signals to alter nuclear gene expression. This cross-talk between mitochondria and the nucleus affects many cellular functions and is assumed to have a critical role in the aging process. There are multiple signaling cascades that involve the mitochondria, including release of ROS from the mitochondria, Ca^{2+} signaling, which activate downstream effectors pathways and transcription factors and the nutrient sensing mechanistic target of rapamycin (mTOR) pathway that regulates growth and cellular metabolism. Recent studies suggest that longevity is regulated by both cell-autonomous and non-autonomous mitochondrial stress pathways triggered by mild mitochondrial impairment.(49) According to this model, adaptive mitochondrial retrograde pathways relay mitochondrial stress signals to nucleus, activating genes involved in maintenance of mitochondrial integrity and cellular function.(46)

Human body has a great ability for extensive and sustained tissue renewal throughout a lifetime. Reservoirs of somatic tissue stem cells are responsible in maintaining this continuous self-renewal ability.(50,51) These tissue stem cells have garnered increasing attention in ageing and regenerative research given accumulating evidence that age-associated physiological decline, particularly in highly proliferative organs, parallels blunted proliferative responses and misdirected differentiation of resident tissue stem cells. Tissues have largely different levels of baseline proliferative activity and regenerative potential. In the high-turnover tissues, it is known that the resident stem cells have the ability to generate great numbers of specialized cell progeny and thereby sustaining tissue cellularity and functionality over a lifetime. Intuitively, it would seem reasonable to posit that preserving an adequate pool of tissue stem cells with robust potential for renewal would be vital to maintaining organ function with advancing age. That kind of thought is supported by the premature ageing phenotypes observed in mice with conditional deletion of the genes encoding ataxia telangiectasia and Rad3-related (ATR), forkhead box O (FOXO) transcription factors, or ataxia telangiectasia mutated (ATM). These mice exhibit tissue stem-cell defects or diminished oxidative defense and ROS-induced stem-cell depletion.(52)

The failure of stem/progenitor cell failure due to p53-mediated cellular checkpoints may underlie compromise of highly proliferative organs. But this mechanism seems not enough to describe the profound physiological decrease in more quiescent tissues, for example, heart (cardiomyopathy) and liver (reduced detoxification capacity, glucose intolerance). These pathologies indicate that telomere dysfunction elicits a degenerative state via additional mechanisms beyond the classical senescence and apoptosis checkpoints.(53,54)

Telomere-dysfunction-induced repression of the PGC network is associated with mitochondrial dysfunction as evidenced by compromised OXPHOS and respiration, decreased ATP generation capacity, and increased oxidative stress. Importantly, given evidence of non-telomere-related functions of TERT (55,56), this also demonstrated that, with onset of telomere dysfunction, *TERT*^{-/-} mice (normal TERT expression) experience degenerative phenotypes indistinguishable of those in the *TERT*^{-/-} model.(57,58) The indistinguishable mitochondrial and energy profiles of TERT and TERC models indicate that telomere dysfunction per se is the principal factor driving these phenotypes.(53) Multiple levels of evidence establish telomere dysfunction-induced p53 represses PGC-1 α and PGC-1 β , thereby

linking telomeres to mitochondrial biology, oxidative defense, and metabolism. This telomere-p53-PGC pathway expands our understanding of how telomere dysfunction may compromise organ function and contribute to age-related disorders.(53)

Figure 2 shows that the core telomere-p53 axis integrates well with almost all genetic elements proven to be important in the aging process of genotoxic stress model of ageing.(52) First, it accounts for the premature aging phenotypes common to both telomere-dysfunctional mice and those with germline p53 hyperactivation.(59,60) Secondly, it explains why mice lacking sirtuin (SIRT)1 or SIRT6, which are proteins that attenuate p53 activity, tend to develop premature aging (61). Third, it could account for the observed connections between mitochondria and key aging factors such as PGC-1 α , PGC-1 β and FOXO proteins. Mice null for each of the genes encoding these proteins experience accelerated tissue degeneration and

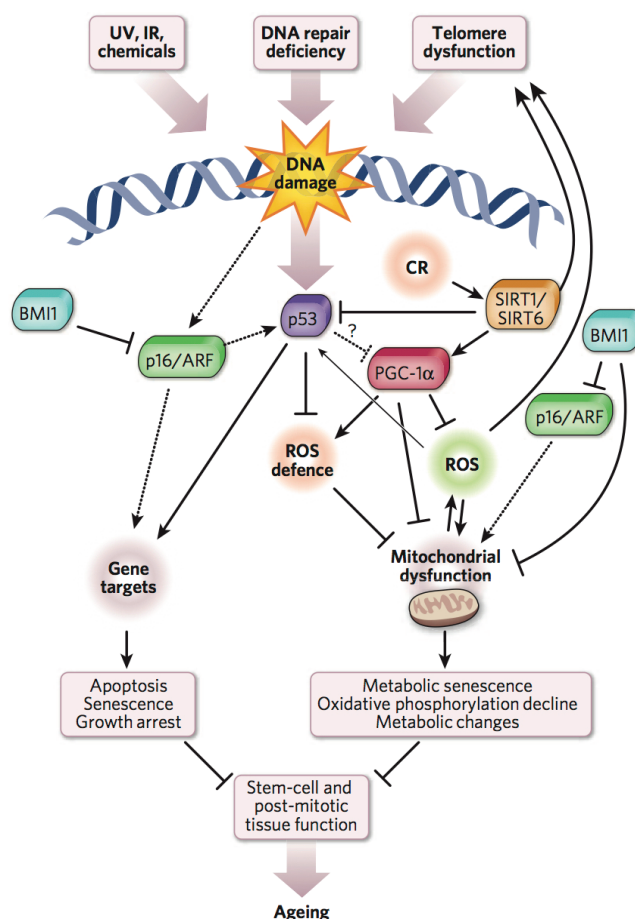


Figure 2. A model of interaction between DNA damage, p53 activation and mitochondrial dysfunction.(50) (Adapted with permission Nature Publishing Group, 2010). UV: ultraviolet; IR: infrared; BMI1: B lymphoma Mo-MLV insertion region 1.

mitochondrial dysfunction. Integration of mitochondria into this core 'axis of aging' is supported by the premature aging conditions shared by telomere-dysfunctional or hyper-p53 mice, as well as mice that have excessive mitochondrial DNA mutation or are deficient in PGC-1 α or PGC-1 β , which are the master regulators of mitochondrial biogenesis and metabolism (62-64), although the precise molecular basis for this commonality in premature ageing phenotypes remains to be elucidated.(52)

Cardiovascular Aging

With aging, cardiac function declines. Cardiac reserve, *i.e.*, the difference between the peak cardiac pumping level and the normal baseline resting level, is reduced. Cardiomyocyte loss, left-ventricular hypertrophy, changes in ventricle chamber diameter, and an accumulation of extracellular matrix lead to reduced cardiac output, decreased left-ventricular end-systolic pressure, fractional shortening, and decreased heart rate.(65-67) These facts clearly stress that the heart ages, indicating that the maintenance and repair potential of the heart is limited.

Cardiac aging is a complex process which includes aging and deposition of extracellular matrix, aging of the

coronary vasculature, aging of cardiac fibroblasts, and aging of the contractile apparatus of the heart.(68-70) Constituting the core of cardiac function and the contractile apparatus, cardiomyocytes display a number of physiological and morphological features, which are affected by the aging process, and these changes are thought to give rise to reduced cardiac function and heart disease.(71)

In old cardiomyocytes, there is a general tendency towards: 1) a reduced ability to cope with stress, *e.g.*, via reduced expression of heat shock proteins (HSP70) and anti-oxidative enzymes (hemeoxygenase-1), 2) reduced and altered function of the mitochondrial respiratory chain (*e.g.*, reduced expression of cytochrome c oxidase), which causes electron leakage and oxidative damage, 3) increased stiffness and reduced contractility/decelerated relaxation, related to downregulation of sarcoplasmic reticulum Ca²⁺-ATPase, increased expression of cytoskeletal proteins, and a transcriptional switch (caused by de-differentiation) of contractile protein isoforms, *e.g.*, from fast (type V1) to slow (type V3) myosin (72,73), and 4) a shift from proliferation and survival signaling towards cell death signaling (reduced expression of survivin, modulation of the Bcl2 rheostat towards a pro-apoptotic state).(74,75) Figure 3 shows the age-dependent changes to cardiovascular tissues. Both the heart and vasculature undergo numerous alterations during

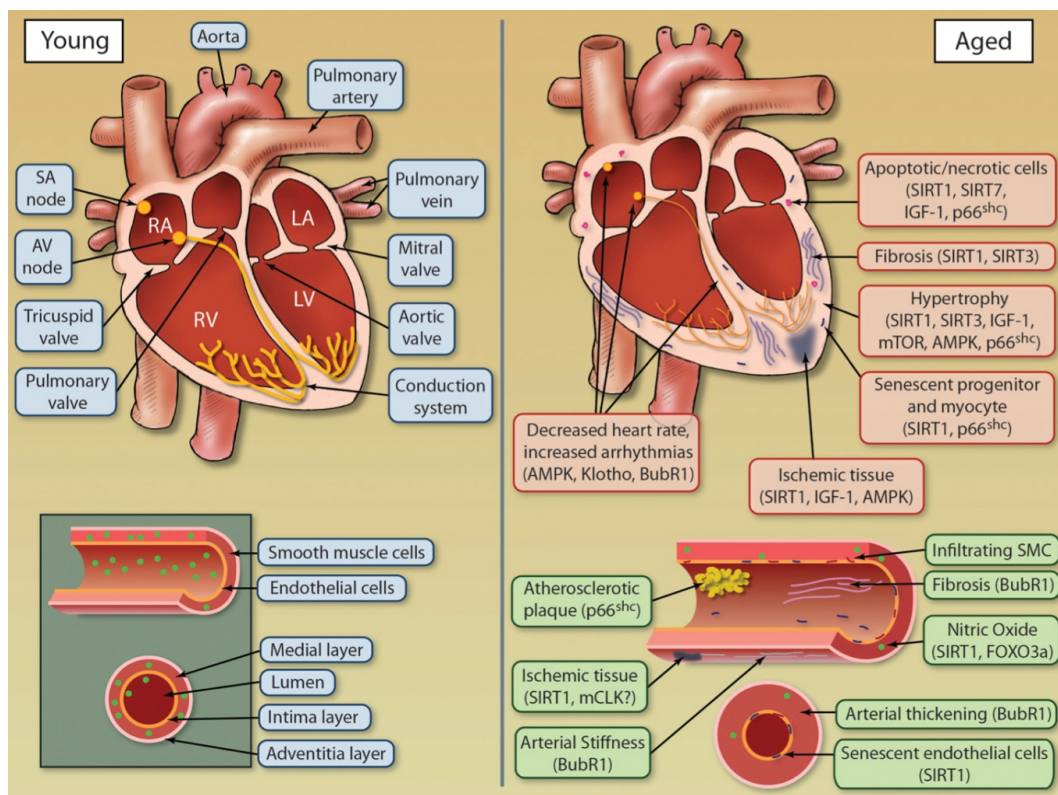


Figure 3. Age-dependent changes to cardiovascular tissues.(74) (Adapted with permission from American Heart Association).

aging as a result of deregulation of molecular longevity pathways, leading to compromised function.(76)

The molecular mechanisms involved allow time for accumulated damage to occur and include free radicals, advance glycation end-products, apoptosis and senescence. The accumulation of DNA damage and telomere attrition may increase cells' senescence in tissues and organs, and decreasing their functions, providing an explanation for the lower threshold to express the clinical manifestation of heart failure.(77)

The shortening of telomeres and telomere attrition has been shown to contribute crucially to a number of factors associated with cardiac aging like oxidative stress, and the finding of different telomeric lengths in old and young cardiomyocytes suggests that cell division and consequent telomeric shortening may play a role. As a matter of fact, old cardiomyocytes show a reduction in TL from 30 to 15 kb.(31,78) The paradigm that all cardiomyocytes are terminally differentiated has been challenged. Recent experiments using human left ventricular myocardial cells and carbon-dating techniques have established that DNA of cardiomyocytes continues to be synthesized many years after birth, indicating that cells in the human heart do renew well into adulthood.(79) Nevertheless, cardiomyocyte DNA synthesis decreases with age. At the age of 25, a mathematical modeling predicts that cardiomyocyte renewal rate is around 1%. Meanwhile at the age of 75, the cardiomyocyte renewal rate is 0.45%. Considering this turnover rate, at the age of 50 years, 55% of the cardiomyocytes stay from the time around birth.(77)

Therefore, some therapeutic strategies to boost myocardial function and outcome in CHF are crucially needed, and novel medicines are rapidly being introduced lately.(80-82) Telomere biology might be involved in the biology of aging and age-associated pathology. Telomeres are connected to the basic biology of aging and trigger cellular senescence.(77)

Telomeres, Mitochondria and Stem Cells in The Aging Heart

Morbidity and mortality rates associated with CVD continue to be high and remain a tremendous burden for the national health care system. In 2015 alone, CVD-related costs were estimated to be \$155 billions.(83) A long-term solution to this social and health care crisis will require a better understanding of how aging per se drives cardiovascular decline. This solution would help to develop the effective

preventive and therapeutic strategies. Telomeres, repetitive TTAGGG sequences at the ends of chromosomes, have been significantly involved in the aging process. It is known that short telomeres precipitate functional decline in different tissues, which includes the cardiovascular system. This is showed by many studies in humans with telomere maintenance disorders and telomerase knock-out mice have that have been done.(52)

The numbers of telomere lost during each cell division are different among people. Prior evidence showed that increased oxidative stress and chronic inflammation are related to higher telomere loss and accelerated telomere shortening.(33) Several common risk factors for CVD (84) such as smoking (34), diabetes mellitus (85), hypercholesterolemia (86), hypertension (87), obesity (88), physical inactivity (89), alcohol consumption (90) and psychosocial problems (91) have been associated with short TL. Telomere shortening process is associated with these risk factors through increased tissue inflammation and oxidative stress.(92-94)

Mechanistically, telomere dysfunction-driven tissue compromise is thought to be secondary to the activation of DNA damage signaling pathways that converge on p53, a central executor of the DNA damage response pathway.(95) The activation of p53 induces senescent and apoptosis pathways, particularly in stem cell and progenitor compartments of highly regenerative organs. The elimination of stem and progenitor cells is assumed to be the leading force in the development of tissue defects.(96)

Cardiovascular stem cells and cardiovascular progenitor cells are known to be insufficient to protect against cardiovascular disease in older individuals. Since new evidence suggests that cardiovascular stem cells and cardiovascular progenitor cells are subject to age-associated changes which impair their function, these changes may contribute to the dysregulation of endogenous cardiovascular repair mechanisms in the aging heart and vasculature.(97)

Human endothelial cells and vascular smooth muscle cells (VSMC) express telomerase activity, which is drastically activated by mitogenic stimuli via a protein kinase C-dependent pathway (98), yet its activity declined with *in vitro* aging because of a decrease in expression of TERT, which cause the telomere shortening and cellular senescence.(99,100) Introduction of telomerase extends the lifespan of both endothelial cells and VSMCs (100-102), suggesting a critical role of telomere and telomerase in vascular cell senescence.(103)

Atherosclerosis is a complex inflammatory process involving adaptive and innate immune mechanisms.(104-

109) Some researches also associating short telomeres with atherosclerosis.(110) Endothelial cell dysfunction triggered by atherogenic stimuli (*e.g.*, elevated plasma cholesterol level, hypertension, diabetes, and smoking) is of central importance in the pathogenesis of atherosclerosis. These *in vitro* studies resulting in the implication of telomeres and telomerase in endothelial cell function. *In vivo*, age-dependent telomere shortening has been reported in endothelial cells from iliac, thoracic, and coronary arteries.(102,111-113) Once fatty streaks are formed, activated neointimal leukocytes produce a plethora of inflammatory mediators that contribute to atheroma growth by provoking excessive VSMC proliferation and migration.(104,105,113-115) Telomerase has been implicated as an important regulator of VSMC proliferation *in vitro*, because TERT activation extends the lifespan of cultured VSMCs and, conversely, telomerase inhibition abrogates VSMC proliferation in a dose-dependent manner.(98,99,116) Regulation of VSMC proliferation by targeting telomerase activity appears to be independent of telomere length, because VSMC growth arrest occurs early after telomerase inhibition (98,117) and telomerase expression alone is capable of rescuing

the senescent phenotype of human plaque VSMCs despite short telomeres.(118) A role of telomerase on the control of VSMC growth has been also proposed *in vivo*. Telomerase activation and telomere maintenance appear to be critical for increased VSMC hyperplastic growth in hypertensive rats.(119)

A deficiency of mitochondrial energetics has been documented in human and experimental animals with heart failure (Figure 4).(120) Mechanisms may include mitochondrial biogenesis that does not keep up with the increasing demand (121), mitochondrial uncoupling and decreased substrate availability,(122) and increased mitochondrial DNA deletions.(123)

Studies have demonstrated that telomere dysfunction-activated p53 directly leads to mitochondrial and metabolic compromise through the repression of the master regulators of mitochondrial biogenesis and function, peroxisome proliferator-activated receptor gamma coactivator (PGC)-1 α and PGC-1 β .(53) PGC repression is associated with a profound compromise in mitochondrial biogenesis and function and subsequent decline in ATP generation, indicating that a fundamental problem of energy maintenance

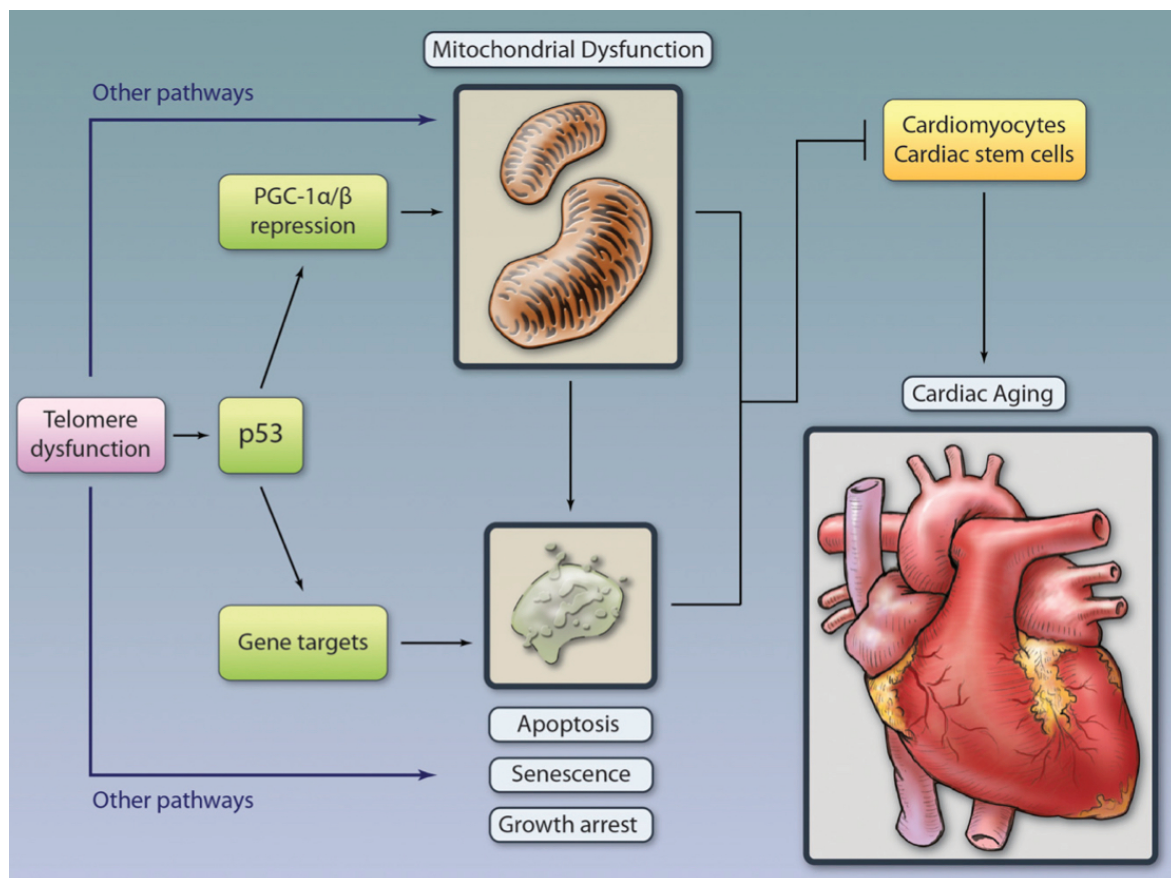


Figure 4. Model of how telomere dysfunction and other pathways cause cardiac aging through either cellular or metabolic compromise.(94) (Adapted with permission from American Heart Association).

drives the aging process.(96) Another potentially important link between mitochondrial oxidative stress and vascular aging is the induction of apoptosis.(124) Oxidative stress in aging is associated with an increased rate of endothelial apoptosis (125,126), which may contribute to microvascular rarefaction impairing the blood supply of the heart (127) and the brain (128). Cerebrovascular endothelial cells are rich in mitochondria, and normal mitochondrial function is essential for maintaining the integrity of the blood-brain barrier. On the basis of the available data with mitochondrial inhibitors (129), we posit that age-related mitochondrial dysfunction may contribute to breakdown of the blood-brain barrier, promoting neuroinflammation in aging.(130)

Lately, there has been an experimental evidence that associate the mitochondrial free radical and telomere-shortening theories of aging.(53) These abnormalities are associated with p53-mediated repression of PGC-1 α and PGC-1 β , and their downstream targets, nuclear respiratory factor-1 (NRF-1) and mitochondrial transcription factor A (TFAM). Thus, age-related telomerase dysfunction might represent a primary instigator of mitochondrial decay, which in turn would cause the decrease of bioenergetic efficiency and increase of ROS production via sustained p53 activation and further repression of PGC signaling.(131)

Optimal regulation of mitochondrial autophagy is crucial for the maintenance of cell homeostasis. This is especially true for cardiomyocytes because of their post-mitotic nature and their high reliance on mitochondrial oxidative metabolism for energy supply. Over their lifespan, cardiac cells are exposed to a high burden of mitochondria-derived oxidative damage, which cannot be diluted through cell proliferation. This shows that the maintenance of a healthy pool of mitochondria and the removal of damaged organelles are important to preserve cardiomyocyte function and viability. Autophagy supplies this essential homeostatic function. This suggests that optimization of the housekeeping function of autophagy may be harnessed as a therapeutic means against heart senescence.(21)

Some dietary and lifestyle factors such as marine omega-3 fatty acid (132), antioxidants (133), vitamin intake (134), physical activity (90), and healthy lifestyle (135) were reported to decrease rates of LTL shortening. These factors might have roles in reducing ROS, inhibiting inflammation, increasing the activity of endothelial nitric oxide synthase (eNOS), and increasing telomerase activity. A human study also reported that alterations in comprehensive lifestyle significantly increased telomerase activity and consequently telomere maintenance ability in human immune system

cells.(135) Consequently, telomere shortening can be used as a reflection of cellular aging and a marker of the health status of the aging population.(136) Absolute TL at birth is determined by genetic materials from both parents. During the process of aging, the mean TL declines with cell replication and turnover. The process of telomere shortening is accelerated by the exposure to disease-promoting factors, such as smoking, obesity, and psychosocial stress. Furthermore, the activation of telomerase has been assumed as a possible target for reversing the telomere shortening. (18) Figure 5 shows the relation of telomere length to CVD.

In patients with CAD, LTL can be used as a prognostic tool. A prospective cohort study with 780 patients were conducted for a follow-up period of 4.4 years reported an association of decreased LTL with all-cause mortality, with an adjusted hazard ratio of 1.8 in the lowest TL quartile compared with the highest TL quartile.(137) Moreover, LTL has been observed to be shorter in patients with premature acute MI (aged <50 years) than in healthy, age-matched controls.(23) In a clinical study of 803 patients, LTL was decreased by approximately 40% in patients with heart failure, and TL in the patients with heart failure was associated to the disease severity.(138) There was a study investigating the association of a lower left ventricular ejection fraction with decreased TL, which reported the association of one SD decrease in TL with a 5% lower ejection fraction.(139) Moreover, LTL was significantly associated with cardiovascular outcomes in patients with ischemic heart failure (140).

Telomerase as A Therapeutic Target in Cardiovascular Disease

From the implications of current understanding of telomere biology, potential therapeutic interventions, such as the maintenance of TL and modulation of telomerase activity to reverse telomere attrition and cellular senescence, is emerging as a new strategy for treating atherosclerosis and CVD.(140)

Experimental studies have reported that the manipulation of telomerase activity and TL enhances or reverses senescence and aging-associated phenotypes.(141-143) For example, the incidence of ischemic heart failure in mice can successfully be prevented by the telomerase activation therapy after MI. The treatment of adeno-associated viruses with the cardiac-specific telomerase expression resulted in elongated telomeres, attenuated cardiac dilation, improved ventricular function, and smaller

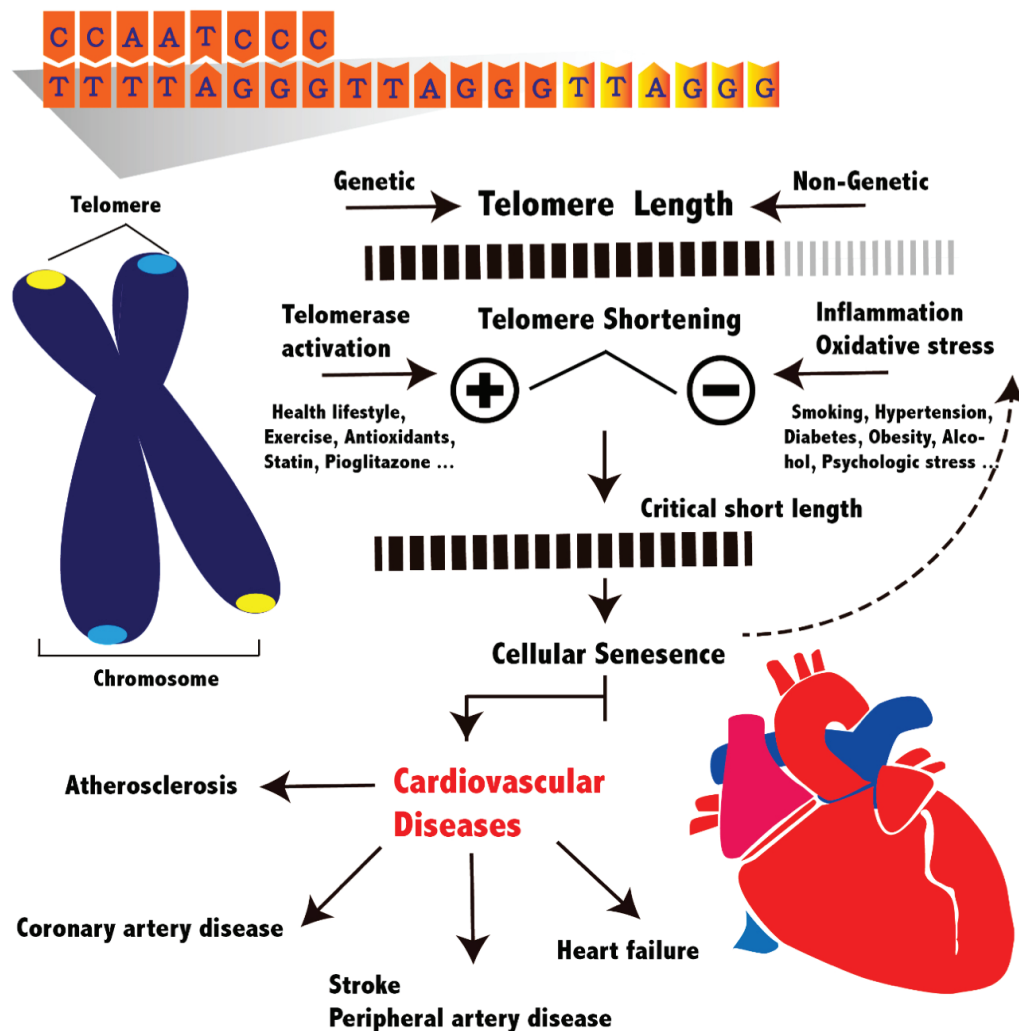


Figure 5. Schematic overview of telomere length and cardiovascular diseases.(18) (Adapted with permission from Molecular Diversity Preservation International)

infarct scars as well as improved the survival by 17% compared to the controls.(144)

The effect of TA-65®, a natural product-derived telomerase activator, on metabolic markers and cardiovascular health. In addition to apparent positive immune remodeling in these patients, TA-65® treatment has shown an improvement of metabolic markers with a decrease in the fasting glucose, insulin levels, total cholesterol and low-density lipoprotein cholesterol. In parallel, the systolic and diastolic blood pressures of these patients were significantly ameliorated after treatment. These results suggest that telomerase activation is a rejuvenation strategy for age-associated diseases such as cardiovascular diseases and might prove a therapeutic adjunct or alternative in this setting.(145) The use of thiazolidinediones (TZD) may hold real promise for a solution to the differential role of telomerase in the intimal and medial layers through

activation of telomerase in ECs and inhibition of telomerase in VSMCs. In the past 15 years, peroxisome proliferator-activated receptor gamma (PPAR- γ), a member of the nuclear receptor superfamily, has emerged as an important player in vascular protection. PPAR- γ is expressed in both vascular endothelial and smooth muscle cells, and shown to be critically involved in the development of vascular complications and inflammation and hypertension.(146-149) In fact, the anti-proliferative, anti-atherosclerosis properties of PPAR- γ have been shown to suppress VSMC proliferation, which could be at least in part mediated by its effects on suppression of telomerase activity (pro-proliferation). This is supported by the findings that PPAR- γ activation suppresses telomerase in cultured VSMC (117).

Other reports showed that resveratrol, a type of natural phenol present in some fruits, activates the catalytic subunit of telomerase in human aortic SMC and pulmonary

microvascular endothelial cells. Similar observations were obtained in resveratrol treated C57BL/6J mouse heart and liver tissues.(149) Resveratrol has been shown to produce changes associated with longer lifespan, including increased insulin sensitivity, PGC-1 α activity and mitochondrial number.(150) Interestingly, elderly mice fed with resveratrol showed a marked reduction in signs of aging with decreased inflammation and vascular endothelium senescence, and increased aortic elasticity.(126) Despite the facts that the exact mechanism by which resveratrol induces TA remains unknown, these findings suggest a strong link with its beneficial effect in anti-aging processes in cardiovascular cells affected by disease.

In the last few years, another chemical compound, AGS-499, has been started to show its neuroprotective effects in the amyotrophic lateral sclerosis (ALS) disease animal model via increased TA. *In vivo* treatment with AGS-499 has increased significantly TA in these animals and improved their life-span.(151) Furthermore, AGS-499 treatment, without altering their functionality, protected stem cells from apoptosis and DNA damage produced by long-term exposure to oxidative stress (152). Many studies have also demonstrated that acute activation of telomerase using AGS-499 restored NO bioavailability and limited ROS production in micro-vessels from subjects with CAD.(153)

Conclusion

Telomere shortening and dysfunction play a crucial role in the pathogenesis of aging-associated CVDs. Crucially short telomeres can cause cellular senescence and apoptosis, which contribute to the development of atherosclerosis and predispose people to plaque instability. Both genetic and environmental factors have been associated with individual variations in TL. Cardiovascular risk factors, such as smoking, diabetes mellitus, hypertension, obesity, sedentary lifestyle and stress, have been assumed to increase the oxidative stress or inflammation, which consequently accelerates TL shortening. Furthermore, targeting telomerase or additional telomere-associated proteins may provide a novel therapeutic strategy for neovascularization in patients with ischemic heart diseases and for restoring replicative capacity in those with HF. Both additional basic and well-designed clinical studies are needed to validate these observations and further expand our knowledge the complexities of telomere dynamics in humans.

References

- Gerhard M, Roddy MA, Creager SJ, Creager MA. Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. *Hypertension*. 1996; 27: 849-53.
- Avolio AP, Deng FQ, Li WQ, Luo YF, Huang ZD, Xing LF, *et al*. Effects of aging on arterial distensibility in populations with high and low prevalence of hypertension: Comparison between urban and rural communities in China. *Circulation*. 1985; 71: 202-10.
- Lakatta EG. Cardiovascular aging research: The next horizons. *J Am Geriatr Soc*. 1999; 47: 613-25.
- Lakatta EG, Levy D. Arterial and cardiac aging: Major shareholders in cardiovascular disease enterprises: Part II: The aging heart in health: Links to heart disease. *Circulation*. 2003; 107: 346-54.
- McGrath BP, Liang YL, Teede H, Shiel LM, Cameron JD, Dart A. Age-related deterioration in arterial structure and function in postmenopausal women: Impact of hormone replacement therapy. *Arterioscler Thromb Vasc Biol*. 1998; 18: 1149-56.
- Tanaka H, Dinunno FA, Monahan KD, Clevenger CM, DeSouza CA, Seals DR. Aging, habitual exercise, and dynamic arterial compliance. *Circulation*. 2000; 102: 1270-5.
- Vaitkevicius PV, Fleg JL, Engel JH, O'Connor FC, Wright JG, Lakatta LE, *et al*. Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation*. 1993; 88: 1456-62.
- Kitzman DW, Sheikh KH, Beere PA, Phillips JL, Higginbotham MB. Age-related alterations of Doppler left ventricular filling indexes in normal subjects are independent of left ventricular mass, heart rate, contractility and loading conditions. *J Am Coll Cardiol*. 1991; 18: 1243-50.
- Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part I: aging arteries: a "set up" for vascular disease. *Circulation*. 2003; 107: 139-46.
- Port S, Cobb FR, Coleman RE, Jones RH. Effect of age on the response of the left ventricular ejection fraction to exercise. *N Engl J Med*. 1980; 303: 1133-7.
- Dai DF, Rabinovitch PS. Cardiac aging in mice and humans: the role of mitochondrial oxidative stress. *Trends Cardiovasc Med*. 2009; 19: 213-20.
- Antelmi I, de Paula RS, Shinzato AR, Peres CA, Mansur AJ, Grupi CJ. Influence of age, gender, body mass index, and functional capacity on heart rate variability in a cohort of subjects without heart disease. *Am J Cardiol*. 2004; 93: 381-5.
- Fluckiger L, Boivin JM, Quilliot D, Jeandel C, Zannad F. Differential effects of aging on heart rate variability and blood pressure variability. *J Gerontol A Biol Sci Med Sci*. 1999; 54: B219-24.
- Schwartz JB, Gibb WJ, Tran T. Aging effects on heart rate variation. *J Gerontol*. 1991; 46: M99-106.
- Lakatta EG. Cardiovascular regulatory mechanisms in advanced age. *Physiol Rev*. 1993; 73: 413-67.
- Fontana L, Vinciguerra M, Longo VD. Growth Factors, nutrient signaling, and cardiovascular aging. *Circ Res*. 2012; 110: 1139-50.
- Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flegal K, *et al*. Heart disease and stroke statistics—2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*. 2009; 119: 480-6.
- Yeh JK, Wang CY. Telomeres and telomerase in cardiovascular disease. *Genes (Basel)*. 2016; 7: e58. doi: 10.3390/genes7090058.
- Jahangir A, Sagar S, Terzic A. Aging and cardioprotection. *J Appl Physiol*. 2007; 103: 2120-8.

20. Marzetti E, Wohlgemuth SE, Anton SD, Bernabei R, Carter CS, Leeuwenburgh C. Cellular mechanisms of cardioprotection by calorie restriction: state of the science and future perspectives. *Clin Geriatr Med.* 2009; 25: 715-32.
21. Dutta D, Calvani R, Bernabei R, Leeuwenburgh C, Marzetti E. Contribution of impaired mitochondrial autophagy to cardiac aging mechanisms and therapeutic opportunities. *Circ Res.* 2012; 110: 1125-38.
22. Fitzpatrick AL, Kronmal RA, Gardner JP, Psaty BM, Jenny NS, Tracy RP, *et al.* Leukocyte telomere length and cardiovascular disease in the cardiovascular health study. *Am J Epidemiol.* 2007; 165: 14-21.
23. Brouillette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol.* 2003; 23: 842-6.
24. D'Mello MJJ, Ross SA, Briel M, Anand SS, Gerstein H, Paré G. Association between shortened leukocyte telomere length and cardiometabolic outcomes: Systematic review and meta-analysis. *Circ Cardiovasc Genet.* 2015; 8: 82-90.
25. O'Donnell CJ, Demissie S, Kimura M, Levy D, Gardner JP, White C, *et al.* Leukocyte telomere length and carotid artery intimal medial thickness R the framingham heart study. *Arterioscler Thromb Vasc Biol.* 2008; 28: 1165-71.
26. Epel ES, Merkin SS, Cawthon R, Blackburn EH, Adler NE, Pletcher MJ, *et al.* The rate of leukocyte telomere shortening predicts mortality from cardiovascular disease in elderly men. *Aging.* 2009; 1: 81-8.
27. Burnett-Hartman AN, Fitzpatrick AL, Kronmal RA, Psaty BM, Jenny NS, Bis JC, *et al.* Telomere-associated polymorphisms correlate with cardiovascular disease mortality in Caucasian women: The Cardiovascular Health Study. *Mech Ageing Dev.* 2012; 133: 275-81.
28. Blackburn EH. Switching and signaling at the telomere. *Cell.* 2001; 106: 661-73.
29. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet.* 2005; 6: 611-22.
30. Chatterjee S. Telomeres in health and disease. *J Oral Maxillofac Pathol.* 2017; 21: 87-91.
31. Fuster JJ, Andres V. Telomere biology and cardiovascular disease. *Circ Res.* 2006; 99: 1167-80.
32. Slagboom PE, Droog S, Boomsma DI. Genetic determination of telomere size in humans: a twin study of three age groups. *Am J Hum Genet.* 1994; 55: 876-82.
33. von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci.* 2002; 27: 339-44.
34. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, *et al.* Obesity, cigarette smoking, and telomere length in women. *Lancet.* 2005; 366: 662-4.
35. Oikawa S, Tada-Oikawa S, Kawanishi S. Site-specific DNA damage at the GGG sequence by UVA involves acceleration of telomere shortening. *Biochemistry.* 2001; 40: 4763-8.
36. Karlseder J, Smogorzewska A, de Lange T. Senescence induced by altered telomere state, not telomere loss. *Science.* 2002; 295: 2446-9.
37. Poss KD, Wilson LG, Keating MT. Heart regeneration in zebrafish. *Science.* 2002; 298: 2188-90.
38. Richardson GD, Breault D, Horrocks G, Cormack S, Hole N, Owens WA. Telomerase expression in the mammalian heart. *FASEB J.* 2012; 26: 4832-40.
39. Blasco M. Telomere length, stem cells and aging. *Nat Chem Biol.* 2007; 3: 640-9.
40. Erusalimsky JD, Kurz DJ. Cellular senescence in vivo: its relevance in ageing and cardiovascular disease. *Exp Gerontol.* 2005; 40: 634-42.
41. Campisi J. Cellular senescence: putting the paradoxes in perspective. *Curr Opin Genet Dev.* 2011; 21: 107-12.
42. Ridout SJ, Ridout KK, Kao HT, Carpenter LL, Philip NS, Tyrka AR, *et al.* Telomeres, early-life stress and mental illness. *Adv Psychosom Med.* 2015; 34: 92-108.
43. Baragetti A, Palmen J, Garlaschelli K, Grigore L, Pellegatta F, Tragni E, *et al.* Telomere shortening over 6 years is associated with increased subclinical carotid vascular damage and worse cardiovascular prognosis in the general population. *J Intern Med.* 2015; 277: 478-87.
44. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res.* 2002; 30: e47. doi: 10.1093/nar/30.10.e47.
45. Hammadah M, Mheid IA, Wilmot K, Ramada R, Abdelhadi N, Alkhoder A, *et al.* Telomere shortening, regenerative capacity, and cardiovascular outcomes. *Circ Res.* 2017; 126: 1130-8.
46. Dai DF, Rabinovitch PS, Ungvari Z. Mitochondria and cardiovascular aging. *Circ Res.* 2012; 110: 1109-24.
47. Harman D. The biologic clock: the mitochondria? *J Am Geriatr Soc.* 1972; 20: 145-7.
48. De Almeida AJPO, Ribeiro TP, de Medeiros IA. Aging: Molecular Pathways and Implications on the Cardiovascular System. *Oxid Med Cell Longev.* 2017; 2017: 7941563. doi:10.1155/2017/7941563.
49. Durieux J, Wolff S, Dillin A. The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell.* 2011; 144: 79-91.
50. Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. *Nature Rev Mol Cell Biol.* 2007; 8: 703-13.
51. Rossi DJ, Jamieson CHM, Weissman IL. Stems cells and the pathways to aging and cancer. *Cell.* 2008; 132: 681-96.
52. Sahin E, DePinho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature.* 2010; 464: 520-8.
53. Sahin E, Colla S, Liesa M, Moslehi J, Muller FL, Guo M, *et al.* Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature.* 2011; 470: 359-65.
54. Meiliana A, Dewi NM, Wijaya A. Telomere in aging and age-related disease. *Indones Biomed J.* 2017 (in press).
55. Maida Y, Yasukawa M, Furuuchi M, Lassmann T, Possemato R, Okamoto N, *et al.* An RNA-dependent RNA polymerase formed by TERT and the RMRP RNA. *Nature.* 2009; 461: 230-5.
56. Sarin KY, Cheung P, Gilson D, Lee E, Tennen RI, Wang E, *et al.* Conditional telomerase induction causes proliferation of hair follicle stem cells. *Nature.* 2005; 436: 1048-52.
57. Farazi PA, Glickman J, Horner J, Depinho RA. Cooperative interactions of p53 mutation, telomere dysfunction, and chronic liver damage in hepatocellular carcinoma progression. *Cancer Res.* 2006; 66: 4766-73.
58. Erdmann N, Liu Y, Harrington L. Distinct dosage requirements for the maintenance of long and short telomeres in mTert heterozygous mice. *Proc Natl Acad Sci USA.* 2004; 101: 6080-5.
59. Maier B, Gluba W, Bernier B, Turner T, Mohammad K, Guise T, *et al.* Modulation of mammalian life span by the short isoform of p53. *Genes Dev.* 2004; 18: 306-19.
60. Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H, *et al.* p53 mutant mice that display early ageing-associated phenotypes. *Nature.* 2002; 415: 45-53.
61. Finkel T, Deng CX, Mostoslavsky R. Recent progress in the biology and physiology of sirtuins. *Nature.* 2009; 460: 587-91.
62. Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, *et al.* Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature.* 2004; 429: 417-23.
63. St-Pierre J, Drori S, Uldry M, Silvaggi JM, Rhee J, Jäger S, *et al.* Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell.* 2006; 127: 397-408.

64. Vianna CR, Huntgeburth M, Coppari R, Choi CS, Lin J, Krauss S, *et al.* Hypomorphic mutation of PGC-1 β causes mitochondrial dysfunction and liver insulin resistance. *Cell Metab.* 2006; 4: 453-64.
65. Chang KC, Peng YI, Dai SH, Tseng YZ. Age-related changes in pumping mechanical behavior of rat ventricle in terms of systolic elastance and resistance. *J Gerontol A Biol Sci Med Sci.* 2000; 55: B440-7.
66. Fleg JL, O'Connor F, Gerstenblith G, Becker LC, Clulow J, Schulman SP, *et al.* Impact of age on the cardiovascular response to dynamic upright exercise in healthy men and women. *J Appl Physiol.* 1995; 78: 890-900.
67. Lakatta EG. Why cardiovascular function may decline with age. *Geriatrics* 1987; 42: 84-7.
68. Anversa P, Palackal T, Sonnenblick EH, Olivetti G, Meggs LG, Capasso JM. Myocyte cell loss and myocyte cellular hyperplasia in the hypertrophied aging rat heart. *Circ Res.* 1990; 67: 871-85.
69. Hacker TA, McKiernan SH, Douglas PS, Wanagat J, Aiken JM. Age-related changes in cardiac structure and function in Fischer 344 x Brown Norway hybrid rats. *Am J Physiol* 2006; 290: H304-11.
70. Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone system. *Circulation* 1991; 83: 1849-65.
71. Bernhard D, Laufer G. The aging cardiomyocyte: a mini review. *Gerontology.* 2008; 54: 24-31.
72. Pandya K, Kim HS, Smithies O. Fibrosis, not cell size, delineates beta-myosin heavy chain reexpression during cardiac hypertrophy and normal aging in vivo. *Proc Natl Acad Sci USA.* 2006; 103: 16864-9.
73. Nag AC, Cheng M. Biochemical evidence for cellular dedifferentiation in adult rat cardiac muscle cells in culture: expression of myosin isozymes. *Biochem Biophys Res Commun.* 1986; 137: 855-62.
74. Bodyak N, Kang PM, Hiromura M, Suljoadikusumo I, Horikoshi N, Khrapko K, *et al.* Gene expression profiling of the aging mouse cardiac myocytes. *Nucl Acids Res.* 2002; 30: 3788-94.
75. Park SK, Prolla TA. Gene expression profiling studies of aging in cardiac and skeletal muscles. *Cardiovasc Res.* 2005; 66: 205-12.
76. North BJ, Sinclair DA. The intersection between aging and cardiovascular disease. *Cir Res.* 2012; 110: 1097-108.
77. Wong LSM, van der Harst P, de Boer RA, Huzen J, van Gilst W, van Veldhuisen DJ. Aging, telomeres and heart failure. *Heart Fail Rev.* 2010; 15: 479-86.
78. Leri A, Franco S, Zacheo A, Barlucchi L, Chimenti S, Limana F, *et al.* Ablation of telomerase and telomere loss leads to cardiac dilatation and heart failure associated with p53 upregulation. *EMBO J.* 2003; 22: 131-9.
79. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, *et al.* Evidence for cardiomyocyte renewal in humans. *Science.* 2009; 324: 98-102.
80. Lipsic E, Westenbrink BD, van der Meer P, van der Harst P, Voors AA, van Veldhuisen DJ, *et al.* Low-dose erythropoietin improves cardiac function in experimental heart failure without increasing haematocrit. *Eur J Heart Fail.* 2008; 10: 22-9.
81. Westenbrink BD, Lipsic E, van der Meer P, van der Harst P, Oeseburg H, Du Marchie Sarvaas GJ, *et al.* Erythropoietin improves cardiac function through endothelial progenitor cell and vascular endothelial growth factor mediated neovascularization. *Eur Heart J.* 2007; 28: 2018-27.
82. Westenbrink BD, Oeseburg H, Kleijn L, van der Harst P, Belonje AM, Voors AA, *et al.* Erythropoietin stimulates normal endothelial progenitor cell-mediated endothelial turnover, but attributes to neovascularization only in the presence of local ischemia. *Cardiovasc Drugs Ther.* 2008; 22: 265-74.
83. Heart Disease and Stroke Prevention [Internet]. Taking on the Nation's Leading Killers [cited 2017 Jun 2]. Available from: https://www.cdc.gov/dhds/docs/dhds_factsheet.pdf.
84. Bekaert S, De Meyer T, Rietzschel ER, De Buyzere ML, De Bacquer D, Langlois M, *et al.* Telomere length and cardiovascular risk factors in a middle-aged population free of overt cardiovascular disease. *Aging Cell.* 2007; 6: 639-47.
85. Sampson MJ, Winterbone MS, Hughes JC, Dozio N, Hughes DA. Monocyte telomere shortening and oxidative DNA damage in type 2 diabetes. *Diabetes Care.* 2006; 29: 283-9.
86. Strandberg TE, Saijonmaa O, Tilvis RS, Pitkälä KH, Strandberg AY, Salomaa V, *et al.* Telomere length in old age and cholesterol across the life course. *J Am Geriatr Soc.* 2011; 59: 1979-81.
87. Benetos A, Okuda K, Lajemi M, Kimura M, Thomas F, Skurnick J, *et al.* Telomere length as an indicator of biological aging: The gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension.* 2001; 37: 381-5.
88. Müezziner A, Zaineddin AK, Brenner H. Body mass index and leukocyte telomere length in adults: a systematic review and meta-analysis. *Obes Rev.* 2014; 15: 192-201.
89. Cherkas LF, Hunkin JL, Kato BS, Richards JB, Gardner JP, Surdulescu GL, *et al.* The association between physical activity in leisure time and leukocyte telomere length. *Arch Intern Med.* 2008; 168: 154-8.
90. Strandberg TE, Strandberg AY, Saijonmaa O, Tilvis RS, Pitkälä KH, Fyhrquist F. Association between alcohol consumption in healthy midlife and telomere length in older men: The Helsinki Businessmen Study. *Eur J Epidemiol.* 2012; 27: 815-822.
91. Cherkas LF, Aviv A, Valdes AM, Hunkin JL, Gardner JP, Surdulescu GL, *et al.* The effects of social status on biological aging as measured by white-blood-cell telomere length. *Aging Cell.* 2006; 5: 361-5.
92. Wolkowitz OM, Mellon SH, Epel ES, Lin J, Dhabhar FS, Su Y, *et al.* Leukocyte telomere length in major depression: Correlations with chronicity, inflammation and oxidative stress -- preliminary findings. *PLoS ONE.* 2011; 6: e17837. doi: 10.1371/journal.pone.0017837.
93. Comporti M, Signorini C, Leoncini S, Gardi C, Ciccoli L, Giardini A, *et al.* Ethanol-induced oxidative stress: Basic knowledge. *Genes Nutr.* 2010; 5: 101-9.
94. Strandberg TE, Saijonmaa O, Tilvis RS, Pitkala KH, Strandberg AY, Miettinen TA, *et al.* Association of telomere length in older men with mortality and midlife body mass index and smoking. *J Gerontol A Biol Sci Med Sci.* 2011; 66: 815-20.
95. Chin L, Artandi SE, Shen Q, Tam A, Lee SL, Gottlieb GJ, *et al.* P53 deficiency rescues the adverse effects of telomere loss and cooperates with telomere dysfunction to accelerate carcinogenesis. *Cell.* 1999; 97: 527-38.
96. Moslehi J, DePinho RA, Sahin E. Telomeres and mitochondria in the aging heart. *Circ Res.* 2012; 110: 1226-37.
97. Ballard VLT, Edelberg JM. Stem cells and the regeneration of the aging cardiovascular system. *Circ Res.* 2007; 100: 1116-7.
98. Minamino T, Kourembanas S. Mechanisms of telomerase induction during vascular smooth muscle cell proliferation. *Circ Res.* 2001; 89: 237-43.
99. Minamino T, Mitsialis SA, Kourembanas S. Hypoxia extends the life span of vascular smooth muscle cells through telomerase activation. *Mol Cell Biol.* 2001; 21: 3336-42.
100. Hsiao R, Sharma HW, Ramakrishnan S, Keith E, Narayanan R. Telomerase activity in normal human endothelial cells. *Anticancer Res.* 1997; 17: 827-32.
101. Yang J, Chang E, Cherry AM, Bangs CD, Oei Y, Bodnar A, *et al.* Human endothelial cell life extension by telomerase expression. *J Biol Chem.* 1999; 274: 26141-8.

102. Chang E, Harley CB. Telomere length and replicative aging in human vascular tissues. *Proc Natl Acad Sci USA*. 1995; 92: 11190-4.
103. Minamino T, Komuro I. Vascular cell senescence contribution to atherosclerosis. *Circ Res*. 2007; 100: 15-26.
104. Lusis AJ. Atherosclerosis. *Nature*. 2000; 407: 233-41.
105. Epstein FH, Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med*. 1999; 340: 115-26.
106. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA*. 2002; 287: 2570-81.
107. MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, *et al.* Blood pressure, stroke, and coronary heart disease. Part 1, prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet*. 1990; 335: 765-74.
108. Binder CJ, Chang MK, Shaw PX, Miller YI, Hartvigsen K, Dewan A, *et al.* Innate and acquired immunity in atherogenesis. *Nat Med*. 2002; 8: 1218-26.
109. Greaves DR, Channon KM. Inflammation and immune responses in atherosclerosis. *Trends Immunol*. 2002; 23: 535-41.
110. Toupance S, Labat C, Temmar M, Rossignol P, Kimura M, Aviv A, *et al.* Short telomeres, but not telomere attrition rates, are associated with carotid atherosclerosis. *Hypertension*. 2017; 70: 420-5.
111. Aviv H, Khan MY, Skurnick J, Okuda K, Kimura M, Gardner J, *et al.* Age dependent aneuploidy and telomere length of the human vascular endothelium. *Atherosclerosis*. 2001; 159: 281-7.
112. Ogami M, Ikura Y, Ohsawa M, Matsuo T, Kayo S, Yoshimi N, *et al.* Telomere shortening in human coronary artery diseases. *Arterioscler Thromb Vasc Biol*. 2004; 24: 546-50.
113. Rivard A, Andre's V. Vascular smooth muscle cell proliferation in the pathogenesis of atherosclerotic cardiovascular diseases. *Histol Histopathol*. 2000; 15: 557-71.
114. Dzau VJ, Braun-Dullaeus RC, Sedding DG. Vascular proliferation and atherosclerosis: new perspectives and therapeutic strategies. *Nat Med*. 2002; 8: 1249-56.
115. Andre's V. Control of vascular cell proliferation and migration by cyclin-dependent kinase signalling: new perspectives and therapeutic potential. *Cardiovasc Res*. 2004; 63: 11-21.
116. McKee JA, Banik SS, Boyer MJ, Hamad NM, Lawson JH, Niklason LE, *et al.* Human arteries engineered in vitro. *EMBO Rep*. 2003; 4: 633-8.
117. Ogawa D, Nomiyama T, Nakamachi T, Heywood EB, Stone JF, Berger JP, *et al.* Activation of peroxisome proliferator-activated receptor gamma suppresses telomerase activity in vascular smooth muscle cells. *Circ Res*. 2006; 98: e50-9.
118. Matthews C, Gorenne I, Scott S, Figg N, Kirkpatrick P, Ritchie A, *et al.* Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: effects of telomerase and oxidative stress. *Circ Res*. 2006; 99: 156-64.
119. Cao Y, Li H, Mu FT, Ebisui O, Funder JW, Liu JP. Telomerase activation causes vascular smooth muscle cell proliferation in genetic hypertension. *FASEB J*. 2002; 16: 96-8.
120. Ventura-Clapier R, Garnier A, Veksler V. Transcriptional control of mitochondrial biogenesis: the central role of pgc-1alpha. *Cardiovasc Res*. 2008; 79: 208-17.
121. Goffart S, von Kleist-Retzow JC, Wiesner RJ. Regulation of mitochondrial proliferation in the heart: power-plant failure contributes to cardiac failure in hypertrophy. *Cardiovasc Res*. 2004; 64: 198-207.
122. Murray AJ, Anderson RE, Watson GC, Radda GK, Clarke K. Uncoupling proteins in human heart. *Lancet*. 2004; 364: 1786-8.
123. Dai DF, Johnson SC, Villarin JJ, Chin MT, Nieves-Cintrón M, Chen T, *et al.* Mitochondrial oxidative stress mediates angiotensin II-induced cardiac hypertrophy and g{alpha}q overexpression-induced heart failure. *Circ Res*. 2011; 108: 837-46.
124. Phaneuf S, Leeuwenburgh C. Cytochrome c release from mitochondria in the aging heart: a possible mechanism for apoptosis with age. *Am J Physiol Regul Integr Comp Physiol*. 2002; 282: R423-30.
125. Csiszar A, Ungvari Z, Koller A, Edwards JG, Kaley G. Proinflammatory phenotype of coronary arteries promotes endothelial apoptosis in aging. *Physiol Genomics*. 2004; 17: 21-30.
126. Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, *et al.* Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab*. 2008; 8: 157-68.
127. Anversa P, Li P, Sonnenblick EH, Olivetti G. Effects of aging on quantitative structural properties of coronary vasculature and microvasculature in rats. *Am J Physiol*. 1994; 267: H1062-73.
128. Sonntag WE, Lynch CD, Cooney PT, Hutchins PM. Decreases in cerebral microvasculature with age are associated with the decline in growth hormone and insulin-like growth factor 1. *Endocrinology*. 1997; 138: 3515-20.
129. Kim GW, Gasche Y, Grzeschik S, Copin JC, Maier CM, Chan PH. Neurodegeneration in striatum induced by the mitochondrial toxin 3-nitropropionic acid: role of matrix metalloproteinase-9 in early blood-brain barrier disruption? *J Neurosci*. 2003; 23: 8733-42.
130. Farrall AJ, Wardlaw JM. Blood-brain barrier: ageing and microvascular disease -- systematic review and meta-analysis. *Neurobiol Aging*. 2009; 30: 337-52.
131. Sohal RS, Ku HH, Agarwal S, Forster MJ, Lal H. Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. *Mech Ageing Dev*. 1994; 74: 121-33.
132. Farzaneh-Far R, Lin J, Epel ES, Harris WS, Blackburn EH, Whooley MA. Association of marine omega-3 fatty acid levels with telomeric aging in patients with coronary heart disease. *JAMA*. 2010; 303: 250-7.
133. Xu Q, Parks CG, DeRoo LA, Cawthon RM, Sandler DP, Chen H. Multivitamin use and telomere length in women. *Am J Clin Nutr*. 2009; 89: 1857-63.
134. Haendeler J, Hoffmann J, Diehl JF, Vasa M, Spyridopoulos I, Zeiher AM, *et al.* Antioxidants inhibit nuclear export of telomerase reverse transcriptase and delay replicative senescence of endothelial cells. *Circ Res*. 2004; 94: 768-75.
135. Ornish D, Lin J, Daubenmier J, Weidner G, Epel E, Kemp C, *et al.* Increased telomerase activity and comprehensive lifestyle changes: A pilot study. *Lancet Oncol*. 2008; 9: 1048-57.
136. Samani NJ, van der Harst P. Biological ageing and cardiovascular disease. *Heart*. 2008; 94: 537-9.
137. Van der Harst P, van der Steege G, de Boer RA, Voors AA, Hall AS, Mulder MJ, *et al.* Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. *J Am Coll Cardiol*. 2007; 49: 1459-64.
138. Collerton J, Martin-Ruiz C, Kenny A, Barrass K, von Zglinicki T, Kirkwood T, *et al.* Telomere length is associated with left ventricular function in the oldest old: The Newcastle 85+ study. *Eur Heart J*. 2007; 28: 172-6.
139. Haver VG, Mateo Leach I, Kjekshus J, Fox JC, Wedel H, Wikstrand J, *et al.* Telomere length and outcomes in ischaemic heart failure: data from The Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA). *Eur J Heart Fail*. 2015; 17: 313-9.
140. Kajstura J, Rota M, Cappelletta D, Ogorek B, Arranto C, Bai Y, *et al.* Cardiomyogenesis in the aging and failing human heart. *Circulation*. 2012; 126: 1869-81.

141. Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. *Circulation*. 2002; 105:1541-4.
142. Oh H, Taffet GE, Youker KA, Entman ML, Overbeek PA, Michael LH, *et al*. Telomerase reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. *Proc Natl Acad Sci USA*. 2001; 98: 10308-13.
143. Jaskelioff M, Muller FL, Paik J-H, Thomas E, Jiang S, Adams AC, *et al*. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. *Nature*. 2011; 469: 102-6.
144. Bär C, Bernardes de Jesus B, Serrano R, Tejera A, Ayuso E, Jimenez V, *et al*. Telomerase expression confers cardioprotection in the adult mouse heart after acute myocardial infarction. *Nat Commun*. 2014; 5:5863. doi: 10.1038/ncomms6863.
145. Harley CB, Liu W, Flom PL, Raffaele JM. A natural product telomerase activator as part of a health maintenance program: metabolic and cardiovascular response. *Rejuvenation Res*. 2013; 16: 386-95.
146. Halabi CM, Beyer AM, de Lange WJ, Keen HL, Baumbach GL, Faraci FM, *et al*. Interference with PPAR [gamma] function in smooth muscle causes vascular dysfunction and hypertension. *Cell Metab*. 2008; 7: 215-26.
147. Beyer AM, Baumbach GL, Halabi CM, Modrick ML, Lynch CM, Gerhold TD, *et al*. Endothelium-specific interference with peroxisome proliferator activated receptor gamma causes cerebral vascular dysfunction in response to a high-fat diet. *Circ Res*. 2008; 103: 654-61.
148. Beyer AM, Baumbach GL, Halabi CM, Modrick ML, Lynch CM, Gerhold TD, *et al*. Interference with PPAR {gamma} signaling causes cerebral vascular dysfunction, hypertrophy, and remodeling. *Hypertension*. 2008; 51: 867-71.
149. Huang P, Riordan SM, Heruth DP, Grigoryev DN, Zhang LQ, Ye SQ. A critical role of nicotinamide phosphoribosyltransferase in human telomerase reverse transcriptase induction by resveratrol in aortic smooth muscle cells. *Oncotarget*. 2015; 6: 10812-24.
150. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, *et al*. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature*. 2006; 444: 337-42.
151. Eitan E, Tichon A, Gazit A, Gitler D, Slavin S, Priel E. Novel telomerase-increasing compound in mouse brain delays the onset of amyotrophic lateral sclerosis. *EMBO Mol Med*. 2012; 4: 313-29.
152. Tichon A, Eitan E, Kurkalli BG, Braiman A, Gazit A, Slavin S, *et al*. Oxidative stress protection by novel telomerase activators in mesenchymal stem cells derived from healthy and diseased individuals. *Curr Mol Med*. 2013; 13: 1010-22.
153. Beyer AM, Freed JK, Durand MJ, Riedel M, Ait-Aissa K, Green P, *et al*. Critical role for telomerase in the mechanism of flow mediated dilation in the human microcirculation. *Circ Res*. 2016; 118: 856-66.