The roles of senescence and telomere shortening in cardiovascular disease

Frej Fyhrquist, Outi Saijonmaa and Timo Strandberg

Abstract | Cellular senescence, defined as arrest during the cell cycle (G_0), is involved in the complex process of the biological ageing of tissues, organs, and organisms. Senescence is driven by many factors including oxidative stress, the DNA damage and repair response, inflammation, mitogenic signals, and telomere shortening. Telomeres are shortened by each cell division until a critical length is reached and dysfunction ensues. DNA-repair pathways are then recruited and cells enter senescence, losing their capacity to proliferate. In addition to cell division, factors causing telomere shortening include DNA damage, inflammation, and oxidative stress. Both cardiovascular risk factors and common cardiovascular diseases, such as atherosclerosis, heart failure, and hypertension, are associated with short leucocyte telomeres, but causality remains undetermined. Telomere length does not satisfy strict criteria for being a biomarker of ageing, but adds predictive power to that of chronological age, and can be considered a marker of cardiovascular ageing. The 'senescence-associated secretory phenotype' of senescent cells exerts a wide range of autocrine and paracrine activities aimed at tissue repair, but which also fuel degenerative and proliferative alterations that contribute to cardiovascular disease. In this Review, the relationship between telomere shortening, senescence, and cardiovascular disease is discussed.

Fyhrquist, F. *et al. Nat. Rev. Cardiol.* advance online publication 12 March 2013; doi:10.1038/nrcardio.2013.30

Introduction

Cellular senescence, a state characterized by cell-cycle arrest, but maintained metabolic activity, can be induced by various factors. Replicative senescence is considered to be protective against malignant transformation, because senescent cells are unable to divide, and eventually undergo apoptosis. Replicative senescence is driven by shortening and dysfunction of telomeres. Cellular senescence can also be triggered independently of telomere status by DNA damage, mitogenic signals from activated oncogenes, and by stress factors causing chromatin changes (Figure 1). Several lines of evidence suggest causal connections between cellular senescence and age-related cardiovascular disease, as has been reviewed previously;[1–5] however, the question of causality is still debated.

Telomere length is widely considered to be a marker of biological ageing, although the parameter does not satisfy the strict criteria of the American Federation for Aging Research.[6,7] Telomere length is largely inherited, and is modulated by a variety of intrinsic and environmental factors throughout life. The majority of factors that can modulate telomere length are also cardiovascular risk factors. In clinical studies, an association between short leucocyte telomere length (LTL) and cardiovascular disorders, including atherosclerosis, myocardial infarction, heart failure, and hypertension, has been repeatedly shown.[8,9] Despite substantial broadening of our knowledge of telomere biology and mechanisms of senescence, the relationship between telomere length and senescence, and their role in cardiovascular disease, is not fully understood. In this Review, we discuss the roles and inter-relationship of senescence and telomere length in cardiovascular disease.

Competing interests
The authors declare no competing interests.

Cellular senescence

Proliferative arrest is a hallmark of cellular senescence. Two types of cellular senescence have been recognized: replicative senescence and stress-induced premature senescence. Replicative senescence occurs as a function of cell division and is characterized by shortening of the telomeres that protect the ends of chromosomes (Figure 1). Stress-induced premature senescence is triggered by external stimuli, including oxidative stress, mitogenic oncogenes, and irradiation,[10,11] which leads to an acute form of senescence, not usually associated with telomere shortening. Inducers of replicative senescence are factors that accelerate telomere shortening, including genes, cell division, DNA damage, and oxidative stress. These factors elicit DNA damage response signals that arise from detectable nuclear foci or 'DNA-SCARS' (DNA segments with chromatin alterations reinforcing senescence),[12–14] and trigger the tumour-suppressor pathways p16 and p53.[15] Senescent cells display irreversible proliferative arrest and altered morphology, including cellular enlargement, flattening, and vacuolization.[16] Senescent cells also have increased expression of senescence-associated β-galactosidase and the tumour suppressor cyclin-dependent kinase inhibitor 2a (also known as $p16^{Ink4a}$).

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Key points

- Cellular senescence—arrest during the cell cycle (G_0)—is involved in the ageing process, and driven by oxidative stress, DNA damage and repair response, inflammation, mitogenic signals, and telomere shortening
- Cellular senescence parallels the development of atherosclerosis and other pathologies in the vasculature and heart and is, therefore, likely to have a pivotal role in cardiovascular disease
- Telomere length, usually measured as leucocyte telomere length, is widely considered a marker of biological ageing, is largely inherited, and is modulated by various intrinsic and environmental factors throughout life
- Endogenous factors causing telomere attrition include ageing, cell division, genetic factors, DNA damage, inflammation, and oxidative stress; telomere attrition can be retarded by genetic factors, telomerase, oestrogen, and antioxidants
- Environmental factors associated with telomere shortening include poor lifestyle (smoking, excess calories, sedentary lifestyle, alcohol abuse), and severe mental stress, whereas healthy lifestyle is associated with maintenance of long telomeres
- Telomere length seems to have a key role in cardiovascular disease by driving cells into cell-cycle arrest, senescence, and ultimately apoptosis

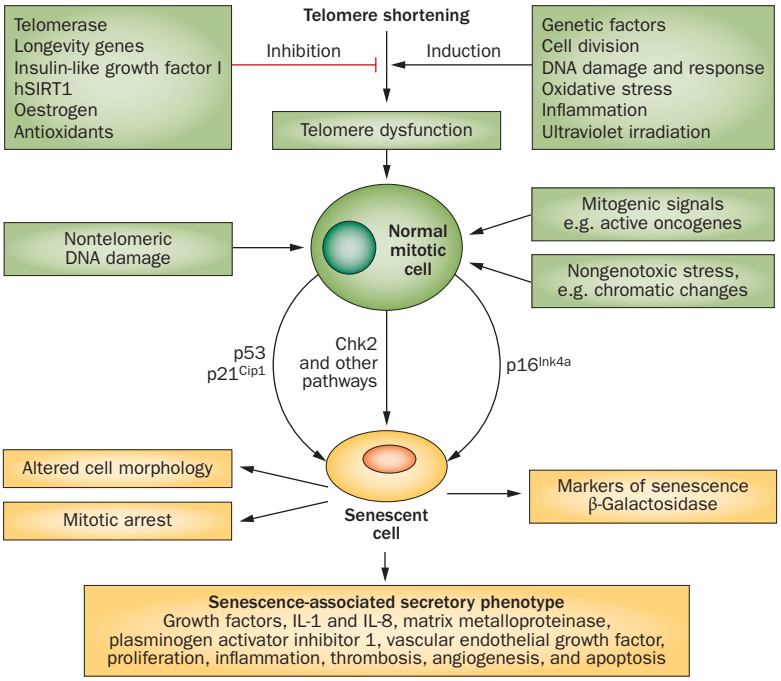

Figure 1 | Telomere shortening and cellular senescence. Factors affecting the rate of telomere shortening are depicted. In addition to telomere shortening, other stressors that induce progression of mitotic cells to senescence include strong mitogenic signals from oncogenes, nontelomeric DNA damage, and structural chromatin alterations. These factors trigger the tumour suppressor pathways p16, $p21^{Cip1}$, and p53. Characteristics of senescent cells include mitotic arrest, altered morphology, and—in the senescence-associated secretory phenotype—many autocrine and endocrine activities potentially involved in tissue repair, proliferation, inflammation, and apoptosis. Chk2 is activated in response to DNA damage and is involved in cell-cycle arrest. Abbreviations: Chk2, checkpoint signalling kinase 2; hSIRT1, NAD-dependent protein deacetylase sirtuin-1.

Cellular senescence occurs *in vivo* in the vasculature, as reviewed previously.[5,17] Increased numbers of senescent cells are found in vascular smooth muscle cells, endothelial cells, and monocytes and macrophages from aged arteries and atherosclerotic plaques.[5,17,18] Shortening of telomere length with age has been shown in tissue and endothelial specimens from various regions of the human vasculature.[19–21] Interestingly, telomere attrition is most pronounced in areas known to develop atherosclerotic lesions, such as the carotid artery[22,23] and aortic wall.[24] Therefore, mounting evidence strongly suggests a link between cellular senescence and atherosclerosis. However, whether senescent cells have a causal role in age-related cardiovascular disease remains unresolved.

Preliminary evidence that senescent cells might, indeed, cause age-related disease comes from a study in which $p16^{Ink4a}$-positive senescent cells were genetically removed from progeroid BubR1 mice. The onset and severity of the premature-ageing phenotype were delayed.[25] However, BubR1 mice do not have a phenotype of cardiovascular disease, so similar studies in animal models that have such a cardiovascular phenotype are awaited.

Cells containing DNA-SCARS of the senescence-associated secretory phenotype (Figure 1) display autocrine and paracrine activities. They secrete IL-6 and IL-8, intercellular adhesion molecule 1, metalloproteases, monocyte attractants, plasminogen activator inhibitor 1, and vascular endothelial growth factor.[13,17] Via these secretory activities, cells with the senescence-associated secretory phenotype contribute to both degenerative and proliferative age-related alterations by causing a chronic state of inflammation, remodelling, and tissue repair. Senescent vascular endothelial cells have a reduced level of nitric oxide synthase,[16,26,27] a change that promotes endothelial dysfunction. The accumulation of senescent cells with age might, therefore, contribute to the initiation and progression of atherosclerosis.

Telomere structure and function

Telomeres are located at the ends of chromosomal DNA (Figure 2). They comprise thousands of tandem repeats of the TTAGGG sequence (9–15 kb in humans) and associated nucleoproteins: adrenocortical dysplasia protein homologue (also known as TIN2-interacting protein), protection of telomeres protein 1, telomeric repeat-binding factors 1 and 2, telomeric repeat-binding factor 2-interacting protein 1, and TERF1-interacting nuclear factor 2.[28–30] These nucleoproteins form the shelterin complex.[31] Another telomere-capping complex comprised of three proteins: Cd13, Stn1, and Ten1 (collectively known as CST), is present in yeast.[32] A CST-like complex has also been found in humans, and genome-wide association studies (GWAS) have shown that the genetic loci of the CST-complex are associated with short telomere length.[33,34] Therefore, the CST-complex is likely to be involved in telomere maintenance in humans.

Telomeres participate in the maintenance of genomic and cellular stability and replication. They protect the genome from degradation, unwanted recombination, and chromosomal fusion.[31] Owing to an inability to maintain the length of the 3' overhang (single-stranded DNA), telomeres shorten by 30–150 base pairs with each cell division.[28] When a critical telomere length is reached, shelterin proteins cannot be adequately recruited to maintain the protective nucleotide T-loop. The DNA damage repair system and cell-cycle inhibitors, including $p16^{Ink4a}$, $p21^{Cip1}$, and p53, are activated (Figure 1). The cell

then enters replicative senescence, which is followed by apoptosis.[35] When only a few telomeres are critically short, they form end-associations, which leads to a DNA-damage signal, and results in replicative senescence, also called the M1 stage.[36] In the absence of cell-cycle checkpoint pathways (such as p53 or p16/retinoblastoma-associated protein, a key regulator of entry into cell division and which acts as a tumour suppressor), cells bypass M1 senescence ('senescence escape'). Consequently, telomeres continue to shorten, which results in crisis, also called the M2 stage. M2 is characterized by chromosome end-fusions, mitotic catastrophe, and multiple apoptotic cells. Telomeres are considered to be a mitotic clock. Notably, not only shortening of the telomere, but also disruption of components and interactions within the shelterin complex can initiate telomere dysfunction and genomic instability.[37,38] Consequently, telomere shortening is not necessarily a prerequisite for telomere dysfunction.

Telomerase reverse transcriptase (commonly referred to as telomerase) is associated with the telomere complex, and catalyses DNA synthesis to maintain telomere length. Germ cells, stem cells, and most cancer cells have a high level of telomerase activity to avoid senescence, whereas somatic cells have a low or undetectable level of telomerase activity. Human telomerase consists of an RNA component (known as TERC), which forms a template, and a catalytic subunit, telomerase reverse transcriptase (TERT). This machinery generates new telomeric DNA repeats.[30,35] In addition to the maintenance of telomere length and function, TERT can affect chromatin structure and promote activation of resting stem cells.[39] Furthermore, TERT can influence Wnt signalling, which is involved in angiogenesis, cardiac hypertrophy, heart failure, and ageing.[40] Like telomere length, telomerase is under both genetic[41] and environmental control.[42] Epigenetic modifications have also been implicated in the transcriptional regulation of TERT.[43]

Measurement of telomere length

The most-commonly used assay of telomere length in clinical and epidemiological studies is to measure LTL in DNA from blood, because leucocytes are easily obtainable. Analysis of the terminal restriction fragment by Southern blot is well suited to studies of large populations, and remains the gold-standard assay,[44,45] but requires large amounts of DNA (2–3 μg per assay) and is time consuming. The coefficient of variation with this method can be as low as 2%.[44,45] Southern blotting offers the advantage of measuring not only mean LTL, but also the proportion of very short LTL, which can add useful information.[48,49] The shortest telomeres are linked more closely with cellular senescence than are the longer telomeres because, regardless of mean telomere length, one critically short telomere can force a cell to enter senescence.[48–50]

Another commonly used method to measure LTL is the quantitative polymerase chain reaction. The telomere signals in experimental DNA samples are measured in one set of reaction wells, and the single copy gene signals are assayed in separate wells, in comparison to reference

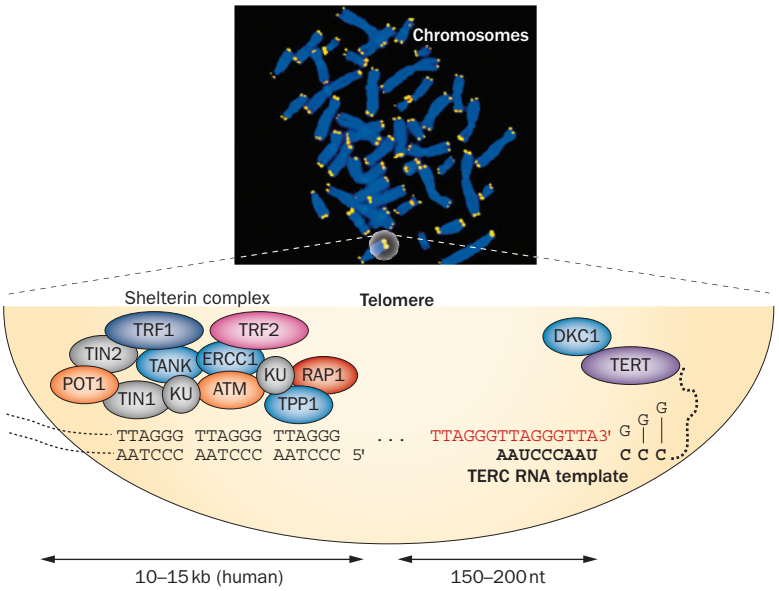

Figure 2 | Human chromosomes and simplified telomere structure. Telomeres, located at the ends of all chromosomes, are shown in yellow using fluorescent *in situ* hybridization. They are composed of thousands of tandem DNA repeats: TTAGGG in the leading 3' strand, and CCCTAA in the lagging 5' strand. Associated protective proteins, forming the shelterin complex, include: POT1, RAP1, TIN1, TIN2, TPP1, TRF1, and TRF2. The 3' end of the telomeric leading strand is a single-strand overhang, which is incompletely reproduced at cell division, which leads to telomere shortening. Abbreviations: ATM, serine-protein kinase ATM (also known as ataxia teleangiectasia mutated); DKC1, H/ACA ribonucleoprotein complex subunit 4 (also known as dyskerin); ERCC1, DNA excision repair protein ERCC-1; KU, Ku70 multifunctional protein involved in telomere maintenance; POT1, protection of telomeres protein 1; RAP1, telomeric repeat-binding factor 2-interacting protein 1; TANK, tankyrase; TERC, telomerase RNA component; TERT, telomerase reverse transcriptase; TIN, TERF1-interacting nuclear protein; TPP1, adrenocortical dysplasia protein homologue (also known as TIN2-interacting protein 1); TRF, telomeric repeat-binding factor.

DNA. The ratio of these two signals is proportional to the average LTL. This method is well suited for the study of large populations,[44] requires less DNA than Southern blotting, and can reach a coefficient of variation <4%.[23] Various techniques of telomere-length measurement, some of them not suitable for studying large numbers of individuals, have been reviewed previously.[44,45,50]

A substantial synchrony exists within individuals between LTL and telomere length in somatic cells.[51] Therefore, LTL can serve as a proxy for telomere length in, for example, vascular cells, whereas LTL might not accurately reflect telomere length in poorly proliferating tissues, such as brain, fat, and liver.

Regulation of telomere length

Genetic factors

Telomere dynamics are complex and strongly influenced by genetic factors.[52] Hereditability of human telomere length has been demonstrated in several studies, as reviewed previously,[17,52] which partly explains inter-individual variation in telomere length. GWAS have identified loci associated with LTL on chromosomes 18q12.2 and 3q26, located near *TERC* and a component of the telomere-maintenance complex (CST complex

Box 1 | Endogenous factors affecting LTL

Induction of telomere shortening
- Genetic factors[33,43,52–56]
- Cell division[19,28,30]
- Age[61]
- Oxidative stress[66,67,72]
- Inflammation[1,13,66,129,131]
- Renin–angiotensin system activity[59,60]
- Total-cholesterol level (increased long term)[68]

Inhibition of telomere shortening
- Telomerase reverse transcriptase[10,29,30,35,39]
- Oestrogen[69]
- Antioxidants[66]

Abbreviation: LTL, leucocyte telomere length.

subunit CTC1), respectively.[33,34] However, GWAS have explained only a small proportion (about 1.6%) of interindividual variation in LTL.[52–54] Mutations in the telomerase components TERC or TERT are linked to dyskeratosis congenita,[11] and are associated with short telomeres and premature ageing, but not necessarily with early cardiovascular disease.[55,56] Patients with Werner syndrome have mutations in the *WRN* gene, which is involved in telomere maintenance, manifest premature ageing, myocardial infarction, and cancer at a young age.[56]

The NAD-dependent protein deacetylase sirtuin (SIRT) 1–7 family of proteins promotes survival, stress resistance, and longevity.[57] Decreased expression of SIRT1 is associated with endothelial dysfunction in arteries from aged mice and humans.[27] Interestingly, the *SIRT1* locus was positively associated with both LTL and longevity in individuals from the Louisiana Healthy Aging Study,[58] which suggests a possible link between telomere length and longevity. Short LTL was associated with the D allele of the angiotensin-converting enzyme I/D polymorphism in 1,249 patients with left ventricular hypertrophy and hypertension,[59] in accordance with the reported association between short LTL and increased activity of the renin–angiotensin system in the Framingham Heart Study.[60]

LTL throughout life

LTL is highly variable between individuals both at birth and throughout life. LTL at birth and telomere attrition during ageing are major determinants of LTL dynamics. LTL is at its longest at birth, shortens rapidly until adolescence, and then shortens at a reduced rate until old age.[61] In a study from the UK, LTL at birth was related to maternal LTL, but not to birth weight.[62] Conversely, investigators in a birth cohort study in Bangladeshi children reported that lower birth weight was associated with shorter LTL.[63] Importantly, a study involving three Finnish cohorts showed no association between body weight at birth and LTL in adult life.[64] LTL is similar in male and female neonates, and highly correlated with telomere length in umbilical artery and foreskin.[65]

Endogenous factors

Telomere attrition

Telomeres in somatic cells are shortened by each cell division.[10,28] Therefore, ageing is the main cause of telomere attrition (Box 1). Additionally, inflammation and oxidative stress are important causes of telomere shortening, and are also implicated in the ageing processes.[66] An important contributing factor to vascular senescence might be oxidized LDL cholesterol.[67] In a life-course study, a high serum level of total cholesterol in midlife (even with a subsequent low level of cholesterol in old age) was associated with reduced LTL in older men (mean age 76 years).[68]

Telomere maintenance

Telomerase counteracts telomere shortening in stem cells, germ cells, and also in cancer cells, by replacing telomere repeats at the ends of chromosomes. The majority of cancer cells have active telomerase and maintain long telomeres, which allows continued proliferation.[10,30] Oestrogen activates telomerase,[69] and inhibits telomere shortening, which probably explains why women have longer leukocyte telomeres than age-matched men. Endogenous antioxidants, such as superoxide dismutase, are thought to inhibit ageing processes and leucocyte telomere shortening.[66]

Environmental factors

Short LTL

Environmental factors associated with short LTL are related to lifestyle and cardiovascular risk factors (Box 2). Smoking and obesity might induce telomere shortening by augmenting tissue inflammation and oxidative stress.[70–72] However, in one study on cultured endothelial cells isolated from the internal mammary artery of smoking or nonsmoking patients with coronary artery disease (CAD), cellular senescence was independent of telomere length, but was clearly related to oxidative damage and markers of inflammation.[73] In smokers, therefore, endothelial senescence might be stress-induced rather than replicative. Additionally, we observed a dose-dependent, linear, inverse relationship between LTL in old age (mean 78 years) and the alcohol consumption of 622 men during the 38-year follow-up of the Helsinki Businessman Study.[74] The mechanism of telomere shortening associated with alcohol intake is currently unknown. Possible explanations include alcohol-induced oxidative stress and inflammation.[75]

An interesting novel aspect of telomere biology is the reported association between severe mental stress and both short LTL and decreased leucocyte telomerase activity.[76] Lifetime exposure to depression has been shown to be associated with short LTL and increased oxidative stress and inflammation.[77] Moreover, individuals who have suffered physical abuse during childhood have shorter mean LTL than do control individuals.[78] Psychological stress, depression, and anxiety disorders have been linked to increased oxidative stress (as reviewed previously[79]), which offers a possible explanation for short LTL in these conditions. However, the number of studies on the relationship between LTL and mental stress and disorders is still limited, the number of study participants is usually low, and the mechanisms by which LTL can be affected by mental stress remain hypothetical.

Long LTL

Environmental factors associated with a reduced rate of LTL shortening have been reported in several studies. In patients with CAD, an increased level of marine ω-3 fatty acid was associated with a decreased rate of LTL shortening over 5 years.[80] Furthermore, increased blood concentrations of vitamin C, 25-hydroxyvitamin D, and vitamin E were related to reduced LTL shortening.[81,82] These observations were thought to reflect antioxidant activity (vitamins C and E) or inhibition of inflammation (vitamin D).

In a large study involving 2,401 white twins (249 men and 2,152 women), higher-intensity physical activity was associated with longer LTL.[83] In mice randomly allocated to voluntary running or no access to a running wheel for 3 weeks, exercise upregulated telomerase activity in the aorta and circulating mononuclear cells, increased vascular expression of telomeric repeat-binding factor 2, and reduced the expression of vascular apoptosis regulators.[84] The investigators reported that endothelial nitric oxide synthase and TERT synergize to confer resistance to endothelial stress after physical activity. Furthermore, they showed similarly favourable changes in circulating leucocytes from well-trained track and field athletes compared with untrained control individuals. They concluded that physical activity regulates telomere-stabilizing proteins in mice and humans.

A greater number of self-reported years of healthy life was associated with longer LTL in a population-based study.[85] In a cross-sectional study of 994 outpatients with stable CAD, poor physical fitness was related to short LTL.[86] A pilot study in 30 patients with prostate cancer showed that an intensive improvement in lifestyle lasting 3 months increased leucocyte telomerase activity, which provides further support that lifestyle improvement can protect telomeres.[87] Conversely, lowering of the LDL-cholesterol level with statin treatment attenuated the association between short LTL and increased risk of CAD in middle-aged men (aged 45–64 years) at risk of cardiovascular disease.[88] Short LTL, which has been shown to be associated with CAD and atherosclerosis,[8,9,22] might, therefore, predict those individuals who will particularly benefit from statin treatment.

Box 2 | Environmental factors affecting LTL

Induction of telomere shortening
- Physical violence during childhood[78]
- Smoking[70,71]
- Alcohol abuse[74]
- Obesity[70,71]
- Sedentary lifestyle[83]
- Mental stress[76,77]

Inhibition of telomere shortening
- Healthy lifestyle[85–87]
- Oestrogen treatment[69]
- Vitamins C, D, and E[81,82]
- ω-3 Fatty acids[80]
- Statin treatment[88,135]

Abbreviation: LTL, leucocyte telomere length.

Stem cells

Stem cells have an important role in maintaining tissue integrity by replenishing senescent cells or repairing tissue damage. Exhaustion of the stem-cell pool contributes to the process of ageing by reducing the efficiency of repair processes in vascular tissues and the heart.[89] The loss of stem-cell function through telomere attrition has been extensively documented.[90] A high degree of synchrony in telomere length exists between leucocytes throughout the human lifespan.[91] Therefore, individuals with a short mean LTL also have short telomeres in subsets of leucocytes, and mean LTL reflects telomere length in both haematopoietic stem and progenitor cells. Shortening LTL is, therefore, likely to reflect declining haematopoietic stem-cell reserves, which are important for tissue repair.[52] Germ cells, but not stem cells, have sufficient telomerase activity to prevent telomere shortening with age.[83] Therefore, shortening of LTL might mirror decreased stem-cell telomerase activity, leading to reduced starting telomere length and deterioration of haematopoietic stem-cell reserves.

Initially, characteristic features of stem cells include the expression of active telomerase and a stable telomere length.[92] However, telomere attrition can also occur in stem cells, for example in CAD[93] or heart failure. Shortening of telomeres has been shown in cardiac stem cells from human failing hearts.[94,95] Cardiac stem cells with the shortest telomeres, but not those with longer telomeres, expressed $p16^{Ink4a}$—a DNA-repair protein and a marker of cellular senescence. Therefore, telomere attrition seems to be an important trigger of cardiac stem-cell senescence, which, in turn, leads to a reduced cardiac stem-cell pool and is related to a diminished rate of cardiomyocyte regeneration with increasing age.[96]

Elongation of telomeres

In some longitudinal studies, LTL elongation instead of shortening with time has been observed in some individuals. In a longitudinal Swedish study involving repeated sampling of DNA from 50 individuals, LTL fluctuated over time, which lead the investigators to postulate an oscillation hypothesis of LTL.[97] In the Heart and Soul Study,[98] LTL was measured at baseline and after 6 years in 608 patients with CAD. Mean LTL decreased by 0.2 kb, but only half of the patients experienced telomere shortening.[98] These observations might reflect the large variety of endogenous and environmental factors that affect telomere length. Methodological factors and the varying composition of blood leucocytes with heterogeneous LTL should also be considered.[45] In addition to other confounding factors, the cross-sectional nature of most studies on LTL, and the often limited number of individuals involved in these studies, necessitates caution when interpreting results.

LTL and cardiovascular disease

Animal experiments

Terc knock-out ($Terc^{-/-}$) mice are an interesting model for studying the consequences of accelerated telomere attrition in cardiovascular disease.[99] $Terc^{-/-}$ mice inbred for five generations develop hypertension and impaired left ventricular function, along with gradually shortening telomeres with each generation.[99] However, $Terc^{-/-}$ mice

are a poor model for human atherosclerosis and ageing, because their comparatively favourable lipoprotein profile (high HDL-cholesterol level, and low LDL-cholesterol and VLDL-cholesterol levels) and short lifespan render them resistant to atherosclerosis.[100,101] Mice deficient in apolipoprotein E (*Apoe*$^{-/-}$) develop lesions that resemble human atherosclerotic lesions, and which are exacerbated by feeding the mice with a high-cholesterol, Western-type diet. However, *Apoe*$^{-/-}$/*Terc*$^{-/-}$ mice display even less atherosclerosis than *Apoe*$^{-/-}$ mice.[102] This observation underscores the problem with translating results obtained in mice to humans, and precludes making an analogy between these species regarding ageing and atherosclerosis. Conversely, studies in *Terc*$^{-/-}$ mice showed that these animals develop cardiomyopathy with systolic and diastolic dysfunction, heart failure, increased expression of p53, and cardiomyocyte apoptosis.[103] However, differences in telomere biology between mice and humans should be remembered when interpreting these results.

Human cultured cells

In human cultured aortic endothelial cells, introduction of the telomerase component telomeric repeat-binding factor 2 extended the cellular lifespan and inhibited functional alterations associated with cellular senescence.[16] This elegant study indicates an important role for telomere dysfunction in the triggering of senescence in vascular endothelial cells. Telomere dysfunction and vascular senescence are associated with increased formation of reactive oxygen species, decreased production of nitric oxide, and elevated levels of proinflammatory adhesion molecules and β-galactosidase.[104,105] In human cultured endothelial cells from umbilical cord, telomere length was shortened by 90 base pairs per population doubling, along with an increase in the number of senescent cells expressing β-galactosidase, which suggests a causal link between telomere shortening and cellular senescence.[106]

Human vascular tissues

In vascular tissue biopsies, reduced telomere length was related to the presence of atherosclerosis. Longer telomeres were observed in the walls of saphenous veins and mammary arteries than in aortic samples.[20,23,24] Furthermore, shorter telomeres were found in arterial wall samples with atherosclerotic lesions than in arterial wall samples without atherosclerosis.[23,24] Moreover, endothelial cells from atherosclerotic plaques express an increased level of β-galactosidase, and have shorter telomeres than control cells.[16,105] These results suggest that local factors associated with haemodynamics and atherosclerosis regulate telomere length in the arterial wall. Accordingly, shortened telomere length has been found in atherosclerotic lesions from regions of increased haemodynamic stress.[19,20,22,23]

Heart tissue

The myocardium has previously been considered a postmitotic organ, composed of terminally differentiated myocytes. However, observations of active telomerase in the left ventricular myocardium of neonatal, young adult, and senescent rats,[108] and reports of telomerase activation and telomere erosion in human myocardium,[94–96] have prompted a reinterpretation of cardiac biology.[109] Several studies have clearly indicated that the heart undergoes some regeneration of cardiomyocytes throughout life.[109–111] This observation implies the recruitment and proliferation of cardiac stem cells, and a role for cardiac telomeres. The importance of the telomere–telomerase axis and cellular senescence in cardiac disease has been emphasized by several lines of evidence.[2,109,111,112] Therefore, the ageing heart contains an increased number of senescent cardiomyocytes, as defined by short telomeres and the expression of p16, p21, p53, and β-galactosidase.[112] Furthermore, endomyocardial biopsies from patients with heart failure have revealed shortened telomeres, and increased cellular senescence and cell death,[94] in accordance with the finding of short LTL in patients with heart failure.[113]

A substantial decline in mitochondrial function, including compromised oxidative phosphorylation, takes place in the ageing heart.[114] Cardiomyocytes have high energy requirements and are exceptionally rich in mitochondria. According to the mitochondrial hypothesis, heart failure is a consequence of mitochondrial dysfunction.[115,116] The hypothesis of telomere dysfunction might converge with that of mitochondrial dysfunction. The ageing heart contains increased numbers of senescent cardiomyocytes that express p16, p21, and p53, and which have short telomeres.[112] Ageing mitochondria produce increasing amounts of oxidative radicals,[114] which contribute to cardiac telomere shortening and cellular senescence. These changes are paralleled by a decrease in cardiomyocyte regenerative activity from 1% per year at the age of 20 years to 0.4% per year at the age of 75 years.[96] The resulting loss of functioning cardiomyocytes might result in age-related heart diseases, such as diastolic heart failure, atrial fibrillation, and ischaemia.[117,118] Telomere dysfunction and cellular senescence might, therefore, have key roles in heart disease.

Clinical studies

Associations between shortened LTL and atherosclerosis,[8,9,22,119] risk of myocardial infarction,[120,121] and mortality in patients with CAD[121] have been reported in several studies. LTL was shorter in patients aged <50 years with a myocardial infarction than in healthy, age-matched, control individuals.[120] In patients with congestive heart failure, particularly of ischaemic aetiology, LTL was again shorter than in age-matched, healthy controls.[113] An association with short LTL has also been reported in individuals with high pulse pressure,[122] women with transient ischaemic attack,[123] and patients with stroke.[124] However, other researchers have reported no association between LTL and stroke.[123,125]

The telomere hypothesis

As discussed above, short LTL in neonates is associated with short maternal LTL,[61] which implies heritability of

LTL. Heritability is also indicated by the observation of short LTL in the healthy offspring ($n = 45$) of individuals with CAD in a pilot study.[126] This finding has been partially confirmed in a study that showed shorter LTL in the offspring of patients with ischaemic heart failure than in the offspring of healthy controls, but no difference between the two groups in telomere length in CD34+ mononuclear cells or buccal cells.[127] These data lend some support to the telomere hypothesis that short telomeres might be causally linked to cardiovascular disease.[88] However, investigators in the large ($n = 2,509$), longitudinal Asklepios Study[128] reported that telomeres were not shortened in individuals with a family history of cardiovascular disease. Importantly, a study in three Finnish cohorts showed no association between body size at birth and LTL in adult life.[64] The outcomes of these last two studies cast serious doubt on the validity of the telomere hypothesis. We conclude that LTL is unlikely to be a link between body size at birth and age-related cardiovascular disease later in life.

Association between LTL and cardiac disease

The vast majority of clinical studies have shown an association between shortening LTL with age and cardiovascular disease and risk factors. However, association does not prove causality, and could also indicate that cardiovascular diseases cause telomere attrition. Alternatively, both telomere attrition and cardiovascular disease might be caused by common risk factors (smoking, hypertension, high total cholesterol level, obesity, physical inactivity), which contribute to inflammation and oxidative stress.[66,72] We endorse a unifying hypothesis, partly proposed previously,[129] that leukocyte telomere shortening caused by ageing (a major risk factor for atherosclerosis) is accelerated by cardiovascular risk factors and disease, and reflects the overall burden of inflammatory, oxidative, and mechanical stress induced by increased heart rate on the cardiovascular system.[130] In addition to age-associated remodelling of the vascular wall, endothelial function declines, the production of nitric oxide decreases, and the formation of oxidative species increases with age,[131] which could cause further telomere attrition.

In a study on cultured endothelial cells from patients with CAD, the early onset of endothelial senescence was more-closely associated with years of exposure to cardiovascular risk factors—particularly hypertension—than with the age of the donor.[129] This study showed an association been cellular senescence and 'biological', rather than chronological, age. Cardiovascular risk factors seemed to accelerate the biological ageing of endothelial cells via both telomere-dependent replicative senescence and nontelomere-dependent stress-induced pathways, both likely to promote the development of atherosclerosis. In a subsequent study, cultured endothelial cells from internal mammary arteries of patients with CAD were treated with a combination of an antioxidant (*N*-acetylcysteine) and telomerase (*TERT*) overexpression.[132] The results indicated that oxidative stress and DNA damage are responsible for endothelial cell senescence, which telomerase overexpression could not override.[132] These studies emphasize the importance of the cumulative burden of cardiovascular risk and oxidative stress to the development of endothelial senescence and its likely contribution to atherosclerosis.

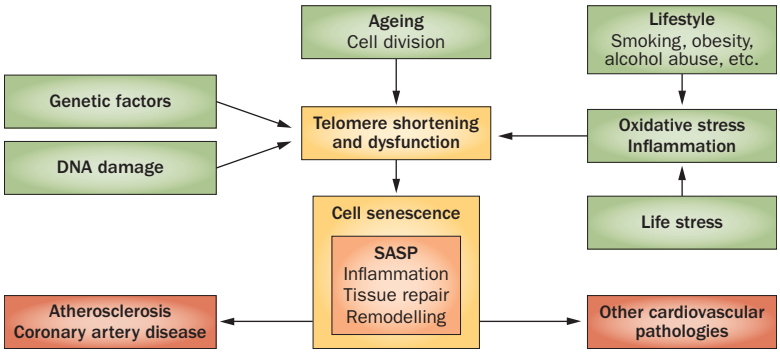

Figure 3 | Telomere attrition, cellular senescence, and cardiovascular disease. Telomere attrition and cellular senescence are potentially involved in the pathway between cardiovascular risk factors and cardiovascular disease. SASP-generated inflammation and oxidative stress promote cardiovascular pathologies. Arrows indicate the direction of actions and associations, not proven causality. Abbreviation: SASP, senescence-associated secretory phenotype.

Therapeutic considerations

Healthy lifestyle is a cornerstone of the prevention and treatment of most cardiovascular diseases, is cheap, and has no adverse effects. The beneficial effects of a healthy lifestyle have been convincingly demonstrated in common, age-related diseases, including atherosclerosis, CAD, hypercholesterolaemia, hypertension, and type 2 diabetes mellitus—all of which are associated with short LTL. But are these beneficial effects mediated by improved telomere maintenance and function? No definite response can currently be given, but preliminary studies suggest that the answer might be 'yes'.[87] Additional prospective intervention studies are needed to clarify the role of a healthy lifestyle in the maintenance of telomere function and cardiovascular health.

Drugs that target the maintenance of telomeres without unacceptable adverse effects might, in theory, offer novel strategies for the prevention and treatment of age-related cardiovascular disease. In fact, such drugs have been used with great success for decades. Inhibitors of the renin–angiotensin–aldosterone system[133,134] and statins[88,135] have been associated with improved maintenance of telomere length in some studies. Whether these drugs exert some of their beneficial effects via mechanisms related to telomere maintenance is unknown. Alternatively, they might delay endothelial senescence by reducing the cumulative burden of cardiovascular risk. Targeting telomerase seems logical, but is controversial because cancer cells express active telomerase, and inhibitors of telomerase have been developed for the treatment of cancer.[136] Therefore, drugs that activate telomerase systemically might increase the risk of cancer.

Conclusions

Although conclusive evidence is lacking, cellular senescence is likely to have a pivotal role in cardiovascular

disease. Cellular senescence is driven by telomere attrition and dysfunction, but also by factors independent of telomere length. Senescence results in mitotic and growth arrest and evokes a chronic inflammatory state, including increased oxidative stress, expression of growth factors, cytokines, adhesion molecules, and proteases—all intended at tissue and functional repair, but often more detrimental than beneficial (Figures 1 and 3). Cells with the senescence-associated secretory phenotype produce an array of autocrine and paracrine factors that promote cellular proliferation, migration, invasion, tissue remodelling, inflammation, and oxidative stress. These processes, mostly aimed at tissue repair, might drive cardiovascular pathologies, such as endothelial dysfunction, arterial stiffening and fibrosis, intimal thickening, atherosclerosis, cardiovascular remodelling, arrhythmias, and heart failure.

Telomere length is widely considered to be a marker of both general and cardiovascular ageing, although does not satisfy the strict criteria for markers of biological ageing. The main roles of telomere length in cardiovascular disease are as a 'mitotic clock', shortening with each cell division, and as a sensor of other factors that affect telomere length. Both endogenous and environmental cardiovascular risk factors mediate detrimental telomere shortening. Telomere attrition and dysfunction have a central role in the machinery that drives mitotic cells into senescence and might, therefore, be regarded as a link between cellular senescence and cardiovascular disease. However, the extent to which telomere attrition is a cause or consequence of cardiovascular disease is debatable. Modification of environmental factors that affect telomere maintenance, such as a detrimental lifestyle and mental stress, is likely to be beneficial. Carefully designed, longitudinal studies to examine the relationship between telomere length and cardiovascular disease are required.

Review criteria

A search for original articles was performed in the PubMed database using the following key terms: "senescence", "telomere", and "cardiovascular disease", either alone or in combination. All articles selected were English language, full-text papers, with no restriction applied to the date of publication.

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Author contributions

F. Fyhrquist and O. Saijonmaa researched data for the article, and all the authors contributed substantially to discussion of its content. F. Fyhrquist and O. Saijonmaa wrote the article, and all the authors reviewed and edited the manuscript before submission.