Reviews

Telomeres and Cardiovascular Disease
Does Size Matter?

Antonio L. Serrano, Vicente Andrés

Abstract—Telomeres—the specialized DNA-protein structures at the ends of eukaryotic chromosomes—are essential for maintaining genome stability and integrity and for extended proliferative life span in both cultured cells and in the whole organism. Telomerase and additional telomere-associated proteins are necessary for preserving telomeric DNA length. Age-dependent telomere shortening in most somatic cells, including vascular endothelial cells, smooth muscle cells, and cardiomyocytes, is thought to impair cellular function and viability of the aged organism. Telomere dysfunction is emerging as an important factor in the pathogenesis of hypertension, atherosclerosis, and heart failure. In this Review, we discuss present studies on telomeres and telomere-associated proteins in cardiovascular pathobiology and their implications for therapeutics. (Circ Res. 2004;94:575-584.)

Key Words: telomeres ■ atherosclerosis ■ heart disease ■ hypertension ■ diabetes

Telomeres are specialized DNA-protein complexes located at the ends of linear chromosomes of eukaryotes that preserve genome integrity and stability by preventing the recognition of chromosomal ends as double-stranded DNA breaks. The telomeric complex is composed of noncoding double-stranded repeats of G-rich tandem DNA sequences (TTAGGG in humans) that are extended several thousand base pairs and end in a 3' single-stranded overhang, the enzyme telomerase, and several associated proteins with structural and regulatory roles that participate in the control of telomere length and capping (ie, TRF1, TRF2, and Ku86) (Figures 1A and 1B). Telomerase has two components, a catalytic telomerase reverse transcriptase (TERT) and a telomerase RNA component (Terc) that serves as a template for the synthesis of new telomeric DNA repeats (Figure 1B). The telomeric structure and the complex regulation of telomere dynamics is thoroughly discussed elsewhere.1,2

Most adult somatic cells exhibit low or absent telomerase activity and thus experience progressive telomere attrition with each mitotic cycle, both in cell culture as a function of population doublings and during aging of the whole organism3-5 (Figure 1C). In contrast, germ and tumor cells maintain high telomerase activity and long telomeres and thus have an extended proliferative potential. Notably, forced overexpression of TERT inhibits replicative senescence and extends life span in numerous cell types. The Table summarizes studies on telomeres and telomerase in the biology of cultured cardiomyocytes, smooth muscle cells (SMCs), and endothelial cells (ECs). Notably, telomere shortening is accelerated in human premature aging syndromes (ie, Werner syndrome, ataxia telangiectasia, and dyskeratosis congenita).

The validity of telomere length by itself as an indicator of cell viability or aging has been challenged by the present model of the telomere complex, which postulates a dynamic switch between a protected or capped state and a temporarily uncapped state.6 Telomere homeostasis is regulated through mutually reinforcing mechanisms, such as its precise protein composition, telomere length, and telomerase activity level. The probability of telomere uncapping increases when one or more of these parameters are critically altered and cannot be compensated by the others. For instance, telomerase is dispensable in cells with sufficiently long telomeres, but cells with critically short telomeres that lack telomerase lose their ability to proliferate (replicative senescence). Telomere dysfunction can provoke chromosomal fusions and apoptotic cell death.

Telomerase expression and activity and telomere length are tissue-regulated and developmentally regulated, with generally greater telomerase activity during embryonic development and low or undetectable levels soon after birth. For instance, telomerase activity in rat embryos is highest in liver and lowest in brain and becomes undetectable in all adult organs examined but in liver,7 and telomere length decreases with aging in rat kidney, liver, pancreas, and lung but not in brain.8 In mice, similar telomere length was found in liver, brain, testis, kidney, and spleen of newborns, but this parameter differed among tissues in adults.9 Significant age-dependent telomere shortening in Mus spreitus was found in spleen and brain but not in liver, testis, or kidney, and telomerase activity was abundant in adult liver and testis but weak to undetectable in spleen, kidney, and brain.10 Although
human telomere reduction rates of 29 to 60 bp per year have been estimated in the liver, renal cortex, and spleen, telomere length is maintained in cerebral cortex.11

Individual differences in telomere length in rodents9,10 and humans11–15 suggest that this parameter is genetically determined. Moreover, human and animal studies revealed higher telomerase activity16 and longer telomeres8,10,17 in females versus males, and estrogens may contribute to these gender differences (see below). It is also noteworthy that human TERT (hTERT) is alternatively spliced in specific patterns in different tissue types during human development, and this mechanism often leads to the expression of hTERT protein lacking functional reverse-transcriptase domains.18

The impact of telomere ablation in the whole organism has been rigorously assessed in Terc-deficient mice.19–26 Notably, the breeding of successive generations of Terc-null mice is necessary to reach critical telomere ablation, leading to increased chromosome end-to-end fusions and alterations characteristic of premature aging and disease, such as infertility, graying of hair, alopecia, impaired wound healing,

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Main Findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac myocyte</td>
<td>Overexpression of hTERT induces hypertrophy and inhibits apoptosis in culture</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>TRF2 inactivation causes telomere erosion, Chk2 activation, and increases apoptosis in cultured rat ventricular myocytes</td>
<td>71</td>
</tr>
<tr>
<td>Smooth muscle cell (SMC)</td>
<td>Hypoxia-induced human SMC growth correlates with telomerase activation</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Telomerase activity in primary SMCs correlates with cell proliferation, and telomerase inhibition diminishes cell growth</td>
<td>62, 95</td>
</tr>
<tr>
<td></td>
<td>Forced expression of hTERT extends life span of rat SMCs</td>
<td>87</td>
</tr>
<tr>
<td>Endothelial cell (EC)</td>
<td>Limited proliferative capacity of passaged human ECs correlates with telomere attrition</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Inhibition or forced expression of telomerase in human aortic ECs affects cell life span</td>
<td>54, 96</td>
</tr>
<tr>
<td></td>
<td>Constitutive hTERT expression enhances the regenerative capacity of endothelial progenitor cells</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Estrogen activates the PI3K/Akt pathway in human ECs, and both PI3K inhibition and dominant-negative Akt mutant significantly reduce telomerase activity in EC cultures; in contrast, Akt activation enhances human telomerase activity</td>
<td>39, 40, 41</td>
</tr>
<tr>
<td></td>
<td>Oxidized low-density lipoproteins inactivate Akt and diminish telomerase activity in ECs</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Estrogen stimulates nitric oxide production in vascular ECs, which in turn induces telomerase in these cells</td>
<td>39, 42</td>
</tr>
</tbody>
</table>

Figure 1. Telomeres, telomerase, and cellular proliferation. A, left, Telomeres (yellow) at both ends of chromosomes (blue) revealed by Q-FISH. Right, Scheme showing the sequence of the tandem repeat present in human telomeric DNA. B, Protein composition of the human telomerase ribonucleoprotein complex has been only partly characterized. The scheme shows the DNA and RNA (Terc) telomeric components, the catalytic telomerase reverse transcriptase (TERT) subunit, and additional telomerase-associated proteins. C, Telomerase activity is low or absent in most somatic cells; therefore, progressive telomere erosion occurs in these cells with each mitotic cycle during normal aging or passage in culture. Germ and tumor cells display high telomerase activity, thus ensuring the maintenance of telomere length and an extended proliferative potential. Accelerated telomere attrition is associated with human premature aging syndromes.
small intestine and spleen atrophy, reduced proliferation of T and B lymphocytes, and hematopoietic disorders. These alterations, which are most prominent in highly proliferative organs, are associated with a significant reduction in life span. Studies using this animal model that are relevant for cardiovascular pathobiology will be discussed in the next sections.

**Telomeres and Neovascularization**

The restoration of blood flow into ischemic territories in the adult organism depends on the development of new collateral vessels from established vascular networks (angiogenesis) and on de novo vessel formation by endothelial progenitor cells (vasculogenesis). New capillaries composed by a monolayer of ECs are stabilized and mature into fully functional vessels on the recruitment of SMCs and pericytes. Hypoxia is a fundamental angiogenic stimulus that induces TERT protein expression and phosphorylation in cultured SMCs. Telomerase inhibition shortened the life span of hypoxic cultures, and constitutive TERT expression extended life span under normoxia, suggesting that hypoxia-mediated telomerase activation promotes long-term SMC growth. Whether chronic hypoxia also leads to higher telomerase activity in ECs remains to be established.

Aging leads to endothelial dysfunction, reduced vascular endothelial growth factor expression, and diminished angiogenesis in a rabbit model of limb ischemia, although advanced age does not preclude augmentation of collateral vessel development in response to the supply of exogenous angiogenic factors. Late-generation Terc-null mice with short telomeres disclosed a sharp decrease in angiogenesis in both Matrigel implants and murine melanoma grafts, and this correlated with diminished tumor cell proliferation, increased tumor cell apoptosis, and a lower tumor growth rate. Collectively, these studies suggest that telomere ablation likely impairs angiogenesis in the aged organism.

Pallini et al found a direct correlation between hTERT mRNA expression in ECs of newly formed vessels and the histological grade of human tumors, thus additionally supporting a role of telomerase in angiogenesis. They detected endothelial hTERT expression in 29%, 56%, and 100% of low-grade astrocytomas, anaplastic astrocytomas, and advanced glioblastomas multiforme, respectively. Whereas hTERT mRNA expression and the proliferation rate of human ECs are dissociated in low-grade and anaplastic astrocytomas, these parameters correlated in glioblastomas multiforme. Remarkably, diffusible factors produced by glioblastoma cells in vitro upregulate hTERT mRNA and protein expression and telomerase activity in ECs.

Human dermal microvascular ECs transduced with hTERT have increased capacity to form more durable microvascular structures when subcutaneously xenografted in severe combined immunodeficiency mice. Likewise, constitutive hTERT expression in cultured human endothelial progenitor cells enhances their mitogenic and migratory activity, improves survival, and augments neovascularization in a murine hind limb ischemia model.

**Telomeres and Cardiovascular Disease**

The cardioprotective effects of estrogens via indirect actions on lipoprotein metabolism and through direct effects on vascular ECs and SMCs are likely to contribute to the lower incidence of cardiovascular disease observed in premenopausal women compared with men. Of note in this regard, women have a decelerated rate of age-dependent telomere exhaustion over men. Moreover, estrogen activates in human ECs the phosphoinositol 3-kinase (PI3K)/Akt pathway, which in turn enhances human telomerase activity through TERT phosphorylation. In contrast, PI3K inhibition or dominant-negative Akt diminishes telomerase activity in ECs. Collectively, these findings suggest that estrogen activates endothelial telomerase via PI3K/Akt signaling. Conversely, Akt inactivation by proatherogenic oxidized low density lipoproteins diminishes telomerase activity in ECs. Estrogen also stimulates nitric oxide production in vascular ECs, which in turn induces telomerase in these cells.

Atherothrombosis is frequently the cause of myocardial infarction (MI) and consecutive heart failure. Atheroma development is a complex multifactorial process that involves distinct cell types and molecular events, including both adaptive and innate immune mechanisms. Endothelial dysfunction in response to atherogenic stimuli (ie, elevated plasma cholesterol level, hypertension, and diabetes) is accepted as one of the earliest manifestations of atherosclerosis at sites of predispotion to atheroma formation. The damaged endothelium promotes the adhesion and transendothelial migration of circulating leukocytes. Early fatty streaks contain mostly highly proliferative macrophages that avidly uptake lipoproteins to become lipid-laden foam cells. Activated intimal leukocytes produce a plethora of inflammatory mediators that promote SMC proliferation and migration, thus additionally contributing to atheroma growth. Plaque rupture or erosion at advanced disease stages can lead to thrombus formation, resulting in MI or stroke. In the next sections, we will discuss studies on telomeres and telomere-associated proteins in cardiovascular pathobiology, including alterations in telomere homeostasis induced by atherogenic stimuli, cardiovascular aging, and heart failure, as well as the cardiovascular phenotype of mice with altered telomerase function (Figure 2).

**Telomeres and Atherosclerosis**

Aging is a major cardiovascular risk factor. ECs from human abdominal aorta display age-dependent telomere shortening and increased frequency of aneuploidy. A greater rate of telomere attrition has been estimated in human ECs from iliac arteries compared with iliac veins (102 versus 47 bp per year, respectively), and age-dependent intimal telomere loss is greater in the iliac artery versus the internal thoracic artery (147 versus 87 bp per year, respectively). A vessel subjected to less hemodynamic stress. Similarly, Okuda et al reported a higher rate of age-dependent telomere attrition in both the intima and media of the distal versus proximal human abdominal aorta. They also found a negative correlation between telomere length and atherosclerotic
grade, although this relationship was not statistically significant after adjustment for age. Collectively, these studies suggest that telomere attrition contributes to age-dependent endothelial dysfunction and reveal a higher rate of telomere attrition in aged vascular beds with increased shear wall stress and enhanced cellular turnover.

ECs with senescence-associated phenotypes are present in human atherosclerotic lesions. This phenotype can be induced in cultured human aortic ECs by overexpression of a dominant-negative mutant of telomere repeat binding factor 2 (TRF2), and replicative senescence of these cells can be prevented by TERT transduction.54 Interestingly, age-dependent telomere shortening of cultured human umbilical vein ECs is slowed down by enrichment of intracellular vitamin C, which reduces by 53% the level of proatherogenic reactive oxygen intermediates.55

Leukocytes play important roles in all phases of atheroma development. Patients with vascular dementia, a disorder that is frequently associated with progressive cerebrovascular atherosclerosis and consecutive stroke, have significantly shorter telomeres in blood circulating leukocytes compared with three age-matched control groups, namely cognitively competent patients suffering from cerebrovascular or cardiovascular disease alone, patients with probable Alzheimer’s dementia, and apparently healthy control subjects.56 Likewise, average telomere length in leukocytes of 10 patients with severe coronary artery disease (CAD) was significantly shorter than that of 20 controls with normal coronary angiograms after adjustment for age and sex.57 In a larger study comparing 203 cases of premature MI and 180 controls, age- and sex-adjusted mean terminal restriction fragment (TRF) length of patients was significantly shorter than that of controls, and this difference was not accounted for by other coronary risk factors.58 Compared with subjects in the highest quartile for telomere length, subjects with shorter than average telomeres had between 2.8- and 3.2-fold higher risk of MI. In another study of 143 healthy unrelated individuals older than 60 years of age, shorter telomere length in blood DNA correlated with poorer survival that was attributable in part to a 3.18- and an 8.54-fold higher mortality rate from heart and infectious disease, respectively.59

The above studies raise the possibility that telomere attrition may be a primary abnormality that renders the organism more susceptible to cardiovascular risk factors. However, because the rate of telomere shortening augments in most somatic cells with increasing cell division, reduced leuko-
cyte telomere length in patients with cardiovascular and cerebrovascular diseases may be a mere consequence of increased cell turnover induced by the chronic inflammatory response underlying atherogenesis. To add insight into this question, we assessed the impact of telomere attrition on atherogenesis induced by dietary cholesterol in apolipoprotein E (apoE)-deficient mice, a well-established model of experimental atherosclerosis that recapitulates important aspects of the human disease.60 We found that late-generation mice doubly deficient in apoE and Terc had shorter telomeres and were protected from atherosclerosis compared with apoE-null mice with an intact Terc gene, and this beneficial effect of short telomeres correlated with impaired proliferative capacity of lymphocytes and macrophages.26 Additional studies are warranted to ascertain whether telomere shortening affects other key processes implicated in atherosclerosis (ie, leukocyte recruitment, SMC proliferation, and migration). If our findings in Terc-apoE doubly deficient mice are applicable to humans, telomere shortening in blood circulating leukocytes is unlikely to represent a factor predisposing to atherosclerosis in humans. However, a conclusive answer to this chief question must await the results of epidemiological studies to ascertain if individuals with significantly shorter telomeres in circulating leukocytes at birth are at higher risk of developing CAD in adulthood independently of known cardiovascular risk factors. Of note in this regard, Okuda et al12 reported high variability of telomeric DNA length in cord blood leukocytes in relation to birth weight. We showed that birth weight affected telomere length independently of gender, suggesting that genetic and environmental determinants that start exerting their effect during embryonic development are key determinants of telomere length. These authors also suggested that shorter telomeres in adult women result from a slower rate of telomere attrition compared with men.60,61 Because human aging is associated with telomere erosion in most somatic cells,4,5 the higher prevalence of atherosclerosis within the elderly seems to challenge the conclusion made in mice that short telomeres protect from atherosclerosis.26 These seemingly conflicting findings might be reconciled if accepting that accumulation of cellular damage imposed by prolonged exposure to cardiovascular risk factors ultimately prevails over protective mechanisms, including telomere shortening. Remarkably, we have shown that 4- to 5-year-old rabbits exhibit a marked reduction in the size of atherosclerotic lesions compared with 4- to 5-months-old counterparts despite comparable hypercholesterolemia induced by the same dietary regimen.61

Telomeres, Hypertension, and Diabetes

Hypertension is a major cardiovascular risk factor.46 In spontaneously hypertensive rats (SHR), both telomerase protein expression and activity are induced in the aorta but not in other tissues before the onset of hypertension, and this correlates with telomere lengthening and increased medial SMC proliferation.62 TERT antisense RNA delivery increased apoptosis in cultured SMCs by a mechanism that was reversed by p53 overexpression. The authors concluded that selective TERT activation and subsequent telomere lengthening in aortic medial SMCs is the driving force for the imbalance between cell proliferation and apoptosis that ultimately results in the vascular remodeling seen in genetic hypertension. Compared with age-matched normotensive rats, the kidney of SHR undergoes a transient hyperplasic response during the first 2 weeks of postnatal life.63 Because shorter telomeres are detected in the kidney of SHR at all ages examined, it was suggested that kidney cells from these animals are subjected to increased turnover, potentially leading to their accelerated aging.

In a study performed on 49 twin pairs that included 38 men and 60 women 18 to 44 years of age, TRF length in WBCs correlated positively with diastolic blood pressure but negatively with systolic blood pressure, suggesting a negative relation between TRF length and pulse pressure.15 Moreover, the correlation between telomere length and pulse pressure was independent of gender, and both parameters appeared highly heritable. Benetos et al17 also investigated WBC telomere length and blood pressure parameters that are associated with stiffness of large arteries (pulse pressure and pulse wave velocity) in French subjects who were not taking any antihypertensive medications (120 men and 73 women; mean age, 56±11 years). Although telomere length negatively correlated with age in both sexes, multivariate analysis showed that telomere shortening significantly contributed to increased pulse pressure and pulse wave velocity only in men. Both studies found age-adjusted longer telomeres in women, suggesting that biological aging is more advanced in men than in women.

Patients with diabetes mellitus are at higher risk for microvascular and macrovascular disease.45 Jeanclos et al64 reported reduced telomere length in WBCs from patients with insulin-dependent diabetes mellitus compared with age-matched nondiabetic subjects. Because this parameter was undistinguishable when comparing patients with non–insulin-dependent diabetes mellitus and nondiabetic controls, the authors suggested that telomere shortening occurs in subsets of WBCs that play a role in the pathogenesis of insulin-dependent diabetes mellitus. The observation that CAD patients with hypercholesterolemia and diabetes mellitus have shorter telomeres in peripheral blood mononuclear cells than healthy controls provides additional support implicating telomere exhaustion as a mechanism contributing to coronary atherosclerosis under some circumstances of metabolic disorders.65

Telomeres and Heart Pathobiology

Similar telomere length in the human heart was found in autopsy samples from 168 individuals in the age range of 0 to 104 years.11 Examination of crude heart extracts revealed a maximum of telomerase activity in embryos, which then declines to become very low or undetectable shortly after birth and throughout adulthood in rodents7,66–68 and humans.69–71 Telomerase downregulation in the adult heart has been eluded in transgenic mice engineered to express hTERT specifically in cardiac myocytes, and this was sufficient to prevent telomere attrition in adult myocardium.68 Although the ventricle of 2-week-old transgenic mice displayed increased DNA synthesis and myocyte density, the ratio of
heart weight to body weight did not increase because transgenic cardiomyocytes at this age were smaller than wild-type controls. By 12 weeks, however, there was a concentric hypertrophy of both ventricles and increased myocyte size, without evidence of cell loss or alteration of mechanical heart dysfunction as an explanation. It is noteworthy that this biphasic response occurred despite sustained telomerase activity throughout the period of time examined, suggesting that hTERT initially delays cardiac myocyte cell-cycle exit and then induces late-onset cell hypertrophy in mice. In contrast, hTERT overexpression in primary cultures of postmitotic rat ventricular myocytes did not elicit DNA synthesis but triggered hypertrophic growth.

Leri and colleagues\textsuperscript{16,72} confirmed the postnatal downregulation of cardiac telomerase activity by analyzing highly pure preparations of rat and dog ventricular myocytes obtained by enzymatic dissociation. Notably, these authors detected telomerase activity in a restricted population of cardiomyocytes throughout adulthood and argued that contamination from fibroblasts, EC, and SMC nuclei lacking telomerase is the most likely cause of the reported lack of telomerase activity in the whole myocardium from 20-day-old rats.\textsuperscript{7} Telomerase expression in adult somatic cells has been also reported in cycling primary presenescent human fibroblasts,\textsuperscript{73} previously believed to lack telomerase activity and expression. Analysis of telomere length in myocyte nuclei isolated from the left ventricle of fetal, neonatal, and adult rats (up to 27 months of age) additionally supports the notion of cellular heterogeneity within the adult heart.\textsuperscript{74} Although this parameter was preserved during aging in most cells, telomere shortening increased with age in a subgroup of myocytes that constituted 16\% of the entire cell population in aged hearts. Because telomere attrition is generally more prominent in proliferating cells, these findings have been interpreted as an indication that replicative-competent cardiac myocytes exist throughout life and that these cells may counteract the continuous death of cells in the aging mammalian rat heart.\textsuperscript{16,74} Indeed, contrary to the generally held concept that adult cardiomyocytes irreversibly exit the cell cycle, several studies reported the presence of proliferating ventricular myocytes in the normal and pathologic adult mammalian heart of several species, including humans.\textsuperscript{75–79}

Recent studies have explored the putative role of telomeres and telomerase in heart pathology. As discussed above, reduced telomere length in circulating leukocytes has been associated with increased risk of MI and heart disease and higher mortality rate.\textsuperscript{58,59} In an experimental model of progressive deterioration of cardiac performance and dilated cardiomyopathy in young dogs, telomerase activity and protein level in left ventricle myocytes, but not in ECs, SMCs, and fibroblasts, increased 3 weeks after the onset of the disease and then were reduced 1 week later.\textsuperscript{72} Notably, the percentage of telomerase-competent cardiomyocytes coexpressing the proliferation marker Ki67 increased during disease progression, and their level of telomerase activity seemed sufficient to preserve telomere length in this model of acute cardiac failure. In contrast, age-related cardiomyopathy in humans (characterized by an increase of cell senescence markers, moderate hypertrophy, and cardiac dilatation) correlated with enhanced apoptosis and telomere shortening despite a 14-fold increase in the number of telomerase-competent myocytes and twice as much telomerase activity with respect to age-matched hearts.\textsuperscript{80} Likewise, heart tissue from patients affected by cardiac hypertrophy consecutive to aortic stenosis with a mean duration of 3 years exhibited increased telomerase activity and a 90- to 120-fold increase in the number of telomerase-positive cells but a 2.7-fold decrease in telomere length.\textsuperscript{81} Thus, unlike acute dilated cardiomyopathy in young dogs,\textsuperscript{72} these human studies demonstrate telomerase shortening during age-related heart disease and cardiac hypertrophy despite enhanced telomerase activity. Oh et al\textsuperscript{71} also reported age-dependent telomere attrition in patients with end-stage heart failure at the time of cardiac transplantation, although they did not detect telomerase activity in the diseased heart.

In addition to cardiac telomere shortening,\textsuperscript{71,81} patients with heart failure disclose induction of the DNA damage-activated checkpoint kinase Chk2, downregulation of TRF2 expression, and increased frequency of myocyte apoptosis compared with control hearts.\textsuperscript{71} TRF2 downregulation was not seen in patients with hypertrophic obstructive cardiomyopathy, a disease that does not affect ventricular function. Biomechanical stress induced by 1 week of partial aortic constriction in mice reproduced the findings made in patients with heart failure, including telomere shortening, TRF2 downregulation, Chk2 activation, and increased apoptosis in myocardial tissue.\textsuperscript{71} Importantly, cardiac-specific hTERT expression in transgenic mice resulted in maintenance of TRF2 protein expression, blockade of Chk2 activity, and diminished cardiac apoptosis after ischemic (coronary ligation) and biomechanical (partial occlusion of the thoracic aorta) injury, and these effects correlated with reduced area of MI and less fibrosis and preservation of systolic function, respectively.\textsuperscript{68,71} Moreover, TRF2 inhibition caused telomere erosion, Chk2 activation, and increased apoptosis in cultured rat ventricular myocytes, and TERT and TRF2 overexpression reduced the apoptotic rate and increased oxidative stress induced by serum withdrawal in these cells.\textsuperscript{71} Collectively, these studies strongly suggest that TRF2 downregulation and Chk2 activation contribute to increased cardiomyocyte apoptosis in both human and murine cardiac failure.

The examination of successive generations of Terc-null mice additionally supports the importance of telomere attrition in cardiac pathology.\textsuperscript{24} Telomere length in isolated cardiomyocytes was progressively reduced up to the fifth generation of Terc-null mice (G5Terc-null). Moreover, old G5Terc-null mice exhibited shorter telomeres in cardiomyocytes than did younger counterparts, and this led to ventricular dilation, thinning of the myocardium, cardiac dysfunction, and sudden death. Compared with wild-type mice, heart sections from G5Terc-null mice revealed increased level of expression of the tumor suppressor protein p53, reduced proliferation and increased apoptosis, and a 50\% reduction in the number of left ventricular myocytes. Moreover, a strong correlation between p53 protein expression and telomere shortening was found in cardiomyocytes of G5Terc-null mice. It remains to be established whether systemic alterations in response to telomere attrition in other organs may
have contributed to cardiac hypertrophy and heart failure in this experimental model.

**Potential Therapeutic Applications of Telomerase Gene Transfer for Cardiovascular Disease**

As discussed in the previous sections, telomerase attrition is likely to play an important role in cardiovascular disease. Thus, telomerase-based gene therapy could be of value for the treatment of these disorders. Importantly, Samper et al. demonstrated that critically short telomeres can become fully functional by restoration of telomerase. They mated heterozygous Terc- mice to late-generation Terc-null mice, which have short telomeres, unstable chromosomes, and signs of premature aging. Analysis of the progeny revealed chromosomes with detectable telomeres, absence of chromosomal instability, and no signs of premature aging in the telomerase-reconstituted mice.

The exogenous supply of angiogenic cytokines promotes therapeutic neovascularization in animal models of peripheral and myocardial ischemia and has shown promising clinical results. Ex vivo expanded human endothelial progenitor cells can also serve as a "supply-side" strategy for therapeutic neovascularization in experimental animals, and in vivo transplantation of hTERT-transduced endothelial progenitor cells have improved capillary density and limb salvage in a murine hind limb ischemia model. It is noteworthy, however, that telomere-independent barriers may limit the transplantation capacity of hematopoietic stem cells. Indeed, although TERT overexpression in murine hematopoietic stem cells prevented telomere attrition in these cells during serial transplantation, this strategy did not extend their transplantation capacity.

hTERT overexpression in human aortic SMCs increased telomere length and extended life span compared with control cells. Late-passage hTERT-transduced SMCs retained a normal morphology and a differentiated, nonmalignant phenotype, and engineered vessels containing human umbilical vein ECs and hTERT-SMCs disclosed markedly improved cellular viability and were architecturally and mechanically superior to vessels generated from control SMCs. Thus, the production of tissue-engineered human arteries for bypass surgery may be facilitated by TERT transduction.

Proof of principle for the notion that telomerase reconstitution may prevent or rescue heart disease was provided by the observation that cardiac-restricted expression of TERT in transgenic mice protects from age-dependent myocardial telomere shortening and apoptosis and alleviates the consequences of ischemic and biomechanical injury (ie, reduced MI area, less fibrosis, and preservation of systolic function). Isolation and ex vivo expansion of telomerase-competing replicating cardiomyocytes found in adult heart could also support myocardial regeneration. Moreover, recent studies have reported the isolation of murine and rat adult heart–derived cardiac progenitor cells that are capable of homing to injured myocardium when injected either intravenously or directly into the ischemic heart. Grafted cardiac stem cells undergo cardiac differentiation with and without fusion to host cells and can encompass as much as 70% of the injured ventricle. Current issues regarding the potential use of stem cell transplantation for the treatment of ischemic heart disease have been comprehensively discussed elsewhere.

Despite encouraging results of gene therapy in preclinical and clinical studies, major efforts are still required to overcome the current practical barriers and limitations placed on most clinical trials before gene therapy strategies exhibit wide application, including the development of safer gene delivery vectors, improvement of transgene expression, and development of efficient systems for conditional expression. Aside from these general considerations, it is worth considering some issues specifically related to gene therapy for telomere-length restoration. First, the protection against heart injury observed in cardiac-specific TERT transgenic mice was achieved in animals that expressed the transgene throughout development, but telomerase gene transfer would most likely be administered in adult patients whose heart contains a large fraction of nondividing cardiomyocytes. Of note in this regard, cardiac-specific TERT transgenic mice displayed cardiac hyperplastic growth by 2 weeks of age and concentric hypertrophy of both ventricles and increased myocyte size at 12 weeks of age. Additional animal studies are thus warranted to assess whether telomerase reconstitution after injury is of therapeutic value. A second aspect is the potential of unwanted effects brought about by telomerase gene transfer that should be considered in the risk-benefit analysis of this approach. For instance, indiscriminate proliferation of telomerized cells within the heart may promote cardiac fibrosis and cancer. Moreover, because neovascularization is enhanced by TERT overexpression and proliferation of vasa vasorum promotes atherosclerosis, grafting of telomerized cells into coronary vessels may worsen atherogenesis in patients with CAD. This potential risk, as well as additional unwanted effects attributable to the homing of grafted telomerized cells in sites other than the heart, may be prevented by the use of cardiac-restricted promoters to drive TERT expression.

**Concluding Remarks**

Telomere dysfunction is emerging as an important factor in the pathogenesis of age-related cardiovascular disease. Critically short telomeres, an imbalance in the relative level of telomere-associated proteins, low telomerase activity, or a combination of these factors can lead to cellular senescence or apoptosis. Both genetic and environmental factors seem to control human telomere length, which shows a high degree of individual variability. It is also notable that estrogens may contribute to longer telomeres in women compared with age-matched men, and this correlates with lower incidence of cardiovascular disease in premenopausal women.

Although a consistent finding in humans is the correlation between short telomeres in blood circulating leukocytes and hypertension, diabetes mellitus, CAD, and MI, whether telomere exhaustion is cause or consequence of these pathologies remains to be established. On the other hand, assessing whether telomere erosion is an independent cardiovascular risk factor will require prospective epidemiological studies. Another important issue is to what extent the observations made in genetically modified mice affecting telomeric components are valid in humans. For instance, although shorter
telomeres have been detected in human arterial tissue from atherosclerosis-prone vascular beds and in blood circulating leukocytes from patients with CAD, telomere shortening in hypercholesterolemic mice significantly reduced atherosclerosis. Furthermore, although cardiac-specific TERT transgenic mice give proof of principle for telomerase reconstitution as a valid therapy for myocardial regeneration, additional studies are required to ascertain whether this strategy may be applicable to humans.

In conclusion, although the role of telomere dysfunction in cardiovascular disease appears evident, more research is needed before telomerization can be translated effectively into clinical practice. Because most reports have focused on telomerase, future studies aimed at assessing the role of additional telomere-associated proteins in cardiovascular pathobiology and their potential implications for therapeutics are warranted.

Acknowledgments

Work in the laboratory of V.A. is supported in part by the Ministry of Science and Technology of Spain and Fondo Europeo de Desarrollo Regional (grants SAF2001-2358 and SAF2002-1443) and from Instituto de Salud Carlos III (Red de Centros C03/01). A.L.S. is the recipient of a Marie Curie postdoctoral fellowship from the European Union. We thank M.J. Andrés-Manzano for preparing the figures and M.A. Blasco for providing the Q-FISH photomicrograph of Figure 1A.

References


44. Lusis AJ. Atherosclerosis.


47. Oh H, Taffet GE, Youker KA, Entman ML, Overbeek PA, Michael LH, Schneider MD. Telomere reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. *Proc Natl Acad Sci U S A.* 2001;98:10308–10313.


59. Pravenec M, Kunes J, Sun Y, Tremblay J. Excess growth and telomere shortening is slowed down by enrichment of intracellular endogenous vitamin C via suppression of oxidative stress.


61. Samper E, Flores JM, Blasco MA. Restoration of telomerase activity with short telomeres.


Telomeres and Cardiovascular Disease: Does Size Matter?
Antonio L. Serrano and Vicente Andrés

Circ Res. 2004;94:575-584
doi: 10.1161/01.RES.0000122141.18795.9C
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/94/5/575

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/