Telomere dysfunction in hypertension

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Aging is a major risk factor for hypertension and associated cardiovascular disease. In most proliferative tissues, aging is characterized by shortening of the DNA component of telomeres, the specialized genetic segments that cap the end of eukaryotic chromosomes and protect them from end-to-end fusions. By inducing genomic instability, replicative senescence and apoptosis, telomere shortening is thought to contribute to organismal aging and to the development of age-related diseases. Here, we review animal and human studies that have investigated the possible links between telomere ablation and the pathogenesis of hypertension and related target organ damage. Although evidence is mounting that alterations in telomerase activity and telomere shortening may play a role in the pathogenesis of hypertension, additional studies are required to understand the molecular mechanisms by which telomere dysfunction and hypertension are functionally connected. As our knowledge on this emerging field grows, the challenge will be to ascertain whether all this information might translate into clinical applications. *J Hypertens* 25:2185–2192 © 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Parallel structural and functional changes in the large arteries (stiffness), cardiac mass (hypertrophy), and myocardial relaxation and filling (diastolic dysfunction) occur in normotensive aging and hypertension at any age. This continuum of age-related change is simply accelerated in individuals with chronic hypertension, so that the same changes occur at an earlier age or to an exaggerated degree. In this regard, the traditional clinical distinction between normotension and hypertension is quite arbitrary, although it may be useful with regard to cardiovascular risk stratification. The similarities between aging and hypertension are so striking that aging can be considered to be 'muted hypertension', whereas hypertension can be likened to 'accelerated aging'. It is imperative, therefore, to introduce biological indicators of aging into models developed to provide a better understanding of the pathophysiology of essential hypertension. One of these indicators may well be the age-dependent telomere length in somatic cells.

Telomeres are specialized chromatin structures that cap the ends of eukaryotic chromosomes and prevent the recognition of chromosomal ends as double-stranded DNA breaks. Functional telomeres are thus essential to avoid a DNA damage cellular response resulting from chromosome recombination and degradation. Telomeres contain a large number of non-coding double-stranded repeats of G-rich tandem DNA sequences (TTAGGG in vertebrates) spanning 10–15 kb in humans and 25–40 kb in mice, which end in a 150–200 nucleotide 3′ single-stranded overhang (G-strand overhang) [1,2]. Telomere-associated proteins include the telomerase components TERC (telomerase RNA component, which serves as a template for the synthesis of new telomeric repeats) and TERT (telomerase reverse transcriptase component, which catalyses the synthesis of new telomeric repeats). Typically, human adult somatic cells display low or absent telomerase activity, except in cell populations with high proliferative potential, such as activated lymphocytes and certain types of stem cells [3–5]. As a result of the so-called 'end replication problem', cells with scarce or absent telomerase activity display progressive telomere attrition with each mitotic cycle, thus telomere length in somatic cells reflects their replicative history and can predict their remaining proliferative potential. Cells with critically short telomeres undergo chromosomal end-to-end fusions, replicative senescence, and apoptosis [6,7].

Telomere length is highly variable among individuals of the same age, both in rodents [8,9] and humans [10–14]. Although evidence exists suggesting that individual telomere length is influenced by genetic factors [11,13,15], evidence is mounting that the effects of environmental factors on the rate of telomere exhaustion may also be of great importance in determining telomere length in adulthood [16]. It has also been shown that females

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display higher telomerase activity [17] and longer telomeres [8,13,18,19] in various adult tissues compared with age-matched males, possibly caused, at least partly, by the estrogen-dependent activation of telomerase [20,21].

The consequences of telomere ablation at the organismal level have been rigorously assessed in *TERC*-deficient mice, which experience progressive telomere shortening with each generation, and lose viability when they reach critically short telomeres (typically after three to five generations). Remarkably, late generation *TERC*-null mice display premature aging symptoms and associated disorders [22–29], thus supporting the concept that progressive telomere shortening might be involved in the pathogenesis of age-related human disorders. Of note in this regard is the fact that telomerase activity is impaired, or telomere attrition is accelerated, in various human premature aging syndromes, such as dyskeratosis congenita [30], Werner syndrome [31] or ataxia telangectasia [32]. The importance of telomerase deficit on the pathogenesis of these disorders is emphasized by the observation that the ectopic expression of telomerase in cultured cells obtained from dyskeratosis congenita patients rescues telomere defects [33].

Relationships between human telomere length and blood pressure parameters

Several population-based studies have assessed the relationship of blood pressure parameters with telomere length in white blood cells (WBC). In a study that included 49 normotensive twin pairs (38 men and 60 women, 18–44 years of age), Jeanclos *et al.* [13] found that telomere restriction fragment length in WBC correlated positively with diastolic blood pressure (DBP) but negatively with both systolic blood pressure (SBP) and pulse pressure (pulse pressure = SBP − DBP). Telomere length and pulse pressure were highly familial and the correlation observed between these parameters was sex independent. By analysing 120 men (SBP 134.8 ± 1.5 mmHg; DBP 85.2 ± 0.9 mmHg; mean age 55 ± 1 years) and 73 women (SBP 131.2 ± 1.9 mmHg; DBP 81.3 ± 1.2 mmHg; mean age 56 ± 1 years) who were not on any hypertensive medication, Benetos *et al.* [18] found a negative correlation between age and telomere length in both sexes. Shorter telomeres, however, appeared to contribute to increased pulse pressure and arterial stiffness only in men [18]. More recently, Demissie *et al.* [34] corroborated the association between hypertension and shorter leukocyte telomere length in 327 men from the Framingham Heart study, and suggested that this relationship is largely caused by insulin resistance, a disorder frequently associated with hypertension [35,36].

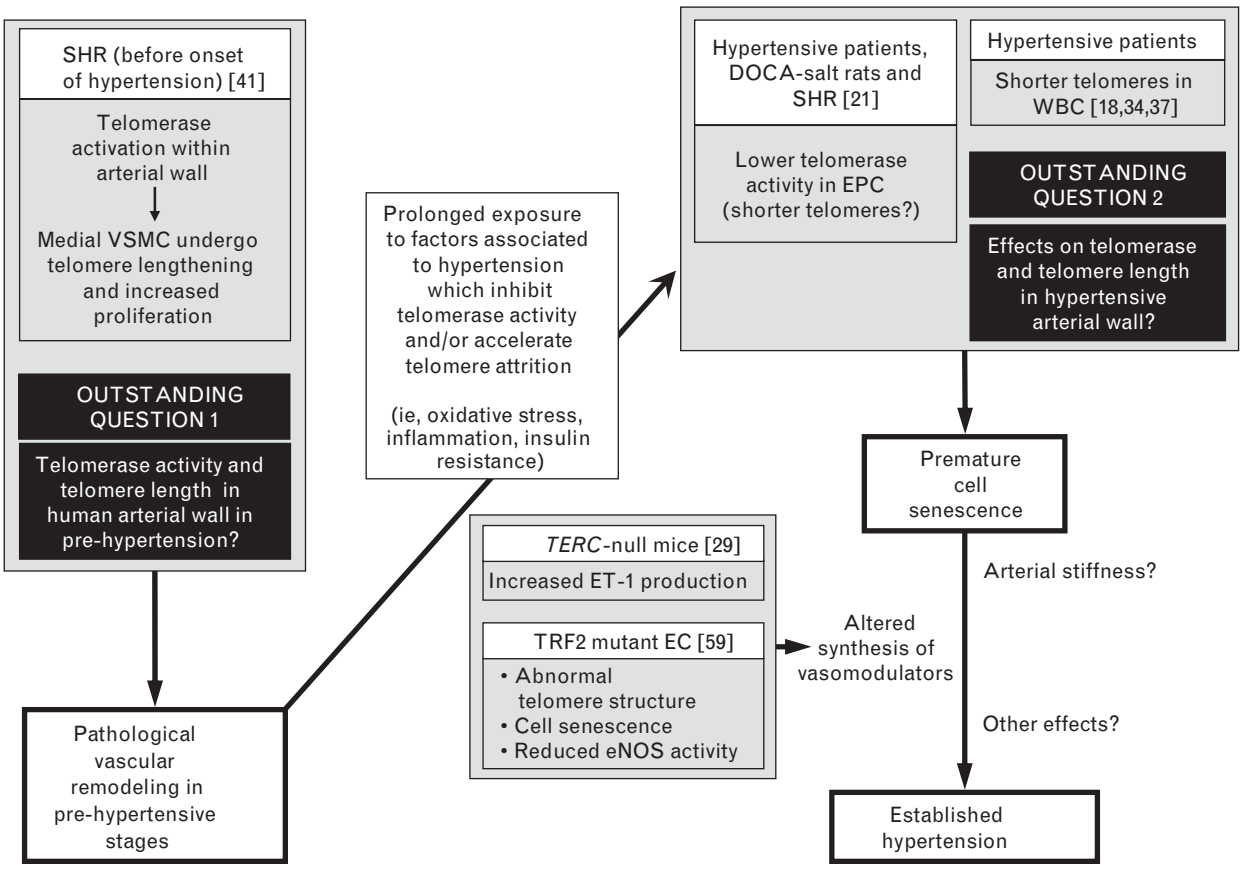
Recent studies in 419 older adults (mean age 74.2 ± 5.2 years) from the Cardiovascular Health Study cohort followed over 10 years showed a borderline inverse association (*P* value of 0.06) between WBC telomere length and DBP [37]. Although the association pointed in the direction one would expect if longer telomeres corresponded with a better blood pressure status, it is clear that additional longitudinal studies are required to investigate further the connections between telomeres and hypertension. Of note is the fact that establishing statistically significant differences in cross-sectional studies will require large cohorts because telomere length is highly variable among humans. However, smaller sample sizes may be adequate in longitudinal studies designed to evaluate the possible differences in telomere attrition rates. These and additional considerations in designing telomere-related epidemiological studies are thoroughly discussed elsewhere [38].

Mechanistic insight into the role of telomerase and telomeres in hypertension

The following section discusses evidence obtained from human and animal studies supporting the notion that alterations in telomerase activity and telomere length may play a role in the pathogenesis of hypertension.

Both endothelial and vascular smooth muscle cells (VSMC) from human vascular tissues undergo age-dependent telomere attrition [39,40]. Cao *et al.* [41] reported that TERT expression and telomerase activity are induced in the aorta, but not in other tissues, of spontaneously hypertensive rats (SHR) at ages preceding the establishment of hypertension. Although it remains to be established whether this is accompanied by increased telomere length and proliferation within aortic cells *in vivo*, primary cultures of medial VSMC obtained from the aorta of SHR displayed increased telomerase activity and telomere length as well as augmented proliferation compared with control VSMC from Wistar–Kyoto rats (WKY). Moreover, lowering telomerase activity reduced proliferation and induced death in SHR but not in WKY VSMC [41]. On the other hand, endothelial progenitor cells (EPC) from hypertensive patients and from SHR and deoxycorticosterone acetate-salt hypertensive rats exhibit reduced telomerase activity and accelerated senescence [42], and angiotensin II-infused hypertensive rats exhibit in EPC reduced telomerase activity, accelerated senescence and decreased mitogenic activity [43]. On the basis of these observations, it is tempting to speculate that increased medial VSMC proliferation caused by early telomerase activation and increased telomere length may contribute to the initial phases of vascular remodeling associated with hypertension (e.g. medial hypertrophy). The prolonged exposure to some factors accompanying hypertension may, however, ultimately promote cell senescence, at least partly as a consequence of reduced telomerase activity and accelerated telomere erosion (Fig. 1). Among these factors, inflammation, oxidative stress, and insulin resistance are

Fig. 1



Hypothetical model of telomere and telomerase alterations in different stages of hypertension. The schematic represents a working model based on a limited number of animal and human studies. Early telomerase activation and telomere lengthening within the artery wall may promote pathological vascular remodeling before the establishment of hypertension in the spontaneously hypertensive rat (SHR) model. At later stages, and as a result of the chronic action of risk factors that are frequently associated with high blood pressure and are known to inhibit telomerase activity, accelerated telomere exhaustion may cause phenotypic alterations that contribute to the development of hypertension (e.g. cell senescence, increased arterial stiffness, altered synthesis of vasomodulators). Validation of this model requires human studies to assess whether prehypertension stages are associated with arterial telomerase activation and telomere lengthening, as has been shown in the SHR model. Moreover, it needs to be determined whether telomere attrition and ensuing cell senescence occur within the artery wall in hypertensive patients and animals. Reference numbers are shown. DOCA, Deoxycorticosterone acetate; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; EPC, endothelial progenitor cell; ET-1, endothelin 1; TERC, telomerase RNA component; TRF2, telomere repeat binding factor 2; VSMC, vascular smooth muscle cell; WBC, white blood cell.

probably the most important, because they are all linked to hypertension [35,36,44–49] and have been proved to accelerate telomere erosion [50–54]. Certainly, additional human and animal studies are required to investigate the possible relationships between telomerase activity/telomere length and insulin resistance, oxidative stress and inflammation markers at different stages of hypertension, both in arterial and circulating cells. It is noteworthy that angiotensin II, which is central to the development of hypertension [55], can induce VSMC senescence without reducing telomere length [56], thus suggesting that telomere-independent mechanisms of vascular senescence might also contribute to hypertension. An inverse relationship has been found between the plasma aldosterone concentration and WBC telomere length in normotensive and hypertensive men [57]. Inappropriately high concentrations of this hormone, as seen in different forms of human hypertension [58], may be linked to a higher rate of telomere attrition and perhaps increased biological aging in these patients.

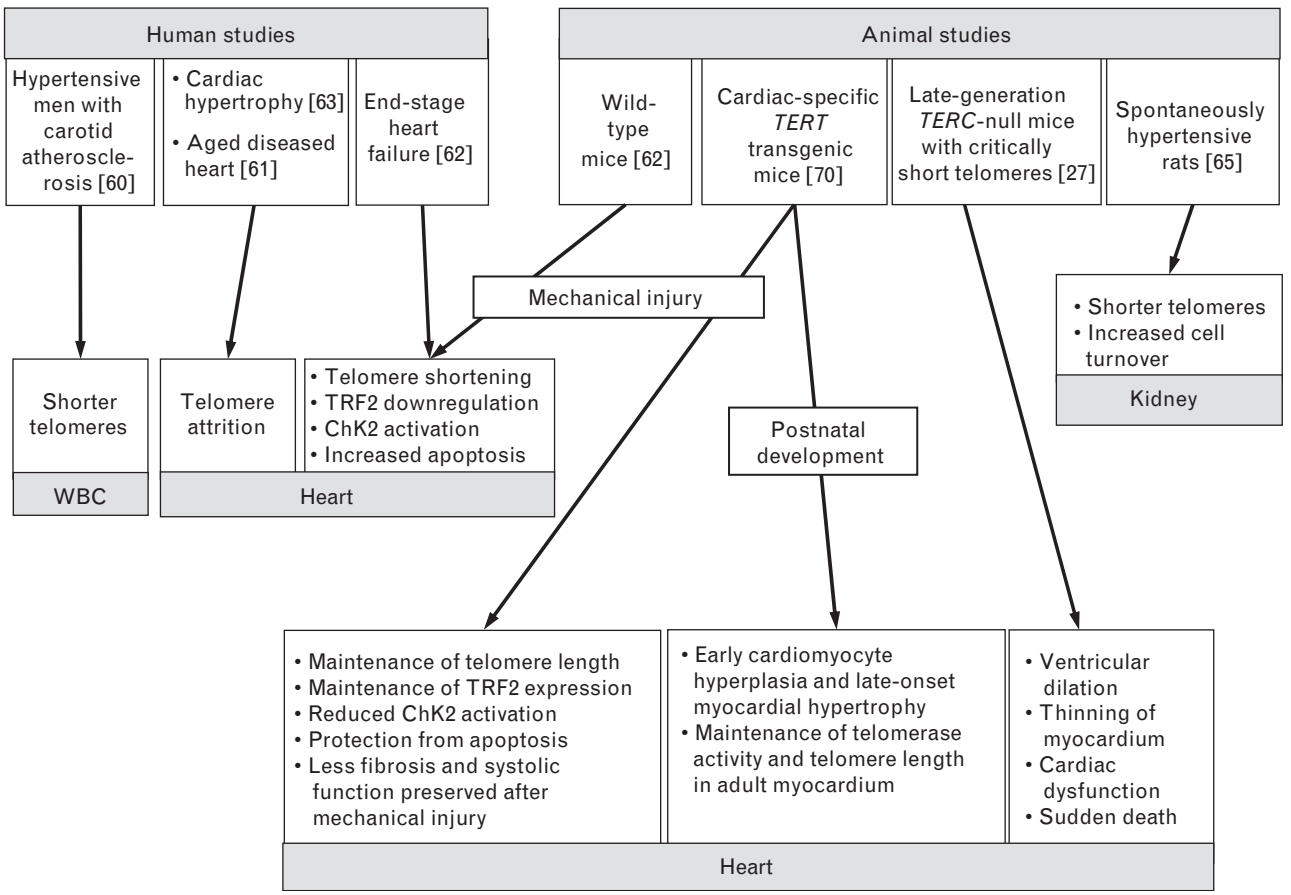
Supporting the notion that telomere dysfunction and hypertension are causally linked, Perez-Rivero *et al.* [29] found that the first and third generations of *TERC*-deficient mice exhibit higher blood and urinary levels of the endothelium-derived vasoconstrictor peptide endothelin 1 and develop hypertension. As no differences in the expression of the precursor prepro-endothelin 1 were detected in the aorta and renal cortex of *TERC*-null

mice, it was postulated that increased levels of circulating endothelin 1 may be caused by the increased expression of the endothelin-converting enzyme (ECE-1), which converts prepro-endothelin 1 into biologically active endothelin 1. ECE-1 messenger RNA expression was significantly higher in *TERC*-deficient mice than in their wild-type counterparts, and ECE-1 promoter activity was increased in murine embryonary fibroblasts obtained from *TERC*-deficient mice. These cells also displayed an enhanced production of reactive oxygen species and their treatment with antioxidants, such as catalase and N-acetylcysteine, reduced ECE-1 promoter activity. These findings suggest a causal link between the synthesis of reactive oxygen species and endothelin 1 levels and support a role for oxidative stress in telomere erosion in hypertension. It was also shown that the expression of a telomere repeat binding factor 2 (TRF2)-dominant negative mutant, which destroys telomere structure, induces in endothelial cells a senescent phenotype and diminished endothelial nitric oxide synthase activity [59]. Collectively, these observations suggest that telomere dysfunction may induce premature senescence and modify the phenotypic characteristics of vascular cells in a way that favours the development of hypertension (e.g. altering the production of vasomodulators) [29].

Telomeres, telomerase and target organ damage in hypertension

Human and animal studies that have demonstrated relationships between telomere dysfunction and target organ damage in hypertension are summarized in Fig. 2. Changes in the composition of cardiac tissue develop in arterial hypertension and lead to structural remodeling of the myocardium. Structural remodeling is the consequence of a number of pathological processes, mediated by mechanical, neurohormonal and cytokine routes, occurring in the cardiomyocyte and the non-cardiomyocyte compartments of the heart. It is classically admitted that cardiomyocyte hypertrophy leading to left-ventricular hypertrophy provides the adaptive response of the heart to pressure overload in an attempt to normalize systolic wall stress. Recent experimental and clinical

Fig. 2



Human and animal studies linking telomere dysfunction to target organ damage in hypertension. Reference numbers are shown. TERC, Telomerase RNA component; TERT, telomerase reverse transcriptase component; TRF2, telomere repeat binding factor 2; WBC, white blood cell.

studies have also provided evidence for the stimulation of cardiomyocyte apoptosis leading to either cell death or dysfunction in the hypertensive heart [66]. Furthermore, the available findings suggest that cardiomyocyte apoptosis precedes the impairment in ventricular function, and its exacerbation accompanies the development of heart failure in hypertensive patients with cardiac hypertrophy.

The role of telomerase in cardiac pathophysiology is highlighted by studies in late-generation *Terc*-null mice with critically short telomeres, which exhibit ventricular dilation, thinning of the myocardium, cardiac dysfunction and sudden death, as well as the reduced proliferation and increased apoptosis of cardiomyocytes [27]. Challenging the classic dogma considering the adult heart as a postmitotic tissue, evidence is mounting to suggest the presence of telomerase-expressing multipotent cardiac stem cells in adult myocardium, which may support regeneration of the damaged heart [63,67–69]. Moreover, new myocyte formation during aortic valve stenosis-induced cardiac hypertrophy may arise from the differentiation of telomerase-positive cardiac stem cells [63]. Remarkably, cardiac-specific *TERT*-transgenic mice exhibit early cardiomyocyte hyperplasia and late-onset myocardial hypertrophy [70].

Taken together, the aforementioned studies suggest a role of telomerase activation in the adaptative changes of cardiomyocytes in the hypertensive heart. Telomere dysfunction may, however, also contribute to maladaptive cardiac hypertrophy and ensuing heart failure. First, telomere attrition has been detected in the heart of patients with cardiac hypertrophy consecutive to aortic stenosis with a mean duration of 3 years, in spite of increased telomerase activity [63]. Likewise, augmented telomerase activity in the aged diseased human heart does not prevent telomere attrition [61]. Second, both in a murine model of cardiac hypertrophy and heart failure induced by severe mechanical overload for 1 week and in patients experiencing end-stage heart failure, cardiac tissue exhibits diminished levels of TRF2, shortened telomeres and activated Chk2 [62]. Similarly, TRF2 inactivation in cultured cardiomyocytes rapidly induced telomere shortening, activation of Chk2 and apoptosis, and exogenous TRF2 protected cardiomyocytes from oxidative stress. The in-vivo responses to mechanical overload were inhibited by ectopically expressing TERT at levels normal for the embryonic heart, which also reduced replacement fibrosis and preserved systolic function. In the absence of heart failure, however, the hypertrophied heart did not display telomere attrition and TRF2 downregulation [62].

Oxidative stress plays an important role in cardiac hypertrophy and its transition to heart failure [64,71], and accelerates telomere erosion [50–52,54]. In line with these observations, cardiac stem cells and cardiomyocytes from mice with streptozotocin-induced diabetes exhibit shorter telomeres associated with oxidative stress [72]. Telomere attrition was not observed in cardiomyocytes from diabetic $p66^{shc}$-deficient mice with the attenuated production of reactive oxygen species [72–74], thus suggesting a link between telomere shortening in the heart, oxidative stress and diabetes.

It is not yet known whether aging is inevitably accompanied by a decline in renal function or how rapidly it might occur. It is, however, accepted that morbid conditions, such as hypertension, facilitate and accelerate age-related renal deterioration. A role for telomere length as one of the molecular mechanisms regulating such a relationship has been proposed [75]. An analysis of surgical samples from 24 human kidneys has revealed that telomeres shorten in the aging kidney, particularly in the renal cortex [76]. In this conceptual framework, Hamet *et al.* [65] found shorter telomeres in kidneys from SHR compared with normotensive rats at all ages examined. As the half-life of cells in the kidney is actually decreased by approximately 50% in SHR compared with normotensive rats [77], it is possible that the hypertensive kidney is characterized by accelerated senescence with increased cell turnover. The potential pathophysiological relevance of this possibility is supported by two facts: (i) individuals with essential hypertension are at increased risk of a particular form of chronic kidney disease (e.g. nephroangiosclerosis) [78]; and (ii) the kidney of patients with nephroangiosclerosis exhibits pathological features similar to the microscopic changes seen in the kidney of normotensive elderly subjects [79].

It is well established that hypertensive individuals are at a greater risk of atherosclerosis. Not all hypertensive patients, however, ultimately manifest atherosclerotic complications. The reasons for this interindividual diversity are unknown, but may reflect differences in environmental or genetic factors, such as oxidative stress, inflammation, and other molecular and cellular mechanisms that are related to aging. A number of data suggest that individuals with shorter telomeres in leukocytes have a higher prevalence of atherosclerotic lesions and an elevated risk of cardiovascular events related to atherosclerosis [80]. In this regard, it was shown that telomere length in WBC is shorter in hypertensive men with carotid artery plaques compared with hypertensive men without plaques [60]. Multivariate analysis showed that in addition to age, telomere length is a significant predictor of the presence of carotid artery plaques. The findings from that study suggest that in the presence of chronic hypertension, which is a major risk factor for atherosclerosis, shorter telomere length in WBC is associated with an increased predilection to carotid artery atherosclerosis. The possible role of telomere dysfunction in atherosclerosis and how cardiovascular risk factors affect

telomerase activity and telomere length has been comprehensively discussed elsewhere [16].

Conclusion

Telomere biology is emerging as an important issue in the pathogenesis of hypertension. Telomere length is highly variable among individuals of the same age, and is determined by both genetic and environmental factors. In the SHR model, telomerase activation was observed in the aorta before the onset of hypertension, and telomeres were longer in primary cultures of aortic medial VSMC obtained from these animals compared with cells from WKY controls. Several studies have, however, shown a connection between established hypertension and low telomerase activity or short telomeres: (i) compared with normotensive subjects, hypertensive patients exhibit shorter telomeres in WBC; (ii) lower telomerase activity was detected in EPC from hypertensive rats and patients with essential hypertension, which may contribute to premature cell senescence; and (iii) *TERC*-null mice exhibit augmented expression of the vasoconstrictor peptide endothelin 1 and develop hypertension. Increased arterial telomerase activity in prehypertensive stages may thus contribute to the onset of pathological vascular remodeling in SHR by inducing hyperplastic growth of arterial VSMC. Whether these alterations also occur in humans is unknown. Based on the findings in WBC and EPC, it can be suggested that the prolonged exposure to risk factors that are frequently associated with high blood pressure and are known to inhibit telomerase activity and accelerate telomere shortening (e.g. oxidative stress and insulin resistance) may ultimately provoke VSMC senescence and favour disease progression (e.g. by increasing arterial stiffness or inducing the synthesis of vasoconstrictor molecules, such as endothelin 1; Fig. 1). Therefore, it is of utmost importance to investigate whether telomerase activity and telomere length are also altered in arterial cells from hypertensive patients and experimental animals.

Regarding target organ damage (Fig. 2), both telomerase activation and telomere attrition have been observed in hypertension-related heart disease: (i) telomerase activity is necessary for cardiac stem cell proliferation and may thus support new cardiomyocyte formation during cardiac hypertrophy; and (ii) telomere shortening may contribute to the transition from maladaptive cardiac hypertrophy to heart failure. In support of this notion, telomere exhaustion occurs in cardiac hypertrophy consecutive to aortic stenosis and in the aged diseased heart, in spite of the presence of telomerase activity. Moreover, mechanical injury in the heart downregulates TRF2, shortens telomeres and activates DNA damage-induced apoptosis in cardiac tissue. As in the vascular wall, oxidative stress appears to contribute significantly to telomere erosion in the diseased heart. On the other hand, accelerated telomere attrition of cortex cells may be one of the factors involved in the accelerated aging of the kidney in hypertension. and this, in turn, may facilitate the development of nephroangiosclerosis.

As our knowledge on telomeres and cardiovascular disease grows, the challenge will be to ascertain whether all this information might translate into clinical applications. In particular, whether targeting the telomere and associated proteins is a suitable therapeutic strategy for the treatment of hypertension and related target organ damage is currently unknown. Of interest is the fact that it has been reported that the rate of senescence and telomerase activity in EPC were significantly higher and lower, respectively, in rats treated with angiotensin II alone than in rats treated with angiotensin II plus the angiotensin II type 1 receptor blocker valsartan [43]. As these two parameters were unchanged in rats receiving angiotensin II plus hydralazine, it can be hypothesized that angiotensin II type 1 receptor blockade may have specific beneficial effects on telomere dysfunction in hypertension. On the other hand, it has been shown that thiazolidinediones, which are antidiabetic agents that can reduce restenosis [81], cause VSMC growth arrest via TERT inhibition [82]. Very recently, Brouilette *et al.* [83] reported that the risk of developing coronary artery disease was increased by approximately twofold in individuals with shorter WBC telomere length, and pravastatin completely attenuated this telomere-related risk. The mean leukocyte telomere length could thus identify individuals who would benefit most from statin treatment. Likewise, recent data suggest that shorter telomere length in hypertension may be one of the factors that explain why some hypertensive patients are more prone than others to developing atherosclerotic lesions. Whether 'telomerization' strategies may find therapeutic application to prevent or ameliorate target organ damage in hypertension remains to be explored.

In conclusion, more basic research is needed to shed light on the relationships between telomere pathobiology and hypertension. As most studies have focused so far on telomerase, it is necessary to explore the role in hypertension of additional telomere-associated proteins. Research in this field would benefit from the generation of genetically modified mice exhibiting tissue-specific alterations in telomere-associated proteins. Furthermore, large cross-sectional and longitudinal population-based studies are required to address two unresolved questions: (i) does short telomere length at birth predispose to hypertension and related disease? and (ii) given that an accelerated rate of telomere shortening may be expected from the chronic exposure to factors frequently associated with high blood pressure, is exaggerated telomere exhaustion a surrogate marker of hypertension and related diseases? If so, measuring telomere attrition rates may become a useful tool to identify hypertensive patients who are more prone to experience target organ damage.

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