REVIEW

Telomere length and cardiovascular disease

Longueur des télomères et maladies cardiovasculaires

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Summary Telomeres are structures composed of deoxyribonucleic acid repeats that protect the end of chromosomes, but shorten with each cell division. They have been the subject of many studies, particularly in the field of oncology, and more recently their role in the onset, development and prognosis of cardiovascular disease has generated considerable interest. It has already been shown that these structures may deteriorate at the beginning of the atherosclerotic process, in the onset and development of arterial hypertension or during myocardial infarction, in which their length may be a predictor of outcome. As telomere length by its nature is a marker of cell senescence, it is of particular interest when studying the lifespan and fate of endothelial cells and cardiomyocytes, especially so because telomere length seems to be regulated by various factors notably certain cardiovascular risk factors, such as smoking, sex and obesity that are associated with high levels of oxidative stress. To gain insights into the links between telomere length and cardiovascular disease, and to assess the usefulness of telomere length as a new marker of cardiovascular risk, it seems essential to review the considerable amount of data published recently on the subject.
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MOTS CLÉS
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Résumé Les télomères sont des structures nucléoprotéiques protégeant l'extrémité des chromosomes, et dont la longueur est susceptible de se raccourcir au cours des divisions cellulaires. Ils ont fait l'objet de très nombreuses études, particulièrement dans le domaine de la cancérologie mais depuis peu, un intérêt croissant est porté sur leur rôle dans l'installation, le développement et le pronostic des maladies cardiovasculaires. En effet, il a déjà été démontré que ces structures sont potentiellement altérées au cours de l'installation du processus athéromateux, dans l'initiation et le développement de l'hypertension artérielle ou encore au cours de l'infarctus du myocarde pour lequel leur longueur pourrait jouer un rôle pronostique. Comme la longueur des télomères constitue par nature un marqueur de la sénescence cellulaire, leur intérêt est certain lorsque l'on étudie l'espérance de vie et le devenir des cellules endothéliales et des cardiomyocytes. De plus, la longueur des télomères semble régulée par différents facteurs, notamment certains facteurs de risque cardiovasculaires comme la consommation de tabac, le genre ou encore l'obésité pour laquelle un stress oxydatif important est rencontré. Pour cela, il est intéressant de réaliser un bilan bibliographique sur les nombreuses données récentes concernant la longueur des télomères dans le cadre des pathologies cardiovasculaires et ainsi d'apprécier leur intérêt en tant que nouveau marqueur de susceptibilité cardiovasculaire.
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Background

The length of telomeres, which are located at the ends of chromosomes, reflects the lifespan of a cell. Telomere length decreases with each cell division and this process is necessary for appropriate DNA replication in eukaryotes. The regular shortening of telomere length will eventually lead to exposure of the genome and trigger the expression of proteins involved in apoptosis. These events make up the phenomenon called cellular senescence. Cellular senescence is associated with the onset and development of certain diseases. In this context, it is interesting to explore telomere ''dynamics'' in ischaemic and non-ischaemic cardiovascular diseases, and to determine whether these structures could be potential pharmacological targets (destroyed to treat a tumour or restored in the case of heart failure to preserve the integrity of myocardial cells).

Telomere biology

Location and function of telomeres

Telomeres are composed of nucleotides and are located at the end of chromosomes in eukaryotic cells. They cap the termination of the double strands of DNA and thus preserve the integrity and stability of the genome during replication [1]. Telomeres are made up of a repetitive sequence of six nitrogenous bases rich in guanine (TTAGGG). This sequence is repeated over several thousand base pairs at the 3' end of DNA (4 to 15 kilobases in humans). In most human somatic cells, the length of the telomeres decreases by 20 to 200 base pairs with each cell division. This loss of genetic material corresponds to the phenomenon called ''the end replication problem''. If this shortening of telomeres is not repaired, it eventually leads to cessation of the cell cycle and cell death by apoptosis [2]. The synthesis of telomere DNA requires the activity of specialized enzyme complexes: telomerases. These complexes are made up of

various proteins (TRAF1, TRAF2, Ku86, TIN2, etc.) [3]. Their function is to lengthen telomeres by the synthesis of two supplementary TTAGGG sequences at their ends. Typically, telomerase activity is diminished or even absent in most adult somatic cells, the exception being cells with a strong potential for division, like active lymphocytes and certain types of stem cells [4].

Regulation of telomere length

The maintenance of telomere length depends on several factors, including the composition in associated proteins, the level of oxidative stress and the level of telomerase activation as well as telomere length itself [1,5]. This is why telomere length varies considerably from one species to another and from one individual to another. Moreover, it seems that telomere length may be affected by certain genetic factors, notably linked to chromosome X [6]: this was shown in a study by Nawrot et al. in 2004 involving a cohort of families.

Telomerase activity, however, seems to be one of the key elements in the maintenance of telomere integrity. Indeed, cells with short telomeres and an absence of telomerase activity become senescent and go into apoptosis more quickly than do cells with telomeres that are long enough not to require telomerase activity to survive [2,3]. Inversely, some cells are deficient in TERC, TERT and other proteins necessary for telomerase function. This is illustrated by the transfection of primary B and T lymphocytes from patients with dyskeratosis congenita with exogenous TERC, which restored telomerase activity and increased telomere length [7].

Moreover, the role of the Rad54, which is involved in DNA repair, in the regulation of telomere length was brought to light recently. Indeed, Rad54-deficient mice presented severe telomere shortening, and this in the absence of any modification in telomerase activity [8]. These findings tend to show that Rad54 protein is involved in a mechanism

that maintains the integrity of telomeres independently of telomerases.

The regulation of telomere length also depends on the level of methylation of certain histones, namely histones H3 and H4, associated with subtelomeric regions. The methylation of these histones decreases access to telomere sequences and thus diminishes telomerase activity [9]. Therefore, proteins that play a role in the regulation of these methylations have an impact on telomere length. For example, proteins of the retinoblastoma family increase the methylation of subtelomeric regions and thus diminish telomere length [10]. In contrast, retinoblastoma protein 2, which depresses the activity of DNA methytransferase, responsible for the methylation of subtelomeric regions, plays a role in increasing telomere length [11,12].

The existence of telomeric RNA (called TERRA or TelRNA), which is transcribed by RNA polymerase II, has been shown recently. These telomeric RNA transcriptions may have a negative impact on telomere length [13]. Finally, one of the major mechanisms of telomere shortening is the activity of exonucleases 5′-3′ [5,14]. Indeed, the role of these exonucleases is to degrade the RNA primer used in the replication of the DNA necessary for DNA polymerase activity. This deterioration creates lesions in which the DNA is in the single strand form within the replication loop. The presence of single-strand DNA prevents the formation of Okazaki fragments and thus elongates the DNA, but can lead to an increase in: damage to DNA; the risk of fusion of chromosome extremities [15]; and the activation of p53-dependent responses to DNA damage [16].

The maintenance of telomere length within eukaryotic cells is thus a complex phenomenon that involves a wide range of factors. Several mechanisms acting in a synergistic fashion thus appear to stabilize telomere length. The different mechanisms mentioned above involved in the regulation of telomere length are shown schematically in Fig. 1.

Oxidative stress

One of the principal mechanisms involved in telomere shortening is represented by the level of free radical oxidative stress. Oxidative damage to telomeric DNA appears as the formation of an adduct of guanine, 8-oxodG, which is involved in the initiation of disturbances in the maintenance of telomere length. Moreover, ROS, and especially the hydroxyl radical, induce breaks in DNA and deteriorate DNA base repair [17]. Unlike the rest of the genome, telomeres seem to be unable to repair breaks in single-strand DNA [18]. Because of this, telomeres are particularly sensitive to the accumulation of the guanine oxide adduct [19]. This sensitivity to oxidation in telomeres was revealed by Oikawa et al. in two studies. The first, in 1999 [20], concerned the exposure of DNA from calf thymus to hydrogen peroxide associated with copper (II). The results showed the presence of DNA lesions especially at the level of the 5′-GGG-3′ triplet. The second study, in 2001 [21], showed that the exposure of fibroblasts to ultraviolet A also induced damage, highlighted by the presence of 8-oxodG localized at telomeres. Moreover, the presence of non-matched bases within the telomeric sequence interferes with the DNA replication mechanisms necessary for the maintenance of structure

integrity. Oxidative stress may thus induce premature shortening of telomeres independently of age [22]. Telomeres are unable to repair oxidized DNA, which therefore accentuates the damage caused by ROS. Petersen et al. [18] showed that lesions caused by hydrogen peroxide were repaired slowly and incompletely at the level of the telomeres, which is not the case at the level of the mini-satellites. One of the hypotheses put forward is that TRF2 binding at the level of the telomere could prevent DNA repair enzymes from reaching the site [23]. Moreover, TRF2 interacts with polymerase β and thus has a potential negative effect on the repair of DNA damage [23]. TRF2 also inhibits ataxia telangiectasia muted kinase phosphorylation, which is involved in the initiation process of DNA repair [24].

One important point, which must always be underlined, is the in vivo concomitance between increased production of ROS and the development of an inflammatory process [25]. In this context, the proinflammatory cytokines produced can cause telomere shortening directly. In this field, several studies have shown that telomerase activity correlated inversely with levels of tumour necrosis factor alpha. The latest, via the activation of two transcription factors (nuclear factor-kappa B and activator protein 1), is responsible for an increase in the expression of proinflammatory genes [26,27]. In this context, the studies of Beyne-Rauzy et al. [26] showed that the reduction in telomere length induced by exposure of cells to tumour necrosis factor alpha brought about a negative regulation in the level of expression of human TERT.

Telomeres and cardiovascular disease in humans

A reduction in and/or loss of function in cells that make up the myocardium or vessels is at the root of both acute and chronic onset of dysfunction that occurs during normal or pathological ageing [28]. Because they shorten gradually according to the number of cell cycles, telomeres can be considered markers of the cellular senescence [29].

Atherothrombosis and cardiovascular risk factors

Independently of age, telomeres may be involved in the initiation and/or progression of cardiovascular disease. The studies of Brouilette et al. [30] and Samani et al. [31] showed that patients with CAD or early myocardial infarction had shorter telomeres compared with control subjects of the same age and sex [32]. This relationship was confirmed in other studies [32,33], although it was not possible to determine whether the shortening of telomere length was a cause or a consequence of the onset of the CAD. The aim of these studies was to determine telomere length in circulating cells, such as white cells, which does not necessarily reflect telomere ''dynamics'' in tissues that are affected directly by the disease (e.g., the myocardium and the coronary vessels). Recently, a study by Wilson et al. answered this question in part; they showed a significant relationship between telomere length in leukocytes and telomere length in vascular tissues, in this case in vascular cells from a human ascending aorta aneurysm [34].
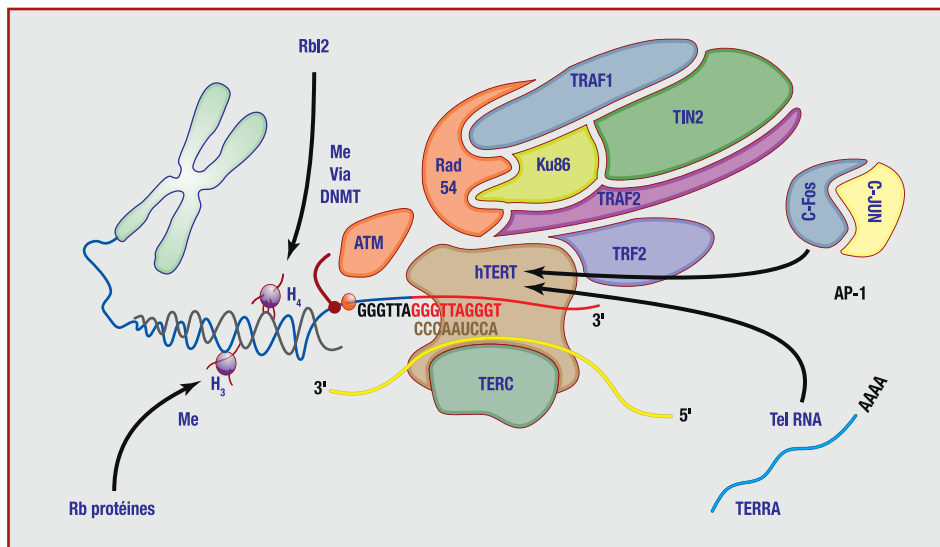
Figure 1. Schematic representation of telomere structure in the eukaryotic cell and the principal mechanisms involved in the regulation (positive or negative) of telomere length. AP-1: activator protein 1; ATM: ataxia telangiectasia muted; c-Fos: cellular proto-oncogene belonging to the immediate early gene family of transcription factors; c-Jun: a gene, which in combination with c-Fos, forms the AP-1 early response transcription factor; DNMT: DNA methyltransferase; h-TERT: human telomerase reverse transcriptase; Me: methyl; Rad54: eukaryotic homologue of the prokaryotic RecA protein 54; Rb: retinoblastoma; Rbl2: retinoblastoma protein 2; RNA: ribonucleic acid; TERC: telomerase RNA component; TERRA or TelRNA: telomeric RNA; TIN2: TATA binding protein-related factor 1 (TRF1)-interacting nuclear protein 2; TRAF1: tumour necrosis factor receptor-associated factor 1; TRAF2: tumour necrosis factor receptor-associated factor 2; TRF2: TATA binding protein-related factor 2.

In many cases, CAD appears in the context of atherothrombosis and cellular senescence, both of which have been the subject of many studies in recent years [28]. It has been suggested that telomere length in vascular cells may play a critical role in the development of CAD, by setting up a particular phenotype of senescence in smooth muscle cells and endothelial cells [28,33]. Brouilette et al. [35], in a case-control study of 104 subjects (45 presenting with a family history of CAD and 59 control subjects), showed an association between a family history of CAD and telomere shortening. These results suggest that the presence of short telomeres is a principal anomaly in atherosclerotic coronary diseases. With regard to hypertensive patients, a recent study showed that such patients had shorter telomeres than healthy subjects [36].

Other cardiovascular risk factors also appear to be associated significantly with leukocyte telomere length in humans. In a cohort of 1122 women, Valdes et al. showed a significant negative association between leukocyte telomere length, a history of smoking ($r = -0.087$) and BMI ($r = -0.077$) [37]. As for metabolic-type risk factors, it appears that the level of homocysteine correlates negatively with leukocyte telomere length. This was studied by Richards et al. in 2008 in a cohort of 1319 subjects [38]. Concerning the effect of smoking, the study by Morla et al. in 2006 [39] confirmed the results of Valdes et al. in 2005 [37] on the negative effect of smoking on leukocyte telomere length. The aim of the preliminary study by Morla et al. was to determine the impact of smoking on leukocyte telomere length in the onset of chronic obstructive pulmonary disease (50 smokers vs 26 non-smokers). The results, however, showed no difference in leukocyte telomere length between subjects with chronic obstructive pulmonary disease and those without. They confirmed the results of Valdes et al. by showing that exposure to cigarette smoke shortened leukocyte telomeres ($r = -0.45$). With regard to the risk induced by obesity, the association between BMI and telomere length was confirmed recently in a study by Nordfjall et al. in 2008 [40]. There was a negative association between BMI and leukocyte telomere length in women but not in men ($r = -0.106$).

Going beyond the hypothesis that telomere shortening could be involved in the onset and development of CAD, recent studies have shown that telomere length is also associated with increased mortality, independently of other cardiovascular risks factors in patients with stable CAD [41]. This suggests that, on the one hand, telomere length could be used in risk stratification, and on the other hand, leukocyte telomere length is a marker that incorporates a wide range of environmental and genetic factors, which alone or in combination cause cellular stress. One study has shown that telomere length, although correlating with mortality, was in no way associated with other markers such as C-reactive protein, BMI or the taking of supposedly protective treatments such as inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA reductase (statins) [41]. These results, however, are not in keeping with other recently published studies [32,37].

To summarize, at the present time, it is still difficult to define with any degree of certainty the role of telomeres in and the impact of telomere length on the atherosclerotic process. It is, however, accepted that the degree of telomere shortening is related to the likelihood of developing atherosclerotic plaques and is a predictor of mortality in CAD patients.

Heart failure

Another field of interest for the study of telomere length in CAD is chronic or ischaemic heart failure. Indeed, cer-

tain studies have tended to show that patients presenting with chronic heart failure have shorter leukocyte telomeres than do healthy subjects (ratio T/S respectively 0.64 vs 1.05, $p < 0.001$). In addition, this association seems to be more marked in more severe forms of heart failure, according to the study of Van der Harst et al. [42]. Moreover, telomere length was inversely proportional to the grade of heart failure according to the New York Heart Association classification (ratio T/S of 0.67 for grade II, 0.63 for grade III and 0.55 for grade IV, $p < 0.05$). These studies also showed that the presence of ischaemic aetiologies (coronary, cerebral or peripheral artery disease) reinforced this association (ratio T/S 0.72 for subjects with no ischaemic aetiologies, 0.65 for one aetiology, 0.48 for two aetiologies and 0.43 for three aetiologies, $p < 0.001$).

In an RNA telomeric Terc-deficient (Terc$^{-/-}$) mouse model, which has decreased telomere length [43], an association was found between the apparition of heart failure and telomere shortening [44] from the fifth generation of knockout mice onwards. This suggests that the mechanisms that lead to the onset of heart failure and telomere shortening are closely linked. In the same way, the studies by Van der Harst et al. [42] brought to light a hereditary component of telomere length suggesting the existence of a phenotype that was predisposed to the onset of heart failure.

Another study conducted in a population of individuals over 85 years of age (the Newcastle 85+ study, reported by Collerton et al.), showed a positive association between left ventricular function and telomere length [29]. This association also seems to be independent of a history of ischaemia or any other cardiovascular risk factor.

Although the mechanisms thought to be involved in the association between telomere shortening and heart failure have not yet been elucidated totally, apoptotic phenomena have to be considered, and cell ageing as well as oxidative stress probably play a key role in these pathophysiological cardiovascular processes.

Cellular senescence and the vascular endothelium

Vascular ageing, which involves endothelial cells, has been the subject of many studies since physiological function is deteriorating [45]. Indeed, this endothelial dysfunction is found in young subjects who present cardiovascular disease [46]. At the cellular level, the ageing of healthy endothelial cells leads to a state of ''stasis'', which is characterized by metabolic activity over several months, but the inability of the cell to respond to mitotic stimuli [47]. This cellular senescence may be accelerated by successive cell divisions via the progressive and cumulative shortening of the telomere. When telomere length falls below a certain threshold, cells go into senescence and a process of apoptosis [35]. However, these phenomena of cellular senescence may occur prematurely in the wake of exposure to various factors, including oxidative stress [48].

Further perspectives of research

Lessons from experimental studies have shown that oxidative stress induces telomeric DNA base damage, and could represent a major pathway of telomere attrition in vitro. However, the physiopathological mechanisms linking levels of oxidative stress and telomere homeostasis are far from being elucidated fully. Moreover, it is not known whether ROS-induced 8-oxoguanine lesions or other oxidative guanine lesions could accumulate in telomeres in vivo. Interesting studies in 8-oxoguanine glycosylase null (OGG1$^{-/-}$) mice have suggested recently that oxidative damage can arise in telomeric DNA, and that repairing guanine oxidative damage is required in the maintenance of telomere integrity in mammals [49]. Furthermore, emerging data suggested paradoxically a beneficial role for the oxidation product of guanine under given conditions (low levels of oxidative stress) [50]. As telomere shortening has been linked to human ageing, cancer and cardiovascular diseases, whether these phenomena could be involved in such pathologies remains a major challenge for future research. Moreover, observational and randomized studies analysing the influence of treatments interfering with oxidative stress, such as statin therapy or lifestyle intervention (e.g., Mediterranean diet or physical activity), on telomere length are required to better understand the pathophysiology of CAD and the role of biological ageing.

Conclusion

In conclusion, telomeres provide an overall index of global exposition of the body to inflammatory and oxidative phenomena. The mechanisms that link the onset and/or progression of cardiovascular disease to the integrity of telomeres need further investigation. At the moment, there is insufficient evidence to validate the associations and determine whether telomere shortening is a cause or consequence of disease. The non-specific modulation of telomere length by modifiable and non-modifiable risk factors suggests a limited potential as a ''biomarker'' of cardiovascular disease.

Conflict of interest statement

There is no conflict of interest to disclose.

References

[1] Blackburn EH. Switching and signaling at the telomere. Cell 2001;106:661—73.

[2] Edo MD, Andres V. Aging, telomeres, and atherosclerosis. Cardiovasc Res 2005;66:213—21.

[3] Collins K. Mammalian telomeres and telomerase. Curr Opin Cell Biol 2000;12:378—83.

[4] Liu K, Schoonmaker MM, Levine BL, et al. Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes. Proc Natl Acad Sci U S A 1999;96:5147—52.

[5] Houben JM, Moonen HJ, van Schooten FJ, et al. Telomere length assessment: biomarker of chronic oxidative stress? Free Radic Biol Med 2008;44:235—46.

[6] Nawrot TS, Staessen JA, Gardner JP, et al. Telomere length and possible link to X chromosome. Lancet 2004;363:507—10.

[7] Kirwan M, Beswick R, Vulliamy T, et al. Exogenous TERC alone can enhance proliferative potential, telomerase activity and telomere length in lymphocytes from dyskeratosis congenita patients. Br J Haematol 2009;144:771—81.

[8] Jaco I, Munoz P, Goytisolo F, et al. Role of mammalian Rad54 in telomere length maintenance. Mol Cell Biol 2003;23:5572—80.

[9] Blasco MA. The epigenetic regulation of mammalian telomeres. Nat Rev Genet 2007;8:299—309.

[10] Schoeftner S, Blasco MA. A 'higher order' of telomere regulation: telomere heterochromatin and telomeric RNAs. EMBO J 2009;28:2323—36.

[11] Benetti R, Gonzalo S, Jaco I, et al. A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases. Nat Struct Mol Biol 2008;15:998.

[12] Okano M, Xie S, Li E. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat Genet 1998;19:219—20.

[13] Azzalin CM, Reichenbach P, Khoriauli L, et al. Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends. Science 2007;318:798—801.

[14] von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. Ann N Y Acad Sci 2000;908:99—110.

[15] van Steensel B, Smogorzewska A, de Lange T. TRF2 protects human telomeres from end-to-end fusions. Cell 1998;92:401—13.

[16] Karlseder J, Broccoli D, Dai Y, et al. p53- and ATM-dependent apoptosis induced by telomeres lacking TRF2. Science 1999;283:1321—5.

[17] Fotiadou P, Henegariu O, Sweasy JB. DNA polymerase beta interacts with TRF2 and induces telomere dysfunction in a murine mammary cell line. Cancer Res 2004;64:3830—57.

[18] Petersen S, Saretzki G, von Zglinicki T. Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. Exp Cell Res 1998;239:152—60.

[19] Kawanishi S, Oikawa S. Mechanism of telomere shortening by oxidative stress. Ann N Y Acad Sci 2004;1019:278—84.

[20] Oikawa S, Kawanishi S. Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. FEBS Lett 1999;453:365—8.

[21] Oikawa S, Tada-Oikawa S, Kawanishi S. Site-specific DNA damage at the GGG sequence by UVA involves acceleration of telomere shortening. Biochemistry 2001;40:4763—8.

[22] von Zglinicki T. Oxidative stress shortens telomeres. Trends Biochem Sci 2002;27:339—44.

[23] Richter T, Saretzki G, Nelson G, et al. TRF2 overexpression diminishes repair of telomeric single-strand breaks and accelerates telomere shortening in human fibroblasts. Mech Ageing Dev 2007;128:340—5.

[24] Karlseder J, Hoke K, Mirzoeva OK, et al. The telomeric protein TRF2 binds the ATM kinase and can inhibit the ATM-dependent DNA damage response. PLoS Biol 2004;2:E240.

[25] Floyd RA, Hensley K, Jaffery F, et al. Increased oxidative stress brought on by pro-inflammatory cytokines in neurodegenerative processes and the protective role of nitrone-based free radical traps. Life Sci 1999;65:1893—9.

[26] Beyne-Rauzy O, Prade-Houdellier N, Demur C, et al. Tumor necrosis factor-alpha inhibits hTERT gene expression in human myeloid normal and leukemic cells. Blood 2005;106:3200—5.

[27] Rahman I, Gilmour PS, Jimenez LA, et al. Oxidative stress and TNF-alpha induce histone acetylation and NF-kappaB/AP-1 activation in alveolar epithelial cells: potential mechanism in gene transcription in lung inflammation. Mol Cell Biochem 2002;234—235:239—48.

[28] Matthews C, Gorenne I, Scott S, et al. Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: effects of telomerase and oxidative stress. Circ Res 2006;99:156—64.

[29] Collerton J, Martin-Ruiz C, Kenny A, et al. Telomere length is associated with left ventricular function in the oldest old: the Newcastle 85+ study. Eur Heart J 2007;28:172—6.

[30] Brouilette S, Singh RK, Thompson JR, et al. White cell telomere length and risk of premature myocardial infarction. Arterioscler Thromb Vasc Biol 2003;23:842—6.

[31] Samani NJ, Boultby R, Butler R, et al. Telomere shortening in atherosclerosis. Lancet 2001;358:472—3.

[32] Brouilette SW, Moore JS, McMahon AD, et al. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. Lancet 2007;369:107—14.

[33] Ogami M, Ikura Y, Ohsawa M, et al. Telomere shortening in human coronary artery diseases. Arterioscler Thromb Vasc Biol 2004;24:546—50.

[34] Wilson WR, Herbert KE, Mistry Y, et al. Blood leucocyte telomere DNA content predicts vascular telomere DNA content in humans with and without vascular disease. Eur Heart J 2008;29:2689—94.

[35] Brouilette SW, Whittaker A, Stevens SE, et al. Telomere length is shorter in healthy offspring of subjects with coronary artery disease: support for the telomere hypothesis. Heart 2008;94:422—5.

[36] Lung FW, Ku CS, Kao WT. Telomere length may be associated with hypertension. J Hum Hypertens 2008;22:230—2.

[37] Valdes AM, Andrew T, Gardner JP, et al. Obesity, cigarette smoking, and telomere length in women. Lancet 2005;366:662—4.

[38] Richards JB, Valdes AM, Gardner JP, et al. Homocysteine levels and leukocyte telomere length. Atherosclerosis 2008;200:271—7.

[39] Morla M, Busquets X, Pons J, et al. Telomere shortening in smokers with and without COPD. Eur Respir J 2006;27:525—8.

[40] Nordfjall K, Eliasson M, Stegmayr B, et al. Telomere length is associated with obesity parameters but with a gender difference. Obesity (Silver Spring) 2008;16:2682—9.

[41] Farzaneh-Far R, Cawthon RM, Na B, et al. Prognostic value of leukocyte telomere length in patients with stable coronary artery disease: data from the Heart and Soul Study. Arterioscler Thromb Vasc Biol 2008;28:1379—84.

[42] van der Harst P, van der Steege G, de Boer RA, et al. Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. J Am Coll Cardiol 2007;49:1459—64.

[43] Wong LS, Oeseburg H, de Boer RA, et al. Telomere biology in cardiovascular disease: the TERC−/− mouse as a model for heart failure and ageing. Cardiovasc Res 2009;81:244—52.

[44] Leri A, Franco S, Zacheo A, et al. Ablation of telomerase and telomere loss leads to cardiac dilatation and heart failure associated with p53 upregulation. EMBO J 2003;22:131—9.

[45] Donato AJ, Eskurza I, Silver AE, et al. Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. Circ Res 2007;100:1659—66.

[46] Cohen RA. The role of nitric oxide and other endothelium-derived vasoactive substances in vascular disease. Prog Cardiovasc Dis 1995;38:105—28.

[47] Chen J, Goligorsky MS. Premature senescence of endothelial cells: Methusaleh's dilemma. Am J Physiol Heart Circ Physiol 2006;290:H1729—39.

[48] Toussaint O, Medrano EE, von Zglinicki T. Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. Exp Gerontol 2000;35:927—45.

[49] Wang Z, Rhee DB, Lu J, et al. Characterization of oxidative Guanine damage and repair in mammalian telomeres. PLoS Genet 2010;6:e1000951.

[50] Radak Z, Boldogh I. 8-Oxo-7,8-dihydroguanine: links to gene expression, aging, and defense against oxidative stress. Free Radic Biol Med 2010;49:587—96.