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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Abbreviations: 8-oxodG, 8-oxo-7,8-dihydroguanine; BMI, body mass index; CAD, coronary artery disease; DNA, deoxyribonucleic acid; Rad54, eukaryotic homologue of the prokaryotic RecA protein 54; RNA, ribonucleic acid; ROS, reactive oxygen species; TERC, telomerase ribonucleic acid component; TERT, telomerase reverse transcriptase; TRF2, TATA binding protein-related factor 2.

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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 (TRA1, 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 methyltransferase, 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 mutated 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 Brouillette 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].
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 risk 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 T erc-deficient (T erc\(^{-/}\)) 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 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 \[48\].

**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 \[48\].

**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**

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