Original Article

Telomere Attrition in White Blood Cell Correlating with Cardiovascular Damage

Hidekatsu NAKASHIMA, Ryoji OZONO, Chizuko SUYAMA, Taijiro SUEDA*,
Masayuki KAMBE, and Tetsuya OSHIMA

Aging is a major risk factor for cardiovascular disease. Chronological aging does not always parallel biological aging, but there is no reliable biomarker for the latter. In the present study, we tested the hypothesis that telomere attrition in white blood cells is related to endothelial dysfunction and the extent of atherosclerosis, and thus may serve as a useful marker for biological aging. We evaluated telomere lengths in white blood cells by measuring the mean telomere restriction fragment length (mTRFL), as well as endothelial function by flow mediated dilatation (FMD) in the brachial artery, in patients with various degrees of cardiovascular damage and in normal subjects. Cardiovascular damage was assessed by a cardiovascular damage (CVD) score, with 1 point being given for the presence of each cardiovascular risk factor (hypertension, hyperlipidemia and diabetes) and for each event (angina, myocardial infarction, cerebrovascular event and peripheral vascular disease). Subset analysis of CVD score groups revealed that mTRFL and FMD decreased in the rank order of CVD score. Although mTRFL was inversely correlated with age, telomere index, defined as the ratio of TRFL to TRFL predicted by age, also decreased with increase in CVD score. These results indicate that telomere attrition in white blood cells is more closely associated with endothelial damage and atherosclerosis than is chronological aging, supporting the hypothesis that mTRFL in white blood cells is a useful marker for biological aging of the cardiovascular system. (*Hypertens Res* 2004; 27: 319–325)

Key Words: telomere, aging, risk factors, cardiovascular disease, endothelium

Introduction

The incidence and prevalence of cardiovascular diseases increase steeply with advance of age (1), and thus advancing age unequivocally confers the major risk for cardiovascular diseases. However, chronological age does not always reflect biological aging, which may be influenced by genetic and environmental factors (2), and age-associated cardiovascular risks therefore need to be assessed by some measures for biological aging (3, 4). Telomeres are nucleoprotein complexes that cap the ends of linear chromosomes protecting the

genome from end-to-end fusion and genetic recombination (5). Telomeric DNA is composed of several thousand double-stranded repeats of the sequence TTAGGG (5), which erodes progressively with each round of cell division (5, 6). Attrition of the telomere to a specific length triggers a proliferative arrest called cellular senescence, which accompanies age-associated cellular dysfunction. This feature allows the telomere to be used as a “molecular counting device” (7). Senescent vascular cells have been shown to accumulate in atherosclerotic lesions of human (7) and rabbit (8) coronary arteries, supporting the hypothesis (9) that biological aging is associated with atherogenesis and that a shortening of telo-

From the Department of Clinical Laboratory Medicine and *Department of Surgery, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan.

This study was supported by Grants-in-Aid for Scientific Research (14572183 and 15390177) from the Ministry of Education, Culture, Sports, Science and Technology, Japan and a grant from the Charitable Trust Clinical Pathology Research Foundation of Japan.

Address for Reprints: Ryoji Ozono, M.D., Department of Clinical Laboratory Medicine and *Department of Surgery, Hiroshima University Graduate School of Biomedical Sciences, 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8551, Japan. E-mail: ozono@hiroshima-u.ac.jp

Received November 11, 2003; Accepted in revised form January 16, 2004.

mere length may be a biomarker for biological aging.

To test this hypothesis, we investigated the relationship between telomere length in white blood cells and the extent of cardiovascular damage in subjects with various degrees of cardiovascular risks and diseases. The telomere length of white blood cells, although not a direct measure from cardiovascular tissues, represents a genetically mediated difference in telomere length (10) and is related to immunological function (11). Although atherosclerotic disease is focal, there is increasing evidence that it is accompanied by more generalized perturbations, and particularly systemic inflammation (12).

Methods

Subjects

Subjects aged over 40 years (total $n = 83$) were recruited from among patients being followed up at Hiroshima University Hospital for treatment of cardiovascular risk factors (hypertension, hypercholesterolemia, and diabetes) and already known events of vascular diseases (coronary heart disease, peripheral artery disease, and cerebrovascular disease). Healthy volunteers and those who visited Hiroshima University Hospital for clinical investigations and were proven to be free of the risks and diseases were also included. Hypertensives were defined as those subjects having a sitting systolic blood pressure (SBP) >140 mmHg and/or diastolic blood pressure (DBP) >90 mmHg on three different occasions or those subjects taking anti-hypertensive drugs. Hyperlipidemia was defined as a low-density lipoprotein level >130 mg/dl. Diabetes was defined according to the criteria of the World Health Organization (13). Coronary heart disease (CHD) and peripheral vascular disease were all angiographically diagnosed. Diagnosis of CHD was based on the presence of at least one atherosclerotic lesion ($>75\%$ diameter) in coronary vessels and/or infarcted myocardium detected by left ventriculography. Patients with vasospasm without any atherosclerotic lesion were excluded. Diagnosis of cerebrovascular disease was based on symptoms (stroke, including transient ischemia) and/or the presence of an ischemic focus in brain magnetic resonance images. Informed consent for participation in the study was obtained from all subjects, and the study was approved by the Hiroshima University Human Ethics Committee for Human Genomic Research.

Assessment of Presumed Atherosclerotic Load

Presumed atherosclerotic load was assessed by a cumulative cardiovascular damage (CVD) score, with 1 point being given for the presence of each risk factor (hypertension, hypercholesterolemia, and diabetes mellitus) or cardiovascular disease (coronary heart disease, peripheral vascular disease, and cerebrovascular disease).

Measurement of the Telomere Lengths of White Blood Cells

The mean length of telomeres in white blood cells was calculated as the telomere restriction fragment length (TRFL) using the Southern blot technique as previously described (4, 14). Genomic DNA was extracted from whole blood by the standard technique. The DNA samples and molecular markers were resolved on 0.6% agarose gel and transferred to nylon membranes (Amersham, Upsala, Sweden), and then the membranes were hybridized with a telomeric probe (TTAGGG) (6), which was 3'-end labeled with digoxigenin (Roche, Mannheim, Germany). The probe was detected using a DIG Luminiscent detection kit (Roche) and exposed to X-ray film. The image of the film was digitalized and analyzed using Scion Image software. The mean TRFL (mTRFL) was calculated by covering the intensities of signals (OD_i) to molecular size based on DNA molecular size markers using the formula $TRFL = \sum OD_i / (\sum OD_i / L_i)$, where OD_i is the integrated signal at position i and L_i is the length of the DNA fragment in position i (6).

Telomere Index

It is known that mTRFL in white blood cells shortens as a function of age (14) and that women's telomeres are longer than men's (15). Thus, attrition of mTRFL was also assessed by telomere index defined as the ratio of mTRFL relative to "normal" mTRFL predicted from the subject's age and sex. We predicted the normal mTRFL for the subject's age on the basis of a simple regression analysis between age and mTRFL in subjects from an independent cohort free of any disease or risk factors (predicted mTRFL = $-94 \times \text{age} + 15,399$ bp; age range: 24 to 83; $r = 0.74$). For female subjects the calculated value was multiplied by 1.035, based on the report by Cawthon *et al.* (15) that women's telomeres were 3.5% longer than men's.

Assessment of Vascular Endothelial Function

Vascular endothelial dysfunction precedes atherosclerosis and uncovers potential cardiovascular damage (16, 17). We assessed endothelial function by flow-mediated dilatation (FMD) of the brachial artery using a previously described method (18, 19). High-resolution ultrasound (25 MHz probe; Sonos, Hewlett Packard Co., Palo Alto, USA) was used to measure changes in the diameter of the brachial artery in response to hyperemia and to nitroglycerin. After baseline measurement of brachial artery diameter in a longitudinal view, a blood pressure cuff around the upper limb was inflated to a pressure of 250 mmHg and then deflated after 3 min. The change in the arterial diameter after cuff deflation was measured over the following 90 s. The maximum percent change in the diameter relative to the baseline value was defined as FMD, *i.e.*, endothelial function. After the arterial di-

Fig. 1. Reproducibility of white blood cell mean telomere restriction fragment length (meanTRFL). The left panel shows representative Southern blots of white blood cell telomeres from two subjects. DNA samples were taken twice within one subject on two different occasions. The right panel shows the correlation between the two measurements. CAD, coronary artery disease; HT, hypertension.

ameter had decreased to the baseline value (10 min), endothelium-independent vasodilation response was similarly measured following administration of nitroglycerin spray (0.3 mg). All measurements and analyses were performed by the same investigator, who was blinded to angiographic and clinical data. FMD is expressed as the percent change in diameter after reactive hyperemia relative to the baseline value.

Statistical Analysis

Repeated measurement reproducibility data and the relationship between age and mTRFL were analyzed using linear regression analysis. Comparisons among different cumulative CVD score subgroups were performed by ANOVA followed by Fisher's protected least significant difference (PLSD) test using the Stat View program (Abacus Co., Berkeley, USA). Correlations of CVD score and with FMD, mTRFL and telomere index were analyzed by logistic regression analysis. The values were expressed as the mean $\pm$ SD. The level of statistical significance was set at $p<0.05$.

Results

Reproducibility of mTRFL Measurement and Effect of Age

Figure 1 shows the correlation between the two measurements of white blood cell mTRFL in each of 10 subjects on two different occasions. The two values correlated well ($r = 0.84$, $p<0.001$), indicating that white blood cell mTRFL is a reproducible measure.

Fig. 2. Relationships between age and white blood cell mean telomere restriction fragment length (mTRFL) in subjects with at least one cardiovascular risk factor (hypertension, hyperlipidemia, and diabetes) and/or known history of cardiovascular event (coronary artery disease, cerebral artery disease, and peripheral artery disease) and in subjects without any risks or known events. mTRFL was inversely and significantly correlated with age in both groups, whereas the r value was greater in healthy subjects than in the other group, reflecting the fact that mTRFL in subjects with cardiovascular risks/events is shortened for their age. , subjects without any cardiovascular risks or events. $p < 0.0002$, $r = 0.78$. , patients with cardiovascular risks and/or known history of cardiovascular events. $p < 0.006$, $r = 0.33$.

Table 1. Prevalence of Cardiovascular Risks/Events by the Cardiovascular Damage Score

	Cumulative number of cardiovascular damage score				
	0	1	2	3	4
Total number	18	19	21	20	5
Hypertension (<i>n</i> (%))	0	11 (65)	14 (70)	20 (91)	5 (100)
Hyperlipidemia (<i>n</i> (%))	0	3 (18)	11 (55)	16 (73)	3 (60)
Diabetes (<i>n</i> (%))	0	0 (0)	3 (15)	7 (32)	4 (80)
Coronary artery disease (<i>n</i> (%))	0	1 (6)	8 (40)	15 (68)	4 (80)
Stroke/ TIA/ lacuna infarction (<i>n</i> (%))	0	0 (0)	3 (15)	6 (27)	3 (60)
Peripheral artery disease (<i>n</i> (%))	0	2 (12)	1 (5)	2 (9)	1 (20)

TIA, transient ischemic attack.

Table 2. Characteristics of Subjects Grouped by Cardiovascular Damage Score

	Cumulative number of cardiovascular damage score				
	0	1	2	3	4
Total number	19	17	20	22	5
Age (years)	61.4 ± 12	66.4 ± 14	61.2 ± 10	66.7 ± 9	69.6 ± 8
Sex (Male/Female)	9/10	11/6	12/8	14/8	4/1
Body mass index (kg/m ²)	24 ± 3.6	22.3 ± 4	23.7 ± 3	22.8 ± 7	23.7 ± 1
SBP (mmHg)	113.7 ± 7*	127.6 ± 16	129.1 ± 13	120.6 ± 11	133.3 ± 6
DBP (mmHg)	70.4 ± 11	70.9 ± 8	75.5 ± 12	70 ± 8	75 ± 9
Total cholesterol (mg/dl)	188 ± 22	199 ± 25	197 ± 24	196 ± 26	191 ± 20
HDL cholesterol (mg/dl)	57 ± 15	60 ± 14	58 ± 17	59 ± 20	48 ± 12
Triglyceride (mg/dl)	107 ± 30	112 ± 36	122 ± 51	134 ± 67	148 ± 64
HbA1c (%)	5.2 ± 0.3	5.1 ± 0.2	5.2 ± 0.4	5.6 ± 0.5 [†]	6.4 ± 1 [§]

Data are expressed as mean ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HbA1c, glycosylated hemoglobin. * $p < 0.05$ vs. groups 1, 2, and 3; [†] $p < 0.05$ vs. groups 0, 1 and 2; [§] $p < 0.05$ vs. 0, 1, 2 and 3.

Figure 2 shows the correlations between age and white blood cell mTRFL in patients with at least one cardiovascular risk/event ($r = 0.33$, $p < 0.006$) and in subjects free of any risks/events ($r = 0.78$, $p < 0.0002$). mTRFL was significantly correlated with age in both groups. Notably, comparing the regression lines between the two groups, it is evident that mTRFLs in subjects with cardiovascular risks/events were shorter than expected from their age. Taken together, the results indicate that mTRFLs are influenced both by chronological age and the presence of cardiovascular damage.

Relationship between Telomere Shortening and Potential/Existing Cardiovascular Damage

Subjects were divided into five subgroups based on the cumulative CVD score (designated as CVD-S groups 0, 1, 2, 3, and 4). The prevalence of cardiovascular risks/events in each subgroup is shown in Table 1. There were no statistically significant differences among the five subgroups in mean values of age, body mass index, DBP, total cholesterol, and high-density lipoprotein (HDL) cholesterol (Table 2). The SBP in CVD-S group 0 was significantly lower than those in CVD-S groups 1, 2, and 3, but was not different among

CVD-S groups 1, 2, 3, and 4. The plasma level of glycosylated hemoglobin (HbA1c) in CVD-S group 3 was significantly higher than those in CVD-S groups 0, 1, and 2 ($p < 0.05$), and that in CVD-S group 4 was again significantly higher than those in the other groups.

Figure 3 shows the mean values of FMD in the five CVD-S groups. The FMDs in CVD-S groups 2, 3, and 4 were significantly ($p < 0.05$) lower than that in CVD-S group 0, and the FMD in CVD-S group 4 was significantly ($p < 0.01$) lower than those in the other groups. The FMDs and the subject's CVD scores were inversely and significantly correlated ($r = -0.37$, $p < 0.01$), indicating that FMD tended to decrease with increase in CVD score. On the other hand, the vasodilatory response to nitroglycerine (NTG), as an index of endothelium-independent vasodilation, was not statistically different in the five CVD-S groups (the responses to NTG being 10.8 ± 2.4 , 11 ± 4.2 , 10 ± 3.5 , 9.5 ± 3.3 and $7.5 \pm 1.6\%$ in CVD-S groups 0, 1, 2, 3 and 4, respectively), indicating that the decrease in FMD in the present study reflects endothelial dysfunction. The finding that FMD decreased with increase in CVD-S indicates that assessment of cardiovascular damage by this scoring system is valid.

Figure 4 shows the mean values of mTRFL in the five

Fig. 3. Flow mediated vasodilation (FMD) and the cumulative cardiovascular damage (CVD) score. FMD, an index of endothelial function, decreased with increasing CVD score, indicating that the assessment of vascular damage by this scoring method was valid. The values are the mean $\pm$ SD. * $p < 0.05$ vs. the group with a CVD score of 0; § $p < 0.01$ vs. the groups with CVD scores of 0, 1, 2 and 3.

CVD-S groups. The mean values of white blood cell mTRFL in CVD-S groups 0, 1, 2, 3 and 4 were 9.56 ± 1.7 , 8.63 ± 1.4 , 7.92 ± 1.2 , 7.83 ± 1.2 and 7.28 ± 1.2 kb, respectively. The mTRFLs in CVD-S groups 1, 2, 3 and 4 were significantly smaller than that in CVD-S group 0, and the mTRFLs in CVD-S groups 3 and 4 were significantly smaller than that in CVD-S group 1. A regression analysis revealed a significant inverse correlation between the cumulative CVD score and mTRFL ($r = -0.45$, $p < 0.0001$), indicating that mTRFL decreased with increase in CVD score.

Since mTRFL decreases with normal aging, we evaluated white blood cell telomeres by the telomere index in the five CVD-S groups (Fig. 5). The telomere index in CVD-S group 0 was 0.98 ± 0.13 , which is consistent with a normal telomere length. The telomere indexes in CVD-S groups 1, 2, 3 and 4 were 0.95 ± 0.13 , 0.84 ± 0.14 , 0.86 ± 0.13 and 0.83 ± 0.18 , respectively. The telomere indexes in CVD-S groups 2, 3 and 4 were significantly smaller than those in CVD-S groups 0 and 1. A regression analysis revealed that there was a significant inverse correlation between CVD score and telomere index ($r = -0.39$, $p < 0.001$), suggesting that the telomere index decreases with increase in CVD score.

Discussion

In the present study, we tested the hypothesis that white blood cell telomere length is a marker for biological aging of the cardiovascular system. We found that mTRFL in white blood cells decreased with increase in CVD score, suggesting that progression of atherosclerosis and accelerated attrition of white blood cell telomeres may share some common pathogenic mechanisms. The telomere index also decreased with increase in CVD score, indicating that attrition of white blood cell telomeres in subjects with cardiovascular damage

Fig. 4. White blood cell mean telomere restriction fragment length (mTRFL) and the cumulative cardiovascular damage (CVD) score. mTRFL decreased with increasing CVD score. The values are the mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$ vs. the group with a CVD score of 0; † $p < 0.05$ vs. the group with a CVD score of 1.

Fig. 5. Telomere index and the cumulative cardiovascular damage (CVD) score. The telomere index decreased with increasing CVD score, indicating that attrition of mTRFL in subjects with more severe cardiovascular damage is independent of their age. The telomere index was calculated as the ratio of mTRFL to the age- and sex-predicted mTRFL as described in the Methods section. * $p < 0.05$ vs. the group with a CVD score of 0; † $p < 0.05$ vs. the group with a CVD score of 1.

was age- and sex-independent. The subjects in the present study included subjects who had no clinical signs of cardiovascular events but who were susceptible to vascular disease due to cardiovascular risk factors. Most of those subjects were categorized into CVD score group 2 (Table 1), in which mTRFL was smaller than that in CVD score group 0, suggesting that the telomere has already shortened when vascular damage is in a subclinical stage. Measurement of white blood cell telomeres provides biochemical information on aging-associated cardiovascular dysfunction that is not known from the subject's age.

Recently, Cawthon *et al.* (15) demonstrated that individuals with shorter telomeres in their white blood cells had poorer survival, with higher mortality rates from heart disease and from infectious disease. Similarly, Brouillette *et al.*

(20) have shown that patients with coronary heart disease had shorter white blood cell telomere length compared with control subjects. Our present observation is consistent with these reports. We add novel findings that shortening of telomeres in white blood cells was correlated with the degree of cardiovascular damage, and that such a relationship between cardiovascular damage and telomere length was observed in patients with various types of atherogenic disorders, and not only in those with coronary heart disease.

Although the clinical data are still accumulating, the pathogenic significance of telomere shortening in white blood cells in the mechanism of atherosclerosis remains uncertain. It is thought that telomere lengths in white blood cells may accord with those in vascular cells. However, it is also possible that telomere attrition in white blood cells is secondary to chronic inflammation rather than an aging of vascular tissue. Recent studies indicate that inflammation is involved in the mechanism of atherosclerosis (12), and the presence of chronic inflammation may accelerate white blood cell turnover, leading to telomere erosion of these cell populations. Recently, bone marrow-derived cells, including endothelial progenitor cells, have been suggested to contribute to pathological remodeling of vasculature (21). Therefore, telomere attrition of white blood cells might be the result of the exhaustion of immuno-responsive cells or bone marrow-derived cells, which would lead to altered vascular repair and atherosclerosis (22). Another problem with the clinical use of white blood cell telomeres is that there may be a large inter-individual variation. Further studies will be needed to clarify the causative links between telomere attrition in white blood cells and atherosclerosis, and to establish white blood cell telomeres as a risk factor of atherosclerosis or a clinical marker for vascular aging.

Consistent with the notion that endothelial dysfunction precedes atherosclerosis and reflects potential as well as already existing atherosclerosis (19), we found a decline in FMD, an index of vascular endothelial function, with increase in CVD score. This is analogous to the decrease in mTRFL with increase in the CVD score, although a direct correlation between FMD and mTRFL or telomere index was not found. Given that an interaction between the vascular endothelium and white blood cells, particularly monocytes, or cytokines produced by white blood cells is an important determinant of atherogenesis (12), the parallel decreases in white blood cell mTRFL and FMD depending on the presumed cardiovascular damage may indicate that telomere shortening in white blood cells is to some extent involved in the mechanisms of vascular endothelial dysfunction.

Atherosclerosis, obviously, develops with advance of age. Recent studies have revealed that the aging process may be altered by exogenous as well as by endogenous stresses (2). Oxidative stress, which is produced in cells subjected to stress and also in normal cells, is the most important determinant of cellular aging (2). Hypertension (23), hyperlipi-

demia (12), and diabetes (24) are all implicated in excessive oxidative stress. Recent evidence (12) indicates that atherosclerosis is initiated by repeated mechanical, hemodynamic, and/or immunological injury, probably involving oxidative stress, to the mural and focal regions of the endothelium (response-to-injury hypothesis). Such insults may cause augmented cell turnover in certain cell populations or tissues (9, 25), rendering the cells older and nearer to their maximum replicative capacity. It has been shown that human vascular endothelial cells lose telomeres as a function of replicative age and that the telomere loss is greater in tissues susceptible to atherogenesis (9). Although we did not study telomeres in vascular tissues, it is plausible that the vascular damage revealed by the CVD scoring and FMD is associated with biological aging of the cardiovascular system and that telomere attrition in white blood cells, at least in part, reflects the biological aging status.

Perspectives

We demonstrated that white blood cell telomere length shortens in parallel to a decline in endothelial function and presumed cardiovascular damage. White blood cell telomere length may be a marker for biological aging of the cardiovascular system, and use of this marker, rather than simply chronological age, is expected to provide a new diagnostic and therapeutic approach to narrow the target of critical biochemical events leading to the age-associated development of atherosclerosis.

References

1. Lakatta EG, Levy D: Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part I: aging arteries: a "set up" for vascular disease. *Circulation* 2003; **107**: 139–146.
2. Finkel T, Holbrook NJ: Oxidants, oxidative stress and the biology of ageing. *Nature* 2000; **408**: 239–247.
3. Aviv A: Chronology *versus* biology: telomeres, essential hypertension, and vascular aging. *Hypertension* 2002; **40**: 229–232.
4. Benetos A, Okuda K, Lajemi M, *et al*: Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension* 2001; **37**: 381–385.
5. Blackburn EH: Telomere states and cell fates. *Nature* 2000; **408**: 53–56.
6. Harley CB, Futcher AB, Greider CW: Telomeres shorten during ageing of human fibroblasts. *Nature* 1990; **345**: 458–460.
7. Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I: Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. *Circulation* 2002; **105**: 1541–1544.
8. Fenton M, Barker S, Kurz DJ, Erusalimsky JD: Cellular senescence after single and repeated balloon catheter denudations of rabbit carotid arteries. *Arterioscler Thromb Vasc*

- Biol* 2001; **21**: 220–226.
9. Chang E, Harley CB: Telomere length and replicative aging in human vascular tissues. *Proc Natl Acad Sci USA* 1995; **92**: 11190–11194.
 10. Slagboom PE, Droog S, Boomsma DI: Genetic determination of telomere size in humans: a twin study of three age groups. *Am J Hum Genet* 1994; **55**: 876–882.
 11. Effros RB: Replicative senescence in the immune system: impact of the Hayflick limit on T-cell function in the elderly. *Am J Hum Genet* 1998; **62**: 1003–1007.
 12. Ross R: Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999; **340**: 115–126.
 13. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications: part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998; **15**: 539–553.
 14. Takauchi K, Tashiro S, Ohtaki M, Kamada N: Telomere reduction of specific chromosome translocation in acute myelocytic leukemia. *Jpn J Cancer Res* 1994; **85**: 127–130.
 15. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA: Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* 2003; **361**: 393–395.
 16. Nakagawa K, Higashi Y, Sasaki S, Oshima T, Matsuura H, Chayama K: Leptin causes vasodilation in humans. *Hypertens Res* 2002; **25**: 161–165.
 17. Higashi Y, Sasaki S, Nakagawa K, et al: Sodium chloride loading does not alter endothelium-dependent vasodilation of forearm vasculature in either salt-sensitive or salt-resistant patients with essential hypertension. *Hypertens Res* 2001; **24**: 711–716.
 18. Teragawa H, Kato M, Kurokawa J, Yamagata T, Matsuura H, Chayama K: Usefulness of flow-mediated dilation of the brachial artery and/or the intima-media thickness of the carotid artery in predicting coronary narrowing in patients suspected of having coronary artery disease. *Am J Cardiol* 2001; **88**: 1147–1151.
 19. Gaeta G, De Michele M, Cuomo S, et al: Arterial abnormalities in the offspring of patients with premature myocardial infarction. *N Engl J Med* 2000; **343**: 840–846.
 20. Brouillette S, Singh RK, Thompson JR, Goodall AH, Samani NJ: White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol* 2003; **23**: 842–846.
 21. Sata M, Saiura A, Kunisato A, et al: Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. *Nat Med* 2002; **8**: 403–409.
 22. Rauscher FM, Goldschmidt-Clermont PJ, Davis BH, et al: Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation* 2003; **108**: 457–463.
 23. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Oshima T, Chayama K: Endothelial function and oxidative stress in renovascular hypertension. *N Engl J Med* 2002; **346**: 1954–1962.
 24. Zhang L, Zalewski A, Liu Y, et al: Diabetes-induced oxidative stress and low-grade inflammation in porcine coronary arteries. *Circulation* 2003; **108**: 472–478.
 25. Kim SH, Kaminker P, Campisi J: Telomeres, aging and cancer: in search of a happy ending. *Oncogene* 2002; **21**: 503–511.