Longer Telomere Length in COPD Patients with $\alpha_1$-Antitrypsin Deficiency Independent of Lung Function

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Abstract

Oxidative stress is involved in the pathogenesis of airway obstruction in $\alpha_1$-antitrypsin deficient patients. This may result in a shortening of telomere length, resulting in cellular senescence. To test whether telomere length differs in $\alpha_1$-antitrypsin deficient patients compared with controls, we measured telomere length in DNA from peripheral blood cells of 217 $\alpha_1$-antitrypsin deficient patients and 217 control COPD patients. We also tested for differences in telomere length between DNA from blood and DNA from lung tissue in a subset of 51 controls. We found that telomere length in the blood was significantly longer in $\alpha_1$-antitrypsin deficient COPD patients compared with control COPD patients ($p = 1 \times 10^{-29}$). Telomere length was not related to lung function in $\alpha_1$-antitrypsin deficient patients ($p = 0.3122$) or in COPD controls ($p = 0.1430$). Although mean telomere length was significantly shorter in the blood when compared with the lungs ($p = 0.0078$), telomere length was correlated between the two tissue types ($p = 0.0122$). Our results indicate that telomere length is better preserved in $\alpha_1$-antitrypsin deficient COPD patients than in non-deficient patients. In addition, measurement of telomere length in the blood may be a suitable surrogate for measurement in the lung.

Introduction

Chronic obstructive pulmonary disease (COPD) is a complex trait with both genetic and environmental risks factors that is characterized by non-reversible airway obstruction and chronic inflammation. The morphologic manifestations of this disorder include small airway remodeling and emphysema. The predominant environmental risk factor for COPD is cigarette smoking, although other factors such as air pollution and respiratory infections play a role. The genetic component of COPD has been demonstrated by twin studies of disease status and lung function. The search for the genes responsible for this disorder has involved the investigation of candidate genes as well as genome-wide association studies of COPD and lung function in the general population, all of which have been associated with premature senescence. COPD patients are at increased risk for cardiovascular disease, osteoporosis, depression, and skin wrinkling, all of which have been associated with premature senescence.

Telomeres shorten with each round of cell division and this results in replicative cell senescence. Telomere length is reduced during DNA replication because of the “end replication problem”, i.e., the 5’ end of the lagging strand is unable to be replicated. This loss of telomeric DNA is predicted to be ~10 base pairs per cell cycle. However, the observed rate of loss can be higher and in humans has been estimated to be 50–200 bp per division. Oxidative stress is one of the main factors in causing this higher rate of loss.

Several studies have examined telomere length in the context of COPD but there is little consistency in the results and the relationship between telomere length and lung function in $\alpha_1$-antitrypsin deficiency has not been previously studied. Oxidative stress plays an important role in the pathogenesis of airway obstruction in $\alpha_1$-antitrypsin deficient patients. Furthermore, $\alpha_1$-antitrypsin has anti-apoptotic effects and anti-inflammatory effects on cytokine production. Therefore, cell...
senescence due to reduction in telomere length may be particularly important in patients with α1-antitrypsin deficiency. We investigated telomere length in a group of COPD patients with α1-antitrypsin deficiency and a group of COPD controls. We also determined whether the length of telomeres in peripheral blood DNA is correlated with that in lung tissue samples in order to test the hypothesis that COPD is a “systemic” disease, and that telomeric shortening in this condition affects both lung and the hematopoietic systems.

Methods

Subjects

We studied 217 α1-antitrypsin deficient patients and 217 COPD control patients (Table 1). Approval for the project was obtained from the University of British Columbia - Providence Health Care Research Ethics Board (REB H09-02042 and H11-02780). The α1-antitrypsin deficient patients were selected from the Alpha-1 Foundation (AATF) DNA and Tissue Bank located at the University of Florida (IRB 659-2002). The COPD controls were selected from the Lung Health Study (LHS), a clinical trial sponsored by the National Heart, Lung and Blood Institute [51]. Participants in the LHS were cigarette smokers between 35–60 years of age with mild to moderate airflow obstruction, defined by an FEV1 between 55–90% of predicted. Additional 51 patients were selected from the lung tissue biobank at the James Hogg Research Centre (JHRC). For the JHRC samples, both lung tissue and blood samples were obtained from individuals in 20

DNA samples

A sample of peripheral blood DNA was used to measure telomere length in the AATF DNA and Tissue Bank samples. Measurement of telomere length in the LHS samples was performed as previously reported [43]. For the JHRC samples, we measured telomere length in DNA samples from both blood and lung tissue in 51 subjects. DNA was extracted from these tissues using the QiAamp DNA Mini Kit (Qiagen, Mississauga, ON, Canada).

Measurement of Telomere Length

Telomere length was measured using a previously published qPCR based protocol [43, 52]. Briefly, DNA samples were quantified using the Nanodrop 8000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Telomere length measurement was performed in triplicate using 5 ng of DNA. Intra-plate coefficients of variance (CV) were calculated between the replicates, and samples with CV ≥ 5% were excluded from further analysis. Reference DNA samples obtained from the Coriell Institute (Camden, NJ) were assayed as calibrator samples in triplicate on each PCR plate to control for variation between plates. Inter-plate CV for the calibrator sample was calculated to be 16%. 36B4 was used as a reference gene. The primer sequences used were: tel 1: GGTTTGGGCTGAGGCTGAAGGTT-GAGGGGTAGG; tel 2: TCCGACACTATCCTACCC-TATTTATCCCCTATCCCCTA; 36B4u: CAGCAAGTGGA-GGTTGTAATCCC; and 36B4d: CCCATTCTATGATCA-AACGCGTACAA. Six qPCR reactions (i.e. triplicates of the telomere and reference gene assays) were performed for each individual in 20 μL reactions including 10 μL Quantitect SYBR Green PCR Master Mix (QIAGEN), and final primer concentrations of tel 1: 270 nM, tel 2: 900 nM, 36B4u: 300 nM, and 36B4d, 500 nM. Reactions were performed on the Viia 7 Real-Time PCR Instrument (Life Technologies). Cycling conditions for the measurement of telomere length were as follows: 50°C for 2 min, 95°C for 2 min, 40 cycles of 95°C for 15 sec and an annealing temperature of 54°C for 2 min. Cycling conditions for measurement of the 36B4 reference gene were the same except 35 cycles, with an annealing temperature of 58°C for 1 min were used. Telomere length was calculated as a ratio of telomere to 36B4, using Cawthon’s formula [32].

Statistical Analysis

Telomere length measurements were log10 transformed to approximate a normal distribution. Student’s t-test was used to compare mean telomere length between groups. Multiple linear regression was performed to test for the effect of α1-antitrypsin deficiency on telomere length and lung function, with adjustments for significant confounders including age, gender and pack years. JMP software (SAS, Cary, NC, USA) was used for all statistical analyses.

Results

Effect of α1-antitrypsin deficiency on telomere length

To test for association between α1-antitrypsin deficiency and telomere length in peripheral blood cells, telomere length was

Table 1. Demographic and genotypic characteristics of subjects from the Alpha-1 Foundation DNA and Tissue Bank and the Lung Health Study.

|                        | Alpha-1 Foundation DNA and Tissue Bank | Lung Health Study |
|------------------------|----------------------------------------|-------------------|
| **Age**                | 53.7±0.45                              | 53.7±0.45         |
| % Male                 | 51.1                                   | 51.1              |
| **Pack Years**         | 22.8±1.28                              | 23.2±0.92         |
| **Average FEV1% predicted** | 40.0±1.68                              | 79.9±0.59         |
| **Genotype**           |                                        |                   |
| MM                     | 0                                      | 217               |
| MZ                     | 0                                      | 0                 |
| ZZ                     | 217                                    | 0                 |

*Mean ± standard error of the mean.

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measured in 217 α₁-antitrypsin deficient patients and 217 control patients matched for ethnicity, gender, age and pack years. Mean telomere length was compared between the two groups using Student’s t-test. Median telomere length (untransformed) was 2.45 fold longer in peripheral blood DNA from COPD patients with α₁-antitrypsin deficiency compared with COPD controls (p = 1 × 10⁻²⁸) (Figure 1). The mean log₁₀ transformed telomere lengths in α₁-antitrypsin deficient patients and COPD controls were −0.1892 with a standard deviation of 0.2074 and −0.3693 with a standard deviation of 0.2074, respectively. The difference in telomere length between α₁-antitrypsin deficient patients and COPD controls remained statistically significant after adjustment for age, gender and pack years. Mean telomere length was again significantly longer in the α₁-antitrypsin deficient patients compared with COPD controls (p = 1 × 10⁻³⁵).

Relationship between tissue type and telomere length

To test for a correlation between telomere length in the blood and in the lungs, telomere length was measured in lung and blood DNA from 51 patients from the JHRC. There was a significant correlation between telomere length in the blood and telomere length in the lungs (Pearson’s r = 0.348, p = 0.012) (Figure 2). On average, however, median telomere length was 1.53 fold shorter in the blood when compared with the lungs (p = 0.008) (Figure 3).

Relationship between telomere length and lung function

Lung function data were available for 157/217 α₁-antitrypsin deficient patients and all COPD controls. The effect of telomere length in the blood on FEV₁ % predicted was tested. There was no significant association between telomere length in the blood and lung function in either the α₁-antitrypsin deficient patients (p = 0.3122), or in the controls with adjustment for age and pack years (p = 0.2503). In addition, lung function data were available for 49 patients from the JHRC with telomere length measurements in the lung. There was no association between telomere length in the lung and FEV₁ % predicted (p = 0.8057).

Discussion

The most important finding of this study was that COPD patients with α₁-antitrypsin deficiency have longer telomere lengths in peripheral leukocytes compared with COPD patients who do not have α₁-antitrypsin deficiency. However, there was no significant relationship between telomere length in blood and lung function as measured by FEV₁ % predicted in α₁-antitrypsin deficient patients or in COPD controls. We also found that within subjects, there was a significant relationship of telomere length in peripheral leukocytes with that in lung tissue, although on average the telomere length of peripheral leukocytes was shorter than that in lung tissue.

Telomere length has been positively correlated with lung function in some studies [26,40,44,45] but not others [41,43]. A recent study examined 46,396 individuals and the results suggested that the association of telomere length with lung function was, though significant, only modest after correction for confounding factors such as age [44]. Shorter telomere length has also been associated with COPD in some studies [39,41,42,44,45] but not in others [25,38]. This is the first study to examine the role of telomere length in α₁-antitrypsin deficient patients, a group who we hypothesized may be particularly susceptible to accelerated reduction in telomeres and the subsequent cellular senescence.

The role of premature aging in COPD has been shown by studies of explanted lung fibroblasts from emphysema patients that showed reduced proliferation rate in vitro [24] and markers of cellular senescence [25]. Similarly, alveolar type II cells and endothelial cells from emphysema patients showed elevated levels of senescence markers including shortened telomeres [26].

In our study we found a relationship between α₁-antitrypsin deficiency and telomere length. However, the direction of effect...
Telomere Length in α1-Antitrypsin Deficiency

was contrary to our hypothesis. Telomere length was longer in patients with α1-antitrypsin deficiency, despite the fact that they are likely exposed to higher levels of oxidative stress than usual COPD patients, as measured by oxidation of nucleic acids [46]. Oxidation of DNA is a general marker of oxidative stress and may directly promote telomere shortening [35] and therefore our results appear counterintuitive. On the other hand, patients with α1-antitrypsin deficiency have lower levels of myeloperoxidase (MPO) and neutrophil counts in sputum than non-deficient COPD patients [54]. MPO is the most abundant protein in neutrophils and catalyzes the formation of hypochlorous acid, a potent oxidant. Therefore, MPO likely plays an important role in oxidative stress in the lung and the lower MPO levels in α1-antitrypsin deficient patients [54] may explain the longer telomere length we observed in these patients.

We found that there was a significant correlation between telomere length in the blood and telomere length in lung tissue. Many studies of telomere length are performed using DNA from peripheral blood cells, and results are extrapolated to biological processes occurring in other tissues. For example, the majority of studies investigating telomere length in COPD patients have been performed in DNA from blood cells [24,38,45,47,48]. Our results indicate that telomere length in the blood is correlated with telomere length in the lungs, suggesting that telomere length in the blood may be an appropriate surrogate for telomere length in the lungs. The correlation between blood and lung telomere length may reflect the nature of COPD as a systemic disease [55]. Thus, exposure to cigarette smoke in the lungs may affect leukocyte telomere length due to translocation of proinflammatory mediators [56] and reactive oxygen species from the lung into the circulation.

We also demonstrated that the telomere length of peripheral leukocytes was shorter than that in lung tissue. Telomere length is known to vary between different human tissues [57] with leukocyte telomeres generally shorter than those in other tissues [58], presumably reflecting greater rates of proliferation in blood cells. Interestingly, Daniali et al. [50] studied adults (age >18 years) and the rate of telomere shortening was similar between the tissue types, suggesting that the length differences between tissues were established in childhood. This may explain why the telomeres in the lung samples in our patient samples were longer than those in blood cells, despite the presumably greater exposure of the lung tissue to oxidative stress via cigarette smoke. The telomere length differences established early in life may overwhelm any effect of exposure to smoke occurring mainly in adulthood.

One limitation of our study is that lung function was only measured at one time point; therefore we could not test the effect of telomere length on rate of decline in α1-antitrypsin deficient patients. Another limitation is that all of the α1-antitrypsin deficient patients included in this study were current or ex-smokers. Therefore, it was not possible to test for the effect of α1-antitrypsin deficiency in non-smokers compared with smokers. Finally, our telomere length measurements in the lung were performed using only a small piece of lung tissue, therefore the telomere length measured may not reflect the whole lung.

Our data indicate that in α1-antitrypsin deficient patients, replicative senescence does not appear to play a significant role in the pathogenesis of COPD. Importantly, for the respiratory community, we found that telomere length of peripheral leukocytes is a good biomarker of telomere length in lung tissue.

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Author Contributions

Conceived and designed the experiments: DJS AJS. Performed the experiments: AS JL. Analyzed the data: AS. Contributed reagents/materials/analysis tools: FNR MLJB. Wrote the paper: AS AJS.

References

1. Antó JM, Vermeire P, Vestbo J, Suryer J (2001) Epidemiology of chronic obstructive pulmonary disease. Eur Respir J 17: 982–994.
2. Loke A, Lange P, Scharling H, Fabricius P, Vestbo J (2006) Developing COPD: a 25 year follow up study of the general population. Thorax 61: 955–959.
3. Andersen ZJ, Heidberg M, Jensen SS, Kriel M, Lofh S, et al. (2011) Chronic obstructive pulmonary disease and long-term exposure to traffic-related air pollution: a cohort study. Am J Respir Crit Care Med 183: 453–461.
4. Hansel NN, McCormack MC, Belli AJ, Matsui EC, Peng RD, et al. (2013) In-home air pollution is linked to respiratory morbidity in former smokers with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 187: 1085–1090.
5. Sethi S, Murphy TF (2008) Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med 359: 2355–2365.
6. Ingebrigtsen T, Thomsen SF, Vestbo J, van der Sluis S, Kyvik KO, et al. (2010) Genetic influences on chronic obstructive pulmonary disease - a twin study. Respir Med 104: 1890–1895.
7. Redline S, Tohler PV, Lewitter FI, Tager IB, Munoa A, et al. (1987) Assessment of genetic and nongenetic influences on pulmonary function: a twin study. Am Rev Respir Dis 135: 215–227.
8. Ingebrigtsen TS, Thomsen SF, van der Sluis S, Kyvik KO, et al. (2010) Genetic influences on chronic obstructive pulmonary disease - a twin study. Respir Med 104: 1890–1895.
9. Bosset Y (2012) Updates on the COPD gene list. Int J Chron Obstruct Pulmon Dis 7: 607–631.
10. Cho MH, Boutaoui N, Klanderman BJ, Sylvia JS, Ziniti JP, et al. (2010) Variants in FAM13A are associated with chronic obstructive pulmonary disease. Nat Genet 42: 200–202.
11. Pillai SG, Ge D, Zhu G, Kong X, Shianna KV, et al. (2009) A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. PLoS Genet 5: e1000421.
12. Hancock DR, Eigelsheim M, Wilk JB, Gharib SA, Loebr LR, et al. (2010) Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. Nat Genet 42: 45–52.
13. Repapi E, Sayers I, Wain LV, Burton PR, Johnson T, et al. (2010) Genome-wide association study identifies five loci associated with lung function. Nat Genet 42: 36–44.
14. Graham SM, Wain LV, Shine N, Kaldheker N, Artigas MS, et al. (2011) A comprehensive evaluation of potential lung function associated genes in the SpiroMeta general population sample. PLoS One 6: e19382.
15. Soler Artigas M, Loth DW, Wain LV, Gharib SA, Obeidat M, et al. (2011) Genome-wide association and large-scale follow up identify 16 new loci influencing lung function. Nat Genet 43: 1082–1090.
16. Wilk JB, Chen TH, Gottlieb DJ, Walter RE, Nagele MW, et al. (2009) A genome-wide association study of pulmonary function measures in the Framingham Heart Study. PLoS Genet 5: e1000429.
17. Wilk JB, Shine NR, Loebr LR, Zhao JH, Manichaikul A, et al. (2012) Genome-wide association studies identify CHRNA5/3 and HTR4 in the development of airflow obstruction. Am J Respir Crit Care Med 186: 622–632.
18. Laurent CC, Eriksson S (1963) The electrophoretic α1-globulin pattern of serum in α1-antitrypsin deficiency. Scand J Clin Lab Invest 15: 192–140.
19. Eriksson S (1964) Pulmonary emphysema and α1-antitrypsin deficiency. Acta Med Scand 175: 197–203.
20. Lomas DA, Evans DL, Finch JT, Carrell RW (1992) The mechanism of Z α1-antitrypsin accumulation in the liver. Nature 357: 605–607.
21. Ekeowa UI, Marciniak SJ, Lomas DA (2011) α1-antitrypsin deficiency and inflammation. Expert Rev Clin Immunol 7: 243–252.
22. Silverman EK, Pierce JA, Province MA, Rao DC, Campbell EJ (1989) Variability of pulmonary function in alpha-1-antitrypsin deficiency: clinical correlates. Annals of Internal Medicine 111: 982–991.
23. Derene DL, Sandhaus RA, Barker AF, Branth ML, Eden E, et al. (2007) Determinants of airflow obstruction in severe alpha-1-antitrypsin deficiency. Thorax 62: 806–813.
24. Holz O, Zühlke I, Jakobst E, Müller KC, Wilker L, et al. (2004) Lung fibroblasts from patients with emphysema show a reduced proliferation rate in culture. Eur Respir J 24: 575–579.
25. Müller KC, Wilker L, Paesch K, Feindt B, Erpenbeck VJ, et al. (2006) Lung fibroblasts from patients with emphysema show markers of senescence in vitro. Respir Res 7: 32.
26. Tsuji T, Aoshika K, Nogai A (2006) Alveolar cell senescence in patients with pulmonary emphysema. Am J Respir Crit Care Med 174: 896–893.
27. Van Eeden S, Leipsic J, Paul Man SF, Sin DD (2012) The relationship between lung inflammation and cardiovascular disease. Am J Respir Crit Care Med 186: 11–16.
28. Miller J, Edwards LD, Agusti A, Bakke P, Calverley PM, et al. (2013) Comorbidity, systemic inflammation and outcomes in the ECLIPSE cohort. Respir Med 107:1376–1384.
29. Atlanticis E, Fehily P, Cochrane B, Smith S (2013) Bidirectional associations between clinically relevant depression or anxiety and chronic obstructive pulmonary disease (COPD): a systematic review and meta-analysis. Chest: 766–777.
30. Patel BD, Loo WJ, Tasker AD, Scraton NJ, Burrows NP, et al. (2006) Smoking related COPD and facial wrinkling: is there a common susceptibility? Thorax 61: 569–571.
31. Fyhrquist F, Saijonmaa O, Strandberg T (2013) The roles of senescence and telomere shortening in cardiovascular disease. Nat Rev Cardiol 10: 274–283.
32. Valdes AM, Richards JB, Gardner JP, Swaminathan R, Kimura M, et al. (2007) Telomere length in leukocytes correlates with bone mineral density and is shorter in women with osteoporosis. Osteoporos Int 18: 1203–1210.
33. Horn PW, de Jonge P, Na BY, Farzaneh-Far R, Epel E, et al. (2011) Depression and leukocyte telomere length in patients with coronary heart disease: data from the Heart and Soul Study. Psychosom Med 73: 541–547.
34. Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during ageing of human fibroblasts. Nature 345: 458–460.
35. Counter CM, Avilion AA, LeFeuvre CE, Stewart NG, Greider CW, et al. (1992) immortal cells which express telomerase activity. EMBO J 11: 1921–1929.
36. Lansdorp PM (2005) Major cutbacks at chromosome ends. Trends Biochem Sci 30: 398–399.
37. Houwen JM, Moonen HJ, van Schooten FJ, Hageman GJ (2008) Telomere dysfunction causes sustained inflammation in chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol 37: 144–151.
38. Ikari Y, Mulvihill E, Schwartz SM (2001) Proteinase inhibitor, anti-antichymotrypsin, and -2-macroglobulin are the antipapoptotic factors of vascular smooth muscle cells. J Biol Chem 276: 11780–11803.
39. Connett JE, Yan J, Mui T, et al. (2012) The relationship between telomere length and mortality in chronic obstructive pulmonary disease (COPD). PLoS ONE 7: e35567.
40. Rode L, Bojesen SE, Weicher M, Vestbo J, Nordestgaard BG (2013) Short telomere length, lung function and chronic obstructive pulmonary disease in 46,396 individuals. Thorax 68: 429–435.
41. Arbecht E, Sillanpaa E, Karrasch S, Alves AG, Codd V, et al. (2013) Telomere length in circulating leukocytes is associated with lung function and disease. Eur Respir J: Dec 5. [Epub ahead of print].
42. Deale G, Woods JC, Moore C, Conradi SH, Gierada DS, et al. (2009) Oxidative damage to nucleic acids in severe emphysema. Chest 135: 965–974.
43. Ikar Y, Mulvihill E, Schwartz SM (2001) -1-Proteinase inhibitor, -1-antichymotrypsin, and -2-macroglobulin are the antipapoptotic factors of vascular smooth muscle cells. J Biol Chem 276: 11780–11803.
44. Petrache I, Fijalkowska I, Medler TR, Skirball J, Cruz P, et al. (2006) antitrypsin inhibits caspase-3 activity, preventing lung endothelial cell apoptosis. Am J Pathol 169: 1153–1166.
45. Chung A, Wang X, Wang RD, Meinizer SC, Pyralid EL, et al. (2007) antitrypsin suppresses TNF- and MMP-12 production by cigarette smoke-stimulated macrophages. Am J Respir Cell Mol Biol 37: 144–151.
46. Kato GB, Chan ED, Dinarello CA, Shapiro L (2009) antitrypsin is an endogenous inhibitor of proinflammatory cytokine production in whole blood. J Leukoc Biol 85: 896–899.
47. Anthonisen NR, Connett JE, Kiley JP, Altose MD, Bailey WC, et al. (1994) Effects of smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of decline of FEV1. The Lung Health Study. JAMA 272: 1497–1505.
48. Cawthon RM (2002) Telomere measurement by quantitative PCR. Nucleic Acids Res 30: e47.
49. Oikawa S, Kawanishi S (1999) Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. FEBS Lett 453: 363–366.
50. Stone H, McNab G, Wood AM, Stockley RA, Sapey E (2012) Variability of antitrypsin, and in COPD. Thorax 68: 429–435.
51. Albrecht E, Sillanpaa E, Alves AG, Codd V, et al. (2013) Telomere length in circulating leukocytes is associated with lung function and disease. Eur Respir J: Dec 5. [Epub ahead of print].
52. Ikari Y, Mulvihill E, Schwartz SM (2001) -1-Proteinase inhibitor, -1-antichymotrypsin, and -2-macroglobulin are the antipapoptotic factors of vascular smooth muscle cells. J Biol Chem 276: 11780–11803.
53. Oikawa S, Kawanishi S (1999) Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. FEBS Lett 453: 363–366.