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Association between oxidative DNA damage and telomere shortening in circulating endothelial progenitor cells obtained from metabolic syndrome patients with coronary artery disease

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Abstract

Metabolic syndrome (MS) induces an increase in oxidative stress and may be an important contributory factor for coronary artery disease (CAD). Telomere shortening of endothelial progenitor cells (EPCs) may be the key factor in endothelial cell senescence. The rate of telomere shortening is highly dependent on cellular oxidative damage. This study analyzed the relationship between telomere shortening and oxidative DNA damage in EPCs obtained from CAD patients with MS and without MS. We analyzed circulating EPCs in peripheral blood obtained from 57 patients with CAD (acute myocardial infarction [AMI], $n=26$; stable angina pectoris [AP], $n=31$) and 21 age-matched healthy subjects (control). Telomere length and telomerase activity were significantly lower in CAD patients than in controls, and were lower in AMI patients than in AP patients. Oxidative DNA damage was higher in CAD patients compared with controls, and oxidative DNA damage in AMI patients was also higher than in AP patients. There was a negative correlation between telomere length and oxidative DNA damage. Telomere length and telomerase activity were lower in CAD patients with MS than in those without MS. Oxidative DNA damage in CAD patients with MS was higher than in those without MS. In our *in vitro* study, oxidative treatments induced telomere shortening and decrease in telomerase activity of EPCs. These results suggest that EPC telomere shortening *via* increased oxidative DNA damage may play an important role in the pathogenesis of CAD. In addition, MS may be related to increased oxidative DNA damage and EPC telomere shortening.

Keywords: Acute myocardial infarction; Flow cytometry; Flow-FISH; Stable angina pectoris; Telomerase

1. Introduction

Metabolic syndrome (MS) is a cluster of insulin resistance, impaired glucose tolerance, dyslipidemia, obesity, and elevated blood pressure that has reached epidemic proportions in industrialized countries [1]. In recent clinical trials, MS was associated with increased risk of coronary artery disease (CAD) [2]. Oxidative stress plays a critical role in the pathogenesis of CAD in patients with MS [3]. Oxidative stress has been implicated in the development of atherosclerosis through a variety of mechanisms, especially those leading to endothelial dysfunction [4,5]. Oxidative stress also induces damage or apoptosis of endothelial cells [6].

Recent studies have identified that normal adults have a small number of circulating endothelial progenitor cells (EPCs) in the peripheral blood [7]. It has also been reported that patients at risk for CAD have a decreased number of circulating EPCs with impaired activity [8]. EPCs are regarded as having a key role in the maintenance of vascular integrity and the replacement of apoptotic or damaged endothelial cells in response to various cardiovascular risk factors, such as MS [9,10]. Cell division is associated with telomere shortening,

Abbreviations: AMI, acute myocardial infarction; AP, angina pectoris; CAD, coronary artery disease; PBMCs, peripheral blood mononuclear cells; TG, triglycerides.

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leading to senescence once telomere length reaches a critical threshold [11]. Telomeres are composed of noncoding double-stranded repeats of G-rich tandem DNA sequence TTAGGG extending over 6–15 kb at the end of eukaryotic chromosomes and are necessary for both successful DNA replication and chromosomal integrity [12]. Telomere shortening is modulated by the rate of cell turnover and oxidative stress [13]. In this study our aim was to determine whether oxidative stress is related to EPC telomere shortening in CAD patients with MS.

2. Materials and methods

2.1. Study population

Peripheral blood samples were obtained from 57 consecutive patients with CAD (26 patients with acute myocardial infarction [AMI] and 31 patients with stable angina pectoris [AP]). All patients with AMI were admitted within 12 h of onset of AMI. The diagnosis of AMI was made on the basis of the presence of prolonged chest pain, typical electrographic changes, and increased concentrations of serum cardiac enzymes. AP according to the exclusion parameters described for AMI and meeting the following criteria: history of typical chest pain on effort, lasting unchanged for more than 3 months and not associated with rest angina; documented exercise-induced myocardial ischemia; and angiographically proven CAD. Patients were excluded from the study if they had clinical signs of acute infection, severe renal failure or rheumatoid disease, or if they were suspected of having a malignant or primary wasting disorder. Peripheral blood samples were immediately taken from AMI patients at the time of admission and before percutaneous coronary intervention (PCI) procedure. Peripheral blood samples were also taken from AP patients before PCI procedure. As a control, peripheral blood samples were obtained from 21 age- and sex-healthy subjects without any evidence of CAD by history and physical examination. Metabolic syndrome was defined as the presence of at least three out of five risk determinants according to the modified NCEP ATP-III report (a waist circumference $\geq$85/90 cm in men/women, fasting triglycerides [TG] >150 mg/dL, low HDL-cholesterol [HDL-C] [men <40 mg/dL, women <50 mg/dL], systolic blood pressure >130 mm Hg and/or diastolic blood pressure >85 mm Hg, and fasting blood glucose level >100 mg/dL) [14,15]. Approval was obtained from the ethical committee of the Iwate Medical University School of Medicine (H17-73), and written informed consent was obtained from all subjects.

2.2. Quantification of circulating EPCs

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples obtained from all subjects by Ficoll–Paque density gradient centrifugation and lymphocyte separation solution (Nacalai Tesque Inc.). PBMCs were resuspended at a final concentration of 1×10^6 cells/mL in RPMI1640 (Sigma). PBMCs were incubated with 10 μL of FITC-conjugated anti-human CD34 m monoclonal antibody (mAb) (Becton Dickinson) and 10 μL of PE-conjugated anti-human KDR mAb (R&D system), followed by incubation at 4 °C for 30 min. After incubation, cells were fixed with 1% paraformaldehyde. Isotype immunoglobulin IgG antibody was used as a control (Becton Dickinson). The number of CD34 and KDR-double positive cells among 1×10^6 cells were counted using a FACScan analyzer (Becton Dickinson).

2.3. Cell culture enrichment of EPCs

PBMCs (8×10^6) were plated on fibronectin-coated culture dishes (Sigma) and maintained in endothelial basal medium-2 (EBM-2; Clonetics, Guelph, Canada) supplemented with EGM-2-MV-SingleQuots (Clonetics) containing 5% fetal bovine serum, 50 ng/mL human vascular endothelial growth factor (VEGF), 50 ng/mL human insulin-like growth factor 1, and 50 ng/mL human epidermal growth factor. To exclude contamination with mature circulating endothelial cells, we carefully removed the culture supernatant 8 h after initial seeding and placed it into new fibronectin-coated culture dishes. After 4 days of culture, nonadherent cells were removed by washing, new medium was applied, and the culture was maintained through day 4. Adherent cells of endothelial lineage were identified by the concurrent binding of FITC-conjugated *Ulex europeaus* agglutinin I (UEA-1, Sigma) and the uptake of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanime (DiI)-labeled acetylated low-density lipoprotein (acLDL, Molecular Probes). Adherent cells were visualized with an inverted fluorescent microscope, and adherent cells that stained for both FITC-UEA-1 and DiI-acLDL were considered to be EPCs. Two independent investigators evaluated the number of EPCs per mm^2 by counting dual-staining cells in 15 randomly selected high power fields using an inverted fluorescent microscope (Olympus, Tokyo, Japan).

2.4. Determination of human telomere length by flow-FISH

Telomere lengths of freshly isolated PBMCs and cultured EPCs were measured using a Dako Telomere peptide nucleic acid kit/FITC for flow cytometry (Dako Cytomation, Ely, U.K.). Relative telomere length (RTL) was determined by comparing isolated EPCs with a control cell line (1301; subline of the Epstein–Barr virus genome negative T-cell leukemia line CCRF-CEM) [16]. A total of 5×10^5 cells were resuspended in 300 μL of hybridization solution containing 70% formamide either with no probe (unstained control) or with FITC-conjugated telomere PNA probe. These cells were heated for 10 min at 82 °C for DNA denaturation. Hybridization was performed overnight at room temperature in the dark. After washing, cells were resuspended in 0.5 mL of

DAKO DNA staining solution and incubated at 4 °C for 2 h in the dark. Each sample was then analyzed by FACScan analyzer (Becton Dickinson) using logarithmic scale FL1-H for probe fluorescence and linear scale FL3-H for DNA staining. Statistical data on these cells were then used to calculate the RTL of the sample cells compared with the control cells, according to the manufacturer's instruction, RTL (%) = (mean FL1-H EPCs with probe − mean FL1-H EPCs without probe) × DNA index control cells (=2) × DNA index EPCs (=1) × 100/(mean FL1-H control cells with probe − mean FL1-H control cells without probe).

2.5. Oxidative DNA damage

A Biotrin OxyDNA test kit (Biotrin, Dublin, Ireland) was used to evaluate oxidative DNA damage in EPCs, following the manufacturer's recommendations. The assay is an *in vitro* fluorescent protein-binding method used to detect oxidative DNA in fixed permeabilized cells. The probe is specific for 8-oxoguanine (as part of the oxidized nucleotide 8-oxoguanosine), which is formed during free radical damage to DNA and is a sensitive and specific indicator of oxidative DNA damage [17]. Briefly, 1×10^6 EPCs were incubated immediately with 1% paraformaldehyde for 15 min on ice, washed once with PBS, and resuspended in 70% ethanol. The cells were incubated for 1 h at 37 °C with 50 μL of Biotrin blocking buffer, washed twice and then incubated for 1 h at room temperature in the dark with 100 μL of FITC-labeled 8-oxoguanine probe. The cells were analyzed by flow cytometry and the mean fluorescent intensity (MFI) of 8-oxoguanine was recorded.

2.6. Telomeric repeat amplification protocol (TRAP) assay

For quantitative analysis of telomerase activity, a TRAP assay was performed using a quantitative telomerase detection kit (Allied Biotech. Ijamsville, MD, USA) according to the manufacturer's protocol [18]. This involved amplification of the telomerase reaction product by real-time PCR (ABI PRISM 7700 sequence detector, PE Biosystem, Foster City, CA, USA). Telomerase activity in the samples was calculated based on the threshold cycle. Quantitative real-time PCR was performed using serial dilutions of positive control template (Allied Biotech.) to generate a standard curve. All samples were run in triplicate, and the lysis buffer was used as a negative control.

2.7. Cell culture with oxidant treatments

Stock solutions of *tert*-butyl hydroperoxide (*t*-BHP, Sigma) and L-buthinonie-[*S*,*R*]-sulphoximune (BSO, Sigma) were freshly made in water and diluted in medium immediately before addition to the cultures. To induce oxidative stress, cultured EPCs from 10 healthy subjects (mean age = 63.2 ± 11.2, male/female = 8/2) were grown in EBM-2 lacking ascorbic acid and exposed at each passage to 0.1 μM *t*-BHP or 10 μM BSO or PBS. These compounds were added to the culture medium 48 h after seeding and then every 2 days at the time of feeding. They were incubated in fibronectin-coated culture dishes (Sigma) for 14 days at 37 °C in 5% CO_2. Two independent cultures were carried out for each condition (*t*-BHP, BSO or PBS) from distinct single donor EPCs. The number of population doublings (PD) was calculated using the formula PD = ($\log_{10}$[number of cells harvested] − $\log_{10}$[number of cells seeded])/log 2. Cell viability was >85% in all experiments as assessed by trypan blue exclusion of EPCs (Gibco BRL). Endotoxin concentrations were tested in all media and buffers used in this study and were <10 pg/mL (Limulus amoebocyte lysate test).

2.8. Statistical analysis

All values are presented as mean ± S.D. Kolmogorov–Smirnov analysis was performed to assess data distribution. Unpaired *t*-test was performed for normally distributed data, and nonparametric Mann–Whitney test was performed when this was not appropriate. Comparisons between three groups (AMI, AP and controls) were analyzed by ANOVA for normally distributed variables and by the Kruskal–Wallis test for non-normally distributed variables. Multivariate linear regression analysis was used to correlate EPC telomere length with cardiovascular risk factors. Pearson's correlation coefficients were used to examine the relationship between RTL and oxidative DNA damage. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Baseline and clinical characteristics

Baseline and clinical characteristics of the study populations are shown in Table 1. There was no significant difference between AMI patients and AP patients in baseline and clinical characteristics (Table 1). Fifteen patients with AMI (57.6%) and 16 patients with AP (51.6%) had MS at the time of admission. There were no cases of MS among the controls.

3.2. Number of EPCs

The number of circulating CD34/KDR double-positive cells was higher in AMI patients than in AP patients and controls (36.0 ± 22.9 vs. 20.3 ± 5.5 vs. 18.6 ± 10.8 per 10^6 events, $P < 0.01$) (Fig. 1A). After 8 days in culture, isolated PBMCs that exhibited a spindle-shaped endothelial cell-like morphology, adherence, and positive staining for both acetylated LDL uptake and UEA1 binding were characterized as endothelial lineage cells (Fig. 1B–D). An increased number of dil-acLDL/UEA1 double-positive cells were counted in patients with AMI compared with AP patients and controls (98.5 ± 37.3 cells/mm^2 vs. 79.5 ± 21.5 cells/mm^2 vs.

Table 1
Baseline and clinical characteristics of study populations

	CAD		Controls ($n=21$)
	AMI ($n=26$)	AP ($n=31$)	
Age (years)	64.1 ± 11.4	65.5 ± 13.1	64.7 ± 9.4
Male, *n* (%)	19 (73.1)	24 (77.4)	16 (76.2)
Waist circumference (cm)	86.9 ± 6.7	90.2 ± 9.1	79.3 ± 5.5
Body mass index (kg/m^2)	24.3 ± 3.1	24.9 ± 2.7	21.3 ± 2.5
Systolic BP (mm Hg)	124 ± 16	131 ± 19	123 ± 13
Diastolic BP (mm Hg)	71 ± 8	74 ± 13	65 ± 7
Smokers (%)	9 (34.6)	11 (35.5)	1 (4.8)*
Components of MS (%)			
High waist circumference	17 (65.4)	21 (67.7)	5 (23.8)*
High triglycerides	12 (46.2)	15 (48.4)	3 (14.3)*
Low HDL cholesterol	13 (50.0)	16 (51.6)	5 (23.8)*
High blood pressure	19 (73.1)	23 (74.2)	4 (19.0)*
High fasting blood glucose	13 (50.0)	16 (51.6)	3 (14.3)*
Fasting blood glucose (mg/dL)	118 ± 22	115 ± 24	97 ± 10*
LDL cholesterol (mg/dL)	114 ± 29	104 ± 42	89 ± 22*
HDL cholesterol (mg/dL)	47 ± 12	50 ± 13	61 ± 18*
Triglycerides (mg/mL)	133 ± 88	152 ± 97	112 ± 65*
hs-CRP (mg/L)	32.5 ± 44.6	16.9 ± 19.3	1.2 ± 0.9*

LDL: low-density lipoprotein-cholesterol, HDL: high-density lipoprotein-cholesterol, hsCRP: high sensitive C-reactive protein and MS: metabolic syndrome.
*$P<0.05$ vs. AMI or AP patients.

68.4 ± 23.7 cells/mm^2, $P<0.01$) (Fig. 1E). The number of these cells was no significant differences between AP patients and controls (all $P>0.05$) (Fig. 1A and E).

3.3. Telomere length, oxidative DNA damage and telomerase activity in EPCs

RTL of EPCs was significantly shorter in CAD patients compared with controls (56.2 ± 14.3% vs. 80.3 ± 13.2%, $P<0.01$). RTL of EPCs was shorter in AMI patients than in AP patients (46.8 ± 10.0% vs. 64.0 ± 11.8%, $P<0.01$). RTL did not differ between freshly isolated PBMCs and cultured EPCs in individual groups (RTL in PBMCs: CAD patients, 55.7 ± 13.9%; controls, 79.6 ± 14.5%, all $P>0.05$ vs. RTL in EPCs).

8-Hydroxyl deoxyguanosine MFI of EPCs was significantly higher in patients with CAD compared with controls (393.6 ± 154.3 vs. 186.7 ± 52.3, $P<0.01$). 8-Hydroxyl

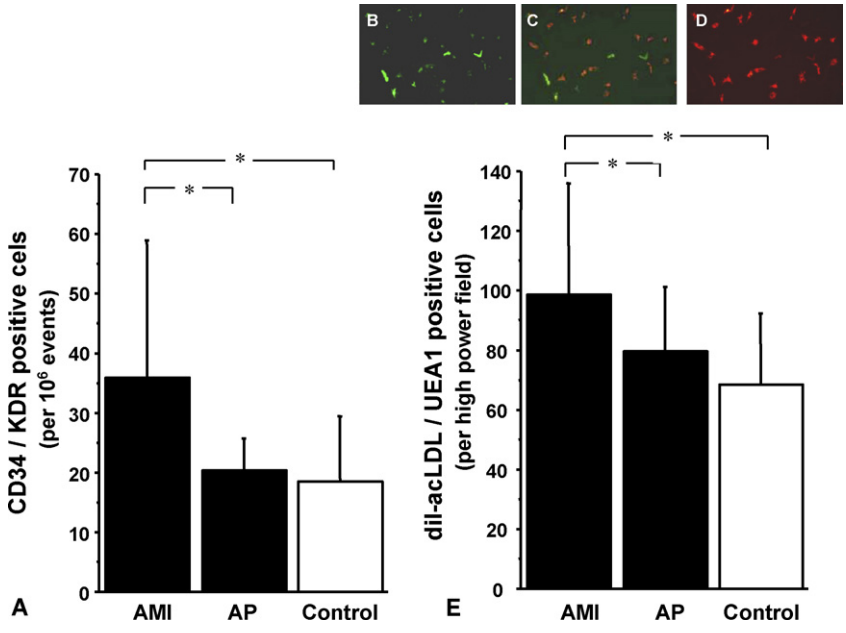

Fig. 1. Number of circulating CD34/KDR-positive cells in CAD and controls (A). EPCs were shown to uptake acetylated LDL (B) and bind UEA-1 (C). Merged images showed dil-acLDL/UEA1 double-positive EPCs (D). Number of dil-acLDL/UEA1 double-positive cells in CAD patients and controls (E). *$P<0.05$.

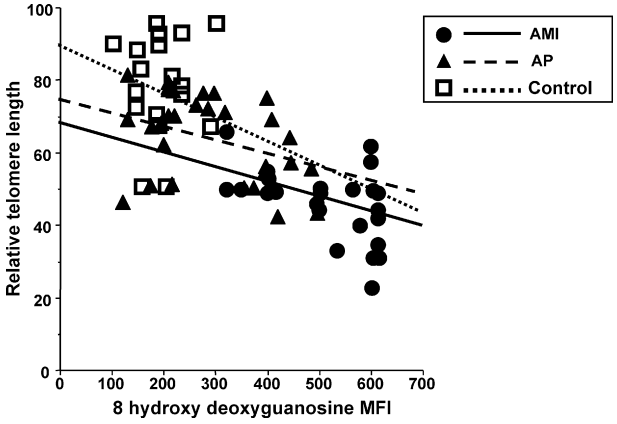

Fig. 2. Correlation between RTL and 8-hydroxyl deoxyguanosine MFI in CAD patients and controls. AMI patients: $r=-0.43$, $P=0.03$. AP patients: $r=-0.43$, $P=0.02$. Controls: $r=-0.65$, $P<0.01$.

deoxyguanosine MFI was also higher in AMI patients than in AP patients (510.7 ± 106.0 vs. 294.9 ± 113.2, $P<0.01$). RTL was weakly negatively correlated with 8-hydroxyl deoxyguanosine MFI in all groups (AMI: $r=-0.43$, $P=0.03$; AP: $r=-0.43$, $P=0.02$; controls: $r=-0.65$, $P<0.01$) (Fig. 2).

Telomerase activity of EPCs was significantly lower in CAD patients than in healthy subjects (34.4 ± 9.8 vs. 55.3 ± 10.5, $P<0.01$). Telomerase activity of EPCs was also lower in AMI patients than in AP patients (29.6 ± 11.2 vs. 39.1 ± 9.9, $P<0.05$).

3.4. Relationship between clinical data, RTL and oxidative DNA damage

Ageing was weakly negatively correlated with RTL in EPCs obtained from all subjects ($r=-0.24$, $P=0.04$). RTL was not statistically correlated with LDL cholesterol (AMI: $r=-0.27$, $P=0.25$; AP: $r=-0.35$, $P=0.17$; controls: $r=-0.18$, $P=0.39$), fasting blood glucose (AMI: $r=0.20$, $P=0.40$; AP: $r=0.02$, $P=0.95$; controls: $r=0.18$, $P=0.58$) or hsCRP (AMI: $r=0.16$, $P=0.51$; AP: $r=-0.30$, $P=0.25$; controls: $r=0.11$, $P=0.47$).

When CAD patients were divided into two subgroups according to the presence or absence of MS, the number of EPCs was significantly higher in CAD patients with MS than in those without MS (Fig. 3A and B). RTL of EPCs was shorter in CAD patients with MS than in those without MS (Fig. 3C). In addition, 8-hydroxyl deoxyguanosine MFI of EPCs was higher in CAD patients with MS than in those without MS (Fig. 3D). Telomerase activity of EPCs was lower in CAD patients with MS than in those without MS (AMI: 25.2 ± 12.1 vs. 32.5 ± 8.9; AP: 35.3 ± 11.4 vs. 43.9 ± 8.9; all $P<0.05$). By multivariate analysis, EPC oxidative DNA damage and the presence of MS remained the only significant independent predictors of telomere shortening of EPCs in CAD patients (Table 2).

3.5. In vitro study with oxidative treatments

To explore the role of oxidative stress in telomere shortening and telomerase activity in EPCs, we used cultured EPCs

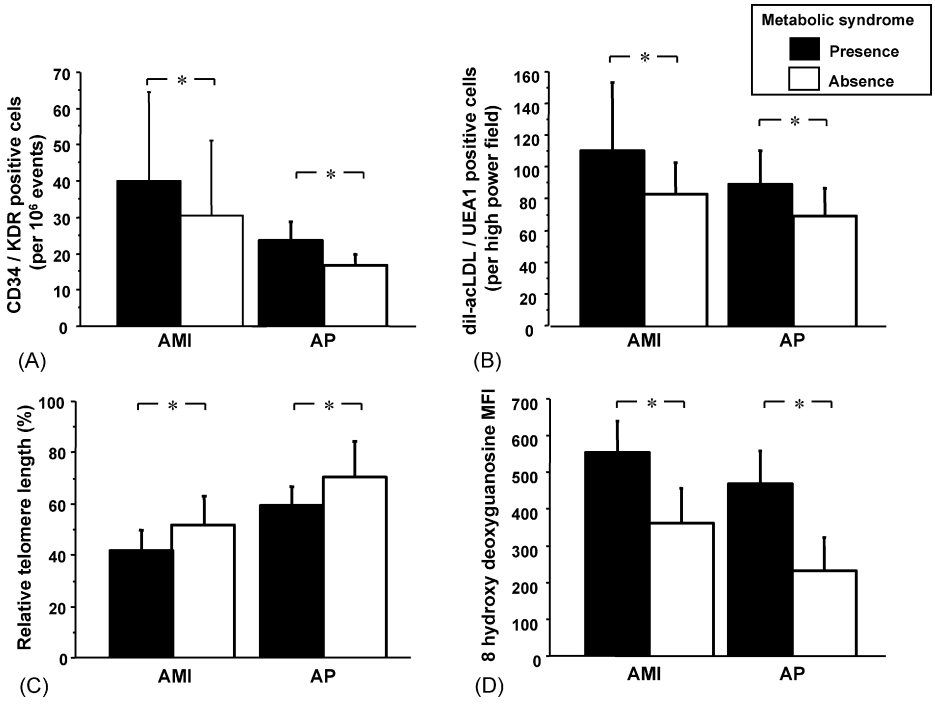

Fig. 3. Comparison of EPC counts (A and B), RTL (C) and 8-hydroxyl deoxyguanosine MFI (D) between CAD patients with MS and those without MS. *$P<0.05$.

Table 2
Multivariate analysis between cardiovascular risk factors, oxidative DNA damage and telomere shortening in EPCs in CAD patients

	β	t	P value
Age	0.26	1.99	0.53
Sex	−3.40	−0.87	0.39
Smoking	0.84	0.26	0.80
Metabolic syndrome	−7.87	−1.76	0.04
Oxidative DNA damage	−0.07	−6.13	<0.001
Significance (ANOVA)			<0.0001

Oxidative DNA damage was presented as 8-hydroxyl deoxyguanosine MFI in EPCs.

treated with *t*-BHP or BSO obtained from healthy subjects. EPCs cultured without oxidative stress ceased to replicate after 14 days, reaching 30 cumulative PD (CPD). Parallel cultures treated with t-BHP or BSO after 14 days reached 25 and 24 CPD, respectively. The *t*-BHP or BSO-stimulated EPCs demonstrated telomere shortening compared with unstimulated EPCs (percent change of RTL with *t*-BHP and BSO vs. PBS: 58.2 ± 18.4 and 60.3 ± 20.1 vs. 91.3 ± 6.5, respectively, $P < 0.01$). In addition the *t*-BHP or BSO stimulations diminished telomerase activity (percent change of telomerase activity with *t*-BHP and BSO vs. PBS: 44.3 ± 13.6 and 46.5 ± 12.2 vs. 97.3 ± 9.4, respectively, $P < 0.01$).

4. Discussion

The most important findings of the present study are (1) EPC telomere length was significantly shorter in CAD patients than in age-matched healthy subjects; (2) EPC telomere length was negatively correlated with oxidative DNA damage; (3) telomere shortening and oxidative DNA damage in EPCs was higher in CAD patients with MS than in those without MS; (4) our *in vitro* study suggests that oxidative stress is involved in telomere shortening of EPCs.

The classic risk factors for atherosclerosis induce endothelial injury, and impaired endothelial function predicts the risks of subsequent cardiovascular events [19]. Endothelial damage may represent an imbalance between the magnitude of injury and the capacity of endothelial cells for repair. Experimental studies have identified a population of EPCs that can be isolated from bone marrow or circulating, blood-derived, mononuclear cells and may contribute to ongoing endothelial repair by incorporating into the sites of neovascularization and homing to sites of endothelial denudation [10,11]. These reports have suggested that the cell senescence of EPCs may affect the progression of atherosclerosis. In the present study, EPC telomere length was shorter in CAD patients than in age-matched healthy subjects. Telomere length was also shorter in AMI patients than in AP patients. To exclude telomere shortening as a cause of premature senescence in EPCs, we measured RTL in PBMCs from individual subjects. RTL did not vary between PBMCs and EPCs, thus eliminating culture-induced telomere erosion as a probable cause of senescence in EPCs. EPC telomere shortening thus indicates a cell population at increased risk of replicative senescence and apoptosis at cell division [11,12]. An in vivo study has reported that telomeres of coronary endothelial cells in CAD patients were markedly shortened compared to non-CAD patients [20]. EPC telomere shortening may therefore accelerate the rate of cell senescence of coronary endothelial cells and may contribute to the pathogenesis of CAD.

Although reduced numbers of circulating EPCs have been shown to predict future cardiovascular events [8], the present study showed that the number of EPCs was higher in AMI patients compared with AP patients and controls. Recent study has reported an increased number of EPCs in the early phase of AMI comparing with AP patients and healthy subjects [21]. Inflammatory cytokines are released from ischemic tissues and may stimulate bone marrow to release EPCs [22]. Indeed, animal model demonstrated that hematopoietic/angiogenic cytokines mobilized EPCs [22,23].

An *in vitro* study using cultured human endothelial cells has demonstrated an association between telomere shortening and chronic oxidative stress [24]. Increased oxidative stress is a key step in the initiation and progression of atherosclerosis [25]. The present study has shown that oxidative DNA damage in EPCs was increased in CAD patients compared to controls. There was an inverse correlation between oxidative DNA damage and EPC telomere length in CAD patients and controls. The present study is therefore the first to isolate and expand EPCs *in vitro* from different cohorts (CAD and healthy subjects) and to relate their telomere length to oxidative DNA damage. We have shown that oxidative stress induces telomere shortening and a decrease in telomerase activity of EPCs. Low dose *t*-BHP and BSO treatment induces intracellular oxidative stress *via* the glutathione redox-cycle but has no cytotoxic or cytostatic effects [25]. From these observations, oxidative DNA damage in EPCs may be one of the triggers for a decrease in telomerase activity, telomere shortening and cell senescence. Oxidative stress, which is mainly superoxide anion ($^{\bullet}O_2^-$), plays a critical role in the pathogenesis of MS parameters [3]. In fact, MS is associated with elevated systemic oxidative stress [26]. This report also demonstrates the relationship between elevated oxidative stress and progression of atherosclerosis [26]. In the present study, CAD patients with MS showed shortened EPC telomere length and increased oxidative DNA damage in EPCs compared to those without MS. In concordance with our findings, MS patients with CAD have been shown to have shorter telomere length in PBMCs than healthy controls [27]. Therefore, MS may induce oxidative stress-related telomere shortening of EPCs and reduce the capacity for endothelial repair, ultimately contributing to the progression of atherosclerosis.

Interestingly, the present study has shown EPC telomere shortening and increased oxidative DNA damage in EPCs in AMI patients compared to AP patients, despite similar frequencies of metabolic syndrome. It has been reported that lipid-laden macrophages, which produce a large

amount of superoxide anion [28], are significantly more abundant in atherosclerotic coronary arteries in patients with AMI than in patients with AP, suggesting that superoxide-producing macrophages may induce an increased oxidative stress in AMI patients [29]. This result permits the speculation that telomere shortening and reduced numbers of EPCs in response to oxidative stress may induce endothelial cell senescence and dysfunction. Damage to the endothelium may thus promote an inflammatory process, such as adhesion and transendothelial migration of circulating leukocytes and monocytes, and induce coronary plaque growth and rupture. It has therefore been speculated that an imbalance in the conditions of enhanced endothelial damage and impaired EPC-mediated endothelial cell repair may contribute to the pathogenesis of coronary plaque instability and rupture in AMI patients.

These results suggest that EPC telomere shortening *via* oxidative DNA damage may play an important role in the pathogenesis of CAD. In addition, MS may be one of the triggers for increased oxidative DNA damage and EPC telomere shortening in CAD patients.

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