Site-Specific DNA Damage at the GGG Sequence by UVA Involves Acceleration of Telomere Shortening†

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ABSTRACT: Telomere shortening is associated with cellular senescence. We investigated whether UVA, which contributes to photoaging, accelerates telomere shortening in human cultured cells. The terminal restriction fragment (TRF) from WI-38 fibroblasts irradiated with UVA (365-nm light) decreased with increasing irradiation dose. Furthermore, UVA irradiation dose-dependently increased the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) in both WI-38 fibroblasts and HL-60 cells. To clarify the mechanism of the acceleration of telomere shortening, we investigated site-specific DNA damage induced by UVA irradiation in the presence of endogenous photosensitizers using ^{32}P 5′-end-labeled DNA fragments containing the telomeric oligonucleotide $(TTAGGG)_4$. UVA irradiation with riboflavin induced 8-oxodG formation in the DNA fragments containing telomeric sequence, and Fpg protein treatment led to chain cleavages at the central guanine of 5′-GGG-3′ in telomere sequence. The amount of 8-oxodG formation in DNA fragment containing telomere sequence [5′-CGC$(TTAGGG)_7$CGC-3′] was approximately 5 times more than that in DNA fragment containing nontelomere sequence [5′-CGC$(TGTGAG)_7$CGC-3′]. Catalase did not inhibit this oxidative DNA damage, indicating no or little participation of H_2O_2 in DNA damage. These results indicate that the photoexcited endogenous photosensitizer specifically oxidizes the central guanine of 5′-GGG-3′ in telomere sequence to produce 8-oxodG probably through an electron-transfer reaction. It is concluded that the site-specific damage in telomere sequence induced by UVA irradiation may participate in the increase of telomere shortening rate.

Telomeres protect chromosome ends against illegitimate recombination and may direct chromosome attachment to the nuclear membrane (*1*−*3*). These functions are mediated by highly conserved repeats, which consist of a characteristic hexameric telomere sequence (TTAGGG), in all vertebrates (*4, 5*). A gradual loss of telomeric repeat sequences with aging previously has been noted in normal adult tissues (*6*−*9*). Hence, telomere shortening has been suggested to be a 'molecular clock' of the aging process (*10, 11*). Recently, Zglinicki et al. reported an increase of the rate of telomere shortening by oxidative stress in human fibroblasts (*12, 13*). It is reasonably considered that acceleration of telomere shortening by increased oxidative stress leads to accelerated proliferative senescence. Furthermore, age-dependent telomere shortening was shown to be slowed by enrichment of intracellular vitamin C via suppression of oxidative stress (*14*). However, the mechanism for an increased rate of telomere shortening by oxidative stress remains to be clarified.

Repeated exposure of human skin to solar UV irradiation leads to skin carcinogenesis and photoaging which involve cell cycle arrest and senescence. It is well-known that the most prevalent DNA lesion induced by UVB irradiation is the pyrimidine dimer, which forms at positions of neighboring pyrimidines (*15, 16*). Kruk et al. have reported that after UVB irradiation with 20 J/cm^2 light and treatment of the purified DNA with T4 endo, a pyrimidine dimer-specific enzyme, telomeres from GM 38A fibroblasts were reduced (*17*). This result suggests that UVB-induced pyrimidine dimers may accelerate telomere shortening in cells. However, it is generally accepted that oxidative damage does not induce pyrimidine dimer formation in telomeric DNA.

Increasing evidence demonstrates that UVA, as well as UVB, irradiation contributes to photoaging (*18, 19*). DNA damage should be produced indirectly through photosensitized reactions mediated by photosensitizers, since UVA can hardly be absorbed by the DNA. We have demonstrated that UVA irradiation induced oxidative DNA damage in the presence of various endogenous photosensitizers (*20*−*23*). In this study, we investigated the shortening rate of telomeres in human WI-38 fibroblasts exposed to UVA irradiation. We also examined the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG)[1] in human cultured cells by using an electrochemical detector coupled to HPLC (HPLC-ECD). Furthermore, we investigated the mechanism for the increase of telomere shortening induced by UVA irradiation using

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[1] Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine (also known as 8-hydroxy-2′-deoxyguanosine); HPLC-ECD, an electrochemical detector coupled to high-pressure liquid chromatography; DTPA, diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid; Fpg, *E. coli* formamidopyrimidine−DNA glycosylase; TRF, terminal restriction fragment; FCS, fetal calf serum.

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^{32}P 5′-end-labeled DNA fragment including the telomeric sequence. The ^{32}P-labeled DNA fragment was exposed to 365-nm irradiation in the presence of riboflavin, as a model of endogenous compound, and subsequently treated with *E. coli* formamidopyrimidine−DNA glycosylase (Fpg). Fpg protein is a DNA glycosylase that removes 8-oxodG from DNA (*24*−*26*). We also measured the photodynamic 8-oxodG formation with riboflavin in DNA fragments including the telomeric sequence by using HPLC-ECD.

MATERIALS AND METHODS

Materials. WI-38 fibroblasts were obtained from the RIKEN Cell Bank (Ibaraki, Japan) at a population doubling level (PDL) of 29. Fetal calf serum was from ICN Biomedicals, Inc. Fpg protein was from Trevigen. Proteinase K was from Merck. Calf thymus DNA, bacterial alkaline phosphatase, and RNase A were from Sigma Chemical Co. [γ-^{32}P]ATP (222 TBq/mmol) was purchased from New England Nuclear. T4 polynucleotide kinase was from New England Biolabs. Acrylamide and bisacrylamide were from Wako Chemical Industries, Ltd., Osaka, Japan. Riboflavin and menadione were from Nacalai Tesque, Inc., Kyoto, Japan. Pterin was from Aldrich Chemical Co. Diethylenetriaminepentaacetic acid (DTPA) was from Dojin Chemicals Co., Kumamoto, Japan. Nuclease P1 (400 units/mg) was from Yamasa Shoyu Co., Chiba, Japan. Alkaline phosphatase from calf intestine was from Boehringer Mannheim GmbH. DNA fragments, 5′-(TAGTAG)$_4$(TTAGGG)$_4$-3′, 5′-(CCCTAA)$_4$(CTACTA)$_4$-3′, 5′-CGC(TTAGGG)$_7$CGC-3′, 5′-GCG(CCCTAA)$_7$GCG-3′, 5′-CGC(TGTGAG)$_7$CGC-3′, and 5′-GCG(CTCACA)$_7$GCG-3′, were synthesized by Sawady Technology Co., Ltd., Tokyo, Japan.

Cell Culture and UVA Irradiation. WI-38 fibroblasts were cultured in minimum essential medium (GIBCO) containing 10% fetal calf serum (FCS) at 37 °C under 5% CO_2 in a humidified atmosphere. The human myelomonocytic leukemia cell line, HL-60, is grown in RPMI 1640 supplemented with 6% FCS. Exponentially growing cells are used throughout all experiments. The cells were irradiated with five 8-W UV lamps (365 nm, UVP, Inc., model TDM-20, San Gabriel, CA) placed at a distance of 3−5 cm. The cells are protected from direct sunlight. After UVA irradiation, cells were immediately harvested by centrifugation at 200*g* for 5 min and washed with phosphate-buffered saline (PBS) 3 times.

Analysis of Terminal Restriction Fragment (TRF) Length. WI-38 fibroblasts were lysed, and proteins were digested in 500 μL of lysis buffer (Applied Biosystems) containing 50 μg of RNase A and 500 μg of proteinase K at 60 °C for 60 min. The genomic DNA was precipitated with ethanol and dissolved in H_2O. The genomic DNA was digested with *Hin*fI and *Rsa*I to generate the terminal restriction fragment (TRF). DNA concentrations were determined by ethidium bromide fluorescence in a Shimazu fluorometer using calf thymus DNA standard. The DNA samples (2.5 μg each) were loaded onto a 0.8% agarose gel and electrophoresed for 5 h at 5 V/cm in TAE buffer. The DNA was transferred to a nylon membrane in 20×SSC overnight and fixed by baking at 120 °C for 20 min. After a 30-min prehybridization, the membrane was hybridized for 3 h at 42 °C with telomere probe using a Telo TAGGG telomere length assay kit (Roche).

Measurement of 8-OxodG in Cultured Cells Irradiated with UVA. DNA was extracted from irradiated WI-38 fibroblasts and HL-60 by using a DNA Extractor WB Kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The DNA was dissolved in H_2O, and treated with 8 units of nuclease P1 and then with 1.2 units of bacterial alkaline phosphatase. The content of 8-oxodG was determined by the method described previously (*20*, *23*).

Preparation of ^{32}P 5′-End-Labeled DNA Fragments. The 48-base fragment 5′-(TAGTAG)$_4$(TTAGGG)$_4$-3′ was phosphorylated with [γ -^{32}P]ATP and T4 polynucleotide kinase according to the method described previously (*27*). The ^{32}P 5′-end-labeled 48-base fragment and the complementary strand 5′-(CCCTAA)$_4$(CTACTA)$_4$-3′ were annealed.

UVA Irradiation to ^{32}P 5′-End-Labeled DNA in the Presence of Endogenous Photosensitizers. The standard reaction mixture in a microtube contained ^{32}P-labeled DNA fragment and 20 μM endogenous photosensitizer in 100 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. The mixtures were exposed to various doses of UVA light using five 8-W UV lamps (365 nm, UVP, Inc., model TDM-20, San Gabriel, CA) placed at a distance of 20 cm. After the irradiation, the DNA fragments were treated with 10 units of Fpg protein in 10 μL of the reaction buffer [10 mM HEPES−KOH (pH 7.4), 100 mM KCl, 10 mM EDTA, and 0.1 mg/mL BSA] at 37 °C for 120 min, and then the Fpg-treated DNA fragments were precipitated. Subsequently, the loading buffer [80% formamide, 0.1% bromphenol blue, 0.1% xylene cyanol, 50 mM Tris−borate (pH 8.3), and 1 mM EDTA] was added to the samples, and the samples were heated at 60 °C for 5 min and fast-cooled to 4 °C. The samples were electrophoresed on a 12% polyacrylamide/8 M urea gel using a DNA-sequencing system (LKB 2010 Macrophor).

The preferred cleavage sites were determined by direct comparison of the positions of the oligonucleotides with those produced by the procedure of Maxam and Gilbert (*28*) using a DNA sequencing system (LKB 2010 Macrophor). A laser densitometer (LKB 2222 UltroScan XL) was used for the measurement of the relative amounts of oligonucleotides from treated DNA fragments.

Analysis of 8-OxodG Formation in Telomeric or Nontelomeric DNA Fragments by UVA Irradiation. Single-stranded DNA fragment containing telomere sequence [5′-CGC(TTAGGG)$_7$CGC-3′] was annealed with the complementary strand 5′-GCG(CCCTAA)$_7$GCG-3′. Another single-stranded DNA fragment containing nontelomere sequence [5′-CGC(TGTGAG)$_7$CGC-3′] was also annealed with the complementary strand 5′-GCG(CTCACA)$_7$GCG-3′. Telomeric or nontelomeric DNA fragments (10 μM per base) were irradiated with various doses of UVA light in the presence of 50 μM riboflavin in 50 μL of 4 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA at 0 °C. After ethanol precipitation, DNA was digested to nucleosides by incubation with nuclease P1 and alkaline phosphatase and analyzed by HPLC-ECD as previously described (*22*, *29*).

RESULTS

Decrease in Telomere Length of WI-38 Fibroblasts by UVA Irradiation. To investigate the effect of UVA irradiation on telomere length, the size of the TRF from WI-38

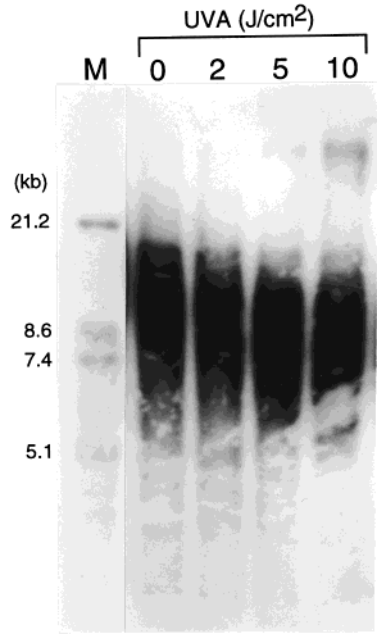

FIGURE 1: Degradation of TRF length in WI-38 fibroblasts irradiated with UVA. WI-38 fibroblasts (1.0×10^6 cells) were irradiated with the indicated dose of UVA light (365 nm). After the irradiation, the cells were lysed, and DNA was extracted. Genomic DNA was digested with *Hin*fI and *Rsa*I, separated by electrophoresis on a 0.8% agarose gel, and hybridized to telomeric probe as described under Materials and Methods. In all cases, 2.5 μg of DNA was loaded. M: size marker DNA.

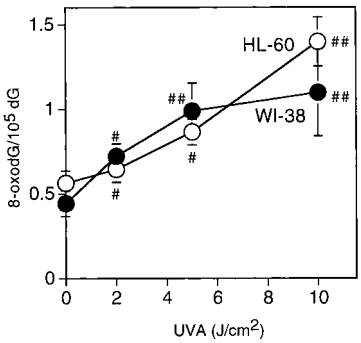

FIGURE 2: Formation of 8-oxodG in cultured cells irradiated with UVA. WI-38 fibroblasts (2.0×10^6 cells) (●) and HL-60 cells (3.0×10^6 cells) (○) were irradiated with the indicated dose of UVA light (365 nm). After the irradiation, the cells were lysed, and DNA was extracted and subjected to enzyme digestion and analyzed by HPLC-ECD as described under Materials and Methods. Results are expressed as means ± SE of values obtained from 3−5 independent experiments. #, $P < 0.01$; ##, $P < 0.05$ compared with nonirradiation; *t*-test.

fibroblasts irradiated with UVA was examined. The TRF was calculated as TRF $= \Sigma(OD_i)/\Sigma(OD_i/L_i)$ where OD_i is densitometer output and L_i is the length of the TRF fragment at position *i* according to the method described (*8*). Figure 1 shows TRF in WI-38 fibroblasts irradiated with UVA. TRF dose-dependently declined with UVA irradiation. This result suggests that telomere shortening is accelerated with increasing irradiation dose.

Formation of 8-OxodG in Cells by UVA Irradiation. Production of 8-oxodG in cellular DNA exposed to 365-nm light was measured as a function of irradiation dose (Figure 2). The formation of 8-oxodG in WI-38 fibroblast cells significantly increased after UVA irradiation. Irradiations

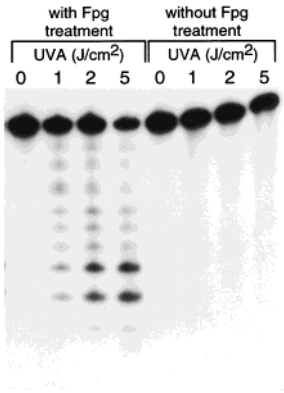

FIGURE 3: Autoradiogram of ^{32}P-labeled DNA fragments exposed to 365-nm light in the presence of riboflavin. The ^{32}P 5′-end-labeled 48-base pair fragment [5′-$(TAGTAG)_4(TTAGGG)_4$-3′] was exposed to the indicated dose of UVA light (365 nm) with 20 μM riboflavin in 100 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. After the irradiation, followed by Fpg protein treatment or without Fpg protein treatment, the treated DNA fragments were electrophoresed on a 12% polyacrylamide/8 M urea gel (12 × 16 cm), and the autoradiogram was obtained by exposing X-ray film to the gel.

with 2, 5, and 10 J/cm^2 light produced 1.64-fold ($P < 0.01$), 2.23-fold, and 2.48-fold ($P < 0.05$) increased 8-oxodG formation in comparison with nonirradiation, respectively. In addition, the formation of 8-oxodG in HL-60 also increased with increasing irradiation dose. These results indicate that UVA irradiation induces oxidative DNA damage in human cultured cells. Irradiation of WI-38 fibroblasts with 0, 2, 5, and 10 J/cm^2 light resulted in a progressive increase in 8-oxodG formation and a decrease in TRF. A progressive increase in 8-oxodG formation by UVA irradiation correlated with a decrease in TRF.

DNA Cleavage by UVA (365-nm) Irradiation in the Presence of Endogenous Photosensitizers. ^{32}P 5′-end-labeled DNA fragments containing the telomere sequence were exposed to 365-nm irradiation in the presence of riboflavin, and the extent of DNA damage was estimated by gel electrophoretic analysis. Figure 3 shows the autoradiogram of double-stranded DNA fragments irradiated with UVA in the presence of riboflavin, followed by Fpg protein treatment. Fpg protein is known to recognize 8-oxodG as well as Fapy residues (*24*−*26*). Photodegradation of DNA with riboflavin increased with irradiation dose. No or little oligonucleotide was produced without Fpg protein treatment. A similar photodegradation was observed in experiments with other endogenous photosensitizers such as pterin and menadione. In addition, when hot piperidine treatment was performed instead of Fpg protein treatment, a similar pattern of DNA damage was observed (data not shown). These results suggest that in the presence of endogenous photosensitizers, UVA irradiation produces not only 8-oxodG but also piperidine-labile residues in DNA fragments containing telomere sequence. Catalase did not inhibit the DNA damage (data not shown), indicating no or little participation of H_2O_2 in DNA damage.

Site-Preference of DNA Cleavage by UVA Irradiation with Riboflavin. The patterns of DNA cleavage caused by UVA irradiation with riboflavin and subsequently treated with Fpg protein were determined with DNA sequences by the Maxam−Gilbert procedure (*28*). The relative intensity of

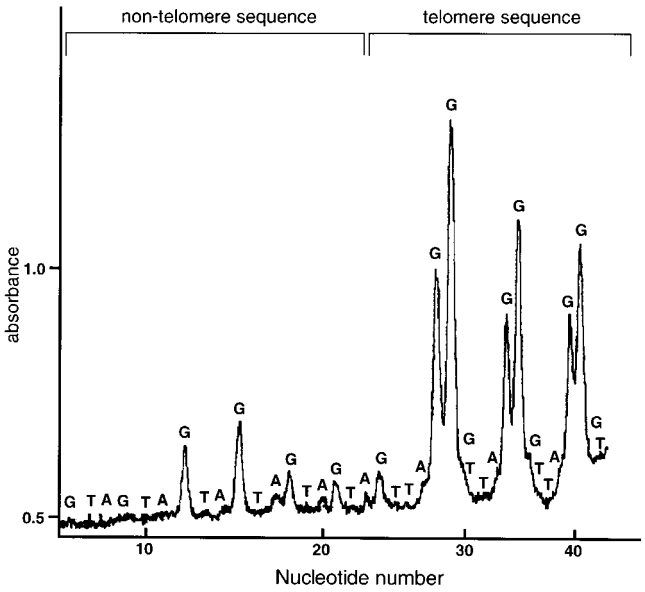

FIGURE 4: Site-preference of DNA cleavage by UVA irradiation in the presence of riboflavin. The ^{32}P 5′-end-labeled 48-base pair fragment [5′-(TAGTAG)$_4$(TTAGGG)$_4$-3′] was exposed to 2 J/cm^2 UVA light (365 nm) with 20 μM riboflavin in 100 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. After Fpg protein treatment, DNA fragments were electrophoresed on a 12% polyacrylamide/8 M urea gel using a DNA-sequencing system, and the autoradiogram was obtained by exposing X-ray film to the gel. The relative amounts of oligonucleotides produced were measured using a laser densitometer. The cleavage sites of the treated DNA were determined by direct comparison with the same DNA fragment after undergoing DNA sequencing reactions according to the Maxam−Gilbert procedure (*28*). The horizontal axis shows the nucleotide number.

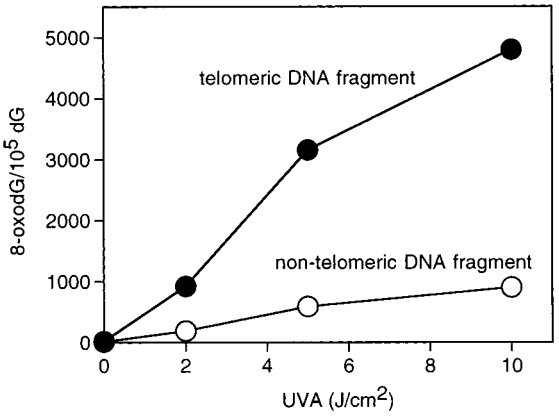

FIGURE 5: Formation of 8-oxodG in DNA fragment irradiated with 365-nm light in the presence of riboflavin. The reaction mixture containing 10 μM per base of DNA fragment containing telomere sequence [5′-CGC(TTAGGG)$_7$CGC-3′] (●) or nontelomere sequence [5′-CGC(TGTGAG)$_7$CGC-3′] (○), 50 μM riboflavin, and 5 μM DTPA in 50 μL of 4 mM sodium phosphate buffer (pH 7.8) was exposed to UVA light (365 nm). After the irradiation, DNA was treated, and the amount of 8-oxodG was measured by the methods described under Materials and Methods.

DNA cleavage obtained by scanning autoradiogram with a laser densitometer is shown in Figure 4. UVA irradiation of double-stranded DNA in the presence of riboflavin induced cleavages specifically at the central guanine of 5′-GGG-3′ in the telomere sequence region in the DNA fragment [5′-(TAGTAG)$_4$(TTAGGG)$_4$-3′]. No or little cleavage was observed at the nontelomere sequence region (Figure 4).

Formation of 8-OxodG in the DNA Fragment Induced by UVA Irradiation with Riboflavin. To confirm 8-oxodG formation, we measured the content of 8-oxodG in 48-base pair DNA fragments containing telomeric sequences [5′-CGC(TTAGGG)$_7$CGC-3′] or nontelomeric sequences [5′-CGC(TGTGAG)$_7$CGC-3′] induced by UVA irradiation. Production of 8-oxodG in DNA fragments containing telomeric sequences and containing nontelomeric sequences exposed to 365-nm light in the presence of riboflavin was compared. The formation of 8-oxodG in DNA fragments containing the telomeric sequences was approximately 5 times more than that generated in DNA fragments containing the nontelomeric sequences (Figure 5). Catalase did not inhibit 8-oxodG formation (data not shown).

DISCUSSION

This study demonstrated for the first time that UVA irradiation caused decreasing telomere length, which is measured as the length of TRF in WI-38 fibroblasts. Furthermore, the formation of 8-oxodG significantly increased in both WI-38 fibroblasts and HL-60 cells irradiated with UVA light dose-dependently. In addition, it has been reported that premutagenic oxidative DNA base damage, 8-oxodG, is induced in human skin fibroblasts and tumoral monocytes by UVA irradiation (*30*, *31*). Therefore, UVA irradiation induces 8-oxodG formation in human cultured cells. Recent studies have reported the age-dependent accumulation of 8-oxodG observed in DNA in several organs (*32*, *33*). We investigated the participation of oxidative DNA damage during the aging process by using 8-oxodG as a marker. The present study indicated that a progressive increase in 8-oxodG formation by UVA irradiation correlated with a decrease in TRF. Human 8-oxodG−DNA glycosylase introduces a chain break in a double-stranded oligonucleotide specifically at an 8-oxodG residue base-paired with cytosine (*34*, *35*). In addition, Doetsch et al. have reported that UV irradiation produces not only 8-oxodG but also formamidopyrimidines, such as piperidine-labile FapyG, in DNA (*36*). These products are excised from DNA by Fpg protein with similar kinetics to those of 8-oxodG excision. Therefore, it is concluded that formation of 8-oxodG and piperidine-labile residues induced by UVA irradiation may participate in the increase of the telomere shortening rate.

To clarify the mechanism of the acceleration of telomere shortening, we investigated site-specific DNA damage induced by UVA irradiation in the presence of endogenous photosensitizers using DNA fragments containing the telomere sequence. UVA irradiation with riboflavin induced 8-oxodG formation specifically at the central guanine of 5′-GGG-3′ in the telomere sequence. In addition, in the presence of riboflavin, UVA irradiation more efficiently induced 8-oxodG formation in the DNA fragment containing the telomere sequence than that in the DNA fragment containing the nontelomere sequence. 8-OxodG may be further oxidized to piperidine-labile imidazolone and oxazolone (*37*, *38*). Catalase did not inhibit these oxidative DNA damages, indicating no or little participation of H_2O_2 in DNA damage. These results suggested that the site-specific photodamage at telomeric DNA was induced through the Type I mechanism. Ito and Kawanishi have reported that the Type I process induces guanine cation radical formation by electron

transfer from the guanine residue to the photoexcited sensitizer (*21*, *22*, *39*). The guanine cation radical formed in DNA predominantly undergoes a hydration reaction to give the C-8 OH adduct radical of guanine, which is well-known to produce 8-oxodG upon oxidation and FapyG upon reduction (*40*). On the basis of our results, the cation radical formed on the central guanine in GGG triplets appears to be the most stable, although HOMO is mainly distributed on the 5′-G (*41*). Furthermore, this cation radical has been reported to be formed through long-range electron transfer initiated by guanine oxidation at a remote site (*42*−*44*). GGG triplets can act as traps in oxidative damage to double-stranded DNA caused by long-range electron transfer (*45*). It is concluded that long repeats of double-stranded telomeric sequence, which include GGG triplets, are good targets for oxidative stress.

Oxidative stress may function as a common trigger for activation of the senescence program (*46*). Our previous studies have shown that oxidative stress, such as oxygen radicals derived from the reaction of H_2O_2 with endogenous metal ions and $ONOO^-$ produced from NO and O_2^-, caused cleavage specifically at the polyguanosine sequence in the telomere sequence (*47*). The present study demonstrated that in the presence of riboflavin, UVA irradiation induced cleavage specifically at the central guanine of 5′-GGG-3′ in the telomere sequence. It is concluded that the GGG-specific DNA damage in the telomere sequence induced by oxidative stress may play an important role in increasing the rate of telomere shortening. Recently, it has been reported that endogenous oxidative stress increases the amount of telomere length lost per population doubling (*48*). Collectively, it is concluded that the structure or location of telomeres in the nucleus may increase the susceptibility to DNA oxidation compared with other internal chromosome targets. Recently, McCullough and Berget have reported that an appreciable number of introns were guanine-rich and specifically contained multiple G triplets or quartets (*49*). It is convenient to imagine that noncoding regions such as telomere and intron may protect chromosomes against oxidative stress-induced toxicity.

REFERENCES

1. Artandi, S. E., Chang, S., Lee, S.-L., Alson, S., Gottlieb, G. J., Chin, L., and DePinho, R. A. (2000) *Nature 406*, 641−648.
2. Rudolph, K. L., Chang, S., Lee, H. W., Blasco, M., Gottlieb, G. J., Greider, C., and DePinho, R. A. (1999) *Cell 96*, 701−712.
3. Allsopp, R. C., Vaziri, H., Patterson, C., Goldstein, S., Younglai, E. V., Futcher, A. B., Greider, C. W., and Harley, C. B. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 10114−10118.
4. Adams Martin, A., Dionne, I., Wellinger, R. J., and Holm, C. (2000) *Mol. Cell. Biol. 20*, 786−796.
5. Blackburn, E. H. (1991) *Nature 350*, 569−573.
6. Lanza, R. P., Cibelli, J. B., Blackwell, C., Cristofalo, V. J., Francis, M. K., Baerlocher, G. M., Mak, J., Schertzer, M., Chavez, E. A., Sawyer, N., Lansdorp, P. M., and West, M. D. (2000) *Science 288*, 665−669.
7. Frenck, R. W., Jr., Blackburn, E. H., and Shannon, K. M. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 5607−5610.
8. Harley, C. B., Futcher, A. B., and Greider, C. W. (1990) *Nature 345*, 458−460.
9. Hastie, N. D., Dempster, M., Dunlop, M. G., Thompson, A. M., Green, D. K., and Allshire, R. C. (1990) *Nature 346*, 866−868.
10. Bodnar, A. G., Ouellette, M., Frolkis, M., Holt, S. E., Chiu, C. P., Morin, G. B., Harley, C. B., Shay, J. W., Lichtsteiner, S., and Wright, W. E. (1998) *Science 279*, 349−352.
11. Vogel, G. (2000) *Science 288*, 586−587.
12. von Zglinicki, T., Saretzki, G., Docke, W., and Lotze, C. (1995) *Exp. Cell Res. 220*, 186−193.
13. von Zglinicki, T., Pilger, R., and Sitte, N. (2000) *Free Radical Biol. Med. 28*, 64−74.
14. Furumoto, K., Inoue, E., Nagao, N., Hiyama, E., and Miwa N. (1998) *Life Sci. 63*, 935−948.
15. Tommasi, S., Denissenko, M. F., and Pfeifer, G. P. (1997) *Cancer Res. 57*, 4727−4730.
16. Clingen, P. H., Arlett, C. F., Roza, L., Mori, T., Nikaido, O., and Green, M. H. (1995) *Cancer Res. 55*, 2245−2248.
17. Kruk, P. A., Rampino, N. J., and Bohr, V. A. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 258−262.
18. Berneburg, M., Grether-Beck, S., Kurten, V., Ruzicka, T., Briviba, K., Sies, H., and Krutmann, J. (1999) *J. Biol. Chem. 274*, 15345−15349.
19. Takeuchi, T., Uitto, J., and Bernstein, E. F. (1998) *J. Invest. Dermatol. 110*, 343−347.
20. Tada-Oikawa, S., Oikawa, S., and Kawanishi, S. (2000) *Methods Enzymol. 319*, 331−342.
21. Ito, K., Inoue, S., Yamamoto, K., and Kawanishi, S. (1993) *J. Biol. Chem. 268*, 13221−13227.
22. Ito, K., and Kawanishi, S. (1997) *Biochemistry 36*, 1774−1781.
23. Tada-Oikawa, S., Oikawa, S., and Kawanishi, S. (1998) *Biochem. Biophys. Res. Commun. 247*, 693−696.
24. David-Cordonnier, M. H., Laval, J., and O'Neill, P. (2000) *J. Biol. Chem. 275*, 11865−11873.
25. Boiteux, S., Gajewski, E., Laval, J., and Dizdaroglu, M. (1992) *Biochemistry 31*, 106−110.
26. Tchou, J., Kasai, H., Shibutani, S., Chung, M. H., Laval, J., Grollman, A. P., and Nishimura, S. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 4690−4694.
27. Yamamoto, K., and Kawanishi, S. (1991) *J. Biol. Chem. 266*, 1509−1515.
28. Maxam, A. M., and Gilbert, W. (1980) *Methods Enzymol. 65*, 499−560.
29. Kasai, H., Nishimura, S., Kurosawa, Y., and Hayashi, Y. (1987) *Carcinogenesis 8*, 1959−1961.
30. Pouget, J. P., Douki, T., Richard, M. J., and Cadet, J. (2000) *Chem. Res. Toxicol. 13*, 541−549.
31. Kvam, E., and Tyrrell, R. M. (1997) *Carcinogenesis 18*, 2379−2384.
32. Lee, H. C., Lu, C. Y., Fahn, H. J., and Wei, Y. H. (1998) *FEBS Lett. 441*, 292−296.
33. Fraga, C. G., Shigenaga, M. K., Park, J. W., Degan, P., and Ames, B. N. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 4533−4537.
34. Rosenquist, T. A., Zharkov, D. O., and Grollman, A. P. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 7429−7434.
35. Roldan-Arjona, T., Wei, Y. F., Carter, K. C., Klungland, A., Anselmino, C., Wang, R. P., Augustus, M., and Lindahl, T. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 8016−8020.
36. Doetsch, P. W., Zasatawny, T. H., Martin, A. M., and Dizdaroglu, M. (1995) *Biochemistry 34*, 737−742.
37. Cadet, J., Berger, M., Buchko, G. W., Joshi, P. C., Raoul, S., and Ravanat, J.-L. (1994) *J. Am. Chem. Soc. 116*, 7403−7404.
38. Kino, K., Saito, I., and Sugiyama, H. (1998) *J. Am. Chem. Soc. 120*, 7373−7374.
39. Ito, K., and Kawanishi, S. (1997) *J. Biol. Chem. 378*, 1307−1312.
40. Burrows, C. J., and Muller, J. G. (1998) *Chem. Rev. 98*, 1109−1151.
41. Yoshioka, Y., Kitagawa, Y., Takano, Y., Yamaguchi, K., Nakamura, T., and Saito, I. (1999) *J. Am. Chem. Soc. 121*, 8712−8719.
42. Lewis, F. D., Liu, X., Liu, J., Miller, S. E., Hayes, R. T., and Wasielewski, M. R. (2000) *Nature 406*, 51−53.

43. Nunez, M. E., Hall, D. B., and Barton, J. K. (1999) *Chem. Biol. 6*, 85−97.
44. Hall, D. B., Holmlin, R. E., and Barton, J. K. (1996) *Nature 382*, 731−735.
45. Saito, I., Nakamura, T., Nakatani, K., Yoshioka, Y., Yamaguchi, K., and Sugiyama, H. (1998) *J. Am. Chem. Soc. 120*, 12686−12687.
46. Finkel, T., and Holbrook, N. J. (2000) *Nature 408*, 239−247.
47. Oikawa, S., and Kawanishi, S. (1999) *FEBS Lett. 453*, 365−368.
48. Xu, D., Neville, R., and Finkel, T. (2000) *FEBS Lett. 470*, 20−24.
49. McCullough, A. J., and Berget, S. M. (1997) *Mol. Cell. Biol. 17*, 4562−4571.

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