Original Article

Identification of Two Novel Mitochondrial DNA Deletions Induced by Ionizing Radiation*

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Abstract

Objective We identify ionizing radiation-induced mitochondrial DNA (mtDNA) deletions in human lymphocytes and their distribution in normal populations.

Methods Long-range polymerase chain reactions (PCR) using two pairs of primers specific for the human mitochondrial genome were used to analyze the lymphoblastoid cell line following exposure to 10 Gy ^{60}Co γ-rays. Limited-condition PCR, cloning and sequencing techniques were applied to verify the mtDNA deletions detected with long-range PCR. Human peripheral blood samples were irradiated with 0, 2 and 6 Gy ^{60}Co γ-rays, and real-time PCR analysis was performed to validate the mtDNA deletions. In order to know the distribution of mtDNA deletions in normal population, 222 healthy Chinese adults were also investigated.

Results Two mtDNA deletions, a 7455-bp deletion (nt475-nt7929 in heavy strand) and a 9225-bp deletion (nt7714 -nt369 in heavy strand), occurring between two 8-bp direct repeats, were identified in lymphoblastoid cells using long-range PCR, limited-condition PCR and sequencing. These results were also observed for ^{60}Co γ-rays irradiated human peripheral blood cells.

Conclusion Two novel mtDNA deletions, a 7455-bp deletion and a 9225-bp deletion, were induced by ionizing radiation. The rate of the mtDNA deletions within a normal population was related to the donors' age, but was independent of gender.

Key words: Mitochondrial DNA deletion; Ionizing radiation;Lymphocytes; Chinese adults

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INTRODUCTION

Mithondria are cellular "powerhouses" that play a critical role in maintaining normal cell functions[1-3]. Human mitochondrial DNA (mtDNA) is a unique extra-nuclear genetic material in that it is not protected by histones and non-histones, or DNA repair mechanisms, thereby rendering it sensitive to oxidative damage associated with physical and

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chemical factors[4-7]. Mitochondrial genome dysfunction is related to a broad spectrum of human diseases, including deafness, diabetes mellitus, Leber's hereditary optic neuropathy, and degenerative diseases[8-18]. Research into mitochondrial genome dysfunction and related diseases is becoming a top priority in medical biology.

Ionizing radiation is an important physical factor, affecting both genomic DNA and mtDNA[19-21]. Ionizing radiation can cause point mutations, insertions and deletions in mtDNA, resulting in changes to mitochondrial function[22-27]. Recent studies indicate that deletions in mtDNA could be induced by ionizing radiation in a dose-dependent fashion. For instance, mtDNA deletions of 4977-bp, 4934-bp, 4881-bp, and 889-bp are related to the level of exposure[28-34]. A small number of radiation-induced mtDNA deletions persist for 24 hours[31]. Studies show that real-time PCR used to quantify mtDNA deletions[35-39], is an ideal tool for rapid biological dose estimation during radiation accidents and nuclear terrorism, where large populations are exposed to ionizing radiation.

Of the mtDNA deletions identified, the 4977-bp deletion (termed common deletion, CD) is often studied. Kubota et al. revealed that CD might be a reflection of the cellular sensitivity to ionizing radiation[28]. Recent findings indicate that CD accumulation in a range of human cell lines is associated with the degree of radiation exposure[31]. It is postulated that a CD biomarker may be used for retrospective radiation dose estimation[30]. According to Prasanna et al. the interphase CD level of lymphocytes analyzed with *in situ* PCR accurately estimates acute exposure in the range of 0.25-2 Gy[30]. Ionizing radiation-induced CD levels, quantified by real-time PCR, were not changed significantly with an increase in radiation dose between 0-5 Gy (as shown by our unpublished data). Other mtDNA deletions have not yet to be delineated.

It is therefore debatable whether CD is a sensitive biomarker for radiation damage. It is essential to investigate the new types of mtDNA deletions specifically induced by radiation, and which are applicable to dose estimation studies. In the present study, long-range PCR was used to screen for novel mtDNA deletions in human cells irradiated with ^{60}Co γ-rays. Irradiated human peripheral blood samples were also used to validate the deletions and to investigate the occurrence of these mtDNA deletions in normal populations.

MATERIALS AND METHODS

Materials

All materials used in cell cultures were provided by Invitrogen (Carlsbad, CA, USA). Reagents for DNA extraction and PCR were obtained from Takara Bio Inc. (Kyoto, Japan). Reagents for long-range PCR were purchased from Roche Applied Science (Mannheim, Germany) and all primers were synthesized by Sangong Biological Engineering & Technology Services Co. Ltd. (Shanghai, China). The E.Z.N.A.^{TM} Gel Extraction Kit was obtained from Omega Bio-Tek Inc. (Norcross, GA, USA).

Subjects

AHH-1 human lymphoblastoid cells were used to screen for mtDNA deletions. The cell line was cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Invitrogen) supplemented with 15% heat-inactivated fetal bovine serum (Invitrogen), 100 U/mL of penicillin and 100 μg/mL of streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

Three peripheral blood samples were collected to validate the radiation-induced mtDNA deletions. This work was conducted at the National Institute for Radiological Protection (NIRP), Chinese Center for Disease Control and Prevention. The scope of the study was explained to each subject and written informed consents were obtained. The Ethics Committee of the NIRP approved all experiments in the present study. All subjects were 20-30 years old, healthy, and did not have any history of chronic disease, substance abuse or toxic chemical exposure. No radiation exposure or viral infection during the months preceding the study was documented. Six-milliliter peripheral blood samples for each subject were collected and divided into three equal aliquots.

Blood donors at the Chinese PLA General Hospital were recruited to study the distribution of mtDNA deletions in a normal population. The eligibility of the 222 healthy adults (108 males and 114 females) aged 23-66 years, was evaluated using questionnaires and regular medical procedures. Once written informed consent was obtained, approximately 2 mL peripheral blood samples were collected from each subject by venipuncture into vacutainers containing lithium heparin (Becton Dickinson, Oxford, UK).

Irradiation

Exponentially growing AHH-1 cells were

irradiated with 0 Gy (sham-irradiation) or 10 Gy at room temperature using a Cobalt-60 γ-ray source, provided by the Second Standard Dosimetry Laboratory of the NIRP, Chinese Center for Disease Control and Prevention. The source radioactivity was 1.3×10^{14} Bq and the homogeneous irradiation field was 10 cm ×10 cm. The dose rate was 0.41 Gy/min. The cells were incubated at 37 °C for 24 h prior to collection for mtDNA extraction. Human peripheral blood samples were subjected to similar conditions of irradiation, although the absorbed doses varied between 0 Gy, 2 Gy, and 6 Gy. The irradiation tests were conducted in triplicate for the lymphoblastoid cells and blood samples.

Extraction of mtDNA

For peripheral blood samples, the red blood cells were discarded firstly. The white blood cells subjected to the following procedure similar to AHH-1 cells. The mtDNA was extracted as previously described[40]. Briefly, the cell pellet was resuspended on ice in buffer A (50 mmol/L glucose, 25 mmol/L Tris·HCl, 30 mmol/L EDTA $Na_2\cdot2H_2O$, pH 8.0), and mixed gently with fresh buffer B (0.2 mol/L NaOH and 1% SDS) and cold buffer C (3 mol/L KAC, 5 mol/L HAC, pH 5.4), then the cellular membrane and nuclear DNA were removed after centrifuge. The mtDNA was purified after mixed with RNase A (100 μg/mL) and proteinase K (20 mg/mL). The size and purity of mtDNA was determined by 1% (w/v) agarose gel electrophoresis, and the agarose gel analyzed using the Multi-image Light Cabinet and software (Alpha Innotech Corporation, San Leandro, CA, USA). The remaining DNA sample was stored at -20 °C.

Long-range PCR

Long-range PCR was conducted using the Expand Long Template PCR System (Roche Applied Science, Indianapolis, IN, USA). PCR reaction systems were prepared using Expand Long Template buffer 2 (27.5 mmol/L $MgCl_2$) according to the manufacturer's instructions. Two pairs of primers specific for the human mitochondrial genome sequence (http://www.mitomap.org) were designed (Table 1) using Primer Premier 5.0 software (PREMIER Biosoft International, Palo Alto, USA). The total length of their amplicons was longer than the human mitochondrial genome (Table 1). The PCR reactions were performed in thin-walled 0.5 mL tubes (Axygen Scientific, Union City, CA, USA). The amplification profile for mtDNA nt153-nt7958 in heavy chain consisted of an initial period of DNA denaturation at 93 °C for 2 min, followed by 10 cycles of 93 °C for 50 s, 54.9 °C for 50 s, and 68 °C for 8 min. An additional 20 cycles were then performed with the extension time for each cycle 20 s longer than for the previous cycles, with a final extension at 68 °C for 7 min. The amplification profile for mtDNA nt7617-nt399 in heavy chain were as for mtDNA nt153-nt7958 as stated above, with the exception that the annealing temperature was increased to 55.6 °C. The amplicons were electrophoresed in a 1.0% (w/v) agarose gel at 100 V for 30 min, stained with ethidium bromide (final concentration 0.5 μg/mL) and analyzed with the Multi-image Light Cabinet and software (Alpha Innotech Corporation, San Leandro, CA, USA).

Table 1. Primer Sets Used for PCR

Position in Heavy Chain		Sequence of Primers
nt153-nt7958	Forward	ATTATTTATCGCACCTACGTTCAATATT
	Reverse	GGGGAAGTATGTAGGAGTTGAAGATTAG
nt7617-nt399	Forward	ACGCTACTTCCCCTATCATAGAAGAGCT
	Reverse	AATTTGAAATCTGGTTAGGCTGGTG
nt3628-nt4061 (ND1 gene)	Forward	CCTCTAGCCTAGCCGTTTAC
	Reverse	GGGAGAGTGCGTCATA

Limited-condition PCR

Limited-condition PCR was carried out once the short fragment products were found in long-range PCR. The PCR reaction system included 50 ng of template mtDNA, 1 μL of each primer (20 μmol/L), 0.5 μL of each dNTP (2.5 mmol/L), 2.5 U of Taq DNA polymerase, and 5 μL of 10×reaction buffer. The amplification profile for mtDNA nt153-nt7958 primers included incubation at 93 °C for 2 min, followed by 40 cycles of 93 °C for 30 s, 54.9 °C for 30 s and 72 °C for 30 s, with a final extension at 72 °C for 7 min. The reaction for mtDNA nt7617-nt399 primers was as per nt153-nt7958 primers above, with the exception that the annealing temperature was increased to 55.6 °C. The amplification products were analyzed by electrophoresis in 1.3% (w/v) agarose gels and stained with ethidium bromide.

Cloning and Sequencing of PCR Products

The fragments resulting from possible deletions were excised from the agarose gel and purified with E.Z.N.A.™ Gel Extraction Kits (Omega), according to the manufacturer's instructions. The purified PCR products were ligated with pGEM-T Vector (Promega, Madison, WI, USA) and the ligation mixture transformed into DH5α competent cells. Positive

clones (white colonies) were isolated using blue/white color screening and standard ampicillin selection techniques. The positive clones were sent to Beijing SinoGenoMax Co., Ltd. (Beijing, China) for sequencing after bacterial enrichment. The sequence homology analysis was conducted with NCBI human nuclear and mitochondrial genome complete sequence on GeneBank using the Basic Local Alignment Search Tool (BLAST).

Real-time PCR

In the present study, the $2^{-\Delta\Delta Ct}$ method of relative quantification was adopted. Individual real-time PCR reactions were carried out in 20 μL volumes in a 96-well plate (Applied Biosystems™, USA) containing 0.4 μL of each forward and reverse primer (each 10 μmol/L) (Table 1), 2 μL mtDNA, 7.2 μL distilled water and 10 μL 2×SYBR Green I Mix (Applied Biosystems™). Real-time PCR was performed with the 7500 Fast Real-time PCR System (Applied Biosystems™), using the default thermocycler program for all genes. The reaction was incubated at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. At the end of each reaction, cycle threshold (Ct) was manually set up at the level that reflected the best kinetic PCR parameters, and melting curves were acquired and analyzed. The cumulative fluorescence of the mtDNA deletion products was normalized to that of the ND1 gene which was rarely deleted from the same sample (Table 1). A minimum of three experiments was carried out for each sample. Each experiment included individual samples in triplicate and the Ct of each well was recorded at the end of the reaction. The average and standard deviation (SD) of the three Cts was calculated.

Statistical Analysis

Statistical analysis was performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). One-way ANOVA was used to analyze the deletion level of different dose groups. The chi-square test was used to compare differences in the proportion of mtDNA deletions between different sex and age groups of normal adults. Statistical significance was defined as $P<0.05$.

RESULTS

Two Novel mtDNA Deletions were Detected in 10 Gy Cobalt-60 Gamma-ray Irradiated AHH-1 Cells

Long-range PCR products of two primer pairs in sham-irradiated AHH-1 cells were of length 7806-bp and 9352-bp. The amplicon of primers mtDNA nt153-nt7958 was approximately 8-kb (Figure 1A), while primers mtDNA nt7617-nt399 produced an amplicon greater than 8-kb in length (Figure 1B). In addition to the 7806-bp or 9352-bp amplicon, there was a new shorter band for each pair of primers in 10 Gy ^{60}Co γ-ray-irradiated AHH-1 cells (Figure 1C). For primers nt153-nt7958, there was an additional band between 230-bp and 396-bp. A band that shorter than 230-bp was detected in the amplicon of mtDNA nt7617-nt399 primers. The two shorter bands indicated the presence of two potential mtDNA deletions.

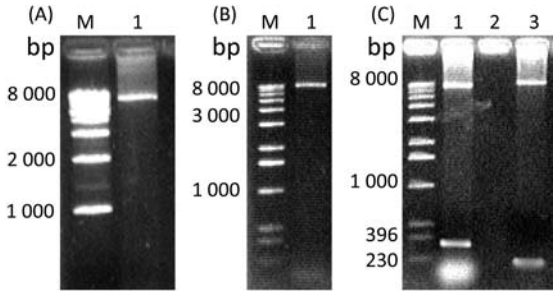

Figure 1. The long-range PCR amplification products of lymphoblastoid cell mtDNA following sham-irradiation and 10 Gy ^{60}Co γ-rays irradiation. A and B: The PCR product for sham-irradiated cells with two pairs of primers was as expected. Primers of mtDNA nt153-nt7958 yielded an amplicon less than 8-kb in length (A) while the amplicon for mtDNA nt7617-nt399 primers exceeded 8-kb (B); M: 1-kb DNA ladder. C: Long-range PCR products of irradiated cells with two pairs of primers. The mtDNA nt153-nt7958 primers generated two bands, one at about 8-kb and the other, a shorter band between 230 and 396-bp (Lane 1). The mtDNA nt7617-nt399 primers produced two bands, with the shorter band less than 230-bp (Lane 3); Negative control in Lane 2. M: 1-kb DNA ladder.

To validate the possible mtDNA deletions detected in long-range PCR, we used limited-condition PCR to analyze the mtDNA sample exposed to 10 Gy ^{60}Co γ-rays. The primers were same as used for long-range PCR, and the PCR reaction system included common PCR buffer and Taq DNA polymerase. The amplification profiles differed from that for long-range PCR, with a shorter extension

time ensuring that the length of the PCR products were no greater than 5-kb. The result (Figure 2A) demonstrated that the size of the amplicon for the nt153-nt7958 primer pair was between 300-bp and 400-bp, and that of the nt7617-nt399 primer pair was between 100-bp and 200-bp.

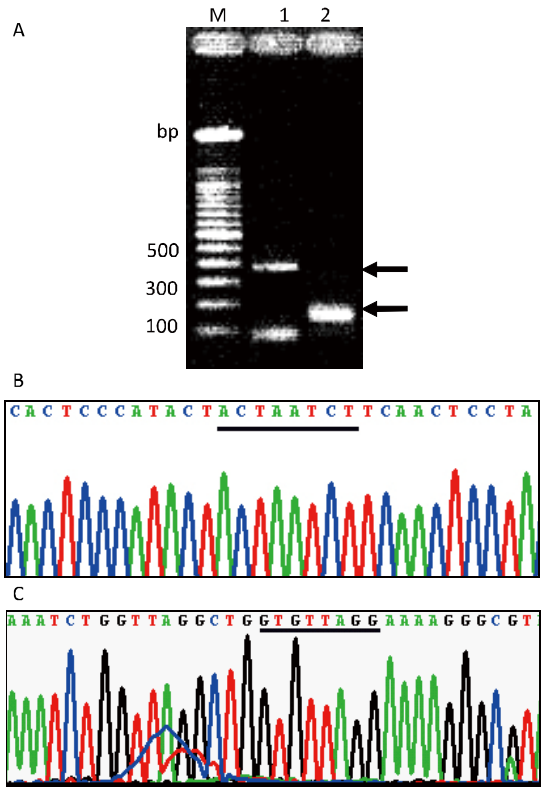

Figure 2. Limited-condition PCR amplification products of lymphoblastoid cell mtDNA following irradiation with 10 Gy ^{60}Co γ-rays and partial sequencing. A. PCR products of irradiated cells with two pairs of primers. PCR products with mtDNA153-7958 primers included two bands. The upper band was between 300 and 400-bp, and the lower band, below 100-bp, was shown to be a primer dimer (Lane 1). PCR product of mtDNA 7617-399 primers was between 100 and 200-bp (Lane 2). M: 100-bp ladder. B and C. Partial sequences of PCR products with two pairs of primers. Partial sequence of product of mtDNA 153-7958 primers showed the flanking 8-bp repetitive sequence in heavy chain: ACTAATCT (B). Partial sequence of PCR products with mtDNA 7617-399 primers indicated that another 8-bp repetitive sequence in light chain (GTGTTAGG) resulted in the 9225-bp deletion (C).

The PCR products of limited-condition PCR and the shorter bands of long-range PCR were purified, transferred to pGEM-T Vector, and then cloned and sequenced. The sequencing data was analyzed using BLAST analysis based on GenBank (GenBank #J01415.0 gi: 337188). It was discovered that the PCR product for the nt153-nt7958 primer pair was 351-bp, which indicated the deletion of 7455-bp. The 7455-bp deletion occurred between nt475 and nt7929 in human mtDNA heavy chain, and the nt475-482/ nt7930-7937 was an 8-bp direct repetitive sequence ACTAATCT (Figure 2B). The product of the nt7617-nt399 primer pair was 127-bp in length, which indicated the occurrence of a 9225-bp deletion. The 9225-bp deletion was between nt7714 and nt369 in human mtDNA heavy chain, resulting from nt7714-7721/nt370-377, which was an 8-bp direct repetitive sequence CCTAACAC (Figure 2C). It was revealed that the 7455-bp and 9225-bp deletions were both novel mtDNA deletions after a MITOMAP (http://www.mitomap.org) and NCBI library search.

Increased Deletion Levels in mtDNAs of Peripheral Blood Samples Following ^{60}Co Gamma Irradiation

We used three peripheral blood samples (A, B, and C) to validate ^{60}Co γ-ray induction of mtDNA deletions in AHH-1 cells. Both the 7455-bp and 9225-bp deletions were detected in all 0, 2, and 6 Gy irradiated peripheral blood samples. The mtDNA deletion levels were significantly enhanced after ^{60}Co γ-ray irradiation (P<0.05, Figure 3), with the mtDNA deletion levels in 6 Gy irradiated samples higher than those for 2 Gy (P<0.05). For each dose level, the relative 9225-bp deletion levels induced by radiation were higher than those for the corresponding 7455-bp deletion (P<0.05).

The different radiosensitivity as reflected in the induced mtDNA deletion levels among the three samples was also indicated for 2 and 6 Gy doses of ^{60}Co γ-ray irradiation. But only the 9225-bp mtDNA deletion levels at 6 Gy between A and B were significantly different (P<0.05).

Distribution of Two mtDNA Deletions in the Peripheral Blood of a Normal Population

To determine the proportion of 7455-bp and 9225-bp mtDNA deletions in normal adults and the underlying factors thereof, we analyzed 222 normal adults, aged between 23 and 66 years. The proportion of the two mtDNA deletions in a normal population were compared by sex and age (refer to Table 2 and Table 3). The 7455-bp mtDNA deletions

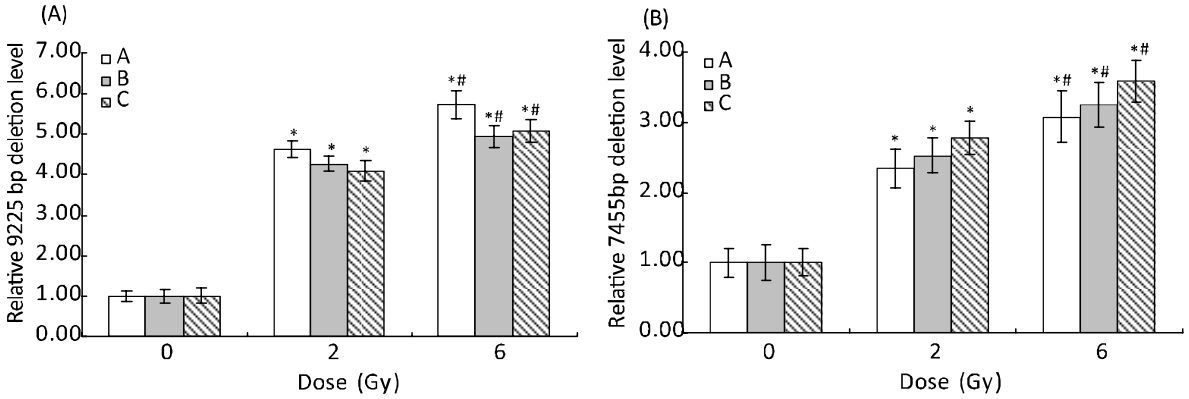

Figure 3. The mtDNA deletion levels for mtDNA 9225 and 7455-bp in three ^{60}Co γ-ray-irradiated peripheral blood samples. SYBR Green real-time PCR quantification of mtDNA 9225-bp deletion (A) and 7455-bp deletion (B) for these samples: both deletions exhibited a dose-dependent enhancement in response to ^{60}Co γ-rays. The deletion levels were normalized to that of the reference ND1, and shown relative to the deletion level in the sham-irradiated sample (designated as 1). The values represent the mean±SD of three independent experiments. *:*P*<0.01, compared with the corresponding 0 Gy. #:*P*<0.05, compared with the corresponding 2 Gy.

Table 2. Analysis of 7455-bp mtDNA Deletions in a Normal Population

Age Group (years)	Male			Female			Percent of Deletions in Both Genders
	No. with deletions	No. of analysis	Percent of deletion (%)	No. with deletions	No. of analysis	Percent of deletion (%)	
21-	2	21	9.5	2	20	10	9.8
31-	3	30	10	3	27	11.1	10.5
41-	4	25	16	5	30	16.6	16.4
51-	5	19	26.3	5	21	23.8	25
61-70	5	13	38.4	6	16	37.5	37.9
Total	19	108	17.6	21	114	18.3	18

Table 3. Analysis of the 9225-bp mtDNA Deletions in a Normal Population

Age Group (year)	Male			Female			Percent of Deletion in Both Gender
	No. with deletion	No. of analysis	Percent of deletion (%)	No. with deletion	No. of analysis	Percent of deletion (%)	
21-	1	21	4.8	1	20	5	4.9
31-	2	30	6.7	2	27	7.4	7
41-	4	25	16	4	30	15.4	14.5
51-	4	19	21.1	4	21	19	20
61-70	3	13	23.1	4	16	23.5	24.1
Total	14	108	13	15	114	13	8.6

occurring in 17.6% of males and 18.3% of females, did not differ significantly (χ^2 =0.02, ν=1, *P*>0.05). The proportion of subjects with a 7455-bp deletion for each age group was combined for the two genders, and the difference between age groups was found to be significant (χ^2 =13.26, ν=4, *P*<0.05). The relationship between the proportion of deletions and age fitted a linear quadratic model (y=0.019x^2-1.04x+23.43, R^2 =0.9994, in which y is the proportion of deletion, and x is the age).

The proportion of males and females with the 9225-bp mtDNA deletion was 13.0% each, and was

not considered significantly different (χ^2=0.002, ν=1, P>0.05). The combined proportion of individuals with a 9225-bp deletion for each age group was statistically significantly different (χ^2=37.93, ν=4, P<0.005). The relationship between the proportion of deletion and age was delineated with a linear quadratic model (y=0.0014x^2+0.39x-6.42, R^2=0.9795, in which y is the proportion of deletion, and x is the age).

DISCUSSION

In the current study two novel mtDNA deletions of 7455-bp and 9225-bp were detected using long-range PCR and limited-condition PCR. There are several methods which could be used to detect mtDNA deletions, including regular PCR[41], nested-PCR[42], long-range PCR[43], primer shift PCR[44-45], serial dilution PCR[46], kinetic PCR[47], competitive PCR[48], *in situ* PCR[30], and fluorescence *in situ* hybridization[49]. Generally, these PCR-based techniques are limited as the PCR primers are designed for a specific deletion and cannot be used to screen for unknown deletions. Long-range PCR, which generally requires several primer pairs to produce amplicons spanning the entire mtDNA genome, facilitates the detection of new mtDNA deletions[17,43]. The occurrence of deletions is determined by the size of the amplicons.

Two pairs of primers with the ability to perform amplifications greater than the length of the entire human mitochondrial genome (16569-bp) were used in this study. The results demonstrated the presence of two smaller sized products in irradiated cells, in addition to the expected 7.8-kb or 9.4-kb products. These results suggested the induction of large mtDNA deletions in irradiated cells. Limited-condition PCR was used to confirm the mtDNA deletions detected by long-range PCR. A reduction in extension times meant that the 7.8-kb or 9.4-kb products could not be amplified, and only amplicons less than 5-kb (indicative of large mtDNA deletions) could be detected. The PCR products were cloned and sequenced following purification. BLAST analysis was used to search the sequences, with the results confirming mtDNA deletions of 7455-bp (nt475-nt7929) and 9225-bp (nt7714-nt369). The present study identified two novel mtDNA deletions of length 7455-bp and 9225-bp. In addition, normal human peripheral blood samples[50] were used to validate the induction of these deletions by ionizing radiation. The combined methods of long-range PCR and limited-condition PCR as used in this study, may be efficient methods for the screening of novel mtDNA deletions in future studies.

More than 150 types of human mtDNA deletions have been identified (http://www.mitomap.org, 2004)[51]. Deletions can be formed by errors in the repair of double-strand breaks, replication slippage or other replication errors[52]. These mtDNA deletions vary in size and position in the heavy chains, with most deletions ranging between 1-kb and 8-kb, and only a few with several base pairs or exceeding 10-kb[53]. Most human mtDNA deletions are flanked by two homologous direct repeat sequences, one of which is eliminated in the deletion process. A few deletions are flanked by imperfect repeats while others are not associated with direct repeats[54-55]. The 7455-bp and 9225-bp deletions identified in this study resulted from 8-bp direct repetitive sequences, ACTAATCT and CCTAACAC, respectively.

To further validate the induction of mtDNA 7455-bp and 9225-bp deletions in human somatic cells by ionizing radiation, we measured the mtDNA deletion levels in ^{60}Co γ-ray-irradiated human peripheral blood cells using real-time PCR. The results showed that the 7455-bp and 9225-bp deletion levels in human peripheral blood cells were significantly elevated after irradiation with 2 and 6 Gy of ^{60}Co γ-rays. The findings confirm that ionizing radiation induces 7455-bp and 9225-bp deletions in human somatic cells. Furthermore, differences in mtDNA deletion levels among the three samples at each radiation dose level, suggest variations in radiation-induced mtDNA deletion levels in human blood cells. We also found that the radiation-induced levels of 9225-bp mtDNA deletion at 2 and 6 Gy were higher than those for the 7455-bp mtDNA deletion.

To investigate the distribution of the two mtDNA deletions in normal adults, the positive rate of 7455 bp or 9225 bp deletions was analyzed in the current study. The results demonstrated that the mtDNA deletions correlated with age and not gender[56-57], with the proportion of 7455-bp deletions at each age group higher than for the corresponding 9225 bp deletion. The 9225-bp mtDNA deletion has the ideal characteristics for potential use as a radiation biodosimeter and as such, will require further investigation in the near future.

In summary, the use of long-range PCR in combination with limited-condition PCR and sequencing techniques enabled two novel mtDNA

deletions of 7455-bp and 9225-bp flanked by an 8-bp direct repeat to be identified in irradiated human lymphoblastoid cells and peripheral blood lymphocytes.

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