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Repair of UV-induced Cyclobutane Pyrimidine Dimers in Human Mitochonrial DNA-less Cells

Takaji Ikushima*, Ning Gu and Yuichi Tanizaki
Laboratory of Molecular Genetics, Biology Division, Kyoto University of Education, Kyoto 612-8522, Japan

UV-induced DNA damage causes cell killing and mutations leading to carcinogenesis. In normal human cells, UV damage such as cyclobutane pyrimidine dimers (CPDs) and primidine-prymidone (6-4) photoproducts are mainly repaired by nucleotide excision repair mechanism. The molecular processes have been well characterized recently. To know the influence of mitochondrial genome on the nucleotide excision repair mechanism against CPDs, we comparatively examined the production of CPDs by UVC irradiation and their repair kinetics in human cells completely lacking mitochondrial DNA (mtDNA) and the parental HeLa S cells. Whole DNA extracted from the cells exposed to UVC was treated with T4-endonuclease V to break the phosphodiester bond adjacent to CPDs. The DNA was electrophoresed in a denaturing agarose gel, which was visualized by ethidium bromide staining. The relative amount of CPDs was determined by image analysis using NIH Image software. MtDNA- less (rho-0) cells were apparently more sensitive to UVC than HeLa S cells, while the level of induction of CPDs in rho-0 and HeLa cells was comparable. The repair of CPDs was less efficient in rho-0 cells compared with HeLa cells. The residual amount of CPDs after 24-h repair was larger in rho-0 cells than in HeLa cells where more than 90 % of CPDs were repaired by then. The non-repaired CPDs would lead to apoptosis in rho-0 cells. These results suggest that mitochondrial genome may contribute to some ATP-dependent steps in nucletide excision repair by supplying sufficient ATP which is generated through a respiratory chain in mitochondria.

Key words: UV, pyrimidine dimer, nucleotide excision repair, mitochondrial DNA, HeLa cells, rho-0 cells, ATP

INTRODUCTION

UV-induced DNA damage causes cell killing and mutations leading to carcinogenesis. In normal human cells, the nucleotide excision repair (NER) system is the principal repair pathway for UV-induced DNA damage such as cyclobutane pyrimidine dimers (CPDs), primidine-prymidone (6-4) photoproducts and other helix distorting DNA lesions. The molecular process of NER has been recently characterized well in human cells [1]. The three basic steps in the molecular mechanism of NER are damage recognition, excision, and repair synthesis. In humans, damage recognition and dual incision are accomplished by six repair factors including xeroderma pigmentosum A (XPA), the trimeric replication protein A (RPA), XPC, TFIIH (which contain XPB and XPD), XPG, and XPF-ERCC1 [2]. The XPG and XPF-ERCC1 nucleases incise at the 3' and 5' junctions of a DNA lesion, respectively and release a 24 to 32 nt-long repair patch. The resulting single-strnaded DNA gap is filled by DNA polymerase δ or ε, and ligated. It is also well established that many lesions that are recognized and repaired by the NER pathway are repaired in a transcription coupled and strand specific manner [3].

In the process of NER pathway, ATP-dependent steps are involved. Most of the total ATP requirement is supplied by mitochondrial oxidative phosphorylation. Here, we studied the influence of mitochondrial genome on NER and/or other repair system against UVC-induced CPDs. A comparative analysis of their repair kinetics was carried out between human cells completely lacking mitochondrial DNA (mtDNA) and its parental HeLa cells, employing CPD-specific T4-endonuclease V.

*To whom correspondence should be addressed.
E-mail: ikushima@kyokyo-u.ac.jp

MATERIALS AND METHODS

HeLa cells were grown in α-MEM supplemented with 10% fetal bovine serum and antibiotics. Rho-0 cells (mtDNA-less cells) were grown in α-MEM supplemented with 4% glucose, 50 μg/ml uridine and 100 μg/ml pyruvate in addition. Cell survival was determined by colonogenic assay. Cells were trypsinized and appropriate numbers were plated in Falcon plastic dishes. Prior to the exposure to UVC generated from a germicidal lamp, attached cells were rinsed with PBS. Visible colonies were stained and counted after one-week growth.

Cellular DNA was extracted from UVC-irradiated and non-irradiated control cells using SDS/proteinase K treatment followed by phenol/chloroform extraction. After treatment with ribonuclease A, DNA was suspended in TE buffer and the concentration was dtermined spectrophotometricaly. The treatment of DNA with T4 Endonuclease V (Endo V, Epicentre Tech.) was done for more than one hour at 37 °C according to the manufacture's instructions. The digestion products were electrophoresed in a denaturing 1% agarose gel which was stained with ethidium bromide and photographed under UV illumination. Photograph profiles were scanned by NIH Image ver. 1.6.1 and the relative amount of CPDs was estimated semi-quntitatively.

The lack of mtDNA in rho-0 cells was confirmed by PCR-amplification of D-loop region (281 bp) in mtDNA using a pair of specific 20-mer primers: TACTTGACCACCTGTACTAC (16140~16159) and TGATTTCACGGAGGATGGTG (16401~16420). PCR-amplified products were analyzed by agarose gel electrophoresis.

RESULTS AND DISCUSSION

Mitochondrial DNA was completely lost in rho-0 cells as shown in Fig. 1.

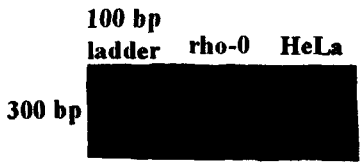

Fig. 1. Complete loss of mtDNA in rho-0 cells. PCR-amplified products were analyzed by agarose gel electrophoresis.

The survival curves of this mtDNA-less cells and the parental HeLa cells after UVC irradiation were given in Fig. 2. Rho-0 cells were about two to three-fold more sensitive to UVC than HeLa cells.

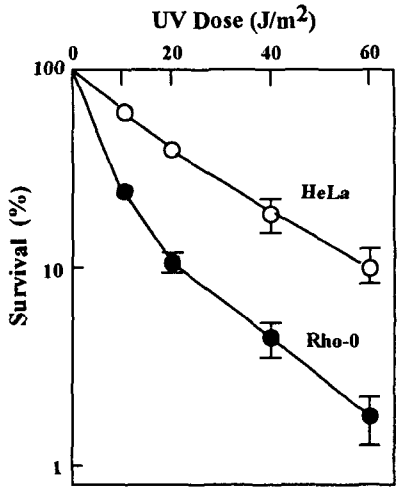

Fig. 2. UVC-sensitivity of rho-0 and HeLa cells.

The UVC-induced CPDs were detected by Endo V digestion of UVC-damaged DNA and subsequent alkaline agarose gel electrophoresis. Endo V binds CPD, cleaves the N-glycosidic bond of the 5'-pyrimidine of the dimer and the 3' phosphodiester bond, rersulting in breakage of the DNA strand [4]. Strand breakage is evident during subsequent electrophoresis. The initial yield of CPDs induced by UVC increased dose-dependently and was at the same level in rho-0 and HeLa cells (data not shown). After irradiation with 30 J/m^2 UVC, rho-0 and HeLa cells were cultured for further 24 h to repair CPDs. The remaining CPDs were monitored by Endo V treatment and subsequent gel electrophoresis during this period. The results obtained in HeLa and rho-0 cells were shown in Fig. 3 and 4, respectively.

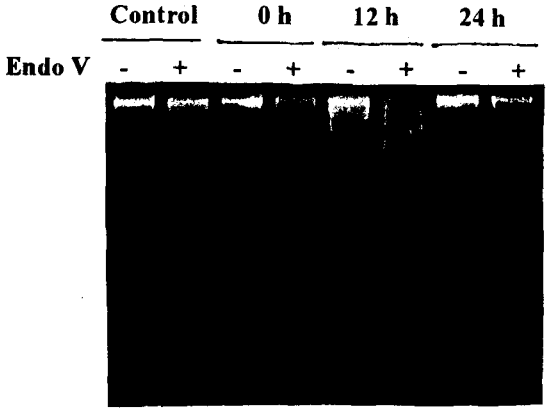

Fig. 3. Repair kinetics of UVC-induced CPDs in HeLa cells.

It was shown that there was extensive Endo V

degradation of the UV-exposed DNA immediately after irradiation, but very little damage to the control cells. With increasing repair time, the degraded DNA was remarkably recovered to the intact size in HeLa cells, indicating the speedy removal of CPDs from UV-damaged DNA strands (Fig. 3).

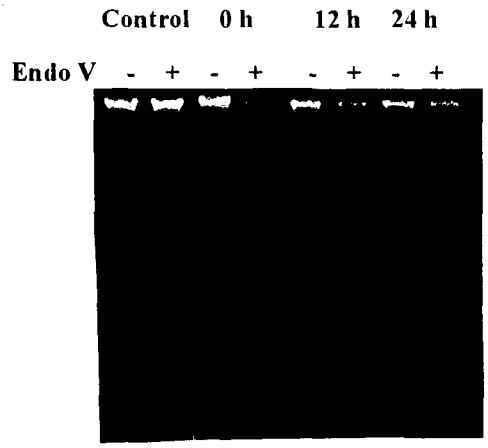

Fig. 4. Repair kinetics of UVC-induced CPDs in rho-0 cells.

On the contrary, a slow recovery in the DNA size was observed in rho-0 cells (Fig. 4). Without Endo V treatment, the UV-damaged DNA showed the degraded pattern after gel electrophoresis, reflecting the incision of DNA strands by exinucleases in the NER process (Fig. 3 and 4) [1]. As shown in Fig. 5, the repair of UVC-induced CPDs was slower or less efficient in rho-0 cells lacking mtDNA, compared with its parental HeLa cells.

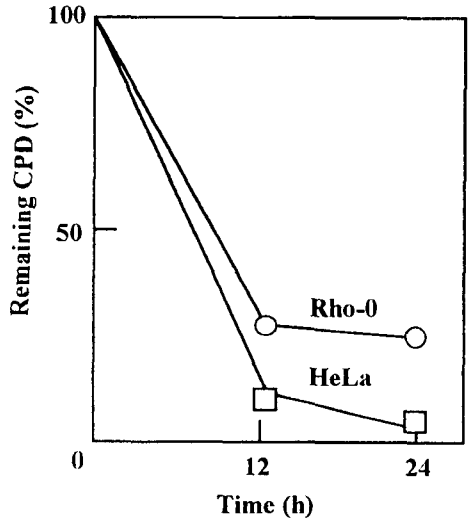

Fig. 5. Repair kinetics of CPDs induced by 30 J/m^2 UVC in HeLa and rho-0 cells.

It has been reported that the yield of CPDs in mammalian cells exposed to UVC is 22/Mbp/j·m^{-2} [5]. Using this value, the number of CPDs per cell can be estimated to be 6.6x10^4 after 30 J/m^2 in human (with genome size of 3000 Mbp). The results obtained in this study indicate that the complete loss of mtDNA results in less efficient NER of UVC-induced CPDs, leading to a high cell killing sensitivity to UVC. An abundant ATP supply may be required for efficient excision repair of UVC-induced CPDs, which is essentially achieved by the integrity of mitochondrial genome[6]. Our experiments also demonstrated that Endo V can be used to detect and quantify UV damage to DNA in human cells, based upon its ability to break the phosphodiester bond adjacent to dimers.

REFERENCES

1. Bessho, T. and A. Sancar (1998) Nucleotide excision repair in man. *Nucl. Acids. Mol. Biol.* 12, 1141-155.

2. Mu, D., D. S. Hsu and A. Sancar (1996) Reaction mechanism of human DNA repair excision nuclease. *J. Biol. Chem.* 271, 8285-8294.

3. Hanawalt, P. C. (1994) Transcription-coupled repair and human disease. *Science* 266, 1457-1458.

4. Friedberg, E. C., G. C. Walker and W. Siede (1995) DNA Repair and Mutagenesis, ASM Press, Washigton D.C.

5. Perdiz, D., P. Grof, M. Meszina, O. Nikaido and E. Noustacchi (2000) Distribution and repair of bipyrimidine photoproducts in solar UV-irradiated mammalian cells. *J. Biol. Chem.* 275, 26732-26742.

6. Garcia, J. J., I. Ogilvie, B. H. Robinson and R. A. Capaldi (2000) Structure, functioning and assembly of the ATP synthase in cells from patients with the T8993G mitochondrial DNA mutation. *J. Biol. Chem.* 275, 11075-11081.