<Original Article>

Decreased Ionizong Radiation-induced DNA Damage Repair Function of Cultured Fibroblasts Derived from Patients with Xeroderma Pigmentosum Variant

Toshihiro OTSUKA[1], Asako J. NAKAMURA[2], and Shinichi MORIWAKI[1]

1 Department of Dermatology, Division of Medicine for Function and Morphology of Sensory Organs, Faculty of Medicine, Osaka Medical and Pharmaceutical University, Takatsuki, Osaka 569–8686, Japan
2 Department of Biological Sciences, College of Science, Ibaraki University, Mito, Ibaraki 310–8512, Japan

Key words: DNA repair, double-strand break, γ-H2AX, ionizing radiation, xeroderma pigmentosum variant

ABSTRACT

Although it is obvious that patients with xeroderma pigmentosum (XP) have a deficient ability to repair ultraviolet radiation-induced DNA damage, the ionizing radiation (IR)-induced DNA damage repair function has not been elucidated. We sought to evaluate the post-IR DNA damage repair function in XP variant (XP-V) with deficient DNA polymerase η, which is possibly effect to the efficient DNA damage repair responses.

We assessed the post-IR DNA repair ability in skin fibroblasts derived from 11 XP-V patients using immunofluorescence staining with an antibody to phosphorylated H2AX, which is a marker of DNA double-strand break. The focus formation of XP-V cells after radiation was compared to that of normal and ataxia telangiectasia cells. The post-IR DNA damage, in any group, was most prominently determined in the 30 minutes after radiation exposure and recovered over the next four hours. Post-IR DNA damage repair occurred immediately after radiation exposure in XP-V cells as it did in normal cells; however, the rate of DNA repair was decreased in all XP-V cells.

These results suggest that the DNA damage repair function of XP-V patients was impaired after IR exposure. To further analyze their radiation-induced DNA damage repair function is important.

INTRODUCTION

Xeroderma pigmentosum (XP) is an autosomal recessive, intractable genodermatosis characterized by extraordinary sensitivity to ultraviolet (UV) radiation, resulting in a very high frequency of cutaneous malignant tumors on sun-exposed areas. There are eight-genetically different groups, XP A–G with deficient nucleotide excision repair (NER) and a

Address correspondence to:
Toshihiro Otsuka, M.D., Department of Dermatology, Division of Medicine for Function and Morphology of Sensory Organs, Faculty of Medicine, Osaka Medical and Pharmaceutical University, 2–7 Daigakumachi, Takatsuki, Osaka 569–8686, Japan
Phone: +81−72−683−1221 (ex.3558) E-mail: toshihiro.otsuka@ompu.ac.jp

variant form (XP-V) with deficient translesion synthesis (TLS) due to a mutation of DNA polymerase η [1]. Among them, XP-A and XP-C are the most common in Japan and in Western countries, respectively. XP-V is the second most common group in Japan (frequency: XP-A, approximately 55 %; XP-V, approximately 25 %) and patients with XP-V live longer because their condition is not complicated by neurological symptoms, unlike XP-A cases [2].

Previous studies assessed the disability of the UV-induced DNA damage repair function using fibroblasts derived from XP patients. In contrast to UV radiation, there have been a few reports describing the response to ionizing radiation (IR) in XP cells and radiosensitivity in XP is still controversial.

Arlett and Harcourt analyzed 6 XP cell strains together with 2 other NER-deficient photosensitive genodermatoses: Cockayne's syndrome (CS) cell strains showed normal radiosensitivity, indicating an absence of cross-sensitivity between ultraviolet light and gamma-irradiation [3]. However, Arlett et al. reported that XP14BR cells, an XP-C cell strain, show defective repair of IR-induced double strand breaks (DSB), which was restored after the transfection of XPC cDNA to the cells [4]. Their group also examined the post-IR survival of 33 XP cell strains and compared cell survival to that in 53 normal fibroblast lines, 7 CS cell strains and 4 XP/CS cell strains. They concluded that CS cells—but not XP or XP/CS cells—tended to show slight hypersensitivity to IR [5]. Mogi et al. reported that polymerase η, which is responsible for the pathogenesis of XP-V, reduced the H2AX response to psoralen plus UVA-induced crosslinks [6]. X-ray inspection is often used for diagnostic purposes and radiation therapy is often performed to treat skin cancer patients, and may be performed for XP-V cases; however, the radiation-induced DNA damage repair function in XP cases has not been elucidated. Therefore, we questioned whether skin cancer in XP-V patients can be safely treated by radiation therapy and sought to evaluate the post-IR DNA damage repair function of cultured fibroblasts derived from XP-V patients with deficient translesion DNA synthesis. In the present study, we evaluated the radiation-induced DNA DSB level and its repair kinetics in 11 XP-V patients by immunofluorescence staining with an antibody to γ-H2AX, a highly sensitive marker of DSB [7,8].

MATERIALS and METHODS

Eleven cell strains, derived from Japanese patients diagnosed with XP-V were analyzed in this study. The average age of the patients was 54.4 years (16–82 years) (**Table 1**). We also used normal primary fibroblasts (N-24, N-25) established in our laboratory. In addition, we newly purchased a primary fibroblast strain from an ataxia telangiectasia (AT) patient (AT2KY) with deficient DSB repair from the Japanese Cancer Research Resources Bank (JCRB) Cell Bank (Osaka, Japan). The number of passages in each of the cells used was about 5–10 times at the time of the experiment. All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with penicillin G/streptomycin and 10 % fetal bovine serum (FBS). A soft X-ray generator M-150WE (SOFTEX, Tokyo, Japan) was used for cell irradiation (Dose rate: 4.30 Gy/min)

We assessed the post-irradiation DNA damage repair function in skin fibroblasts derived from XP-V patients and compared it to that in normal, and AT cells using immunofluorescence staining with an antibody to phosphorylated H2AX (γ-H2AX), as described previously [7]. Briefly, 2×10^5 cells were plated in a 4-well chamber (Thermo Fisher Scientific KK, Tokyo, Japan), followed by treatment with 1 Gy irradiation. Before and 30 minutes to 4 hours after irradiation, the cells were fixed with 2 % paraformaldehyde at each point. After blocking with bovine serum albumin (BSA), the cells fixed over time were reacted with the primary antibody (Anti-phospho-Histone H2A.X (Ser139) Antibody) (05-636: Merck Millipore, Tokyo Japan), and then reacted with fluorescently labeled secondary antibody (Alexa Fluor® 488-Affini Pure Fab Fragment Goat Anti-Mouse IgG (H + L)) (Thermo Fisher Scientific KK, Tokyo, Japan). The specimen is completed by reacting with Ribonuclease A to separate it from DNA, and then mounted by a Vector shield containing a nuclear stain (propidium iodide [PI]) to prevent fading of the fluorescent stain. Finally, we examined the focus formation per field as a marker of the function of DSB after IR exposure in each specimen under a laser microscope ((LSM500 META, Carl Zeiss, Tokyo, Japan) & (TCS SP8, Leica, Tokyo, Japan)), by counting the number of foci as labeled DNA damage sites. γ-H2AX foci were counted by eye in a blinded fashion in 100 randomly chosen cells. This study was approved by the Medical Ethics Committee of Osaka Medical College and was conducted in accordance with the principles of the Declara-

Table 1
The XP-V patients in the present study. The patients included 9 men and 2 women who were 16 to 82 years of age at the diagnosis of XP-V.
n.d.; not determined

	Age	Sex	*XPV* gene mutation
XP-V1	39	F	C725G/A1766C
XP-V2	82	M	G490T (homo)
XP-V3	69	M	G916T (homo)
XP-V4	67	M	G490T (homo)
XP-V5	45	M	del 1661A/n.d.
XP-V6	53	M	C725G (homo)
XP-V7	68	M	C1066T (homo)
XP-V8	31	F	G490T (homo)
XP-V9	16	M	C907T/C1643A
XP-V10	54	M	G490T (homo)
XP-V11	74	M	G490T (homo)

tion of Helsinki.

Differences in the number of γ-H2AX foci were analyzed by Student's *t*-test. Fisher's exact test was applied to analyze significant differences in γ-H2AX responses.

RESULTS

The focus formation of XP-V cells after IR was compared with that of normal and AT cells. In all groups, the number of foci, showing post-irradiation DNA damage most prominently appeared at 30 minutes after IR treatment (**Figure 1**) and gradually decreased over the next 4 hours, which revealed the gradual repair of post-IR DNA damage (**Figure 2**). The repair rate in normal cells within 4 hours is faster than that in AT cells; however, the repair rate in XP-V cells varies among XP-V cell strains and the changes of the level of post-irradiation γ-H2AX focus formation of each XP-V cell strain were generally indicated to be close to those of normal cells or between normal cells and AT cells.

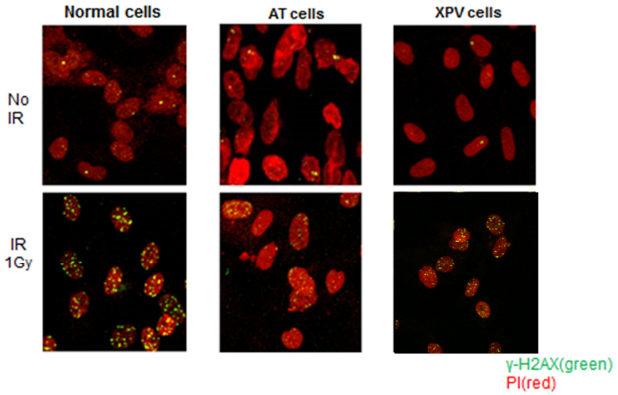

Figure 1
Representative images of DSB focus formation in normal cells (N-24), AT cells (AT2KY), and XP-V cells (XP-V3). DSB focus formation was evaluated 30 minutes after 1 Gy IR treatment.

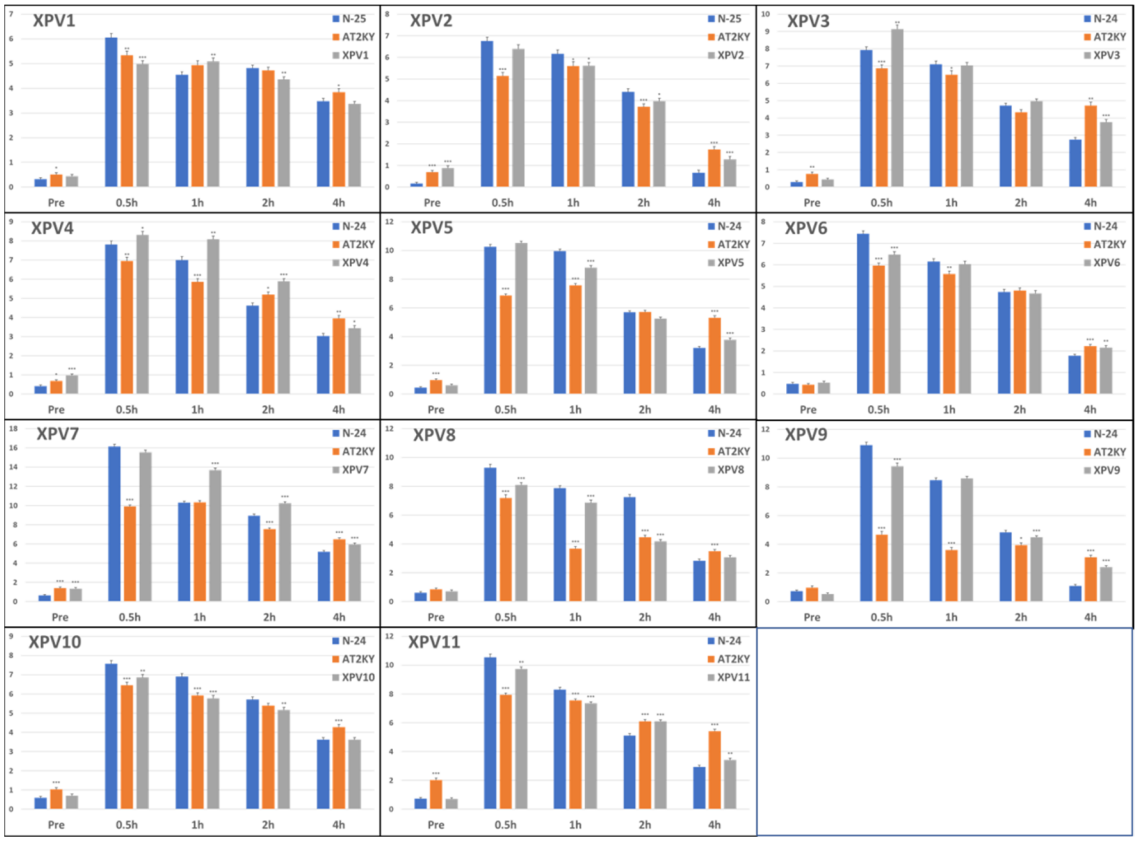

Figure 2
Changes in DSBs in each cell (normal cells, AT cells, and XP-variant group patient-derived cells) over time up to 4 hours after irradiation (1 Gy).
Vertical axis: γ-H2AX foci per cell, Horizontal axis: Time after irradiation.
Error bars signify standard error.
Asterisk indicate statistically significant difference by *t*-test: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Table 2

The DSB repair function in each XP-V or AT cell strain. The DSB repair function was evaluated as follows: First, the number of foci per cell in pre-irradiation normal (N-24, N-25), XP-V, or AT (AT2KY) cells was subtracted from the number of foci in cells after IR (0.5Hr-4Hr) in each experiment (**Figure 2**). Then, the difference between the resultant number after 0.5 Hr (pre-repair DSB foci) and that after 4 Hr (post-repair DSB foci) for each strain was obtained, and the difference in XP-V or AT cells was divided by that in normal cells. The ratio of < 1 indicates that the DSB repair ability of XP-V or AT cells is impaired in comparison with that of normal cells.

	XP-V1	XP-V2	XP-V3	XP-V4	XP-V5	XP-V6	XP-V7	XP-V8	XP-V9	XP-V10	XP-V11	Average
XP-V / Normal	0.82	1	0.91	1.03	0.95	0.89	0.95	0.91	0.82	0.93	0.9	0.92±0.02
AT / Normal	0.69	0.85	0.52	0.74	0.48	0.83	0.61	0.78	0.54	0.71	0.55	0.66±0.04

To evaluate the DNA repair function, the number of foci after 4 hours was subtracted from the number of foci after 30 minutes of IR irradiation in XP-V and AT cells, and the ratio to the value in normal cells was calculated. The average ratio was 0.92 (XP-V) and 0.66 (AT) (**Table 2**), and the relative levels of DSB repair in XP-V cells to normal cells were significantly lower (ratio: < 1) in 9 of 11 XP-V cell strains (a ratio value close to 1 indicates that the cells have the same DNA repair function as normal cells). In contrast, there was variability in the level of post-IR foci formation in AT cells among each experiment using DSB repair-deficient control cells; however, the ratio of focus formation in AT cells to normal cells, showed a much greater decrease in comparison to XP-V cells.

DISCUSSION

In the present study, we assessed the post-IR DNA damage repair function using primary skin fibroblasts derived from Japanese XP-V patients by immunofluorescence staining with anti-γ-H2AX antibody, a highly sensitive marker of IR-induced DNA damage; DSB. Our findings suggest that the repair function of DNA damage of the XP-V patients was decreased not only after UV irradiation but also after IR exposure. In addition, the post-irradiation DNA damage repair occurred immediately after radiation in normal and XP-V cells, but the rate of post-IR DNA damage repair in XP-V cells was not fully sufficient in comparison to normal cells. However, the abnormal pattern in XP-V cells was not as severe as that in AT cells, which are known to be unusually hypersensitive to IR due to *ATM* gene mutation, resulting in an impaired cell cycle checkpoint kinase. Looking at 11 XP-V patients, there was diversity in the DSB repair function, which may not have been related to the severity of the clinical symptoms of the XP-V and *XP-V* genotype.

NER, which is commonly abnormal in XP group A–G, is normal in XP-V patients with abnormal TLS due to DNA polymerase η deficiency, which is possibly related to the γ-H2AX response to DSB [6]. The mechanism of decreasing the repair capacity of XP-V cells after IR is unknown, but we imply the possible involvement of polymerase η in part of the cell cycle checkpoint pathways. The UV sensitivity of XP-V cells is often enhanced by a low caffeine concentration. The increased lethal effect of caffeine to UV is known to be due to the inhibition of caffeine-sensitive recombinant repair process, which results in interference in the intra-S phase and G2 DNA damage checkpoint [9,10,11]. TLS is a conserved function in which DNA polymerases are involved and DNA polymerase η is responsible for error-free DNA repair of UV-induced DNA damage, such as cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts [12,13]. DNA polymerase η is also important for the tolerance of oxidative stress-induced DNA damage, including 8-oxoguanine and thymine glycol and other genotoxic agents [10]. In addition, DNA polymerase η plays a partial role in homologous recombination other than TLS [11]. Our results imply that the homologous recombination system may be disabled in XP-V cells.

The present study was associated with a limitation in that the number of the XP-V patients in this study was relatively low, and further studies are needed to explore the relationship between the DSB repair ability and the clinical severity of XP-V as well as the responsiveness of skin cancers in XP-V patients to radiation therapy.

We first revealed the decreased DSB repair ability in XP-V by immunofluorescence staining with anti-γ-H2AX antibody, a highly sensitive marker of DSB. We observed that the post-irradiation DNA damage repair function is slightly lower in XP-V cells and that the cells from XP-V patients seem to be highly radiosensitive. In the future, the biological effects of radiation in XP-V cells should be investigated in greater detail and the safety of using X-rays when XP-V patients are managed in the clinical setting should be assessed.

Because guidance on radiation use for XP patients has not been established [2], it is important to analyze their radiation-induced DNA damage repair function. Moreover, the changes in the skin condition after examinations and therapies using IR should be checked in XP patients, especially in re-

lation to the management of XP-V patients in daily practice because XP-V is a cutaneous type of XP with a relatively long-lived phenotype [2].

REFERENCES

1. Moriwaki S. Human DNA repair disorders in dermatology: A historical perspective, current concepts and new insight. J Derm Sci. 2016;81:77–84.
2. Moriwaki S, Kanda F, Hayashi M, Yamashita D, Sakai Y, Nishigori C. Xeroderma pigmentosum clinical guideline. J Dermatol. 2017;44:1087–96.
3. Arlett CF, Harcourt SA. Survey of radiosensitivity in a variety of human cell strains. Cancer Res. 1980;40:926–32.
4. Arlett CF, Plowman PN, Rogers PB, Parris CN, Abbaszadeh F, Green MH, McMillan TJ, Bush C, Foray N, Lehmann AR. Clinical and cellular ionizing radiation sensitivity in a patient with xeroderma pigmentosum. Br J Radiol. 2006;79:510–7.
5. Arlett CF, Green MH, Rogers PB, Lehmann AR, Plowman PN. Minimal ionizing radiation sensitivity in a large cohort of xeroderma pigmentosum fibroblasts. Br J Radiol. 2008;81:51–8.
6. Mogi S, Butcher CE, Oh DH. DNA polymerase η reduces the γ-H2AX response to psoralen interstrand crosslinks in human cells. Exp Cell Res. 2008;341:887–95.
7. Nakamura AJ, Suzuki M, Redon CE, Kuwahara Y, Yamashiro H, Abe Y, Takahashi S, Fukuda T, Isogai E, Bonner WM, Fukumoto M. The causal relation between DNA damage induction in bovine lymphocytes and the Fukushima nuclear power plant accident. Radiat Res. 2017;185:630–6.
8. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem. 1998;273:58–68.
9. Osman F, McCready S. Differential effect of caffeine on DNA damage and replication cell cycle checkpoints in the fission yeast Schizosaccharomyces pombe. Mol Gen Genet. 1998;260:319–34.
10. Lerner LK, Francisco G, Soltys DT, Rocha CR, Quinet A, Vessoni AT, Castro LP, David TI, Bustos SO, Strauss BE, Gottifredi V, Stary A, Sarasin A, Chammas R, Menck CF. Lerner LK. Predominant role of DNA polymerase eta and p53-dependent translesion synthesis in the survival of ultraviolet-irradiated human cells. Nucleic Acids Res. 2017;45:1270–80.
11. Kawamoto T, Araki K, Sonoda E, Yamashita YM, Harada K, Kikuchi K, Masutani C, Hanaoka F, Nozaki K, Hashimoto N et al. Dual roles for DNA polymerase eta in homologous DNA recombination and translesion DNA synthesis. Mol Cell. 2005;20:793–9.
12. Tanioka M, Masaki T, Ono R, Nagano T, Otoshi-Honda E, Matsumura Y, Takigawa M, Inui H, Miyachi Y, Moriwaki S, Nishigori C. Molecular analysis of DNA polymerase eta gene in Japanese patients diagnosed as xeroderma pigmentosum variant type. J Invest Dermatol. 2007;127:1745–51.
13. Masaki T, Ono R, Nagano T, Funasaka Y, Tanioka M, Moriwaki S, Nishigori C. Four types of possible founder mutations are responsible for 87 % of Japanese patients with xeroderma pigmentosum variant type J Derm Sci. 2008;52:144–8.

Received August 19, 2021
Accepted September 17, 2021