Xeroderma Pigmentosum Diagnosis Using a Flow Cytometry-Based Nucleotide Excision Repair Assay

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org and at https://doi.org/10.1016/j.jid.2017.08.016.

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TO THE EDITOR

UVR induces dipyrimidine photoproducts such as cyclobutane pyrimidine dimer (CPD) and 6-4 pyrimidine–pyrimidone photoproduct (6-4PP), which cause distortions in the double helix (Lagerwerf et al., 2011). These dipyrimidine photoproducts are repaired through the nucleotide excision repair (NER) pathway. Xeroderma pigmentosum (XP) is a rare, autosomal recessive, hereditary disease characterized by hypersensitivity to sunlight and is associated with a high incidence of skin cancer; some patients also experience neurological symptoms. XP is classified into eight subclinical types: seven genetic complementation groups (A through G) deficient in an NER pathway and the XP variant (XP-V) type, which is deficient in translesion synthesis (DiGiovanna and Kraemer, 2012; Masutani et al., 1999). Because of NER or translesion synthesis deficiency, patients with XP cannot remove or overcome the dipyrimidine photoproducts. Therefore, to diagnose XP, the DNA repair ability and host cell reactivation have been assayed in clinical settings (Moriwaki and Kraemer, 2001). The unscheduled DNA synthesis (UDS) assay measures the cellular ability of NER (Lehmann et al., 1975). However, conventional

Abbreviations: 6-4PP, 6-4 pyrimidine-pyrimidone photoproduct; CPD, cyclobutane pyrimidine dimer; CS, Cockayne syndrome; NER, nucleotide excision repair; UDS, unscheduled DNA synthesis; XP, xeroderma pigmentosum; XP-V, xeroderma pigmentosum variant

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Table 1. Clinical features, UDS and 6-4PP removal, and cell cycle analysis in XP-V

Patient	Genotype	Age/Sex	Age of Onset			UDS (%)	6-4PP Removal (%)[1]			
			BCC	SCC	MM		Total	G1	S	G2
XPV23KO	c.349G>C	75/M	71	73	—	85.7	89.0 ± 10.4	93.1 ± 12.7	73.1 ± 6.1	89.5 ± 11.0
XPV24KO	c.490G>T	60/M	57	—	—	93.9	92.2 ± 5.5	94.8 ± 4.0	71.5 ± 10.6	91.2 ± 6.0
XPV25KO	c.490G>T	59/M	22	—	—	78.5	85.2 ± 4.5	88.9 ± 2.0	70.6 ± 10.0	89.4 ± 2.6
XPV4KO	c.725C>G	66/M	44	64	44	88	91.2 ± 1.5	92.3 ± 1.3	85.0 ± 2.9	91.2 ± 1.4
XPV6KO	c.916G>T	65/F	—	54	—	84.8	91.0 ± 0.4	94.4 ± 1.5	71.5 ± 8.6	92.7 ± 1.1
XPV14KO	c.1191delA	82/F	—	71	—	100.0	83.0 ± 6.6	90.9 ± 6.4	62.1 ± 7.0	89.0 ± 8.7
XPV13KO	c.1191delA	78/F	36	—	66	83.3	82.1 ± 5.6	88.4 ± 4.7	55.1 ± 6.2	85.7 ± 6.4
Normal-1	—	36/M	—	—	—	100	96.3 ± 5.0	97.1 ± 5.2	94.4 ± 6.3	94.2 ± 1.7
Normal-2	—	47/F	—	—	—	93.5	98.8 ± 3.1	95.9 ± 3.2	95.4 ± 2.3	96.7 ± 4.4

Abbreviations: 6-4PP, 6-4 pyrimidine −pyrimidone photoproduct; BCC, basal cell carcinoma; F, female; M, male; MM, malignant melanoma; SCC, squamous cell carcinoma; UDS, unscheduled DNA synthesis; XP, xeroderma pigmentosum; XP-V, xeroderma pigmentosum variant.
[1]Results shown are the average 6-4PP removal rates 6 h after UV irradiation in the whole cell cycle and in each phase of the cell cycle, with standard deviations.

UDS measurement can be time consuming and involves many complicated steps with autoradiography. Furthermore, the levels of UDS in XP-V cells are approximately normal, making XP-V diagnosis difficult (Tanioka et al., 2007). Recently, a flow cytometry-based NER assay was reported (Rouget et al., 2008). The same group reported that 6-4PP removal was specifically decreased during the S phase in patients with XP-V (Auclair et al., 2008). In this study, we assessed the clinical performance of the flow cytometry-based NER assay. We also developed a method to identify XP-V using cell cycle analysis using this new assay.

The Medical Ethics Committee of Kobe University approved this work, which was conducted in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all patients for participation in this study. Fibroblasts derived from healthy volunteers were irradiated with UVC and stained with anti-6-4PP and anti-CPD antibodies at several time points (Material and Methods are described in the Supplementary Materials online). The 6-4PP generated after UVC irradiation was detected using anti-6-4PP antibodies, and its signal intensity was analyzed by flow cytometry (see Supplementary Figure S1a and b online). In normal cells, 6-4PP was removed in a time-dependent manner; most of the 6-4PP was removed 6 hours after UVR irradiation (see Supplementary Figures S1a, c, and e). CPD removal was slower than 6-4PP removal; approximately 40% of CPDs were removed at 24 hours (see Supplementary Figures S2a and c). However, neither 6-4PP nor CPD was removed in XP-A cells (see Supplementary Figures S1b, d, and e and S2b and c). Based on these results, we examined 6-4PP removal at 6 hours and CPD removal at 24 hours after UVR exposure to measure the DNA repair ability in various DNA repair disorders, including XP and Cockayne syndrome (CS). As shown in Supplementary Table S1 online, 6-4PP was not removed in two XP-A cell strains with the Japanese founder mutation, IVS3-1G>C, in *XPA* (Nishigori et al., 1994). However, about 20% of 6-4PP was removed in cells derived from an XP-A patient with mild clinical manifestations. More than 90% of 6-4PP was removed in cells from patients with XP-V and CS and from normal cells. When CPD removal in normal cells was set as 100%, most cell strains showed similar CPD and 6-4PP removal levels. We compared the results of the flow cytometry-based NER assay with those of the conventional UDS measurement to validate the accuracy of the new method and its suitability as an alternative NER assay. Both 6-4PP and CPD removal measured by flow cytometry were strongly correlated with those measured by UDS. In particular, the R^2 value for 6-4PP removal by the two methods was 0.978 (see Supplementary Figure S3 online).

We examined 6-4PP removal in each phase of the cell cycle to evaluate whether a cell cycle-specific flow cytometry-based NER assay could diagnose XP-V. We examined patients with homozygous mutations in *POLH*. All patients had skin cancer and showed UDS levels that were approximately 80−100% of the normal control levels (Table 1). Results of 6-4PP removal in the whole cell cycle were similar to those of the UDS. However, 6-4PP removal rates at the S phase of the cell cycle were less than the total rate and those at the G1 and G2 phases in all patients, regardless of the genotype. In other XP complementation groups, no significant differences between cell cycle phases were observed (Figure 1).

Some nonradioactive methods for UDS such as BrdU-based (Hashimoto et al., 2009) and alkyne-conjugated thymidine analog 5-ethynyl-2′-deoxyuridine (i.e., EdU)-based UDS were reported as potential agents for UDS. For high-throughput measurement, a method using flow cytometry or automated plate scanners with a fluorescent microscope and charge-coupled device camera was proposed (Nakazawa et al., 2010); however, these methods require expensive equipment and, more importantly, could not evaluate cell cycle-specific DNA repair activity. In this study, we showed that the removal of UVR-induced DNA damage as measured by monoclonal antibodies using flow cytometry was well correlated with the results of conventional UDS measurements. The 6-4PP removal was particularly strongly correlated.

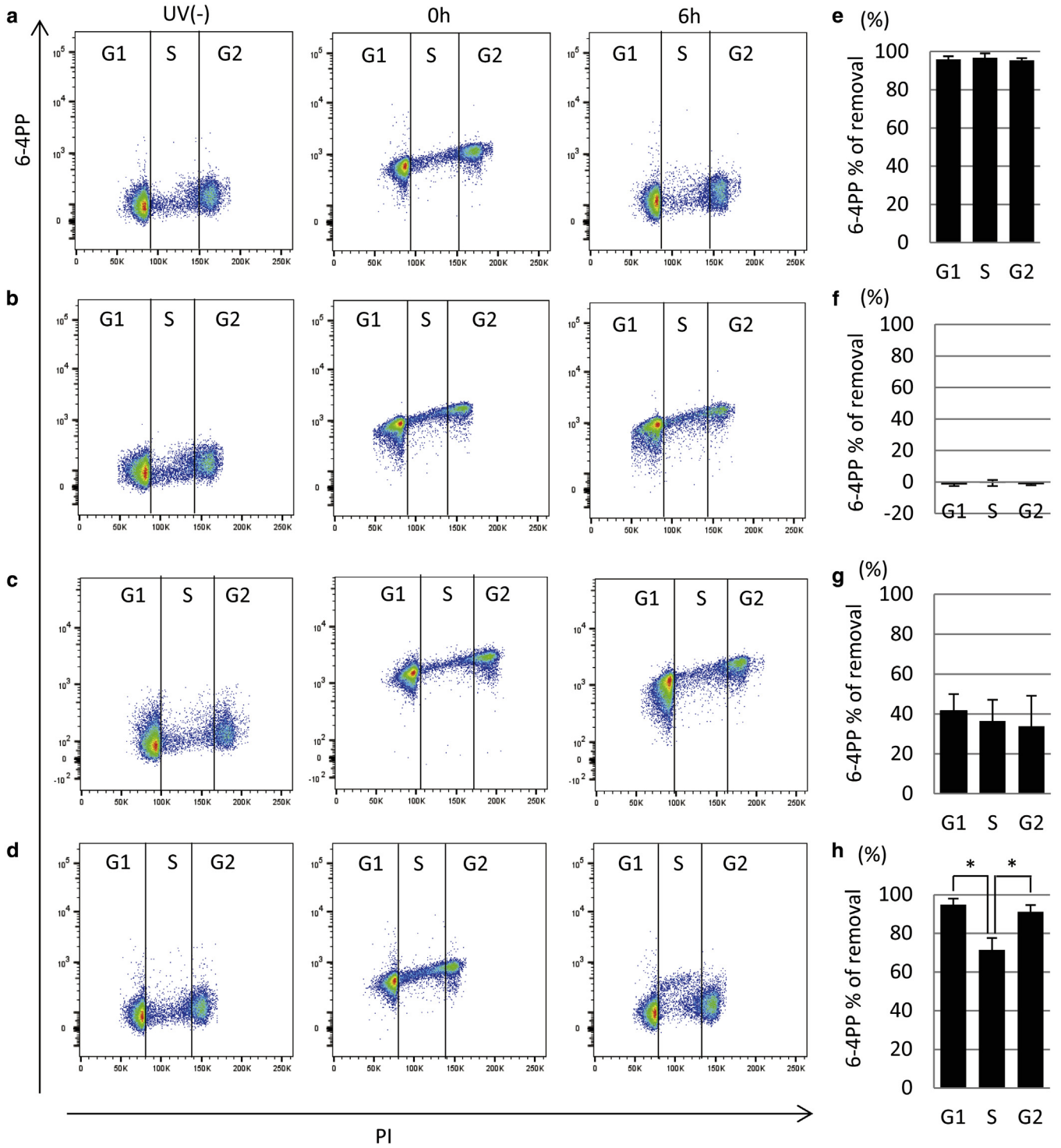

Figure 1. Removal of 6-4PP during the cell cycle in normal and XP cells. Representative bivariate dot plots of 6-4PP and DNA content in the G1-, S-, and G2-phases in (**a**) normal (normal-1), (**b**) XP-A (XP116KO), (**c**) XP-F (XP118KO), and (**d**) XP-V (XPV24KO) cells. Graphical representation of 6-4PP removal 6 h after UV irradiation in each cell cycle phase in (**e**) normal, (**f**) XP-A, (**g**) XP-F, and (**h**) XP-V cells. Values represent the mean ± SE of three or more independent experiments. *$P < 0.05$, analyzed by Student *t* test.

XP-V is caused by a POLH deficiency, and patients with XP-V are proficient in NER, rendering UDS measurement insufficient to diagnose XP-V. Although the repair of UVR-induced DNA damage in XP-V manifests as a strong attenuation, specifically in the S phase (Auclair et al., 2008), it is not known whether this attenuation occurs in patients with any mutations in *POLH*. Recently, it was reported that either ATR kinase or POLH deficiency caused profound NER inhibition, exclusively during the S phase. We examined NER activity in each phase of the cell cycle in NER-deficient XP and CS. POLH deficiency caused a decrease in NER activity during the S phase, regardless of the genotype. Our results suggested that the decreased NER activity during the S

phase might be indicative of XP-V, based on observations in patients clinically diagnosed with XP. XP-V cells are known to be hypersensitive to UVR in the presence of caffeine (Tanioka et al., 2007). Therefore, in the future, more sensitive assay using caffeine or more feasible methods using blood cells are expected to be developed.

Taken together, the flow cytometry-based NER assay using monoclonal antibodies to detect UVR-induced DNA damage can be an attractive alternative for UDS measurement. It is also useful for diagnosing XP-V through evaluation of 6-4PP removal in the S phase.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2017.08.046.

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