DNA Repair Characteristics of a Hybrid Cell Clone between Xeroderma Pigmentosum and *Potorous tridactilis*

KENJI IDA

Department of Experimental Radiology and
Department of Gynecology and Obstetrics
Faculty of Medicine, Kyoto University
Kyoto 606

(Received October 28, 1985)
(Revised version, accepted June 19, 1986)

**INTRODUCTION**

Mammalian cells may be divided into at least two groups with respect to DNA repair. One group is represented by human cells, in which excision repair is predominant; the other, such as in rodent cells, in which presumably postreplication DNA repair predominates. Cells of *Potorous tridactilis* have characteristics in between these two types. We fused xeroderma pigmentosum (XP) cells defective in excision repair and *Potorous* cells, and isolated a hybrid clone. The hybrid clone (PX1) showed repair characteristics intermediate between those of the parent cell lines. Detailed characteristics of the PX1 clone are presented in this paper with a discussion of the comparison of DNA repair in the two parental cell strains.

**MATERIALS AND METHODS**

1) Cells

XP20S(SV)neo, a fibroblast cell line derived from an XP patient belonging to complementation group A, transformed by SV40 virus, was made resistant to G418, a neomycin derivative.
compound, by transfection of pSV2neo\textsuperscript{3}). Pt K2 is an established kidney cell line of \textit{Potorous tridactilis}\textsuperscript{4}) obtained from Flow Laboratories (Rockville, MD, USA). VA13 (kindly supplied by Dr. S. Goto) and KTSH are normal human cell lines, the former being transformed by SV40.

Cells were cultured and maintained in α-modified minimum essential medium (α-MEM) (Irvine Science, Santa Ana, Cal. USA) supplemented with 15% fetal calf serum without antibiotics at 37°C in air containing 5% CO\textsubscript{2}.

2) Cell fusion

XP20S(SV)neo and Pt K2 lines, 3 x 10\textsuperscript{7} cells of each, were fused in 50% polyethylene glycol (PEG) 6000 (Koch-lite, England) with 10% DMSO (dimethyl sulphoxide). After cells were sedimented in a test tube, 0.5 ml of PEG solution was added and mixed thoroughly by forceful withdrawing and repipetting for 60 seconds. Then PEG was thoroughly washed out with the culture medium supplemented with 5% calf serum, and 6 x 10\textsuperscript{6} cells per 10 cm plastic dish were plated.

3) UV survival and Photoreactivation (PR)

The appropriate numbers of cells in the exponential growth phase were plated in 6 cm dishes and 15 h later irradiated with UV at a fluence rate of 0.5–1.0 J/m\textsuperscript{2}/sec. PR light exposure was carried out after UV irradiation as described above for appropriate lengths of time. After incubating for 13–21 days, colonies were fixed, stained with a Giemsa solution, and colonies with 50 cells or more were scored as survivors.

4) Measurement of unscheduled DNA synthesis (UDS)

Cells (1 x 10\textsuperscript{5}) were inoculated on a cover glass placed in a 2 cm dish and incubated for 18 h. After the medium was removed, cells were washed with phosphate buffered saline (PBS), and cells were irradiated with UV doses of 0, 10, 20 and 30 J/m\textsuperscript{2}. \textsuperscript{3}H-thymidine (10 µCi/ml, 24 Ci/mmole; Amersham) in culture medium was added, followed by incubation for 3 h. Cells were fixed with methanol and then washed with 5% trichloroacetic acid (TCA) at 4°C. Dried slides were dipped into NTB3 nuclear emulsion (Kodak, Rochester, NY, USA), exposed for 10 days at 4°C, developed and stained with Giemsa solution. The average number(s) of grains over lightly labelled cell nuclei, excluding S-phase cells, were scored\textsuperscript{5}).

5) Host cell reactivation (HCR) assay

Cell monolayers were prepared by inoculating 5 x 10\textsuperscript{5}–1 x 10\textsuperscript{6} cells/dish into 6 cm plastic dishes and incubated for 2–3 days. One ml Herpes simplex virus (HSV) type 1 suspension (approximately 10\textsuperscript{6} PFU/ml) was irradiated with UV in a plastic dish. After appropriate dilutions in PBS, 0.5 ml of virus suspension was added to the cell monolayer which had been pre-rinsed with PBS, and viruses were permitted to adsorb for 90 min. at 37°C in 5% CO\textsubscript{2} in air. After adsorption, 5 ml of fresh medium containing 0.25% human γ-globulin were added. On the 2nd or 3rd day, cells were fixed and stained for scoring the number of plaques\textsuperscript{2}).
6) Caffeine treatment

After inoculation and irradiation with UV light, cells were treated with 2.0 mM caffeine (Eastman, Rochester, NY, USA) for 72 h at 37°C, and then the cells were thoroughly washed with PBS. Cells were incubated for 14–20 days without medium change. Colonies were fixed, stained and scored as described above.

7) Chromosome analysis

Exponentially growing cells were treated for 3–8 h with colcemid (GIBCO, Grand Island, NY USA) at a final concentration of 0.05 μg/ml for XP20S(SV)neo and PX1, and 0.2 μg/ml for Pt K2. The harvested cells were treated with a hypotonic solution, of 0.075 M KC1, fixed with a acetic acid-methanol (1:3) solution, and stained in a Giemsa solution after trypsin treatment for G-banding.

8) DNA analysis

Cloning of Pt K2 repetitive DNA sequence on plasmid pBR322 was carried out by the "Shotgun method". After extraction, Pt K2 DNA was digested by EcoRI, ligated to pBR322 by DNA ligase, and transformed in E coli which were plated on an agar plate. By colony hybridization and cesium chloride centrifugation, 5 highly repetitive and 1 moderately repetitive Pt K2 DNA sequences were obtained. 7 μg each of DNA from XP20S(SV)neo, PX1, and Pt K2 were digested by one of the 3 restriction enzymes (EcoRI, HindIII, BamHI) and were searched for the presence of transferred genome by the Southern blot hybridization. Each repetitive sequence of Pt K2 DNA described above was digested with EcoRI and used as a probe. Sheared Pt K2 genomic DNA was also used as a probe. Each probe was labeled with 32P by nick translation procedure to a specific activity of 1–5 x 10⁸ cpm/ng.

RESULTS

1) Isolation of PX1 clone

XP20S(SV)neo and Pt K2 cells were fused as described in Materials and Methods. On the third day, 300 μg/ml of G418 was added to the medium to eliminate the unfused Pt K2 cells. On the 13th day, XP20S(SV)neo was killed by UV irradiation (2.5 J/m²). Since Pt K2 cells possess an efficient capacity for photoreactivation (PR), the cells were irradiated with visible light after UV so that hybrid clones retaining the capacity of PR might appear and could be rescued. The source of visible light was by five 6W fluorescent lamps filtered by window glass and vinyl film (kindly supplied by M. Ikenaga, Kyoto Univ.) to cut off wave lengths shorter than 390 nm. Illumination was for 1–4 h. During the exposure, Hapes buffer without phenol red supplemented with 15% FCS was used to avoid a photodynamic effect. For further selection of the hybrid cells, 5 J/m² UV irradiation and 1 h of PR treatment on the 17th day, and 5 J/m² UV irradiation and 2 h of PR treatment were done on the 31st day. Only one colony survived after the selection procedure on the 42nd day, and the clone was designated as PX1.
2) UV sensitivities and effects of caffeine

Survival curves for the two parent cell lines and the hybrid PX1 are shown in Figure 1. The $D_0$ value of PX1 was 2.5 J/m$^2$, which is 9 times as resistant as that of XP20S(SV)neo ($D_0$=0.27 J/m$^2$) and twice as sensitive as that of Pt K2 ($D_0$=4.7 J/m$^2$). SV40 transformed human fibroblast VA13 exhibited the same resistance as did Pt K2 ($D_0$=4.9 J/m$^2$).

With visible light illumination for 4 h, survival of Pt K2 cells increased from a $D_0$ value of 4.7 J/m$^2$ to 6.8 J/m$^2$. PX1 and XP20S(SV)neo showed no detectable PR with the same dose of PR light.

UV survival curves of cells treated with 2.0 mM caffeine for 72 hours after UV irradiation are also shown in Figure 1. The reductions of $D_0$ values after caffeine treatment are from 4.7 to 3.3 J/m$^2$ in Pt K2, from 2.5 to 1.7 J/m$^2$ in PX1, and from 0.27 to 0.09 J/m$^2$ in XP20S(SV)neo.

Relative caffeine enhancement was compared by taking the ratio of the $D_0$ value of UV survival without caffeine ($D_0$) to that with caffeine treatment ($D_0$ caf.).

$$\text{Caffeine Enhancement Ratio (CER)} = \frac{D_0}{D_0 \text{ caf.}}$$

CERs for Pt K2, PX1 and XP20S(SV)neo were 1.43, 1.47 and 3.03 respectively (Table 1). CER
Table 1. D₀ values of UV survival curves with and without caffeine treatment

<table>
<thead>
<tr>
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<th>D₀ (J/m²)</th>
<th>D₀ caf. (J/m²)</th>
<th>D₀/ D₀ caf.</th>
</tr>
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<tbody>
<tr>
<td>Pt K2</td>
<td>4.7</td>
<td>3.3</td>
<td>1.43</td>
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<tr>
<td>PX1</td>
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<td>1.7</td>
<td>1.47</td>
</tr>
<tr>
<td>XP20S(SV)neo</td>
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</tr>
<tr>
<td>VA13</td>
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<td>3.3</td>
<td>1.49</td>
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<td>KTSH</td>
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for VA13 amounts to 1.49, D₀ being 4.9 J/m² and D₀ caf. being 3.3 J/m².

3) Unscheduled DNA synthesis (UDS) after UV irradiation

UDS after UV irradiation in each cell clone is shown in Figure 2. XP20S(SV)neo showed practically no UDS, and the level of UDS in Pt K2 was the same as in normal human cells (KTSH). It is characteristic that PX1 showed an intermediate UDS value between those of Pt K2 and XP20S(SV)neo. VA13 cells showed the highest UDS level, presumably arising from the increased chromosome numbers due to SV40 transformation. The approximate ratio of UDS in (VA13, Pt K2, PX1 and XP20S(SV)neo) is 3:2:1:0.
4) Host cell reactivation (HCR)

The result of the HCR experiment reflecting the capacity of excision repair is shown in Figure 3. The PX1 curve lies intermediate between those of the two parent cell lines, Pt K2 and XP20S(SV)neo. This intermediate HCR activity of PX1 is consistent with the partial increase in clonogenic UV survival (Fig. 1) and UDS (Fig. 2).

5) Karyological studies

Histograms of the chromosome numbers in the 3 cell lines are shown in Figure 4. Karyotype of Pt K2 is mostly 14XY with a few cells with 13 and 15 chromosomes. Since XP20S(SV)neo is transformed by SV40 virus, as is the hybrid PX1, the karyotypes of XP20S(SV)neo and PX1 were so diverse with many chromosomes with structural changes that karyological analysis was difficult. The modal chromosome numbers of XP20S(SV)neo and PX1 were 70–74 and 80–84, respectively. Karyotype analysis showed that the majority of chromosomes of PX1 was identical with those of XP20S(SV)neo. In addition, PX1 cells had many unidentified chromosomes with characteristic morphology (Fig. 5), but no single Pt K2 chromosome was observed in PX1 chromosomes (Fig. 5). The possibility cannot be excluded that small fragments of Pt K2 chromosomes were translocated to the XP20S(SV)neo chromosomes. The
search for Pt K2 chromosomes and fragments using differential staining by the Giemsa-11 method and by the quinaquin staining method was not successful.

6) DNA analysis

The search for Pt K2 DNA in PX1 DNA was carried out by the Southern hybridization method. With the cloned Pt K2 repetitive sequences or with the whole genomic DNA of Pt K2 as probes, the presence of Pt K2 DNA was not evident in the PX1 DNA under the conditions of these experiments.

**DISCUSSION**

Our aim at the beginning of the experiment was to fuse the two kind of cells which both lack excision repair activity, namely XP20S(SV)neo and Pt K2, and to transfer the PR gene of Pt K2 so it might be expressed in the fusion cells. Though we failed in the attempt to induce the PR gene in the exc- human fibroblast, we detected excision repair capacity in the PX1 cells.
which led us to recheck the repair capacity of the Pt K2, the kidney fibroblast of the rat kangaroo. As is shown in Figure 2 (UDS) and Figure 3 (HCR), we found that Pt K2 which were reported to lack excision repair capacity nevertheless possess a considerable amount of excision repair capacity according to our results. Shaeffer et al. reported that rat kangaroo kidney line (RKK) exhibited neither UV nor X-ray induced UDS. Kato et al. also determined the UDS of kidney cell of rat kangaroo (Pt K1) and reported that the UDS after UV irradiation was 18.1% of human cells. The difference in UDS between their reports and this study may be attributable
to the strain differences.

Hybrid cells between XP and normal human or other mammalian cells with normal repair capacity have been reported to retain the normal repair capacity. Goldstein and Lin reported that hybrid cells between XP and hamster cells exhibited the normal UV survival level of hamster cells. Ganesan et al. found that hybrid cells between XP (group A) and mouse cells exhibited the normal UV survival level of the mouse cells. By introducing micronuclei from normal human cells to XP (group A) cells, the normal level of clonogenic UV survival was recovered.

In the present study, PX1, a hybrid cell line between group A XP20S(SV)neo and Pt K2, showed an intermediate level of repair capacity not only in clonogenic UV survival but also in UDS and HCR. Similarly, Shiomi et al. described a mouse intraspecies hybrid between a UV-4NQO resistant cell and a UV-4NQO sensitive mutant which exhibited an intermediate 4NQO sensitivity in colony forming ability, despite normal UV survival. Thompson et al. showed that a hybrid between CHO UV20, a UV sensitive mutant, and a group A XP cell strain (XP2CA) was somewhat less resistant to killing by UV than the normal CHO cells, but much more resistant than the parental CHO UV20. They observed the appearance of sensitive clones at relatively high frequencies in parallel to the loss of a human chromosomes responsible for the complementation.

Why does PX1 attain a level of DNA repair only intermediate to that of Pt K2? The possibilities are: 1) Pt K2 possesses two or more excision repair genes but only one of them is retained in PX1 while the others are lost, 2) all the repair genes exist in the PX1 cells but factors arising from interspecies hybridization suppress the expression of the repair genes, 3) PX1 may be a revertant.

Ganesan et al. and Royer-Porka and Haseltine reported the isolation of numerous UV resistant clones after DNA transfection of group A XP12RO(SV) cells and identified them as revertants. Although we failed to identify any single Pt K2 chromosome in the PX1 cells, the possibility of isolating a UV-resistant back mutant of XP20S(SV) is very unlikely, because XP20S(SV) that has been used in our laboratory since 1973 has never reverted following UV treatment. Isozyme patterns of esterase D, acid phosphatase, phosphoglucomutase (PGM), glucose-6-phosphate dehydrogenase (G6PD) and phosphogluconate dehydrogenase (PGD) of PX1 cells were consistent with those of XP20S(SV)neo (data not shown), and there were no indications of the presence of Pt K2 repetitive sequences in PX1. These results suggest that transfer of a small DNA segment may have occurred, although the possibility of reversion cannot be completely excluded.

ACKNOWLEDGEMENTS

The author is grateful to Drs. Hiraku Takebe, Masao S. Sasaki, Kanji Ishizaki, Ohtsura Niwa and Kouichi Tatsumi for their advice and discussions, and to Dr. Takahide Mori for providing opportunity to pursue this work. Help in experiments by Mr. Takenao Nagare and Mr. Minoru Miyazono is greatly appreciated. This work was supported by a Grant-in-Aid from Ministry of Education, Science and Culture.
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