Assignment of A Chinese Xeroderma Pigmentosum Patient from Taiwan to Complementation Group C

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A 2 years and 7 months-old Chinese boy with severe skin symptoms was diagnosed as xeroderma pigmentosum (XP) at Chang Gung Memorial Hospital in Taipei, Taiwan. Skin fibroblasts derived from the patient (patient identification number, XP1CTA) were used for genetic complementation analysis by the conventional cell-fusion technique followed by measurement of ultraviolet light (UV)-induced unscheduled DNA synthesis (UDS). The level of UDS in XP1CTA cells measured by autoradiography was about 20% of that in normal cells. When XP1CTA cells were fused with cells of a representative strain from each of the complementation groups A, D, E, F, G, and H, binuclear cells showed UDS levels in the range of normal cells, demonstrating a clear complementation between XP1CTA strain and either one of these strains. XP1CTA cells failed to complement with all the five reference strains belonging to group C. From these results, the XP1CTA was unambiguously assigned to complementation group C. Sensitivity of XP1CTA cells to UV, as measured by colony-forming ability, also fell within a range of variation in UV sensitivities of these group C XP cell strains.

INTRODUCTION

Xeroderma pigmentosum (XP) is an autosomal recessive human disease characterized

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by severe skin lesions in the areas exposed to sunlight and high frequency of skin cancers\textsuperscript{1,2).} The disorder comprises at least eight genetic complementation groups, A through H, and a variant form\textsuperscript{3).} In addition to the skin symptoms, most of group A and many of group D XP patients have progressive neurological abnormalities\textsuperscript{2,3).} Cells from patients belonging to groups A to H are defective in excision repair of ultraviolet (UV)-induced pyrimidine dimers in DNA, and are hypersensitive to various degrees to the killing effect of UV\textsuperscript{3,4).}

In Japan, about half of the excision-defective XP patients so far reported belong to group A\textsuperscript{5),} while in Europe and the USA, the group C XP patients are most common\textsuperscript{3).} This indicates that the distribution of XP complementation groups may be different between Oriental and Caucasian, as observed with various polymorphic genetic traits\textsuperscript{6).} Information concerning the complementation group assignment of XP patients in other Asian countries has been limited at present.

In the present study, we have made a genetic complementation analysis of an XP cell strain derived from an XP patient in Taiwan by cell-fusion techniques and assigned the strain to complementation group C.

**MATERIALS AND METHODS**

**Cell strains and culture conditions**

Fibroblast cultures were prepared from a skin biopsy taken from the Chinese XP patient, XP1CTA. The names, their characteristics and origins of fibroblast strains used as references for the complementation analysis are presented in Table 1.

All cells were grown in Dulbecco's modified minimum essential medium supplemented with 10\% fetal calf serum (Hyclone Lab., Logan, UT, USA) and antibiotics as described\textsuperscript{8,9).} Cultures were maintained at 37°C in an atmosphere of 10\% CO\textsubscript{2} in air.

**Complementation analysis and measurement of unscheduled DNA synthesis**

Two different methods were employed to assign the complementation group of XP1CTA cells, depending on the residual DNA repair activity of each reference XP strain, although the both methods were essentially the same in a sense that they involved cell fusion technique followed by measurement of UV-induced unscheduled DNA synthesis (UDS). For the complementation analysis with the reference strains having low UDS levels, such as those belonging to groups A, C, D, F and G, we used a conventional method as described previously\textsuperscript{7,8,18).} Briefly, $5 \times 10^4$ cells each of XP1CTA and reference cells were mixed, seeded on a coverslip with a culture medium, incubated overnight at 37°C, and then fused with 45\% polyethylene glycol (#6,000, Nacalai Tesque, Kyoto). About 36 hr after the treatment, the cells were irradiated with 20 J/m\textsuperscript{2} of 254 nm UV, incubated with culture medium containing 0.37 MBq/ml of [methyl\textsuperscript{3}H]thymidine (25 GBq/mM, Amersham Intern., Buckinghamshire, UK) at 37°C for 3 hr, and further incubated for 1 hr in medium containing 5 \mu g/ml of nonradioactive thymidine. Autoradiography was performed using Konica NR-M2 emulsion (Konica, Tokyo), and samples were exposed for 2 weeks in the dark at 4°C.
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Table 1. XP complementation groups and relative amounts of UDS of strains used in the present study

<table>
<thead>
<tr>
<th>Cell strains</th>
<th>Complementation group (references)</th>
<th>UDS level (% of normal cells)</th>
<th>Source of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>XP1CTA</td>
<td></td>
<td>18</td>
<td>Our cell stock</td>
</tr>
<tr>
<td>XP350S</td>
<td>A (7,8)</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Our cell stock</td>
</tr>
<tr>
<td>GM3176 (XP1AA)</td>
<td>C (9)</td>
<td>21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Dr. Kraemer</td>
</tr>
<tr>
<td>XP3BE (GM0030A)</td>
<td>C (2,4)</td>
<td>32</td>
<td>Camden Bank&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>XP8BE (GM0671)</td>
<td>C (2,4)</td>
<td>23</td>
<td>Dr. Kraemer</td>
</tr>
<tr>
<td>XP8CA (GM2996)</td>
<td>C (10)</td>
<td>18</td>
<td>Camden Bank&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>XP20MA</td>
<td>C&lt;sup&gt;e&lt;/sup&gt; (11,12)</td>
<td>19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Dr. Arase</td>
</tr>
<tr>
<td>XP7BE</td>
<td>D (2,4)</td>
<td>17</td>
<td>Dr. McCormick</td>
</tr>
<tr>
<td>XP24KO</td>
<td>E (13)</td>
<td>55</td>
<td>Dr. Fujiwara</td>
</tr>
<tr>
<td>XP101OS</td>
<td>F (14)</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Our cell stock</td>
</tr>
<tr>
<td>XP2BI</td>
<td>G (15)</td>
<td>4.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Dr. Fujiwara</td>
</tr>
<tr>
<td>GM3248</td>
<td>H (16)</td>
<td>22</td>
<td>Dr. Robbins</td>
</tr>
<tr>
<td>Normal (CHFU)</td>
<td>(17)</td>
<td>100</td>
<td>Our cell stock</td>
</tr>
</tbody>
</table>

<sup>a</sup>) The strain XP20MA was originally assigned to group I by Fischer et al. (1985)<sup>11</sup>, but recently it was reassigned to group C by Bootsma et al. (1989)<sup>12</sup>.

<sup>b</sup>) The data taken from Chang et al. (1989)<sup>9</sup>, and the rest of UDS levels were determined in the present study.

<sup>c</sup>) Human Genetic Mutant Repository, Camden, NJ, USA.

The numbers of grains per nucleus were counted in lightly labeled 50 binuclear cells (100 nuclei).

For the fusion of XP1CTA and groups E (XP24KO) and H (GM3248) strains which have higher residual UDS levels, the test and reference cytoplasms were separately labeled with different sizes of Latex beads (Sigma Chemical Co., St. Louis, MO, USA) for easy identification of heterodikaryons as described by Fujiwara et al<sup>13</sup>). One million cells each of XP1CTA and the reference strains were incubated for 24 hr with large beads (0.913 μm in diameter) and small beads (0.482 μm), respectively. Then unadsorbed beads were extensively washed with medium, and incubated with fresh culture medium for 24 hr. The test and the reference cells were then trypsinized, mixed, seeded on a coverslip and processed by the same manners as the conventional method described above. After the development of the autoradiographic samples, the numbers of grains per nucleus were counted only with heterodikaryons having different sizes of Latex beads.

**Survival assay of ultraviolet-irradiated cells**

Appropriate numbers of cells were inoculated into 6-cm dishes with 5 ml each of culture
medium. After 16–18 hr incubation, cells were washed once with phosphate-buffered saline and irradiated with UV at different dose rates. The cells were then incubated for 2 weeks with medium changes of twice a week, and colonies were counted after staining to determine survival fractions.

RESULTS

Clinical features

A 2 years and 7 months-old Chinese boy, without apparent neurological abnormalities, exhibited severe photosensitivity with numerous hyper/hypopigmented spots and keratotic papules on sun-exposed areas of the skin. About 3 months after birth, he developed freckle-like pigmentation on his nose and cheeks on minimum sun exposure. At the age of 1 year and 7 months, he was diagnosed as XP at Chang Gung Memorial Hospital, Taipei, Taiwan; at that time skin biopsy from an ulcerated nodule on the scalp had already disclosed a squamous cell carcinoma.

Discussion with the patient’s parents indicated that their ancestors had immigrated into Taiwan from Fu-Chien province in mainland China, about 4 centuries ago. The parents are first cousins. Three elder sisters of the patient have no indication of XP, but a 3 month-old younger sister displays freckle-like pigmentation on the tip of nose and cheeks.

Unscheduled DNA synthesis and complementation analysis

Figure 1 shows the distribution of the numbers of grains per nucleus in normal and XP1CTA cells after irradiation with 20 J/m² of UV. The amount of UDS in XP1CTA cells, as expressed by average number of grains per nucleus, was about 20% of that in normal cells. The rates of UDS in the reference XP strains used for the present complementation analysis were similarly determined and summarized in Table 1.

Figure 2 shows the distribution of the number of grains per nucleus in fused binuclear cells. When XP1CTA cells were fused with cells of a representative strain from groups A (XP35OS), D (XP7BE), F (XP101OS) and G (XP2BI), about half of the binuclear cells showed UDS levels in the range of normal cells, indicating that XP1CTA neither belongs to XP group A, D, F nor G. In contrast to these reference strains having low levels of UDS, group E (XP24KO) and group H (GM3248) strains showed high levels of inherent UDS (Table 1). Therefore, the complementation was identified by the beads incorporated in the cytoplasms. As shown in Figs. 2d and 2g, heterodikaryons derived from fusion between XP1CTA and XP24KO as well as fusion between XP1CTA and GM3248 displayed the normal levels of UDS, demonstrating that XP1CTA complemented with XP groups E and H strains. In contrast, XP1CTA neither complemented with an authentic group C XP strain GM3176 (Fig. 2b), nor with other four group C strains (Fig. 3). These results clearly demonstrate that XP1CTA belongs to group C.
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Fig. 1. Distribution of the number of grains per nucleus in UV-irradiated normal and XP1CTA fibroblast cells. Cells on coverslips were irradiated with 20 J/m² UV, labeled with [³H]thymidine for 3 hr, and processed for autoradiography as described in the text. The numbers of grains per nucleus were scored with 100 nuclei per sample. Mean numbers of grains per nucleus are also shown.

Ultraviolet survival

Figure 4 shows the survival curves of UV-irradiated XP1CTA cells, as measured by colony-forming ability, in comparison with those of the reference XP strains belonging to groups A, C, D and F. Post-UV colony-forming ability of XP1CTA cells was intermediate between that of normal cells and the most sensitive group A XP35OS cells. The UV sensitivity of XP1CTA strain was almost identical to those of two representative group C XP strains, GM3176 and XP8CA, although they were slightly more sensitive to UV than two other authentic group C strains, XP3BE and XP8BE.
Fig. 2. Complementation analysis of XP1CTA strain with reference strains belonging to groups A, C, D, E, F, G and H. The XP1CTA and reference cells were fused, subjected to autoradiography as described in the text, and the number of grains per nucleus was counted over 50 fused binuclear cells. For fusion between XP1CTA and XP24KO cells (d) and between XP1CTA and GM3248 cells (g), the numbers of grains were scored only with heterodikaryons labeled with different sizes of Latex beads (see text in detail).
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DISCUSSION

A Chinese XP patient in Taiwan, XP1CTA, who exhibits severe skin lesions and had already developed a squamous cell carcinoma at the age of 1 year and 7 months, was assigned to complementation group C by assaying UV-induced UDS in cultured skin fibroblasts. Group C XP (XP-C) cells have some unique DNA repair phenotypes compared with other groups of XP cells. Firstly, XP-C strains show the greatest variation in sensitivity to killing by UV, and this was also confirmed by our present study (Fig. 4). Secondly, the residual excision repair in XP-C cells is domain limited, namely, occurring in clustered regions of the genome. Also, unlike the XP-A gene products, the XP-C gene products do not readily diffuse out of the nucleus, and the XP-C cells complement poorly in the absence of protein synthesis. Therefore, some changes in cultural conditions may cause an alteration of the DNA repair pattern specifically in XP-C cells, which then might give rise to a misleading complementation result, as seen in a certain case. Accordingly, it is recommended to use at least 2 different group C strains as references for assigning a test XP strain to group C, or even to a new complementation group.
In Japan, XP-C patients are relatively rare\textsuperscript{5}; only 8 patients are assigned so far to group C among 69 excision-defective XP patients whose complementation groups are identified\textsuperscript{24}. Patients in Japan are dominated by group A (35 patients) and variant (30 patients)\textsuperscript{5,24}, while group C is the most common complementation group in the USA and Europe\textsuperscript{3}. For Chinese XP patients, to the best of our knowledge, there is no detailed report on the complementation assignment of XP. However, some information is available, such as in a quotation in a literature and in an abstract form. A Chinese XP cell strain derived from a 6 year-old patient with severe skin lesions has been assigned to complementation group C by Cleaver\textsuperscript{25}, who also assigned 2 other Chinese XP strains to variant form (Cleaver, J.E., personal communication), one (GM6090) of which is registered in the Human Genetic Mutant Repository, Camden, NJ, USA. Recently, Wang et al.\textsuperscript{26} have identified 3 patients belonging to group C and a patient to group F. Altogether, there seem to be 5 group C patients including the present case, one group F and 2 variant forms among the Chinese XP patients. Furthermore, in a survey of Chinese literatures dealing with 44 XP cases, Takebe et al.\textsuperscript{5} found that 7 patients, all under 20 years old, had some neurological abnormalities, suggesting that most of these patients belong to group A. All these data
indicate that the distribution of different XP complementation groups may be different, to some extent, between Japanese and Chinese populations. More studies are needed to identify the complementation groups of many Chinese XP patients, so that we may be able to elucidate the different defects of XP between Japanese and Chinese.

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REFERENCES


