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Chromosomal Instability in Cell Lines Derived From Patients with Xeroderma Pigmentosum[1] (38725)

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Patients with the rare skin disease, xeroderma pigmentosum (XP), an inherited autosomal recessive mutation, are extremely sensitive to ultraviolet (uv) light (1, 2). Various types of skin cancer are very common among XP patients (1, 2). Cell cultures derived from skin or peripheral blood of XP patients are also sensitive to uv irradiation (2–5) and to treatment by some chemical carcinogens and mutagens (6, 7), possibly due to defective DNA-repairing mechanism (8).

Cytogenetic data on XP cells is scarce. Reed *et al.* (1) reported the findings of normal karyotypes in lymphocyte cultures from four XP patients. On the other hand, German *et al.* (9, 10) detected a very low incidence of chromosomal rearrangement in three of six fibroblastic cultures derived from one XP patient. Bloch-Shtacher *et al.* (11) studied the chromosomes of skin taken from different areas of an XP patient. They reported that the percentage of cells with normal diploid karyotype in unexposed areas was 84%. This reduced to 57% for cells from exposed areas, while the culture derived from skin which had developed squamous cell carcinoma had only 10%.

In an over-all study of cytogenetic stability of cell lines derived from patients with various genetic defects as well as sensitivity of such lines to different agents, we have studied the chromosomes of four cell lines derived from patients believed to be homozygous for the XP genes. The results are presented in this paper.

Materials and Methods. A total of eight cell lines, four XP and four normal, were used (Table I). CRL lines were obtained from the American Type Culture Collection (12) and GM lines from the Institute for Medical Research, Camden, NJ. CRL lines were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum (HI-FCS). GM lines were grown in Eagle's minimum essential medium with 2X amino acids and vitamins and supplemented with 10% HI-FCS. The growth of the cultures varied from line to line. Generally, at the early passages, the XP lines grew better than those of corresponding controls. At the late passages, the reverse was true. Cultures were subcultured usually in one to two split, about every 6–8 days.

Cultures in active growth were harvested for chromosome analysis. Flasks were first treated with Colcemid at a concentration of 0.05 μg/ml of medium for 2–6 hr. cells were then trypsinized off the flask; treated with a hypotonic solution (1% sodium citrate), and fixed in 1:3 acetic acid/methanol. Flame-dried slides were stained with Giemsa.

Distribution of chromosome counts, incidence of cells with aberrations, and polyploid rate, were studied. Chromosome counts were usually made directly under a microscope. In some instances, however, metaphases were photographed and the number of chromosomes were counted in prints. In every passage, 5 to 20 metaphases were selected for karyotype analysis. Clear chromatid or chromosome breaks, dicentrics, exchanges, fragments were recorded. Polyploid rates in both XP and control cultures were determined after examining 1000–2000 randomly selected metaphases under a low objective. In control cultures, 50–100 metaphases from each passage were counted. In early passages (no. 11 or earlier) G-banding patterns of cells with modal chromosome number were studied by a technique described by Seabright (13). Karyotype and G-banding pattern of control cultures were not studied.

Results. Table II shows the distribution of chromosome counts, rate of polyploidy, and

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TABLE I. CELL LINES DERIVED FROM PATIENTS WITH XERODERMA PIGMENTOSUM (XP) AND FROM NORMAL PERSONS.

Cell line	Donor's age/sex	Passage no. when obtained	Diagnosis
CRL-1158[a]	12/M	4	XP
CRL-1161[a]	12/M	4	XP
CRL-1125	13/F	3	Normal
GM-179	13/M	5	Normal
CRL-1166[a]	22/M	3	XP
CRL-1170	27/F	4	XP
CRL-1105	29/M	6	Normal
GM-43	32/F	5	Normal

[a] Siblings. CRL-1158 and 1161 were derived from skin biopsies of identical twins.

incidence of chromosome aberrations in the 4 XP cell lines at various passages. The cytogenetic data of the corresponding controls at comparable passages to the XP lines (not listed in Table II) were as follows. Cells with diploid chromosome number of 46 were 85% or over, polyploid rates were 4% or less, and cells with a single aberration per cell were 5% or less.

In the early passages, all the XP lines generally maintained a normal chromosome constitution with relatively low levels of polyploidy and chromosome aberrations. Karyotypic analysis as well as G-banding studies of cells at passage no. 11 or earlier in these lines revealed no obvious deviation from the normal. In the later passages however, very high levels of polyploidy and chromosome abnormalities were observed in two XP lines, CRL-1161 and 1166. In the other two lines, CRL-1158 and 1170, the incidence of cells with chromosome aberrations was much less, but still higher than those of controls.

Chromosome studies in later passages than those listed in Table II in all four XP lines were attempted but with no success. The cultures grew much slower than corresponding controls; consequently, mitosis was rare. These signs of aging became prominent around 30th passage.

Cell lines CRL-1158 and 1161 were derived from skin biopsies of identical twins; however, the chromosomes observed were quite different. CRL-1158 had a relatively stable chromosome constitution in most of the passages studied. The rate of polyploidy and incidence of chromosome aberrations were relatively low except in the last sample of passage 26, in which the incidence of cells with aberrations was higher than the control. Karyotype analysis of all samples showed a normal male constitution except, again, in the last sample. A total of 15 metaphases were karyotyped in this sample, eight were found to be normal, six cells had the long arm of one chromosome in B-4 which was substantially longer than its homologue, one cell had one extra chromosome in group F and lacked one in group C.

Line CRL-1161 had extensive chromosome abnormalities. Except in the first sample of passage 10, all other samples had high rate of polyploidy and high incidence of cells with chromosome aberrations. Samples from passages 19 and 21 had high rate of hypodiploid cells and the percentage of cells with the modal number was less than 50%.

The prevailing aberration was of the dicentric type (Figs. 1, 2). For instance, in 100 randomly selected metaphases at diploid region in the sample of passage 19, 79 cells had chromosome aberrations of which 75 were dicentrics, involving nonspecific chromosomes. Dicentrics were also common in polyploid cells. In 100 randomly selected metaphases at tetraploid region in the sample of passage 17, 76 cells had up to five dicentrics per cell (Fig. 2). Chromosome or chromatid breaks were also frequently seen. Ring chromosomes were observed but infrequently (Figs. 3, 4). Another type of aberration which was rarely seen, is pulverization of part of a chromosome complement (Fig. 1). These partially pulverized metaphases did occur in both diploid and polyploid cells. Karyotype analysis of cells with modal chromosome number in the first four samples, i.e., passages 10, 17, 19, and 21 all showed normal constitution. In passage 24, a total of 20 metaphases with model number were karyotyped. In nine of these cells, one chromosome placed in B-4 seemed larger than its partner as described in CRL-1158. Moreover, there were other nonspecific changes in four of the nine cells.

TABLE II. CHROMOSOME COUNTS AND INCIDENCES OF POLYPLOIDY AND CHROMOSOME ABERRATIONS OF FOUR XP CELL LINES

Cell line	Passage no.	Total cells	Chromosome counts (%)					Polyploidy (%)	Cells with aberrations (%)
			44	45	46	47	49		
CRL-1158	7	76	2.6	6.6	85.5	5.3		1.1	2.6
	11	50	6.0	22.0	72.0			1.1	2.0
	17	20	10.0	15.0	75.0			NA[a]	0.0
	22	50		4.0	96.0			1.0	2.0
	25	20	5.0	15.0	80.0			NA[a]	5.0
	26	110	3.6	7.3	86.4	2.7		1.2	7.3
CRL-1161	10	67		7.5	91.0	1.5		3.5	4.0
	17	48	12.5	12.5	71.0	2.0	2.0	25.0	23.0
	19	43	37.2	16.3	46.5			50.0	79.0[b]
	21	116	28.5	13.8	49.1	4.3	4.3	48.2	70.0
	24	63	7.9	15.9	71.4	1.6	3.2	28.8	51.0[b]
CRL-1166	6	84		3.6	89.3	7.1		0.5	2.4
	14	26	4.0		96.0			NA[a]	3.8
	24	46	30.4	15.2	50.0	2.2	2.2	44.0	65.0[b]
CRL-1170	6	63		4.8	90.4	4.8		1.6	1.6
	10	62	1.6	6.5	83.9	8.0		2.0	3.2
	18	50		12.0	86.0	2.0		0.6	8.0
	20	50	2.0	18.0	80.0			1.8	8.0
	22	50	20.0	6.0	72.0	2.0		0.8	12.0
	24	50	6.0	6.0	86.0	2.0		1.2	8.0

[a] Not available.

[b] Percentages of cells with chromosome aberrations in 100 randomly selected metaphases at diploid region.

The remainder of the 11 cells studied were normal.

Line CRL-1166 had high levels of polyploidy and chromosome aberrations in the sample harvested at passage 24 (Table II). The extent and type of aberrations were similar to those described for CRL-1161. No karyotype analysis was made at passage 24. In the passages 6 and 14, however, the chromosomes were found to be normal.

The chromosomes of all passages studied in line CRL-1170 were relatively stable except in the last four samples from passages 18, 20, 22, and 24, in which the incidence of cells with aberrations was higher than controls. Again most of the aberrations were of the dicentric type. Karyotype analysis of cells in this line from various passages were found to be normal.

Discussion. The chromosomes of four XP lines studied were less stable than those of corresponding controls at late passages. The degree of chromosomal instability, however, varied greatly among the lines. The reasons for such variation are not clear, especially since three of the four XP lines were derived from skin biopsies of three brothers and two of them were identical twins (2, 12). Parrington *et al.* (5) studied the effect of uv irradiation on the chromosomes in cell cultures derived from three XP patients. They found that the XP cultures had a higher rate of cells with chromosome aberrations than controls, but the degree of chromosome aberrations among XP lines also varied.

It is known that XP cells are defective in DNA repair and the repair capacity varied greatly from one XP line to another (2–5, 8). It is possible, therefore, that a different degree of repair capacity may give a different degree of chromosome instability. The DNA repair rate of the four XP lines at the late passages was not determined. At early passages, however, they all had about 15–25% of normal DNA repair rate (2). Clear correlation of chromosome aberrations and DNA repair capacity in the various

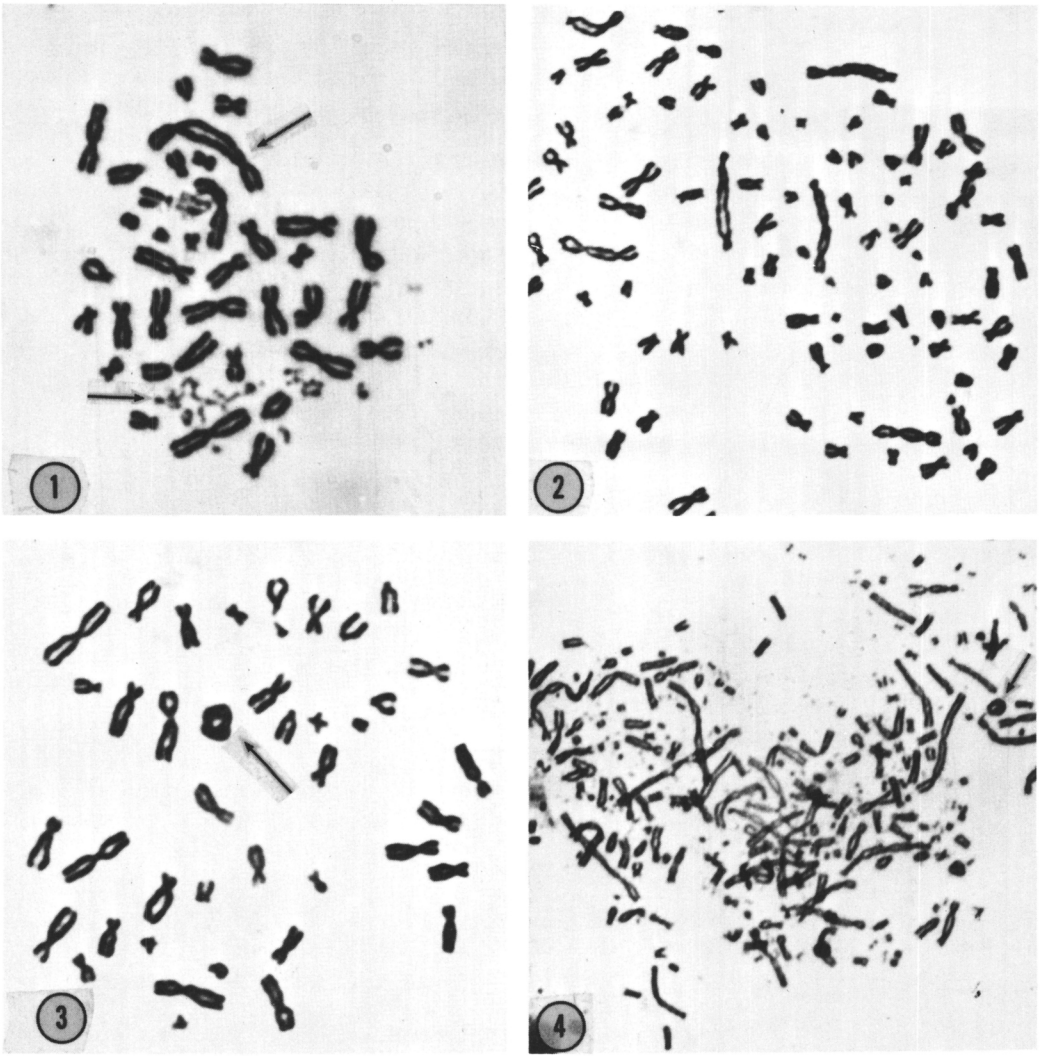

FIG. 1. A metaphase with partial pulverization and a dicentric. CRL-1161, passage 19.
FIG. 2. Part of a polyploid cell with five dicentrics. CRL-1161, passage 19.
FIG. 3. Part of a metaphase with a ring chromosome. CRL-1161, passage 21.
FIG. 4. Part of a polyploid cell with numerous fragments and a ring chromosome (arrow). CRL-1161, passage 21.

XP cell lines have been demonstrated after treatment with uv irradiation, chemical carcinogens, and infection with adenovirus type 12 (5–7, 14).

No special precautions against the exposure of cultures to ambient light, during routine manipulation, were made. However, all cultures were handled in a similar fashion. It is possible that a differential sensitivity to the repeated exposure to the room light might result in a variation of chromosome instability among the XP lines at the late passages.

Another possible explanation of the differential chromosome stability observed in this study may be due to the variation in skin areas where the biopsies were taken. As mentioned earlier, Bloch-Shtacher *et al.* (11) found different chromosome constitutions among cultures derived from skin biopsies at different areas of the same patient.

The incidences of cells with 44 and 45 chromosomes in many samples of the four XP lines are much higher than the corresponding controls (Table II). For instance, in some preparations of lines CRL-1161 and 1166, over 40% of cells counted were hypodiplody and in lines CRL-1158 and 1170, over 25% of the cells were hypodiploidy. The reason for such a high incidence of hypodiploidy was very likely due to the hypotonic treatment which caused a large number of metaphases to rupture and the resulting loss of one or two chromosomes. Although we treated both XP and control cells identically (1% sodium citrate for about 15 min) the high incidence of hypodiploidy observed with XP cells indicated that they were more sensitive to osmotic shock. This may be a characteristic of XP cells.

German *et al.* (10) studied the chromosome of an XP culture at the 4th passage and found a very low percentage of cells with a specific structural rearrangement, but no fragments, dicentrics, or chromatid exchanges were seen. In the early subcultures of all four XP lines studied here, we found no specific chromosomal change by either karyotype analysis or by G-banding studies. The rates of aberrations at early passages were also well within the range of what we found in the control cultures. The number of metaphases selected for analyses were relatively low; therefore, the possible existence of small fraction of cells with a mutant karyotype in such cultures could not be completely ruled out.

In the late passages, cells with structural chromosome changes were observed in two of the four XP lines studied, i.e., CRL-1158 and 1161. In passage 26 of CRL-1158 and passage 24 of CRL-1161, a relatively high incidence of metaphases analyzed (six out of 15 in CRL-1158 and nine out of 20 cells in CRL-1161), had a substantially longer chromosome in the B group. It is difficult to ascertain whether this pseudodiploid occurred *in vitro* or was already present in the patients.

Summary. The chromosomes of four cell lines derived from skin biopsies of four patients with xeroderma pigmentosum (XP) were studied and compared with corresponding controls. In the early passages, all XP lines had normal chromosome constitution with rates of polyploidy and chromosome aberrations within the range found in the controls. In the later passages, the XP cell lines had higher levels of chromosome abnormalities. The degree of abnormalities varied greatly among the XP lines; two lines had very high level of polyploidy (up to 50%) and cells with chromosome aberrations (up to 79%), the other two XP lines had a normal level of polyploidy and a slightly higher incidence of cells with chromosome aberrations than normal. The most common type of aberration was dicentrics.

1. Reed, W. B., Landing, B., Sugarman, G., Cleaver, J. E., and Melnyk, J., J. Amer. Med. Assoc. **207**, 2073 (1969).
2. Robins, J. H., Kraemer, K. H., Lutzner, M. A., Festoff, B. W., and Coon, H. G., Ann. Intern. Med. **80**, 221 (1974).
3. Bootsma, D., Mulder, M. P., Pot, F., and Cohen, J. A., Mutat. Res. **9**, 507 (1970).
4. Kleijer, W. J., Weerd-Kastelein, E. A. D., Sluyter, M. L., Keizzer, W., Wit, J. D., and Bootsma, D., Mutat. Res. **20**, 417 (1973).
5. Parrington, J. M., Delhanty, J. D. A., and Baden, H. P., Ann. Hum. Genet. **35**, 147 (1971).
6. Stich, H. F., San, H. C., and Kawazoe, Y., Mutat. Res. **17**, 127 (1973).
7. Stitch, H. F., Stich, W., and San, R. H. C., Proc. Soc. Exp. Biol. Med. **142**, 1141 (1973).
8. Cleaver, J. E., *in* "Nucleic Acid-Protein Interactions—Nucleic Acid Synthesis in Viral Infection" (D. W. Ribbons, J. F. Woessner, and J. Schultz, eds.), p. 87. North-Holland, Amsterdam (1971).
9. German, J., *in* "Progress in Medical Genetics" (A. G. Steinberg, and A. G. Bearn, eds.), Vol. 8, p. 61. Grune & Stratton, New York (1972).
10. German, J., Gilleran, T. G., Setlow, R. B., and Regan, J. D., Ann. Genet. **16**, 23 (1973).
11. Bloch-Shtacher, N., Slor, H., and Goodman, R. M., Jerusalem Chromosome Conference p. 1, Sept., 1972.
12. American Type Culture Collection. List of human skin fibroblasts. Rockville, Md., Feb. 1973.
13. Seabright, M., Chromosoma **36**, 204 (1972).
14. Stich, H. F., Stich, W., and Lam, P., Nature (London) **250**, 599 (1974).

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