[CANCER RESEARCH 49, 1927–1930, April 15, 1989]

Reduced Levels of UV-induced Unscheduled DNA Synthesis in Epidermal Keratinocytes of Patients with Xeroderma Pigmentosum and Correlation with Development of Skin Neoplasms[1]

Seiji Kondo,[2] Yoshiaki Satoh,[3] and Toshio Kuroki

Department of Dermatology, School of Medicine, Tokyo Medical and Dental University, Yushima, Bunkyo-ku, Tokyo 113 [S. K., Y. S.]; and Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108 [T. K.], Japan

ABSTRACT

Primary epidermal keratinocytes obtained from 25 patients with xeroderma pigmentosum (XP) (nine with XP-A, one with XP-C, two with XP-D, five with XP-E, and eight with XP-variant) exhibited less UV-induced unscheduled DNA synthesis (UDS) than did those from 34 normal subjects. Levels of UDS depended greatly on the type of XP; *i.e.*, 3–17% of the control in XP-A, 14% in XP-C, 33–53% in XP-D, 38–77% in XP-E and 58–98% in XP-variant. The extent of UDS in epidermal keratinocytes was almost the same as that in dermal fibroblasts in XP-C, D, and E, but in three out of eight of the XP-variant the level of UDS in epidermal keratinocytes was significantly lower than that in normal subjects.

Clinically, three out of nine XP-A patients developed skin neoplasms before 20 years of age. Both patients with XP-D developed skin neoplasms around 40 years of age. In the five XP-E patients, two developed multiple basal cell epithelioma on sun-exposed areas during the forth decade, and one of them also developed squamous cell carcinoma at the age of 50. Four out of the eight patients with the XP-variant developed various skin neoplasms during their 20s and 30s.

These results suggest that a defect in UV-induced UDS in epidermal keratinocytes of XP patients is responsible for skin carcinogenesis and the extent to which this defect occurs tends to relate to the age of onset of skin neoplasms.

INTRODUCTION

XP[4] is a rare autosomal recessive disease and is characterized by hyperphotosensitivity and high incidence of skin neoplasms in areas of skin exposed to sun (1). Cleaver (2) discovered in 1968 that the cells from XP patients are defective in repairing UV radiation-induced damage to their DNA. Since then, a number of studies have demonstrated defective repair of DNA damage in XP cells following induction by UV and certain chemicals (3–8). Most of these studies have been carried out by using 254-nm UV (UVC). Accumulated DNA damage due to repair defects may be a cause of human skin cancers in XP patients (9–12).

In order to investigate the relationship between DNA repair defects and skin carcinogenesis, it is obviously important to study the DNA repair of epidermal keratinocytes, because a great majority of human malignant tumors arise from epidermal keratinocytes. In spite of this fact, most information on DNA repair including that of XP has been obtained from studies on cultured dermal fibroblasts. DNA repair in epidermal keratinocytes in culture has been reported previously from several laboratories (13–16). In these studies, epidermal keratinocytes were dissociated by trypsin and cultured to form monolayers, and then DNA repair was assayed. The culture methods in these studies, however, required relatively large samples of skin for isolation and cultivation of epidermal keratinocytes. We have previously established a new explant-outgrowth method for culturing epidermal keratinocytes from small biopsy specimens of skin (17). This method, however, has a limitation that assay methods other than UDS are hardly applicable because of a small amount of cells available.

In the previous paper, we demonstrated a defect in the repair of DNA in epidermal keratinocytes from eight XP patients (17). Here, we confirm and extend our previous observations using 25 cultures of XP epidermal keratinocytes. The results suggest that the observed repair deficiency in XP keratinocytes tends to relate to the age of onset of skin neoplasms.

MATERIALS AND METHODS

Materials. [*Methyl*^{3}H]thymidine (specific activity, 25 Ci/mmol) was purchased from Amersham, Buckinghamshire, UK. NR-M2 emulsion was purchased from Konishiroku Photo Co., Tokyo, Japan.

Culture of Epidermal and Dermal Cells. We obtained skin samples by biopsies from the elbows of XP patients and from discarded materials in fresh surgical specimens of normal subjects. These samples were cultured by the explant-outgrowth method as described previously using Eagle's minimum essential medium supplemented with 10% fetal calf serum (17). In brief, skin samples were cut into 0.5–1.0-mm^2 pieces and each skin fragment was placed, dermal side down, on a round glass cover slip. Another cover slip was placed on the top of the tissue fragments and culture medium was filled between two cover slips. Sandwiched tissue fragments were placed in a 35-mm dish wetted with the medium and cultured at 37°C in a humidified atmosphere of 5% CO_2. On Day 3 the top cover slip was removed and the medium was added. UV irradiation to epidermal keratinocytes was carried out between Days 7 and 14 when a sheet of epidermal keratinocytes with about 30 cell layers had been formed from explants. One of the explant cultures was used for isolation of dermal fibroblasts, which began to grow around the epidermal sheet after 3 weeks in culture. Fibroblasts were selectively transferred by light trypsinization and seeded on a cover slip (18-mm diameter) placed in a 35-mm dish at a concentration of 10^5 cells/dish. UV irradiation of fibroblasts was done 2 days after seeding.

Measurement of UDS. The cells were washed once with phosphate buffered saline and irradiated at doses of 5–20 J/m^2 of UV at a wavelength of 254 nm from a 15-W GL lamp (Toshiba). UV doses at 254 nm were determined with a UVR-254 UV radiometer (Tokyo Kogaku Kikai Co., Tokyo, Japan). UV-irradiated and nonirradiated cells were labeled by exposure to 5 μCi/ml of [*methyl*^{3}H]thymidine for 3 h at 37°C. The labeled cells were processed for autoradiography using a Sakura NR-M2 emulsion and exposing for 7 days at 4°C. After development, numbers of grains were counted on 30–50 lightly labeled cells. To avoid complications due to cell differentiation at central regions, UDS was measured at peripheral regions of the cell sheet where cells grew actively. The amount of UDS in XP cells was expressed as a percentage of that in normal cells which were assayed in parallel.

Received 8/8/88; revised 12/1/88; accepted 1/19/89.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by Grants-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan.

[2] To whom requests for reprints should be addressed, at Department of Dermatology, Tokyo Medical and Dental University 5-45, Yushima 1-Chome, Bunkyo-ku, Tokyo 113, Japan.

[3] Present address: Department of Dermatology, Dai-Ni Hospital, Tokyo Women's Medical College, Nishi-oku, Arakawa-ku, Tokyo 116, Japan.

[4] The abbreviations used are: XP, xeroderma pigmentosum; UDS, unscheduled DNA synthesis; SCC, squamous cell carcinoma; BCE, basal cell epithelioma.

RESULTS AND DISCUSSION

UV-induced UDS in Normal Epidermal Keratinocytes and Dermal Fibroblasts. Cells from a total of 34 normal subjects were investigated for UV-induced UDS as a control for the XP cells. Each experiment included XP and normal cells measured in parallel. In most cases, normal subjects from which control cells were derived were age-matched to XP patients. Furthermore, we and others reported that levels of DNA repair were independent of ages of donors (15, 17).

In both epidermal keratinocytes and dermal fibroblasts, UDS was induced dose dependently on UV irradiation at doses of 5–20 J/m^2. The mean grain numbers of epidermal keratinocytes of these 34 subjects were 30.4 ± 3.3 at 5 J/m^2, 43.9 ± 5.3 at 10 J/m^2, and 54.0 ± 4.9 at 20 J/m^2. The range of UDS is shown as a shaded area in Figs. 1–3. In dermal fibroblasts, the number of grains was about 2.5 times greater than that in epidermal keratinocytes; *i.e.*, 68.7 ± 7.7 at 5 J/m^2, 100.4 ± 11.5 at 10 J/m^2, and 129.1 ± 10.8 at 20 J/m^2. This difference between epidermal and dermal cells is in good agreement with the observation obtained for the corresponding mouse cells (18) and may be a result of the complexity of the factors involved, *e.g.*, size of the intracellular pyrimidine pool, size and thickness of cells, amount of intracellular organelles including keratin and cell-type specific sensitivity.

UV-induced UDS in XP-A. XP-A is the most common form in Japan. XP-A patients are characterized by extreme sensitivity to sunlight and high frequency of cutaneous malignancies at a very early age. Most XP-A patients exhibit neurological abnormalities. We examined nine cases of XP-A in patients ranging from 11 months to 17 years (Table 1). Epidermal keratinocytes isolated from these patients displayed very low levels of UDS with an average of 6.6 ± 4.0% (range, 3.2–17.2%) of that of normal keratinocytes (Fig. 1). UDS in dermal fibroblasts was also low being 1.8 ± 1.0% of the control cells (Fig. 1).

UV-induced UDS in XP-C. XP-C is the most common complementation group in the United States and Europe but is rarely seen in Japan (23). XP-C patients have often cutaneous and ocular abnormalities but are neurologically normal. Epidermal keratinocytes and dermal fibroblasts were cultured from a single case of XP-C (Table 1). In both types of cells, levels of UDS were 14–16% of the control cells (Fig. 2).

UV-induced UDS in XP-D. XP-D is as rare as XP-C in Japan (21, 24, 26). Skin symptoms of Japanese XP-D patients appear moderate, and there are seldom neurological abnormalities. In two cases of XP-D, both epidermal and dermal cells exhibited relatively high levels of residual DNA repair; *i.e.*, 33 and 53% in epidermal keratinocytes and 42 and 36% in dermal fibroblasts (Fig. 2).

UV-induced UDS in XP-E. XP-E patients are relatively rare, and are characterized by late onset of skin malignancies and mild clinical signs, such as moderate sensitivity to sunlight, pigmentation or depigmentation of sun-exposed areas (22). We examined epidermal and dermal cells isolated from five cases of XP-E (Table 1). Like XP-D, these cells also retained a moderate capacity for UDS, *i.e.*, 50.9 ± 14.2% (range, 38–77%) in epidermal keratinocytes and 44.7 ± 5.4% (range, 40–55%) in dermal fibroblasts (Fig. 2).

UV-induced UDS in the XP-variant. The XP-variant is as common as XP-A in Japan. XP-variant patients manifest a delayed onset of pigmented freckles as an initial symptom that occurs within 10 years of age and without acute sun erythema. It is postulated that XP-variant cells are proficient in the first incision step of excision repair but have a defect in the later polymerization/ligation step of excision repair (19, 27, 28) rather than a defect in postreplication repair (29). In our previous work with two patients (17), we observed a defect in UDS of epidermal keratinocytes but not in dermal fibroblasts

Table 1 *UDS and skin neoplasms of XP patients*

Group[a]	Patient[b]	UDS (% of normal cells)[c] Keratinocytes	Fibroblasts	Skin neoplasm Age[d]	Histology
A	17TO F 13	6.7	2.8		
	23TO M 15	5.7	1.2	3	SCC
	24TO F 7	3.3	2.9		
	35TO M 8	6.8	0.6	6	BCE
	76TO F 17	6.2	3.2	16	SCC
	78TO F 11m[e]	6.2	1.9		
	79TO M 4	17.2	2.6		
	84TO M 1	3.8	0.2		
	87TO F 1	3.2	1.1		
	Mean ± SD	6.6 ± 4.0 (3.2–17.2)[f]	1.8 ± 1.0 (0.2–3.2)		
C	21SE F 11	14.0	16.0		
D	77TO M 64	52.5	41.8	44	SCC
	85TO F 36	32.6	36.0	36	BCE
E	70TO F 5	76.7	55.0		
	80TO F 50	37.7	42.5	46, 50	BCE, SCC
	81TO F 42	37.9	39.5	41	BCE
	82TO F 41	51.2	44.3		
	83TO F 43	51.2	42.0		
	Mean ± SD	50.5 ± 14.2 (37.7–76.7)	44.7 ± 5.4 (39.5–55.0)		
Va	4TO F 32	83.6[g]	99.3	24, 33	SCC, BCE
	5TO F 36	69.6	99.6	21	BCE
	50TO F 42	89.6[g]	103.6		
	53TO F 15	97.9[g]	104.2		
	71TO M 22	85.8[g]	99.6		
	86TO F 14	57.5	101.0		
	88TO F 51	60.3	98.2	19	BCE
	91TO M 35	81.2[g]	100.8	32, 35	BCE, MM
	Mean ± SD	78.2 ± 13.4 (57.5–97.9)	100.8 ± 2.0 (98.2–104.2)		

[a] Types of XP were determined using fibroblasts separately by complementation of fused heterodikaryons (24).
[b] XP4TO and XP5TO were reported by Fujiwara and Satoh (19); XP70TO by Kawada *et al.* (20); XP77TO by Mamada *et al.* (21); XP80TO, XP81TO, and XP82TO by Kondo *et al.* (22). The others are unpublished. TO, Tokyo; SE, Sendai.
[c] Values, mean UDS on UV irradiation at 5 to 20 J/m^2.
[d] Age at skin biopsy.
[e] m, months. Age is in years unless otherwise indicated.
[f] Values in parentheses, ranges of UDS.
[g] Not significantly lower from control cells of normal subjects. The rests are all significant ($P < 0.05$).

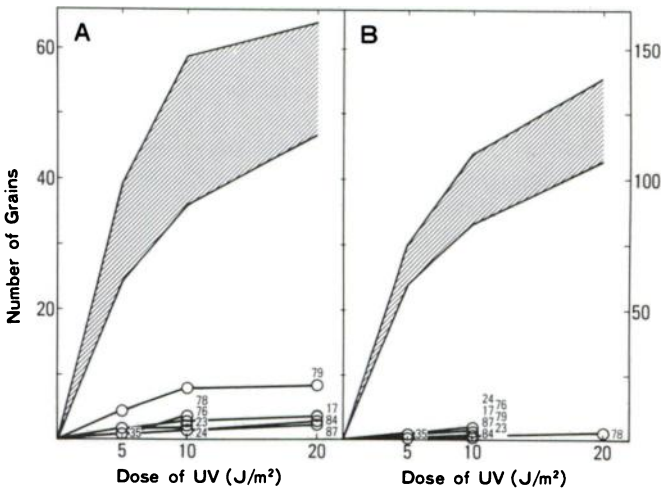

Fig. 1. Dose-dependent induction of UDS by UV-irradiation in XP-A (○). *A*, epidermal keratinocytes; *B*, dermal fibroblasts. The range of UDS in normal subjects is indicated by the shaded area. Each line represents one patient: identification numbers are given without suffixes "TO" or "SE."

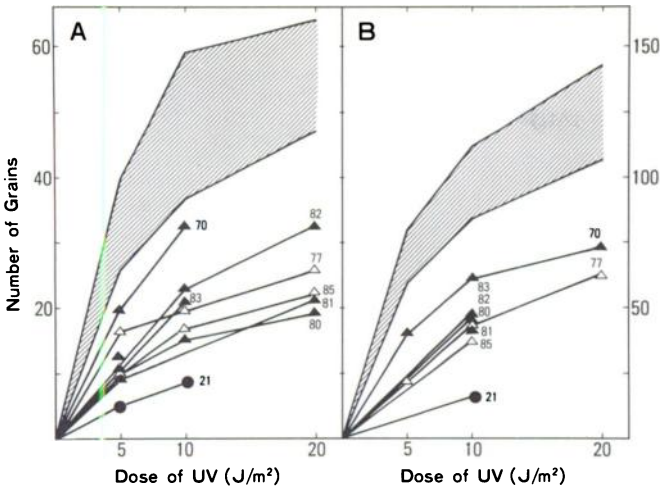

Fig. 2. Dose-dependent induction of UDS by UV-irradiation in XP-C (●), D (△), and E (▲). *A*, epidermal keratinocytes; *B*, dermal fibroblasts.

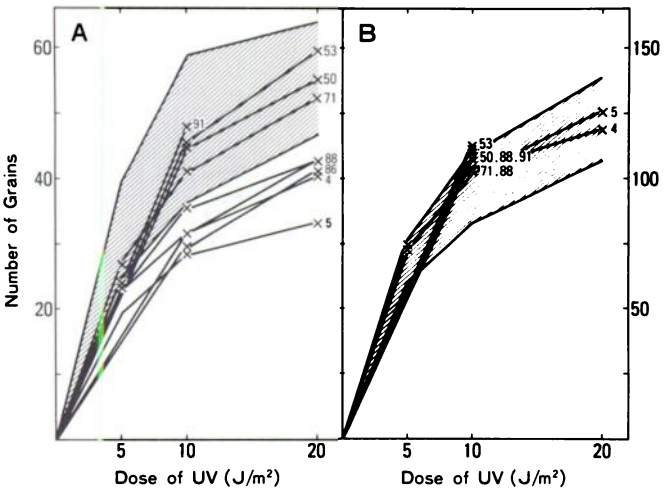

Fig. 3. Dose-dependent induction of UDS by UV-irradiation in XP-variant (X). *A*, epidermal keratinocytes; *B*, dermal fibroblasts.

of XP-variant. Here, we have confirmed this observation by examining eight cases of XP-variant including the two cases previously described (Table 1). UDS in epidermal keratinocytes was 78.2 ± 13.4% (range, 58–98%) of the control: three of eight cases (5TO, 86TO, and 88TO) showed a significantly lower level of UDS than the normal cells ($P < 0.05$). In contrast, dermal fibroblasts from all of these XP-variant showed normal levels of UDS (100.4 ± 2.1%; range, 98.2–104.2%) (Fig. 3), consistent with the previous findings (17). In keeping with the present observation, Wood *et al.* (30) demonstrated a defect in the excision repair of lymphoid cell lines derived from the XP-variant using a cell-free system.

Relationship of Defect of UDS in Epidermal Keratinocytes to Onset of Skin Neoplasms. We examined skin malignancies in all patients investigated in the present study with particular respect to any relationship that might exist between UDS level of XP epidermal keratinocytes and age at onset of skin neoplasms. Three of nine cases of XP-A developed skin neoplasms (XP23TO: SCC on the upper lip at age 3 and on the top of the nose at age 10; XP35TO: BCE on the nose at age 6; XP76TO: SCC on the chin at age 16). Epidermal keratinocytes from these three patients exhibited 6–7% UDS. Similar figures for UDS in the epidermal keratinocytes of other XP-A patients who had not yet developed skin neoplasms were also observed, however.

A single case of XP-C whose epidermal keratinocytes also showed a severe defect of UDS had not developed neoplasms yet, while both of the XP-D patients had developed skin neoplasms (XP77TO: SCC on the lower lip at age 64; XP85TO: multiple BCE at age 36).

Two cases out of five XP-E patients developed skin neoplasms (XP80TO: multiple BCE on sun-exposed areas from age 46 and one SCC on the lower lip at age 50; XP81TO: multiple BCE on the face at age 41). The epidermal keratinocytes from these patients exhibited a lower amount of UDS (38%) than did the epidermal keratinocytes from the other three XP-E patients without skin neoplasms.

Four out of eight patients with XP-variant developed skin neoplasms (XP4TO: SCC on the nose at age 24 and two BCE on the nose at age 33; XP5TO: multiple skin neoplasms over the face from age 21, which revealed four SCC and eight BCE; XP88TO: multiple BCE on the face at age 19; XP91TO: multiple BCE at age 32 and one lentigo maligna melanoma on the face at age 35).

In the present study, the average age at the onset of skin neoplasms was 8.3 years in XP-A, 40.0 years in XP-D, 43.5 years in XP-E, and 24.0 years in XP-variant. These ages were in accordance with the results of a comprehensive survey of Japanese patients by Takebe *et al.* (31), with the exception that the mean development time of the XP-variant was 38.1 years in their study as compared to a much younger age in the present study.

Levels of epidermal UDS tend to correlate with age at onset of skin neoplasms. This correlation was statistically significant for the seven cases of XP-A, XP-D, and XP-E that developed tumors (correlation coefficient, 0.94; $P < 0.005$). No significant correlation was obtained, however, when cases of the XP-variant were included (correlation coefficient, 0.41; $P > 0.05$).

The absence of skin neoplasms in certain XP patients in the present study seems due to the relatively shorter periods of follow-up. For example, patients lacking skin neoplasms but with low levels of UDS were in general younger than the average onset ages (*e.g.*, 84TO, 87TO, 21SE, and 86TO). Furthermore, ages of the onset of skin neoplasms may be associated with exposure history to UV-light or other environmental carcinogens.

In conclusion, epidermal keratinocytes of XP patients have various extents of defect in UDS depending on the type of XP and this defect may be associated with development of skin neoplasms.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Y. Fujiwara (Department of Radiation Biophysics, Kobe University School of Medicine) for his cooperation in the determination of the complementation groups of the cases reported here.

REFERENCES

1. Robbins, J. H., Kraemer, F. H., Lutzner, M. A., Festoff, B. W., and Coon, H. C. Xeroderma pigmentosum: an inherited disease with sun sensitivity, multiple cutaneous neoplasms, and abnormal DNA repair. Ann. Intern. Med., *80:* 221–248, 1974.
2. Cleaver, J. E. Defective repair replication of DNA in xeroderma pigmentosum. Nature (Lond.), *218:* 652–656, 1968.
3. Setlow, R. B., Regan, J. D., German, J., and Carrier, W. L. Evidence that xeroderma pigmentosum cells do not perform the first step in the repair of ultraviolet damage to their DNA. Proc. Natl. Acad. Sci. USA, *64:* 1035–1041, 1969.
4. Takebe, H., Furuyama, J., Miki, Y., and Kondo, S. High sensitivity of xeroderma pigmentosum cells to the carcinogen 4-nitroquinidine-1-oxide. Mutat. Res., *15:* 98–100, 1972.
5. Robbins, J. H., and Burk, P. G. Relationship of DNA repair to carcinogenesis

in xeroderma pigmentosum. Cancer Res., *33:* 929–935, 1973.
6. Cleaver, J. E., and Bootsma, D. Xeroderma pigmentosum: biochemical and genetic characteristics. Ann. Rev. Genet., *9:* 19–38, 1975.
7. Goth-Goldstein, R. Repair of DNA damaged by alkylating carcinogens is defective in xeroderma pigmentosum-derived fibroblasts. Nature (Lond.), *267:* 81–82, 1977.
8. Robbins, J. H. Significance of repair of human DNA: evidence from studies of xeroderma pigmentosum. J. Natl. Cancer Inst., *61:* 645–656, 1978.
9. Takebe, H., Miki, Y., Kozuka, T., Furuyama, J., Tanaka, K., Sasaki, M. S., Fujiwara, Y., and Akiba, H. DNA repair characteristics and skin cancers of xeroderma pigmentosum patients in Japan. Cancer Res., *37:* 490–495, 1977.
10. Pawsey, S. A., Magnus, I. A., Ramsay, C. A., Benson, P. F., and Giannelli, F. Clinical, genetic and DNA repair studies on a consecutive series of patients with xeroderma pigmentosum. Q. J. Med., *48:* 179–210, 1979.
11. Takebe, H. Xeroderma pigmentosum: DNA repair defects and skin cancer. *In:* T. Sugimura, H. Endo, T. Ono, and H. Sugano (eds.), Progress in Cancer Biochemistry, Gann Monograph on Cancer Research 24, pp. 103–117. Japan Scientific Society Press, Tokyo, 1979.
12. Kraemer, K. H., Lee, M. N., and Scotto, J. DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. Carcinogenesis (Lond.), *5:* 511–514, 1984.
13. Taichman, L. B., and Setlow, R. B. Repair of ultraviolet light damage to the DNA of cultured human epidermal keratinocytes and fibroblasts. J. Invest. Dermatol., *73:* 217–219, 1979.
14. Hanawalt, P. C., Liu, S-C., and Parsons, C. S. DNA repair responses in human skin cells. J. Invest. Dermatol., *77:* 86–90, 1981.
15. Liu, S-C., Parsons, C. S., and Hanawalt, P. C. DNA repair responses in human epidermal keratinocytes from donors of different age. J. Invest. Dermatol., *79:* 330–335, 1982.
16. Liu, S-C., Parsons, C. S., and Hanawalt, P. C. DNA repair in cultured keratinocytes. J. Invest. Dermatol., *81:* 179s–183s, 1983.
17. Kondo, S., Satoh, Y., and Kuroki, T. Defect in UV-induced unscheduled DNA synthesis in cultured epidermal keratinocytes from xeroderma pigmentosum. Mutat. Res., *183:* 95–101, 1987.
18. Hosomi, J., and Kuroki, T. UV-induced unscheduled DNA synthesis in cultured human epidermal and dermal cells. Gann, *76:* 1072–1077, 1985.
19. Fujiwara, Y., and Satoh, Y. Age-dependent changes in fibroblast cultures from a xeroderma pigmentosum variant. J. Invest. Dermatol., *76:* 215–220, 1981.
20. Kawada, A., Satoh, Y., and Fujiwara, Y. Xeroderma pigmentosum complementation group E: a case report. Photodermatology, *3:* 233–238, 1986.
21. Mamada, A., Kondo, S., Kawada, A., Satoh, Y., and Fujiwara, Y. Delayed sensorineural deafness and skin carcinogenesis in a Japanese xeroderma pigmentosum group D patient. Photodermatology, *5:* 83–91, 1988.
22. Kondo, S., Fukuro, S., Mamada, A., Kawada, A., Satoh, Y., and Fujiwara, Y. Assignment of three patients with xeroderma pigmentosum to complementation group E and their characteristics. J. Invest. Dermatol., *90:* 152–157, 1988.
23. Fujiwara, Y., Ichihashi, M., Uehara, Y., Matsumoto, A., Yamamoto, Y., Kano, Y., and Tanakura, Y. Xeroderma pigmentosum groups C and F: additional assignments and a review of the subjects in Japan. J. Radiat. Res., *26:* 443–449, 1985.
24. Fujiwara, Y., and Satoh, Y. Assignment of two Japanese xeroderma pigmentosum patients to complementation group D and their characteristics. Gann, *76:* 162–166, 1985.
25. Ichihashi, M., Yamamura, K., Hiramoto, T., and Fujiwara, Y. No apparent neurologic defect in a patient with xeroderma pigmentosum complementation group D. Arch. Dermatol., *124:* 256–260, 1988.
26. Ichihashi, M., and Fujiwara, Y. Clinical and photobiological characteristics of Japanese xeroderma pigmentosum variant. Br. J. Dermatol., *105:* 1–12, 1981.
27. Cleaver, J. E., Thomas, G. H., and Park, S. D. Xeroderma pigmentosum variants have a slow recovery of DNA synthesis after irradiation with ultraviolet light. Biochim. Biophys. Acta, *564:* 122–131, 1979.
28. Dunn, W. C., and Regan, J. D. Inhibition of DNA excision repair in human cells by arabinofuranosyl cytosine, effect on normal and xeroderma pigmentosum cells. Mol. Pharmacol., *15:* 367–374, 1979.
29. Lehmann, A. R., Kirk-Bell, S., Arlett, C. F., Paterson, M. C., Lohman, P. H., de Weerd-Kastelein, E. A., and Bootsma, D. Xeroderma pigmentosum cells with normal levels of excision repair have a defect in DNA synthesis after UV-irradiation. Proc. Natl. Acad. Sci. USA, *72:* 219–233, 1975.
30. Wood, R. D., Robins, P., and Lindahl, T. Complementation of the xeroderma pigmentosum DNA repair defect in cell-free extracts. Cell, *53:* 97–106, 1988.
31. Takebe, H., Nishigori, C., and Satoh, Y. Genetics and skin cancer of xeroderma pigmentosum in Japan. Gann, *78:* 1135–1143, 1987.