Cultured Skin Fibroblasts from Patients with Porokeratosis Are Hypersensitive to the Lethal Effects of X-radiation

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Porokeratosis is an autosomal dominant inherited skin disorder. The lesions are characterized by localized abnormal keratinization and may develop into malignant tumors. To determine the cellular basis of the cancer susceptibility associated with this skin condition, we examined the colony-forming ability of X-ray or ultraviolet (UV) light irradiated, cultured fibroblasts derived from porokeratosis patients' normal-appearing skin. Four fibroblast strains derived from four porokeratosis patients' skin were significantly hypersensitive to the lethal effects of X-radiation. However, they all showed a similar sensitivity to strains from normal donors to 254 nm UV light. The hypersensitivity to X-ray radiation in cultured skin fibroblasts from porokeratosis patients suggests an inherent instability of cellular DNA and may probably be associated with the cancer-prone nature of this skin condition.

Key words: Porokeratosis — Cancer-prone genodermatosis — DNA-damaging agent — X-radiation

Porokeratosis is a rare genetic skin disorder that may present with one, several, or even hundreds of skin lesions, varying from a few millimeters to several centimeters in diameter. The lesions usually show an atrophic center surrounded by a raised keratotic wall. The histology of porokeratosis is characterized by a unique abnormality in the process of maturation of the epidermal keratinocytes.

Clinical evidence has accumulated of malignant epithelial tumors, squamous cell carcinoma, Bowen's disease (squamous cell carcinoma *in situ*), and occasionally basal cell carcinoma developing on porokeratosis skin lesions.^{1,2)} The association has been calculated to occur in 7% of porokeratosis patients.³⁾ Although the pathogenesis still remains to be elucidated, Reed and Leone⁴⁾ postulated, on the basis of histologic features, that clones of abnormal epidermis may be responsible. Our recent reports have demonstrated, using microfluorometric methods, that abnormal DNA ploidy is frequently detected in cells of the epidermis of porokeratosis skin lesions, suggesting the existence of neoplastic and probably precancerous clones responsible for the development of malignant tumors.^{5,6)} Thus, porokeratosis skin lesions are now regarded as arising from a neoplastic process characterized by a unique abnormality in keratinization.

Hypersensitivity of cultured cells to the lethal effects of DNA-damaging agents has been reported in several kinds of genetic disorders that show a predisposition to malignant tumors.⁷⁾ Porokeratosis is also known to be inherited in an autosomal dominant fashion, occasionally

occurring sporadically.^{8,9)} Furthermore, ionizing radiation or ultraviolet (UV) light may provoke new skin lesions and/or malignant tumors in porokeratosis patients.^{3,10,11)} Therefore, we turned our attention to the sensitivity of cultured cells from porokeratosis patients to DNA-damaging agents in an attempt to explain the development of neoplastic or precancerous clones in the epidermis, and to elucidate the cellular abnormalities of this skin condition.

MATERIALS AND METHODS

Cells and culture All the cells are fibroblasts derived from the skin. Four strains of porokeratosis and five strains of normal controls have been established in our laboratory. Four strains of porokeratosis were derived from the normal-appearing skin of four different, genetically unrelated patients, PK1TO (64, F), PK2TO (43, M), PK3TO (46, F) and PK4TO (62, M). Five strains of normal controls were derived from the normal-appearing skin of five different, non-porokeratotic patients with localized benign skin tumors, NL-1 (73, F), NL-2 (61, M), NL-3 (46, F), NL-4 (42, M) and NL-5 (29, M). A strain of ataxia telangiectasia (AT2KY) was obtained from the Japanese Cancer Research Resources Bank. A xeroderma pigmentosum strain (XP24TO, group A) was a generous gift from Dr. Y. Sato (Tokyo).

Fibroblasts were cultured in Eagle's MEM medium containing 15% fetal bovine serum (Hyclone, Logan, Utah), and grown in humidified conditions of 5% CO₂, 95% air, at 37°C. A single lot of serum was used for the cultures for both maintenance and the experiments.

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Experimental procedures Experiments were carried out with cells at passage levels two to ten. Cells in the logarithmic phase of growth were X-ray or UV-irradiated. For the X-ray experiments the cells were irradiated, with air as the gas phase, by a therapeutic 50 kVp X-ray machine (Siemens) at the dose rate of 100 rads/min. For the UV experiments, cells in 0.76 mm depth of culture medium lacking fetal bovine serum were irradiated with 254-nm UV radiation from a germicidal lamp (Toshiba GL 15). Immediately following X-ray or UV-irradiation, cells were trypsinized, resuspended in culture medium containing fetal bovine serum, and then counted with a hemocytometer. After appropriate serial dilutions, experimental cells were seeded into 60 mm Petri dishes. After being cultured for two weeks, the cells were fixed and stained, and the number of colonies containing at least fifty cells was counted. Three different cell concentrations for each dose, and 3 plates for each cell concentration of a specific dose, were prepared. All the experiments were repeated at least three times. The colony-forming efficiency was defined as the number of colonies obtained, divided by the number of cells plated.

Colony-forming ability (CFA) was calculated by dividing the colony-forming efficiency of a strain's cells irradiated at a given dose, by the colony-forming efficiency of the same strain's unirradiated cells in the same experiment. For the evaluation of survival curves, D_0 (dose reducing survival to 37% in the exponential region) and D_{10} (dose reducing survival to 10%) were obtained. Comparison of parameters among groups of cell strains were based on the analysis of the variance.

Cytogenetic studies were done at low passage levels (2–6 passages) for 4 porokeratosis strains to check their karyotype according to the usual method.

RESULTS

X-ray survival experiments The colony-forming efficiency ranges of untreated strains (i.e., plating efficiency) were 14–31% in five normals, 5.4–19% in four porokeratosis, 6.1% in AT2KY, and 17% in XP24TO. Figure 1 shows post-X-ray survival curves for the fibroblast cell strains used in our experiment. At each of the doses tested, the four porokeratosis strains (PK1TO–PK4TO) had lower survival than the five normal controls (NL-1–NL-5) ($P < 0.01$). D_0 and D_{10} values are listed in Table I. Both parameters of the four porokeratosis strains (D_0 : 92–110 rads, D_{10} : 207–253 rads) were significantly lower than those of the normal controls (D_0 : 120–166 rads, $P < 0.025$, D_{10} : 316–384 rads, $P < 0.005$) and higher than those of AT2KY (D_0 : 73 rads, D_{10} : 170 rads).

UV experiments Post-UV survival curves are shown in Fig. 2. Survival curves for the four porokeratosis strains were similar to those of normal controls. D_0 values of

Fig. 1. Dose-response curves showing the post-X-ray survival of fibroblast strains. Each symbol represents the mean post-X-ray colony-forming ability in replicate experiments performed on a strain. Five normal control strains are shown with broken lines: NL-1, ○; NL-2, □; NL-3, △; NL-4, ▽; NL-5, ○. Four porokeratosis strains are shown with solid lines: PK1TO, ●; PK2TO, ■; PK3TO, ▲; PK4TO, ▼. An AT strain is shown with a dotted broken line: AT2KY, ◇.

porokeratosis (3.4–4.3 J/m²) were not significantly different from those of normal controls (3.2–5.6 J/m²) (Table I).

Cytogenetic study The four porokeratosis strains presented the normal karyotype of 46 XY or 46 XO without ploidy abnormalities or significant structural changes. A karyogram from PK4TO is shown in Fig. 3.

DISCUSSION

Our results show that cultured skin fibroblasts derived from normal-appearing skin of porokeratosis patients are hypersensitive to the lethal effects of X-radiation but not UV-radiation. The sensitivity was similar among porokeratosis strains, and was intermediate between normal control strains and an AT strain (AT2KY). Similarly, decreased survival following treatment with DNA-damaging agents has been reported in several other

Table I. D_0 and D_{10} Values of Fibroblast Strains after X-ray or UV Irradiation

Cells	X-ray irradiation		UV irradiation
	D_0 (rads)	D_{10} (rads)	D_0 (J/m^2)
PK1TO	107	240	3.9
PK2TO	108	247	3.5
PK3TO	92	207	4.3
PK4TO	110	253	3.4
Mean $\pm$ SD	104 ± 7.2 ($P < 0.025$)	237 ± 17.8 ($P < 0.005$)	3.8 ± 0.356
NL-1	166	364	3.2
NL-2	120	316	3.4
NL-3	123	320	4.8
NL-4	158	384	5.6
NL-5	133	327	4.1
Mean $\pm$ SD	140 ± 18.6	342 ± 27.0	4.2 ± 0.891

The D_0 value is the dose reducing survival to 37% in the exponential portion of a survival curve. The D_{10} value is the dose reducing survival to 10%.

Fig. 2. Fluence-response curves showing the post-UV survival of fibroblast strains. Each symbol represents the mean post-UV colony-forming ability in replicate experiments performed on a strain. Symbols are as given in the legend to Fig. 1 except for an XP strain (dotted broken line: XP24TO, $\diamond$).

Fig. 3. Normal karyotype (46 XY) of a cultured fibroblast from PK4TO.

cancer-prone genetic disorders: xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome, and Fanconi's anemia.^{12,13} It is not always clearly understood how *in vitro* radiosensitivity is involved in increased susceptibility to cancer in these genetic disorders or porokeratosis. However, a type of instability of cellular DNA, whether due to a DNA repair defect or not, is probably the basis for the cellular vulnerability to genome alteration by DNA-damaging agents. The possible DNA instability may also be responsible for chromosomal instability, which was not detected in the four fibroblast strains used in our study but was found in nearly half of the other porokeratosis fibroblast strains studied by other investigators.^{14,15} The development of neoplastic or precancerous clones in the epidermis may result from similar events in epithelial cells with or without external factors.

It has been found clinically that porokeratosis skin lesions can be induced by exposure to ionizing radiation or to sunlight.^{10,11} Ionizing radiation, which was occasionally used in the early days of treatment of this skin condition, not only induces the formation of new porokeratosis skin lesions but also the growth of skin cancers thereon.³ This clinical evidence suggests an important role of ionizing radiation in the pathogenesis of porokeratosis and in the development of malignant tumors. On the other hand, porokeratosis skin lesions have been reported to be induced or exacerbated by sunlight,¹⁰ a common DNA-damaging agent for the skin in exposed areas. Sunlight-induced or exacerbated porokeratosis, a clinical subtype known as disseminated superficial actinic porokeratosis (DSAP),^{9,10} however, rarely

develops malignant tumors.²⁾ Thus, UV-radiation is expected to play some role in the induction or exacerbation of porokeratosis skin lesions, but it is not likely to participate in the major developmental process of malignant skin tumors. In fact, no cellular abnormalities have been found in cultured skin fibroblasts from porokeratosis skin lesions in terms of post-UV survival or post-UV unscheduled DNA synthesis function.¹⁵⁾ Our results, a decreased survival after X-irradiation and a normal survival after 254 nm UV-irradiation of patients' cultured cells (the latter of which is compatible with a

previous report¹⁵⁾), agree with the accumulated information that points to DNA-damaging agents as being related to the development of malignant tumors on porokeratosis skin lesions.

In conclusion, our study demonstrated the existence of cellular hypersensitivity of porokeratosis to X-radiation. This finding suggests the instability of cellular DNA of porokeratosis cultured cells, and may provide a cellular basis for the increased susceptibility to cancer of patients with this unique cancer-prone genodermatosis.

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