Porokeratosis with Large Skin Lesions

Histologic, Cytologic and Cytogenetic Study of Three Cases

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Three porokeratosis patients with large skin lesion(s) are reported. The histopathology of the large lesions revealed that the epidermis 1) frequently presented slight or marked acanthosis and/or elongation of the rete ridge, and 2) contained abnormal cells, e.g. hyperchromatic, large, multinucleated, and/or irregular shaped nuclei. The DAPI-DNA microfluorometric study revealed DNA polyploidy and/or an increased population of epidermal cells with hyperdiploid and/or tetraploid DNA content. These results indicate the proliferating potential of the epidermis and the existence of a neoplastic clone or clones therein. This finding may explain the enlargement of skin lesions and possibly the development of malignancy, as sometimes occurs in large skin lesions. Furthermore, cultured skin fibroblasts from a patient's skin lesion or its surrounding skin revealed various kinds of chromosomal structural abnormalities, which may serve as a basis for the development of abnormal neoplastic clones in the porokeratotic epidermis. ***Key words: DNA ploidy; Chromosomal abnormality; Large porokerartosis skin lesion.***

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Porokeratosis (PK) is a rare cancer-prone genodermatosis with one, several, or even hundreds of skin lesions varying from a few millimetres to several centimetres in diameter. A large PK skin lesion (or lesions) sometimes develops. When such a lesion is quite large it can be called a giant PK (1). Although large PK lesions have been characterized clinically, their biological characteristics have not yet been detailed.

We recently examined 3 PK patients with a large skin lesion or lesions. We performed histopathologic, DAPI-DNA microfluorimetric analyses of the epidermis of the patients' skin lesions and a chromosome analysis of cultured dermal fibroblasts from one patient. The clinical and histologic features ofthe 3 cases and the cytologic and cytogenetic results are described.

CASE REPORTS

Case 1

A 58-year-old man noticed four small skin lesions on his knees and legs, 6 years ago. They enlarged to the size of around 7 cm in diameter, in which keratotic papules were scattered. He recently developed small PK lesions on his right arm (Fig. 1).

Case 2

A 66-year-old woman noticed a small skin lesion on her left elbow 20 years ago. It gradually enlarged to measure 13 × 9.5 cm^2. The lesion was a well demarcated, scaly, slightly atrophic erythema, in which slightly elevated keratotic areas scattered.

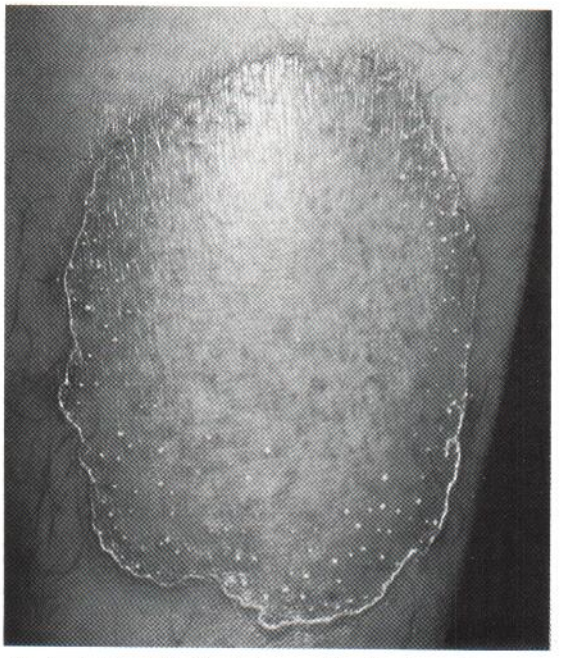

Fig. 1. A large PK skin lesion on the shin (case 1).

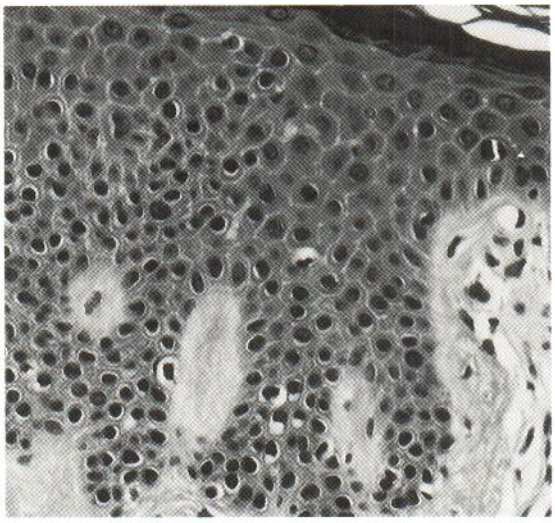

Fig. 2. Histologic features of a large PK lesion (case 2) show acanthosis of the epidermis containing hyperchromatic nucleated cells.

Case 3

A 62-year-old man noticed a small exanthema of the size of a grain of rice on his right buttock when he was around 20 years old. It gradually increased to 3.3 × 4.1 cm^2 during the ensuing 40 years. The skin lesion featured slightly elevated, keratotic, erythematous and pigmented plaque with a small satellite lesion.

Histopathology

Ten specimens (two from case 1, five from case 2, and three from case 3) were taken from a large skin lesion on each of the patients. The five were from the periphery which included marginal keratotic wall, and the other five were from the non-peripheral intralesional part. All the skin lesions featured cornoid lamellae at their border. In cases 1 and 2, the lesional epidermis was slightly atrophic in some specimens and slightly or markedly acanthotic with or without elongation of the epidermal rete ridge in others (Fig. 2). In case 3, the three specimens showed marked acanthosis with rete ridge elongation. The feature was fundamentally compatible with the clinical appearance of the skin specimen taken. Apparent dysplasia was not seen in any specimens but existed a small or large number of cells with hyperchromatic, large, or irregular shaped nuclei (Fig. 2). Atrophic lesional epidermis sometimes had such abnormally nucleated cells, while acanthotic epidermis often contained a number of such cells.

Microfluorimetric analysis of cellular ploidies in terms of DNA content

Ten porokeratosis skin lesion specimens and 11 normal-appearing skin specimens from 4 healthy individuals and 4 patients with localized benign skin tumours were used for the study. The procedural details have been described elsewhere (2, 3). Briefly, however, paraffin-embedded 50-μm specimens were deparaffinized. The epidermis was trimmed and loosened, then sonified to produce a cell suspension. The cells were stained by 4,6-diamidino-2-phenylindole (DAPI). The fluorescence intensity produced by coupling DAPI to the adenine-thymine bond of DNA was measured by microfluorimetry. Stromal lymphocytes

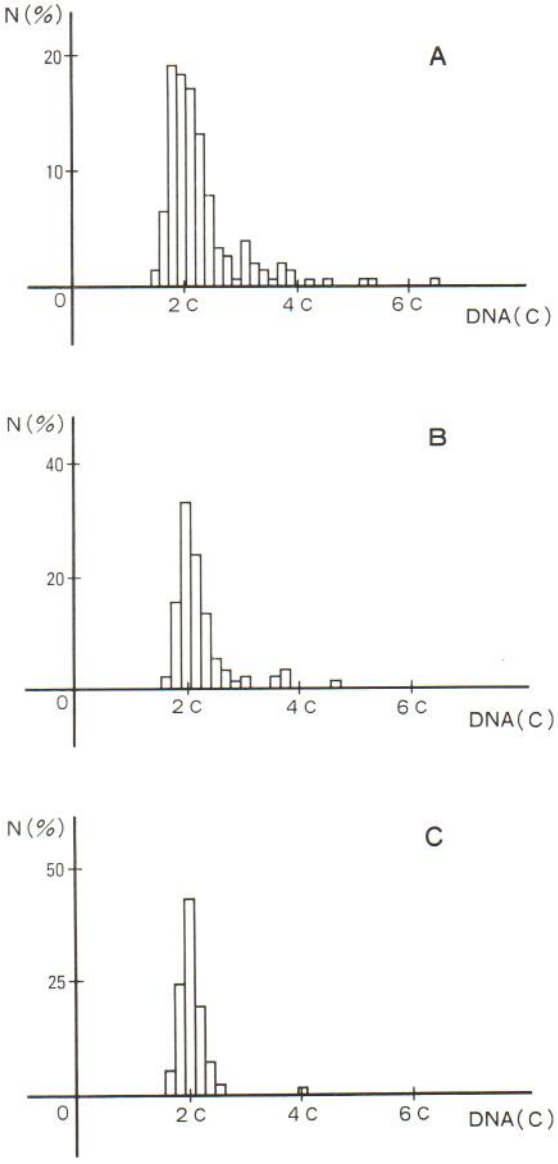

Fig. 3. Distribution histogram of DNA content per cell. Abscissa: DNA content; ordinate: percentage of total nuclei measured. *A:* Small number of polyploid cells and an increased cell population in the hyperdiploid and tetraploid DNA content area (specimen from case 3). *B:* Diploid pattern with increased proportion of cells containing DNA content around hyperdiploidy or tetraploidy (specimen from case 3). *C:* A representative diploid pattern in 11 normal skin specimens from control donors.

Table I. *Summary of chromosomal abnormalities of cultured cells derived from the patient in case 3.*

Specimen site	Abnormal karyotype
Central part of lesion	46, XY, t(1p−; Cq+) 46, XY, t(Dq−; Dq+) 46, XY, Cq− 47, XY, +C
Periphery of lesion	46, XY, t(Fq−; Gp+) 46, XY, −E+F
Normal appearing skin adjacent to lesion	46, XY, r(C)

were used as the control for the normal diploid content. Some 100–300 intact epidermal cell nuclei were observed and measured to obtain DNA distribution histograms. Polyploid cells were defined as those having a DNA content exceeding three times the diploid DNA content. The proportion of cells with hyperdiploid or tetraploid DNA content was calculated, assuming a normal distribution of each peak and was defined as increased when it exceeded the mean value of the cell population of normal control skin specimens by more than a standard margin of error.

A PK specimen from case 3 showed a polyploid pattern, a diploid pattern accompanying a small number of polyploid cells (Fig. 3 A). Most of the other PK specimens showed a diploid DNA histographic pattern with increased proportions of cells with the DNA content of hyperdiploidy and/or tetraploidy (Fig. 3 B). PK spcimens with an acanthotic change had significantly higher DNA index values (1.16 ± 0.0367) than those with non-acanthotic epidermis (1.10 ± 0.0360, $p < 0.05$). Specimens including cornoid lamella had 1.09 ± 0.032. All the normal-appearing skin from control donors showed a diploid pattern without an increased proportion of such cells (Fig. 3 C), of which the DNA index values were 1.03 ± 0.0245.

Chromosome analysis of dermal fibroblasts

Cultured were three different dermal fibroblast strains from different parts of the PK lesion and a strain from normal-appearing skin surrounding a lesion in case 3. Cultured cells, passaged twice, were incubated overnight with 0.02 μg/ml colcemid. Chromosome preparations were then made. The cells were treated with 0.075 M potassium chloride, fixed in a 3 : 1 methanol and glacial acetic acid solution, and stained with Giemsa stain. G-banded (trypsin treatment) and/or Q-banded (quinacrine mustard dihydrochloride staining) karyotypes were studied when necessary for the detailed analysis.

The results are summarized in Table I. The major karyotype of cultured dermal fibroblasts from the patient was 46XY. No clonal proliferation of cells with an abnormal karyotype could be found, although several different kinds of structural abnormalities were present in a few cells.

DISCUSSION

PK sometimes features a solitary large skin lesion, or a few large lesions with or without small ones. Large PK lesions usually take many years to develop, from 6 to 40 years in our 3 cases and more than 20 years in cases in our Japanese literature survey (4). Although our 3 cases have not yet developed malignant skin tumours, large PK lesions have been reported to be a frequent precursor of malignant changes (4, 5).

The histopathology revealed that some part of the patients' large PK lesional epidermis was slightly or markedly acanthotic, with or without the elongation of the rete ridge, which is different from the usual atrophic feature of small typical PK skin lesions. Cells having hyperchromatic, large, or irregular shaped nuclei lay in the epidermis, which can be regarded as slightly dysplastic cells, or which give the impression of abnormal clonal cells. Cellular DNA ploidy abnormalities, such as polyploid cells and an increased proportion of cells having hyperdiploid and/or tetraploid DNA content, also suggest that an abnormal clone or clones, which has been presumed to exist (6) and has recently been demonstrated in our previous study (2), is present in the epidermis of the large skin lesions in the present study. Since these DNA ploidy abnormalities suggest the existence of abnormal neoplastic cells responsible for mitotic irregularities, and/or an increase in proliferating cells in the S or G_2/M phase range of the cell cycle, greater abnormalities of DNA ploidy, which were reflected in the increased DNA index values of acanthotic epidermis suggest a greater number of abnormal cells and/or proliferative activity. Although the greater DNA index values were not at the margin but in the large skin lesion, such proliferating potential may contribute to the enlargement of skin lesions. Furthermore, it is possibly a reflection of an early malignant codition, since great DNA ploidy abnormalities are usually a characteristic of malignant tumours rather than a benign condition. This view may support the literature evidence that malignancy often develops in the non-peripheral area of large PK lesions (5).

PK is known to be inherited in an autosomal dominant fashion, although sporadic cases are often encountered, the latter of which have been presumed to result from somatic mutation (7). Neoplastic and potentially malignant clones may derive from the genetically determined predisposition. Chromosomal instability has been reported in cultured dermal

fibroblasts derived mainly from PK skin lesions, which suggests a relationship to malignant tumour development (8, 9). Although we examined chromosomal abnormalities in cells from various sites in the lesion and the normal-appearing skin of case 3, no clonal proliferation of such abnormal cells could be detected, nor could the specific site of such abnormalities be identified. However, various kinds of structural abnormalities were found in fibroblasts of this patient's skin. These abnormal cells may explain the development of abnormal clones in the epidermis of the PK skin lesion, even though the mechanism or process has not been clarified.

Our present study suggests that large PK skin lesions contain proliferating clones in the epidermis, which are probably responsible for the formation of large skin lesions.

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