Cell Cycle Analyses of Extramammary Paget's Disease

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Summary: The cell cycle of Paget cells in extramammary Paget's disease was investigated using microscopic analysis of the DNA content, and using immunohistochemistry for 5-bromodeoxyuridine (BrdU) incorporation. Stripped skin was treated with tetrasodium ethylenediamine tetraacetate dihydrate (EDTA) and trypsin, and epidermal cells suspensions were obtained. Microscopic analysis of the DNA content in the cells was performed. The resulting DNA histogram was close to the normal ploidy. Specimens from the involved skin were incubated with BrdU, and sections were immunostained with anti-BrdU monoclonal antibody. A few nuclei showed positive staining for BrdU. The present study showed that the proliferative potential of Paget cells in extramammary Paget's disease was low. This may explain why lesions of extramammary Paget's disease enlarge rather slowly.

Key words: Paget cells—immunohistochemistry—BrdU—DNA histogram—epidermal cell suspension

Introduction

Extramammary Paget's disease usually appears as a well-marginated erythematous patch or plaque whose surface may be scaly, crusted or eroded. It is most likely a primary intraepidermal apocrine carcinoma that occurs most commonly in the anogenital region. Alternatively, Jones et al. (1979) have suggested that extramammary Paget's disease may represent a secondary involvement of the skin and its adnexa by underlying carcinomata of the gastrointestinal and genitourinary tracts. Extramammary Paget's disease commonly spreads well beyond its obvious topographic borders for several years before it causes concern. Thus, the lesions enlarge rather slowly. We have previously examined the DNA distribution pattern in three cases of extramammary Paget's disease (Mori et al. 1993). The results obtained showed that there were no significant differences between Paget cells and normal keratinocytes.

In 1982, Gratzner reported a novel approach to detection of cells in the S (synthetic)-phase of the cell cycle, based on the use of monoclonal antibody specific for the thymidine analogue 5-bromodeoxyuridine (BrdU). Accordingly, it was of interest to examine S-phase Paget cells in extramammary Paget's disease using anti-BrdU antibody. The observations may support our previous study by the DNA histograms, and may explain the slow enlargement of the eruptions in the disease.

Materials and Methods

Skin samples were obtained from a 78
A 70-year-old man with extramammary Paget's disease. The patient had a scaly erythematous area on the scrotum, penis, and adjacent skin. A biopsy of the lesion was taken and histopathologic confirmation of the finding of extramammary Paget's disease was carried out. Immunohistochemically, the Paget cells were positive for carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA), and were negative for ras p21 (Mori et al. 1989, 1990). Wide excisions, 5 cm away from the clinical limits of the eruption including both superficial inguinal lymphadenectomy, were performed.

**Immunostaining**

Specimens (1 mm in diameter and about 1 mm thick) were washed in cold phosphate-buffered saline (PBS) and floated, dermal face downward, in a 5 ml glass bottle containing 2 ml of RPMI 1640 medium (Nissui, Tokyo, Japan) with 20% FCS (fetal calf serum) and 400 µM BrdU (SIGMA, St Louis, MO) for 1 h at room temperature in air at 2 atmospheres pressure. The specimens were rinsed in PBS, fixed in 70% ethanol for 24 h, embedded in paraffin and cut into sections of 4 µm. The sections were deparaffinized, denatured with 2 N hydrochloric acid for 1 h at room temperature and rinsed with PBS.

Immunostaining for BrdU was performed using the avidin-biotin complex (ABC) technique described by Hsu et al. (1981). Endogenous peroxidase activity was blocked by incubating the sections with methanol that contained 0.3% hydrogen peroxide at room temperature for 20 min. After 20 min incubation with 1/20 diluted normal goat serum, the sections were labeled with antibody against BrdU (Becton-Dickinson, Mountain View, CA) diluted 1/25 with PBS at 4°C for 18 h. After washing with PBS, sections were allowed to react with biotinylated goat anti-mouse IgG for 20 min. After rinsing with PBS, the sections were incubated with avidin biotinylated horseradish peroxidase complex (ABC) (Vector Labs, Burlingame, CA) for 20 min. The sections were then treated with a solution containing 20 mg of 3-amino-9-ethylcarbazol (Sigma) dissolved in 5 ml of N,N-dimethylformamide, 95 ml of 0.1 mol/l acetate buffer at pH 5.2, and 0.1 ml of 30% hydrogen peroxide for 5 min. The sections were counterstained with Mayer's hematoxylin and mounted with glycogen jelly without dehydration.

**DNA cytofluorometry**

Epidermal cell suspensions were prepared by the previously described method with some modifications (Mori et al. in press). Briefly, split skin sections were taken with a keratome set at 0.5 mm. The tissue sections were cut into small pieces (approx. 5×1 cm) and incubated at 37°C for 60 min in an isotonic solution of 0.01 M phosphate-buffered saline (PBS, pH 7.3) containing 20 mM of tetrasodium ethylenediamine tetraacetate dihydrate (EDTA). Epidermal sheets were separated from the dermis with forceps, and rinsed in Hanks balanced salt solution (HBSS). The epidermal sheets were incubated in a 0.25% solution of lyophilized trypsin (1:250, Difco, MICH) in HBSS at 20°C for 20 min, and then filtered through a fine mesh nylon net (0.2 mm pore size). The resulting cell suspensions were washed three times in HBSS.

For staining DNA, the Feulgen method was used. Details of the technique used have been published earlier (Sasai et al. 1984).

**Results**

Histological examination revealed no evidence of dermal invasion and metastases of regional lymph nodes in the case.
Immunostaining

In hair follicles, several labeled nuclei were observed, whereas in Paget cells only a few nuclei were positive for BrdU (Fig. 1). In the epidermis rare nuclei of suprabasal keratinocytes showed positivity for BrdU.

DNA cytofluorometry

In the whole epidermal cell suspension, the purity of Paget cells, identified on the basis of a positive reaction for CEA, was about 35%. The majority of the cells contained 20 AU (arbitrary unit) of DNA. A relatively small number of cells exhibited approximately 40 AU (Fig. 2). There were no significant differences between this case and other cases reported earlier (Mori et al. 1993).

Discussion

This study showed that the proliferative potential of Paget cells in extramammary Paget's disease was low as determined immunohistochemically with anti-BrdU monoclonal antibody, and also cytofluorometrically with the Feulgen method. We had previously reported on the DNA content of Paget cells microscopically in three cases of extramammary Paget's disease, and found that the DNA histograms were close to the normal ploidy. In this case, the DNA distribution pattern was also almost similar to the previous reports. We investigated in this study whole epidermal cell suspension without density gradient centrifugation with Percoll, since no differences were
noted in the distribution of Paget cells among each fraction, nor between each fraction and whole epidermal cell suspension in our previous study (Mori et al. in press). As a result, the purity of Paget cells was not very high. However, in cytofluorometry, one can select Paget cells by the characteristic appearance. Accordingly, whole epidermal cell suspension may be available on cytofluorometry of Paget cells instead of the fractions obtained by density gradient centrifugation.

In the present study, we examined a case of extramammary Paget's disease without any dermal invasion or metastases of regional lymph nodes. Further investigations are necessary to determine whether or not the Paget cells with dermal invasion or metastases exhibit a different cell cycle. In addition, it will be necessary to determine the rate of cell loss via apoptosis, since this clearly also contributes to the observed growth rate. Studies to this end are in progress.

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**References**


