Paget cells express cytokeratins typical of glandular epithelia

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SUMMARY

The expression of cytokeratins in Paget cells in mammary and extramammary Paget's disease was studied using different keratin antibodies and immunofluorescence microscopy. Antibodies to epidermal keratin did not react with the Paget cells but stained the surrounding epidermis. Two monoclonal cytokeratin antibodies (PKK1 and RGE 53), which reacted typically with simple glandular epithelia in normal tissues, brightly stained the Paget cells and left the surrounding epithelial cells unstained. The results indicate that Paget cells are derived from mammary or sweat duct epithelium rather than from epidermal cells.

Cytokeratins, the subunit proteins of the intermediate filaments of epithelial cells, are a group of proteins consisting of several different polypeptides, which are expressed specifically in different epithelial tissues. Thus, simple glandular epithelia of parenchymal organs contain cytokeratins different from those of stratified squamous epithelium (for a review, see Moll et al., 1982; Osborn & Weber, 1983). Unexpectedly, Mazoujian, Pinkus and Haagensen (1984) failed to demonstrate keratin positivity in Paget cells, which both ultrastructurally (Belcher, 1972) and immunohistochemically (Nadji et al., 1982; Kariniemi et al., 1984) present features compatible with an apocrine epithelial origin. In this study, using different cytokeratin antibodies, we show the presence of cytokeratins in Paget cells of both mammary and extramammary Paget's disease. This shows that Paget cells originate from simple glandular epithelium.

METHODS

Paraffin-embedded specimens from six cases of mammary and seven cases of extramammary Paget's disease were collected from the Department of Dermatology, Helsinki University Central Hospital. In addition, specimens, snap-frozen in liquid nitrogen, from two cases of

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mammary Paget's disease were also studied. All cases of mammary Paget's disease were associated with an intraductal or ductal adenocarcinoma of the breast, whereas no underlying malignancy was found in any of the extramammary cases, which were located in the skin of vulva, pubis or axilla.

Keratin antibodies

Rabbit antibodies to epidermal keratins. Prekeratin polypeptides were isolated from human plantar callus as described earlier (Virtanen et al., 1981). Antibodies were raised in rabbits and were rendered monospecific using immunoadsorption (Virtanen et al., 1981).

Monoclonal antibodies. Monoclonal cytokeratin antibodies were raised against cytoskeletal polypeptides of a pig kidney epithelial cell line (LLC-PK, American Type Culture Collection) by the hybridoma technique of Köhler, Howe and Milstein (1976) as described earlier (Holthöfer et al., 1983). The PKK1 antibodies react in human tissues with all simple glandular epithelia, but do not react with the epidermis. The other mouse monoclonal cytokeratin antibody, RGE 53, was raised against HeLa cell cytoskeleton and human callus keratins as described earlier (Ramaekers et al., 1983). This antibody shows a fibrillar staining with HeLa cells, but does not react with human epidermal cells. Instead, the RGE 53 antibodies specifically bind only to simple glandular epithelia of parenchymal organs and also to adenocarcinomas derived from them. These antibodies appear to react solely with cytokeratin 18, according to the nomenclature of Moll et al. (1982) (F. Ramaekers, unpublished results).

Staining procedures
The formalin-fixed, paraffin-embedded sections were deparaffinized, exposed to 0.4% pepsin in 0.01 N HCl (2 h, 37°C) and washed with phosphate-buffered saline (PBS). The frozen sections

![Figure 1. Extramammary Paget's disease in the pubic skin. (a) Staining with epidermal keratin antibodies reveals positivity in all epidermal cells, while Paget cells lack positivity. (b) Phase contrast (original magnification ×400).](image-url)
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were fixed in cooled methanol at $-20^\circ$C for 10 min. The sections were incubated with the different keratin antibodies for 30 min, washed thoroughly and further exposed either to fluorescein isothiocyanate (FITC)-coupled rabbit anti-mouse IgG or goat anti-rabbit IgG antisera (Cappel Laboratories, Cochranville, U.S.A.). The sections were washed three times, then mounted in Veronal-glycerol (1:1, pH 8.4). A Zeiss Standard microscope equipped with the epi-illuminator IIIRS and filters for FITC fluorescence was used.

Figure 2. Extramammary Paget’s disease in the vulva. (a) Staining with PKK1 cytokeratin antibodies reveals positivity in Paget cells, whereas epidermal cells remain negative. (b) Phase contrast (original magnification $\times 400$).

Figure 3. Frozen section of mammary Paget’s disease stained with RGE 53 cytokeratin antibodies. (a) Positive staining result is only seen in Paget cells. (b) Phase contrast (original magnification $\times 400$).
RESULTS

Both in paraffin and frozen sections the Paget cells were easily recognized in phase-contrast microscopy as large, pale cells. They were not connected to the surrounding keratinocytes by typical saw-like intercellular bridges, as the epidermal cells were. The epidermal keratin antibodies did not stain the Paget cells either in formalin-fixed or frozen sections in any case of mammary and extramammary Paget's disease, whereas the surrounding epidermal cells were positive (Fig. 1a,b). Instead, the PKK1 antibodies stained brightly the Paget cells, but did not react with the surrounding epidermis (Fig. 2a,b). The PKK1 antibodies bound also to mammary and sweat gland and duct epithelial cells both in paraffin and frozen sections.

In two cases of mammary Paget's disease the RGE 53 antibodies, reacting only in frozen sections, brightly stained both the Paget cells and the mammary epithelium, but did not bind to epidermal cells (Fig. 3a,b).

DISCUSSION

Many recent studies have shown that different cell types in mammalian tissues show a cell type-specific expression of intermediate filaments, both mesenchymal cells, muscle cells, epithelial cells, glial cells and neuronal cells containing a distinct intermediate filament type, vimentin, desmin, keratin, glial fibrillary acidic protein or neurofilaments, respectively (Osborn & Weber, 1983). Tumour cells appear to maintain the expression of the specific intermediate filament type (Osborn & Weber, 1983). Thus, demonstration of keratin in malignant tumours can be used to confirm the epithelial origin of the tumour. However, keratins are a large family of polypeptides, different epithelia expressing specifically different polypeptides (Moll et al., 1982). Epidermal keratin antibodies in particular, which are most often used in these studies, typically react only with stratified epithelia and often do not stain simple glandular epithelial cells or adenocarcinomas. The monoclonal cytokeratin antibodies PKK1 (Virtanen et al., 1984) and RGE 53 (Ramaekers et al., 1983) used in this study bind to all simple glandular epithelia, but not to epidermal cells. In addition, the RGE 53 antibodies react only with various malignant neoplasms of glandular origin (adenocarcinomas) but not with squamous cell carcinomas or tumours of mesenchymal origin (Ramaekers et al., 1983). The distribution of cytokeratins recognized by the monoclonal PKK1 antibodies seems to be wider in malignant epithelial neoplasms, because in addition to adenocarcinomas, various squamous cell carcinomas also react with these antibodies (Holthöfer et al., 1983; Miettinen et al., 1984; Virtanen et al., 1984).

Our results show that the Paget cells, reacting with the PKK1 and RGE 53 antibodies, both in mammary and extramammary Paget's disease express the same types of cytokeratins, which are present in mammary and sweat gland and duct epithelia, but not in stratified squamous epithelium. Instead, epidermal keratin-like immunoreactivity could not be shown in Paget cells. Our findings thus explain the surprising results of Mazoujian et al. (1984), indicating lack of keratin in Paget cells. Their antibodies, staining the surrounding epidermal cells, but not the Paget cells, were raised against epidermal keratins, thus failing to react with Paget cells in line with our results. The expression of cytokeratins typical of simple epithelia in Paget cells together with earlier results with other differentiation markers (Nadji et al., 1982; Kariniemi et al., 1984; Mazoujian et al., 1984) suggest that the Paget cells originate from mammary or sweat duct epithelium and not from epidermal cells.

Extramammary Paget's disease may be difficult to differentiate histologically from Bowen's
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disease or pagetoid malignant melanoma. Demonstration of the simple epithelium-type of
cytokeratins and absence of epidermal keratins in Paget cells may be helpful in differential
diagnosis, because squamous cell carcinoma contains epidermal keratin-like immunoreactivity,
whereas malignant melanoma does not contain keratin, but expresses vimentin (Osborn &

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REFERENCES

of Pathology, 94, 59.

origin and differentiation of renal carcinomas. A fluorescence microscopic study with kidney-specific antibodies,
antiintermediate filament antibodies and lectins. Laboratory Investigation, 49, 317.


cell lines. European Journal of Immunology, 6, 292.

origin. An immunoperoxidase study of gross cystic disease fluid protein-15, carcinoembryonic antigen, and keratin
proteins. American Journal of Surgical Pathology, 8, 43.

proteins in thyroid gland and thyroid tumors. Laboratory Investigation, 50, 262.

patterns of expression in normal epithelia, tumors and cultured cells. Cell, 34, 11.


surgical pathology. Laboratory Investigation, 48, 372.

to keratin filaments, specific for glandular epithelia and their tumors. Use in surgical pathology. Laboratory
Investigation, 49, 353.

VIRTANEN, I., LEHTO, V.-P., LEHTONEN, E., VAKTO, T., STEINMAN, S., KURKI, P., WAGNER, P., SMALL, J.V., DAHL, D. &