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Toker cells are probably precursors of Paget cell carcinoma: a morphological and ultrastructural description

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Abstract The present paper documents an investigation of the morphology, immunohistochemistry, and ultrastructure of Toker cells (TC), aiming for a better definition of these elements and better understanding of their histogenesis. We studied 12 nipples removed for nipple adenoma from twelve patients and a case of supernumerary nipple. In addition four cases of Paget's carcinoma (PC) restricted to the nipple without underlying tumor were studied for comparison. All cases were stained with hematoxylin and eosin (H&E), Alcian blue pH 2.5 and periodic acid-Schiff (PAS) preceded by diastase digestion and with immunohistochemistry using antisera anti cytokeratin 7, cytokeratin 20, protein S100, GCDFP-15, c-Erb-B2, CAM 5.2, and epithelial membrane antigen (EMA). Two cases from the nipple adenoma series were studied by electron microscopy. In seven cases within the series of 12 nipple adenomas as well as in the case of supernumerary nipple, keratin 7 antibody highlighted numerous cells located within the nipple epidermis which in three cases showed dendritic processes. These same elements were also positive with CAM 5.2. All these same elements were negative with Alcian Blue (AB), PAS and the other antisera employed. Ultrastructural examination demonstrated that these cells differed from keratinocytes while they presented the same features as the glandular cells seen in the related nipple adenoma. The cells constituting Paget's carcinoma showed more irregular nuclei and were more easily seen in the context of the epidermis. The immunocytochemical profile of the cancer cells was similar to that of TC, but in addition the neoplastic cells were c-Erb-B2 and EMA positive in all cases, and one case also displayed numerous cells immunoreactive with anti GCDFP-15 antibody. Keratin 7 highlighted dendritic cells in two cases and AB, PAS was negative in all patients. The immunocytochemical profile and the ultrastructural features of TC are similar to those of the glandular cells constituting the ducts and the adenoma. These findings together with the localization of TC near or around the openings of the lactiferous sinuses indicate that TC might be ductal cells with a dendritic aspect and migrate through the galactophorous ostia. PC cells not related to ductal carcinomas have a similar but not superimposable immunohistochemical profile to TC, and in two cases the neoplastic elements were also dendritic which suggests that these same cells are likely to be the neoplastic counterpart of TC.

Keywords Toker cells · Ultrastructure · Paget cells · Nipple · Breast

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Introduction

Toker cells (TC) were described in full detail by Toker [16], although they had been previously observed but not recognized by Orr and Parish [10]. TC have a roundish, bland nucleus and paler cytoplasm than the surrounding keratinocytes and are difficult to identify at the haematoxylin and eosin (H&E) level. They are smaller than typical PC cells, but larger than their squamous neighbors [16]. TC are histologically evident at the summit of normal nipples as well as in the skin from the areola, although they are most numerous immediately above the opening of lactiferous sinuses [7]. At the H&E level they were present in 9% of surgical material and in 23 nipples out of a series of 190 nipples (12%) from 101 post-mortems; their number varied from "scattered individuals" to

many clear cells [16]. Use of keratin 7 antibody raised the incidence of TC to 83% in a series of non neoplastic post-mortem patients [7]. TC are a diagnostic problem since their distinction from Paget cells is difficult, especially in those cases in which they are numerous when associated with nipple adenoma [18]. Immunohistochemistry is useful though not distinctive since TC share with PC antigenic properties such as immunopositivity for keratins (7 and CAM 5.2) and inconsistently for EMA [6], as well as negativity for keratin 20 and S100 protein [7]. GCDFP-15 is occasionally positive in PC while it is negative in TC [7]. Probably the only helpful immunostain to distinguish the two cell types is c-Erb-B2 antibody which are negative in TC [18] but consistently positive in PC [1]. Therefore, as repeatedly stressed by Toker, the differential diagnosis resides on absence of cytological stigmata of malignancy and absence of carcinoma in lactiferous ducts [16]. The purpose of the present paper is to draw attention to the histogenesis of TC, whether they are cellular extensions from the epithelium of the ducts or remnants from embryonic tissue [14, 16]. We chose a series of nipple adenomas, since Toker cells are frequent in this condition. Furthermore, the first ultrastructural description of these elements is provided. Finally, TC are compared to a series of Paget's carcinoma in which no underlying tumor was present. This was undertaken to investigate whether a true relation exists between TC and PC.

Materials and methods

Cases and clinical data

We studied 12 nipples removed for nipple adenoma and randomly selected. Patient ages ranged from 29 to 69 years (median 50), and one patient was male. In addition a supernumerary nipple from a 22-year-old woman was included in the study. The cases of nipple adenoma were treated with complete nipple excision in nine cases, and wedge biopsy followed by tylectomy in the remaining patients. Follow-up (FU) ranged from 3 to 14 years (mean 8.2). Neither recurrences nor metastases were seen.

Four cases of Paget's carcinoma restricted to the nipple without underlying tumor as demonstrated in multiple blocks after mastectomy (from four women aged 77, 70, 66, and 60) were also included in the study. FU ranged from 2.5 to 12 years (average 7.8). No recurrence was seen in these four.

The cases came from the files of the Section of Anatomic Pathology, of the Department of Oncology, University of Bologna, at Ospedale Bellaria, Bologna, Italy, from the files of the Department of Pathology, Institute of Oncology of Ljubljana, Slovenia, and from the Department of Pathology of the Netherlands Cancer Institute of Amsterdam.

Tissues were formalin fixed and routinely processed to paraffin. Blocks were serially cut and stained with H&E, Alcian blue pH 2.5 and periodic acid-Schiff (PAS) preceded by diastase digestion. The ABC immunohistochemical method was followed. An epitope retrieval method was employed as as now described. Before immunostaining, sections were steamed in citrate buffer for 5 min and cooled for 5 min. Source and dilutions of the antisera in use were: cytokeratin 7 [clone OVTL12/30, Dako (Glostrup, Denmark), dilution 1:100], cytokeratin 20 (clone Ks20.8, DAKO, dilution 1:40), protein S100 (DAKO, polyclonal, dilution 1:1500), GCDFP-15 [clone D6, (DBA, Milan, Italy) dilution 1:300], c-Erb-B2 [clone CB11, (Neomarkers, Freemont, Calif., USA) dilution 1:50], Cam 5.2 [clone CAM 5.2, (Becton Dickinson, Erembogen-Aalst, Belgium) prediluted], and EMA (clone E29, DAKO, dilution 1:80).

Ultrastructural study

Two nipple adenoma cases (cases 10 and 12),were studied by electron microscopy (EM).

Tissue from each case was obtained from de-waxed paraffin blocks. After staining with keratin 7 antibody, small blocks were microdissected where TC were most numerous as seen at the immunohistochemical level. In case 12 tissue from the adenoma was obtained for comparison.

The small biopsies were routinely de-waxed by immersion overnight in xylol and rehydrated to phosphate buffer, through a graduated series of alcohol dilutions. Biopsies were then re-fixed in buffered glutaraldehyde and post-fixed in OsO4, dehydrated in alcohol, and embedded in Epon 812 (Fort Washington, Pa., USA). Thick sections stained with toludine blue were used to select areas with major numbers of TC. Thin sections were stained with uranyl acetate and Reynold's lead citrate, and observed in a Philips CM 10 TEM (Eindhoven, Netherlands).

Results

Light microscopy

The 12 cases of adenoma of the nipple displayed the features of the glandular proliferation of the upper portion of the nipple beneath the squamous epithelium of the epidermis. Eight cases were characterized by florid epithelial hyperplasia while the remaining were represented by the sclerosing adenosis pattern [11]. Squamous differentiation extended also to the superficial part of glands of the "adenomatous" proliferation.

In three of these cases single cells were seen within the squamous epithelium of the nipple with a round, bland nucleus and cytoplasm paler and slightly more copious than that of the surrounding keratinocytes (Figs. 1, 2). Their number did not exceed six pale cells per case and most were individually located along the suprabasal layer. In the epidermis of the supernumerary nipple no cells that differed from the keratinocytes were evident at H&E.

The four cases of PC were characterized by "easily" visible cells located along the epidermis. These cells were larger than keratinocytes, were individually located within the epidermis, but occasional small nests of them were evident. They appeared located anywhere within the Malpighian layer, although the suprabasal location was most frequent. Their cytoplasm was eosinophilic and abundant; the nuclei were large, irregular, with evident nucleoli (Fig. 3).

Immunohistochemistry

In eight cases of the 12 nipple adenomas (67%) keratin 7 antibody stained numerous individually located cells in the epidermis (or arranged in small nests in one case) (Fig. 4), as well as staining the cells lining the glandular

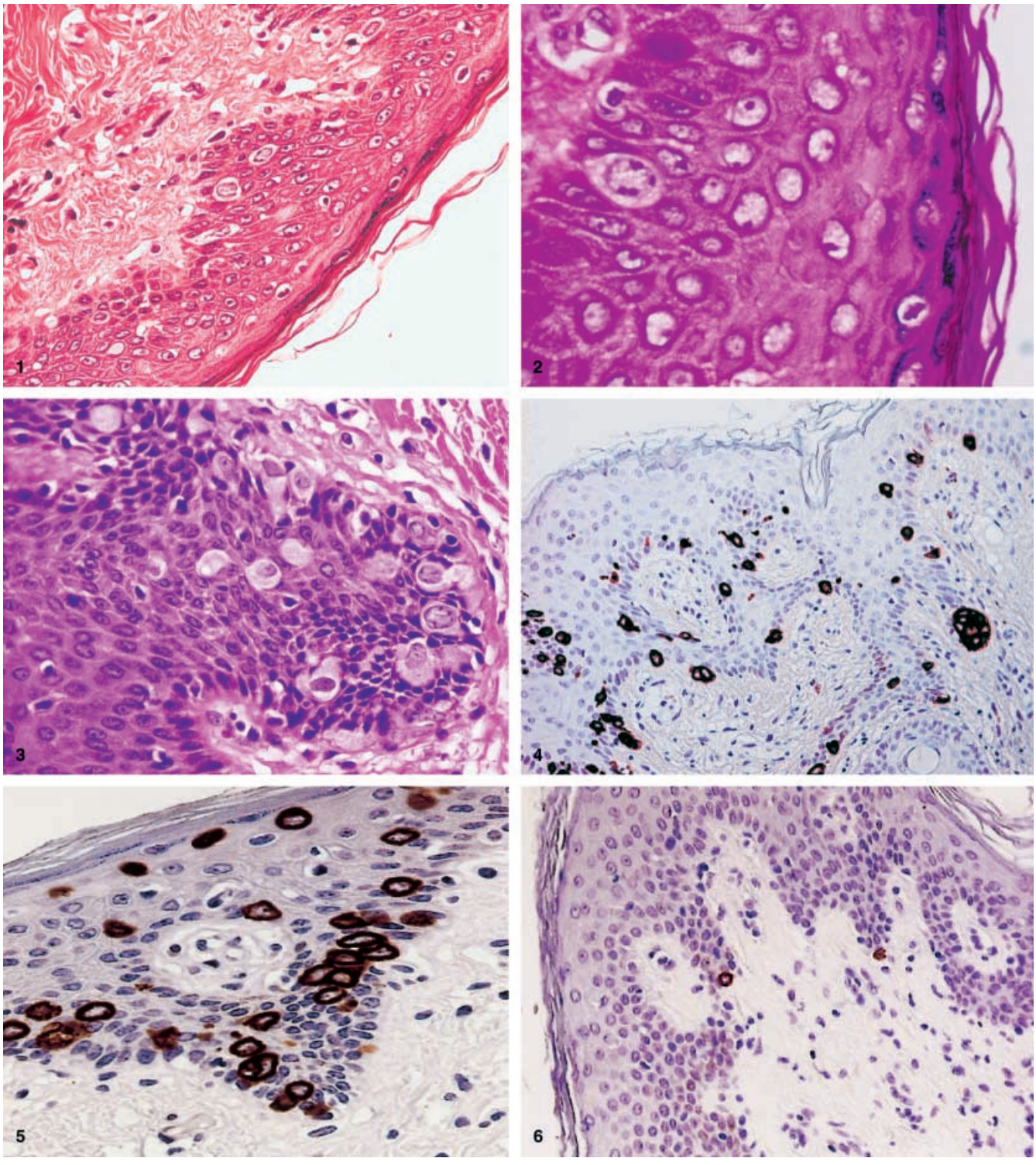


Fig. 1 Case 2: Toker cells: sparse cells with cytoplasm, paler than that of the keratinocytes, are immersed within the Malpighian layer (H&E, ×200, original magnification)

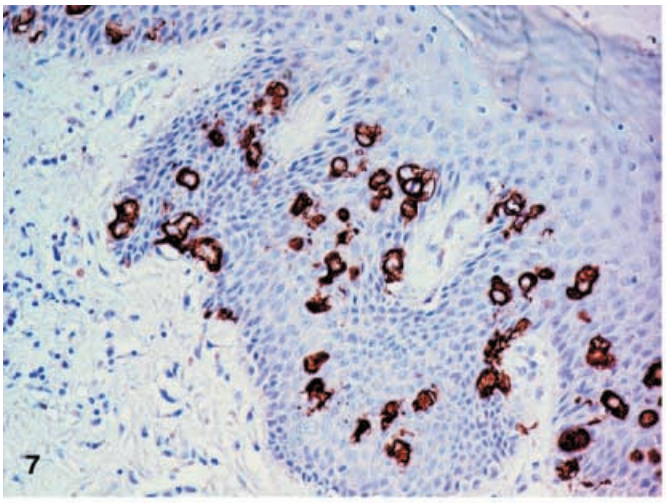
Fig. 2 Case 2: These Toker cells show round nuclei with dispersed chromatin (H&E ×400, original magnification)

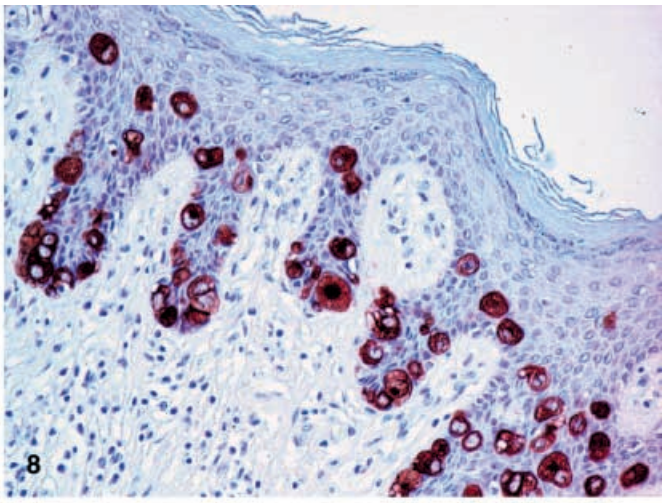
Fig. 3 Case 14: PC cells: the intraepidermal cells of this case of PC show abundant cytoplasm and irregular nuclei (H&E ×250, original magnification)

Fig. 4 Case 10: Toker cells: in this case numerous cells are stained by keratin 7. The cells are individually located within the epidermis or are arranged in small nests. Notice thin dendritic processes. This case figures Toker cell hyperplasia (ABC immunoperoxidase, ×175)

Fig. 5 Case 10: Toker cells: keratin 7 stains globoid elements that are mostly located in suprabasal position (ABC immunoperoxidase, ×250, original magnification)

Fig. 6 Case 4: Merkel cell: one cell only, basally located, is stained by keratin 20 (ABC immunoperoxidase, ×200, original magnification)





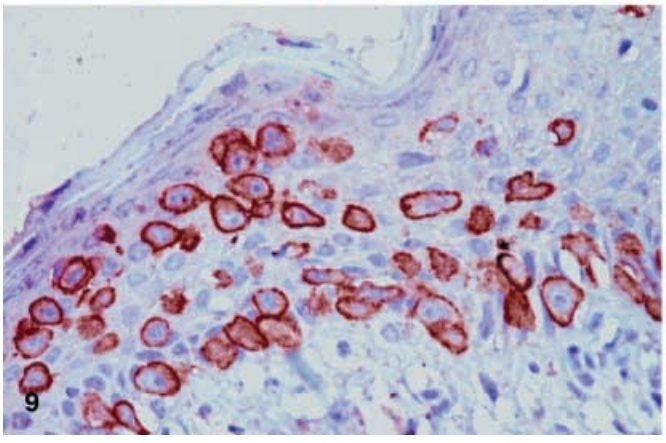


Fig. 7 Case 14: Numerous cells of this PC are stained by Keratin 7. Occasional cells display cytoplasmic "dendritic" projections (ABC immunoperoxidase, ×175 original magnification)

Fig. 8 Case 16: PC. Numerous cells appear stained for EMA (ABC peroxidase, ×250, original magnification)

Fig. 9 Case 16: PC: the cytoplasmic membrane is nicely outlined by the anti c-ErB-2 antibody (ABC peroxidase, ×400, original magnification)

Table 1 Histochemical and immunohistochemical profile of intraepidermal cells. *NA* nipple adenoma, *SN* supernumerary nipple, *PC* Paget's carcinoma, *nd* not done, *CK* cytokeratin, *APO* GCDFP–15, *AB* Alcian blue, *NEU* c–Erb–B2

Cases	CK7	CK20	CAM5.2	NEU	EMA	APO	AB PAS	S100
1 NA	–	–	–	–	–	–	–	–
2 NA	+	–	+	–	–	–	–	–
3 NA	–	–	–	–	–	–	–	–
4 NA	+	+	–	–	–	–	–	–
5 NA	–	–	–	–	–	–	–	–
6 NA	+	–	+	–	–	–	–	–
7 NA	–	–	–	–	–	–	–	–
8 NA	+	–	+	–	–	–	–	–
9 NA	+	–	+	–	–	–	–	–
10 NA	+	–	+	–	–	–	–	–
11 NA	+	–	+	–	–	–	–	–
12 NA	+	–	+	–	–	–	–	–
13 SN	+	–	+	–	–	–	–	–
14 PC	+	–	+	+	+	+	–	–
15 PC	+	–	nd	+	+	–	–	–
16 PC	+	nd	nd	+	+	–	–	–
17 PC	+	–	+	+	+	–	–	–

adenomatous proliferation and those of the large ducts (Table 1). The myoepithelial cell layer appeared unstained. The same pattern of staining was obtained by CAM 5.2 antibody which appeared less sensitive than keratin 7 as it stained the intraepidermal cells in only seven cases.

Most of the stained cells located in the epidermis were globoid (Fig. 5), but in three cases occasional cells showed short dendritic cytoplasmic processes (Fig. 4). All these cells were immersed within the Malpighian layer, mostly located in the suprabasal position with occasional elements dispersed in the upper layer of the epi-

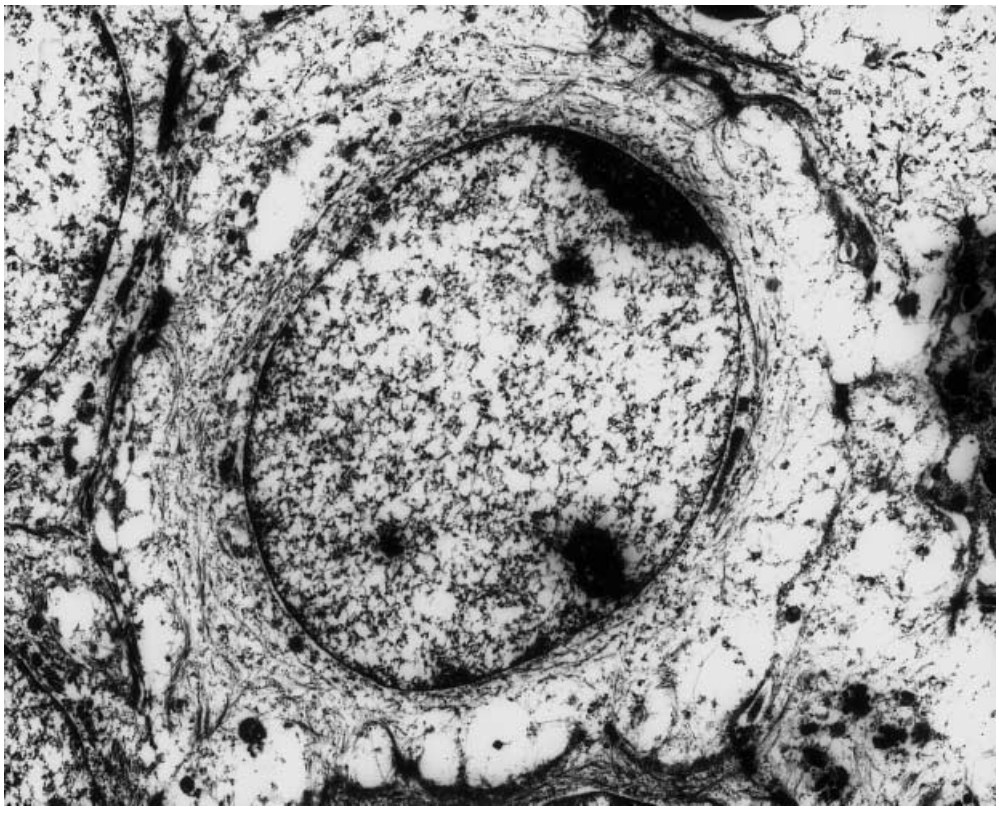


Fig. 10 Case 10: This cell has a round nucleus with three small nucleoli. The cytoplasm contains thin filaments and the cell membrane shows a festoon-like polycyclic profile (×15,500, *bar* 1 µm)

dermis. TC averaged one positive cell for every 70 keratinocytes in the affected epidermis. The tip of the nipple was the site of the major concentration of these elements, but positive cells were also found individually scattered through the entire nipple epidermis. In two cases, positive cells were also seen along the walls of lactiferous sinuses, especially at the ductal-epidermal junction. All other antibodies tested (keratin 20, c-Erb-B2, S-100 protein, EMA, GCDFP-15) were found to be consistently negative.

Case 4 was characterized by rare cells situated along the basal layer of the epidermis that were positive both for keratins 7 and 20, while dendritic keratin 7-positive cells located within the epidermis were not observed in this case (Fig. 6).

In the supernumerary nipple rare keratin 7 and CAM 5.2 cells were scattered in the nipple epidermis showing the same distribution as observed in the other cases.

In 10 out of 12 cases of nipple adenoma, EMA stained the adenomatous cells. The staining was confined mostly to the luminal border of the cells while most of the remaining proliferating cells were unstained.

Paget's carcinoma

The pale neoplastic cells were strongly positive for keratin 7, CAM 5.2, c-Erb-B2, and EMA in all cases (Figs. 7, 8, 9), and GCDFP-15 was positive in only one case (case 14). All the other antibodies were consistently negative as was the AB PAS stain. PC cells, mostly globoid and averaging one for every ten keratinocytes, were spread along a long tract of the affected epidermis. In addition, in two cases occasional cells showed short dendritic processes (Fig. 7).

Electron microscopy

The intraepidermal clear cells from both cases that were examined by EM showed round to ovoid nuclei with one to three nucleoli and dispersed chromatin. The cells were round to elongated and one of them displayed short dendritic projections. The cytoplasmic membrane showed a festoon-like polycyclic profile with blunt extraflexions and rare hemidesmosomes. Rare organelles, sparse cytoskeletal filaments, and occasional dense bodies were present. These elements were surrounded by epidermal keratinocytes showing darker cytoplasm characteristically filled with thick bundles of keratin (Fig. 10).

In the adenomatous proliferation three types of cells were visible: 1- luminal cells with microvillous projections; 2- myoepithelial cells located mostly at the edge of the glands; and 3- elements showing the same features seen in the epidermal clear cells, except for the festooned and dendritic appearance. These last were the most numerous elements (Fig. 11).

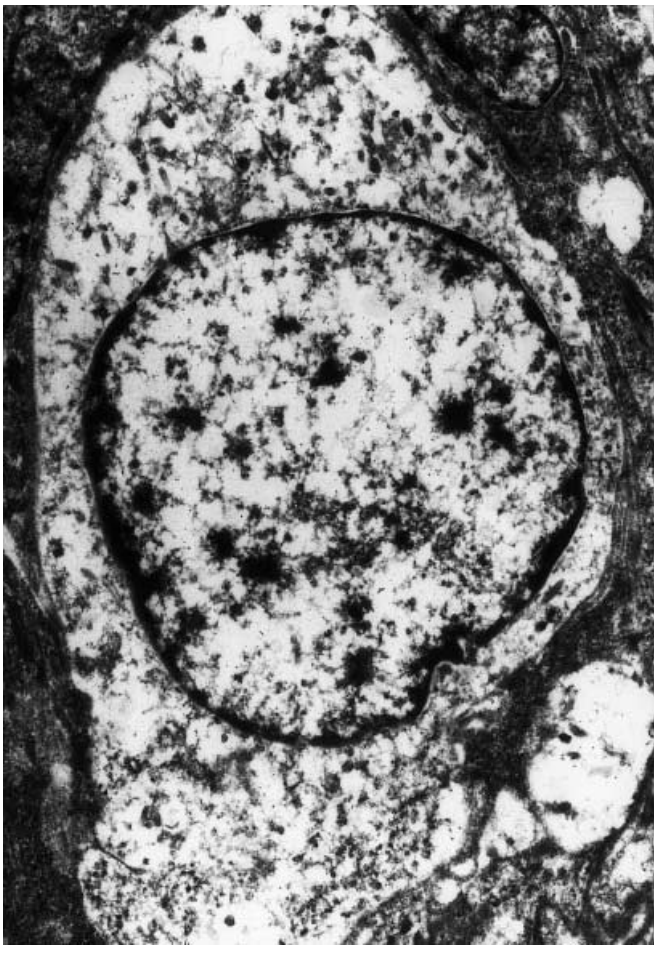


Fig. 11 Case 12: Cellular proliferation within the adenoma of the nipple. A lighter cell remarkably similar to the intraepidermal clear cells (×11,000, *bar* 1 μm)

Discussion

The clear cells of the nipple identified by Toker in 1970 [16] are still in need of histogenetic clarification. These cells, "normal" constituents of the nipple epidermis, occasionally constitute a diagnostic pitfall because of their similarity to PC cells, from which they must be distinguished. In our series of nipple adenomas and one supernumerary nipple, cells having the same histological and immunohistochemical features of TC as defined by Toker [16] and subsequently by Lundquist et al. [7] were seen in eight cases. TC were apparent on routine histology in three cases, mostly seen as individual elements located within the epidermis in a suprabasal position. Keratin 7 and CAM 5.2 selectively stained these cells and revealed positive elements in an additional five cases which had remained undetected with H&E. These cells were generally globoid and in two cases showed dendritic processes. Most keratin 7 and CAM 5.2 positive cells were located close to the lactiferous sinuses. All the other antibodies were consistently negative. One case (case 4) presented occasional cells situated along the basal layer. These showed Merkel cell features, being positive only to keratins 7 and 20 [8, 2].

Both cases of nipple adenomas studied ultrastructurally revealed that TC were easily distinguished from the surrounding keratinocytes, Langerhans cells, melanocytes, and Merkel cells. This was true because they lacked the features specific of each of these other cells, such as thick bundles of keratin, indented nuclei, Birbeck bodies, abundant melanosomes, or endocrine-like granules. Toker cells were globoid or showed dendritic cytoplasmic projections. Rare hemidesmosomes were evident and no microvillous cytoplasmic surface was seen, the latter a feature of PC [12]. In addition, when TC were compared to cells of the adenomatous glandular structures of case 12, no distinguishing features were observed other than dendritic features. The ultrastructure of these intraepidermal cells have not previously been illustrated; they are reminiscent only of the clear basal cell as described by Toker in normal breast ducts [15]. These elements do not have microvilli, but have thin cytoskeletal filaments. The lack of microvilli might explain the EMA negativity in TC.

In view of the fact that ductal (clear basal cells) and TC share similar immunohistochemical and ultrastructural features, and considering that TC are found predominantly along the opening of lactiferous sinuses, it seems likely that ductal cells migrate from the lactiferous sinuses into the epidermis. This is also suggested by the dendritic features of TC occasionally observed with cytokeratin 7, and probably, by analogy with PC as suggested by De Potter et al., reflects release of cytokines from keratinocytes [3, 4]. Schelfhout et al. [13] have shown that keratinocytes produce heregulin-alpha, a motility factor. When this factor is added in vitro to breast carcinoma cells, these form long thin plasma membrane protrusions and pseudopodia, and move apart. However, a malformative aberrant line of differentiation of epidermal keratinocytes towards ductal "secretory" cells cannot be excluded [9], especially from those cases that do not harbor proliferative adenomatous lesions or, as in one case of the present series, TC are present in a supernumerary nipple.

The four cases of PC were characterized by cells that had an immunocytochemical profile similar to that of TC, with the exception that the PC cases were all consistently EMA and c-Erb-B2 positive. In addition, one PC case was also positive with GCDFP-15. Therefore, it seems that in this respect negativity with c-Erb-B2 antibody together with (as stressed by Toker) absence of cytological stigmata of malignancy militate against the diagnosis of PC.

Toker has suggested that mammary Paget's carcinoma arises either from duct neoplastic cells that migrate from neoplastic ducts or are the malignant counterpart of intraepidermal cells [16]. This is especially the case for the rare patients with PC without associated carcinoma in the rest of the breast. The case of mammary Paget's carcinoma confined to the areola and associated with multifocal TC hyperplasia [17] is consonant with this

view as well as the case of Paget's carcinoma of a supernumerary nipple that was difficult to distinguish from TC hyperplasia as reported by Decaussin et al. [5]. Finally the dendritic features (indicating motility of the cell) of two of the present cases of PC, similar to those seen in TC, together with the fact that the four cases did not have an associated carcinoma, are all features consonant with a strict relationship between the two processes.

In conclusion, the ultrastructural examination of two cases of nipple adenoma revealed that these TC have the same features as some ductal elements. We suggest that TC are ductal elements migrated to the nipple epidermis. PC unrelated to duct carcinoma are probably the malignant counterpart of TC.

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