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Intraepidermal Cytokeratin 7 Expression Is Not Restricted to Paget Cells But Is Also Seen in Toker Cells and Merkel Cells

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Histologically, extramammary Paget's disease and mammary Paget's disease (MPD) are characterized by large atypical cells distributed throughout the epidermis. Although classic examples of these disorders are easily diagnosed on morphologic grounds, some cases may cause differential diagnostic problems. Immunohistology with a wide variety of antibodies has been used as an aid for the identification of Paget cells, for their distinction from other entities, and for investigation of the origin or nature of the disorder. Recently, cytokeratin 7 has been proposed as a specific and 100% sensitive marker for Paget's disease. We studied 22 cases of mammary Paget's disease and 22 cases of extramammary Paget's disease with and without an underlying malignancy for their reactivity with monoclonal antibodies to cytokeratin 7 (CK7) and cytokeratin 20 (CK20). Our studies show that anti-CK7 is an effective but not 100% sensitive marker for Paget cells, staining 21 of 22 cases of mammary Paget's disease and 19 of 22 cases of extramammary Paget's disease, whereas CK20 stained 0 of 17 cases of mammary Paget's disease and 6 of 19 cases of extramammary Paget's disease. We also demonstrate that CK7, but not CK20, highlights intraepidermal clear cells with bland nuclear features (Toker cells) that have been reported in 11% of normal nipples. By using CK7 as a marker, however, we were able to identify Toker cells in most of the nipples we studied: 8 of 15 nipples from mastectomy patients without Paget's disease, and 15 of 18 autopsy cases (both male and female) with normal breasts and nipples. It also permitted us to perform more extensive phenotyping on them, showing that Toker cells share similar antigens with Paget cells and with cells lining the underlying normal lactiferous ducts. In 7 of 15 cases containing CK20-positive Merkel cells, CK7 was also seen to stain Merkel cells. In infrequent cases, Toker cells or Merkel cells may be so numerous focally that a CK7 stain may raise the possibility of involvement of the nipple by Paget's disease. An awareness of the CK7 reactivity of Toker cells and Merkel cells as well as attention to the cytologic features of the case should avoid this problem.
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Histologically, extramammary Paget's disease (EMP) and mammary Paget's disease (MPD) are characterized by large atypical cells distributed throughout the epidermis. Although classic examples of these disorders are easily diagnosed on morphologic grounds, some cases may cause differential diagnostic problems. Because of small biopsy size, paucity of atypical cells, or atypical presentation, the Paget cells may not be easily recognized or the lesion may engender a broad differential diagnosis. Lesions in the differential diagnosis range from the clinically insignificant incidental finding of Toker cells (TC) to malignant neoplasms such as melanoma, leading to clinical distinctions of great consequence.[9,10]

Mammary Paget's disease is almost always associated with an underlying malignancy and is generally considered to represent migration of tumor cells into the epidermis from the underlying breast carcinoma. Extramammary Paget's disease most commonly occurs in the vulva and perianal region but also has been reported in other apocrine gland–bearing regions including the axilla, eyelids, external ear canal, penis, and scrotum.[7] The origin of the cells in EMP is more controversial than in MPD because an underlying malignancy is identified in only 15–33% of cases.[7]

Toker cells are cells with bland cytologic features and clear cytoplasm that have been identified by standard light microscopic means in ~10% of normal nipples.[14,22,24] They have been proposed as a cell of origin for the rare cases of MPD without an underlying carcinoma[14,22,24] but have not been described in extramammary sites.

Immunohistology has been used as an aid for the identification of Paget cells, for their distinction from other entities, and for investigation of the origin or nature of the disorder. Antibodies previously used for these purposes include those reactive with epithelial markers, tissue-restricted markers, and tumor markers.[3,6,10,12,15,16,18,21,23] These various antibodies are not

100% sensitive and do not, in every case, distinguish Paget's disease or EMP from other processes in the differential diagnosis. Recent reports have proposed cytokeratin 7 (CK7) expression as a specific and 100% sensitive marker for the cells of both mammary and extramammary Paget's disease.[1,5,20]

We report here our studies demonstrating that although CK7 is an effective marker for Paget cells in MPD and EMP in paraffin-embedded tissue, it is not 100% sensitive. It must also be interpreted with a knowledge of its other reactivities, because CK7 also proves to be a sensitive marker for Toker cells, and an occasional marker of Merkel cells.

MATERIALS AND METHODS

Case Selection and Clinical Features

Twenty-two cases of EMP and 22 cases of MPD were obtained from the surgical pathology files of Stanford University Hospital (40 cases), Palo Alto Veterans Affairs Medical Center (two cases), Santa Clara Valley Medical Center (one case), and Cornell University Medical Center (one case). Formalin-fixed, paraffin-embedded skin biopsy specimens were stained with hematoxylin-eosin (H&E) and the original diagnoses were confirmed.

The clinical features of the 21 cases of EMP are given in Table 1. All but case 15 have been previously reported in a study of GCDFP15 expression.[11] In summary, the patients ranged in age from 59 to 88 years (median, 69 years). In 16 patients with EMP, an underlying malignancy was excluded clinically by cytoscopy, sigmoidoscopy, and physical examination. Of these 16 patients, EMP occurred on the vulva in 12, the scrotum in 3, and the anus in 1. The remaining six cases (four male, two female) were examples of EMP with an underlying malignancy, five in the perianal area and one on the penis. The underlying tumors in each case are given in Table 1.

All 22 cases of MPD cases were associated with underlying ductal carcinoma in situ or invasive ductal carcinoma. The patients ranged in age from 34 to 67 years (median, 52 years).

We also examined 15 normal nipples from patients with breast carcinoma on whom a mastectomy was performed (cases selected from Stanford University Hospital). In one patient a bilateral mastectomy was performed, and we were able to examine both nipples. There was no clinical or histological evidence of Paget's disease in any of the cases. The patients ranged in age from 26 to 85 years of age (median, 54 years).

Finally, both nipples from eight random autopsy cases (Stanford University Hospital) and a single nipple from two cases (Palo Alto Veterans Affairs Medical Center) were removed and entirely submitted for histological examination. Four patients were male (ages 39, 66, 30, 72) and six were female (ages 7, 45, 68, 77, 45, 81). None of the patients had Paget's disease or carcinoma except for a 77-year-old woman who had primary fallopian tube carcinoma but clinically and histologically normal breasts/nipples.

Immunohistochemistry

Immunohistochemical studies were performed on paraffin sections using an indirect biotin-avidin method on a

TABLE 1. *Extramammary Paget's disease*

Case no.	Sex/age (years)	Site	Underlying malignancy	CK7	CK20	GCDFP15
1	F/65	Vulva	No	–	++f	–
2	F/80	Vulva	No	+	–	+++
3	F/78	Vulva	No	++	–	++
4	F/71	Vulva	No	++	–	+++
5	F/77	Vulva	No	++	–	++
6	F/77	Vulva	No	++f	+f	–
7	F/67	Vulva	No	+++	–	++
8	F/65	Vulva	No	+++	–	+
9	F/62	Vulva	No	+++	ND	+++
10	F/62	Vulva	No	+++	–	+++
11	F/79	Vulva	No	+++	–	+++
12	F/65	Vulva	No	+++	–	–
13	M/67	Scrotum	No	++	ND	++
14	M/NA	Scrotum	No	+++	–	+++
15	M/58	Scrotum	No	+++	–	+++f
16	M/61	Anus	No	++	++	–
17	F/81	Anus	Adenoca anus	–	++f	–
18	M/NA	Anus	Adenoca rectum	+++	++f	–
19	F/70	Anus	Adenosquamous anus	–	–	–
20	M/NA	Anus	Adenoca rectum	++	+++	–
21	M/88	Anus	Adenoca rectum	+++	–	+++
22	M/72	Penis	TCCA bladder	+++	ND	–

Adenoca, adenocarcinoma; f, focal; NA, not available; ND, not done; TCCA, transitional cell carcinoma.

Ventana 320 automated immunohistochemistry system (Ventana Medical Systems, Tucson, AZ). Sections were cut at 4 μm and heated at 65°C for 20 minutes before staining. The automated method used primary antibody incubation at a temperature of 37°C for 32 minutes. The following primary mouse monoclonal antibodies were used: anti-cytokeratin 7 (clone OVTL/30, Dako, Carpinteria, CA), anti-cytokeratin 20 (clone Ks20.8, Dako), anti-carcinoembryonic antigen (CEA, clone 0062, Boehringer Mannheim, Indianapolis, IN), anti-estrogen receptor (ER, clone 6F11, Ventana), antiprogesterone receptor (clone 1A6, Ventana), anti-epithelial membrane antigen (EMA, clone E29, Dako), anti-GCDFP15 (clone BRST2, Signet, Dedham, MA), and anti-keratins AE1 (Boehringer Mannheim), CAM5.2 (Becton Dickinson, Mountain View, CA), and 34BE12 (Dako). S-100 was detected with a polyclonal rabbit antiserum (Dako). The Ventana DAB Detection System (Ventana Medical Systems) was used for detection of antibody reactivity. The sections were counterstained with hematoxylin, dehydrated, and coverslipped. Tissues known to express the determinants of interest were used as positive controls. The data presented are based on evaluation of a single slide stained with each antibody.

RESULTS

Light Microscopy Findings

All of the cases of EMP and MPD contained Paget cells with atypical round/oval nuclei and abundant pale cytoplasm. On light microscopy, the Paget cells in EMP and MPD were identical. In the majority of cases, the nuclei were centrally placed, but there were two cases of EMP in which the Paget cells had eccentrically placed nuclei (signet ring–type cells). The Paget cells were present as single cells or small clusters and were located throughout all layers of the epidermis. The mitotic activity was variable. In the cases of EMP without an underlying malignancy, the Paget cells were confined to the epidermis or adnexal structures except for one case, which showed dermal invasion. In cases of EMP with an associated malignancy, the underlying invasive carcinoma was variably located either directly underneath and contiguous with the Paget cells, or present in a different area of the specimen, or not represented in the biopsy specimen.

Sections of histologically normal nipples from the mastectomies and autopsies were examined by conventional microscopy for TC. On H&E stains (without knowledge of the immunophenotypic results), 4 of 32 nipples contained cells with the appearance of TC: round nuclei, abundant pale cytoplasm, and scant nuclear chromatin.[22] These cells were larger than the surrounding keratinocytes and were located predominantly in the basal half of the epidermis (but only infrequently in the basal layer itself) and occasionally in the upper half of the epidermis. Architecturally, the cells were present as single cells or infrequently arranged in clusters or small groups with abortive tubule formation (Fig. 1). Toker cells were clearly distinguished from Paget cells because they lacked the large, pleomorphic, and cytologically atypical nuclei characteristically seen in Paget's disease. There was no evidence of inflammation, disturbance of the maturation pattern, or disruption of the epidermis to suggest that the TC affected the surrounding epidermis.

Immunohistochemical Findings

A summary of the immunohistochemical data is shown in Tables 1 and 2. In 21 of 22 cases of MPD, >95% of the Paget cells were strongly CK7 positive, whereas anti-CK20 was negative in all 17 cases tested. The one CK7-negative MPD case was positive for EMA, CEA, and keratins detected by AE1. We stained a prior biopsy specimen from this patient that showed underly-

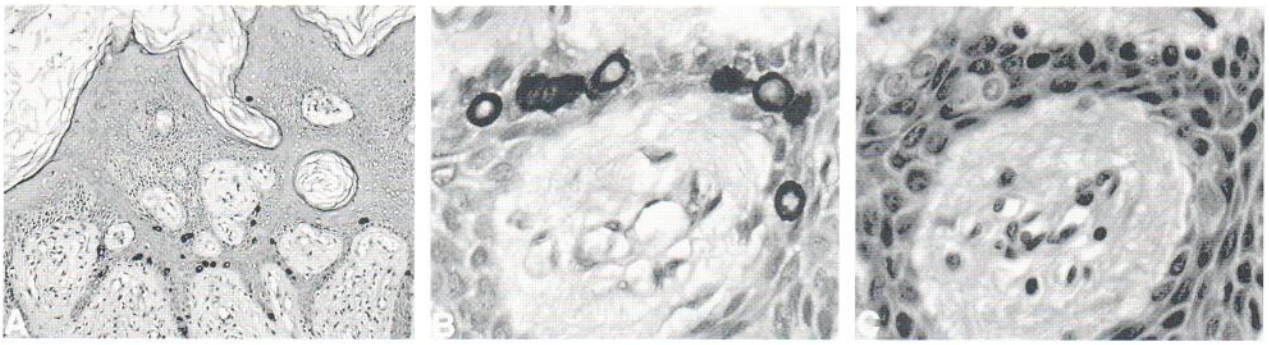

FIG. 1. Normal nipple from a 45-year-old woman; autopsy specimen. **(A)** Low-power view of CK7 stain with light hematoxylin counterstain shows many positive Toker cells scattered singly and in small groups. **(B)** High-power view of CK7 stain from the same case shows cells with moderate amounts of cytoplasm generally located near but not in the basal layer. **(C)** Serial section to that shown in (B) stained with hematoxylin and eosin. Some of the Toker cells are identifiable by their clear cytoplasm and pale nuclei.

TABLE 2. *Results summary*

Case group	CK7 positive	CK20 positive
Mammary Paget's disease	21/22	0/17
Extramammary Paget's disease (total)	19/22	6/19
No underlying malignancy	15/16	3/14
With underlying malignancy	4/6	3/5

ing intraductal carcinoma and found the neoplastic cells to be negative for CK7 and CK20 (normal epithelium in the same section was CK7 reactive). None of the pathologic material we have located from this patient has shown invasive carcinoma.

Twenty-two cases of EMP were tested for CK7: 17 were strongly reactive in >95% of cells, 2 cases were focally or weakly reactive, and 3 were negative. Nineteen cases were tested for CK20; six were reactive, four focally and two extensively. CK7 reactivity was present in 15 of 16 EMP cases without an underlying/deep carcinoma compared with 4 of 6 cases with associated malignancy. Of the three CK7-negative EMP cases, two were positive for CK20.

While staining nipples from patients with Paget's disease with anti-cytokeratin 7, we occasionally observed an intense cytoplasmic staining of scattered cytologically bland cells within the nipple epidermis at sites distant from the clearly malignant cells. These cells exhibited the appearance and distribution of TC. We then collected a series of normal nipples from mastectomies without nipple involvement and from autopsies from patients without breast carcinoma. Using CK7 as a marker, we were able to identify cells with the characteristics of TC in 8 of 15 nipples from breast cancer patients without MPD and in 15 of 18 autopsy nipples without breast carcinoma (Figs. 1 and 2). Using CK7 to localize TC, we reviewed parallel sections stained with H&E and were able to identify cells consistent with TC in the same regions of the specimens in many cases. These CK7-positive cells were present individually or in small clusters or in groups of up to 10–55 cells. Although they were present primarily in the lower half of the epidermis (but rarely in the basal layer itself), they were not infrequently seen in the upper half also. In the 23 nipples containing CK7-positive TC, 60 foci of 1–10 cells were identified. Of these, 9 were located in the ostia of lactiferous ducts, 38 were above or near the ducts, and 13 could not be directly related to the ducts. Twenty-one foci of 10–55 TC were identified in 10 nipples. Nine were located in the ostia of lactiferous ducts, 11 were above or near the ducts (Fig. 2), and one could not be directly related to the ducts. (Note that these data are derived from examination of a single CK7-stained section for each nipple.) Cells with the characteristics of TC were not identified in the epithelium adjacent to any of the cases of EMP studied.

Further immunophenotypic analysis in five nipples with larger clusters of TC (Table 3) showed that TC in each case also expressed EMA, and keratins detected by CAM 5.2 and AE1, but did not stain with antibodies to cytokeratin 20, GCDFP15, high-molecular-weight keratin (34BE12), or S-100. The TC stained variably for ER, progesterone receptor, CEA, and cerbB2. Toker cells were easily identified as they contrasted with the nipple skin, which was negative with CK7, CAM5.2, and EMA and variably reactive with AE1. (AE1 epidermal reactivity was occasionally intense enough to partially obscure the TC reactivity.) The lining cells of the underlying lactiferous ducts exhibited an immunophenotype similar to TC, with abrupt strong CEA expression seen in the most apical portion of the dermal segment of the ducts, which were otherwise CEA negative. The variable ER and progesterone receptor expression noted in TC correlated with the variable expression of the same antigens in the underlying ducts in each case.

As noted above, CK20 did not label TC. In 15 of 22 normal nipples, however, cells with the characteristics of Merkel cells were identified on the CK20 stains. These cells were restricted to the basal layer of the epidermis, possessed small round nuclei and scant cytoplasm, and were never identified in cohesive groups or in the necks of lactiferous ducts (Fig. 3). Merkel cells could not be discerned on H&E stains. In 13 of the 15 cases contain-

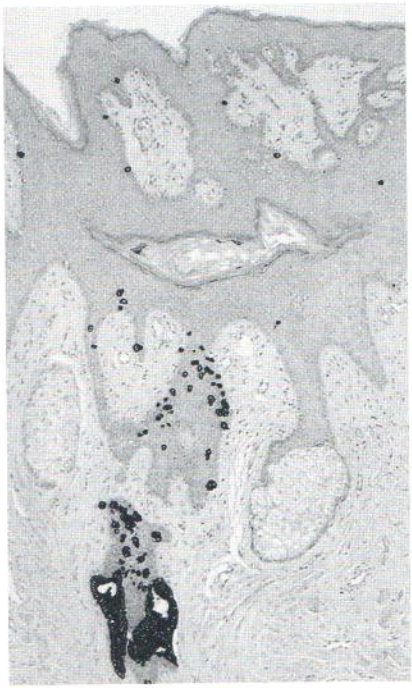

FIG. 2. Normal nipple from a 26-year-old woman; surgical specimen. CK7 stain labels numerous Toker cells immediately above the opening of a lactiferous sinus. Scattered single cells are identified with increasing distance from the duct orifice.

TABLE 3. *Comparative phenotype of toker cells*

Cell type	Immunohistochemical markers											
	CK7	CK20	AE1	CAM5.2	34BE12	EMA	ER	PR	GCDFP15	CEA	cerbB2	S-100
Toker cells												
Case 1	+++	–	+++	+	–	+++	++f	–	–	+f	+++	–
Case 2	+++	–	+++	+	–	+	+	+f	–	+	++	–
Case 3	+++	–	+++	+++	nd	+++	–	–	–	nd	–	–
Case 4L	+++	–	+++	+++	–	ND	–	–	–	–	faint	ND
Case 4R	+++	–	+++	+++	–	ND	–	–	–	+f	faint	ND
Paget cells	+++	rare	+++	+++	rare	+++	var	var	var	var	++	var
Lactiferous duct	+++	–	+++	var	var	var	var	var	var	+++f*	+++	–
Epidermis	–	–	var	–	basal	var	–	–	–	–	++	–†

f, focal; ND, not done; neg, negative; var, variable. Antibody and antigen abbreviations are as given in the text. Cases 1, 2, and 3 were derived from surgical mastectomy specimens and cases 4L and 4R were obtained at autopsy. See Kohler et al.[10] and Ordonez et al.[16] for data on Paget cell phenotype not derived from this study.

* CEA staining detected only in the high dermal segment of the ducts.

† S-100 positive Langerhans cells present.

ing labeled Merkel cells, the cells were present as scattered cells, usually single cells but occasionally in foci of three to seven cells per 0.5 mm. On average, there was fewer than one cell per millimeter of epidermis. Two nipples, however, contained a single focus each with large numbers of CK20-reactive cells (14 and 28 cells) with all of the above characteristics of Merkel cells. Even though numerous in these two foci, the cells remained basally located and did not form cohesive groups. CK7 identified Merkel cells in 7 of 32 normal nipples tested. All 7 were among the 15 nipples with CK20-positive Merkel cells. Even in these seven specimens, fewer Merkel cells were identified on the CK7 stains than on the CK20 stains. Among both the solitary and clustered Merkel cells, occasional cells could be seen to stain for both markers on serial sections (Fig. 3). CK7-positive Merkel cells were distinguished from TC based on the above-stated characteristics and their frequent solitary nature, occurring far from the usually clustered TC. The occasional Merkel cell that was coincidentally located

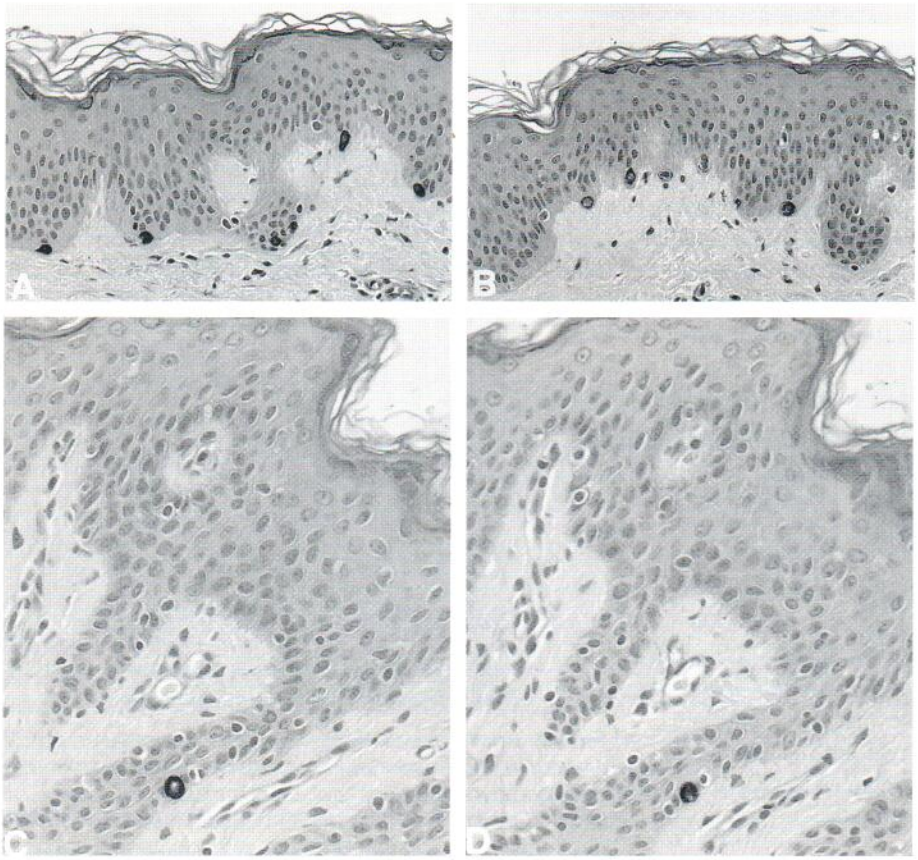

FIG. 3. Normal nipple from a 39-year-old woman; surgical specimen. **(A)** CK20 labels several small cells with scant cytoplasm exclusively located in the basal layer, features typical of Merkel cells. This is the typical density of Merkel cells in a haarscheibe. **(B)** CK7 stain of the same region on a parallel section identifies cells with similar features. **(C)** High-power view of CK20-stained Merkel cell. **(D)** CK7 stain on serial section to (C) labels the same cell.

within a cluster of TC could not be reliably distinguished on the CK7 stain.

DISCUSSION

Numerous studies have shown that in addition to similar histologic and cytologic features, Paget cells in EMP and MPD have similar immunophenotypes, with the large majority of cases expressing CEA, GCDFP15, EMA, and cytokeratins detected by CAM 5.2 and AE1.[10] CEA, EMA, and CAM5.2 have commonly been used to confirm the diagnosis of Paget's disease and EMP, but they have not been found to stain every single case. Recent studies have found CK7 to be a sensitive marker in paraffin sections for both types of Paget's disease, staining 16 of 16 cases of EMP,[1] 9 of 9 MPD, and 7 of 7 EMP[20] and 16 of 16 cases of EMP.[5] Using a series containing a large number of cases of both mammary and extramammary Paget's disease, we confirm the finding that CK7 is a sensitive marker for the cells of both types of Paget's disease but that occasional cases of each type may be nonreactive. In addition, we found that normal nipples contain CK7-positive TC and CK7-positive Merkel cells.

It is not surprising that occasional cases of Paget's disease should be CK7 negative. Although not a common event, CK7-negative breast carcinomas are described in the literature, comprising 4 of 64 cases reported in three large studies.[19,25,26] It should be expected that MPD would reflect the immunophenotype of its underlying carcinoma. The one CK7-negative case of MPD in our series was associated with an underlying intraductal carcinoma that was also CK7 negative. It is even less surprising that some cases of EMP also are negative for CK7. Extramammary Paget's disease is a heterologous disorder, ranging from a primary, noninvasive process to a locally invasive carcinoma to a manifestation of deep adenocarcinomas of various origins. For example, adenocarcinomas of large intestinal origin are infrequently CK7 reactive (16 of 82 in three series[19,25,26]); cases of EMP associated with such carcinomas should be expected to be CK7 negative. Extensive data on CK7 reactivity in apocrine neoplasms are not currently available.

Immunophenotypic heterogeneity may correlate with clinicopathologic differences in EMP. Recent studies have demonstrated that expression of GCDFP15 in a patient with EMP may indicate a low probability of an underlying malignancy.[1,5,11] In Kohler's study, 80% of EMP cases without an associated malignancy strongly expressed GCDFP15, whereas only 17% of EMP cases with an underlying malignancy stained with the GCDFP15 antibody. Using the same EMP patients, our current research reveals a similar but less significant association between the presence of an underlying malignancy in EMP and differential antigen expression, in this case CK7 and CK20 (Table 2). Although almost every case (15 of 16) of EMP disease without an underlying malignancy stained for CK7, only 4 of 6 cases of EMP with an underlying malignancy expressed this antigen. Cytokeratin 20 reactivity was seen in 3 of 5 cases with underlying malignancy, whereas only 3 of 14 cases without carcinoma were positive. Compared with GCDFP15, CK7 was a more sensitive marker for Paget cells in cases of EMP with an underlying malignancy and labeled 3 cases that failed to stain for GCDFP15. This spectrum of antigen expression and clinical features further supports the hypothesis that extramammary Paget's disease is a heterogeneous disorder that in some instances may be an in situ cutaneous adenocarcinoma with apocrine differentiation, whereas in others it may represent epidermal involvement by an underlying malignancy.

Mammary Paget's disease is almost always associated with an underlying breast carcinoma and is considered to represent migration of the tumor cells into the epidermis.[4,8] Most cases of EMP, on the other hand, are not associated with an underlying malignancy, and the origin of the Paget cells is controversial. In the cases of Paget's disease (MPD and EMP) without an underlying malignancy, it has been proposed that the Paget cells arise from TC,[14,22,24] even though they have only been described in the nipple. Toker cells are intraepidermal clear cells first described by Toker[22] that are identified by standard histology in ~11% of normal nipples. By light microscopy, TC have round eccentric nuclei with abundant pale cytoplasm. The cells are predominantly located in the basal layer but extend into the upper epidermis and in some instances clump together and form small lumina. In contrast to Paget cells, the TC demonstrate no nuclear atypia, have a low nuclear/cytoplasmic ratio, and show no evidence of mitotic activity. There are very little data on the immunophenotype of TC. Nagle et al.[14] found identical cytokeratin antibody staining (using clone KA4, reactive with keratins of 40, 46 and 54 kDa) in all of their cases of mammary Paget's disease (4 of 4 cases) and in TC from clinically normal breasts (4 of 36 cases). Pierard-Franchimont et al.,[17] studying one case, demonstrated that TC express EMA and CAM5.2. Overall, these immunohistochemical studies have shown some similarities between TC and Paget cells, but they used very small numbers of nipples and were limited to either noncommercial or widely reactive antibodies.

Using CK7, we were able to identify rare to scattered intraepidermal cells with clear cytoplasm in 8 of 15 nipples from patients with carcinoma but no clinical or histological evidence of Paget's disease, and in 15 of 18 nipples from autopsy cases without breast carcinoma. We identified the labeled cells as TC based on the cytologic features of the stained cells, the identification of TC in the same region on parallel sections stained with H&E,

their presence in nipples from breasts without underlying carcinoma, and their absence in the normal epidermis adjacent to EMP in the specimens examined. The labeled cells were differentiated from Merkel cells because the latter are smaller, limited to the basal layer of the epidermis, do not form cohesive clusters, and on serial sections can be seen to express CK20.[13]

Identification of TC with CK7 permitted more complete immunophenotyping in a subset of cases. In addition to CK7, TC express EMA and keratins detected by CAM5.2 and AE1. CK7 was the most effective marker for TC, because CAM5.2 and EMA stained them only faintly or focally in some cases and AE1, while providing uniformly strong staining of TC, also stained regions of the surrounding epidermis in most cases. The phenotype of TC is similar to that of Paget cells, but differs primarily in the lower incidence of GCDFP15 reactivity. As summarized in Table 3, the immunophenotypes of TC and the lining cells of the lactiferous ducts are quite similar, differing only in the relative lack of CEA expression by TC. Using anti-CEA clone 0062, TC were seen to be only faintly and focally reactive. The deeper portion of the lactiferous ducts was completely negative but the upper dermal portion of the ducts abruptly expressed strong CEA reactivity.

The use of CK7 as a marker of TC resulted in a much higher incidence of these cells than the 11–12% of nipples reported in other studies.[14,22] Several factors may contribute to this discrepancy. First, the majority of positive cases only contained four to five TC, which frequently were difficult or impossible to identify on adjacent H&E-stained sections. Second, although the surgical specimens were collected retrospectively, the autopsy nipples were prospectively collected and entirely submitted with careful orientation to permit identification of the ostia of the lactiferous ducts. This increased the probability of identification of rare foci of TC. Overall, it appears that the majority of people have TC in their nipple epithelium independent of gender and age (within the range studied). It is also plausible that with exhaustive serial sectioning and immunohistologic staining, it may be possible to identify TC in nipples from every breast.

While the majority of cases contained only rare to scattered TC (~4–6 per slide), in 10 nipples we found clusters of 10 to 55 TC. In all but one case, these clusters were observed in or near the orifices of the lactiferous ducts. This phenomenon was noted by Toker in his original report, and was also described by Pierard-Franchimont et al. in one case.[17,22] As originally suggested by Toker, scattered individual cells may in many cases represent outliers from larger groups out of the plane of the section. This frequent localization of groups of TC to lactiferous duct ostia as well as their similar immunophenotype suggest that TC may be derived form lactiferous duct lining cells. Their scattered presence in the epidermis could be caused by simple entrapment or by active migration. Although we cannot disprove it, we find no support for the suggestion that TC represent embryonic rests of precursor cells.[14]

In two nipples, foci containing large numbers of Merkel cells were identified. Such foci have been termed ''haarscheibe,'' or hair discs, and have been described in various locations on the body.[2] Even in such foci, the Merkel cells are exclusively located in the basal layer. Normal Merkel cells are known to be CK20 positive,[13] but to our knowledge, have not been tested for CK7 reactivity. Our findings indicate that both solitary Merkel cells and those in the haarscheibe may express detectable CK7 in paraffin sections, although with lower frequency than CK20. The absence of clusters, restriction to the basal layer, bland cytology, and strong CK20 reactivity all serve to differentiate Merkel cells from Paget cells.

Overall, our results confirm that CK7 is a very sensitive marker for Paget cells. In our experience, it stains almost all cases of MPD and the majority of EMP cases. However, in the nipple one must be cautious in interpreting CK7 staining because TC strongly express CK7 and can be present focally in an identical location and in sufficient numbers to mimic mammary Paget's disease. Groups of Merkel cells may also be identified on CK7 stains and should be differentiated from Paget cells. Morphologic correlation is necessary to distinguish both of these types of benign cells from the malignant cells of Paget's disease. □

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