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A comparative immunohistochemical study of mammary and extramammary Paget's disease and superficial spreading melanoma, with particular emphasis on melanocytic markers

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Abstract A comparative immunohistochemical study was performed on Paget's disease of the nipple (PDN), extramammary Paget's disease (EMPD) and cutaneous superficial spreading melanoma (SSM) using antibodies to S100, NK1-C3 and HMB45, cytokeratin (CAM 5.2) and c-erb B2 oncoprotein (21N). Conventional histochemical stains for intracytoplasmic mucin and melanin were also done. Of the 20 cases of PDN, positivity was seen in 12 with S100, 16 with NK1-C3, none with HMB45, 20 with CAM 5.2 and 19 with 21N. All 5 cases of EMPD were CAM 5.2 positive and HMB45, S100 and 21N negative. Three EMPD were NK1-C3 positive. All 10 cases of SSM were S100, NK1-C3 and HMB45 positive and all were CAM5.2 and 21N negative. Mucin was demonstrable in 11 cases of PDN and all of EMPD but none of SSM. Melanin was seen in 2 PDN, 3 EMPD and all SSM cases. Identification of mucin and melanin, therefore, proved an unreliable means of distinguishing these diseases. Immunohistochemistry for cytokeratin and HMB45 appear to be the most specific markers in differentiating Paget's disease and SSM. Antibodies to c-erb B2 may also be valuable in this situation.

Key words Immunohistochemistry · Melanocytic markers · Paget's disease · Melanoma

Introduction

In the absence of underlying mammary carcinoma the diagnosis of Paget's disease of the nipple (PDN) can occasionally be problematic. The main conditions to be considered in the differential diagnosis are superficial spreading (pagetoid) melanoma (SSM) and Bowen's disease (intra-epidermal squamous cell carcinoma), although the latter usually only poses a diagnostic problem when it shows the clonal or so-called Borst Jadassohn phenomenon. SSM, on the other hand, characteristically shows upward migration (pagetoid spread) of tumour cells within the epidermis, frequently also spreading along the epithelium of pilosebaceous structures in a manner similar to both PDN and extramammary Paget's disease (EMPD). Fortunately, both SSM and Bowen's disease are rare in the nipple and, therefore, only infrequently present a problem at this site in a routine diagnostic situation.

Application of recognised histological criteria will, in the majority of instances, allow differentiation of Paget's disease from SSM [16], but occasionally the differential diagnosis can be difficult. Identification of intracytoplasmic mucin and melanin in Paget's disease and melanoma respectively was, in the past, also considered a useful means of further distinguishing the two lesions. However, only a small number of cases of PDN (in contrast to EMPD) show evidence of mucin secretion and, when present, it is usually very patchy in distribution [1, 12, 27, 29, 33]. Furthermore, melanin pigment has occasionally been found within the neoplastic cells of PDN [16, 27], and mucin within melanoma cells [12, 16]. These latter lesions together with amelanotic melanomas could, therefore, be misdiagnosed on the basis of morphological features and histochemistry only, resulting in inappropriate patient management.

With the advent of immunohistochemistry the use of antibodies to S100 protein, a marker of melanocytes as well as other cells [26], was initially recommended in the differential diagnosis, as it was positive in SSM but negative in both PDN and EMPD [1, 12, 27, 30]. However, in later studies, S100 expression has been reported in a few cases of PDN [6, 12].

Other antibodies championed as useful in the diagnosis of Paget's disease include those to low-molecular

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weight cytokeratins [1, 5, 6, 15, 30, 32], carcinoembryonic antigen [1, 6, 18, 35], human milk fat globulin [18, 31, 35], epithelial membrane antigen [6, 14, 15, 31] and, more recently, c-erb B2 oncoprotein [14, 15, 19]. Only a few of these published studies, however, included a comparative study of Paget's disease and melanoma [1, 15, 30, 32].

In view of the increasing reliance on immunohistochemistry in diagnostic histopathology and the finding of S100 protein expression in the intra-epidermal malignant cells of some cases of mammary Paget's disease in a previous study by two of the authors [11], we decided to conduct a comparative immunohistochemical study of mammary and extramammary Paget's disease and primary cutaneous malignant melanoma, employing the other commonly used "melanocytic markers", namely NK1-C3 and HMB45. As far as we are aware, these two markers have not been extensively evaluated in either PDN or EMPD.

Materials and methods

We retrieved 20 cases of PDN seen over the past 12 years, together with 5 cases of EMPD seen over the past 8 years and 10 cases of primary cutaneous SSM seen over the past 3 years. All 20 cases of PDN were associated with poorly differentiated, large cell ("comedo") ductal carcinoma in situ (DCIS) of the breast, and in 7 cases there was also invasive carcinoma. In 14 cases DCIS was present in the same section as the nipple but in the remaining 6 cases a separate, although representative, block of tumour was retrieved for the study. All the cases of EMPD were from either the vulval or the perianal region (from 2 female and 3 male patients). None was associated with internal malignancy.

The SSMs had been excised from various sites (although none was from the skin of the breast or nipple) and showed wide variation in size and thickness. The haematoxylin and eosin stained sections of all the lesions were reviewed and the diagnosis confirmed in every case. Sections were stained immunohistochemically with antibodies to S100 protein, NK1-C3, HMB45, low-molecular-weight cytokeratin (CAM 5.2) and c-erb B2 (21N) (Table 1).

All staining was carried out on 3 μm sections of paraffin-embedded, formalin-fixed material using a peroxidase-conjugated streptavidin biotin technique.

Additional sections from all selected cases were stained histochemically with the Masson-Fontana argentaffin reaction for melanin pigment and a combined alcian blue (at pH 2.5) and periodic-acid–Schiff stain with diastase digestion (AB-dPAS) for mucin.

Results

Immunohistochemistry

The results of immunohistochemical staining for the three diseases are summarised in Table 2.

A total of 12 cases of PDN showed immunoreactivity for S100 protein. In 8 of these, there was strong nuclear and cytoplasmic staining of the neoplastic cells within the epidermis; in 5 of these 8 cases, uniform staining of all tumour cells was noted, the remaining 3 showing a more patchy staining pattern. Weaker staining (which was more often nuclear than cytoplasmic) was noted in the other 4 cases. The tumour cells were clearly S100 negative in the remaining 8 cases of PDN. NK1-C3 positive staining of the Paget's cells was observed in no less than 16 of the 20 cases. In 12 of these there was strong cytoplasmic staining, the remaining 4 showing usually weak, patchy staining. None of the cases of PDN examined showed any staining for HMB45, whilst all 20 cases showed, as expected, strongly positive staining of the neoplastic cells with CAM 5.2. All but 1 case showed strong membrane staining with 21N (Fig. 1). In all cases of PDN, the keratinocytes in the epidermis failed to stain with any of the melanocytic markers. Great caution was exercised so as not to misinterpret the positively staining melanocytes, and dendritic (Langerhan's) cells within the epidermis.

In every case of PDN, the associated breast carcinoma and the Paget's cells within the epidermis showed similar immunoreactivity, although there was slight to moderate variation in the intensity of staining with NK1-C3 and S100. Within some of the infiltrating carcinomas, a scattering of S100-positive dendritic cells was

Table 2 Proportion of positive immunohistochemical staining in Paget's disease of the nipple (PDN), extramammary Paget's disease (EMPD) and superficial spreading melanoma (SSM)

	S100	NK1-C3	HMB45	CAM 5.2	21N
PDN	12/20	16/20	0/20	20/20	19/20
EMPD	0/5	3/5	0/5	5/5	0/5
SSM	10/10	10/10	10/10	0/10	0/10

Table 1 List of antibodies used and the antigens they bind with, together with their source and respective dilutions

Antibody	Antigen/description	Source	Dilution
CAM 5.2 (monoclonal)	Low-molecular weight keratins[a]	ICRF Laboratories London, UK	Neat
S100 (polyclonal)	S100 protein	Dako, High Wycombe, UK	1/500
HMB45 (monoclonal)	Melanoma-specific cytoplasmic antigen	Dako, High Wycombe, UK	1/100
NK1-C3 (monoclonal)	Melanoma-associated antigen	Euro-Path, Bude, UK	1/50
21N (polyclonal)	c-erb B2	Prof W Gullick ICRF Clinical Oncology Group[b], Hammersmith Hospital, London, UK	1/50

[a] Keratins of 8, 18, 19, 39, 43 and 52 kDa molecular weights normally present in glandular epithelia

[b] *ICRF* Imperial Cancer Research Fund

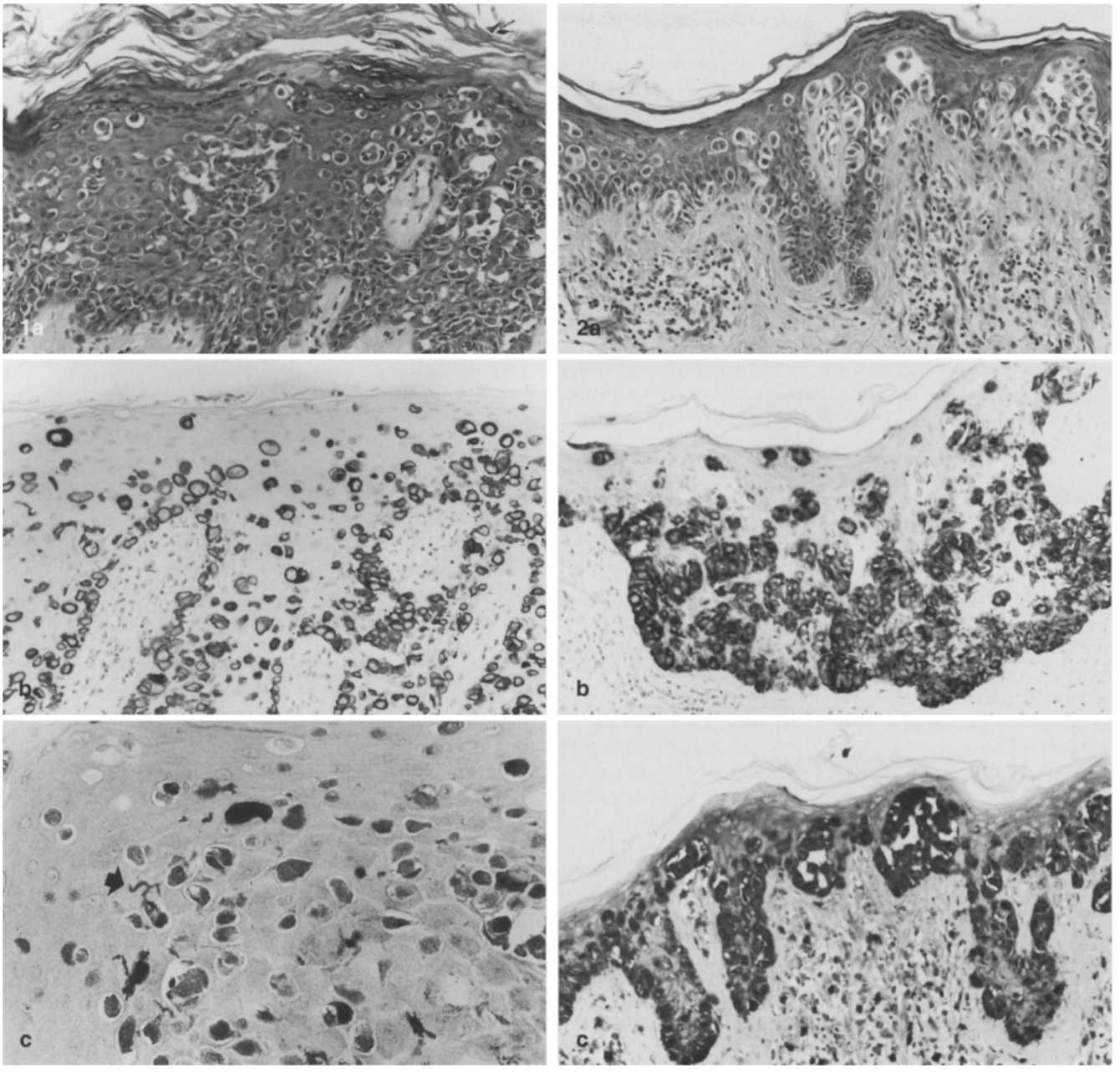

Fig. 1a–c Mammary Paget's disease. **a** Typical malignant (Paget's) cells are found throughout the epidermis, both singly and in small nests. H&E, ×400. **b** CAM 5.2. Strongly positive membrane staining of Paget's cells, but not of surrounding keratinocytes. Immunoperoxidase, ×400. **c** S100. Diffuse, strong nuclear and cytoplasmic staining of Paget's cells. The dendritic cells in the surrounding epidermis are also stained (*arrow*), but not the keratinocytes. The pattern of staining was similar with NK1-C3. Immunoperoxidase, ×800

Fig. 2a–c Superficial spreading melanoma. **a** Irregular lateral spread of nests and single malignant melanocytes along the dermo-epidermal junction. Upward (pagetoid) migration of similar cells in to the superficial epidermis is also evident. H&E, ×200. **b** HMB45. Positive membrane staining of melanoma cells. Immunoperoxidase, ×200. **c** S100. Strongly positive nuclear and cytoplasmic staining of melanoma cells. A similar result was seen with NK1-C3. Immunoperoxidase, ×200

noted and myoepithelial cells surrounding foci of DCIS were also positively stained in most cases with this antibody.

As with PDN, all cases of EMPD were strongly CAM 5.2 positive. None showed any immunoreactivity with HMB45, S100 or 21N. Three showed NK1-C3 positivity, which was classified as strong in 2 cases. In 1 of the latter and in the weak case staining was both nuclear and cytoplasmic. In the other stronger staining case it was mainly cytoplasmic.

Amongst the SSMs, there was strong nuclear and cytoplasmic staining for S100 protein as well as NK1-C3 and HMB45 in all 10 cases. All were unequivocally negative with CAM 5.2 and 21N (Fig. 2).

Interestingly, melanin was identified within the cytoplasm of tumour cells in 2 cases of PDN and 3 of EMPD. This was prominent in 1 case of EMPD but sparse in all other cases. Only 11 cases of PDN (55%) showed evidence of intracellular mucin (which was AB or dPAS positive), and this was very focal in distribution. However, all cases of EMPD showed large amounts of intracellular mucin, which was predominantly AB positive with only a focal admixture of dPAS positivity.

Amongst the SSMs, 8 (80%) showed significant amounts of melanin pigment within the neoplastic cells; 2 (20%) were entirely amelanotic, although in 1 of these numerous melanophages were identified in the dermis. None demonstrated any intracellular AB or dPAS positive material.

Discussion

S100 protein expression has been identified in neurogenic tumours, melanomas and mammary carcinomas as well as a variety of other, both benign and malignant, neoplasms [8–10, 26, 34]. It appears, therefore, that this marker is not specific for cell type. Previous studies of S100 protein expression in Paget's disease (both mammary and extramammary variants) have shown variable results, most initial reports being negative [1, 12, 29, 30]. A previous study by two of the present authors reported S100 positivity in 18% of cases of mammary Paget's disease studied [11], and this was followed by another more recent report of a 35% incidence of S100 positivity [6]. Our study shows the highest reported incidence of S100 protein expression so far, that of 60%. This is neither surprising nor unexpected , as there are published studies of S100 protein expression in a large number of mammary carcinomas (up to 84%) [8, 9, 11, 34]. Indeed, it would be more surprising, in view of the immunophenotypic correlation between Paget's cells and the underlying mammary carcinoma, if S100 were not detected in the former.

Although NK1-C3 was originally recommended as a useful melanocytic marker, to be used in conjunction with S100, it was also noted to be positive in some breast and prostatic carcinomas [20]. This monoclonal antibody was synthesised by The Netherlands Cancer Institute and raised against a melanoma-associated cytoplasmic antigen. Subsequent studies have also reported NK1-C3 expression in many nonmelanocytic tumours, notably adenocarcinomas and even lymphomas [10]. Strong immunoreactivity for NK1-C3 was seen in all our melanomas; it was also variably expressed in 90% of cases of PDN (as well as the associated mammary carcinomas) and 60% of EMPD. We are aware of only one reported case of NK1-C3 positivity in PDN [2].

Early studies using antibodies to HMB45 reported 100% specificity, with this melanoma-specific marker, in the diagnosis of melanoma [13, 36]. The high level of specificity was also confirmed in a more recent study where various nonmelanocytic markers were evaluated [38]. To our knowledge, only 8 cases of EMPD and 4 cases of PDN have been studied with HMB45, all with negative results [2, 4, 36]. In our study, all the melanomas were strongly HMB45 positive and all cases of EMPD and PDN, together with the underlying mammary carcinomas that were associated with the latter, were negative. Although there have been recent reports of aberrant HMB45 expression in mammary carcinoma with a single positive case in each of two different studies [2, 4], we did not obtain any positive results with our cases. All our cases of SSM were positive with HMB45, but the sensitivity of this antigen has been shown to be lowered in spindle-cell (desmoplastic) melanoma [36] and in recurrent or metastatic melanoma [3].

Our study, in agreement with some others [8, 15, 30, 32], did not show discrepant cytokeratin expression in any of the primary melanomas included. This is in contrast to other studies, where aberrant cytokeratin expression (with CAM 5.2) was observed in a few melanomas [3, 10, 11, 39]. The incidence of immunoreactivity was, however, found to be lower in formalin-fixed, paraffin-processed material than in frozen sections and, furthermore, many of the positive cases were recurrent or metastatic, rather than primary, melanomas and would readily be distinguished from Paget's disease on both clinical and morphological grounds.

The high incidence (95%) of c-erb B2 expression in PDN, both in the Paget's cells and the underlying mammary carcinoma, in this and other studies confirms its usefulness as a marker in the investigation of PDN [14, 15, 19, 37]. PDN is almost exclusively associated with underlying mammary carcinoma composed of poorly differentiated DCIS, with or without associated infiltrating carcinoma. As poorly differentiated DCIS is nearly always c-erb B2 positive [23], the high incidence of positivity in Paget's cells is to be expected. Indeed, De Potter et al. [7] suggest that Paget's cells spread through the epidermis due to motility induced by a chemotactic factor released by epidermal keratinocytes mediated by the c-erb B2 protein. No c-erb B2 overexpression was noted in any of our melanomas. However, a larger group of these tumours needs to be evaluated with this marker before the true frequency of activation of this oncogene in melanomas can be determined. It is interesting that, in contrast to PDN, none of our EMPD cases expressed c-erb B2. A similar absence of c-erb B2 overexpression in EMPD has been noted in one other study [21], but overexpression in a small proportion of cases has been reported in three papers [17, 28, 37]. In one of the latter reports [17] staining was noted to be weaker in EMPD than in PDN. The different level of overexpression of c-erb B2 oncoprotein in PDN and EMPD possibly indicates that there are different mechanisms involved in the pathogenesis of Paget's disease at the different sites.

In this study we found mucin (AB or dPAS positive) in 55% of PDN. This is a similar incidence to those previously reported [12, 15, 33]. A higher rate of mucin pro-

duction has been recorded in EMPD [12], which is in agreement with our study where all cases of EMPD showed abundant, predominantly AB positive mucin. One previous study also found PAS and mucicarmine positive mucin in 20% of cases of SSM studied [12], but we did not find any AB or dPAS positive mucin in our series. The presence of melanin in PDN and EMPD has been well documented [16, 24, 27], with a reported incidence as high as 60% in PDN. It is generally considered that the melanin is transferred from adjacent melanocytes [24]. We found it to be present in 2 of our 20 PDN cases and 3 of 5 EMPD cases. In the differential diagnosis of Paget's and SSM, although conventional histochemical stains for mucin and melanin may be used as a first line investigation, it should always be borne in mind that they may be present or absent in either of the two diseases.

In conclusion, the results of our study indicate that in the assessment of 'pagetoid' lesions of the skin, when the differential diagnosis includes SSM and Paget's disease, HMB45 is the most useful melanocytic marker. Other conventional melanocytic markers, namely S100 protein and NK1-C3, are unreliable as they often show some positive staining of Paget's cells. Immunohistochemical staining for low-molecular-weight cytokeratin and HMB45 are the most specific markers for Paget's disease (both PDN and EMPD) and SSM respectively, each showing 100% specificity in our hands. Antibodies to c-erb B2 oncoprotein (such as 21N) are also of value in PDN.

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