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Distribution of melanoma specific antibody (HMB-45) in benign and malignant melanocytic tumours

An immunohistochemical study on paraffin sections*

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Summary. The distribution of a recently produced melanoma specific antibody (HMB-45) has been evaluated histochemically on paraffin sections in a large panel of melanocytic and non melanocytic tumours. Results have been compared with the presence of S-100 protein. HMB-45 was shown to be a highly specific antibody being present only in melanomas, junctional melanocytes and histogenetically related neoplasms such as melanocytic neuroectodermal tumour of infancy and, at low levels, on a proportion of peripheral nerve sheath tumours. The high specificity of HMB-45 antibody, coupled with the greater sensitivity of S-100, makes the combined use of these markers practical in the differential diagnosis of skin tumours and of metastatic lesions of uncertain primary site.

Key words: Malignant melanoma – Anti-human-melanoma monoclonal antibody – Immunohistochemistry.

Introduction

The differential diagnosis between malignant melanoma and benign melanocytic proliferations or neoplasms other than melanomas can occasionally be a difficult task on morphological grounds alone. Malignant melanoma can be confused with malignancies of different histogenesis, especially when first presenting as metastatic lesion.

A variety of antibodies recognizing melanoma-associated molecules have been developed to overcome these diagnostic problems (Koprowski et al. 1978; Dippold et al. 1980; Natali et al. 1981; Wilson et al. 1981; Imai et al. 1982; Hellstrom et al. 1983; Natali et al. 1982; Hayashibe et al. 1986; Maeda and Jimbow 1987). However, the practical usefulness of most of these antibodies is restricted by their low specificity and/or technical limitations (e.g. the need for frozen tissues). (Johnson and Riethmuller 1982; Lloyd et al. 1982; Suter et al. 1983; Kageshita et al. 1985; Godal et al. 1986; Vanstapel et al. 1986).

Recently, a monoclonal antibody (HMB-45) has been described which recognizes a so far undefined molecule which is apparently confined to normal and neoplastic melanocytes. The practical usefulness of HMB-45 is further increased by the fact its immunoreactivity can successfully be demonstrated on formalin fixed and conventionally embedded tissues. HMB-45 not only discriminates between melanocytic and non-melanocytic tumours but also shows, among benign melanocytic lesions, a peculiar distribution, being present in junctional nevi and absent in intradermal ones (Gown et al. 1986).

To evaluate the practical usefulness of this antibody further we have tested a large number of benign and malignant melanocytic lesions and 79 non-melanocytic tumours immunohistochemically. All cases have been also studied for the presence of S-100 protein, a broadly employed marker for melanocytes, and results have been compared.

In addition, a series of different normal human tissues have been used as controls to evaluate the specificity of this antibody.

Material and methods

We studied 115 pigmented lesions of the skin and 12 lymph nodes with metastatic melanoma. In addition 79 cases of non-melanocytic tumours of the skin and other organs were also studied (Table 1).

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Table 1. Immunocytochemical reactivity of 79 various tumours other than melanomas with HMB-45 and anti S-100 antibodies

	HMB-45		S-100		n° cases
	+	−	+	−	
Carcinomas					
Breast	0	3	1	2	3
Colon	0	5	0	5	5
Colon, carcinoid	0	2	0	2	2
Lung, carcinoid	0	1	1	0	1
Pancreas	0	2	0	2	2
Thyroid, medullary	0	2	0	2	2
Endometrium	0	4	0	4	4
Liver, hepatoblastoma	0	1	0	1	1
Kidney	0	2	0	2	2
Rhinopharynx, undifferentiated	0	1	0	1	1
Skin, basal cell	0	6	0	6	6
All sites, squamous cell	0	2	0	2	2
All sites, anaplastic cell	0	2	0	2	2
Lymphomas					
B-large cell lymphomas	0	5	0	5	5
Tumors histogenetically related to melanocytes					
Neurofibroma	0	2	0	2	2
Neurilemmoma	1[a]	3	4	0	4
Malignant schwannoma	2[a]	2	1	3	4
Melanocytic neuroectodermal tumor of infancy, maxilla	1	0	0	1	1
Miscellanea					
Chemodectoma, carotic body	0	2	0	2	2
Granular cell tumor, esophagus	0	2	2	0	2
Ewing's sarcoma, bone	0	1	0	1	1
Chondrosarcoma, bone	0	1	1	0	1
Chordoma, clivus	0	1	1	0	1
Fibroushistiocytoma, skin	0	3	0	3	3
Cylindroma, skin	0	2	0	2	2
Neuro-endocrine carcinoma, skin	0	5	0	5	5
Kaposi's sarcoma, skin	0	2	0	2	2
Cystadenoma, ovary	0	1	0	1	1
Hydatidiform mole, uterus	0	1	0	1	1
Seminoma, testis and mediastinum	0	2	0	2	2
Leiomyosarcoma	0	3	0	3	3
Rhabdomyoma	0	1	0	1	1
Malignant histiocytosis	0	2	0	2	2
Histiocytosis X	0	1	1	0	1

[a] scant positivity in a minority of neoplastic cells

Tissues were fixed in 10% formalin and conventionally embedded in paraffin. Consecutive 4 μ thick sections were cut for histology and immunohistochemical studies. Benign and malignant melanocytic lesions of the skin comprised 5 lentigo simplex, 5 junctional nevi, 45 compound nevi, 22 intradermal nevi, 9 blue nevi, 5 Spitz nevi, 1 pigmented spindle cell nevus, and 23 malignant melanomas (Table 2). The histologic types of malignant melanomas were categorized according to the criteria of Clark (Clark et al. 1969) and Reed (Reed 1976) as lentigo maligna melanoma (4 cases), superficial spreading malignant melanoma (8 cases), nodular malignant melanoma (8 cases) and acral lentiginous melanoma (3 cases).

Non-melanocytic tumours of different histogenesis, selected from the files of the Department of Surgical Pathology of Verona University, are listed in Table 1. These neoplasms were diagnosed by conventional H&E morphology and, where needed, confirmed by immunophenotypical analysis. The range of diagnostic markers included common leucocyte antigen, low molecular weight cytokeratins, vimentin, etc.

Normal human tissues representative of skin, liver, lung, gallbladder, pancreas, gastrointestinal tract, kidney, breast, thyroid, uterus, testis, peripheral nervous system and lymph nodes were also studied (Table 3).

Immunohistochemistry. Melanoma associated monoclonal antibody HMB-45 was obtained from Enzo Biochem, IN (New York, USA). Rabbit antiserum against S-100 protein was obtained from Ortho Diagnostic System (Raritan, New Jersey, USA).

Peroxidase anti-peroxidase (PAP) and alkaline phosphatase anti-alkaline phosphatase (APAAP) techniques have been used to detect HMB-45 immunoreactivity; PAP technique has been used for S-100 antisera. The techniques have been previously described (Sternberger 1974; Chilosi et al. 1981; Cordell et al. 1984). The great variability of HMB-45 positivity prompted us to correlate the percentage of positive cells with histological aspects such as growth phase (horizontal and vertical), presence of inflammatory infiltrates, Clark's level of invasion and presence of melanin. The amount of inflammatory infiltrate and the presence of melanin were independently graded by two observers.

Results

The two immunohistochemical methods used (PAP and APAAP) demonstrated comparable sensitivity, but APAAP method was clearly superior for the more evident distinction of the final red product of the reaction as compared with the brown immunostaining of PAP method which could occasionally be confused with melanin pigment.

HMB-45 failed to react with melanocytes in normal skin (10 specimens), but clearly showed melanocytes at the dermo-epidermal junction, recognizable by their peculiar dendritic morphology and basal location, in apparently normal skin adjacent and overlying melanocytic lesions without a junctional component (9 blue nevi and 22 dermal nevi).

Interestingly, we found the same pattern in several non-melanocytic lesions (including basal cell carcinomas, neuro-endocrine carcinoma of the skin, cylindromas, fibroushistiocytomas and Kaposi's sarcoma). This phenomenon was independent of the histotype and nature, benign or malignant, of the adjacent lesion.

A granular HMB-45 immunoreactivity was evi-

Table 2. Immunocytochemical positivity of 115 benign and malignant melanocytic lesions of the skin with HMB-45 and Anti S-100 antibodies

	n° cases	Intraepidermal cells		Junctional cells		Intradermal cells	
		S-100	HMB-45	S-100	HMB-45	S-100	HMB-45
Lentigo simplex	5	2	–	5	5	–	–
Junctional nevi	5	3	–	5	5	–	–
Compound nevi	45	21	–	45	45	45	–
Intradermal nevi	22	12	–	22	22[a]	22	–
Blue nevi	9	4	–	4	9[a]	8	8
Spitz nevi	5	4	4	5	5	5	2
SCPN[b]	1	1	1	1	1	–	–
LMM[c]	4	4	4	4	4	4	4
SSMM[d]	8	7	8	8	8	7	5[e]
MM[f]	8	8	8	7	7	8	8
ALM[g]	3	3	3	3	3	3	3

[a] Only rare junctional melanocytes stained positively
[b] Pigmented spindle cell nevus
[c] Lentigo maligna melanoma
[d] Superficial spreading malignant melanoma
[e] Three cases were level I of Clark
[f] Malignant melanoma nodular type
[g] Acral lentiginous melanoma

dent in the cells forming the secretory terminal tract of sweat glands (Table 3).

Antiserum against S-100 protein recognized the melanocytes at the dermo-epidermal junction, Langerhans cells in the *"stratum spinosum"* and *"stratum granulosum"*, and the nerve sheaths occasionally found within the dermis.

All the normal tissues analyzed in this study were negative for the presence of HMB-45 with the exception of sweat glands, as referred to above (see Table 3).

Melanocytes of lentigo simplex (5 cases), junctional nevi (5 cases) and junctional melanocytes of compound nevi (45 cases) evidenced a comparable positivity, when tested with HMB-45 (Fig. 1 a). Intense immunostaining was evident in all these junctional melanocytes. However, the dermal melanocytes of both intradermal (22 cases), and compound nevi were HMB-45 negative, only rare melanocytes in upper dermis being weakly positive in intradermal nevi (Table 2). In the four Spitz nevi examined a similar pattern was observed, except for the presence of positive melanocytes in the epidermal *"stratum spinosum"* and *"stratum granulosum"* beside the junctional ones.

A significantly different pattern was observed in sections stained with S-100 antisera, where most melanocytes of junctional, compound, intradermal and Spitz nevi evidenced strong positive reaction (Fig. 1 b), independently from their location in the skin (Table 2). A variable number of positive cells with monocytoid and dendritic morphology were also detected in the *"stratum spinosum"* and *"stratum granulosum"* of the epidermis. These latter S-100 positive cells were considered to be Langerhans cells (Table 2).

The spindle-shaped dermal melanocytes of blue nevi (9 cases) in both variants, common and cellular, showed marked positivity when tested with both HMB-45 and S-100 antibodies (Table 2).

All primary malignant melanomas, including lentigo maligna (4 cases), superficial spreading (8 cases), nodular type (8 cases) and acral lentiginous (3 cases) stained positively when tested for HMB-45 (Table 2). The number of positive neoplastic cells varied considerably: in superficial spreading malignant melanoma, in acral lentiginous melanoma and lentigo maligna melanoma almost all neoplastic cells were positive, whereas in malignant melanoma nodular type they ranged from 5% to 90%.

Of particular interest was the strong positive staining observed in malignant melanocytes infiltrating the upper layers of epidermis in all variants of malignant melanoma (Fig. 2). S-100 immunoreactivity was also present in all cases tested and was very strong in the great majority of neoplastic cells of malignant melanomas.

In two cases of superficial spreading malignant melanoma we observed nests of melanocytes stained with S-100 antisera within reticular dermis, but negative when tested for HMB-45 (Table 2): a more accurate study in H&E sections of these melanocytes showed lack of any cytological atypia. We therefore considered them as the dermal component of a preexisting nevus.

Table 3. Immunocytochemical reactivity of normal human tissues with MoAb HMB-45

	HMB-45	
	+	−
Skin		
Langerhans cells	0	10
Melanocytes	0	10
Melanophages	0	10
Keratinocytes	0	10
Hair follicle	0	10
Sebaceous glands	0	10
Sweat glands	8	2
Nerve corpusoles	0	10
Liver		
Hepatocytes	0	3
Bile duct cells	0	3
Lung		
Alveolar epithelium	0	1
Bronchial epithelium	0	1
Gallbladder		
Surface epithelium	0	2
Glands	0	2
Pancreas		
Exocrine acini	0	3
Ducts	0	3
Islets of Langerhans	0	3
Gastrointestinal tract		
Esophagus	0	2
Stomach	0	2
Small intestine	0	2
Large intestine and appendix	0	12
Kidney		
Glomerular epithelium	0	5
Tubules	0	5
Urothelium	0	5
Breast		
Glands	0	5
Ducts	0	5
Thyroid		
Follicular cells	0	2
Parafollicular cells	0	2
Uterus		
Endometrium	0	1
Testis		
Germinal epithelium	0	1
Sertoli cells	0	1
Leydig cells	0	1
Peripheral nervous tissue		
Schwann cells	0	10
Ganglion cells	0	8
Myenteric plexus	0	12
Lymphoid tissue		
Histiocytes	0	10
Dendritic reticulum cells	0	10
Interdigitating reticulum cells	0	10
Lymphocytes	0	10
Mesenchymal cells		
Adipocytes	0	20
Chondrocytes	0	2
Striated muscle	0	2
Smooth muscle	0	18
Endothelial cells	0	37

The majority of lymph node metastases (11/12 cases) of malignant melanoma demonstrated a percentage of cells staining with HMB-45 (Fig. 3) and in only one case, in front of a strong positive reaction with S-100, no positive cell could be observed with HMB-45.

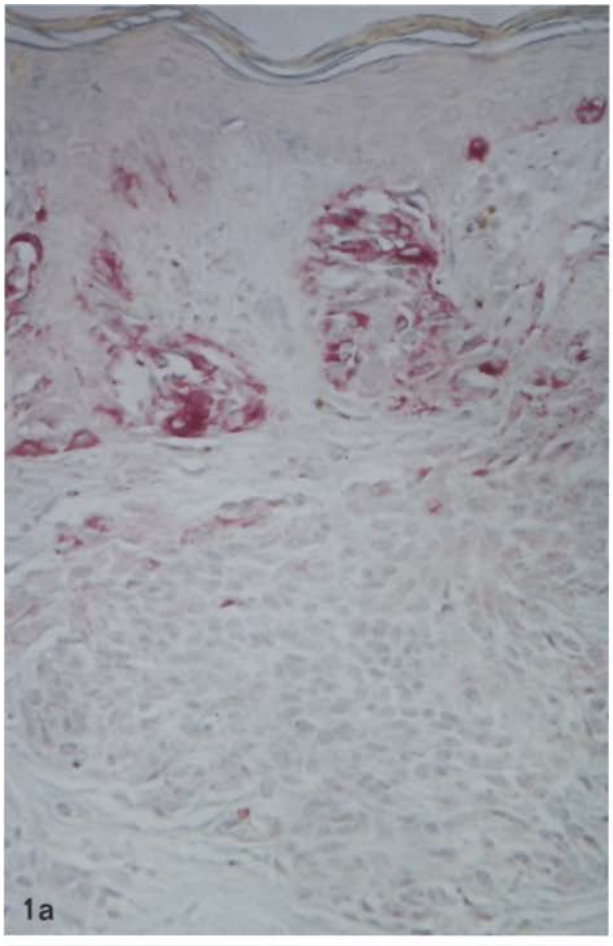
In primary malignant melanomas all cases of horizontal growth phase (superficial spreading malignant melanoma, acral lentiginous melanoma and lentigo maligna melanoma) showed clear positivity of almost all neoplastic cells. However, the vertical growth phase of malignant melanomas (nodular melanoma) evidenced variability of the percentage of HMB-45 positive neoplastic cells, ranging from 5 to 90%.

Interestingly, in all nodular melanoma cases,

Fig. 1a. Compound nevus: a strong positive reaction is evident in junctional melanocytes, while intradermal ones are negative. HMB-45, APAAP, ×100. **1b.** Same case of Fig. 1a: staining with S-100 protein evidences diffuse positive reaction of both junctional and intradermal melanocytes. S-100, PAP, ×100

Fig. 2. Superficial spreading malignant melanoma: staining with HMB-45 evidences positive melanocytes both in the basal layer and infiltrating upper epidermis. HMB-45, APAAP, ×250

Fig. 3. Lymph node metastasis of malignant melanoma: HMB-45 stains intensively the majority of metastatic cells, but is negative in part of them and in lymphocytes. HMB-45, APAAP, ×100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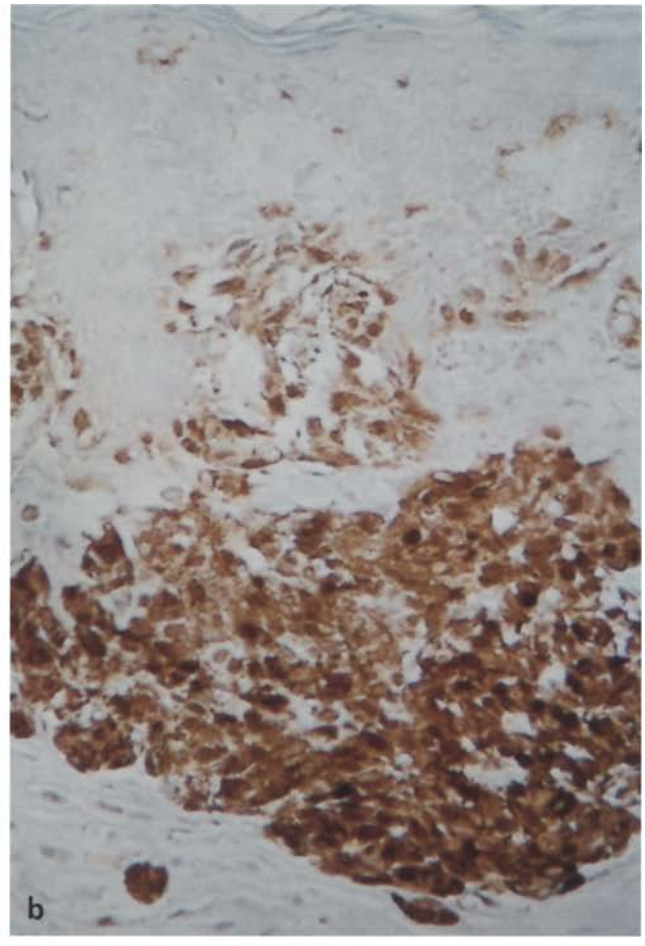
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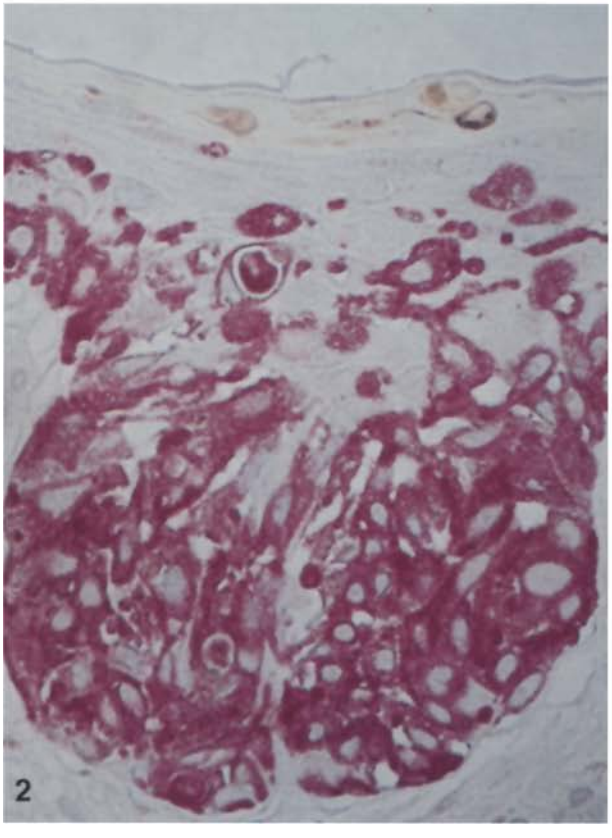
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3

neoplastic melanocytes infiltrating the epidermis were HMB-45 positive. The presence of melanin pigment appeared significantly associated with the expression of HMB-45 reactivity. All heavy pigmented cases of malignant melanoma evidenced a majority of HMB-45 positive cells while in amelanotic melanomas only a minority of cells reacted with the antibody. Even within the same case, melanotic areas contained more positive cells than amelanotic ones.

The presence of melanin appeared significantly associated with the percentage of HMB-45 positive cells also in metastatic malignant melanoma. In fact, the single HMB-45 negative case was totally amelanotic while two cases with 100% of HMB-45 positive cells were heavily pigmented.

Other morphological features, including the presence of inflammatory infiltration and the level of invasion, did not appear to be significantly correlated with the expression of the antigens recognized by HMB-45.

The results of non-melanocytic tumours are summarized in Table 1. The majority of benign and malignant tumours tested were negative for HMB-45. Only the single melanocytic neuroectodermal tumour of infancy stained positively in the cellular aggregates occasionally containing melanin pigment. Interestingly, this case was negative when tested with S-100 antiserum.

In addition, weak positivity was found in a proportion of nerve sheath tumours. In these tumours the immunoreactivity was confined to a minority of cells with spindle morphology.

Discussion

The development of tissue specific antibodies which react on paraffin embedded formalin fixed tissues considerably broadens their practical usefulness. Among antibodies which recognize melanocytes, antisera against S-100 protein have found wide application. Unfortunately, the usefulness of S-100 protein as immunohistochemical marker is significantly decreased by its presence in a very large variety of normal and neoplastic tissues (Vanstapel et al. 1986).

The recently produced monoclonal antibody HMB-45 seems to have the specificity required for an important diagnostic tool. In the report of Gown et al. (1986) not a single positive tissue, among the large number tested, could be found outside melanomas and junctional nevi. In our study only a rare case of melanocytic neuroectodermal tumour of the maxilla, a tumour histogenetically related to melanoma (Dehner et al. 1979), was positively stained by HMB-45 antibody, while lacking S-100 immunoreactivity.

The focal and weak positive reaction found in some cases of neurilemmoma and malignant schwannomas might be considered as expression of the common origin from neural crest of both Schwann cells and melanocytes (Weston 1970).

All the other tumours tested, including neoplasms which can be morphologically confused with melanomas, including seminoma, anaplastic carcinoma, malignant histiocytosis and neuro-endocrine carcinoma of the skin, were negative.

In normal tissues, however, a constant and reliable cytoplasmic positive reaction was observed in the cells lining the secretory terminal portion of sweat glands. This cross reaction does not undermine the practical usefulness of this antibody but should be taken into account in evaluating skin lesions stained with HMB-45.

Among melanocytic lesions, HMB-45 evidenced a peculiar and constant distribution; in fact all primary malignant melanomas and the junctional melanocytes of nevi stained positively while intradermal melanocytes of nevi were constantly negative. In compound nevi, a transition from a positive melanocytic junctional population to an intradermal negative one was constantly observed (Fig. 1 a). The antibody therefore recognizes different stages of the same cell population.

The molecule(s) recognized by HMB-45 is not yet identified but it seems reasonable to think that it may be related to a peculiar stage of melanocyte differentiation. The observed correlation of the number of HMB-45 positive cells with growth phase and melanin pigmentation could give some clues to the molecule's function. It should be noted that melanin production is by itself associated with growth phase, being more present in the horizontal than in the vertical one. Also the distribution of HMB-45 positive cells within benign and malignant melanocytic lesions seems to follow the presence of melanin. This is also suggested by the observation that the only intradermal benign melanocytes with consistent HMB-45 positivity are those found in blue nevi: these lesions are always heavily pigmented.

We therefore suggest HMB-45 be related to melanin production, but to a peculiar stage, not being present in all pigmented melanocytes.

The positive staining in apparently normal melanocytes in epidermal areas adjacent to different skin lesions, in contrast to the absence of HMB-45 reactivity in melanocytes of normal skin, was not noticed in the Gown's report (Gown et al. 1986), and might be an expression of altered melanocytic

turn-over. However, S-100 protein was present in almost all melanocytes independent of their location in the junctional or dermal zone (Fig. 1 b).

In face of a much higher degree of specificity of HMB-45, S-100 protein evidenced a relatively higher sensitivity. In malignant melanomas, primary and metastatic, only a percentage of neoplastic cells (Fig. 3) reacted with HMB-45 while S-100 protein was more widely expressed. In a single case of metastatic melanoma the neoplastic cells were S-100 positive but HMB-45 negative (in Gown's series in 2 out of 60 metastatic melanomas no positive reaction was observed with HMB-45).

In superficial spreading malignant melanoma the antibody was of particular help in diagnosis. In fact an important hallmark of this type of malignant melanoma is the presence of atypical melanocytes invading the upper part of epidermis (Kamino and Ackermann 1981; McGovern 1983), which are often difficult to identify in routine E&H sections. Superficial spreading malignant melanoma may therefore be underdiagnosed (Jones et al. 1981).

Staining with S-100 is of limited use for it also stains Langerhans cells in the epidermis. HMB-45 instead clearly showed single melanocytes invading the epidermis (Fig. 2); HMB-45 positive cells within epidermis were never observed among junctional, compound and intradermal nevi; in contrast Spitz nevus and spindle cell pigmented nevus may present epidermal invasion by melanocytes (Reed et al. 1975; Sagebil et al. 1984; Smith 1987) well shown with HMB-45.

In conclusion, we stress the high specificity of HMB-45 for melanocytic lesions, the only positive neoplasms. Other positive melanotic lesions (such as melanocytic neuroectodermal tumour of infancy and peripheral nerve tumours) are histogenetically related to melanocytes. It seems, from our experience and from the literature, to be the antibody of choice in evaluating melanocytic lesions. The incomplete sensitivity of the antibody should be taken into account – negative staining with the HMB-45 does not exclude the diagnosis of melanoma.

HMB-45 may also be useful in the differential diagnosis between junctional nevi and superficial spreading malignant melanoma.

However HMB-45 is of no use in discriminating between Spitz nevi, blue nevi, pigmented spindle cell nevus and melanomas; it is present in them all.

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