Virchows Archiv A Pathol Anat (1992) 420:121–126

Virchows Archiv A
Pathological Anatomy and Histopathology

Melanoma-associated antigens in tumours of the nervous system: an immunohistochemical study with the monoclonal antibody HMB-45

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Received July 17, 1991 / Received after revision October 14, 1991 / Accepted October 17, 1991

Summary. The aim of this study was to determine the specificity and sensitivity of the commercially available, monoclonal anti-melanoma antibody HMB-45 in brain tumours and peripheral nerve sheath tumours. Hence, a series of 155 different non-melanotic tumours of the central and peripheral nervous system were examined immunohistochemically. The brain lesions consisted of primary tumours and metastases from various carcinomas. Twenty melanotic tumours (cerebral metastases of malignant melanomas, meningeal melanomatosis, meningeal melanocytomas) and dermal blue cell naevi served as controls. All melanotic tumours stained positive. Furthermore, a positive immunohistochemical reaction was observed in the following non-melanotic tumours: gliosarcomas, primitive neuroectodermal tumours, ependymoma, malignant schwannomas and different intracranial hamartomas. Two plasmocytomas and 4 metastatic carcinomas also revealed positive staining for HMB-45. Our results confirm the necessity for cautious interpretation of HMB-45 immunoreactivity as a tool in the immunohistochemical characterization of nervous system tumours.

Key words: Tumours of the nervous system – Non-melanotic tumours – HMB-45 – Immunohistochemistry

Introduction

The distinction between malignant melanomas and carcinomas, non-Hodgkin lymphomas or sarcomas in the differential diagnosis of undifferentiated malignant brain tumours continues to be difficult. In these cases immunohistochemical analysis appears indicated, for example, with HMB-45, a paraffin-compatible monoclonal antibody directed against melanoma antigen. A very high specificity for melanotic tumours has been ascribed to this antibody, although reports of occasional positive HMB-45 labelling of non-melanotic tumours, for example liver (Wang et al. 1991), breast (Bonetti et al. 1989), lung carcinoma (Ordonez et al. 1988) and clear cell tumours of the lung (Gaffey et al. 1991; Pea et al. 1991), suggest caution in interpreting HMB-45 positivity.

We are not aware of any systematic evaluation of HMB-45 immunoreactivity in nervous system tumours. This study was designed to determine the specificity of HMB-45 as a marker for malignant melanoma in a wide range of different primary non-melanotic brain tumours, tumours of the peripheral nervous system and cerebral metastases of various carcinomas. In order to evaluate the sensitivity of HMB-45, the staining of different melanotic brain tumours and dermal blue cell naevi was studied for control purposes.

Materials and methods

We examined a total of 155 primary and secondary brain tumours, selected mainly from the files of our institution. Individual lesions (peripheral schwannomas) were kindly supplied by G. Jautzke (Department of Pathology, University Hospital Rudolf-Virchow, Berlin) and H. Martin (Department of Pathology, Charité, Humboldt University, Berlin). The tissue had been surgically removed in all but 2 cases. The 2 cases of meningeal melanomatosis were incidental findings in autopsy patients. In addition to the tumours listed in Table 1, we examined 4 hamartomas (a gliomesenchymal hamartoma of the lamina quadrigemina, another such lesion located in the temporal lobe, a gliovascular hamartoma of the frontal lobe in a child and a renal angiomyolipoma).

Twenty melanotic tumours were stained with HMB-45 and reviewed for control purposes. The specimens from the 2 meningeal melanocytomas were biopsies obtained from primary lesions of the middle cranial fossa. The 8 dermal blue cell naevi were kindly provided by N. Haas (Department of Dermatology, University Hospital Rudolf-Virchow, Berlin). The site of the primary tumour had been confirmed histologically by biopsy during life or autopsy findings in all brain metastases (carcinomas and melanomas).

All tissue specimens had been fixed in 10% phosphate-buffered formalin, then embedded and stored in paraffin wax. Sections were routinely cut at 5 μm and stained with haematoxylin and eosin,

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Table 1. HMB-45 immunostaining results in different investigated tumours

Diagnosis	HMB-45 pos./neg.
A: Primary tumours of the cerebral and nervous system	
Neuroepithelial tumours:	
Pilocytic astrocytoma	0/3
Astrocytoma (II–III)	0/11
Oligodendroglioma (II–III)	0/10
Ependymoma	1/7
Subependymoma	0/1
Plexus papilloma	0/5
Glioblastoma	0/14
Gliosarcoma	5/6
Pineocytoma	0/2
Cerebral PNET	2/11
Medulloblastoma	0/11
Neuroblastoma	0/2
Desmoplastic infantile ganglioglioma	0/1
Dysembryoplastic neuroepithelial tumour	0/1
Tumours of meningothelial cells:	
Meningioma	0/13
Malignant meningioma	0/1
Meningosarcoma	0/1
Cerebral and peripheral nerve sheath tumours:	
Central schwannoma	0/12
Peripheral malignant schwannoma	3/4
Spinal neurofibroma	0/4
B: Secondary non-melanotic cerebral and spinal tumours	
Metastatic thyroid carcinoma	1/1
Metastatic lung carcinoma	0/11
Metastatic bladder carcinoma	2/2
Metastatic renal carcinoma	0/5
Metastatic colonic carcinoma	0/2
Metastatic gastric carcinoma	1/1
Metastatic liver cell carcinoma	0/1
Metastatic endometrial carcinoma	0/1
Metastatic breast carcinoma	0/4
Metastatic spinal plasmacytoma	2/3
C: Primary and secondary melanotic tumours of the brain and skin	
Metastatic brain melanoma	8/8
Meningeal melanomatosis	2/2
Meningeal melanocytoma	2/2
Dermal blue cell naevi	8/8
Total	37/171

PNET, Primitive neuroectodermal tumours

Nissl, Masson's trichrome and periodic acid-Schiff. Bielschowsky's axon stain, Prussian blue stain for ferric iron and the Masson-Fontana procedure for melanin were also employed.

The following monoclonal antibodies (mAb) and polyclonal antisera (pAs) were obtained from Dakopatts (Hamburg, FRG): mAb-V9 (vimentin, 1:40), D-33 (desmin, 1:100), factor-VIII-related antigen (1:100), thyroglobulin (1:10000), Kp1 (CD68, 1:100), Mac 387 (1:4000), prostate-specific antigen (1:4000) and the pAs-glial fibrillary acidic protein (GFAP) (1:5000) and S-100 (1:5000). Further immunohistological staining was performed employing mAb-neurofilament (Immunotech via Dianova, Hamburg, FRG, 1:5000), KL1 (cytokeratin, Immunotech via Dianova, 1:500), AE1/3 (cytokeratin, Boehringer Mannheim, Mannheim, FRG, 1:100), Lu-5 (cytokeratin, Boehringer Mannheim, 1:100), chromogranin (Hybridtech, Hürth, FRG, 1:5000) and the pAs-type-IV collagen (Medac, Hamburg, FRG, 1:500).

Immunostaining was carried out applying the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique (Cordell et al. 1984) and the avidin-biotin complex (ABC) method (Hsu et al. 1981). The secondary antibody and the APAAP complex were purchased from Dakopatts. The biotinylated secondary antibody and the ABC were supplied by Vectastain via Camon (Wiesbaden, FRG). Prior to application of the primary antibodies, sections to be stained for factor-VIII-related antigen, Lu-5, Mac 387 (pronase E; Merck, Wiesbaden, FRG) and type IV collagen (pepsin; Sigma, Deisenhofen, FRG) were subjected to protease digestion. Positive and negative control tests were conducted concurrently for all antibodies employed.

In order to select representative sections for staining with the melanoma-associated monoclonal antibody HMB-45 (Enzo, New York, USA), all specimens were reviewed and newly classified according to the criteria of the revised WHO classification of CNS tumours (Kleihues et al., in press). Sections (5 μm) were incubated with HMB-45 antibody (working dilution 1:10000) without prior protease digestion. Immunostaining was performed using the APAAP technique. Sections were counterstained with haematoxylin. Replacement of the primary antibody with non-immune serum served as negative control. Malignant melanoma of the skin represented the positive control.

Results

The immunohistochemical results are presented in detail in Table 1.

Single HMB-45-positive tumour cells, frequently arranged in small groups, were detected in 5 of the 6 examined gliosarcomas (Fig. 1). Among the remaining neuroepithelial tumours only 2 of 11 primitive neuroectodermal tumours and a single ependymoma contained randomly distributed individual cells displaying HMB-45 immunoreactivity. All benign meningiomas (WHO grade I), one atypical meningioma and all malignant meningiomas (grade II–III, including a meningosarcoma) revealed negative results for HMB-45 immunostaining.

Upon review of the nerve sheath tumours, 3 of 4 malignant peripheral schwannomas stained positive for HMB-45. The other central schwannomas (12) and spinal neurofibromas (4) showed no HMB-45 positivity. The strongest reaction was observed in a malignant epithelioid schwannoma (Fig. 2). The GFAP staining was also positive in this tumour. A retroperitoneal malignant schwannoma displayed fewer HMB-45-stained cells (Fig. 3). The malignant schwannoma that lacked HMB-45 positive cells was a tumour of questionable status. Although it appeared relatively isomorphic and had a low mitotic index, the adjacent muscle tissue was definitely infiltrated.

In the group of secondary non-melanotic tumours only single HMB-45-positive cells were observed in 6 of the 31 cases. These cells were usually arranged in randomly distributed clusters (at times as few as 2–3 cells). The 2 metastatic bladder carcinomas staining HMB-45 positive were of the small-cell variant. Occasional positive HMB-45 immunoreactivity included 1

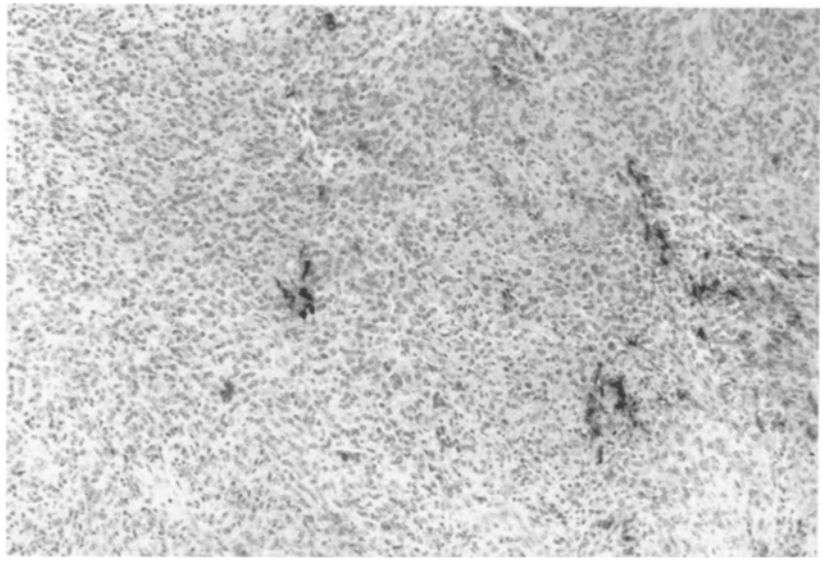

Fig. 1. Small groups of cells showed positive HMB-45 immunostaining in a gliosarcoma. HMB-45, ×200

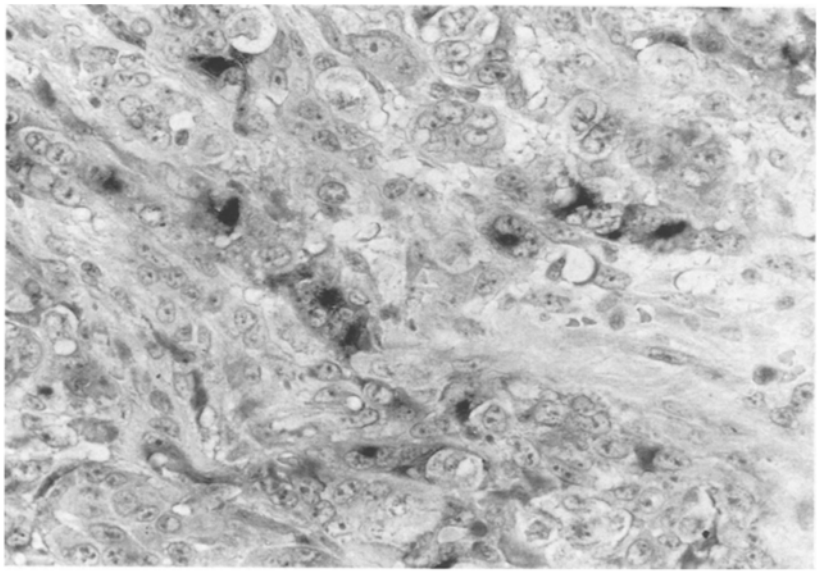

Fig. 2. Some HMB-45-positive cells in a malignant epithelioid schwannoma. HMB-45, ×80

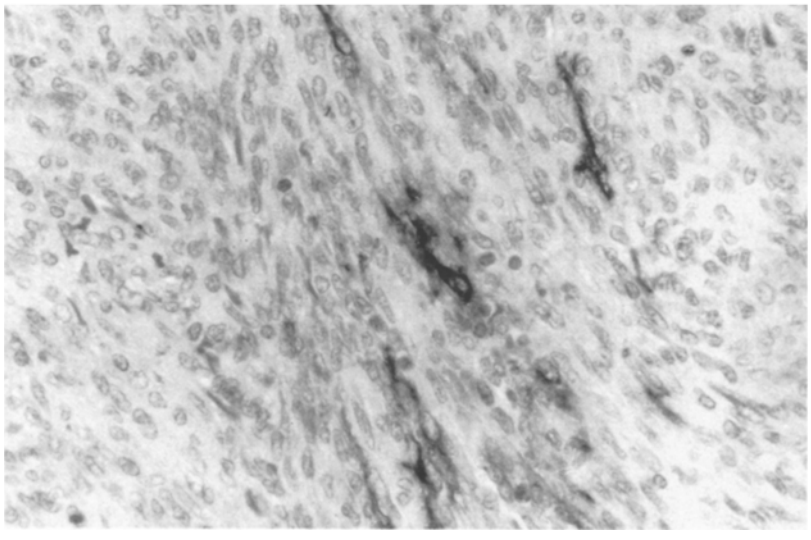

Fig. 3. Few HMB-45-positive tumour cells were observed in a retroperitoneal schwannoma. HMB-45, ×250

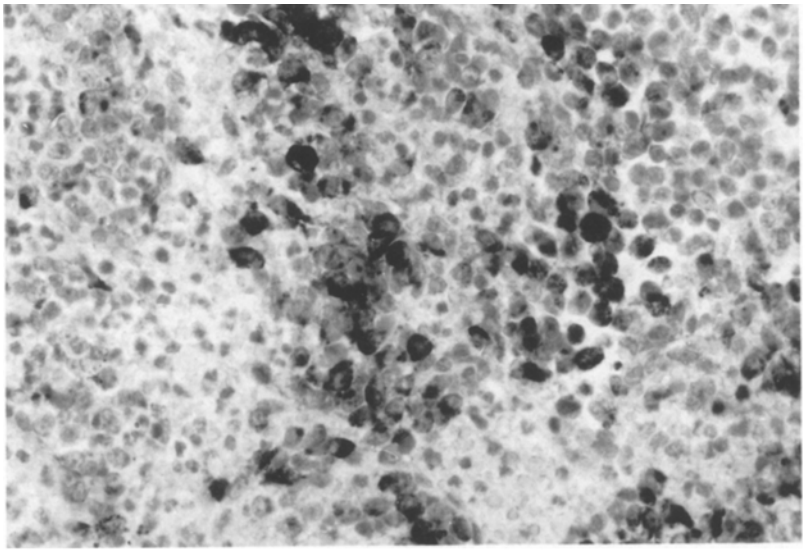

Fig. 4. Widely distributed heavily staining tumour cells in a secondary malignant melanoma of the brain. HMB-45, ×160

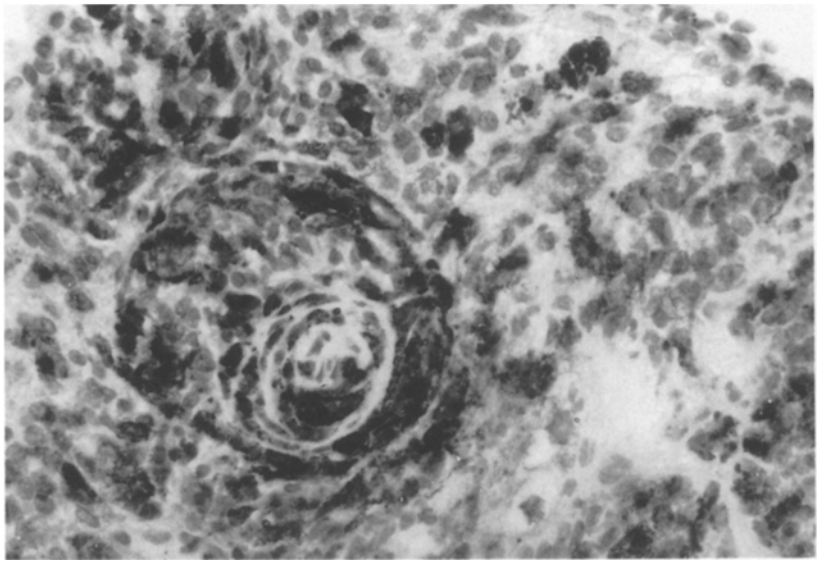

Fig. 5. Numerous HMB-45-stained tumour cells in a meningeal melanocytoma. HMB-45, ×200

gastric and thyroid carcinoma and 2 spinal plasmacytomas.

A surprising finding was the identification of HMB-45-staining cells in 3 of the 4 examined hamartomas. The gliomesenchymal hamartoma of the temporal lobe was the only negative tumour in this group. Again, cells expressing HMB-45 were organized in focal clusters.

Among the primary and secondary melanotic tumours, the HMB-45-positive cells revealed a relatively homogeneous pattern of distribution (Fig. 4). Within these cells, staining was diffuse. Some tumours exhibited focal accumulations of denser HMB-45-positive cells. Occasionally, areas of negative HMB-45 immunoreactivity were observed in metastatic melanomas and dermal blue cell naevi.

Discussion

The histological diagnosis of intracerebral metastases of non-melanotic melanomas may be difficult. The differential diagnosis from anaplastic glial tumours and metastases from undifferentiated carcinomas sometimes poses a considerable problem. In particular tissue specimens from stereotactic brain biopsies produce real difficulties. The success of the Masson-Fontana silver stain as the standard procedure to detect melanin is limited, since lipofuscin or argentaffin cell granules (Stevens 1977) may also stain positively. Immunohistochemical methods, primarily S-100 protein, appear unreliable, since positive immunostaining with this antibody has been observed in a wide range of other tumours, such as schwann cell tumours (Stefansson et al. 1982), chondroblastomas, osteosarcomas, chondrosarcomas (Nakamura et al. 1983), histiocytic tumours (Nakajima et al. 1982a) and various carcinomas (Nakajima et al. 1982b). The vast majority of primary brain tumours, regardless of the histological variant, display S-100 protein positivity (Bonnin and Rubinstein 1984; Russell and Rubinstein 1989). Therefore, a positive reaction for S-100 protein as the sole immunohistochemical tool seems inadequate to diagnose metastatic melanoma in the brain.

Only a combination of an epithelial marker, e.g. keratin, and GFAP may help to elucidate that diagnosis. Consequently, melanoma-specific markers would greatly increase the diagnostic yield of immunohistochemistry. The diagnosis of malignant melanoma has been considerably facilitated with the introduction of immunohistological labelling of a melanoma antigen. A variety of melanoma-associated antibodies have been propagated in the past. Since the majority of these antibodies are directed towards cell-surface antigens, only cryostat sections of the tissue can be employed. Thus, retrospective studies on paraffin-embedded material are impossible. In addition, limited specificity has been ascribed to a large proportion of these antibodies.

These limitations have been overcome through the introduction of HMB-45, a monoclonal antibody labelling melanoma-specific antigen in formalin-fixed, paraffin-embedded sections. It was derived from a lymph node metastasis of a pigmented malignant melanoma by lysate immunization (Gown et al. 1986). To date, the binding antigen has not been isolated. Fetal and neonatal melanocytes, junctional naevus cell naevi, Spitz naevi and metastatic melanoma all stain positively with this antibody, whereas adult melanocytes display no staining (Gown et al. 1986; Palazzo and Duray 1988). Immunoelectron microscopy revealed that the antibody serves as a tool to differentiate between premelanosomes in malignant melanomas and benign melanophages (Walts et al. 1989).

In the literature the antibody is described as highly specific and sensitive. Wick et al. (1988) studied 200 paraffin-embedded cutaneous tumours and found a specificity of 100% and sensitivity of 93%. While none of the 133 non-melanotic tumours reacted with the antibody, 62 of 67 cutaneous melanomas were HMB-45 positive. The 5 non-reactive melanomas were of the desmoplastic variant that commonly stains negative for HMB-45. Nevertheless, high specificity must be doubted for this antibody, since positive staining in non-melanotic tumours has been reported repeatedly. Kornstein and Franco (1990) encountered positive immunostaining of a metastatic adenocarcinoma from the inguinal region (the primary tumour was not mentioned), resulting in the initially false diagnosis of malignant melanoma. Breast carcinomas (Bonetti et al. 1989), liver carcinomas (Wang et al. 1991), clear cell tumours of the lung (Gaffey et al. 1991), plasmacytomas (Leong and Milios 1989), a non-Hodgkin lymphoma (Friedman and Tatun 1991) and olfactory neuroblastomas (Wick et al. 1988) also yielded false positive results.

Our study of central and peripheral nervous system tumours also demonstrates positive HMB-45 immunostaining in several primarily non-melanotic tumours. Both primary and secondary nervous system tumours stained positive. A remarkable finding was the tendency of malignant primary tumours (malignant schwannomas, gliosarcomas) to express HMB-45 positivity more intensively than histologically less malignant tumours (schwannomas, low-grade astrocytomas, meningiomas or oligodrendrogliomas). HMB-45 positive secondary brain tumours come from a wide variety of primary sites. This study demonstrated staining of brain metastases from bladder, gastric and thyroid carcinomas and plasmacytomas. We expect further reports on HMB-45 immunostaining in various secondary non-melanotic brain tumours in the future. There are several possible explanations for the coincidence of HMB-45 immunoreactivity and melanotic differentiation in central and peripheral nervous system tumours. The origin of neuroectodermal tissue and melanocytes from the neural crest should be remembered. Neuroectodermal stem cells are therefore capable of producing melanin, an ability lost by most cells in the process of differentiation, but maintained by melanocytes. Consequently, HMB-45 expression in neuroectodermal tumours of infancy and in neuroblastomas is plausible (Colombari et al. 1988; Wick et al. 1988). The same explanation may hold true for pigmented neuroepithelial tumours, plexus tumours (Boesel and Suhan 1979; Lana-Peixoto et al. 1977), ependymomas and subependymomas (McCloskey et al. 1976), medulloblastomas (Best 1973; Sung et al. 1973), and intracranial teratomas (Russell and Rubinstein 1989). We believe that the immature glioneural components are responsible for the expression of HMB-45 in our cerebral hamartomas.

The positive reaction in the renal angiomyolipoma, however, is a perplexing result. This finding has been previously observed by Pea et al. (1991). These hamartomas are frequently associated with tuberous sclerosis, but, tuberous sclerosis had not been diagnosed in our patient and GFAP was not detected in the tumour. Very few cells gave a positive reaction for S-100 protein, although these might be abortive fat cells. A possible explanation is related to the degree of anaplasia. The production of a protein serving as an epitope for the HMB-45 antibody may occur as an epi-phenomenon during cell anaplasia in tumours. This may contribute to the positive immunostaining of metastases of certain carcinomas. Further clues stem from the fact that several gastrointestinal and respiratory tract tumours contain hormone-secreting cells related to the APUD cell system. These cells are histogenetically linked to the neural crest and, hence, may account for an association with the melanoma antigen.

So-called pigmented meningiomas, often located in the middle cranial fossa, are a rare entity and must be distinguished from malignant melanomas of the leptomeninges. The ultrastructural lack of meningiothelium in the former tumours justifies the term "meningeal melanocytoma" than "melanotic meningioma" (Jellinger et al. 1988; Winston et al. 1987). In view of the positive HMB-45 expression in our meningeal melanocytomas, this marker seems unreliable in the differentiation of these tumours. This may be better accomplished through the fairly benign histology and clinical course of the meningeal melanocytoma.

In conclusion, we observed HMB-45 expression in several non-melanotic, primary and secondary tumours of the nervous system. In accordance with earlier reports was the demonstration of positive HMB-45 immunostaining in all examined melanotic tumours, confirming a high sensitivity. Regarding the specificity of this marker, positive staining must be interpreted with caution. In our experience the positive staining of HMB-45

in a few cells is a very unreliable criterion for the diagnosis of melanotic tumours, whereas large-scale expression seems to be a fairly safe indicator.

Acknowledgements. The authors wish to thank G. Jautzke, M.D. and N. Haas, M.D. [Departments of Pathology (G.J.) and Dermatology (N.H.) University Hospital Rudolf-Virchow, Berlin] and H. Martin, M.D. (Department of Pathology, Charité, Humboldt University, Berlin) for generously providing histological material. We also thank G. Kluge and K. Deparade for skilful technical assistance.

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