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HMB45: a specific marker for melanoma metastases in the central nervous system?

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Abstract The monoclonal antibody HMB45 is used to detect an epitope specific for melanocytes, malignant melanomas and melanoma metastases. Using the PAP method, we observed consistent expression of HMB45 in 19 metastases of melanotic and amelanotic malignant melanomas of the central nervous system, while metastases of 32 adenocarcinomas, 10 squamous cell and 8 small cell carcinomas were negative except for 2 cases of breast cancer. Differential diagnosis between cancer and melanoma metastases can be made using cytokeratins as an additional immunocytochemical marker protein. Ten meningeomas and 5 pineocytomas were also negative. Even though it is not absolutely specific, we consider the HMB45 immunoreaction diagnostic for a metastasis of a malignant melanoma if the tumour is cytokeratin negative and HMB45 positive in a large number of tumour cells.

Key words HMB45 · Brain metastases · Malignant melanoma

Introduction

Metastases account for about 10% of tumours of the central nervous system, and metastases of malignant melanomas occur frequently. The differential dagnosis between malignant melanomas and carcinomas can be difficult [14], especially in tiny tissue samples such as are obtained in stereotactic brain biopsies. The use of immunohistological methods allows the expression of cytokeratins specific for metastatic carcinoma to be identified, and vimentin is found in malignant melanomas. The expression of vimentin, however, is not specific for a given cell type or tumour entity. The same holds true for protein S100 [15].

An immunomorphological marker for malignant melanomas would be of great value, and early reports on the specificity and sensitivity of HMB45 for melanocytes and malignant melanomas were very promising. HMB45 has been shown to react with a neuraminidase-sensitive oligosaccharide side chain of a glycoconjugate present in immature melanosomes. The reactive antigen is present in cutaneous melanocytes, retinal pigment epithelium, and melanoma cells; it is thought to be oncofetal in nature [9].

The present study was undertaken to demonstrate the reliability and specificity of HMB45 expression in metastases of malignant melanomas in contrast with metastatic carcinoma.

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Materials and methods

Paraffin blocks of formaldehyde-fixed tumour biopsies were selected randomly from the files of the institute. The cases comprised metastases from 32 adenocarcinomas, 10 squamous cell carcinomas, 8 small cell carcinomas and 19 malignant melanomas (8 amelanotic, 11 melanotic). The tumours were localised in the brain, the epidural space or the vertebrae. The source of the primary tumour was known in some cases. In addition, metastases from 1 Ewing´s sarcoma, 1 osteoblastoma, 10 meningeomas (endotheliomatous, transitional and fibroblastic) and 5 pineocytomas were analysed. Tumour diagnosis was done on HE- and PAS-stained slides.

To detect the HMB45 antigen, the peroxidase-antiperoxidase (PAP) technique of Sternberger et al. [18] was applied. Briefly, 4- to 6-µm paraffin sections were dewaxed in fresh xylene for 2×10 min at room temperature, rehydrated in ethanol and incubated in 1‰ H_2O_2/methanol (30%) for 20 min to block the endogenous peroxidase activity. After rinsing in phosphate-buffered saline (PBS, pH 7.6) the slides were incubated with 5% normal swine serum (Dako, Hamburg, Germany) for 30 min to reduce background staining. With monoclonal antibody (mab) the four-step PAP method comprised sequentially: (a) mab (DAKO-HMB45, supernatant form; Dako) against HMB45 (diluted 1l:l400) and mab against cytokeratins (clone KL1, 1l:l40; Dianova, Hamburg, Germany) for 1 h at room temperature; (b) rabbit anti-mouse immunoglobulins (1l:l50; Dianova, Hamburg, Germany) for 30 min; (c) swine anti-rabbit immunoglobulins (1l:l20; Dako) for 30 min; and (d) soluble PAP complex (1l:l100; Dako) for 30 min. Control sections were performed without the primary antibody or by employing nonspecific antisera. All incubation steps were carried out at room temperature in a moist chamber. Positive immunoreaction was visualised by 40 mg% 9-amino-ethylcarbazole

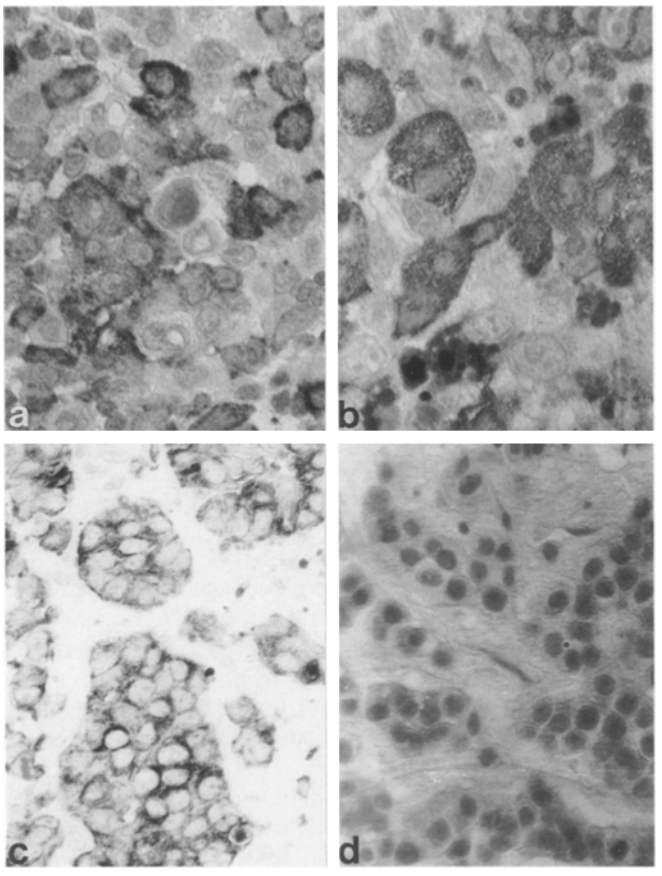

Fig. 1a–d Expression of HMB45 antigen in metastases in the central nervous system. Positive immunostaining in **a** amelanotic and **b** melanotic malignant melanoma, and **c** breast cancer. No reactivity in a metastasis from an adenocarcinoma (**d**). PAP, haematoxylin counterstain; original ×25

Table 1 HMB45 immunoreaction (proportion of cells positive: + under 30%, ++ 30–70%, +++ over 70%)

Tumor entity	*n*	+++	++	+	−
Metastasis					
Malignant melanoma					
Melanotic	11	7	1	3	0
Amelanotic	8	5	3	0	0
Adenocarcinoma					
Kidney	9	0	0	0	9
Breast	5	0	1	1	3
Bronchus	3	0	0	0	3
Colon	2	0	0	0	2
Prostate	2	0	0	0	2
Lip	1	0	0	0	1
Others	10	0	0	0	10
Squamous cell carcinoma	10	0	0	0	10
Small cell carcinoma	8	0	0	0	8
Ewing's sarcoma	1	0	0	0	1
Osteoblastoma	1	0	0	0	1
Brain tumor					
Pineocytoma	5	0	0	0	5
Meningioma					
Endotheliomatous	4	0	0	0	4
Transitional	3	0	0	0	3
Fibroblastic	3	0	0	0	3
Total	86				

Table 2 Comparison of HMB45 and cytokeratin immunoreactions

Metastasis	HMB 45	KL1
Malignant melanoma		
Melanotic	11/11	0/11
Amelanotic	8/8	0/8
Adenocarcinoma	2/32	32/32
Squamous cell carcinoma	0/10	10/10
Small cell carcinoma	0/8	8/8

(Sigma, Deisenhofen, Germany) in 50 mM acetate buffer (pH 5.0) with 0.05% H_2O_2 [8]. Finally, the nuclei were counterstained with haematoxylin. The immunoreactions were evaluated using the following score scale: negative, -; less than 30% of cells positive, +; between 30% and 70% of cells positive, ++; more than 70% of cells positive, +++ [2, 13].

Results

The immunomorphological results are displayed in Tables 1 and 2. All malignant melanoma samples tested, regardless of whether they were from pigmented or non-pigmented malignant melanomas, gave a positive immune reaction. HMB45 immunoreactivity was intracytoplasmic, granular and concentrated at the cell membrane. The intensity of immunoreaction was variable, but this is not taken into account. All metastases of adenocarcinomas (n=32), squamous cell carcinomas (n=10) and small cell carcinomas (n=8) were negative except for two cases. These two positive samples were metastases of breast cancer. The reactivity in these cases has been evaluated as ++ and +, respectively. All carcinoma metastases showed intracytoplasmic cytokeratin immunoreactivity.

Metastases of one Ewing sarcoma, one osteoblastoma and endotheliomatous, transitional and fibroblastic meningiomas (n=10) were completely HMB45 and KL1 negative. The same was true for five pineocytomas, which were also KL1 negative.

Discussion

HMB45 is a monoclonal antibody that reacts with a neuraminidase-sensitive oligosaccharide side chain of a glycoconjugate present in immature melanosomes [9]. It has been demonstrated to have a high sensitivity and specificity for malignant melanomas. As in our series, all reports in the literature demonstrate marked expression of HMB45 in all or almost all malignant melanomas tested [7, 10, 11, 12, 17].

Our aim was to test the specificity of HMB45 expression in malignant melanomas in contrast with the reactivity of other carcinoma metastases in the central nervous system. We found that the overwhelming majority of adenocarcinoma, squamous cell carcinoma and small cell carcinoma metastases were completely negative. However, two out of five metastases of ductal invasive

breast cancer exhibited immunoreactive tumour cells. This phenomenon has been reported previously [3]. HMB-45 immunoreactivity in breast carcinomas should be confirmed by in situ hybridisation. To date, however, mRNA probes are not available for these studies. It is interesting that only breast cancer demonstrated a positive immunoreaction, which has been regarded as a false positive by some [4]. The discrimination could be done by Western blot analysis. This, however, was not possible in our series, because only paraffin-embedded material was available. In the situation of diagnostic immunomorphology, the phenomenon (false positive or not) seems to be irrelevant, because the validity of an immunoreaction cannot be read from a paraffin slide.

Zimmer et al. [20] reported on HMB45 immunoreactivity in a metastatic thyroid cancer. Testing in a large series of tumours of the central nervous system revealed that isolated cases of ependymoma (1/7) and cerebral primitive neuroectodermal tumours (2/11) and most of the gliosarcomas (5/6) were HMB45 positive [20].

All cases of meningioma so far reported, as well as those in our series, have been consistently negative (whether endotheliomatous, transitional or fibroblastic). Some cases of HMB45-reactive tumours, including angiomyolipoma, epitheloid and malignant schwannoma and malignant lymphoma, have been reported in the literature [1, 5, 6, 16, 19, 20]. Pineocytomas (n=5), which can be problematic in differential diagnosis, were also negative. These tumours and malignant schwannomas [20] and malignant epitheloid malignant schwannomas [16] are not really problematic with respect to the differential diagnosis against malignant melanoma. Despite this, it should be borne in mind that some carcinomas or metastases exhibit false-positive HMB45 immunoreactivity or none.

In conclusion, HMB45 is consistently expressed in malignant melanomas and their metastases. HMB45 is not, however, absolutely specific for malignant melanomas.

In tumours of the central nervous system, a real diagnostic problem can arise when it is necessary to differentiate between metastatic carcinoma and melanoma. Positive immunoreaction for HMB45 alone must be regarded with caution, but when it is observed together with the results of cytokeratin staining a diagnosis of malignant melanoma can be made if the tumour is cytokeratin negative and HMB45 positive in a large number of tumour cells.

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