VS38 immunostaining in melanocytic lesions

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Abstract

Aims—To investigate the immunoreactivity of a range of melanocytic lesions, both benign and malignant, with the monoclonal antibody VS38. This was recently described as a marker of reactive/neoplastic plasma cells and, therefore, is useful in the diagnosis of plasmacytoma/myeloma and lymphomas with plasmacytic differentiation. This study was prompted by the recent observation that a plasmacytoid melanoma arising in the nasal cavity was strongly immunoreactive with VS38, which was therefore a potential source of major diagnostic error.

Methods—The Streptavidin-peroxidase complex technique was used on paraffin wax embedded sections of 167 melanocytic lesions. Diaminobenzidine (DAB) was used as chromogen for non-pigmented or lightly pigmented lesions and nickel/DAB for more heavily pigmented lesions.

Results—Positive immunostaining for VS38 was seen in 14.5% (10/69) of benign naevi (including 40% (four of 10) of Spitz naevi), 10.5% (two of 19) of dysplastic naevi/in situ melanomas, 92% (35/38) of primary cutaneous melanomas, 100% (four of four) of primary mucosal melanomas, 91.7% (33/36) of recurrent/metastatic melanomas, and 100% (one of one) of clear cell sarcomas of soft tissues.

Conclusions—VS38 immunostaining is frequently positive in primary and recurrent/metastatic malignant melanoma and is also reactive less commonly with benign naevi. These results should be borne in mind when this recently described marker of normal/neoplastic plasma cells is used to identify tumour lineage, particularly in tumours arising at unusual

sites, such as in the nasal cavity. The possibility of malignant melanoma should be actively considered and excluded in any undifferentiated tumour which shows VS38 immunoreactivity.

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The monoclonal antibody VS38 was described in 1994¹ as an immunohistochemical marker of myeloma/plasmacytoma in bone marrow or other tissues. The antibody is currently marketed by Dako (High Wycombe, UK) as VS38c. Although VS38 has been reported to positively label a melanoma cell line,¹ there has been no systematic investigation of its use in the immunohistochemistry of melanocytic lesions as far as we are aware. The present study was prompted by our observation that VS38 stained a case of amelanotic, focally plasmacytoid malignant melanoma of the nasal cavity in which a plasmacytoma was considered in the differential diagnosis. This led us to explore further the immunohistochemical reactivity of melanocytic lesions, both benign and malignant, with VS38. Our initial pilot study suggested that VS38 only rarely stained benign melanocytic naevi in contrast to malignant melanomas, which were frequently positive. We therefore set about investigating the immunohistochemical profile of this antibody with a wide range of melanocytic lesions.

Methods

The spectrum of melanocytic lesions studied is shown in table 1. They constituted a random selection of cases from within specified SNOMED coded groups which had been biopsied between 1992 and 1995. Lesions had been removed from patients over a wide age range and one was a malignant melanoma from a child. Primary cutaneous malignant melanomas ranged from 0.3 to 9.5 mm in Breslow depth. The metastatic melanomas were within lymph nodes with the exception of two cases, one of which was in skeletal muscle and the other within subcutaneous tissue. All of the recurrent melanomas were in dermis and/or subcutaneous tissue with the exception of one case which recurred as neurotropic melanoma growing along the radial nerve. Another case of recurrent cutaneous melanoma contained cells with a naevocytoid appearance.

Sections were cut at 3 µm onto Polysine-coated slides (Merck Ltd., Lutterworth, UK), dried overnight at 37°C and then melted on at 60°C for 30 minutes. Following blocking in methanol peroxide, sections were placed in

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Table 1 Immunoreactivity of melanocytic lesions with the monoclonal antibody VS38

Lesion	Number of cases	Number positive	Per cent positive
Junctional naevus	9	0	0
Compound naevus	22	1	4.5
Intradermal naevus	21	2*	9.5
Spitz naevus	10	4	40
Common blue naevus	4	2†	50
Cellular blue naevus	1	1	—
Combined intradermal/blue naevus	1	0	—
Combined compound/cellular blue naevus	1	0	—
Dysplastic naevus/in situ melanoma	19	2	10
Invasive melanoma NOS	4	4	100
Superficial spreading invasive melanoma	21	19	90
Nodular melanoma	4	3	75
Mucosal melanoma (invasive)	4‡	4	100
Lentigo malignant melanoma	9	9	100
Recurrent melanoma	13	11**	85
Metastatic melanoma	23	22	96
Malignant melanoma of soft parts (clear cell sarcoma)	1	1	—

NOS=not otherwise specified. * One was a focally positive neuroid intradermal naevus; † both were focally positive; ‡ two nasal and two urethral melanomas; ** one naevocytoid recurrent melanoma and one neurotropic recurrence were negative.

0.01 M citrate buffer (pH 6.0) and were irradiated in a 700 W microwave oven for 23 minutes. The primary antibody was VS38c

Figure 1 Metastatic melanoma in a lymph node showing strong cytoplasmic positivity. Some intra-nuclear cytoplasmic inclusions (arrow) also stained. (VS38c; NBT chromogen.)

Figure 2 Intradermal naevus. Whilst the naevus cells are negative, the overlying squamous epithelium is immunoreactive. (VS38c; NBT chromogen.)

supplied by Dako and a standard Streptavidin-peroxidase complex method was used.

Nickel/diaminobenzidine (DAB) was required in a total of 33 lesions including heavily pigmented benign naevi and deeply pigmented melanomas. For example, all blue naevi required this chromogen to enable proper interpretation. In the majority of cases the distinction of peroxidase staining from melanin was possible without resort to nickel/DAB because of the darker, more granular staining of melanin. Problems were occasionally encountered in cases which had a very fine intracellular dusting of melanin and in these nickel/DAB was used.

Results

The results following immunohistochemical staining with VS38 are shown in table 1. Immunohistochemical staining was seen as diffuse brown cytoplasmic signal where DAB chromogen was used or an intense black cytoplasmic staining where nickel/DAB was required because of heavy melanin pigmentation. Nitroblue tetrazolium (NBT) chromogen substrate was used in order to facilitate photomicrography.

Figure 1 shows a malignant melanoma with strong positivity for VS38. The overall rate of positivity in malignant melanomas, whether invasive primary or recurrent/metastatic lesions, was 92.3% (72/78) compared with 14.5% for benign naevi. The latter figure includes Spitz naevi, 40% (four of 10) of which were VS38 positive. If Spitz naevi are excluded, then only 10.2% (six of 59) of benign naevi were positive. Figure 2 shows a typical intradermal naevus in which the lesional cells were negative. The in situ melanoma/dysplastic naevus group only showed positivity in 10.5% (two of 19) of cases. In addition to cytoplasmic staining, intranuclear cytoplasmic inclusions in some cells were positive. The tumour cells of some melanomas showed subplasmalemmal staining with a perinuclear unstained zone.

Positivity was variable in both intensity and extent between individual lesions ranging from weakly focal to strong and diffuse staining. Positivity confined to less than 25% of the lesional area (focal staining) was observed in 39% of all primary invasive or recurrent/metastatic malignant melanomas. When focal staining was seen it tended to be present in the deeper portions of the tumour in cutaneous primary melanomas or in cells at the tumour edge. The malignant melanoma which had been removed from the back of a child was focally positive. For benign naevi other than Spitz, focal staining in <25% of the lesional area was noted in all positive cases. Three of the four positive Spitz naevi showed more diffuse staining. The fourth positive Spitz naevus showed only isolated positive cells. Comparison of staining intensity between cases is subjective and of limited value. However, in most cases which demonstrated immunoreactivity to VS38, staining was of weak or moderate intensity. Particularly strong positivity was seen in a small number of cases.

Examples included a polypoid nodular malignant melanoma removed from the left elbow of a 69 year old man and bilateral axillary lymph nodes containing metastatic melanoma from a 45 year old man. One clear cell sarcoma (malignant melanoma of soft parts) was strongly and diffusely positive for VS38.

Discussion

In 1994 Turley *et al*¹ established that the monoclonal antibody VS38 recognises both normal and neoplastic plasma cells through affinity for a 64 kilodalton intracytoplasmic antigen. These workers tested VS38 reactivity in histological sections from a range of normal tissues which had been pretreated in a microwave oven. No immunoreactivity could be established without microwave pretreatment. In addition to plasma cells, they noted positivity in tonsillar epithelium, exocrine pancreas, renal distal tubules and Bowman's capsule, adrenal glomerulosa cells, and gut APUD (amine precursor uptake and decarboxylation) cells. In the brain, VS38 highlighted neurons, glial cells and Purkinje cells. Unidentifiable stromal spindle cells were also reported to be strongly positive in some tissues, a finding which we also noted in some of our cases.

VS38 reactivity was also investigated in a number of cell lines and tissue sections of selected neoplastic lesions.¹ Some non-plasma cell tumours such as lymphoplasmacytoid lymphomas were positive, with weak staining also in a proportion of B cell, large cell non-Hodgkin's lymphomas. Weak cross-reactivity with non-neoplastic epithelium as well as with epithelial tumours was also noted,¹ although haematopoietic cells of lineage other than plasma cells were negative. It was shown by western blotting that a breast carcinoma cell line also contained a strong protein band of 64 kilodaltons. Although positivity for VS38 has been noted in single melanoma cell line,¹ ours is the first reported study of VS38 immunoreactivity in melanocytic lesions.

The present study was initiated after we found immunohistochemical positivity for VS38 in a plasmacytoid intranasal malignant melanoma. Further investigation revealed positivity of the same tumour for S-100 protein, NKI/C3 and HMB-45 and a diagnosis of malignant melanoma was therefore made. Reactivity for VS38 in such a situation where malignant melanoma is not initially considered on morphological grounds could potentially lead to an erroneous diagnosis. Whilst this sort of problem may not arise in lesions from cutaneous sites, primary melanomas at less common locations or metastatic tumours could pose problems of interpretation. This is particularly so given the notoriously chameleon-like morphology of malignant melanomas.

The classic immunophenotypic markers of melanocytic lesions are S-100 protein, NKI/C3 and HMB-45 in order of increasing specificity. NKI/C3 is more specific than S-100² but its specificity for melanocytic lesions is not absolute.²⁻⁴ HMB-45 is the most specific marker of malignant melanoma, although there are rare

reports of immunoreactivity in other tumours.⁵ Up to 13% of melanomas are negative for HMB-45 (from pooled literature data).⁶ Malignant melanoma may also express cytokeratins,^{6,7} neurofilament protein,⁷ desmin,^{8,9} α -smooth muscle actin,⁶ KP1 (CD68),¹⁰ and epithelial membrane antigen (EMA).¹¹ It could be argued that VS38 expression which we found in the majority of malignant melanomas represents an extension of the anomalous immunophenotype of melanomas. We did not observe sufficient sequential specimens in the present study to make any valid comment on temporal alterations in VS38 immunoreactivity. The explanation for the focal nature of staining for VS38 in the lesions described is unclear although it is interesting that heterogeneity of staining of melanoma cells for HMB-45 is also well recognised.¹²

Although our study has shown positivity for VS38 in a majority of malignant melanomas and in a minority of benign naevi, it should not be used as a discriminator between difficult benign and malignant melanocytic lesions. Morphological features remain paramount in this distinction in difficult cases because 40% of Spitz naevi were positive.

In conclusion, we would like to emphasise that VS38C is not entirely specific for plasmacytoma/myeloma and is particularly expressed in malignant melanoma. If an undifferentiated tumour is encountered in which VS38 positivity is seen, we recommend a panel of immunohistochemical markers which should include NKI/C3 and HMB-45 to exclude malignant melanoma, even if the tumour has a plasmacytoid appearance suggestive of myeloma/plasmacytoma.

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