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Malignant melanomas contain only the vimentin type of intermediate filaments*

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Summary. Six malignant melanomas have been examined for the type of intermediate filament they contain. All six cases showed positive staining of intermediate filaments with antibodies to vimentin, with cells containing large numbers of melanosomes being stained less strongly in general.

The tumor cells did not react with antibodies to keratin, desmin, neurofilaments or glial fibrillary acidic protein. Thus typing of intermediate filaments can distinguish melanoma from undifferentiated carcinoma, but not from lymphoma or sarcoma. Since melanocytes are known to be vimentin positive, and since most of the samples we studied were from metastases, these results are a further indication that the intermediate filament type typical of the parental cell is retained in the metastases, as well as in the primaries of solid tumours.

The implications of vimentin positivity for the histiogenesis of the melanocyte are also discussed.

Key words: Malignant melanoma – Intermediate filaments – Vimentin – Immunohistochemistry

Melanomas account for approximately 2% of all cancers. In the last decade the incidence of and mortality from malignant melanomas of the skin has increased, although the incidence at other sites of malignant melanoma appears fairly constant. These tumours are considered to originate by aberrant multiplication of the melanocytes which leads to migration of these atypical cells away from their usual position and results in tumour formation. In many cases a diagnosis of malignant melanoma is suspected from an examination of the macroscopic appearance of this usually pigmented

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lesion and can be confirmed by conventional light microscopy. Histochemistry, using specific stains for melanocytes such as the Dopa stain which indicates an active tyrosinase enzyme or one of the silver stains, which indicate the presence of melanin pigment granules (Lever and Schaumburg-Lever 1975) is also valuable. In other cases, for instance when the tumour is amelanotic or presents with metastasis, diagnosis can be much more difficult. Not only is it neccessary to separate true malignant melanomas from other lesions involving pigmented nevi, but it is also important to be able to distinguish malignant melanoma from undifferentiated carcinomas, or from the occasional pigmented carcinoma (for review see Ackerman 1981).

Surprisingly the histological origin of the parent cell, the melanocyte, is still under discussion. Usually it is assumed to arise by migration of cells from the neural crest, and therefore to be of neuroctodermal origin (Du Shane 1948; Bolande 1974; Cove 1979; Pearse 1979; Ackermann 1981). Recently the use of sera to different intermediate filament subtypes has been shown to be of value in the classification both of normal cells and of tumours. Relevant to this study is the fact that melanocytes in primary culture and in situ (Franke et al. 1978; Löning et al. 1982) are characterized by the presence of vimentin, an intermediate filament subtype which is often associated in situ with both cells of mesenchymal origin (Franke et al. 1978; Bennett et al. 1978) and certain other cells of non-epithelial origin. In addition, the use of well-characterized sera each containing only one IF type has shown that certain tumour groups can be differentiated. These are, (i) carcinomas characterized by the presence of cytokeratin filaments, (ii) non-muscle sarcomas characterized by the presence of vimentin filaments, (iii) rhabdomyosarcomas characterized by the presence of desmin filaments, (iv) gliomas by GFA and certain tumours of neural origin which contain neurofilaments (e.g. Gabbiani et al. 1981; Altmannsberger et al. 1981a and b, 1982a and b; Caselitz et al. 1981; Osborn et al. 1982a and b).

To date there is no reported analysis of the intermediate filament type or types present in malignant melanoma. Here we present our results on 6 cases of malignant melanoma. We discuss whether our finding that these tumours are positive for vimentin and negative for other intermediate filaments can be of use in diagnosis, and also discuss the histiogenetic implications of this result.

Materials and methods

The malignant melanomas used in these studies were obtained through the Clinic of Dermatology in the University of Hamburg (Director: Prof. Dr. Nasemann). Some information about the different specimens is given in Table 1. Additionally, two anaplastic carcinomas of the mammary gland were investigated as controls.[1]

Most specimens were collected in an advanced stage of the disease (e.g. lymph node metastasis). The material was divided. One part was fixed routinely in Bouin's solution and subsequently prepared for conventional microscopical techniques including hematoxylin-eosin

[1] We thank Prof. Dr. Stegner for the material. (Gynecological Clinic of the University of Hamburg)

Table 1. Reaction of malignant melanomas with antibodies to the different intermediate filament subtypes

Case No.	Location	Reaction of tumor cells with antibodies to				
		vimentin	keratin	desmin	GFA	Neurofilament
1	skin	+	−	−	−	−[a,b]
2	skin	+	−	−	−	−[a,b]
3	lymph node	+	−	−	−	
4	lymph node	+	−			
5	lymph node	+	−	−	−	−[c]
6	lymph node	+	−	−	−	−[a,c]

[a, b, c] Refer to the particular neurofilament antibodies used (see Material and Methods)

staining, Giemsa's stain and Masson-Fontana stain. The other part was quick frozen in liquid nitrogen and stored at −70° C. Cyrostat sections were cut at −20° C and were either used after 1–24 h at room temperature or were stored at −70° C up to 2 weeks before use.

Antibodies

The following antibodies against the different intermediate filament types, were available each of which recognizes only one intermediate filament type.

1. *Vimentin* antibody, made in guinea pig against vimentin from mouse 3T3 cells (Franke et al. 1979) which had been affinity-purified on Sepharose 4B coupled to vimentin purified from rabbit chondrocytes (Osborn et al. 1982a).

2. *Prekeratin* antibody made in guinea pig against prekeratin purified from cow hoof (Franke et al. 1978) and which was affinity-purified on the same antigen coupled to Sepharose 4B (Osborn et al. 1982a).

3. *Desmin* antibodies made in rabbit against desmin purified from chicken gizzard and affinity-purified on the same antigen coupled to Sepharose 4B (Osborn et al. 1981);

4. Antibody to *glial fibrillary acidic protein* made in rabbits against the protein purified from pig spinal cord (Sharp et al. 1982). This antibody was used as a serum at 1:30 dilution;

5. Antibodies against *neurofilaments* purified from rat brain. Several different preparations made in rabbits were used during this study and were the gift of Dr. G. Shaw: a) Antibody specific for the 200K neurofilament polypeptide; b) antibody specific for the 68K neurofilament polypeptide; c) antibodies which recognize all three neurofilament polypeptides, made by mixing a) and b) with an antibody specific for the 145K neurofilament polypeptide (for details see Shaw and Weber 1981).

6. Antibodies against lysozyme (Dako, Copenhagen, Denmark).

For a review of the properties of these antibodies and for the staining patterns on normal and pathological tumour material see the above references and also Altmannsberger (1981a and b, 1982a and b; Caselitz et al. 1981). Second antibodies were FITC goat anti-rabbit IgG (Miles, Israel), FITC goat anti-guinea pig IgG (Cappell Laboratories, Cochranville, PA., USA) and were usually absorbed on an acetone powder of human large bowel to lower the background.

Immunofluorescence procedures

Sections were fixed in acetone at −10° C for 10 min and air dried. After application of the first antibody sections were incubated for 45 min at 37° C and washed well in phosphate buffered saline. The second antibody was then applied and the specimens incubated for a further 30–45 min at 37° C. After a further wash specimens were mounted in Mowiol 4-88.

In some cases, the indirect immunoperoxidase technique was used. The prcodure was the same as for immunofluorescence, but the second antibody was coupled with peroxidase. For demonstration of the localisation, the DAB reaction was used.

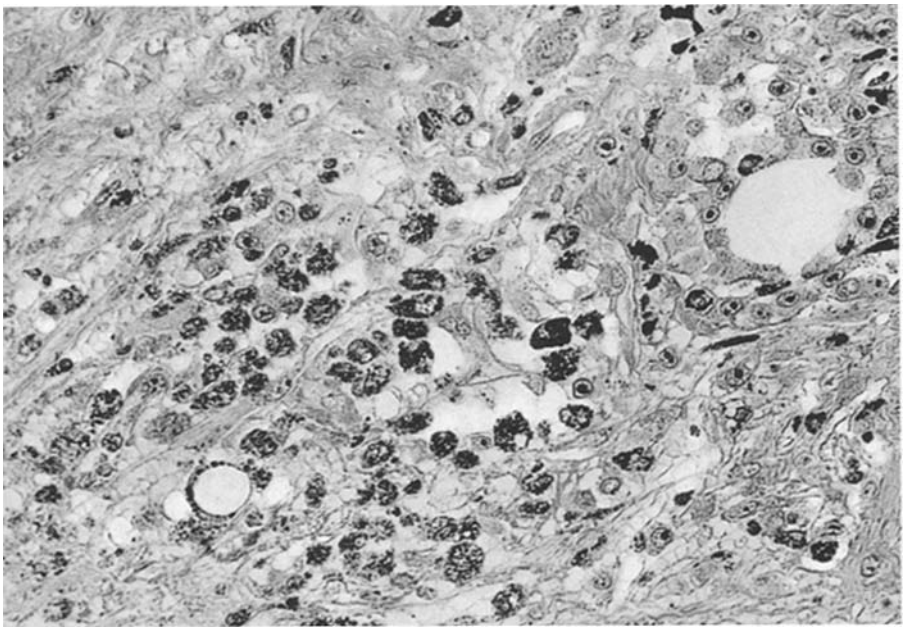

Fig. 1. Case stained by the Masson-Fontana method. Note the strong staining of the melanocytes in this specimen. Magnification × 300

Results

The six cases of melanoma studied here were derived from different sites (Table 1). The tumour cells in the six cases showed differing shapes when conventional histological stains were used: some were oval, some spindle-like and some pleomorphic. Use of the silver staining procedure (Fig. 1), as well as phase microscopy of the frozen sections (Fig. 2b) showed numerous cells in which melanosomes could be visualized. The melanocytes could be easily distinguished from macrophages by their negative lysozyme staining.

The results of using antibodies specific for each type of intermediate filament are summarized in Table 1 and illustrated in Figs. 2 and 3. The tumour cells showed a strong reaction in all six cases with antibodies to vimentin (Fig. 2b, Fig. 3a). On viewing the specimens at higher power such staining was clearly associated with cytoplasmic filament bundles with a distribution similar to that expected for intermediate filaments. Cytoplasmic filaments were not detected when the tumour cells were tested with antibodies to prekeratin (Fig. 3b) although the prekeratin antibody clearly stains epithelia of keratinizing and non-keratinizing origin, as well as tumours derived from these tissues. The tumour cells were also negative when tested with antibodies against chicken gizzard desmin (Fig. 3c) which recognizes skeletal, cardiac and certain vascular smooth muscle cells. In addition no

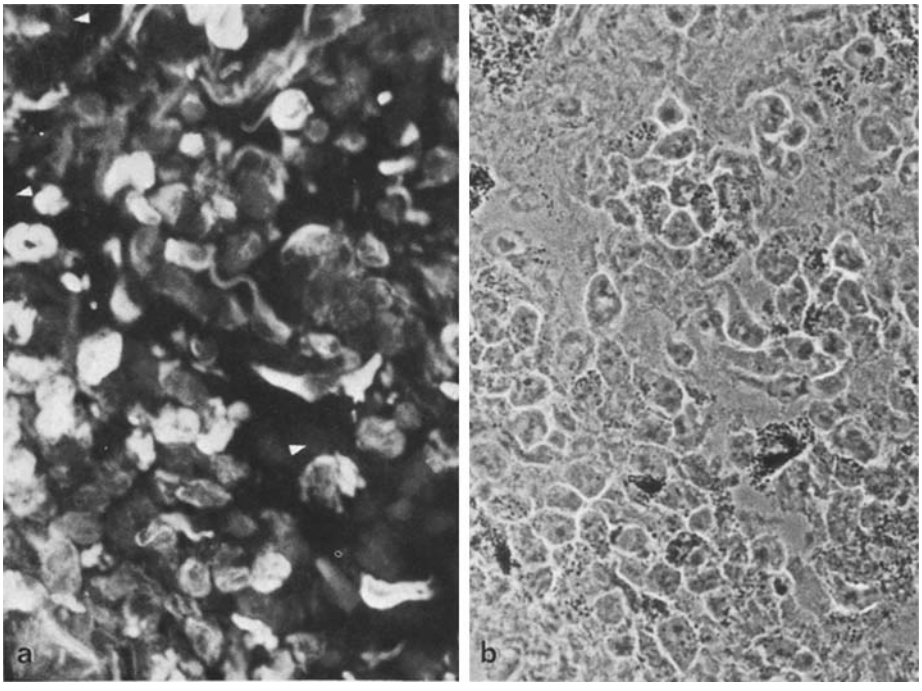

Fig. 2a, b. Frozen sections from case stained with antibodies to vimentin and then photographed either in fluorescence **a** or in phase microscopy **b**. Note the strong staining of cytoplasmic filaments visible in **a**. Note also that the staining appears weaker in the cells with large numbers of melanosomes (compare **a, b,** arrowheads). Magnification × 600

reaction was seen with antibodies to glial fibrillary acidic protein (Fig. 3e) or with two different preparations of antibodies which recognize neurofilaments (Fig. 3f), even though these latter antibodies clearly recognize neurofilaments as well as certain tumours derived from neuronal cells.

By photographing the sections treated with the vimentin antibody, first in fluorescence and then in phase microscopy, it was possible to compare the vimentin staining of cells with melanosomes to those that lacked them. This is illustrated in Fig. 2a, b. Cells that were very strongly stained by the vimentin antibody usually lacked melanosomes. Conversely cells that could be identified from the phase picture as having large numbers of melanosomes usually showed only weak staining with the vimentin antibody. It is not known whether this apparent reduction in intermediate filaments seen in tumour cells containing melanosomes reflects a real reduction in the amount of intermediate filaments as a consequence perhaps of cell differentiation or whether some of the fluorescence is being quanched or rendered non-visible by the melanosomes. Melanomas were also shown to be vimentin-positive using a peroxidase procedure (data not shown).

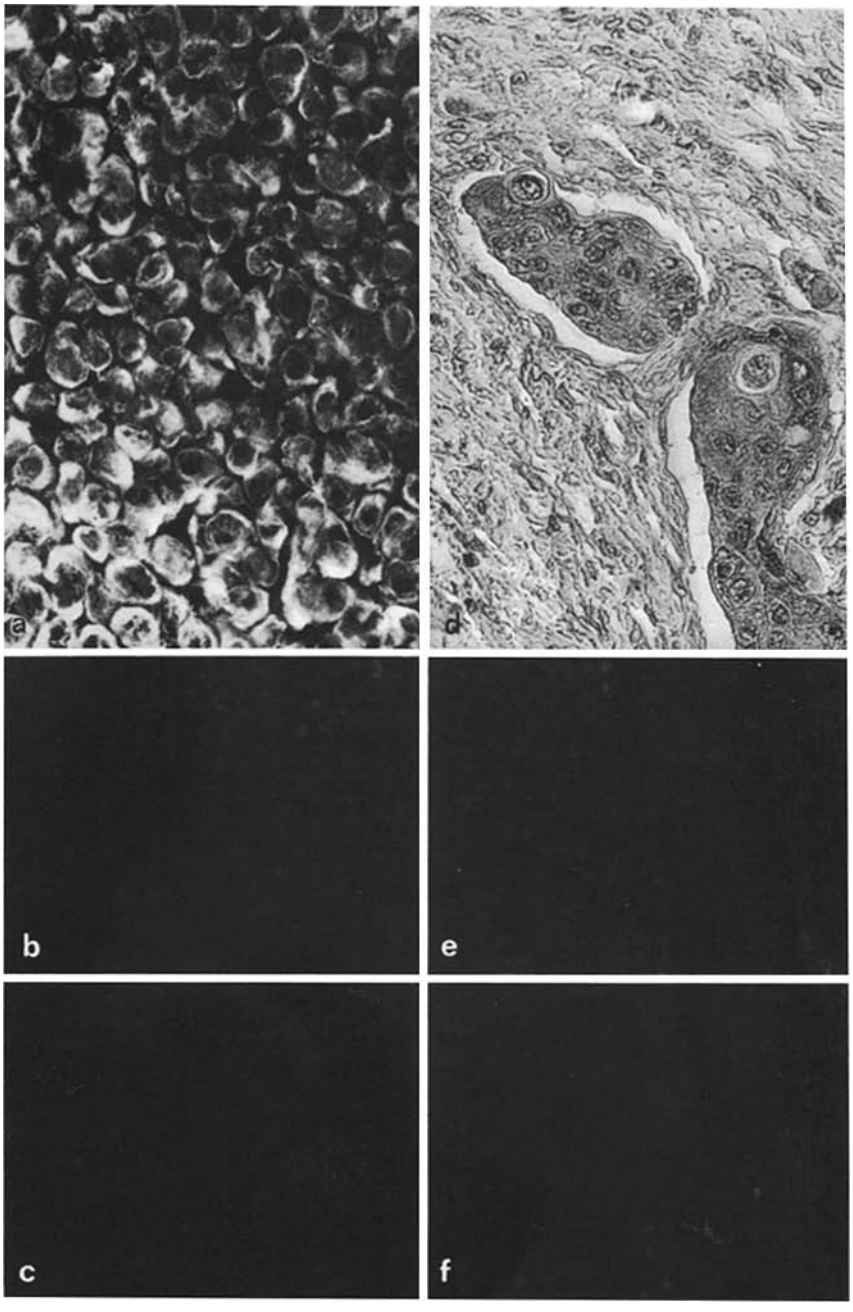

Fig. 3a–c. Frozen sections from case 1 (**a–e**) stained with antibodies to vimentin **a**, keratin **b**, desmin **c**, glial fibriallary acidic protein **d** or neurofilaments **e**. Note that the tumour cells are positive with antibodies to vimentin and negative with antibodies to the other intermediate filament types. Fig. 3f shows a frozen section an additional case of an anaplastic carcinoma stained with antibodies against keratin. Magnification **a** ×410, **b–f** ×250

Discussion

Melanomas contain the vimentin type of intermediate filaments and only this type of intermediate filament. This fact places them into the same category as non-muscle sarcomas including lymphoma. Use of a set of intermediate filament sera each of which recognizes one intermediate filament type can therefore distinguish this group of tumours which are vimentin-positive, from carcinomas which are positive for prekeratin, rhabdomyosarcoma in which tumour cells are desmin-positive, gliomas which are positive for glial fibrillary acidic protein and certain tumours of neuronal origin which are neurofilament-positive (Altmannsberger 1981a and b, 1982a and b; Caselitz et al. 1981; Gabbiani et al. 1981; Osborn et al. 1982b). Thus use of intermediate filament sera – and particularly of sera specific for vimentin and for prekeratin – can be expected to be of help where it is important to separate melanoma from anaplastic carcinoma, or from the rare pigmented carcinoma. Conversely if a tumour of unknown origin is found to be vimentin-positive the possibility that it may be a melanoma should be kept in mind.

Melanoma represents another example of a tumour in which the intermediate filament type typical of the cell of origin – in this case the melanocyte – is retained in the tumour. Melanocytes from cow and rat are known to be vimentin-positive (Franke et al. 1978) and melanocytes from human are also vimentin-positive (Löning et al. 1982). In this study mostly metastatic specimens were used. Thus, as shown in several previous instances for solid tumours (Altmannsberger et al. 1981b and 1982b; Gabbiani et al. 1981), metastatic melanomas also retain the intermediate filament type characteristic of the parent cell and of the primary tumour. No additional intermediate filament type appeared to be acquired in the melanoma metastases.

The finding that malignant melanomas and melanocytes are vimentin-positive has interesting implications for the histogenesis of the melanocyte. The melanocyte is assumed to arise from the neural crest (Hu 1981) and to migrate at a certain stage development to other sites in the body (Schnyder 1979). Vimentin-positive filaments are generally characteristic of mesenchymally derived cells as well as of certain other non-epithelial cell types in the adult. Recent studies on the sequential appearance of the different intermediate filament types during the development of certain species have shown that the precursors of both neurones and glia contain vimentin type intermediate filaments. Later in development neurofilaments and glial filaments can be expressed either in addition to, or instead of, the vimentin intermediate filaments (Tapscott et al. 1981; Schnitzer et al. 1981; Shaw et al. 1981b; Yen and Fields 1981). Thus further studies are required to test the hypothesis that at the developmental stage at which the cells migrate from the neural crest those cells destined to become melanocytes express vimentin filaments.

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