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Complementary expression of melanosomal antigens and constant expression of pigment-independent antigen during the evolution of melanocytic tumours

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Summary. We have generated monoclonal antibodies (MoAbs) against melanosomal proteins (MoAb 1C11 and MoAb HMSA-1) and a cytoplasmic protein strongly synthesized in neoplastic melanocytes but not associated with melanogenesis (MoAb 7H11). An immunohistochemical study of paraffin sections showed that nearly 90% of epidermal neoplastic melanocytes, including melanomas, expressed 1C11 antigen, whereas this antigen was poorly preserved in dermal melanocytic cells except melanomas. HMSA-1 antigen was expressed in a complementary manner to 1C11 antigen, being found in dermal naevus cells but not generally in the epidermal regions, except for dysplastic naevi and melanomas. In contrast, 7H11 antigen was distributed in nearly 90% of melanocytic tumours except solar lentigo and lentigo maligna lesions. The failure of MoAb 1C11 to react with dermal melanocytes may reflect a subtle alteration in melanogenesis during tumour evolution. Overall, the combined use of MoAbs serves as an accurate diagnosis of melanocytic tumours, the pigment-independent MoAb 7H11 being particularly useful for amelanotic and metastatic lesions.

Key words: Monoclonal antibody – Tyrosinase – Malignant melanoma – Metastasis – Immunohistochemistry

Introduction

It is widely accepted that the common melanocytic naevus develops by a step-wise progression (Clark et al. 1984). As an initial step, proliferation of naevo-melanocytes occurs either singly or forming nests in the epidermal basal layer (junctional naevi), then neoplastic melanocytes extend into the dermis with the remains of epidermal components (compound naevi). Finally, tumour cells disappear from the epidermis and subsequent atrophic changes occur in the dermal remnants (intradermal naevi). In a recent ultrastructural study, however, it was suggested that the basement membrane remains intact during the expansion of neoplastic melanocytes into the dermis (Lea and Pawlowski 1986). Suitable molecular markers could therefore help define the changes involved in this process and may assist in the diagnosis of problem lesions if they could be applied to paraffin-embedded tissues.

Tyrosinase activity and melanosome formation, characteristics of the melanocyte, are greatly affected by the cell location. High enzymatic activity and well-developed melanosomes are observed in the epidermis, whereas low activity and minimal melanosomal formation are found in cells deeply located in the dermis. Melanosomal matrix protein (MMP) and tyrosinase are synthesized at different sites in the cytoplasmic organelles, followed by a final complex formation at a certain stage of melanosomal development (Jimbow et al. 1976; Mishima et al. 1979). In addition, it is also well known that both MMP and tyrosinase have an antigenicity which is identified in vitiligo and melanoma patients who reveal halo (depigmented) macules (Naughton et al. 1983). Since the hybridoma technique was introduced (Kohler and Milstein 1975), a large number of monoclonal antibodies (MoAbs) reactive with melanocytic tumours have been developed and reported (Mackie et al. 1984; Ruiter et al. 1985; Hayashibe et al. 1986). MoAbs highly specific to MMP and tyrosinase, however, are still limited to a small number, particularly to the latter enzyme (Tomita et al. 1985; Tomita and Hearing 1986; McEwan et al. 1988).

We previously examined the specificity of MoAb 5C12 (McEwan et al. 1988), reactive with active tyrosinase, and MoAb 1C11 (McEwan et al. 1989) reactive with a tyrosinase-like protein, using human cell lines. In order

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to determine the diagnostic utility of these MoAbs and the relationship between antigenic expression and histogenesis of melanocytic tumours, immunohistochemical studies were carried out on various tumours including those of skin and other tissues. These had been routinely processed by formalin fixation and paraffin embedding. Furthermore, to elucidate the biological significance of the antigens in the processes of malignant transformation and metastasis, comparison was made with MoAb against MMP (MoAb HMSA-1) (Akutsu and Jimbow 1986) and a newly derived MoAb against a cytoplasmic, pigment-independent protein highly synthesized in melanocytic cells (MoAb 7H11).

Materials and methods

The human melanoma MM96E cell line is an early passage of MM96 (Pope et al. 1979) with moderate melanin production, whereas the MM96L cell line is a late passage subline without visible melanin production.

Detailed procedures of hybridoma production have previously been described (McEwan et al. 1988, 1989). BALB/c mice were immunized by intra-peritoneal injection each week for 3 weeks with sonicated and fractionated MM96E cell lysate for MoAb 1C11 or a mixture of live and methanol-fixed MM96L cells (approximately 5×10^6) for MoAb 7H11, followed by a final intravenous injection. Spleen cells of immunized mice were fused with NS-1 myeloma cells, and subsequent selection was done in Hypoxanthine, Aminopterin and Thymidine (HAT) medium. After screening of the supernatants, hybridoma cells which produced antibody selective to melanoma cells were recloned.

Screening of hybridoma cells was carried out first on a mixture of methanol-fixed melanoma cells, HeLa cells and fibroblasts (Table 1). After confirming a selective reactivity with melanoma cells, a second screening with formalin-fixed cells was carried out as follows. Cells were seeded into 96-well multiplates (Nunc, Copenhagen, Denmark) at a density of 10^4 cells/well, incubated overnight at 37° C to allow attachment, washed with phosphate-buffered saline (PBS; pH 7.2) and fixed with methanol for 1 min or 10% buffered formalin solution for 20 min. After extensive washing with PBS, fixed cells were incubated with 50 µl of hybridoma supernatant at 37° C for 30–60 min. After rinsing with water, 50 µl alkaline phosphatase(AP)-conjugated sheep antimouse IgG (Silenus Lab, Sydney, Australia) was added to each well and incubated for 30 min at room temperature. After extensive rinsing with water, 50 µl of 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitroblue tetrazolium (NBT) solution was reacted with cells for 15–60 min. The immunoproduct was recognized as a deep purple colour deposited at the intracellular localization of the MoAb.

Protein samples were solubilized in 10 mM Tris, pH 7.4, 20% glycerol, 10 mM dithiothreitol (DTT) and 1% sodium dodecyl sulphate, followed by application to 6–15% polyacrylamide gels and electrophoresis in 25 mM Tris – 192 mM glycine – 1% sodium dodecyl sulphate, pH 8.3. Molecular weight standards were purchased from Pharmacia (Uppsala, Sweden), and silver staining was carried out as previously described (McEwan et al. 1988, 1989). Immunoblotting was performed by transfer of protein from polyacrylamide gels to nitrocellulose (Burnett et al. 1981), followed by reaction with hybridoma supernatant and visualization with AP-conjugated antimouse IgG and BCIP/NBT substrates, as described above.

Mouse-Typer sub-isotyping kit (Biorad, Sydney, Australia) was used for determination of immunoglobulin subclass.

To characterize the distribution of antigens in normal and pathological tissues, an immunohistochemical study was carried out on paraffin sections. All specimens examined were obtained from the Department of Pathology, Royal Brisbane Hospital. For this study, the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique (Cordell et al. 1984) and the avidin-biotin peroxidase complex (ABC) method using peroxidase (Hsu et al. 1981) were used. Neutral formalin-fixed (10%) and paraffin-embedded tissues were cut 4 µm thick, dewaxed with xylene, hydrated with graded alcohols, washed with PBS and incubated with 5% horse serum in PBS for 20 min at room temperature. Following incubation with hybridoma supernatant (MoAb) for 30 min at room temperature and extensive washing with PBS, biotinylated horse antimouse IgG (1:200) in PBS was applied to the specimens for 30 min at room temperature, washed with PBS, incubated with ABC or ABC-AP reagent (Vector Lab, Burlingame, USA) for 30–60 min at room temperature, washed with PBS, and then allowed to react with diaminobenzidine (DAB) or AP substrate buffered with 0.1 M Tris-HCl, pH 7.2 or 8.2, respectively. As counterstain, Mayer's haemalum and Scott's blue were used.

Results

MoAb 7H11 showed a strong reactivity with heavily-melanotic (MM418), lightly melanotic (MM96E) and amelanotic (MM96L) cell lines, all of which possess tyrosinase activity, as well as with melanoma cell lines containing undetectable levels of tyrosinase (MM127 and MM229). Neither HeLa cells nor neonatal foreskin fibroblasts were significantly reactive with MoAb 7H11 (Table 1).

As a result of immunoblotting, the molecular weight of the antigen recognized by MoAb 1C11 was found to be 56 kDa (McEwan et al. 1989), and the antibody was of IgG_1 subclass. The molecular weight of antigen and immunoglobulin subclass of MoAb HMSA-1 were 35 and 65 kDa and IgG_1, respectively (Akutsu and Jimbow 1986). The immunoglobulin subclass of MoAb 7H11 was IgG_{2b} and the molecular weight of the antigen as determined by Western blotting was 37.5–47 kDa (Table 2). MoAb 7H11 therefore was distinct from MoAb 1C11 and MoAb HMSA-1 in recognizing a different molecular weight antigen and in reacting with all melanoma cell lines tested, including those devoid of tyrosinase activity (MM127 and MM229).

Table 1. Monoclonal antibody specificity on methanol-fixed cultured cells

MoAb	NMC	MM96E	MM96L	MM418	MM127	MM229	MM253cl	HeLa	NFF
1C11	++(100)	+++(100)	++(90)	+++(100)	− (0)	− (0)	++ (70)	−(0)	−(0)
7H11	+ (90)	+++(100)	+++(100)	+++(100)	+(80)	++(40)	+++(100)	−(3)[a]	−(2)[a]

Intensity: −, negative; +, weak; ++, moderate; +++, strong. ( ): percentage of positive cells
NMC = Normal melanocyte; NFF = neonatal foreskin fibroblast

[a] Less than 5% in number, and weakly stained cells being scored as negative

Table 2. Characteristics and distribution of MoAb 1C11, 7H11, and HMSA-1

Property	MoAb		
	1C11	7H11	HMSA-1
Immunogen	MM96E cell (lysate)	MM96L cell (mixture)[a]	Ms fraction of human melanoma
Molecular weight of the antigen	56 kDa	37.5–47 kDa	35 & 65 kDa
Isotype	IgG_1	IgG_{2b}	IgG_1
Tissue preservation	P & F	P & F	P & F
Subcellular localization	Ms	Cp	Ms, Er
Reactivity with normal skin			
Keratinocyte	–	–	–
Melanocyte	+(4/15)	+(2/14)	–
Langerhans cell	–	–	–
Hair follicle	+(3/15)	+(2/14)	–
Sweat gland	–	+(5/14)[b]	–
Sebaceous gland	–	–	–
Peripheral nerve	–	+(1/14)	–
Arrector pili muscle	–	–	–
Fat tissue	–	–	–
Fibroblast	–	–	–
Melanocytes in Sk and BCC	+(3/8)	+(1/8)	–

P = Paraffin-embedded tissue; F = frozen tissue; Ms = melanosome; Cp = whole cytoplasm; Er = endoplasmic reticulum; Sk = seborrhoeic keratosis; BCC = basal cell carcinoma

[a] Mixture of live and methanol-fixed cells

[b] Positive with luminal membrane of sweat gland

Immunohistochemical study

On formalin-fixed and paraffin-embedded tissues, MoAb 1C11 (4/15) and MoAb 7H11 (2/14) showed immunolabelling with normal melanocytes (Table 2). However, low immunoreactivity was sometimes difficult to recognize in melanocytes because of melanin and shrinkage of the cytoplasm. The reactive intensity of MoAb 1C11 was weak with basal melanocytes but strong with those seen in seborrhoeic keratoses and basal cell carcinomas (3/8). No skin appendage except hair follicles (3/15) revealed 1C11 antigen, whereas 7H11 antigen was sometimes expressed in the luminal membrane of the sweat gland (5/14) and peripheral nerve (1/14). No MoAb HMSA-1 positive cell was observed in normal skin (Table 2).

Regarding the common melanocytic naevi (CMN) examined, MoAb 1C11 demonstrated a distinct reactive pattern between epidermal and dermal melanocytic cells. Epidermal melanocytic lesions expressed 1C11 antigen strongly, whereas MoAb 1C11 reacted poorly with dermal lesions and the number of positive cells was less than 10% of dermal naevus cells (Fig. 1a). Interestingly, staining intensity was variable and heterogeneous with Spitz's naevi and dysplastic naevi compared with the homogeneous cytoplasmic reaction of ordinary naevi. In addition, MoAb 1C11 reactivity with dysplastic naevi was stronger on the "shoulder" of the lesions than in the centre (Fig. 2a) and 5 of 18 cases showed an immunoproduct in adjacent keratinocytes.

In contrast, immunohistochemical staining of CMN using MoAb 7H11 showed that this antigen was highly expressed in CMN lesions except for solar lentigines (Table 3). Among 4 cases of solar lentigines, only 1 lesion was weakly reactive with MoAb 7H11. The immunoreactive site of MoAb 7H11 was cytoplasm, and no significant difference in immunolabelling between epidermal and dermal tumour cells was observed (Fig. 1b). Heterogeneous staining of dysplastic naevi was also demonstrated as well as MoAb 1C11 and an immunoproduct within surrounding keratinocytes was observed in 4 of 18 cases examined.

HMSA-1 antigen was expressed in a different manner from that of 1C11 antigen. Among 52 CMN lesions, most of the epidermal melanocytic lesions except dysplastic naevi showed a weak or negative staining with MoAb HMSA-1. However, strong and homogeneous reactivity was observed in dermal melanocytic lesions (Fig. 1c). The immunoreactive pattern of MoAb HMSA-1 with dysplastic naevi was heterogeneous in epidermal components but homogeneous in dermal tumour cells, consistent with a previous report (Akutsu and Jimbow 1986).

In malignant melanomas, including lentigo maligna (9 cases), superficial spreading type (SSM; 11 cases), nodular type (NM; 7 cases) and metastatic lesions of various sites (16 amelanotic and 6 sparsely melanized lesions), immunolabelling by MoAb 1C11 was variable, suggesting heterogeneous expression of this antigen (Fig. 3a). Although the reactive pattern was basically similar to that of benign pigmented lesions, dermal melanoma cells still retained 1C11 antigen. No obvious correlation between immunoreactivity and degree of melanization was observed (Fig. 2b), and immunoproduct observed in adjacent keratinocytes was more prominent than that seen in dysplastic naevi.

Among melanoma specimens studied using MoAb 7H11, only lentigo maligna lesions showed lower reactivity compared with other types of melanomas (Table 3). Interestingly, this reactive pattern was similar to that of solar lentigines studied in benign counterparts. Regarding primary melanomas (SSM and NM), however, there was no significant difference of 7H11 expression between epidermal and dermal tumour cells (Fig. 3b), and no correlation was observed between labelling intensity and tumour cell location, cell shapes (epithelioid and spindle cells), and degree of melanization. In addition, immunoproduct was demonstrated in at least 50% of metastatic tumour cells with heterogeneous intensity.

In contrast to the reaction of MoAb 1C11, MoAb HMSA-1 failed to show positive cells in lentigo maligna lesions (0/4). Among 16 cases of primary melanomas (SSM and NM) studied, however, 12 cases were reactive with MoAb HMSA-1 with heterogeneous staining. The reaction intensity was strong in epithelioid and amelanotic cells and weak in spindle and melanized cells. Simi-

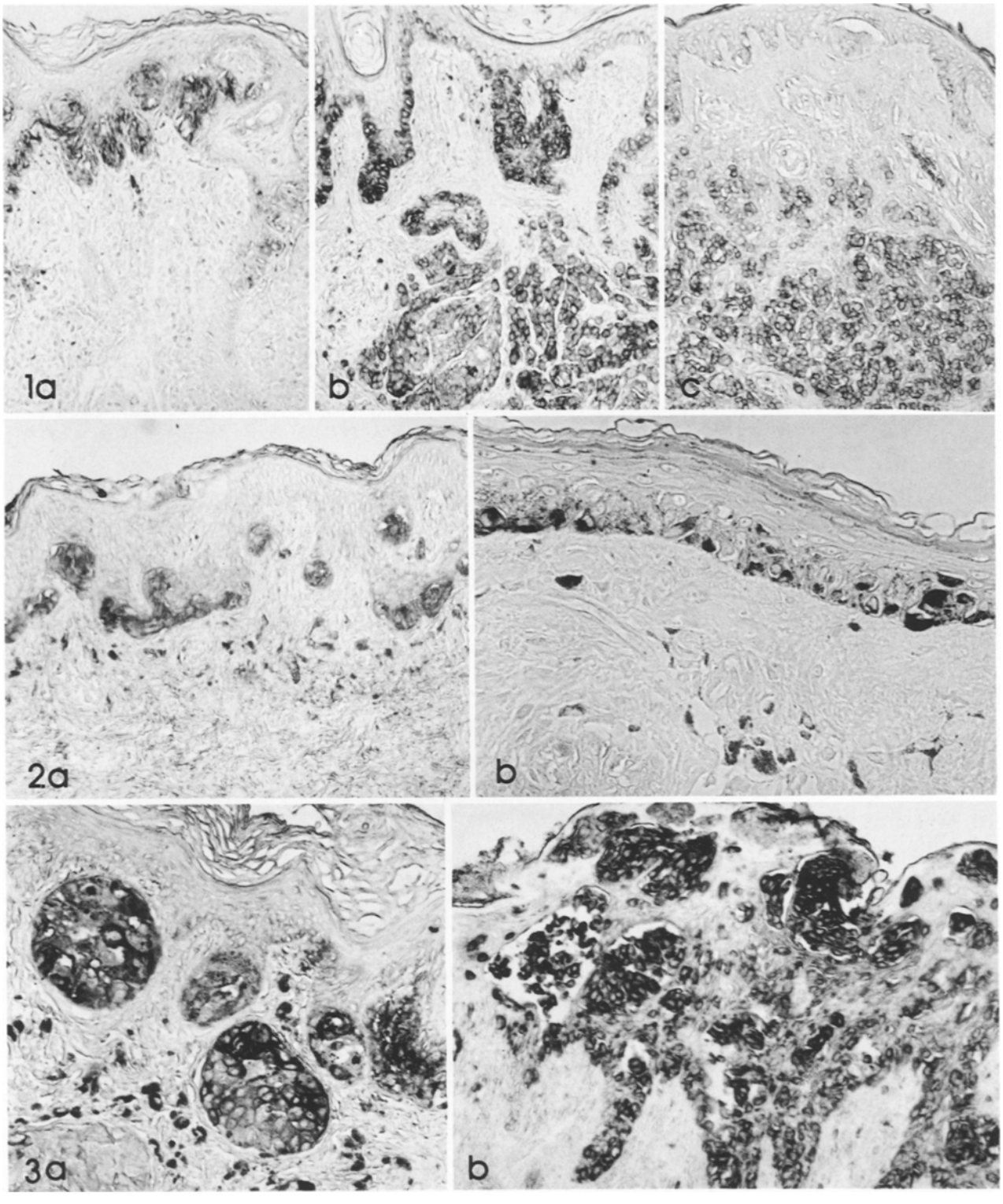

Fig. 1. a Immunoproduct of MoAb 1C11 is observed only with epidermal melanocytic nests but not with dermal tumour cells of a compound naevus. ABC, ×118. **b** 7H11 antigen is expressed in both epidermal and dermal neoplastic melanocytes of a compound naevus. ABC, ×118. **c** Homogeneous immunolabelling with MoAb HMSA-1 is seen only in the dermal component of a compound naevus. Note a negative reaction with epidermal nests of the lesion. ABC, ×118

Fig. 2. a "Shoulder" lesion of dysplastic naevus intensely expresses 1C11 antigen. Melanophages are also recognized in upper dermis. APAAP, ×118. **b** MoAb 1C11 is reactive with lentigo maligna lesion. ABC, ×420

Fig. 3. a Immunoreactivity of MoAb 1C11 is observed with both epidermal and dermal melanoma cells with heterogeneous staining pattern (negative tumour cells in the left field. APAAP, ×240. **b** MoAb 7H11 is strongly reactive with primary melanoma. APAAP, ×240

Table 3. Immunohistochemical study of melanocytic tumours in paraffin sections

Tissue	MoAb			
	1C11	7H11	1C11 & 7H11[a]	HMSA-1
Common melanocytic naevi				
Junctional	3/3	3/3	3/3	1/3
Compound				
(e)	14/15	16/16	15/15	2/10[b]
(d)	2/15[b]	16/16	15/15	8/10
Intradermal	3/20[c]	18/20	18/20	13/13
Blue naevi	1/5	5/5	5/5	2/5
Spitz's naevi				
(e)	4/4	3/4	4/4	0/9
(d)	1/4[b]	3/4	4/4	4/9
Dysplastic naevi				
(e)	15/18	17/18	18/18	9/9
(d)	2/13[b]	13/13	13/13	7/7
Solar lentigines	3/4	1/4	3/4	1/3
Malignant melanomas				
Lentigo maligna	8/9	5/9	9/9	0/4
Primary				
(e)	16/18	16/18	17/18	12/16
(d)	7/15	15/16	15/16	12/16
Metastatic	10/22	22/22	22/22	10/14

e = Epidermal tumour cells; d = dermal tumour cells

[a] Number of cases positive for either MoAb 1C11 or MoAb 7H11

[b] Positive cells $<10\%$

[c] Positive cells were located in superficial dermis

lar reactivity was also observed in metastatic lesions as found previously (Akutsu and Jimbow 1986).

No skin tumours other than those of melanocytic lineage were reactive with MoAbs 1C11, 7H11 and HMSA-1. The specimens examined as skin tumours were 4 eccrine poromas, 3 seborrhoeic keratoses, 5 keratoacanthomas, 4 Bowen's disease, 19 squamous cell carcinomas originating from skin and mucosa, 5 basal cell carcinomas, 5 neurofibromas and schwannomas, 2 Merkel cell tumours of the skin, 1 granular cell tumour, and 1 atypical fibroxanthoma.

The tumours originating from tissue other than skin were tested and included those which tend to metastasize to the skin: carcinomas (3 breast, 3 colon, 3 lung, 4 kidney, 4 stomach, and 2 ovary), sarcomas (5 malignant lymphomas, 1 neurofibrosarcoma, 3 Ewing's sarcomas, and 5 osteosarcomas), 3 osteochondromas, 4 carcinoids and 1 fibroadenoma. All MoAbs showed negative staining with the above tumour tissues except 1 carcinoid showing weak reactivity with MoAb 7H11.

Discussion

In this study, we showed that the antigen recognized by MoAb 1C11 was strongly expressed in cultured melanoma cells which possessed high tyrosinase activity (MM96E, MM96L, MM418, and MM253c1 cells), in epidermal neoplastic melanocytes and in melanocytes present in solar induced lesions. The epidermal melanocyte is easily activated with respect to proliferation and melanization by endogeneous and exogeneous stimuli such as hormones (Lerner et al. 1954), ultraviolet irradiation (Quevedo et al. 1975) and chemical agents (Horikoshi and Carter 1986). In addition, DNA flow cytometry has shown that S and G2 phase melanoma cells have more 1C11 antigen than G1 cells (unpublished data). Our previous study indicated that MoAb 1C11 may recognize a polypeptide epitope in a protein related to tyrosinase (McEwan et al. 1989). It is therefore suggested that the 1C11 antigen is expressed according to the proliferative and/or melanogenic activity of epidermal melanocytes. Although generally absent in dermal naevi, 1C11 antigen appears to be expressed randomly in the post-naeval histogenesis of melanocytic tumours, presumably as a part of the deranged melanogenesis reported in dysplastic naevi and melanomas (Takahashi et al. 1985, 1987; Maeda et al. 1988a; Rhodes et al. 1988).

Regarding immunoreactivity on pathological tissues, MoAb NKI/beteb and MoAb HMB-45 were reported to show a similar labelling pattern to that of MoAb 1C11. The molecular weight of the antigen recognized by MoAb NKI/beteb, however, is 100 kDa and 7 kDa by immunoblotting and is less sensitive on paraffin sections (Vennegoor et al. 1988). The other MoAb, MoAb HMB-45, was generated from immunization with metastatic melanoma and was reactive with paraffin sections (Gown et al. 1986). This MoAb was reported to react with junctional naevo-melanocytes but not as strongly with intradermal naevus cells as MoAb 1C11. Although the molecular weight and subcellular localization of MoAb HMB-45 have not yet been determined, it is possible to discriminate it from MoAb 1C11 in view of there being no reactivity with normal basal melanocytes and much higher sensitivity to dermal melanoma cells.

The tissue distribution of the cytoplasmic 7H11 antigen was rather broader than that indicated by MoAbs against melanosomal proteins. MoAb 7H11 showed immunoreactivity to all cultured melanoma cell lines without any relationship to tyrosinase activity. Of the normal tissue embedded in paraffin, 7H11 antigen was revealed not only in melanocytes but in the luminal membrane of the sweat gland (5/14) and peripheral nerve (1/14). In pathological tissues, however, MoAb 7H11 reacted only with melanocytic tumours, and no significant correlation was found between immunoreactivity and degree of melanization, location of the tumour cells in the skin, or the stages of differentiation including malignant transformation. MoAb 7H11 was poorly reactive with solar lentigines and lentigo maligna lesions (Hutchinson's melanotic freckle) compared to epidermal lesions of primary melanoma. Even in such specimens, however, the combined use of MoAbs 1C11 and 7H11 yielded more than 90% immunoreactivity. Therefore, combined application of panels of MoAbs enables an accurate diagnosis of melanocytic tumours, particularly of oligomelanotic and amelanotic lesions. The antigen recognized

by MoAb 7H11 is unlikely to be S-100 protein for the following reasons. MoAb 7H11 is not reactive with the nucleus, not reactive with Langerhans cells, reactive with a different site in sweat gland from that of S-100 protein (luminal membrane rather than gland cells), not reactive with neurogenic tumours (schwannomas and neurofibromas), and not reactive with osteochondromas which are uniformly reactive with S-100 protein. The antigen which MoAb 7H11 recognizes has not yet been identified and immunoelectron microscopy failed to demonstrate a specific localization to a particular organelle. The cross-reactivity of MoAb 7H11 with peripheral nerve and carcinoid may suggest a neural-crest origin for the antigen. However, because of its high rate of expression in melanocytic tumours and absence in the other neural-crest tumours examined so far, we are considering that 7H11 antigen may be an S-100-like protein which is strongly synthesized in the cells of melanocytic lineage. Neither vimentin nor neuron-specific enolase, both non-specific markers but strongly expressed in melanocytic cells, resemble the 7H11 antigen because of the difference of molecular weight and distribution in normal and pathological tissues. We are now undertaking the purification and further characterization of 7H11 antigen.

The characteristics and tissue distribution of MMP have been well established using MoAbs against human melanosome specific antigen (HMSA) (Akutsu and Jimbow 1986, 1988; Maeda and Jimbow 1987; Maeda et al. 1987, 1988b), being strongly expressed in intradermal naevi with homogeneous reactive pattern despite little or no staining in epidermal neoplastic melanocytes. This labelling pattern is completely the converse of that of MoAb 1C11 which is strongly expressed in epidermal melanocytes.

At present, it is believed that tyrosinase and matrix protein are synthesized at different sites and associate at a certain stage of melanogenesis (Jimbow et al. 1976). Considering the above theory together with the histogenesis of naevus formation (Clark et al. 1984), the present data suggest that the expression of HMSA-1 antigen becomes more apparent from the middle stage of naevus formation while that of 1C11 antigen occurs in an early stage with subsequent inactivation enzymatically and biologically. However, in dysplastic naevi and melanomas, the expression of both antigens becomes unpredictable and heterogeneous, probably because of deranged melanogenesis reflected in the alteration of melanosomal morphology, disturbance of MMP formation and tyrosinase activity, and transfer of such abnormal melanosomes to adjacent keratinocytes. The recent study of Lea and Pawlowski (1986) implies that HMSA-1 and 1C11 reactive cells lie above the basement membrane. The origin of the complementary demarcation of expression of these antigens therefore remains unknown but may be a part of an early stage of melanoma evolution.

MoAb 1C11 and MoAb 7H11 will not discriminate between benign melanocytic tumours and their malignant counterparts, but combined application of those MoAbs (including MoAb HMSA-1) specific for a different melanosomal protein provides an accurate and reliable diagnosis of melanocytic tumours. This is particularly true of oligomelanotic and amelanotic lesions, of tumour extension in radial and/or vertical directions in the skin, and for metastatic lesions. Moreover, in a different manner from MoAb HMSA-1, immunoreactivity for MoAb 1C11 reflects a unique biological characteristic of neoplastic melanocytes.

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References

Akutsu Y, Jimbow K (1986) Development and characterization of a mouse monoclonal antibody, MoAb HMSA-1, against a melanosomal fraction of human malignant melanoma. Cancer Res 46:2904–2911

Akutsu Y, Jimbow K (1988) Immunoelectron microscopic demonstration of human melanosome associated antigens (HMSA) on melanoma cells: comparison with tyrosinase disribution. J Invest Dermatol 90:179–184

Burnett WN (1981) "Western blotting". Electrophoretic transfer of proteins from sodium dodecylsulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal Biochem 112:195–203

Clark WH, Elder DE, Guerry D, Epstein MN, Greene MH, Horn M (1984) A study of tumor progression: the precursor lesions of superficial spreading and nodular melanoma. Hum Pathol 15:1147–1165

Cordell JL, Falini B, Erber WN, Ghosh A, Abdulaziz Z, MacDonald S, Paulford K, Stein H, Mason D (1984) Immunoenzymatic labelling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 32:219–229

Gown AM, Vogel AM, Hoak D, Gough F, McNutt MA (1986) Monoclonal antibodies specific for melanocytic tumors distinguish subpopulations of melanocyte. Am J Pathol 123:195–203

Hayashibe K, Mishima Y, Ichihashi M, Kawai M (1986) Melanosomal antigenic expression on the cell surface and intracellular subunits within melanogenic compartments of pigment cells: analysis by antimelanosome-associated monoclonal antibody. J Invest Dermatol 87:89–94

Horikoshi T, Carter DM (1986) Ultrastructural characteristics and kinetics of tyrosinase of cultured human melanocytes. Struct Funct Melanin 3:95–102

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Jimbow K, Quevedo WC, Fitzpatrick TB (1976) Some aspects of melanin biology: 1950–1975. J Invest Dermatol 67:72–89

Kohler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495–497

Lea PJ, Pawlowski A (1986) Human melanocytic naevi. I. Electron microscopy and 3-dimensional computer reconstruction of naevi and basement membrane zone from ultrathin serial sections. Acta Derm Venereol [Suppl] (Stockh) 127:5–15

Lerner AB, Shizume K, Bunding I (1954) The mechanism of endocrine control of melanin pigmentation. J Clin Endocrinol Metab 14:1463–1490

Mackie RM, Campbell I, Turbitt ML (1984) Use of NK1 C3 monoclonal antibody in the assessment of benign and malignant melanocytic lesions. J Clin Pathol 37:367–372

Maeda K, Jimbow K (1987) Development of MoAb HMSA-2 for melanosomes of human melanoma and its application to immunohistopathologic diagnosis of neoplastic melanocytes. Cancer 59:415–423

Maeda K, Yamana K, Jimbow K (1987) Development of MoAb HMSA-3 and HMSA-4 against human melanoma melanosomes and their reactivities on formalin-fixed melanoma tissues. J Invest Dermatol 89:588–593

Maeda K, Maeda K, Jimbow K (1988a) Positive reactivity of dysplastic melanocytes with a monoclonal antibody against melanoma melanosomes, MoAb HMSA-2. J Invest Dermatol 91:247–250

Maeda K, Maeda K, Jimbow K (1988b) Specification and use of a mouse monoclonal antibody raised against melanosomes for the histopathologic diagnosis of amelanotic malignant melanoma. Cancer 62:926–934

McEwan M, Parsons PG, Moss DJ (1988) Monoclonal antibody against human tyrosinase and reactive with melanotic and amelanotic melanoma cells. J Invest Dermatol 90:515–519

McEwan M, Parsons PG, Moss DJ, Burrows S, Stenzel D, Bishop C, Strutton GM (1989) Monoclonal antibody against a melanosomal protein in melanotic and amelanotic human melanoma cells. Pigment Cell Res 2:1–7

Mishima Y, Imokawa G, Ogura H (1979) Functional and three-dimensional differentiation of smooth membrane structures in melanogenesis. In: Klaus SW (ed) Pigment cell, vol 4. Karger, Basel, pp 277–290

Naughton GK, Elsinger M, Byrstryn J (1983) Antibodies to normal human melanocytes in vitiligo. J Exp Med 158:246–251

Pope JH, Morrison L, Moss DJ, Parsons PG, Mary R (1979) Human malignant melanoma cell lines. Pathology 11:191–195

Quevedo WC, Fitzpatrick TB, Pathak MA, Jimbow K (1975) Role of light in human skin color variation. Am J Phys Anthropol 43:393–408

Rhodes AR, Seki Y, Fitzpatrick TB, Stern RS (1988) Melanosomal alterations in dysplastic melanocytic naevi: a quantitative, ultrastructural investigation. Cancer 61:358–369

Ruiter DJ, Dingjan GM, Steijlen PM, Beveren-Hooyer M, Graaf-Reitsma CB, Bergman W, Muijen GNP, Warnaar SO (1985) Monoclonal antibodies selected to discriminate between malignant melanomas and nevocellular naevi. J Invest Dermatol 85:4–8

Takahashi H, Horikoshi T, Jimbow K (1985) Fine structural characterization of melanosomes in dysplastic naevi. Cancer 56:111–123

Takahashi H, Yamana K, Maeda K, Akutsu Y, Horikoshi T, Jimbow K (1987) Dysplastic melanocytic nevus: electron microscopic observation as a diagnostic tool. Am J Dermatopathol 9:189–197

Tomita Y, Hearing VJ (1986) Monoclonal antibodies produced against murine tyrosinase identify pigmented human melanocytes. Diagn Clin Immunol 4:149–154

Tomita Y, Montague PM, Hearing VJ (1985) Anti-T4-tyrosinase monoclonal antibodies – specific markers for pigmented melanocytes. J Invest Dermatol 85:426–430

Vennegoor C, Hageman PH, Nouhuijs H, Ruiter DJ, Calafat J, Ringens PJ, Rumke P (1988) A monoclonal antibody specific for cells of the melanocytic lineage. Am J Pathol 130:19–92