Radioimmunoscintigraphy and immunohistochemistry with melanoma-associated monoclonal antibodies in choroidal melanoma: a comparison of the clinical and immunohistochemical results

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Abstract

Radioimmunoscintigraphy with monoclonal antibodies (MoAbs) to melanoma associated antigens is a new technique that can be used as an additional test to detect ocular melanomas in clinically difficult cases. Immunoscintigraphy with ^{99m}technetium-labelled monoclonal antibody fragments of MoAb 225-28S in 14 patients with melanoma yielded a positive image in only six cases (43%). The expression of high molecular weight melanoma-associated antigen (HMW-MAA) was immunohistochemically assessed in melanoma tissue obtained from these 14 patients to find a possible correlation between the results of immunoscintigraphy and expression of the HMW-MAA. The melanoma tissues were immunohistochemically stained by a sensitive immunoperoxidase procedure with three different monoclonal antibodies to the HMW-MAA: 225-28S, Mel-14, and AMF-6. Expression of the antigen detected by MoAb 225-28S was found in 13 of 14 melanomas; the MoAb Mel-14 reacted positively with all 14 melanomas; staining with MoAb AMF-6 was achieved in 10 melanomas. No correlation was found between the immunohistochemical staining results, the conventional histopathological findings, and the immunoscintigraphic results. The immunohistochemical staining results suggest that anti-HMW-MAA MoAbs bind to the melanoma tissue and are therefore potentially suitable for immunoscintigraphy.

Since the development of the hybridoma technology¹ several monoclonal antibodies (MoAbs) have been raised against cutaneous melanoma.²⁻⁹ Only a few MoAbs have been developed against uveal melanoma.¹⁰ A major reason for the limited number of antibodies to uveal melanoma is the problem of establishing a uveal melanoma cell line which facilitates the induction and characterisation of uveal melanoma specific MoAbs. Fortunately several MoAbs of cutaneous melanoma show cross reactivity with uveal melanoma tissue.¹¹⁻¹³

Antibodies such as 225-28S are directed to a high molecular weight melanoma-associated antigen (HMW-MAA),³ which is expressed in about 90% of the cutaneous melanomas.^{14 15} Expression of the HMW-MAA in uveal

melanoma was found to occur in 40 to 50% of cases.^{12 16}

The ^{99m}technetium-labelled antibody 225-28S has been used for immunoscintigraphy in uveal melanoma patients and yielded detection percentages from 37% to 64%.¹⁷⁻¹⁹ The use of sophisticated equipment like single photon emission computerised tomography (SPECT) or a 'double pinhole' collimator enhanced the detection percentages to 73% to 92%.¹⁷⁻¹⁹ In view of the results of the relatively low in-situ expression of the HMW-MAA in uveal melanoma in previous studies^{12 16} the detection rates of immunoscintigraphy with MoAb 225-28S in patients seem to be surprisingly high. Since we could obtain tumour tissue from 14 patients who participated in a previous immunoscintigraphic study with MoAb 225-28S¹⁹ we determined the expression of the HMW-MAA immunohistochemically in these tissues. In addition to monoclonal antibody 225-28S to the HMW-MAA, MoAb Mel-14² and MoAb AMF-6²⁰ were used for immunohistochemical analysis.

Patients and methods

IMMUNOSCINTIGRAPHY

Radioimmunoscintigraphy was performed in 14 melanoma patients with MoAb 225-28S from an instant labelling kit (Technemab-K-1; Sorin Biomedica, Italy). For immunoscintigraphy 250-350 µg of antibody labelled with 740 MBq ^{99m}technetium was administered by means of a slow intravenous injection. For more detailed information on the immunoscintigraphy we refer to a previous publication.¹⁹

HISTOPATHOLOGY

Specimens of uveal melanomas from the same 14 patients were obtained after enucleation of the eye. The enucleations were performed at the Leiden University Hospital or at the Academic Medical Centre of Amsterdam. A portion of each specimen was snap-frozen and stored at -20°C for immunohistochemical analysis. The remainder of each tumour was processed for conventional histopathological examination.

The histopathological diagnosis was assessed on paraffin and/or celloidin sections. Cell type was recorded as spindle cell, epithelioid cell, or

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mixed cell type. Pigmentation was scored as absent, less than half of the cells pigmented, more than half of the cells pigmented, or heavily pigmented. In addition invasion was scored as absent, slight (less than 25% of the scleral thickness), moderate (approximately 50%), deep (approximately 75%), or episcleral.

IMMUNOPEROXIDASE STUDY

Frozen sections 4 µm thick were air dried and fixed in acetone for 10 min.²⁰ Sections were incubated for 60 min at room temperature with MoAb diluted in phosphate buffered saline and then for 30 min with rabbit-mouse immunoglobulin-horse-radish peroxidase conjugate (Tago, Sanbio BV, Uden, The Netherlands). Between the two incubations the sections were washed three times with phosphate buffered saline (pH 7.4). Staining was achieved by incubation of sections for 10 min in an acetate buffer solution (pH 5.0) that contained 3-amino-9-ethyl-carbazole (AEC). Alternatively staining was performed by incubation of sections in a phosphate imidazole buffer solution (pH 7.6) that contained diaminobenzidine (DAB). Finally, the sections were washed, counterstained in Mayer's haematoxylin, and mounted in Aquamount (Gurr, BDH Chemicals Ltd, Poole, England). As a negative control a section was incubated with irrelevant MoAbs instead of anti-HMW-MAA MoAbs as the first step. Sections of cutaneous melanoma were used as positive controls.

The results of both the AEC and DAB staining methods were compared. The percentage of stained tumour cells was estimated independently by two observers. Differences in results between the observers were less than 10%. In addition the intensity of cellular staining was assessed. Correlations between expression of different antigens were assessed by the Kendall rank correlation procedure. The results of the two staining methods were analysed by Wilcoxon's paired signed ranks test.

MONOCLONAL ANTIBODIES

The MoAb 225-28S was tested in the form provided as a ready-to-use kit for clinical use by Sorin Biomedica, and in the pure form as

provided by Dr S Ferrone. Prior to immunohistochemistry with MoAb 225-28S from the ready-to-use kit ion exchange chromatography by means of a DEAE-Sephadex A25 column was performed. This procedure is routinely performed after labelling the antibody with ^{99m}Tc -pertechnetate for immunoscintigraphy to reduce the amount of free pertechnetate. Ion exchange chromatography was performed to check its influence on the antibody's immunoreactivity. The two batches of antibody 225-28S were compared by Wilcoxon's paired signed ranks test. Two other antibodies, Mel-14 and AMF-6 to high molecular weight melanoma-associated antigen were obtained from Dr S Carrel and Dr C Figdor.

Results

The results of immunohistochemistry, immunoscintigraphy (planar scintigraphy), and histology are summarised in Table I. With planar scintigraphy six of 14 melanomas could be visualised. On immunohistochemistry (diaminobenzidine staining with 225-28S antibody from the ready-to-use kit) 13 of 14 melanomas (92%) showed expression of the antigen to which MoAb 225-28S is directed. Immunohistochemistry with the pure antibody 225-28S showed expression of the HMW-MAA in 12 of 14 melanomas. The numbers of positively staining cells with the two batches of antibody 225-28S did not differ significantly (Wilcoxon's paired signed ranks test, $p=0.00033$). Expression of the antigens detected by the MoAb Mel-14 and AMF-6 was found in 14 and 10 melanomas, respectively.

The Kendall rank correlation procedure was used to assess a possible correlation between 'detection on planar scintigraphy' and the following parameters: tumour prominence, pigmentation, cell type, expression of MoAb 225-28S in cells, expression of MoAb 225-28S in vessels, expression of Mel-14 cells, and expression of AMF-6 cells. No correlation between any of these parameters and detection on scintigraphy was found. The expression of MoAb 225-28S was positively correlated with the expression of Mel-14 (14 observations, Kendall rank correlation procedure, $p=0.037$). The expression of AMF-6 was not correlated to the expression of either Mel-14 or 225-28S (14 observations).

Table I Immunohistochemistry, scintigraphy and histology

Patient	Sex/ age	DAB staining (Ferrone) 225-28S		DAB staining (Sorin) 225-28S		AEC staining 225-28S (Ferrone)		DAB staining Mel-14		DAB staining AMF-6		Scintigraphy	Tumour prominence (mm)	Cell type	Pigmen- tation	Scleral invasion
		*	†	*	†	*	†	*	†	*	†					
1	F/58	80	1	30	1	1	50	1	100	2	100	2	Neg	5.5	Spindle	1
2	M/75	100	1	100	1	—	10	1	100	2	30	1	Neg	2.2	Spindle	2
3	F/65	90	1	10	1	2	80	2	20	2	0	—	Pos	15	Mixed	1
4	M/42	80	1	50	1	0	40	1	25	1	0	—	Pos	3	Mixed	0
5	M/65	90	2	80	3	2	50	1	100	2	10	1	Pos	7.9	Epithel	2
6	M/76	0	—	5	1	2	5	1	20	1	0	—	Neg	10	Epithel	1
7	F/56	100	1	100	2	2	5	1	100	2	10	1	Neg	9.5	Mixed	1
8	M/76	90	1	100	2	2	40	1	100	2	20	1	Pos	15	Mixed	1
9	M/68	60	1	40	1	3	50	1	100	1	20	1	Neg	11	Mixed	2
10	M/65	70	1	75	1	—	0	—	90	2	70	1	Neg	8	Spindle	3
11	M/75	70	1	80	1	2	30	1	100	1	10	1	Neg	3.5	Mixed	3
12	F/64	80	1	80	2	2	40	1	100	1	10	1	Pos	7.5	Mixed	2
13	F/65	100	2	100	2	2	70	1	100	2	80	1	Neg	4.9	Mixed	2
14	F/69	0	—	0	—	1	0	—	100	1	0	—	Pos	6	Mixed	1

*Percentage of positively staining cells. †Intensity of staining (1=weak, 2=moderate, 3=strong). ‡Intensity of vessel staining (1=weak, 2=moderate, 3=strong, —=not assessable, owing to heavy pigmentation or cell staining). §Could not be determined owing to preparational artefact.

Staining of the tumour vasculature with MoAb 225·28S was found in 11 out of 14 cases. In two cases staining of the vessels could not be determined owing to heavy pigmentation of the tumour or diffuse cellular staining with the MoAb.

Discussion

The expression of the antigen detected by MoAb 225·28S in 13 out of 14 melanomas (92%) in the immunohistochemical study is in agreement with the results of one study (83% expression),¹³ but two other studies showed a much lower expression of the antigen in uveal melanomas (50% and 41% expression respectively).^{12, 16} The percentage of positively staining cells per tumour is also higher in the present study than in a previous study.¹² A major reason for the difference between the results of this study and of the previous study¹² may be the use of diaminobenzidine staining in the present study, which proved to be much more sensitive than 3-amino-9-ethyl-carbazole. The results of this study indicate that the antibody 225·28S may be useful in immunoscintigraphic diagnosis of uveal melanoma, whereas previous studies showed only a limited expression of the HMW-MAA in uveal melanoma.¹²⁻¹⁶

The expression of the HMW-MAA in choroidal and cutaneous melanoma vessels has been previously shown with use of the 225·28S antibody.^{12, 21} An immunoelectron microscopic study revealed that the HMW-MAA is located in the mural pericytes surrounding the endothelial cells.²¹

The expression of antigen detected by Mel-14 in this study is in agreement with a previous study.² The expression of Mel-14 was correlated with the expression of the antigen detected by 225·28S (Kendall rank correlation procedure, $p=0.037$). The high antigen expression makes the Mel-14 antibody suitable for immunodiagnosis.

Expression of the antigen detected by AMF-6, which is at present called NKI-M6, was higher than the expression previously found.²²

No correlation was found between tumour detection on scintigraphy with MoAb 225·28S and antigen expression for MoAb 225·28S. Such a correlation was previously found in cutaneous melanoma.²³ Since we observed expression of the HMW-MAA in this study in 92% of the cases, the detection of six of 14 melanomas by planar scintigraphy with labelled MoAb 225·28S is low. In one melanoma which could be imaged by planar scintigraphy and SPECT no expression of HMW-MAA was seen immunohistochemically (Table I). Heterogenous expression of HMW-MAA in the tumour may explain this difference.¹² On the other hand the expression of HMW-MAA in the tumour vessels may have been responsible for the positive image. Local factors such as vascular perfusion, necrosis, or the presence of an inflammatory infiltrate may influence adequate uptake of antibody in vivo. Necrosis or inflammatory infiltrate was scarcely present in our material.

Scintigraphy results of another study showed a higher detection rate with MoAb 225·28S. An

explanation for this difference may be the performance of three scans after injection of the antibody²⁴ as compared with one scan in our study.¹⁹ Performance of several scans at standard consecutive time intervals may provide information on the distribution of activity in the course of time. A specific activity in the vessels is high directly after injection of the antibody and then decreases, while a specific binding of antibody to the antigen needs some time to become established, and specific tracer uptake will increase on the consecutive images.

An important limiting factor is the high non-specific background activity in the nasopharyngeal region, but the use of single photon emission computerised tomography enables the physician adequately to differentiate the tumour from the nasopharyngeal activity.^{18, 24}

The results of the present study indicate that with a sensitive immunoperoxidase procedure the MoAbs 225·28S and Mel-14 show adequate binding in uveal melanoma tissue. The immunohistochemical study supports the results of some clinical studies indicating the usefulness of antibody 225·28S for immunodiagnostic procedures in uveal melanoma.^{17, 18} However in view of our clinical results as well as recent correspondence in this journal²⁵ the conditions of adequate immunoscintigraphy have to be carefully assessed.

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- 1 Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975; 256: 495-7.
- 2 Carrel S, Accolla RS, Carmagnole AL, Mach JP. Common human melanoma associated antigens detected by monoclonal antibodies. *Cancer Res* 1980; 40: 2523-8.
- 3 Imai K, Molinaro GA, Ferrone S. Monoclonal antibodies to human melanoma associated antigens. *Transplant Proc* 1980; 12: 380-3.
- 4 Stepilewski Z, Herlyn M, Herlyn D, Clark W, Koprowski H. Reactivity of monoclonal anti-melanoma antibodies with melanoma cells freshly isolated from primary and metastatic melanoma. *Eur J Immunol* 1979; 9: 94-6.
- 5 Hellström IH, Garrigues J, Cabasco L, Mosely GH, Brown JP, Hellström KE. Studies of a high molecular weight human melanoma-associated antigen. *J Immunol* 1983; 130: 1467-72.
- 6 Bumol TF, Reisfeld RA. Unique glycoprotein-proteoglycan complex defined by monoclonal antibody on human melanoma cells. *Proc Natl Acad Sci USA* 1982; 79: 1245-9.
- 7 Brown JP, Woodbury RG, Hart CE, Hellström KE. Quantitative analysis of melanoma-associated antigen p97 in normal and neoplastic tissues. *Proc Natl Acad Sci USA* 1981; 78: 539-43.
- 8 Dippold WG, Lloyd KO, Li LTL, Ikeda H, Oertgen HF, Old LJ. Cell surface antigens of human malignant melanoma: definition of six antigenic systems with mouse monoclonal antibodies. *Proc Natl Acad Sci USA* 1980; 77: 6114-8.
- 9 Imai K, Natali PG, Kay NE, Wilson BS, Ferrone S. Tissue distribution and molecular profile of a differentiation antigen detected by a monoclonal antibody (345·134S) produced against human melanoma cells. *Cancer Immunol Immunother* 1982; 12: 159-66.
- 10 Damato BE, Campbell AM, McGuire BJ, Lee WR, Foulds WS. Monoclonal antibodies to human primary uveal melanomas demonstrate tumor heterogeneity. *Invest Ophthalmol Vis Sci* 1986; 27: 1362-7.
- 11 Folberg R, Donoso LA, Atkinson BF, Ernst CS, Herlyn M, Arbizzon VV. An antimelanoma monoclonal antibody and the histopathology of uveal melanomas. *Arch Ophthalmol* 1985; 103: 275-9.

- 12 Van der Pol JP, Jager MJ, de Wolff-Rouendaal D, Ringens PJ, Vennegoor C, Ruiter DJ. Heterogenous expression of melanoma-associated antigens in uveal melanomas. *Curr Eye Res* 1987; 6: 757-65.
- 13 Bomanji J, Garner A, Prasad J, et al. Characterisation of ocular melanoma with cutaneous melanoma antibodies. *Br J Ophthalmol* 1987; 71: 647-50.
- 14 Wilson BS, Imai K, Natali PG, Ferrone S. Distribution and molecular characterization of a cell-surface and a cytoplasmic antigen detectable in human melanoma cells with monoclonal antibodies. *Int J Cancer* 1981; 28: 293-300.
- 15 Natali PG, Bigotti A, Cavaliere R, Nicotra MR, Ferrone S. Phenotyping of lesions of melanocyte origin with monoclonal antibodies to melanoma-associated antigens and to HLA antigens. *J Natl Cancer Inst* 1984; 73: 13-24.
- 16 Natali PG, Bigotti A, Nicotra MR, et al. Analysis of the antigenic profile of uveal melanoma lesions with anti-cutaneous melanoma-associated antigen and anti-HLA monoclonal antibodies. *Cancer Res* 1989; 49: 1269-74.
- 17 Bomanji J, Hungerford JL, Granowska M, Britton KE. Radioimmunoscintigraphy of ocular melanoma with ^{99m}Tc labelled cutaneous melanoma antibody fragments. *Br J Ophthalmol* 1987; 71: 651-8.
- 18 Scheidauer K, Markl A, Leinsinger G, et al. Immunoscintigraphy in intraocular malignant melanoma. *Nucl Med Comm* 1988; 9: 669-79.
- 19 Schaling DF, Van Kroonenburg MJPG, Borsje RA, et al. Radioimmunoscintigraphy with melanoma associated monoclonal antibody fragments in ocular melanoma. *Graefes Arch Clin Exp Ophthalmol* 1989; 227: 291-4.
- 20 Ruiter DJ, Bergman W, Welvaart K, et al. Immunohistochemical analysis of malignant melanomas and nevocellular nevi with monoclonal antibodies to distinct monomorphic determinants of HLA antigens. *Cancer Res* 1984; 44: 3930-5.
- 21 Schlingemann RO, Rietveld FJR, De Waal RMW, Ferrone S, Ruiter DJ. Does the melanoma-associated high-molecular-weight antigen play a role in pericyte function during tumor angiogenesis? *Proc Am Assoc Cancer Res* 1989; 30: 378.
- 22 Ringens PJ, Van Haperen R, Vennegoor C, et al. Monoclonal antibodies in detection of choroidal melanoma. *Graefes Arch Clin Exp Ophthalmol* 1989; 227: 287-90.
- 23 Siccardi AG, Buraggi GL, Callegaro L, et al. Multicenter study of immunoscintigraphy with radiolabelled monoclonal antibodies in patients with melanoma. *Cancer Res* 1986; 46: 4817-22.
- 24 Bomanji J, Nimmon CC, Hungerford JL, Solanski K, Granowska M, Britton KE. Ocular radioimmunoscintigraphy: sensitivity and practical considerations. *J Nucl Med* 1988; 29: 1038-44.
- 25 Tullo AB, Dodd CJ, Noble JL, Owens S, Rao H. Correspondence on ocular melanoma. *Br J Ophthalmol* 1989; 73: 771. Hungerford J: In reply. *Br J Ophthalmol* 1989; 73: 771-2.