Monoclonal antibodies in detection of choroidal melanoma

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Abstract. Three monoclonal antibodies (MoAbs) prepared against cutaneous melanomas were tested against one group of 12 choroidal melanomas with indirect immunofluorescence in frozen sections. A fourth MoAb was tested in paraffin sections of a second group of 47 choroidal melanomas. One MoAb (NKI-M7) did not react with choroidal melanoma, even though it had a high sensitivity for cutaneous melanoma. A second MoAb (NKI-M6) showed a positive reaction with only 2/12 choroidal melanomas. The third MoAb (NKI/beteb) reacted with all choroidal melanomas, regardless of the cell type. MoAb NKI/C-3 was positive with 38/47 (81%) choroidal melanomas. We conclude that NKI/C-3 and NKI/beteb have a high sensitivity for both cutaneous and choroidal melanomas in frozen sections. Of these two antibodies NKI/beteb was the most specific for cutaneous naevi and melanomas.

Introduction

The diagnosis of posterior uveal melanoma is usually not a difficult one, both on the basis of the clinical picture and the various diagnostic procedures available. Serological tests for choroidal melanoma have been employed in order to refine the diagnosis and assessment of prognosis [1, 3, 6, 12].

A major step in tumour diagnosis and therapy was Köhler and Milstein's [15] discovery of a technique to obtain monoclonal antibodies (MoAbs) of predestined specificity in large quantities. MoAbs are now widely used for diagnostic purposes and they are helpful in identifying tumours of unknown origin [4]. Conjugation of MoAbs with cytotoxic and chemotherapeutic agents has already shown promising results in the treatment of tumours [13, 17]. The present role of MoAbs in ophthalmology, has been reviewed by Rodrigues [19].

In uveal melanomas MoAbs have been used for defining cell populations composing a tumour [8], for histopathological diagnosis [10], and for identification of melanoma metastases [7]. Since both ocular and cutaneous melanocytes are derived from the neural crest, it is reasonable to assume that ocular and cutaneous melanomas share common antigens. Folberg and associates [9] were indeed able to demonstrate a common antigen in ocular and cutaneous melano-

mas, a glycoprotein preserved in paraffin-embedded tissue. However, this antibody showed only a reaction with the epithelioid-cell population and not with the spindle cells.

In the Netherlands Cancer Institute, MoAbs have been produced against differentiation antigens and progression markers for human cutaneous melanoma [5, 22, 23]. The present paper describes the reaction pattern of these MoAbs on a series of choroidal melanomas. Both similarities and differences in relation to cutaneous melanomas will be demonstrated.

Materials and methods

Tumours

A punch biopsy specimen was taken from 12 choroidal melanomas within 1 min after enucleation. These eyes had all received two times 4 Gy external radiation on the 2 days prior to enucleation. Paraffin sections revealed: 6 spindle-cell, 3 epithelioid-cell and 3 mixed-cell melanomas (group 1). Paraffin sections were available from 47 additional non-irradiated cases. This series consisted of 23 mixed-cell, 19 spindle-cell and 5 epithelioid-cell melanomas (group 2).

Monoclonal antibodies

Four MoAbs were selected on the basis of specificity and sensitivity for cutaneous melanoma. These were designated NKI/C-3 [22], NKI/beteb [23], NKI-M6 (former name AMF-6 [5]), and NKI-M7 (former name AMF-7 [5]). These four MoAbs were tested on all tumours of group 1, while only NKI/C-3 was used against sections of group 2.

Immunohistochemistry

An indirect immunofluorescence procedure on frozen sections was used. Sections were incubated with 50 µl of the MoAb for 60 min at room temperature. Dilutions were 1/10 (hybridoma supernatants) for NKI/C-3 and NKI/beteb and 1/175 (ascites) for NKI-M6 and NKI-M7. After incubation sections were washed three times with phosphate buffered saline (PBS). The next step was an incubation with 50 µl of an FITC-labelled goat anti-mouse immunoglobulin at room temperature. After 60 min the sections were rinsed again three times with PBS. The tissue was finally embedded in buffered glycerin and the fluorescence pattern was studied by two investigators. Sections incubated with only PBS and stained with FITC served as controls. In addition, a

group of paraffin sections was incubated with only NKI/C-3 (group 2).

Interpretation

Classification of the choroidal melanomas was made by two ophthalmic pathologists. Tumour cells were identified by comparison of parallel frozen sections stained with haematoxylin and eosin. The proportion of positive tumour cells was estimated and expressed as a percentage of the total number of tumour cells present in the tissue sections (more than 10% staining was considered as positive). In the paraffin sections of group 2 the number of positive tumour cells reacting with NKI/C-3 was counted twice. Again, more than 10% staining was considered to be positive.

Results

All choroidal melanomas from group 1 stained with NKI/C-3 and with NKI/beteb (Fig. 1). In all but two tumours more than 60% of the cells showed a positive reaction. In these sections, no reaction was seen with non-tumour cells. In the remaining two tumours (both spindle-cell type melanomas) at least 10% of the cells showed a positive reaction.

The staining was diffuse throughout the cytoplasm, sometimes with a higher concentration around the cell nucleus. An equal staining intensity could be seen throughout each of the tumour sections. NKI/beteb had a marked granular aspect.

Table 1. Reactions of monoclonal antibodies NKI/C3, NKI/beteb, NKI-M6 and NKI-M7 with choroidal melanomas (group 1) and other melanomatous and non-melanomatous tumours

MoAb	NKI-M6 [5]	NKI-M7 [5]	NKI/beteb [23]	NKI/C-3 [11, 16, 21, 22]
Melanoma choroidal ^a	2/12	0/12	12/12	12/12
primary skin	6/6	5/5	19/19	105/117
metastases	35/35	29/29	56/63	176/186
Skin naevi	5/6	8/17	27/28	86/104
Clear cell sarcoma	NT	NT	4/4	3/12
Basal cell ca./ squamous cell ca.	NT	NT	0/11	0/46
Other tumours	0/12	0/12	0/174	106/495

^a The data on choroidal melanomas are from this paper; the other data are from references as indicated. The nominator indicates number of positive reactions, the denominator the numbers of tumours tested

NT, not tested

Two melanomas (one of the epithelioid-cell type and one of the mixed-cell type) revealed a positive reaction with NKI-M6 (results not shown), whereas NKI-M7 did not react at all with any of these tumours.

Reactions of these MoAbs with choroidal melanomas are summarized in Table 1, together with their reactions against various other tumours. In the paraffin sections of

Fig. 1 a-d. Immunofluorescence pattern on frozen sections of spindle cell (a, b), epithelioid cell (c) and mixed cell (d) uveal melanomas with NKI/C-3 (a and d) and with NKI/beteb (b and c); $\times 100$

Table 2. Reactions of MoAb NKI/C-3 with paraffin sections of different types of choroidal melanomas (group 2). See legend Table 1

Type of choroidal melanoma	
Spindle cell	18/19
Epithelioid cell	2/5
Mixed cell	18/23
Total	38/47

group 2 especially the spindle-cell type and mixed-cell type melanomas showed positive reactions with NKI/C-3 (Table 2).

Discussion

The immunohistochemical staining results showed a high sensitivity for differentiation antigens associated with cutaneous melanoma recognized by MoAbs NKI/C-3 and NKI/beteb in choroidal melanoma. This confirms that common antigens can be found in choroidal and cutaneous melanomas as was shown earlier [9, 10]. The staining distribution of the MoAbs used in the current study did not discriminate between spindle-cell and epithelioid-cell areas in group 1. In the paraffin sections of group 2 NKI/C-3 reacted better with spindle than with epithelioid cells, which is in contrast to Folberg's report [9]. The reaction of NKI/C-3 in group 2 is also different from the results with metastases of cutaneous melanomas where NKI/C-3 reacted primarily with epithelioid cells and less with spindle cells [18, 21].

The tumours in group 1 had been irradiated prior to enucleation whereas those in group 2 had not. It has been shown that irradiation changes the expression of HLA antigens in ocular melanomas [14] and it could be that this irradiation also had an effect on the expression of the antigens recognized by the anti-melanoma MoAbs used in this study. In one more study [20] the recognition of antigens by MoAb M.2.2.4 was changed by irradiation but in another study the expression of the antigens recognized by NKI-M7 and NKI/beteb was not affected by irradiation [23]. Thus, the effect of irradiation still needs further clarification.

The shared antigens of choroidal, uveal and cutaneous melanomas reflect their common derivation from the neural crest as does the presence of S-100 protein in ocular melanomas [2]. NKI/beteb proved to be specific for the detection of melanocytes [23] but it was not able to discriminate between cutaneous naevi and melanomas. We were not able to obtain recently enucleated eyes with choroidal naevi suitable for this investigation so that we could not check NKI/beteb and NKI/C-3 against choroidal naevi. It may be assumed that these antibodies react similarly in choroidal and in cutaneous naevi. This will probably restrict the clinical application of NKI/beteb and NKI/C-3 for diagnostic purposes in ophthalmology because they could not differentiate between skin naevi and melanomas. The most difficult differentiation in the case of a choroidal tumour is not so much between a naevus and a melanoma as between an amelanotic melanoma and a metastasis from a systemic tumour. Choroidal metastasis is currently treated by irradiation and not by enucleation; thus, we also did not have sections of choroidal metastasis available for testing these

MoAbs and we cannot predict the future diagnostic use of them. Moreover, as the antigens recognized by NKI/beteb and NKI/C-3 have an intracytoplasmic position they may not be useful for immunotargeting or immunoscintigraphy.

The reactions with MoAbs NKI-M6 and NKI-M7 show that the antigenic phenotype of choroidal melanoma differs from cutaneous melanoma. Monoclonal antibody NKI-M6, which recognizes a high molecular weight antigen often expressed in cutaneous melanoma, was barely detectable in our choroidal melanoma cases. This finding is in agreement with other investigators [17, 20]; Matsui et al. [17] used MoAb 225.28S, which also recognizes a high molecular weight antigen. MoAb NKI-M7, which recognizes a melanoma-associated cellular adhesion molecule that is expressed more extensively in melanoma metastases than in primary cutaneous melanomas [5], did not show any staining with choroidal melanoma. It remains to be seen if metastases from choroidal melanomas will be reactive with these two MoAbs.

In conclusion, NKI/beteb and NKI/C-3 seem to be good histological markers for choroidal melanoma in frozen sections. NKI/C-3 is also useful in paraffin-embedded tissue sections. Whether they differentiate between choroidal melanoma and naevus and whether they will react with metastases from choroidal melanomas remains to be demonstrated.

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