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Immunohistochemical Analysis of Antimelanoma Monoclonal Antibodies, with Special Reference to Fetal Tissue Distribution

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An immunohistochemical study using 2 antihuman melanoma monoclonal antibodies designated as MoAb 225.28S and MoAb 653.40S was carried out on various human skin tumors, including malignant melanoma as well as on normal and fetal tissues by indirect immunofluorescence technique. Specific immunofluorescence was observed not only in malignant melanoma cells but also in cells of pigmented nevi, basal cell epithelioma, normal hair follicles, and some fetal tissues. Both monoclonal antibodies were revealed to be able to recognize the common antigenic determinant shared by several skin tumors, including malignant melanoma, and fetal tissues. Therefore, both monoclonal antibodies might recognize premature antigen of both melanocytic and keratinocytic cell lineage.

In the field of oncology, monoclonal antibody technology was introduced in expectation of finding tumor-, or cancer-specific antigens. In particular, active attention was focused on malignant melanoma because it was believed to express strong tumor-specific antigens. Subsequently, many monoclonal antibodies against melanoma cells have been developed. However, it turned out that most of them recognized melanoma-associated antigen such as a common neuroectodermal antigen [1–9].

In this study, the tissue reactivity of 2 monoclonal antihuman melanoma antibodies designated as MoAb 225.28S and MoAb 653.40S was studied immunohistochemically. As a result of this study, we found that these monoclonal antibodies showed reactivity to more various tissue specimens than ever reported [10–12].

MATERIALS AND METHODS

Tissues

A total of 23 specimens of human primary and metastatic malignant melanoma was used in this study. In addition, various pigmented nevi, several kinds of tumors of non-neural and neural tissue origin, and various parts of human normal and fetal tissue (9–16 weeks of gestation) were also used. All tissues were freshly obtained at biopsy or operation. Human fetal tissues were obtained from artificial abortion materials. These tissues were immediately cut into halves; one was fixed in 10% formalin solution for routine histopathologic examination and the other was quickly frozen in normal hexane at −80°C and stored in a deep freezer until use for immunohistochemical studies.

Monoclonal Antibodies to Human Malignant Melanoma

Two monoclonal antibodies, designated as MoAb 225.28S and MoAb 653.40S, were used in this study and have been described in detail elsewhere [2,3]. The immunoglobulin subclass of each antibody was IgG2a (MoAb 225.28S) and IgG1 (MoAb 653.40S). An indirect inhibition binding assay suggests that both monoclonal antibodies recognize the same or closely associated antigenic determinants [13].

Indirect Immunofluorescence Technique

Cryostat sections (4 μm thick) were cut from frozen tissues and placed on albumin-coated glass slides. After air drying for 30 min, they were washed 3 times with cold phosphate-buffered saline (PBS) (pH 7.2) for 30 min. and reacted with 225.28S or 653.40S monoclonal antibodies (both dilutions 1:40), for 30 min at room temperature in a moist chamber. After washing 3 times with cold PBS, fluorescein isothiocyanate (FITC) labeled sheep antimouse IgG1 or IgG2a (Serotec, England, dilution 1:80) was applied to the slide for 30 min at room temperature in a moist chamber. Then the sections were thoroughly washed with cold PBS and mounted in buffered glycerol (pH 9.5) for observation by a Carl-Zeiss fluorescence microscope. Culture medium of myeloma cells was used for control study.

RESULTS

Melanotic Tumor

As listed in Table I, both monoclonal antibodies reacted to a majority of malignant melanoma, both primary and metastatic lesions including amelanotic type. The specific immunofluorescence for both monoclonal antibodies was observed exclusively on the plasma membrane of the melanoma cells. The intensity of specific immunofluorescence was relatively heterogeneous depending on the area of the tumor. Some tumor cells lacked specific immunofluorescence. In malignant melanomas, the immunoreactivity of MoAb 225.28S was wider than that of MoAb 653.40S. The reactivity of both monoclonal antibodies suggests that there was no correlation with the clinical stage of melanoma, with the degree of invasiveness of primary lesion, with the amount of melanin, or with tumor cell morphology such as spindle-shaped or epithelioid-shaped. But in a case of malignant blue nevus, only epithelioid-shaped tumor cells were reacted and spindle-shaped tumor cells were not (Fig 1). In a case of lentigo maligna, both monoclonal antibodies reacted to some atypical melanocytes in the basal layer of the epidermis, but did not react to melanophages in the papillary layer of the dermis.

Both monoclonal antibodies showed similar reactivity to all types of pigmented nevi except intradermal nevi. Exceptionally, MoAb 653.40S surpassed MoAb 225.28S in the immunoreactivity of intradermal nevi. Specific immunofluorescence was relatively strong and evenly distributed on the cell surface of the nevus cells. However, neither common blue nevi nor cellular blue nevi showed reactivity to either of the monoclonal antibodies. In some cases of intradermal nevus, hyperpigmented basal cells of the epidermis were revealed to react with both monoclonal antibodies.

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Abbreviations:
BCE: basal cell epithelioma
FITC: fluorescein isothiocyanate
ML: malignant lymphoma
MoAb: monoclonal antibody
SCC: squamous cell carcinoma

TABLE I. *Reactivity of MoAb 225.28S and MoAb 653.40S with melanomas and nevi*

Tissue	MoAb 225.28S	MoAb 653.40S
Malignant melanoma:		
Primary melanoma	6/8	2/3
Metastatic melanoma	13/15	2/5
Malignant blue nevus	1/1	1/1
Pigmented nevi:		
Junctional nevi	2/2	1/1
Compound nevi	1/3	1/1
Intradermal nevi	6/20	9/13
Blue nevi:		
Common blue nevi	0/6	0/3
Cellular blue nevi	0/2	0/2

No. of positive cases/no. of total cases.

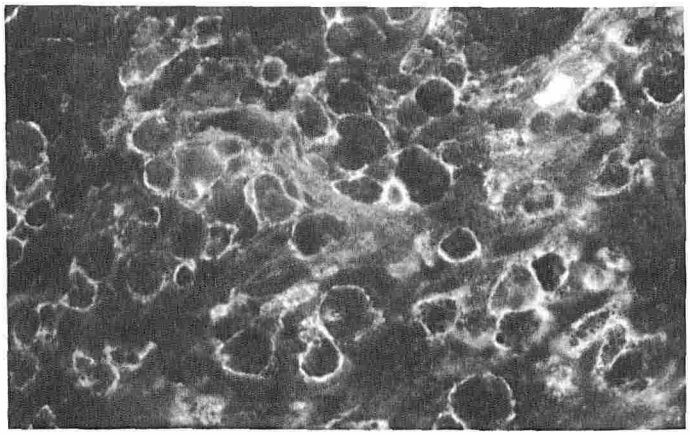

FIG 1. Indirect immunofluorescence staining of malignant blue nevus with the MoAb 225.28S. The staining appears to be restricted to the plasma membrane of epithelioid-shaped tumor cells.

Normal Tissues and Various Tumors of Non-neural and Neural Origin

Both monoclonal antibodies showed no reaction with normal skin, lymph nodes, and tonsils, including melanocytes in the epidermis; however, normal lower hair follicles showed positive staining (Fig 2). As shown in Table II, the reactivity of MoAb 653.40S was greater than the reactivity of MoAb 225.28S in basal cell epithelioma (BCE) and squamous cell carcinoma (SCC) cases. Specific staining of them was observed on the plasma membrane of the tumor cells and the intensity of immunofluorescence was as strong as that of nevus cells.

In the tumors of the nervous system, only schwannoma showed positive staining by MoAb 225.28S.

Fetal Tissues

MoAb 225.28S reacted with the skin of 8 out of 10 feti at from 9–16 weeks' gestation, and the MoAb 653.40S reacted with all cases stained positively with MoAb 225.28S. The positive staining was observed on the plasma membrane of the epidermal cells of the skin, including melanocytes (Fig 3). There was no positive staining in the periderm or immature mesenchymal cells in the dermis. In the fetal kidney of 16 weeks' gestation, only proximal tubules showed positive staining and glomerules and distal tubules did not react with either of the monoclonal antibodies (Fig 4). In the fetal rib bone of 9 weeks' gestation, chondroblasts were stained by both monoclonal antibodies. Other fetal tissues, such as brain, liver, and intestine, did not show any positive staining.

DISCUSSION

There have been many reports concerning the production of monoclonal antibodies against human malignant melanoma [1–9]. The majority of monoclonal antibodies to human malignant melanoma reported previously seemed to be melanoma-associated rather than melanoma-specific because they have been shown to cross-react with various tumors other than melanoma [2–9], pigmented nevi [1], and even some normal human tissues

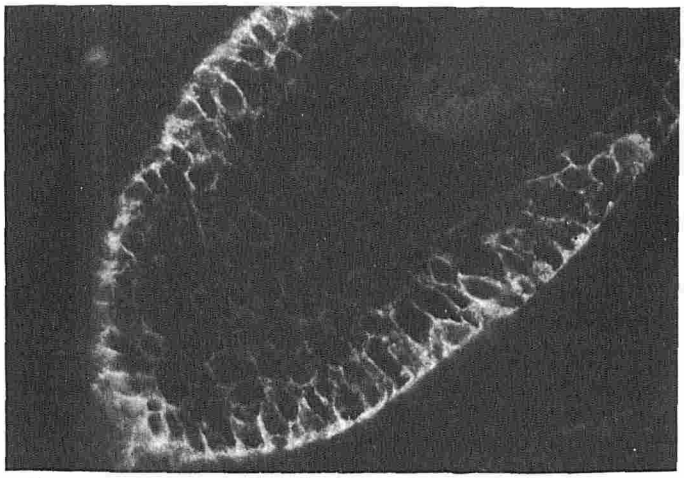

FIG 2. Indirect immunofluorescence staining of a normal hair follicle with MoAb 653.40S. MoAb 653.40S stains only normal outer root sheath cells.

TABLE II. *Reactivity of both monoclonal antibodies to various tumors and normal human tissues*

Tissue	MoAb 225.28S	MoAb 653.40S
Malignant tissues:		
BCE	5/12	9/10
SCC	2/8	4/6
ML	0/3	0/2
Tumors of nervous system:		
Meningioma	0/6	0/1
Glioma	0/1	NT
Schwannoma	1/1	NT
Ependymoma	0/1	0/1
Glioblastoma	0/3	0/3
Normal tissues:		
Epidermis	0/6	0/6
Hair follicle	5/5	5/5
Lymph node	0/3	0/3
Tonsil	0/2	0/2
Fetal tissues:		
Skin (9–16 weeks)	8/10	9/9
Kidney (16 weeks)	2/2	2/2
Brain (9–16 weeks)	0/5	0/5
Intestine (10–11 weeks)	0/1	0/1
Rib bone (9 weeks)	2/2	2/2
Liver (10–11 weeks)	0/3	0/3

No. of positive cases/no. of total cases. NT = not tested.

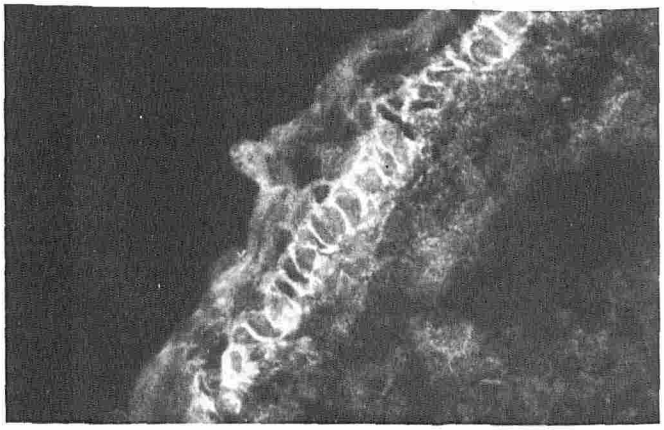

FIG 3. Indirect immunofluorescence staining of the thigh skin of a 16-week fetus. Specific staining is seen only on epidermal cells.

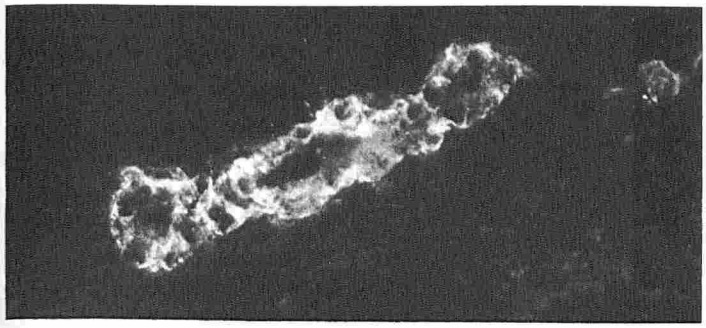

FIG 4. Indirect immunofluorescence staining of the kidney of a 16-week fetus. Specific staining is seen on the epithelium of proximal tubules.

[14]. The monoclonal antibodies used in this study are identical to those used by Wilson et al and Natali et al [10–12] and the results obtained herein were similar to their reports. However several new findings are reported concerning the immunoreactivity of these monoclonal antibodies.

Firstly, the reactivity of these antibodies to malignant blue nevus has not been reported yet. It has been known that both common and cellular blue nevi show no positive reaction to these monoclonal antibodies [12]. Malignant blue nevus originates from the common or cellular blue nevus. Therefore, the expression of the antigen recognized by these antibodies seems to be associated with the malignant transformation of blue nevus cells.

Secondly, although the cross-reactivity of these antibodies to keratinocytic lineage such as BCE and SCC has been previously reported, the cross-reactivity to normal hair follicles and even epidermis of the fetal skin is not known [10–12]. These findings show that the antigen detected by both monoclonal antibodies was expressed on malignant and premature keratinocytes.

Thirdly, the cross-reactivity to fetal tissues was previously reported by many investigators, however they mainly used a radioimmunoassay method for the demonstration of cross-reactivity, and the precise distribution of the antigen reactive to antimelanoma monoclonal antibodies in fetal tissues was not elucidated by immunohistochemical methods [15–17]. This study is the first to demonstrate specific fluorescence in various fetal tissues. Therefore, these monoclonal antibodies seem to have a premature nature at least in both melanocyte and keratinocyte lineage background rather than a common neuroectodermal nature, because of the lack of reactivity to most neural tumors and fetal brain tissues. Further sequential studies are necessary to clarify the nature of the antigen detected by these monoclonal antibodies.

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