

Expression of microphthalmia transcription factor, S100 protein, and HMB-45 in malignant melanoma and pigmented nevi

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Abstract. Malignant melanoma (MM) is a type of malignant tumor, which originates from neural crest melanocytes. MM progresses rapidly and results in a high mortality rate. The present study aims to investigate the expression of microphthalmia transcription factor (MITF), the S100 protein, and HMB-45 in MM and pigmented nevi. A total of 32 MM samples (including three skin metastasis, three lymph node metastasis and two spindle cell MM samples), two Spitz nevus samples, four pigmented nevus samples and two blue nevus samples were collected. The expression levels of S100 protein, HMB-45, and MITF were observed via immunostaining. The S100 protein exhibited high positive rates in MM and pigment disorders (96.7 and 100%, respectively), but with low specificity. The S100 protein was also expressed in fibroblasts, myoepithelial cells, histocytes and Langerhans cells in normal skin samples. HMB-45 had high specificity. Its positive expression was only confined to MM cells and junctional nevus cells. Furthermore, HMB-45 was not expressed in melanocytes in the normal tissue samples around the tumor or in the benign intradermal nevus cells. MITF exhibited high specificity and high sensitivity. It was expressed in the nuclei of melanocytes, MM cells and nevus cells. It was observed to be strongly expressed in metastatic MM and spindle cell MMs. Thus, MITF may present as a specific immunomarker for the diagnosis and differential diagnosis of MM.

Introduction

Malignant melanoma (MM) is a type of malignant tumor, which originates from neural crest melanocytes. MM progresses rapidly and leads to a high mortality rate, and in recent years its incidence has been increasing. The morbidity

rate significantly increases in individuals >60-years-old and the gender ratio of those affected by MM is 1:5 (male:female) (1). The mortality rate associated with MM is 7.6 (2) and 5.7 (3) per 100,000 patients in England and the USA, respectively. Therefore, early and accurate diagnosis of MM is considered to be of great significance in clinical practice (4).

MM presents a variety of pathological manifestations, particularly spindle cell and desmoplastic melanomas, which often require differentiation from mesenchymal malignant tumors. Therefore, immunohistochemical staining is important for the confirmation of MM, as well as its differentiation from other types of malignant tumor (5). Currently, markers that are frequently used for melanocytes include S100 protein and HMB-45. Although the S100 protein has high sensitivity, it lacks specificity and is positively expressed in multiple types of tumor (6-8). In addition to melanocytes, Schwann cells, musculoepithelial cells, adipocytes, chondrocytes, histiocytes and Langerhans cells all demonstrate S100-positive expression. HMB-45 has high specificity, but low sensitivity, particularly to spindle cell and desmoplastic melanomas where it is not expressed (9,10). Microphthalmia transcription factor (MITF) is a nucleoprotein in melanocytes, which is critical in the production and activity of melanocytes (11,12), and serves as an extracellular signal that exerts regulatory and modificatory functions. MITF exhibits satisfactory expression in pigmented diseases and MM with >95% sensitivity and specificity. For this reason, it is considered to be a promising marker of melanocytes. However, MITF expression in pigmented diseases and MM remains controversial. A previous study identified that MITF (D5) cannot function as a sensitive or specific marker for the diagnoses of spindle cell and desmoplastic melanomas (13). MITF has been demonstrated as noticeably advantageous in the diagnoses of spindle cell, desmoplastic and metastatic melanomas (14).

The aim of the present study was to observe the role of MITF in the diagnosis of pigmented diseases, particularly spindle cell and metastatic melanomas, and that of the combination of S100 protein and HMB-45 in the diagnostic improvement of pigmented diseases and MM.

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Materials and methods

Sample preparation and microscopic analysis. Microscope slides were soaked in sulfuric acid, washed with tap water,

soaked in ethanol, dried and coated with polylysine. Thirty-two tissue samples (including four pigmented nevus tissue samples, two blue nevus tissue samples and two Spitz nevus tissue samples) were obtained from patients in the Department of Dermatology at the Kyushu University (Fukuoka, Japan) and the Second Hospital of Jilin University (Changchun, China). The patients were 49-78 years old (mean age, 65 years; 20 males and 12 females) and provided written informed consent. The paraffin-embedded tissues were sectioned (size, 3.5 μm), placed on the prepared slides and dried at 37°C overnight. The samples were dewaxed twice for 5 min with xylene, and dehydrated with absolute ethyl alcohol for 2 min, twice, in 95% alcohol for 2 min and in 80% alcohol for 2 min, then purified with water twice. For HMB-45 (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) immunostaining, the samples were subjected to microwave heating for antigen retrieval. For MITF immunostaining (Novocastra; Leica Microsystems, Ltd., Milton Keynes, UK) the samples were subjected to autoclaving at 95°C for 20 min for antigen retrieval. No antigen retrieval was required for the S100 protein immunostaining (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.).

Immunostaining. The procedure was performed according to instructions. Briefly, the immunostaining SP kits were purchased from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. The samples were incubated at 37°C for 10 min with 3% hydrogen peroxide, then washed three times for 2 min with phosphate-buffered saline (PBS). The samples were incubated at 37°C for 2 h with primary antibodies [S100A mouse anti-human monoclonal antibody (cat. no. TA807339; 1:10; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.), HMB45 mouse anti-human monoclonal antibody (cat. no. GM063402; 1:10, Shanghai Gentech Co., Ltd., Shanghai, China) and MITF mouse anti-human monoclonal antibody (cat. no. TA336406; 1:10; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.)] and the reagent was added. Then the samples were incubated with goat anti-mouse IgG antibody (HRP; cat. no. ZDR-5307; 1:50; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.) at 37°C for 30 min and washed three times for 2 min with tris-buffered saline (TBS; for MITF) and phosphate-buffered saline (PBS; for S100 and HMB-45). The samples were incubated for 10 min with 3-amino-9-ethylcarbazole (AEC) and counterstained with hematoxylin for 5 min. The samples were then decolorized with 1% HCl (alcohol, 1:99) and washed under running water. Finally, the samples were mounted with neutral balsam and dehydrated. The samples were stained with AEC color developing agent and observed under a microscope (U-MDOB3; Olympus Corp., Tokyo, Japan). The immunostaining buffer solution for the MITF antibody was TBS, and was PBS for the S100 protein and HMB-45 antibodies.

Staining classification. Five fields from each section were observed under the microscope (magnification, x400; 150-200 cells were counted). Cells with sepia-stained granules in the cytoplasm were considered to be positive for S100 protein and HMB-45. Sections were classified as follows: >75% positive cells, (+++); >50% positive cells, (++) ; >25% positive cells, (+); <25% positive cells, (-), according to (15).

Table I. Expression of S100 protein, HMB-45, and MITF in MM and pigmented nevus samples.

Sample	n	S100				HMB-45				MITF	
		-	+	++	+++	-	+	++	+++	-	+
MM	32	1	13	7	11	6	16	3	7	1	31
Pigmented nevus	4		4			3	1				4
Blue nevus	2		2			2					2
Spitz nevus	2		2				2				2

>75% positive cells, (+++); >50% positive cells, (++) ; >25% positive cells, (+); <25% positive cells, (-). MITF, microphthalmia transcription factor; MM, malignant melanoma.

Cells with a stained nucleus were MITF-positive, and >10% positive cells was determined as (+).

Results

MM pathological typing. The samples were classified according to previous guidelines (16). Among the 32 MM samples, six were superficial spreading type, five were nodular type, six were malignant lentigo type, nine were acral-lentiginous type, three were skin metastasis type, and three were lymph node metastasis type. According to cytological classification, the majority of the samples were epithelioid cell type and two were spindle cell type.

Expression of S100 protein, HMB-45, and MITF. S100 immunostaining demonstrated that the positive rates of S100 protein in MM and pigmented nevi were 96.8 and 100%, respectively (Table I and Fig. 1). Langerhans cells, musculoepithelial cells, and a small number of fibrocytes in normal tissue samples also exhibited S100 positivity (Fig. 2). In addition, the four pigmented nevus samples, two blue nevus samples and two Spitz nevus samples exhibited S100-positive expression (Fig. 3). HMB-45 immunostaining indicated that the positive rate of HMB-45 in MM was 81.2%. Positive HMB-45 expression was only confined to MM, active pigmented nevus cells and Spitz nevus cells, and was not expressed in the normal Langerhans cells (Fig. 4). MITF immunostaining showed that the positive rates of MITF expression in MM and pigmented nevus cells were 96.8 and 100%, respectively. Positive expression of MITF was not observed in the components of skin tissue samples, apart from in melanocytes, nevocytes and in MM cells.

Discussion

MM is a highly malignant type of tumor. Immunostaining is a particularly important method of diagnosis, in addition to clinical and histopathological analyzes. The present study utilized immunostaining to observe the expression of MITF, S100 protein and HMB-45 in 32 MM samples, four pigmented nevus, two blue nevus, and two Spitz nevus samples. The results showed that S100 protein was highly sensitive for the

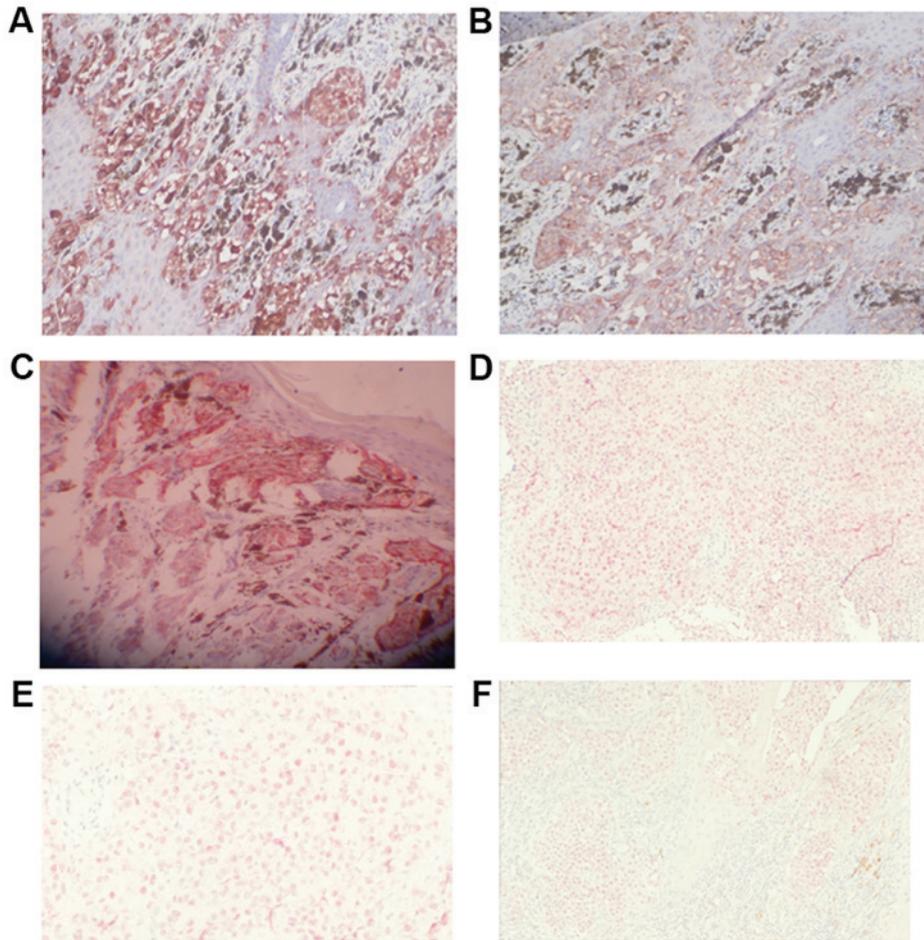


Figure 1. Expression of S100 protein, HMB-45, and MITF in MM and pigmented nevus samples. (A) S100 protein in MM (+++; magnification, x200); (B) HMB-45 in MM (magnification, x400); (C) HMB-45 in Spitz nevus (+++, magnification, x200); (D) MITF in MM (+; magnification, x100); (E) MITF in metastatic MM (+; magnification, x100); (F) MITF in Spitz nevus (+; magnification, x100). MITF, microphthalmia transcription factor; MM, malignant melanoma.

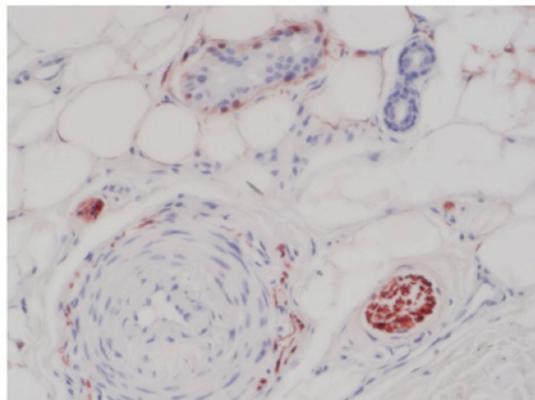


Figure 2. Unspecific staining of S100 protein in hair follicles, sweat glands and fibroblasts (magnification, x400).

diagnosis of MM and pigmented nevus with positive rates of 96.8 and 100%, respectively. This finding is consistent with a previous study (17). However, in addition to melanocytes and MM cells, the S100 protein is expressed in gliocytes, Schwann cells, striated muscle cells, cardiac muscle cells, fat cells and fibroblasts in normal tissues. Therefore, it is also expressed in tumors associated with the above-mentioned cells. In the

present study, S100 protein expression was observed in hair follicle myoepithelial cells and a small number of fibrocytes. This finding indicates that the S100 protein has high sensitivity, but low specificity for the diagnosis of MM and pigmented skin diseases, thus its application in clinical practice is somewhat limited.

HMB-45 immunostaining demonstrated that HMB-45-positive expression was only confined to MM and actively proliferating melanocytes, such as junctional nevus cells and the junctional cells between the dermis and the epidermis in Spitz nevus, whereas no positive reactions were observed in other components of the skin tissues. This finding indicates that HMB-45 has high specificity for the diagnosis of MM (18).

MITF is a nucleoprotein of melanocytes, which is critical in the production and activity of melanocytes. It regulates the morphology, differentiation and survival of melanoblasts (cells where melanocytes originate), as well as melanocytes and MM cells (19,20). MITF encodes a modulin, which exerts the functions of transcription factors and has a basic structure of the helix-loop-helix leucine zipper dipolymer. This modulin (a trans-acting factor) binds with the DNA template before transcription to form a transcription initiation complex, functioning as an important protein regulating

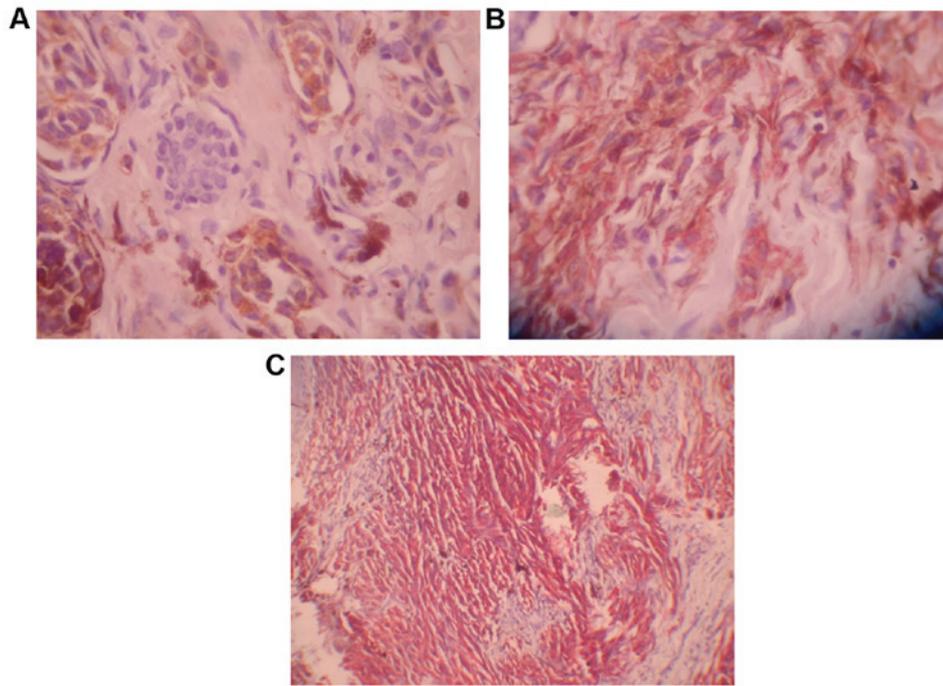


Figure 3. Expression of S100 protein in pigmented nevus, blue nevus, and Spitz nevus samples. S100 protein in (A) pigmented nevus, (B) blue nevus and (C) Spitz nevus (magnification, x400).

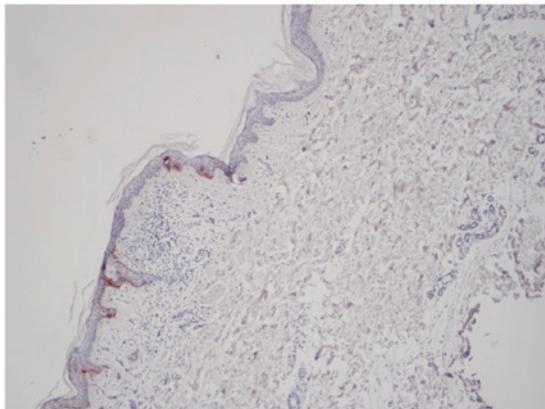


Figure 4. HMB-45 expression in normal Langerhans cells without skin damage caused by melanoma (magnification, x400).

cellular activities. MITF exhibits a different staining mode when compared with other markers; cells display nuclear staining and necrotic tumor tissues are not stained (21). For these reasons, MITF has become a global point of interest. MITF copy number variations are closely correlated with patient survival rate. The larger the MITF copy number is, the lower patient's survival rate will be, indicating a poorer prognosis (22). However, MITF expression in pigment diseases and MM that has been reported in different studies is inconsistent (23). MITF (D5) was not considered to be a sensitive or specific marker for the diagnosis of desmoplastic and spindle cell melanomas (13). Although, MITF exhibited marked advantages in the diagnosis of desmoplastic, spindle cell and metastatic melanomas (14).

In the present study, Ncl-MITF staining indicated that Ncl-MITF was present in almost all MM cells (its positive

rate in MM was 96.8%), pigmented nevus cells, and normal melanocytes in skin tissue samples. It was also expressed in pigmented nevus, blue nevus, and Spitz nevus samples, with a positive rate that was comparable to that of S100 protein in MM and pigmented nevus samples. Furthermore, it was not expressed in other components of skin tissue, which was similar to HMB-45. Therefore, MITF is considered to be a relatively highly sensitive and specific immunologic marker of MM (23,24). In addition, in the present study, MITF-negative expression of S100 protein and HMB-45 was positive in the same patient.

In conclusion, the present study indicates that the diagnosis of MM should be based on comprehensive clinical and histopathological analyzes, in addition to multiple immunohistochemical indices.

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