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HMB-45 may be a more sensitive maker than S-100 or Melan-A for immunohistochemical diagnosis of primary oral and nasal mucosal melanomas

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BACKGROUND: Primary mucosal melanomas (MMs) of the head and neck are a rare entity. Melanomas with characteristic melanin-pigmented tumor cells are easy to diagnose, but those without melanin-pigmented tumor cells, amelanotic melanomas, are difficult to identify and need immunohistochemistry (IHC) to confirm the final diagnosis. In this study, we examined the expression of three melanocytic differentiation markers, HMB-45, S-100, and Melan-A in primary oral and nasal MMs. We tried to evaluate whether HMB-45, S-100, and Melan-A were useful for diagnosis of primary oral and nasal MMs and to find out which marker was the best of the three.

METHODS: This study used IHC to examine the expression of HMB-45, S-100, and Melan-A in 17 formalin-fixed paraffin-embedded specimens of primary oral and nasal MMs. The staining intensities (SIs) and labeling indices (LIs) of HMB-45, S-100, and Melan-A in 17 MMs were calculated and compared between any two markers.

RESULTS: Immunostaining results showed that the positive rate was 94% (16 of 17) for HMB-45, 88% (15 of 17) for S-100, and 71% (12 of 17) for Melan-A in 17 MMs. The SI of HMB-45 was significantly higher than that of S-100 ($P = 0.0011$) or of Melan-A ($P = 0.0034$). In addition, the mean LI of Melan-A (59 ± 43%) was significantly lower than that of HMB-45 (83 ± 28%, $P = 0.0065$) or of S-100 (79 ± 33%, $P = 0.0237$).

CONCLUSIONS: Our results indicate that both HMB-45 and S-100 show a high positive rate and LI in MMs and therefore may be good markers for immunohistochemical diagnosis of primary oral and nasal MMs. In addition, HMB-45 may be a more sensitive marker than S-100 because HMB-45 shows a significantly higher SI than S-100 in this study.

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Introduction

Primary mucosal melanomas (MMs) of the head and neck are far less common than their cutaneous counterparts, comprising 0.4–1.8% of all melanomas (1–3). Melanomas with characteristic melanin-pigmented tumor cells are easy to diagnose, but those without melanin-pigmented tumor cells, amelanotic melanomas, are difficult to identify (4, 5). Amelanotic epithelioid melanoma is easily confused with a poorly differentiated carcinoma or a large cell lymphoma (5, 6). In addition, spindle cell melanomas may be difficult to distinguish from sarcomas, sarcomatoid carcinomas, or an inflammatory scarring process (6, 7). In such cases, the final diagnosis usually requires immunohistochemistry (IHC) with certain antibodies directed against melanocytic differentiation antigens (4–7).

The HMB-45 and S-100 are currently the two most useful immunomarkers to identify melanocytes and melanomas (4–13). Anti-HMB-45, which was first described by Gown et al. (8) in 1986, is an antibody that recognizes the melanosomal glycoprotein gp100. Previous ultrastructural study revealed the localization of HMB-45 antigen in the pre-melanosomes, and HMB-45-positive staining was related to active early melanosome formation (9). Anti-HMB-45 is regarded more specific but less sensitive than S-100 protein (6, 9). It is very

helpful in distinguishing the differentiated melanomas from other non-melanocytic malignancies (9). Expression of HMB-45 has been detected in primary and metastatic melanomas by several investigators (4, 6, 8–12). However, HMB-45 is also expressed in a few tumors other than melanomas, such as breast carcinomas, plasmacytomas (14), angiomyolipomas, and pigmented nerve sheath tumors (6).

The S-100 protein is an acidic, calcium-binding protein that is first extracted from bovine brain by Moore (15) in 1965. It is a very sensitive marker for nevus and melanoma cells (9) but is also widely distributed in both the central and peripheral nervous systems of all vertebrates (16). The major drawback of using S-100 as a diagnostic marker for melanomas is its lack of specificity (6). S-100 protein has been shown to be present in a wide range of tumors, such as peripheral nerve sheath and cartilaginous tumors, chordomas, histiocytosis X, and carcinomas from various sites including the salivary gland, lung, breast, stomach, colon, endometrium, kidney, and ovary (14). Several previous studies have used anti-S-100 antibody for immunohistochemical diagnosis of primary and metastatic melanomas arising from a variety of human body sites (4–6, 8, 9, 11–13, 16). Anti-S-100 antibody has also been found to be valuable in the detection of spindle cell and desmoplastic melanomas (9).

Melan-A is another melanocytic differentiation marker that is identified on melanomas as an antigenic target of cytotoxic T lymphocytes (17). It is a product of the *MART-1* gene, recognizes a 20–22 kDa doublet in Melan-A mRNA-positive melanomas, and is thought to be specific for melanoma cell lines (18, 19). The anti-Melan-A mouse monoclonal antibody A103 was generated from immunizing mice with recombinant Melan-A protein by Jungbluth et al. (10) in 1998. Anti-Melan-A antibody has been shown to stain both benign and malignant melanocytic lesions in a very similar pattern to that of anti-S-100 with the exception of desmoplastic, spindle cell, and metastatic melanomas (6, 9, 10, 20). This antibody is also useful for the distinction of primary epithelioid melanocytic tumors from histologic mimics, such as histiocytomas (6, 20).

Although several studies have investigated the usefulness of HMB-45, S-100, and Melan-A in immunohistochemical diagnosis of primary and metastatic melanomas (4–14, 16, 20), little is known about the expression of these three melanocytic markers in primary oral and nasal MMs (4, 6, 9, 11–13). In this study, we examined the expression of three melanocytic differentiation markers, HMB-45, S-100, and Melan-A in 17 formalin-fixed paraffin-embedded specimens of primary oral and nasal MMs by IHC. The staining intensities (SIs) and labeling indices (LIs) of HMB-45, S-100, and Melan-A in 17 MMs were calculated and compared between any two markers. We tried to evaluate whether HMB-45, S-100, and Melan-A were useful for diagnosis of primary oral and nasal MMs and to find out which marker was the best of the three.

Materials and methods

Formalin-fixed, paraffin-embedded tissue blocks were obtained from 17 patients (nine men and eight women, mean age 59 years, range: 34–76) with primary oral or nasal MM (Table 1). Diagnosis of MMs was mainly based on histologic examination of hematoxylin and eosin-stained tissue sections. In some cases, IHC was needed to confirm the final pathologic diagnosis. All patients were diagnosed and/or treated at the National Taiwan University Hospital during the period from January 1988 to December 2003. Specimens were obtained from either incisional biopsy or total surgical excision of the tumors. Of the 17 MMs, four (23.5%) were located on the hard palate, one (5.9%) on both the hard palate and maxillary gingiva, one (5.9%) on the mandibular gingiva, and 11 (64.7%) on the nasal mucosa. More MMs were found in the right nasal cavity (nine cases) than in the left nasal cavity (two cases; Table 1). None of the patients had received any form of tumor-specific therapy before initial biopsies.

All the specimens for immunohistochemical staining were fixed in 10% neutral formalin, embedded in paraffin, and cut in serial sections of 4 μm. Immunohistochemical staining was performed using a peroxidase-labeled streptavidin-biotin technique. Briefly, tissue sections were deparaffinized, rehydrated, and then heated in a plastic slide holder (Dako, Copenhagen, Denmark) containing 0.1 M citrate buffer (pH 6.0) in a microwave oven for 10 min to retrieve antigenicity. The endogenous peroxidase activity was blocked by immersing the sections in 3% H_2O_2 in methanol for 10 min. After washing in 10 mM Tris-buffered saline (TBS), pH 7.4, sections were incubated with 10% normal goat serum to block non-specific binding. Sections were then incubated overnight at 4°C with the primary antibodies. The specificity, source, and dilution of the primary antibodies used for IHC are listed in Table 2. After washing in TBS, sections were treated with either

Table 1 Clinical features of 17 cases of primary oral and nasal mucosal melanomas

Cases	*Age (years)*	*Gender*	*Primary site*
1	69	Female	Hard palate
2	75	Female	Hard palate
3	62	Male	Hard palate
4	70	Female	Hard palate
5	56	Male	Hard palate and maxillary gingiva
6	54	Female	Mandibular gingiva
7	76	Male	Right nasal cavity
8	34	Male	Right nasal cavity
9	52	Male	Left nasal cavity
10	59	Female	Left nasal cavity
11	69	Female	Right nasal cavity
12	40	Male	Right nasal cavity
13	54	Male	Right nasal cavity
14	68	Male	Right nasal cavity
15	51	Male	Right nasal cavity
16	68	Female	Right nasal cavity
17	52	Female	Right nasal cavity

Table 2 The specificity, source, and dilution of the primary antibodies used for immunohistochemistry

Antibody	*Specificity*	*Source*	*Dilution*
Mouse monoclonal antihuman HMB-45 antibody	Melanocytes and melanoma cells	Dako	1:50
Polyclonal rabbit anticow S-100 antibody	Wide range of normal and neoplastic tissues including melanoma cells	Dako	1:400
Mouse monoclonal anti-Melan-A antibody	Mart-1/Melan-A protein in melanocytes and melanoma cells	BioGenex, San Ramon, CA, USA	1:50

biotinylated goat antimouse immunoglobulin G (IgG; for anti-HMB-45 and anti-Melan-A) or biotinylated goat antirabbit IgG (for anti-S-100), and subsequently with a streptavidin-peroxidase conjugate (Dako). The 0.02% diaminobenzidine hydrochloride (DAB; Dako) containing 0.03% H_2O_2 is used as chromogen to visualize the peroxidase activity. The preparations were lightly counterstained with hematoxylin, mounted with Permount, and examined by light microscopy. Cutaneous melanomas and nevus tissues were used as positive controls and TBS instead of primary antibody was used for negative controls.

A brown cytoplasmic staining was counted as positive for HMB-45 and Melan-A, and a brown nuclear and cytoplasmic staining was considered as positive for S-100 protein. When immunostained sections were carefully compared with negative control sections, it was not difficult to distinguish positively immunostained cells from melanin-pigmented cells. The SI was graded as 0, no staining; 1, weak; 2, moderate; and 3, strong, when compared with the positive control sections of cutaneous melanoma in which the SI was graded as 3. For calculating LIs, the sections were initially scanned at low power, at least three high-power fields were then chosen randomly, and 1000 tumor cells were counted for each case. HMB-45, S-100, and Melan-A LIs were counted as a ratio of positively immunostained tumor cells to the total number of tumor cells counted. An eyepiece graticule was used to ensure that all tumor cells were evaluated once only.

The mean LIs of HMB-45, S-100, and Melan-A in 17 MMs were calculated and the three markers' medians of SI were determined. Because three sets of serial sections of 17 MMs were stained by the antibodies directed against three markers respectively, the data were considered repeated measurements. We performed the Wilcoxon signed rank test to compare the difference in SI between two groups and conducted the paired *t*-test to compare the difference in LI between two groups. A *P*-value of < 0.05 was considered statistically significant.

Results

The immunostaining of HMB-45, S-100, and Melan-A in melanoma cells was mainly cytoplasmic but nuclear staining was also noted in anti-S-100-stained tumor cells. It was not difficult to distinguish the golden brown immunostained melanoma cells from dark brown melanin-pigmented cells (Fig. 1a–e). SIs and LIs of HMB-45, S-100, and Melan-A in 17 primary oral and nasal MMs are shown in Table 3. HMB-45 was expressed in 16 of 17 MMs (94%). The HMB-45 SI was consistent in 13 MMs and variable in three MMs. The SIs for 16 HMB-45-positive MMs were all graded as 3. The LIs for 16 HMB-45-positive MMs varied from 24 to 100% (mean 83 ± 28 for 17 MMs). The distribution of HMB-45-positive tumor cells was even in 13 MMs and patchy in three MMs (Table 3).

Expression of S-100 protein was observed in 15 of 17 MMs (88%). The S-100 SI was consistent in 14 MMs and variable in one MM. Of 15 S-100-positive MMs, SI was graded as 1 in four, 2 in nine, and 3 in two MMs. The LIs for 15 S-100-positive MMs varied from 52 to 100% (mean 79 ± 33 for 17 MMs). The distribution of S-100-positive tumor cells was even in all 15 MMs (Table 3).

Melan-A was expressed in 12 of 17 MMs (71%). The Melan-A SI was consistent in nine MMs and variable in three MMs. Of 12 Melan-A-positive MMs, SI was graded as 1 in one, 2 in four, and 3 in seven MMs. The LIs for 12 Melan-A-positive MMs varied from 22 to 100% (mean 59 ± 43 for 17 MMs). The distribution of Melan-A-positive tumor cells was even in 10 MMs, patchy in one MM, and focal in one MM (Table 3).

Further comparison of the SI between any two markers in 17 MMs showed that the SI of HMB-45 was significantly higher than that of S-100 ($P = 0.0011$) or of Melan-A ($P = 0.0034$). However, no significant difference in the SI between S-100 and Melan-A was found. In addition, the mean LI of Melan-A was significantly lower than that of HMB-45 ($P = 0.0065$) or of S-100 ($P = 0.0237$). However, there was no significant difference in the mean LI between HMB-45 and S-100. Because the sample size of this study was small ($n = 17$), we had no intention to stratify the MMs into oral and nasal MMs and to compare the SI and LI between any two markers in six oral MMs or in 11 nasal MMs.

Discussion

Primary MMs of the head and neck are rare compared with their cutaneous counterparts (1–3). Amelanotic variants can occasionally be difficult to recognize by routine light microscopy. Immunohistochemical studies may be needed for a final diagnosis. Several studies have utilized antibodies directed against melanocytic differentiation markers for immunohistochemical diagnosis of primary or metastatic cutaneous and MMs (4–14, 16, 20). Among the three antibodies, anti-HMB-45, anti-S-100, and anti-Melan-A, are most frequently used for this purpose.

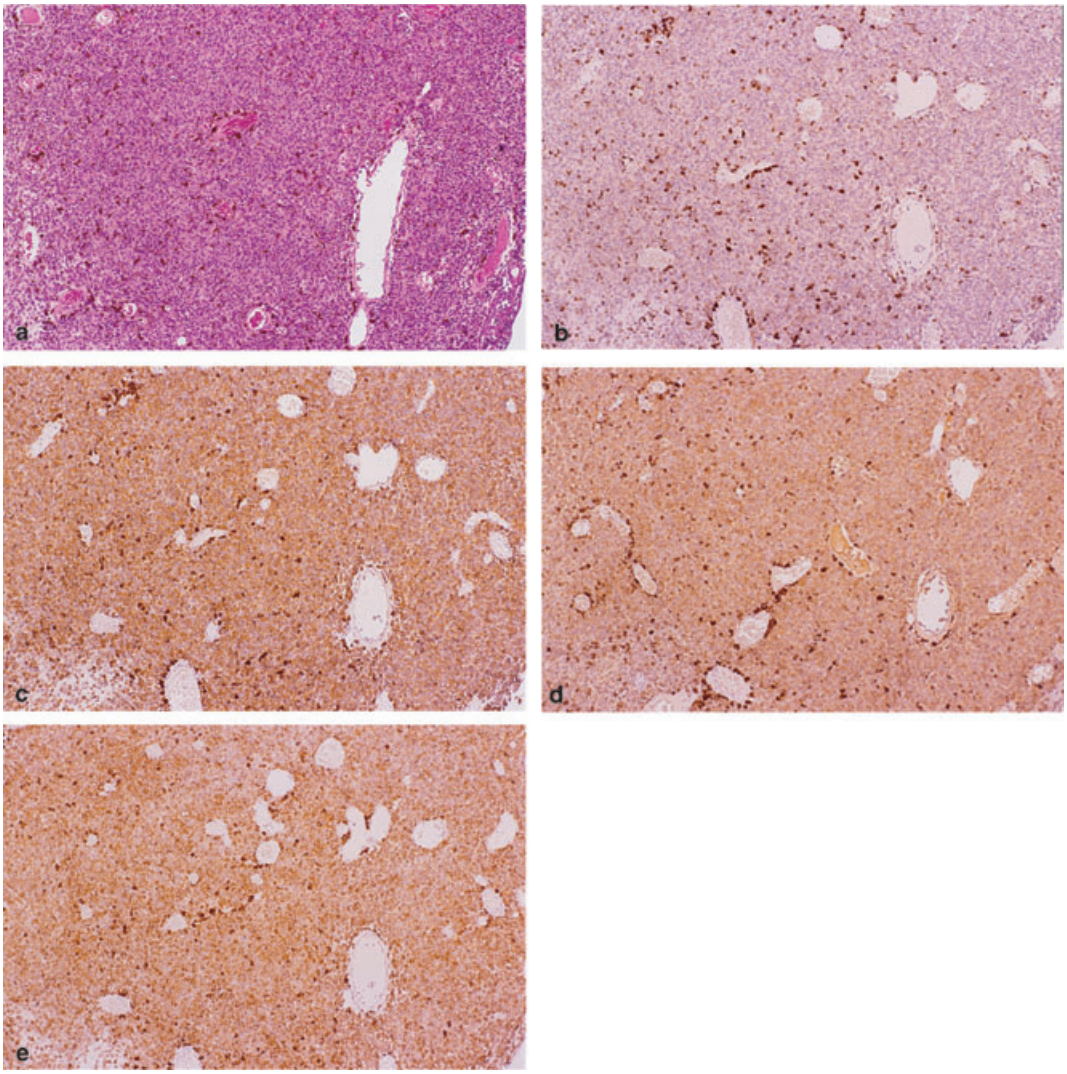

Figure 1 Hematoxylin and eosin-stained and immunostained sections of a nasal mucosal melanoma. (a) A hematoxylin and eosin-stained section showing a sheet of round melanoma cells. Some of these melanoma cells and melanophages contained dark brown melanin pigments. (b) A negative control section showing no staining of melanoma cells. The dark brown melanin-pigmented tumor cells and melanophages were dispersed among negatively stained melanoma cells. (c) A serial section of (b) stained with anti-HMB-45 demonstrating strong golden brown cytoplasmic staining in all melanoma cells. (d) A serial section of (c) stained with anti-S-100 exhibiting moderate to strong golden brown cytoplasmic and nuclear staining in all melanoma cells. (e) A serial section of (d) stained with anti-Melan-A showing strong golden brown cytoplasmic staining in nearly all melanoma cells. It was not difficult to distinguish the golden brown immunostained melanoma cells (c–e) from dark brown melanin-pigmented tumor cells and melanophages (a–e, original magnification: 25×).

Anti-HMB-45, anti-S-100, and anti-Melan-A have been used for diagnosis of site-mixed primary or metastatic cutaneous and MMs in several studies (5, 8–10, 12, 13, 20). For site-mixed primary melanomas, the mean positive rate was approximately 86% (range: 70–100) for anti-HMB-45 (8–10, 12), 95% (range: 86–100) for anti-S-100 (5, 8, 9, 12, 13), and 84% (range: 75–97) for anti-Melan-A (9, 10, 20). For site-mixed metastatic melanomas, the mean positive rate was approximately 72% (range: 43–100) for anti-HMB-45 (9, 10, 12), 94% (range: 83–100) for anti-S-100 (9, 12, 13), and 76% (range: 71–81) for anti-Melan-A (9, 10). These findings indicate that anti-S-100 has a higher sensitivity than anti-HMB-45 or anti-Melan-A and is probably a better marker than the latter two antibodies for immunohistochemical diagnosis of site-mixed primary and metastatic melanomas. In addition, the latter two antibodies have an approximately equal sensitivity for diagnosis of site-mixed primary and metastatic melanomas.

Prasad et al. (6) studied the expression of melanocytic differentiation markers in 27 primary sinonasal MMs by IHC; 26 (96%) were positive for HMB-45, 25 (93%) for S-100, and 27 (100%) for Melan-A. Our study demonstrated that among 11 nasal MMs 10 (91%) were positive for HMB-45, 10 (91%) for S-100, and eight (73%) for Melan-A. The results of these two studies suggest that all the three melanocytic differentiation markers can be used for immunohistochemical diagnosis of primary sinonasal MMs with a sensitivity of approximately 92%.

Anti-S-100 and anti-HMB-45 are the two antibodies most frequently used for the immunohistochemical

Table 3 Staining intensities (SIs) and labeling indices (LIs) of HMB-45, S-100, and Melan-A in 17 primary oral and nasal mucosal melanomas

	HMB-45		*S-100*		*Melan-A*	
Cases	*SI*[a]	*LI (%)*[b]	*SI*	*LI (%)*	*SI*	*LI (%)*
1	3	97	2	96	3	95
2	3	87, patchy	1	95	0	0
3	3	72	0	0	0	0
4	3	90	1	52	2, variable	60, patchy
5	3	90	2	89	3	82
6	3, variable	100	2	100	1	90
7	3	80, patchy	2	100	0	0
8	3, variable	97	0	0	0	0
9	3	97	1	96	3	95
10	0	0	1	79	0	0
11	3	100	2	100	2, variable	100
12	3	100	3	100	3	100
13	3	98	2	98	3	98
14	3	98	2	100	2	99
15	3	90	3	70	2	70
16	3	24, patchy	2	100	3	22, focal
17	3, variable	86	2, variable	70	3, variable	86
Mean		83 ± 28		79 ± 33		59 ± 43
Median	3		2		2	

[a]Staining intensity: 0, no staining; 1, weak; 2, moderate; 3, strong. The SI was either consistent or variable. Those cases without comments on SI represented a consistent SI.
[b]The distribution of positive tumor cells was even, patchy or focal. Those cases without comments on the distribution of positive tumor cells represented an even distribution of positive tumor cells.
Comparison of the SI between two markers in 17 mucosal melanomas using Wilcoxon signed rank test: HMB-45 vs. S-100, $P = 0.0011$; HMB-45 vs. Melan-A, $P = 0.0034$; S-100 vs. Melan-A, $P = 0.6221$.
Comparison of the LI between two markers in 17 mucosal melanomas using one-sided paired *t*-test: HMB-45 vs. S-100, $P = 0.366$; HMB-45 vs. Melan-A, $P = 0.0065$; S-100 vs. Melan-A, $P = 0.0237$.

diagnosis of primary oral MMs. The positive rate was 100% for anti-S-100 (4, 6, 11, 12) and approximately 79% (range: 71–100) for anti-HMB-45 (4, 6, 11–13). In only one study anti-Melan-A used for the detection of 21 primary oral MMs; among this 18 (86%) were positive for Melan-A (6). These results indicate that with respect to the sensitivity S-100 is a more sensitive marker than HMB-45 or Melan-A for immunohistochemical diagnosis of primary oral MMs. However, this study found a 100% positive rate for HMB-45 compared with an 83% positive rate for S-100 and a 67% positive rate for Melan-A in six primary oral MMs. In addition, the SI of HMB-45 was significantly greater than that of S-100 or of Melan-A. Taking these findings together, we suggest that both HMB-45 and S-100 may be good markers for immunohistochemical diagnosis of primary oral MMs.

Prasad et al. (6) assessed the expression of HMB-45, S-100, and Melan-A in 48 primary sinonasal and oral MMs by IHC, and found a positive rate of 85%, 96%, and 94%, respectively. This study showed that HMB-45, S-100, or Melan-A was expressed in 94%, 88%, or 71% of 17 primary nasal and oral MMs. The mean LI of HMB-45 (83 ± 28%) or of S-100 (79 ± 33%) was significantly higher than that of Melan-A (59 ± 43) in 17 primary nasal and oral MMs. Furthermore, the SI of HMB-45 was significantly higher than that of S-100 or of Melan-A. Previous studies also demonstrated a moderate to strong SI for HMB-45 and S-100 in primary sinonasal and oral MMs (4, 6, 11, 12). Therefore, taking the positive rate, LI, and SI into consideration, we suggest that both HMB-45 and S-100 may be better markers than Melan-A for immunohistochemical diagnosis of primary oral and nasal MMs. Moreover, HMB-45 may be a more sensitive marker than S-100 because HMB-45 shows a significantly higher SI than S-100 in this study.

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