
Original Article

The expression of human high molecular weight melanoma-associated antigen in acral lentiginous melanoma

Hazuki Nishi^{1,*}, Yuji Inoue¹, Toshiro Kageshita¹, Minoru Takata², Hironobu Ihn¹

¹Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan;

²Department of Dermatology, Shinshu University School of Medicine, Matsumoto, Japan.

Summary

The high molecular weight melanoma-associated antigen (HMW-MAA) is a membrane-bound chondroitin sulphate proteoglycan that is highly expressed on the surface of melanoma cells. It represents an attractive target for immunotherapy of malignant melanoma. Previously, it was reported that HMW-MAA was detected in about 20-30% of primary acral lentiginous melanoma (ALM) lesions by immunohistochemical staining (IHC) of frozen sections with monoclonal antibodies (mAbs). In the present study, we examined the expression of HMW-MAA in 95 paraffin-embedded, primary ALM lesions and 13 primary superficial spreading melanoma (SSM) lesions. A total of 51 primary ALM lesions (53.6%) were positive for HMW-MAA. Almost all of these positive cases showed a weak staining intensity. On the other hand, all 13 primary SSM lesions were strongly positive for HMW-MAA expression. Our data showed that the staining intensity of HMW-MAA ALM lesions was weaker than that of SSM. Furthermore, the percentage of HMW-MAA positive staining in ALM lesions was higher than previously reported.

Keywords: High molecular weight melanoma-associated antigen (HMW-MAA), acral lentiginous melanoma (ALM)

1. Introduction

The high molecular weight melanoma-associated antigen (HMW-MAA) is a membrane-bound chondroitin sulphate proteoglycan that is highly expressed on the surface of melanoma cells. Recent findings have shown that HMW-MAA is involved in the activation of several signaling pathways modulating melanoma cell adhesion, spreading, migration and invasion (1). The HMW-MAA mediates the interaction of melanoma cells with the extracellular matrix, appears to play a role in the metastatic potential of melanoma cells, and has been shown to promote melanoma invasion through cytoskeletal rearrangements (1).

The HMW-MAA has been used as a target for immunotherapy of melanoma. Induction of humoral

anti-HMW-MAA immunity following immunization with anti-idiotypic mAb MK2-23, which mimics the HMW-MAA, is associated with statistically significant survival prolongation in patients with stage IV melanoma (2). It was also reported that elicited HMW-MAA-specific Abs were able to mediate cell-dependent cytotoxicity and inhibited several HMW-MAA-dependent cellular functions including: spreading, migration, and invasion upon binding to HMW-MAA⁺ melanoma cells (3).

The HMW-MAA is expressed in over 80% of human melanoma lesions and in the majority of human melanoma cell lines (1). The level of HMW-MAA expression is similar among lentigo maligna, nodular and superficial spreading melanoma lesions, but is lower in ALM lesions (20-30%) as detected by immunohistochemical staining (IHC) of frozen sections (1,4).

In Japan, ALM is the most common type of malignant melanoma, accounting for nearly 50% of melanoma patients (5). In this study, we investigated HMW-MAA expression in paraffin embedded, primary ALM and SSM lesions.

*Address correspondence to:

Dr. Hazuki Nishi, Department of Dermatology and Plastic Surgery, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan.
e-mail: 060r5136@st.kumamoto-u.ac.jp

2. Materials and Methods

2.1. Melanoma specimens

Formalin-fixed, paraffin-embedded archival tissue (PEAT) specimens were obtained from a total of 95 primary ALM (42 males and 53 females, mean age 70.9 years, age range 37-97 years) and 13 primary SSM (6 males and 7 females, mean age 62.1 years, range 30-83 years). With respect to ALM cases, 30 patients had stage I melanoma, 45 had stage II, 15 had stage III, and 5 had stage IV. With respect to SSM cases, 4 patients had stage I melanoma, 5 had stage II, and 4 had stage III.

A total of 60 and 13 patients with ALM and SSM, respectively, were from the Department of Dermatology & Plastic Surgery, Kumamoto University Hospital, while 35 patients with ALM were from the Department of Dermatology, Shinshu University Hospital; these patients underwent surgery between 1989 and 2006.

2.2. Monoclonal antibody

The mAb D2.8.5-C4B8 against distinct determinants of HMW-MAA was developed and characterized as described previously (1,6). The mAb is of mouse origin.

2.3. Immunohistochemistry

IHC was performed on 4- μ m sections that had been incubated overnight at 50°C and deparaffinized in xylene. We used the CSA II System (Biotin-Free Catalyzed Amplification System, Dako, Carpinteria, CA, USA) with modifications as previously described (7). Tissue sections were incubated overnight at 4°C with HMW-MAA-specific mAb at 5 μ g/mL. After development with the substrate (VIP Substrate Kit, Vector Labs, Burlingame, CA, USA), tissue sections were counterstained with Mayer's hematoxylin 1 \times (Muto Pure Chemicals, Tokyo, Japan) for 1 min at room temperature, dehydrated and mounted.

Negative controls were performed by replacing the primary antibody with TBS containing Tween 20 buffer (Tris-HCl/NaCl/0.1% Tween 20).

Stained sections were scored according to the percentage of stained melanoma cells: 100-75%, 74-50%, 49-25%, 24-1%, or negative. The staining intensity was scored as strong, intermediate, weak or negative. The staining intensity and percentage of stained melanoma cells in the each section were estimated independently by three investigators (H.N., Y.I., and T.K.).

2.4. Statistical analysis

Chi-square tests were used to evaluate the percentage of

positive HMW-MAA in ALM and SSM lesions. $P < 0.05$ was considered statistically significant.

3. Results

Representative staining patterns are shown in Figure 1. Positive immunoreactivity for HMW-MAA was indicated by purple staining. The staining was observed on the membrane of melanoma cells; there was no

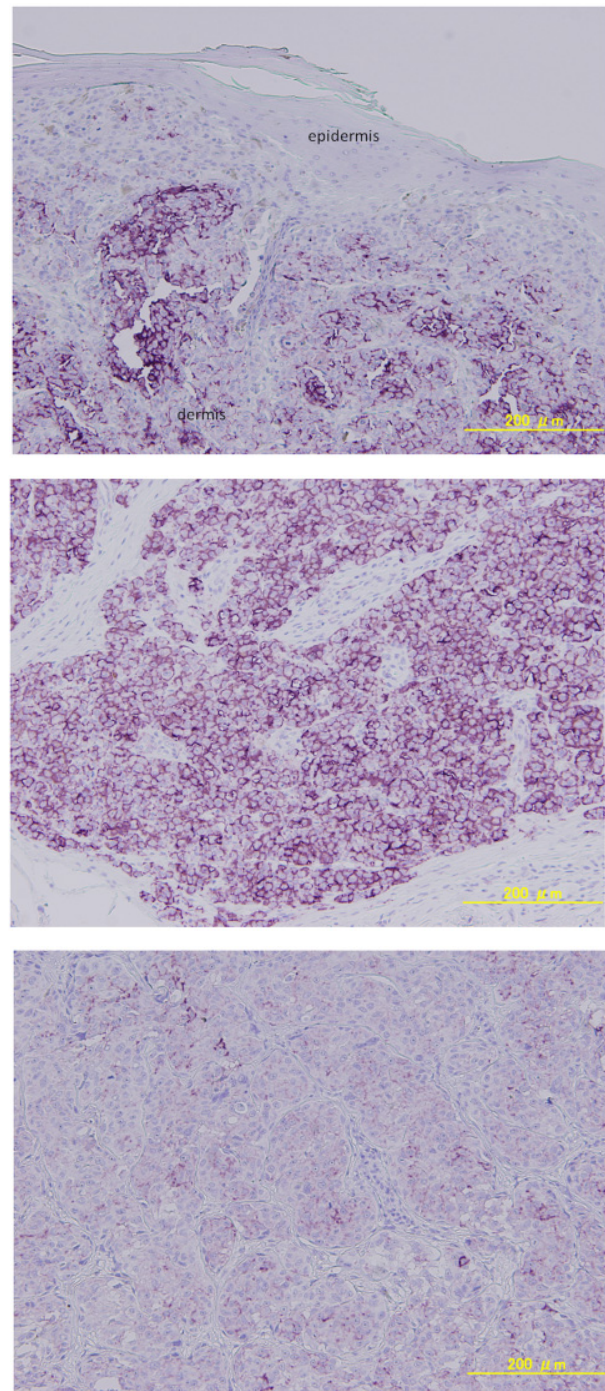


Figure 1. Representative staining patterns for HMW-MAA in primary ALM lesions. (A and B) Immunohistochemical staining for HMW-MAA of primary ALM lesions showing strong staining intensity. (C) The weak staining intensity of HMW-MAA-positive cells in primary ALM lesions.

Table 1. Positive reactivity with anti-HMW-MAA mAb and the percentage of stained melanoma cells in ALM lesions

	Negative	< 25%	25-49%	50-74%	≥ 75%	Total of positive cases (%)
Stage I (n = 30)	11 (36.7%)	2 (6.7%)	8 (26.7%)	3 (10%)	6 (20%)	19/30 (63.3%)
Stage II (n = 45)	25 (55.6%)	7 (15.6%)	5 (11.1%)	5 (11.1%)	3 (6.67%)	20/45 (44.4%)
Stage III (n = 15)	5 (33.3%)	6 (40%)	0	2 (16%)	2 (16%)	10/15 (66.7%)
Stage IV (n = 5)	3 (60%)	0	0	1 (20%)	1 (20%)	2/5 (40%)
Total						51/95 (53.6%)

staining of the surrounding lymphocytes. The staining was heterogeneous in almost all lesions.

The results of IHC of the 95 primary ALM lesions using the anti-HMW-MAA mAb are summarized in Table 1. A total of 51 primary ALM lesions (53.6%) were positive for HMW-MAA; there were 19 (63.3%) positive stage I ALM, 20 (44.4%) positive stage II ALM, 10 (66.7%) positive stage III ALM, and 2 (40%) positive stage IV ALM. The percentage of stained melanoma cells in ALM lesions is also shown in Table 1. A total of 23 (45%) out of the 51 showed positive staining in more than half of the melanoma cells. There was no significant difference among the stages. The intensity of staining of ALM lesions is shown in Table 2. Almost all of the positive cases had a weak staining intensity.

On the other hand, all of the 13 primary SSM lesions reacted with the anti-HMW-MAA mAb. The intensity of staining of SSM lesions is shown in Table 2. The staining intensity of SSM lesions was higher than that in ALM lesions. The percentage of stained melanoma cells in SSM is shown in Table 3. The percentage of stained melanoma cells was < 25% in 2 out of 4 lesions of stage I SSM, and the prevalence of positive HMW-MAA in SSM lesions was higher than that in ALM lesions ($P < 0.01$).

4. Discussion

We showed that 53.6% of PEAT from ALM expressed HMW-MAA, and that almost half of the ALM lesions

showed a weak staining intensity. It has been reported that HMW-MAA was detected in approximately 30% of frozen tissue of primary ALM lesions by IHC using mAb (8). HMW-MAA was detected in a greater percentage of ALM lesions than reported previously; this may be due to the difference in the immunohistochemical staining method (7). It was reported that the staining intensity achieved using HMW-MAA-specific mAbs was stronger than that from MART-1 mAbs for both macro- and micrometastases (7).

In this study, the percentage of positive HMW-MAA in SSM lesions was higher than that in ALM lesions. Furthermore, the immunostaining intensity of HMW-MAA in SSM lesions was higher than that in ALM lesions. This is similar to a previous report that describes a greater percentage of HMW-MAA-positive SSM lesions compared to ALM (1), and that its expression was demonstrated in 80% of primary melanomas except ALM (1).

Our data showed that the staining intensity of HMW-MAA in ALM was weaker than that of SSM, and the percentage of HMW-MAA positive ALM was higher than that previously reported.

Acknowledgement

The authors wish to thank Dr. S. Ferrone for the gift of the anti-HMW-MAA mAb.

References

1. Campoli MR, Chang CC, Kageshita T, Wang X, McCarthy JB, Ferrone S. Human high molecular weight-melanoma associated antigen (HMW-MAA): a melanoma cell surface chondroitin sulfate proteoglycan (MSCP) with biological and clinical significance. *Crit Rev Immunol.* 2004; 24:267-296.
2. Mittelman A, Chen ZJ, Yang H, Wong GY, Ferrone S. Human high molecular weight melanoma-associated

Table 2. Staining intensity of ALM and SSM lesions

Staining intensity	Cases of ALM (%)	Cases of SSM (%)
Strong	4 (4.2%)	4 (30.8%)
Intermediate	9 (9.5%)	6 (46.2%)
Weak	38 (40%)	3 (23.1%)
Negative	44 (46.3%)	0 (0%)

Table 3. The percentage of stained melanoma cells in SSM lesions

	Negative	< 25%	25-49%	50-74%	≥ 75%	Total of positive cases (%)
Stage I (n = 4)	0	2	0	0	2	4/4 (100%)
Stage II (n = 6)	0	0	1	0	5	6/6 (100%)
Stage III (n = 3)	0	0	1	1	1	3/3 (100%)
Total	0	2/13 (15.4%)	2/13 (15.4%)	1/13 (7.7%)	8/13 (61.5%)	13/13 (100%)

- antigen (HMW-MAA) mimicry by mouse anti-idiotypic monoclonal antibody MK2-23: induction of humoral anti-HMW-MAA immunity and prolongation of survival in patients with Stage IV melanoma. *Proc Natl Acad Sci U S A.* 1992; 89:466-470.
3. Luo W, Ko E, Hsu JC, Wang X, Ferrone S. Targeting melanoma cells with human high molecular weight-melanoma associated antigen-specific antibodies elicited by a peptide mimotope: functional effects. *J Immunol.* 2006; 176:6046-6054.
 4. Kageshita T, Nakamura T, Yamada M, Kuriya N, Arai T, Ferrone S. Differential expression of melanoma associated antigens in acral lentiginous melanoma and in nodular melanoma lesions. *Cancer Res.* 1991; 51:1726-1732.
 5. Ishihara K, Saida T, Otsuka F, Yamazaki N. Statistical profiles of malignant melanoma and other skin cancers in Japan: 2007 update. *Int J Clin Oncol.* 2008; 13:33-41.
 6. Giacomini P, Natali P, Ferrone S. Analysis of the interaction between a human high molecular weight melanoma-associated antigen and the monoclonal antibodies to three distinct antigenic determinants. *J Immunol.* 1985; 135:696-702.
 7. Goto Y, Ferrone S, Arigami T, Kitago M, Tanemura A, Sunami E, Nguyen SL, Turner RR, Morton DL, Hoon DS. Human high molecular weight-melanoma-associated antigen: utility for detection of metastatic melanoma in sentinel lymph nodes. *Clin Cancer Res.* 2008; 14:3401-3407.
 8. Kageshita T, Kuriya N, Ono T, Horikoshi T, Takahashi M, Wong GY, Ferrone S. Association of high molecular weight melanoma-associated antigen expression in primary acral lentiginous melanoma lesions with poor prognosis. *Cancer Res.* 1993; 53:2830-2833.

(Received December 2, 2009; Revised March 3, 2010; Accepted March 9, 2010)