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Melan-A expression in non-melanocytic carcinoma: A potential diagnostic pitfall

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Summary. Background. Melan-A/MART-1 is a melanocytic differentiation marker recognized as an antigen on melanoma cells. It is a useful diagnostic marker for pathologists in the diagnosis of melanocytic tumors. However, we recently found that Melan-A can be expressed in some non-melanocytic carcinomas that are rarely reported in the literature.

Methods. We analyzed the expression of Melan-A in 87 non-melanocytic carcinoma tissue samples by immunohistochemistry. Marker positivity was defined as ≥10% positive tumor cells.

Results. In 87 non-melanocytic carcinoma tissue samples, Melan-A was positive in six (6.89%) cases, of which four (66.7%) were male and two (33.3%) were female, with a mean age of 60 years (range 21-82 years). Five (83.3%) of the Melan-A-positive cases had distant metastases. Compared with Melan-A negative cases, Melan-A positive non-melanocytic carcinomas were significantly associated with poor prognosis (P=0.0023).

Conclusions. Melan-A expression is relatively rare in non-melanocytic carcinoma cases. This report highlights a potential diagnostic pitfall in the diagnosis of melanoma, urges pathologists to exercise caution in cases of Melan-A positivity, and illustrates the need for an immunohistochemical marker panel to avoid misdiagnosis.

Key words: Melan-A, Non-melanocytic, Carcinoma, Diagnostic pitfall

Introduction

The Melan-A gene, cloned from the human melanoma cell line SK-MEL-29, encodes a melanoma antigen that is recognized by autologous cytotoxic T cells (Coulie et al., 1994). By analyzing the antigenic targets of tumor-infiltrating lymphocytes from a melanoma sample, the same gene, designated *MART-1*, was independently cloned (Kawakami et al., 1994). The Melan-A/MART-1 protein is a melanocytic differentiation antigen, usually considered to be specific to melanocytic cells (Chen et al., 1996). It is a useful antibody against melanocytic neoplasms and is of interest to clinicians as a potential immunotherapeutic target (Blessing et al., 1998).

In addition to positive expression in melanoma, the Melan-A antigen can also be expressed in adrenal tissue, sex cord-stromal tumors, and MiT family translocation renal cell carcinoma (Stewart et al., 2000; Argani, 2008). However, Melan-A expression in non-melanocytic carcinomas is rarely reported in the literature.

Here, we systematically analyzed the expression of Melan-A in 87 non-melanocytic carcinoma cases, including 27 lung cancer, 13 liver cancer, 12 kidney cancer, 17 gastrointestinal cancer, and 18 nasal cancer cases.

Materials and methods

Patient samples

A total of 87 non-melanocytic samples were collected from the archives of the Second Affiliated Hospital of Nanchang University in Nanchang, China. The samples were collected from January 2017 to December 2022. All specimens were fixed in 10% formaldehyde and embedded in paraffin. Sections were cut at a 4 μm thickness and stained with hematoxylin and eosin.

Immunohistochemical staining

The 4-μm thick sections were immunostained for Melan-A (A-103; ZSGB-BIO), HMB45 (HMB45; ZSGB-BIO), S100 (15E2E2+4C4.9; ZSGB-BIO), Inhibin (AMY82; ZSGB-BIO), cytokeratin (AE1/AE3; ZSGB-BIO), EMA (GP14; ZSGB-BIO), CEA (12-140-

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10; ZSGB-BIO), CD10 (UMAB235; ZSGB-BIO), P63 (UMAB4; ZSGB-BIO), PAX8 (OTI6H8; ZSGB-BIO), and P40 (BC28; ZSGB-BIO) antibodies using the DAKO Omnis automated staining platform. Antibodies were diluted 1:100. Animal serum and buffer were used as negative controls instead of the primary antibody. The antibody was optimized using the EnVision FLEX DAB detection kit and standard quality control procedures were performed. Staining was scored by an experienced pathologist based on the positivity of >10% positive tumor cells. This cut-off of 10% positive tumor cells was chosen to avoid classifying minimal staining (which would likely be background staining) as positive.

Statistical analysis

Differences between categorical variables were tested using the chi-squared test or Fisher's exact test, whereas continuous variables were tested using the nonparametric Kruskal-Wallis test for multiple group comparisons. Correlations between two continuous variables were compared using Spearman's rank correlation. Differences were considered significant when $P<0.05$, $P<0.01$, $P<0.001$, and $P<0.0001$. All analyses and graphs were performed using GraphPad Prism 6 software (GraphPad, La Jolla, CA, USA).

Results

Clinicopathological findings

The 87 non-melanocytic carcinoma cases were analyzed and six showed positive Melan-A expression (Table 1). Of these six non-melanocytic carcinoma patients, four (66.7%) were male and two (33.3%) were female, with a mean age of 60 years (range 21-82 years). As regards six 6 Melan-A positive non-melanocytic carcinoma cases, two (33.3%) were located in the lung, one (16.6%) in the liver, one (16.6%) in the kidney, one (16.6%) in the stomach, and one (16.6%) in the nasal cavity.

Pathological findings

The six Melan-A-positive non-melanocytic carcinoma cases appeared gray-white and gray-red on visual inspection. Under light microscopy, these lesion sections showed epithelioid tumor cells arranged in a prominent nesting, cord, or glandular tubule pattern (Fig. 1A,C). In addition, two of the Melan-A positive non-melanocytic carcinoma specimens showed a prominent nucleus and slightly eosinophilic cytoplasm.

Immunohistochemical results

Immunohistochemical staining for Melan-A was performed on 27 lung, 13 liver, 12 kidney, 17 gastrointestinal, and 18 nasal cancer specimens. Approximately 6.89% (6/87) of non-melanocytic carcinomas showed positive membrane Melan-A staining. In particular, Melan-A immunoreactivity appeared to be weakly positive in two (33.3%) cases (Fig. 1B), partly positive in one (16.7%), focally positive in two (33.3%), and diffusely strongly positive in one (16.7%) (Fig. 1D). HMB45 and S100 were negative in all cases (Fig. 2A,B). Immunohistochemical staining for cytokeratin and inhibin was also performed on all non-melanocytic carcinoma samples, of which 87 (100%) were positive for cytokeratin expression (Fig. 2C) and negative for inhibin (Fig. 2E). In addition, two (33.3%) located in the lung were positive for CK7 and EMA (Fig. 2D), one (16.6%) in the liver was strongly positive for cytokeratin, one (16.6%) in the kidney was positive for PAX-8, CD10, and EMA, one (16.6%) in the stomach was positive for CEA, and one (16.6%) in the nasal cavity was positive for P40 (Fig. 2F) and P63.

Treatment and outcomes

Two patients with non-melanocytic carcinoma underwent surgical tumor resection, one patient underwent transarterial chemoembolization (TACE), and the other three patients underwent histologic biopsy. A

Table 1. Summary of clinicopathologic characteristics of six Melan-A-positive non-melanocytic carcinoma cases.

Case #	Age (y)/sex	Site	Treatment	IHC					Diagnosis	Follow-up
				Melan-A	HMB45	S100	CK	inhibin		
1	77/F	Lung	Biopsy	+	-	-	+	-	NSCLC	2Ms, dead
2	82/M	Lung	WE	+(p)	-	-	+	-	NSCLC	3Ms, dead
3	47/M	Nasal cavity	Biopsy+CT	+(w)	-	-	+	-	NK-NPC	7Ms, alive
4	70/M	Stomach	Biopsy	+(w)	-	-	+	-	AC	6Ms, dead
5	21/F	Kidney	WE	+(f)	-	-	+	-	RCC	12Ms, alive
6	64/M	Liver	TACE	+(f)	-	-	+	-	HCC	8Ms, dead

+ indicates positive; -, negative; F, female; M, male; CT, chemotherapy; NSCLC, non-small cell carcinoma; NK-NPC, non-keratinizing nasopharyngeal carcinoma; AC, adenocarcinoma; RCC, renal cell carcinoma; HCC, hepatocellular carcinoma; WE, wide excision; TACE, transarterial chemoembolization; Ms, months; p, partial; w, weak; f, focal.

total of six Melan-A-positive patients and 51 patients without Melan-A expression were successfully followed up until August 3, 2023. Four patients died and the other two patients were alive at the time of writing (Fig. 3).

Discussion

Melan-A/MART-1 protein is a melanocytic differentiation antigen that is usually considered to be specific for melanocytic cells, but Melan-A expression in non-melanocytic carcinoma is still rarely reported in the literature (Bachmeier et al., 2008; Kriegsmann et al., 2018; Weston and Murphy, 2021). To investigate Melan-A expression in non-melanocytic carcinoma, we analyzed 87 non-melanocytic carcinoma cases.

Of our 87 non-melanocytic carcinoma tissue samples, Melan-A was positive in six (6.89%) cases. The reason why non-melanocytic carcinomas express Melan-A is still under investigation. One possible reason we hypothesize is that it may be associated with melanocytic differentiation in these poorly differentiated carcinomas. Another possible reason we think is that it may just be an abnormal expression without any molecular changes, as we know that the abnormal expression of some antibodies often shows a weak positive expression pattern by IHC. In fact, in our study, Melan-A was weakly positive in five cases, and diffusely strong positive in only one, suggesting the possibility of the abnormal expression of Melan-A in these cases. This reminds us that if Melan-A was weakly positively expressed in a poorly differentiated carcinoma, we should be cautious of a possible abnormal expression.

The differential diagnosis between poorly differentiated non-melanocytic carcinoma and malignant melanoma is challenging, especially in small biopsy specimens. Given the overlap in

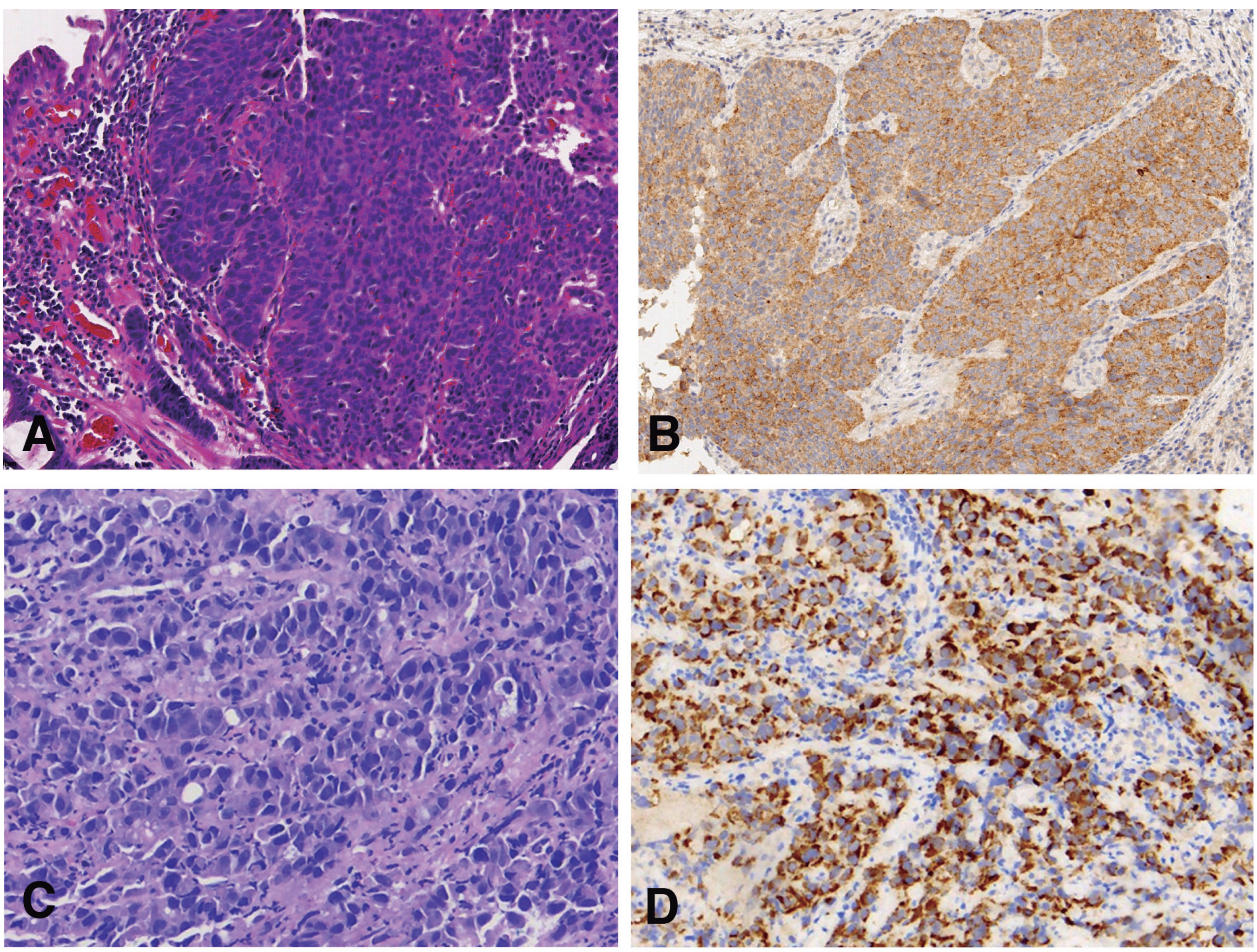

Fig. 1. Morphologic and immunohistochemical features of Melan-A-positive non-melanocytic carcinoma. Histologically, the epithelioid tumor cells were arranged in a prominent nested pattern **(A)** and showed a weak positive expression pattern for Melan-A **(B)**. The tumor cells showed a poorly differentiated glandular pattern **(C)** and strong positive expression for Melan-A **(D)**. x 200.

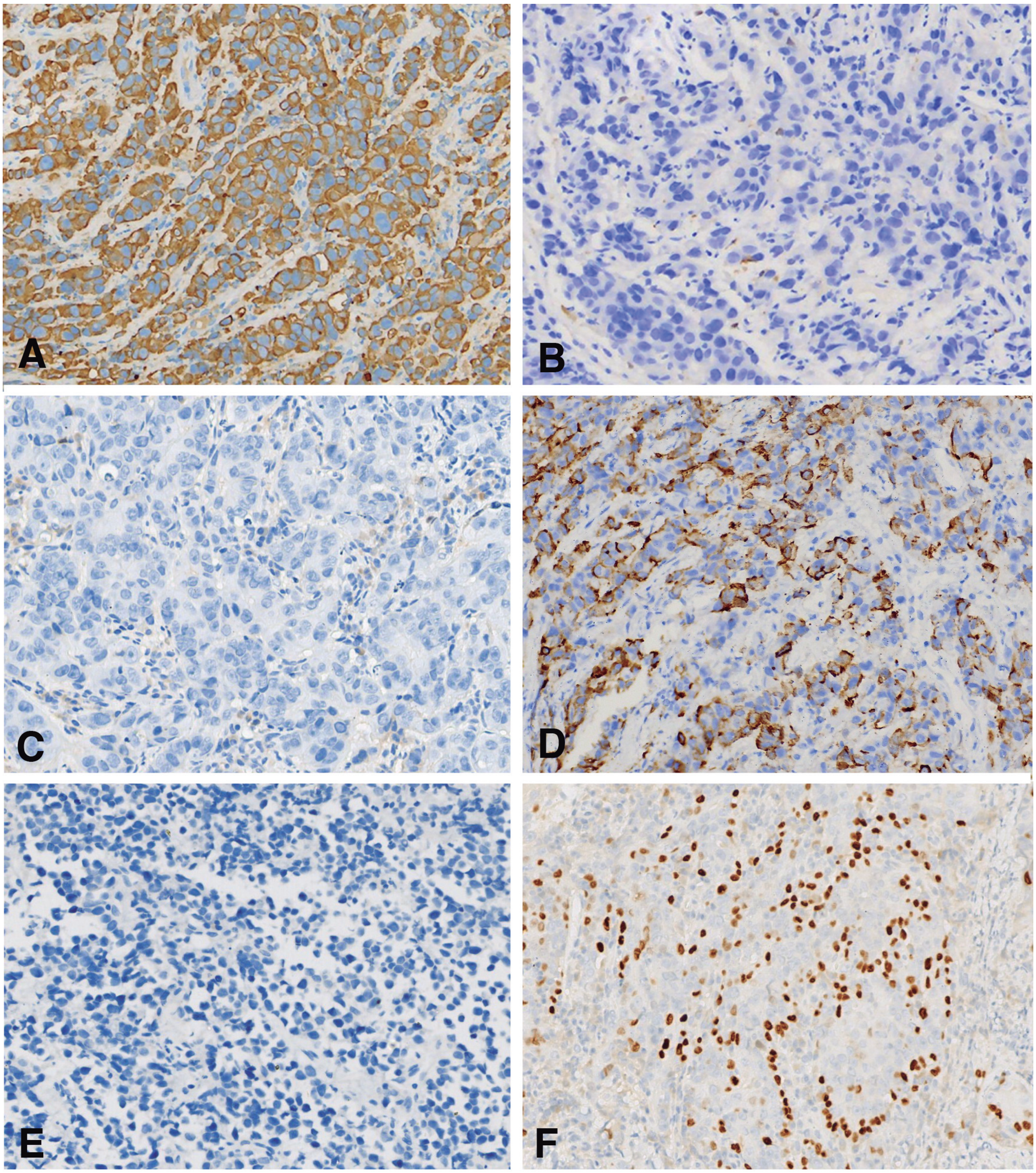

Fig. 2. Immunohistochemical features of Melan-A-positive non-melanocytic carcinoma. Tumor cells showed a diffuse strong positive expression for pancytokeratin **(A)**, and negative expression for S-100 **(B)** and HMB45 **(C)** in all 6 cases. EMA was strongly positively expressed in two cases located in the lung **(D)**. Inhibin was negative in all 6 cases **(E)**, and P40 was positively expressed in the nasal case **(F)**. x 200.

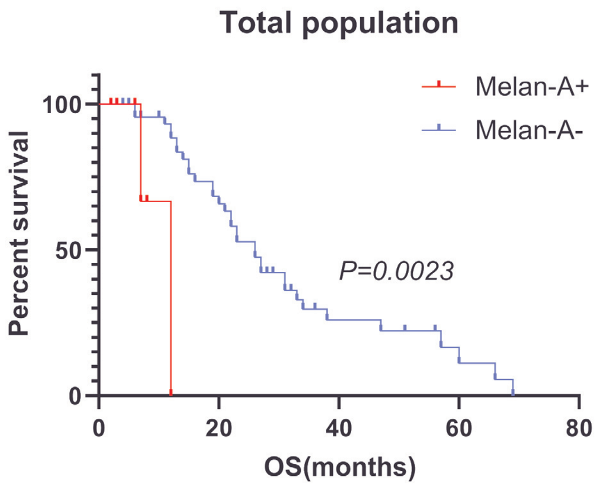

Fig. 3. Survival analyses according to Melan-A expression. Melan-A expression was significantly associated with poor prognosis (P=0.0023).

immunohistochemical expression between poorly differentiated non-melanocytic carcinoma and malignant melanoma carcinoma, the diagnosis should be made with great caution. According to a recent report in the literature, metastatic melanoma may also show a complete loss of immunohistochemical melanocytic markers such as S100, HMB45, Melan-A, and SOX10, and may even express cytokeratin abnormally (Agaimy et al., 2016, 2021), emphasizing the need for further immunohistochemical or molecular investigation to avoid misdiagnosis. In our study, in addition to the strong positive expression of pan-cytokeratin in all six Melan-A-positive cases, we also performed an immunohistochemical marker panel to exclude the possibility of malignant melanoma, and molecular testing was performed in the case of strong positive Melan-A expression. In this patient, we found that there were no mutations in the *BRAF*, *NRAS*, or *MAP2K1* genes, which are closely associated with melanoma (Akabane and Sullivan, 2016; Agaimy et al., 2016).

The biological role of Melan-A expression in non-melanocytic carcinoma tissue samples is rarely reported. In our study, four patients had died at the time of writing, the other two patients were alive and being followed up. Five (83.3%) of the six Melan-A-positive cases had distant metastases, and we further found that Melan-A-positive expression patients were significantly associated with worse survival, suggesting that Melan-A could be a potential marker of poor prognosis in non-melanocytic carcinoma.

In conclusion, we have reported relatively rare cases of non-melanocytic carcinoma with Melan-A expression, which highlights a potential diagnostic pitfall in our daily work, especially in cases with weak Melan-A positivity, and may suggest the poor prognosis of these patients. More cases are needed to further investigate the clinicopathologic features and prognostic value of Melan-A-positive non-melanocytic carcinoma in the future.

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