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Loss of S100 antigenicity in metastatic melanoma

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Summary

Melanoma is a highly malignant disease that may initially present as a poorly differentiated metastatic tumor. Therefore, the S100 immunostain, immunoreactive in 96% to 99% of melanoma, is used to evaluate poorly differentiated malignant tumors. To develop criteria for correctly diagnosing S100-negative melanomas, we studied the immunohistochemical profile of 1553 patients enrolled in ongoing National Cancer Institute clinical trials for melanoma. Seventeen patients (1%) had metastatic melanoma specimens that were negative for S100. Of the 17 S100-negative lesions, 10 (59%) were immunoreactive for both GP100 and MART-1. Of the 17 S100-negative cases, 13 had a documented primary melanoma. Twenty-four percent of the S100-negative cases had an ocular primary, whereas only 6% of all melanomas had an ocular origin. In 11 of the 17 cases with previous surgical specimens, a prior documented S100-immunoreactive specimen was identified in 9 cases (82%). The time interval for loss of S100 immunoreactivity ranged from 3 weeks to 3 years (average, 13.5 months). There was no association between S100-negative status and histological appearance or site of metastasis. We conclude that all S100-negative melanomas could be correctly identified by negative workup for carcinoma, lymphoma, and sarcoma plus (1) GP100/MART-1 immunoreactivity and/or (2) prior documentation of melanoma.

Keywords

S100; Melanoma; Antigenicity; Diagnosis

1. Introduction

Metastatic melanoma is one of the great mimickers in pathology. The diagnosis of metastatic melanoma is complicated by its ability to metastasize to any site and manifest variable histological patterns. In the absence of a known primary melanoma, accurate diagnosis requires a battery of immunostains. Although not specific, the S100 protein is expressed in all melanocytic cells and in 96% to 100% of malignant melanomas [1–4]. Adjunct, but less sensitive, markers of melanoma include GP100 (86%), MART-1 (86%), tyrosinase (90%), and KBA-62 (87%) [3,5,6].

In up to 4% of metastatic melanomas, the S100 immunostain is negative [7–9]. One study that evaluated the immunoreactivity for S100 in 124 cases of metastatic melanoma showed a negative staining pattern in 2.4% of cases [6], whereas another study indicated a possible link between tumor metastasis and progression or atypical morphology with loss of immunoreactivity for S100 [8]. To develop criteria for accurately diagnosing S100-negative melanoma, we studied the evolution of S100 immunoreactivity in 1553 patients enrolled in

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ongoing clinical trials for metastatic melanoma in which multiple specimens were immunophenotyped during the course of their disease. We report on the primary site, histology, and immunophenotype of 17 cases that were negative for S100.

2. Materials and methods

2.1. Selection of cases and immunohistochemistry

Melanoma cases in which S100 immunostaining was performed were identified with a keyword search performed on the National Cancer Institute (NCI) computerized database (1999–2004). Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded tissue sections using heat-induced antigen retrieval. In brief, after deparaffinization, the slides were placed in a microwavable pressure cooker with 1.5 L of a 10 mmol/L concentration of citrate buffer (pH 6.0) containing 0.1% Tween 20 (Sigma-Aldrich, Milwaukee, Wis) and microwaved (model R4A80; Sharp Electronics, Rahway, NJ) for 20 minutes at 700 W.

Immunohistochemical analysis was performed using an automated immunostainer (Ventana Medical Systems, Tucson, Ariz) according to the manufacturer's protocol with minor modifications. Antibodies were incubated at the following titrations: S100, 1:4000 (Biogenex, San Ramon, Calif); GP100 (HMB45), 1:2 (Enzo, New York, NY); MART-1, 1:10 (Rosenberg Laboratory, NCI, Bethesda, Md); KBA-62, neat (Immunotech, Fullerton, Calif); tyrosinase, 1:10 (Novocastra, Newcastle, UK); and NSE, 1:400 (Dako, Santa Barbara, Calif). Primary antibody incubation was performed for 2 hours with antibodies to MART-1, KBA-62, and tyrosinase, and for 32 minutes with the remaining antibodies. Positive and negative controls were included. A positive immunoreaction was defined as at least weak and/or focal positivity. All cases with negative or equivocal staining were reviewed by 2 pathologists, and the immunohistochemical studies were performed or repeated in our laboratory with appropriate positive controls for all negative cases.

3. Results

A search of the NCI computerized database revealed that 2464 cases of melanoma representing 1553 patients were evaluated by immunohistochemical staining for S100 (Table 1). Of these, 19 metastatic lesions representing 17 unique patients were negative for S100. Of these 17 patients, 9 (53%) had a documented primary cutaneous melanoma. Four had a documented primary ocular melanoma (24%), whereas ocular melanoma represented only 6% of all patients reviewed. In the remaining 4 patients (24%) with no documented primary tumor, the diagnosis of melanoma was based on adjunct tests (see below).

For most patients on the NCI trials, serial biopsies of metastatic lesions were immunophenotyped for study purposes (Table 2). Of the 17 patients, 11 had prior material evaluated for S100 staining, of which 9 patients (82%) had at least 1 prior S100-positive melanoma. The pattern of prior S100 staining ranged from focal to diffuse, with strong positivity. The time interval for the loss of S100 immunoreactivity ranged from 3 weeks to 3 years (average, 13.5 months).

In all cases, the diagnosis of melanoma was confirmed by either adjunct immunostains for melanoma markers (GP100, MART-1, KBA-62, and NSE) or a history of melanoma (primary melanoma or prior S100-positive specimens). Immunostains for GP100 and MART-1 (Melan A) were equally positive in 10 (59%) of the 17 lesions (Fig. 1). Of the remaining 7, 5 had a documented primary cutaneous melanoma. Although the remaining 2 of 7 cases had no documented primary lesion, both had a prior metastatic lesion that was immunoreactive for S100. In 4 lesions negative for both GP100 and MART-1, tyrosinase and KBA-62 were immunoreactive in only 1 each.

Negative staining for S100 did not correlate with the histology of the metastatic melanoma (data not shown). Although limited data were available regarding therapeutic history before enrollment at the NCI, treatment and outcome data were available for 8 of the 17 patients in this study, and no correlation with NCI therapy or outcome was noted (data not shown).

4. Discussion

The S100 family of proteins is a member of the calcium-binding EF-hand superfamily. Although the exact function of S100 proteins is not clear, they are involved in the regulation of a diverse host of cellular functions, including contraction, motility, growth, differentiation, cell cycle progression, transcription, and secretion [10,11]. Certain S100 proteins are highly expressed in non-small cell lung cancer, breast cancer, gastric cancer, and lymphoma, whereas reduced levels of other members are found in breast and prostate cancer [10,11]. The association of S100 proteins with neoplastic processes suggests the possibility that different isoforms may act as tumor suppressors, whereas others may act as oncogenes. S100 protein expression may also have prognostic significance in a variety of tumors [12–14]. Aside from the diagnostic importance of S100 protein expression, its relationship with melanoma pathogenesis is unclear [15].

S100 protein is expressed in most metastatic melanoma. In our study, S100 immunoreactivity is present in 99% of melanomas, consistent with prior reports [6,8]. Detection of this antigen is best achieved using monoclonal antibodies because commercially available polyclonal antibodies are not specific [16].

Our study, which follows the evolution of S100 antigenicity in a subset of melanomas, demonstrated that 82% of the cases had prior metastases that were immunoreactive for S100. This change in antigenicity may reflect dedifferentiation of tumor cells or, alternatively, clonal selection of a subpopulation with a growth advantage. Differences in specimen processing may account for loss of antigenicity; however, this is unlikely because other antigens (eg, GP100) were not affected and positive controls were included in each assay. Antigenic loss has been studied in a variety of tumor types and model systems, and is thought to be associated with escape from immune surveillance in some tumors and with dedifferentiated phenotype in others [17–20]. This phenomenon has been studied in metastatic melanoma in association with escape from immunotherapy [21,22].

S100-negative melanomas arising from ocular melanoma were disproportionately high relative to the overall proportion of ocular melanoma. These findings are consistent with a previous study that demonstrated a uniform lack of S100 immunoreactivity in a subset of spindle cell ocular melanomas [23]. This phenotype may reflect inherent differences in uveal versus cutaneous melanocytes.

When confronted with a metastatic poorly differentiated tumor, the initial immunostains should include S100 and, where necessary, the standard panel to rule out carcinoma, sarcoma, and lymphoma. Based on this current study, immunoreactivity for GP100/MART-1 or a prior documentation of melanoma (S100 positivity or primary lesion) should confirm the diagnosis of melanoma if S100 is negative. Prior (and current) tumor specimens should be extensively sampled for S100 because most first or early metastases (82%) were immunoreactive for this antigen.

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Fig. 1.
Flow chart for analyzing S100-negative metastatic tumors.

Table 1

Case data for S100-negative melanoma

Total no. of melanoma cases	1553
S100-negative melanoma (% total)	17 (1.0)
Total no. of ocular melanoma (% total)	92 (6.0)
S100-negative ocular melanoma (% S100-negative)	4 (24)

Table 2

Summary of immunohistochemistry and clinical data

Patient	GP100	MART-1	KBA-62	Tyrosine	NSE	Prior S100-positive	Site of primary	Time interval for loss of S100
1	+	+	+	+	+	+	Ocular	3 wk
2	+	+	+	+	–	+	Ocular	9 mo
3	+	+	+	–	N/A	N/A	Ocular	N/A
4	+	+	–	+	+	N/A	Back	N/A
5	+	+	–	+	+	N/A	Back	N/A
6	+	+	–	+	+	N/A	Temporal	N/A
7	+	+	–	+	–	+	Shoulder	13 mo
8	+	+	–	+	–	N/A	Unknown	N/A
9	+	+	–	N/A	N/A	–	Unknown	N/A
10	+	+	–	–	–	N/A	Ocular	N/A
11	–	–	+	–	+	+	Shoulder	10 mo
12 ^a	–	–	–	+	+	–	Back	N/A
13	–	–	–	–	+	+	Unknown	3 y
14 ^a	–	–	–	–	+	+	Back	2 y
15	–	N/A	–	–	N/A	+	Back	5 mo
16	–	N/A	N/A	N/A	N/A	+	Back	2 y
17	–	–	–	–	–	+	Unknown	11 mo

Abbreviation. N/A, not available.

^aMelanosomes identified by electron microscopy.