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Comparison of Two Prognostic Markers for Malignant Melanoma: MIA and S100 β

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Key Words
Melanoma-inhibitory activity · S100 β · Tumor marker · Malignant melanoma

Abstract
It has recently been shown that the serum level of melanoma-inhibitory protein (MIA) provides useful information for the therapy and follow-up of patients with malignant melanoma. Previously, S100 β has been described as a useful tumor marker for malignant melanoma. In this study, we compare the significance of the two markers in follow-up, therapy outcome and prognosis by measuring MIA and S100 β serum levels in 50 melanoma patients. Serum levels were measured in patients with malignant melanomas of stages I–IV with at least 3 time points of measurement. Serial MIA and S100 β measurements were obtained from 32 patients with stage IV disease in parallel to chemotherapy and from 18 patients with a history of stage I and stage II disease during follow-up. The response to chemotherapy in stage IV disease and relapse of melanoma during follow-up correlated with changes in MIA and S100 β serum levels. In comparison, MIA revealed slightly higher specificity and sensitivity. In conclusion, both markers are useful for detection of progression from localized to metastatic disease during follow-up and for monitoring therapy of advanced melanomas.

Introduction

Proteins strongly expressed or released from tumor cells can be used as markers to monitor the development of disease. For malignant melanomas, two markers have recently been described, MIA and S100 β. Melanoma-inhibitory activity (MIA) has been identified previously within growth-inhibitory activities purified from tissue culture supernatant of malignant melanoma cells in vitro [1, 2]. A single copy gene coding for a secreted 11-kD protein was cloned and mapped to human chromosome 19q13.32–13.33 [3, 4]. Analyses of embryonic and adult tissues have revealed cell type-restricted expression patterns for MIA mRNA. In nonneoplastic tissues, MIA was mainly detected in developing and mature cartilage [5, 6], while in malignant tumors, high MIA mRNA levels were detected in almost 100% of malignant melanoma samples [7, 8]. Comparing the expression in normal skin, benign human skin melanocytes, benign melanocytic nevi and

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malignant melanomas, we observed that MIA mRNA levels reflect the progressive malignancy of melanocytic tumors [7]. Indeed, previous studies revealed that MIA serum levels provided diagnostic information in patients with malignant melanomas [9].

Another serum marker recently established for malignant melanoma, S100 β, was first described for the detection and quantification of damage to the central nervous system. S100 β is a cytoplasmatic, calcium-binding protein with a molecular weight of 21 kD and is expressed mainly in astrocytes, Schwann cells and satellite cells in sympathic ganglia, melanocytes and malignant melanomas [10]. A number of studies describe S100 β as a marker for clinical staging and monitoring of metastatic malignant melanoma [11–16].

The aim of this study was to compare the significance of these two markers for monitoring therapy and follow-up in patients with malignant melanoma, using an ELISA and a chemiluminescent assay (LIA) for serum measurements of MIA and S100 β, respectively.

Methods and Patients

Patients

Four hundred and eighty-five serum samples were obtained from a total of 50 patients with malignant melanoma. At the beginning of the study, 18 of the patients were in stage I with a history of a previously excised primary melanoma and were examined during tumor follow-up. Thirty-two of the patients were in stage III or IV. Diagnosis of melanoma was based on histological examination in all cases and confirmed by S100 and HMB45-immunohistochemistry in all doubtful cases. Tumor thickness was determined according to the criteria of Breslow [17] and tumor stage according to the American Joint Committee on Cancer (stage I: T_{1-2}, N_0, M_0; stage II: T_{3-4}, N_0, M_0; stage III: T_{1-4}, N_{1-2}, M_0; stage IV: T_{1-4}, N_{1-2}, M_1 [American Joint Committee, 1988]). Response to therapy was followed by clinical examination, routine laboratory tests, chest X-rays and tumor size measurements by ultrasound and CAT scans and nuclear magnetic resonance imaging. Response to therapy was defined as a reduction of tumor mass and failure as continuous growth of the tumor.

A reference panel of 212 sera from healthy blood donors (age between 19 and 86 years, 43.9 ± 19.2 years, mean ± SD) was selected based on the following criteria: no use of medication, no record of any metabolic disorder and no record of a malignant tumor. All sera were stored at –80 °C until assayed and determined blind of clinical information.

Serum levels were measured in patients with malignant melanomas of stages I–IV with at least three time points of measurement. Serial MIA and S100 β measurements were obtained from 32 patients with stage IV disease in parallel to chemotherapy and from 18 patients with a history of stage I and stage II disease during follow-up.

MIA and S100 β Assays

MIA was measured by a one-step ELISA (Roche, Germany) using two labeled monoclonal antibodies directed against 14-mer peptide sequences at the N-terminal (MAB 1A12) and C-terminal (MAB 2F7). The assay was performed following the manufacturer's instructions [9]. Briefly, 20 µl serum was incubated with the biotinylated MAB 2F7 and POD-conjugated MAB 1A12 antibodies in streptavidin-coated 96-well plates for 45 min. After washing three times with phosphate-buffered saline (PBS) 200 µl ABTS solution (2,2′-azino-di-[3-ethylbenz-thiazolinesulfonate]; Roche, Germany) was incubated in the wells for 30 min and measured colormetrically at 405 nm. Recombinant MIA purified from stably transfected CHO cells was used for calibration (0.1 and 50 µg/l). Serum samples were diluted to stay in the linear range of the ELISA. A high-dose hook effect was not observed [18]. Reproducibility of test results was confirmed by measuring repeatedly 8 standard sera using different ELISA lots (mean SD = 9.4%).

MIA serum levels in a reference group of 212 healthy blood donors varied between 1.9 and 6.43 ng/ml following a Gaussian distribution. Values above the 97 percentile of 4.5 ng/ml were defined positive.

S100 β was measured by a commercially available LIA (Byk-Sangtec Diagnostics, Dietzenbach, Germany; [19]) using the internal calibrators and controls provided by the manufacturer. Several cutoff values have been reported in the literature. In our study, the cutoff was set at 0.12 ng/ml according to the manufacturer's instructions for the LIA and several published studies [11–16].

Results

Characterization of the Patients

In this study, 485 serum samples from 50 patients with malignant melanoma were analyzed. Twenty-eight of the 50 patients were female. The mean age of the female patients was 52.6 years (± 16.8), of the male patients 55.6 years (± 15.8). Nineteen of the patients had superficial spreading melanoma, 10 nodular malanoma, 6 acral lentiginous melanoma, 4 lentigo maligna melanoma, 2 amelanotic melanoma, 1 ulcerating melanoma, and in 8 the primary tumor was not found.

The patients were followed for at least 5 and a maximum of 21 intervals. The longest follow-up time was 26 months. Eighteen of the patients had a primary melanoma excised before and were controlled regularly in tumor follow-up. Thirty-two of the patients were in stage III and IV, and were controlled during treatment.

Sensitivity and Specificity of MIA and S100 β Detection in Serum

Of the 378 sera obtained from patients with metastatic melanomas and actual tumor load (stage III and IV) 77.7% (MIA) or 55.5% (S100 β) were positive in stage III, and 84.1% (MIA) or 73.9% (S100 β) were positive in stage IV. These data suggest that both enhanced MIA and S100 β serum levels coincide with systemically progressed disease, but indicate a higher sensitivity of MIA ($p <$

Table 1. Clinical detection of metastasis after an increase in MIA or S100 β serum levels above the 97 percentile: all patients with tumor progression during follow-up were evaluated

Follow-up time period months	MIA		S100 β	
	patients	%	patients	%
Coincident	7	33.3	10	47.6
1	5	23.8	4	19.1
2	3	14.4	2	9.6
3	1	4.7	1	4.7
4	3	14.4	2	9.6
5	1	4.7	1	4.7
6	1	4.7	1	4.7

0.005). Evaluating both markers in parallel, we measured enhanced levels of at least 1 marker in all but one patient with stage IV melanoma.

The specificity of both markers and the number of true-negative values to the number of patients with no clinically detectable tumor were compared. MIA revealed a specificity of 81.7%, S100 β of 80.3%. These data indicate that MIA, as a serum marker for systemically progressed malignant melanoma, has a specificity comparable to S100 β. However, enhanced MIA serum levels are a more sensitive indicator of malignant melanoma in stages III and IV when compared to S100 β values.

Monitoring MIA and S100 β Serum Levels during Therapy of Malignant Melanoma

The correlation between tumor burden and MIA or S100 β serum levels was analyzed. In 22 patients with metastatic melanomas undergoing surgical removal of metastases, the MIA and S100 β levels obtained immediately prior to surgery and within 2 weeks after surgery were compared. MIA levels dropped in 20 cases, S100 β levels in 19 cases . In 2 MIA cases and 3 S100 β cases, the levels remained constant due to a large amount of residual tumor burden. In 2 patients with complete removal of tumor burden, serum was obtained before surgery, 6 h and 12 h after surgery. Measuring the stability of MIA in these serum samples revealed a half life of 3.7 h.

Serial MIA and S100 β measurements were obtained from all 32 patients undergoing chemotherapy of stage III and IV melanoma. In 24 patients, a correlation between changes in MIA serum levels and the clinical outcome of therapy was found. Similarly a correlation for S100 β in 20 patients was seen. In 3 patients MIA remained high at more than one time point despite a response to therapy (false positive), and in 5 patients it decreased despite the failure of therapy (false negative). S100 β showed false positives at more than one timepoint in 4 patients and false negatives in 8 patients.

Detection of Metastasis during Follow-up of Melanoma Patients

The clinical significance of MIA and S100 β serum levels in detecting disease progression of patients with surgically excised melanomas or melanoma metastases was analyzed. In 21 patients examined at 3- to 6-month intervals during tumor follow-up, we evaluated if clinical detection of metastasis was preceeded by an increase of serum markers (table 1). During routine follow-up periods of 3 months, metastasis could be detected in 14 (67.7%) patients at least 1 month earlier by MIA serum levels than by routine diagnostic procedures, while the corresponding figure for S100 β assays was 11 (52.4%) patients. In this time period, 6 (MIA) or 5 (S100 β) patients were found to have metastases. Both markers indicated a progression of the disease 2.6 months earlier than routine diagnostic procedures.

Correlation between Overall Survival and Serum Levels

Thirteen of the 50 patients died during the time of the study (26%). The survival curve (fig. 1) shows the correlation between positive or negative serum level and survival. For MIA ($p < 0.0001$) and for S100 β ($p = 0.0015$) both curves differed significantly.

Discussion

Some proteins that are strongly expressed or released from tumor cells have been successfully used as tumor markers in the serum of patients with carcinomas of different histologic origins. However, two new tumor markers have recently been described to detect and monitor disease progression in patients with malignant melanomas, MIA and S100 β. In this study, we compared the sensitivity and specificity of these markers by analyzing 485 serum samples from 50 patients with malignant melanomas. The mean age, the sex ratio and the distribution of melanoma site was representative of melanoma patients from Western Society.

The results presented here define MIA and S100 β as serum markers with almost equivalent clinical significance in staging and monitoring metastatic melanomas.

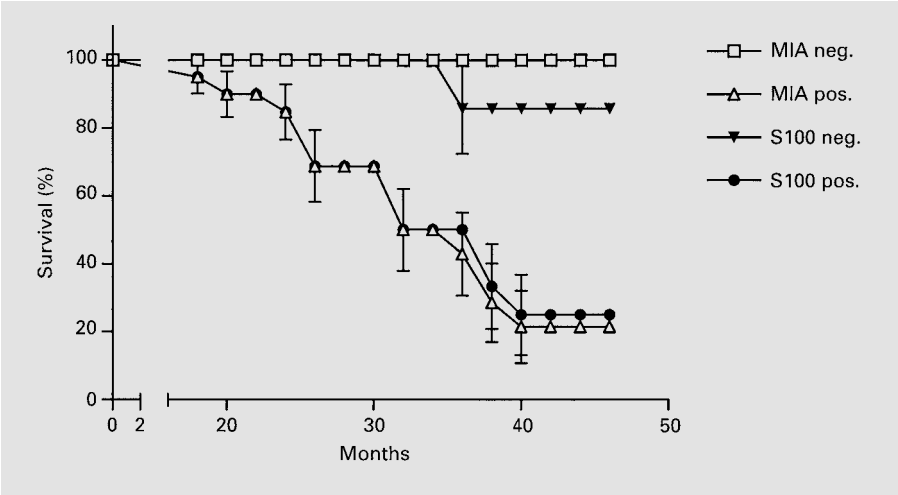

Fig. 1. Kaplan-Meier survival plots for disease-specific survival correlating survival of the patients with serum levels of MIA and S100 β. The cutoff for MIA was set at 4.5 ng/ml and for S100 β at 0.12 ng/ml.

Our data indicate that MIA and S100 β serum levels increase above the 97 percentile of healthy control donors in 77.7%, or 55.5% of patients in stage III, and 84.1 or 73.9% of patients with clinically apparent metastatic melanoma (stage IV), respectively. Other studies using the LIA system have reported similar results for S100 β [10, 12]. Comparing MIA and S100 β levels in these subgroups, we found an equivalent specificity (81.7 or 80.3%), but a significantly higher sensitivity for MIA than for S100 β in the detection of advanced melanomas. Measuring both markers simultaneously, we detected increased levels in all but 1 patient with stage IV melanoma. These data suggest that determination of both proteins in the serum represents a highly reliable surrogate marker for metastasized melanomas. Furthermore, both markers showed a highly significant correlation to the clinical outcome during therapy: an increase in MIA or S100 β serum protein levels during tumor follow-up was a strong indicator of disease progression, while a decrease was correlated with tumor regression.

By measuring MIA and S100 β during tumor follow-up, we were able to detect metastasis on average 2.6 months earlier than by clinical diagnosis. In another study, similar results were obtained analyzing MIA serum levels in melanoma patients under different types of therapy [20]. We also observed a significant correlation between survival and tumor marker levels. While this observation has previously been reported for S100 β [12, 13], to our knowledge, this is the first report indicating a similar correlation for MIA.

We conclude that MIA and S100 β are of clinical value in detecting tumor progression during follow-up of patients with localized melanomas and in monitoring therapy of advanced disease. Further evaluation is needed to address the possible clinical value of MIA and S100 β as independent prognostic markers of stage I and II disease.

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