High Serum Levels of Matrix Metalloproteinase-9 and Matrix Metalloproteinase-1 Are Associated with Rapid Progression in Patients with Metastatic Melanoma

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Abstract

Purpose: Matrix metalloproteinases (MMP) are proteolytic enzymes that play an important role in various aspects of cancer progression. In the present work, we have studied the prognostic significance of serum levels of gelatinase B (MMP-9), collagenase-1 (MMP-1), and collagenase-3 (MMP-13) in patients with advanced melanoma. Experimental Design: Total pretreatment serum levels of MMP-9 in 71 patients and MMP-1 and MMP-13 in 48 patients were determined by an assay system based on ELISA. Total MMP levels were also assessed in eight healthy controls. The active and latent forms of MMPs were defined by using Western blot analysis and gelatin zymography. Results: Patients with high serum levels of MMP-9 (>376.6 ng/mL; n = 19) had significantly poorer overall survival (OS) than patients with lower serum MMP-9 levels (n = 52; median OS, 29.1 versus 45.2 months; P = 0.033). High MMP-9 levels were also associated with visceral or bone metastasis (P = 0.027), elevated serum alkaline phosphatase level (P = 0.0009), and presence of liver metastases (P = 0.032). Serum levels of MMP-1 and MMP-13 did not correlate with OS. MMP-1 and MMP-9 were found mainly in latent forms in serum, whereas the majority of MMP-13 in serum was active (48 kDa) form. MMP-13 was found more often in active form in patients (mean, 99% of the total MMP-13 level) than in controls (mean, 84% of the total MMP-13 level; P < 0.0001). After initiating the therapy, patients with elevated levels of MMP-1 (>29.8 ng/mL, n = 10) progressed more rapidly than patients with lower levels (median, 1.9 versus 3.5 months; P = 0.023). Serum levels of MMP-9 and MMP-13 did not correlate with the time to progression (TTP). In multivariate analysis with age and gender, MMP-9 or MMP-1 turned out to be independent prognostic factors for OS [P = 0.039; hazard ratio (HR), 1.8; 95% confidence interval (95% CI), 1.03–3.3] or TTP (P = 0.023; HR, 2.7; 95% CI, 1.15–6.4), respectively. Conclusions: Our findings provide evidence that MMP-1, MMP-9, and MMP-13 play important roles at different phases of metastatic melanoma spread and that serum MMP-9, in particular, could have clinical value in identifying patients at high risk for melanoma progression.

Matrix metalloproteinases (MMP) are a family of zinc-dependent neutral endopeptidases characterized by their ability to degrade extracellular matrix components. In addition to the various structural components of the extracellular matrix, MMPs are known to degrade many other cell membrane and pericellular proteins, including cell membrane precursor forms of growth factors, growth factor binding proteins, growth factor receptors, cell adhesion molecules, clotting factors, and proteinase inhibitors as well as their own inactive zymogen forms (1–3). The human MMP family currently consists of 23 members, which can be divided into eight structural classes or, based on their substrate specificity and primary structure, into the more familiar subgroups of collagenases, gelatinases, stromelysins, MT-MMPs, and novel MMPs. MMPs are synthesized as inactive zymogens, which are activated predominantly pericellularly by other MMPs or serine proteinases. The activity of MMPs is specifically inhibited by tissue inhibitors of metalloproteinase and by nonspecific proteinase inhibitors (e.g., α2-macroglobulin). The balance between MMPs and their inhibitors is essential in many physiologic conditions where rapid remodeling of extracellular matrix is required and it is disturbed in pathologic conditions such as cancer.

Collagenases (MMP-1, MMP-8, and MMP-13) are the principal extracellular proteinases capable of degrading native
Table 1. Characteristics of patients

<table>
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<th>Variable</th>
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<th>$N^*$</th>
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<td>23</td>
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<td>Gender</td>
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<tr>
<td>Male</td>
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<td>16</td>
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<tr>
<td>Female</td>
<td>19</td>
<td>7</td>
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<tr>
<td>Age (y)</td>
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<tr>
<td>Median (range)</td>
<td>57 (36-75)</td>
<td>40 (16-114)</td>
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<tr>
<td>Disease-free survival (mo)</td>
<td>11 (0-224)</td>
<td>17 (0-58)</td>
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<td>Overall survival (mo)</td>
<td>44 (5-265)</td>
<td>45 (2-75)</td>
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<td>Survival after the initiation of therapy (mo)</td>
<td>9 (0.3-73)</td>
<td>10 (2.4-64)</td>
</tr>
<tr>
<td>TTP (mo)</td>
<td>2 (0.2-70)</td>
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<tr>
<td>Previous treatment</td>
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<td></td>
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<tr>
<td>No treatment</td>
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<td>7</td>
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<tr>
<td>Radiotherapy</td>
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<td>3</td>
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<tr>
<td>Surgery</td>
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<td>15</td>
</tr>
<tr>
<td>IFN-α</td>
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<td>7</td>
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<td>Treatment arms</td>
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<tr>
<td>Dacarbazine + natural IFN-α</td>
<td>12</td>
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<td>Recombinant IFN-α</td>
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<td>DOBC + natural IFN-α</td>
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<td>DOBC + recombinant IFN-α</td>
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<tr>
<td>Tumor burden</td>
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<td>Lung metastases ± soft tissue metastases</td>
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<td>Two or more sites</td>
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<td>Lung</td>
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<td>Skin/subcutis</td>
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<td>7</td>
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<tr>
<td>Other sites</td>
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<td>18</td>
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<td>9</td>
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*An additional group of 23 patients was analyzed for total MMP-9 serum levels.

†Ten patients had both radiotherapy and surgery; one patient had surgery combined with IFN-α and one patient had both radiotherapy and surgery combined with IFN-α. In the additional group of 23 patients, five patients had surgery combined with IFN-α and two patients had both radiotherapy and surgery combined with IFN-α.

**Serum MMP-1, MMP-9, and MMP-13 in Metastatic Melanoma**

Serum levels of MMPs are known to be altered in many human pathologic conditions. Elevated serum levels of MMPs have been reported in, for example, polycystic kidney disease (19), systemic sclerosis (20), rheumatoid arthritis (21), and cancer. In some diseases, such as acute viral hepatitis, however, serum levels of MMPs are reduced (22). In comparison with healthy controls, the serum levels of MMP-9 are elevated in head and neck squamous cell carcinoma (23, 24) and in colon (25), gastric (26), bladder, breast, and prostate cancer (27). Furthermore, elevated MMP-9 serum levels have been shown to correlate with poor survival in stage I to II non–small cell lung cancer patients (28).

There are few studies analyzing the serum levels of MMPs in patients with metastatic melanoma and their results have been partly controversial. Vuoristo et al. (29) studied the serum levels of MMP-2 in 50 patients with advanced melanoma and found that serum MMP-2 had not a role as a prognostic marker. On the other hand, in a study on a larger patient material, Wollina et al. (30) found that the serum levels of MMP-2 were significantly higher in patients with metastatic melanoma than in patients with localized melanoma. In addition, somewhat higher MMP-2 levels were noted in patients with more advanced disease when MMP-2 levels were compared with different tumor stages. In the present work, we have determined the serum levels of MMP-1, MMP-9, and MMP-13 in 71 patients with metastatic melanoma with a special interest to...
determine the levels and prognostic value of their active and latent forms. These MMPs were chosen because of their known role as prognostic factors in our earlier studies with melanoma tumors. In addition, we wanted to see whether proangiogenic MMP-9 serves as a prognostic factor among these patients, which were all treated with IFN-α, a cytokine with antiangiogenic properties.

**Patients and Methods**

**Patients**

The patient population consisted of 48 patients with advanced melanoma, who were recruited into a Finnish randomized multicenter study between 1995 and 1998. The treatment arms were dacarbazine or a four-drug chemotherapy composed of dacarbazine, vincristine, bleomycin, and lomustine, both combined with either recombinant or natural IFN-α. There were 29 male and 19 female patients and their median age was 62 years (range, 25-75). The patients recruited in this study had a progressive, inoperable, histologically verified metastatic melanoma. Some patients had previous metastases, whereas some patients entered the study after the appearance of their first metastasis. Before therapy, a full medical examination, including a chest X-ray, abdominal ultrasound and computerized tomography, a bone scan, and blood biochemistry, was carried out. The evaluation of metastatic sites and tumor burden is based on these studies. Because of interesting results of analysis concerning MMP-9, we expanded the patient population analyzed for total MMP-9 with an additional group of 23 patients. These patients had been attending the ongoing multicenter study during our first-line studies. Patient characteristics are summarized in Table 1. The serum samples were collected before initiating the chemoimmunotherapy. The control samples were collected among three healthy male and five healthy female volunteers ages from 26 to 72 years (median, 41) ranging between the minimum and maximum ages of those in the patient group.

**Determination of serum matrix metalloproteinase-1, matrix metalloproteinase-9, and matrix metalloproteinase-13 levels by ELISA**

Quantitative analysis of serum MMP-1, MMP-9, and MMP-13 was done by using a commercial Human Biotrak ELISA System according to the manufacturer’s instructions (Amersham Biosciences, Buckinghamshire, United Kingdom). The MMP-1 assay recognizes total MMP-1 (i.e., proMMP-1, active MMP-1, and MMP-1/tissue inhibitor of metalloproteinase-1 complex). The MMP-9 assay is specific for proMMP-9 and MMP-9/tissue inhibitor of metalloproteinase-1 complex, whereas the MMP-13 assay recognizes both proMMP-13 and active MMP-13. Briefly, all the kits are based on a two-site ELISA sandwich format. Standards and serum samples were incubated in a microwell plate precoated with anti-MMP-1 or anti-MMP-9 or anti-MMP-13 antibody. Any MMP-1, MMP-9, or MMP-13 present in the samples was bound to the wells, and the excess was removed by extensive washing. These MMPs were then detected by a peroxidase labeled antibody, and the amount of peroxidase was determined by addition of TMB substrate. Reactions were stopped by adding acid solution and the absorbance was read at 450 nm in a microtiter plate spectrophotometer. Serum concentrations were determined from the corresponding standard curves run for each plate separately.

**Determination of active and latent forms of matrix metalloproteinases**

**Sample preparation and positive controls.** The proportions of latent zymogen forms of MMPs and activated MMPs were determined by Western blot analysis and gelatin zymography. Due to the high concentration of proteins, the serum samples were diluted in PBS (1:20). A medium known to contain the studied MMPs was used as a positive control. This medium was obtained from normal human fibroblasts infected with adenovirus to generate MMP-13 expression (31) and then cultured in DMEM containing 0.5% FCS for 4 days. Another positive control for active MMP-1 and MMP-13 was prepared by incubating the control medium with 1 mmol/L APMA (Sigma, St. Louis, MO) for 30 minutes at 37°C to activate latent MMPs. A mixture of control medium and serum was used in a Western blot analysis to obtain a fractionation similar to serum samples. The patient samples were analyzed in a series where one or more samples from the previous gel were run on the following gel together with some new samples.

**Western blot analysis.** Equal aliquots of the diluted serum samples were fractionated on 8.5% SDS-PAGE and transferred to Hybond ECL nitrocellulose membrane (Amersham Biosciences). The amounts of MMP-1 and MMP-13 were determined using a polyclonal anti-MMP-1 antibody (AB806; Chemicon International, Inc., Temecula, CA) and a monoclonal anti-MMP-13 antibody (IM44L; Oncogene Research Products, Cambridge, MA) in dilutions of 1:5,000 and 1:500, respectively. The specifically bound primary antibodies were detected with peroxidase-conjugated secondary antibodies and visualized by enhanced chemiluminescense (Amersham Biosciences).

**Gelatin zymography.** Aliquots of the diluted serum samples were fractionated on 10% SDS-PAGE containing 1 mg/mL gelatin (G-9382; Sigma) and 0.5 mg/mL 2-methoxy-2,4-diphenyl-3(2H)-furanone (64958; Fluka, Buchs SG, Switzerland). The gels were incubated for 30 minutes in 50 mmol/L Tris, 0.02% NaN₃, and 2.5% Triton X-100 (pH 7.5) and for 30 minutes in the same buffer supplement with 5 mmol/L CaCl₂ and 1 μmol/L ZnCl₂ (pH 7.5). Then the gels were incubated at 37°C in 50 mmol/L Tris, 0.02% NaN₃, 5 mmol/L CaCl₂, and 1 μmol/L ZnCl₂ for until the gelatinase bands were developed. After that, the gels were fixed in 50% methanol/7% acetic acid, stained with 0.25% Coomassie brilliant blue G250, and photographed.

**Evaluation of the active and inactive components of matrix metalloproteinase-13**

The area of the gel lanes representing proMMP-13 and active MMP-13 was measured in pixels by using a MCID-M5+ computer program, v 4.0 (Imaging Research, Inc., St. Catharines, Ontario, Canada). A computer-driven illuminator and camera transferred data from the gels. The settings were defined so that the same samples on different gels produced similar results. These values in pixels were used in analysis inside the patient group. For each sample, the percentage of the active MMP-13 from the total MMP-13 was calculated, and this percentage was used in comparison between the patient group and the control group.
Statistical analysis
The results from ELISA and Western blot analysis and clinical data were analyzed with the SAS system for Windows, v 8.2 and the Enterprise Guide, v 2.0 (SAS Institute, Inc., Cary, NC). The Mann-Whitney U test (Kruskal-Wallis test) was used in assessing the associations between MMP levels and different categorized variables such as tumor burden and in assessing the difference in MMP levels between patient and control groups. The two-sample \(t\) test and ANOVA were used when analyzing the association between the active MMP-13 and categorized variables. Cumulative survival curves for overall survival and time to progression were drawn by the Kaplan-Meier method and the difference between the curves was analyzed by the Mantel-Cox (log-rank) test. The Cox’s multivariate analysis was used to further study the effect of MMPs on survival.

Results
Serum levels of matrix metalloproteinase-1, matrix metalloproteinase-9, and matrix metalloproteinase-13 in patient and control samples. The serum levels of MMP-1, MMP-13, and MMP-9 in melanoma patient and healthy control samples were measured by ELISA. A statistically significant difference in MMP-9 and MMP-1 levels was noted between the patient and control groups (Table 2). Higher serum MMP-9 levels were found in patients than in controls (mean, 295.4 versus 132.2 ng/mL; \(P = 0.0024\)), whereas serum MMP-1 levels were higher in controls than in patients (mean, 126.5 versus 28.8 ng/mL; \(P < 0.0001\), Mann-Whitney \(U\) test; Fig. 1; Table 2). No such differences were found in MMP-13 levels between the patient and control groups (Fig. 1; Table 2).

Matrix metalloproteinase-13 is mainly present in active form. The qualitative analysis of the serum samples was done by Western blot analysis and gelatin zymography. In patient and control samples, MMP-13 was detected primarily as the active 48-kDa form, whereas MMP-1 and MMP-9 were primarily in latent form (i.e., as 55-kDa proMMP-1 and 92-kDa proMMP-9; Fig. 2A-C). However, in control samples, the latent 60-kDa form of MMP-13 was more abundant than in patient samples (Fig. 2D). To compare the differences in the amounts of active and latent MMP-13 between patient and control groups, the percentage of the active MMP-13 from total MMP-13 was

Table 2. Serum levels of MMP-1, MMP-9, and MMP-13 in patient and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Minimum (ng/mL)</th>
<th>Maximum (ng/mL)</th>
<th>Median (ng/mL)</th>
<th>Mean (ng/mL)</th>
<th>(P^*)</th>
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<tbody>
<tr>
<td>MMP-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Patient</td>
<td>0</td>
<td>268.2</td>
<td>16.5</td>
<td>28.8</td>
<td>&lt;0.0001</td>
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<tr>
<td>Control</td>
<td>31.5</td>
<td>339.5</td>
<td>104.2</td>
<td>126.5</td>
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<tr>
<td>MMP-9</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>48.4</td>
<td>1074.7</td>
<td>260.1</td>
<td>295.4</td>
<td>0.0024</td>
</tr>
<tr>
<td>Control</td>
<td>45.7</td>
<td>233.6</td>
<td>132.1</td>
<td>132.2</td>
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<tr>
<td>MMP-13</td>
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<tr>
<td>Patient</td>
<td>0.079</td>
<td>2.850</td>
<td>0.410</td>
<td>0.763</td>
<td>NS</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>2.178</td>
<td>0.417</td>
<td>0.646</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

* Mann-Whitney \(U\) test.

Fig. 1. Distribution of total serum levels of MMP-1, MMP-9, or MMP-13 in patient and control groups. \(A\), serum MMP-1 levels were higher in controls \((n = 8)\) than in patients \((n = 48);\ mean, 126.5 versus 28.8 ng/mL; \(P < 0.0001)\). \(B\), serum MMP-9 levels were higher in patients \((n = 71)\) than in controls \((n = 8);\ mean, 295.4 versus 132.2 ng/mL; \(P = 0.0024)\). \(C\), there were no statistically significant differences in serum MMP-13 levels between patients \((n = 48)\) and controls \((n = 8);\ mean, 0.763 versus 0.646 ng/mL; \(P = 0.7967)\).
calculated. In the melanoma patient group, proportion of the active MMP-13 varied from 96% to 100% with a median value of 100% and a mean value of 99%. In the control group, the percentages varied from 65% to 98% with a median and mean value of 84%. The proportion of the active MMP-13 was significantly higher in the patient group than in the control group (mean, 99% versus 84%; \( P < 0.0001 \), Mann-Whitney \( U \) test). In addition, the actual levels of the active MMP-13 were determined from the total MMP-13 levels, measured by ELISA, based on the percentages obtained with the Western blot analysis. The levels of active MMP-13 in melanoma patients varied from 0.079 to 2.850 ng/mL with a median value of 0.410 ng/mL and a mean value of 0.756 ng/mL. In controls, the levels of active MMP-13 varied from 0 to 1.742 ng/mL with median and mean values of 0.300 and 0.530 ng/mL, respectively.

**Serum matrix metalloproteinases and survival.** The most appropriate cutoff points for dividing the serum MMP levels of the patients into two groups were defined by giving different values for the cutoff point and determining the corresponding \( P \) values using the Mantel-Cox (log-rank) test to analyze Kaplan-Meier survival curves. This method has been previously described (32). The cutoff point that corresponded to the lowest \( P \) value was used in the analysis (Fig. 3). This cutoff point was \( \geq 29.8 \) ng/mL for MMP-1, \( \geq 376.6 \) ng/mL for MMP-9, and \( \geq 1.508 \) ng/mL for MMP-13. The pixel values for the active MMP-13 were divided similarly and this cutoff point was \( \geq 421 \) pixels. The cutoff points from this study are planned to be used in further prospective studies. Corresponding patient numbers in each patient group are shown in Table 3.

We found that patients with high serum MMP-9 levels (\( \geq 376.6 \) ng/mL, \( n = 19 \)) had a poorer overall survival (OS) with a median of 29.1 months when compared with patients with lower MMP-9 levels (\( n = 52 \); median OS, 45.2 months; \( P = 0.033 \), log-rank test; Fig. 4A; Table 3). In multivariate analysis including age and gender, MMP-9 turned out to be an independent prognostic factor for OS (\( P = 0.039 \); hazard ratio, 1.8; 95% confidence interval, 1.03-3.3). The serum levels of MMP-1 or MMP-13 or the active MMP-13 did not correlate with OS (Table 3).

The time to progression (TTP) was calculated from the beginning of the chemoimmunotherapy to the diagnosis of progressive disease. We found that patients with higher levels of serum MMP-1 (\( \geq 29.8 \) ng/mL, \( n = 10 \)) had significantly shorter TTP than patients with lower MMP-1 levels (\( n = 38 \); median, 1.9 versus 3.5 months; \( P = 0.023 \), log-rank test; Fig. 4B; Table 3). In multivariate analysis with age and gender, MMP-1 was an independent prognostic factor for TTP (\( P = 0.023 \); hazard ratio, 2.7; 95% confidence interval, 1.15-6.4). The serum levels of MMP-9, proMMP-13, or the active MMP-13 did not correlate with TTP, and none of the studied MMPs correlated with the survival after the initiation of chemoimmunotherapy (Table 3).

**Serum matrix metalloproteinases and tumor site.** Site of metastasis has a major effect on survival of the metastatic melanoma patients. We used a tumor site classification resembling the M1a-c classification in the new American Joint Committee on Cancer melanoma staging system (8). The patients were divided into three groups according to the sites of their tumors. The first group included patients with soft tissue metastases (i.e., skin, s.c., or lymph node metastases), the second group included patients with lung metastases with or without soft tissue involvement, and the third group included patients with other visceral or bone metastases. The serum MMP-9 levels were found to correlate with tumor site. The mean MMP-9 level was 217.6 ng/mL in the first group (\( n = 11 \)), 275.4 ng/mL in the second group (\( n = 17 \)), and 323.3 ng/mL in the third group (\( n = 43 \); \( P = 0.027 \), Kruskal-Wallis test). The levels of MMP-1 (\( P = 0.63 \)) and MMP-13 (\( P = 0.55 \); Kruskal-Wallis test) or the active MMP-13 (\( P = 0.57 \), ANOVA) did not correlate with tumor site.

In addition, we studied whether serum MMP levels were elevated in patients with specific metastatic sites (bone, liver, lung, and skin/subcutis) or whether the levels correlated with the number of metastases (one or more). The serum levels of MMP-9 were found to be significantly higher in patients with liver metastases (\( n = 33 \)) than in patients without them (\( n = 38 \); mean, 331.2 versus 264.4 ng/mL; \( P = 0.032 \), Mann-Whitney
The levels of MMP-1 ($P = 0.71$), MMP-13 ($P = 0.42$, Mann-Whitney $U$ test) or the active MMP-13 ($P = 0.83$, ANOVA) did not correlate with the presence of liver metastases. The serum levels of MMPs did not correlate with other metastatic sites or with the number of metastases (data not shown).

**Serum matrix metalloproteinase levels and the level of serum alkaline phosphatase.** The routine measurements of the patients’ serum levels of alkaline phosphatase and alanine aminotransferase were included in the study. Serum alanine aminotransferase is a quite specific marker for liver failure, whereas serum alkaline phosphatase level is elevated in disease processes of the bone. In melanoma patients, the elevated levels of these serum markers can reflect the presence of liver or bone metastases. The patients were divided into two groups with normal or elevated serum alkaline phosphatase and alanine aminotransferase levels according to the upper limit of the standard laboratory reference value. The patients with elevated S-alp levels ($n = 10$) had higher serum MMP-9 levels than those patients with normal S-alp levels ($n = 59$; mean, 469.7 versus 266.0 ng/mL; $P = 0.0009$, Mann-Whitney $U$ test; S-alp values were missing from two patients). Conversely, patients with elevated S-alp levels ($n = 9$) had lower serum levels of MMP-1 than patients with normal S-alp levels ($n = 39$; mean, 13.5 versus 32.4 ng/mL; $P = 0.028$, Mann-Whitney $U$ test). Serum alkaline phosphatase levels did not correlate with the levels of active ($P = 0.7$, two-sample $t$ test) or total MMP-13 ($P = 0.2$, Mann-Whitney $U$ test). The serum levels of alanine aminotransferase did not associate with MMP serum levels (data not shown).

**Discussion**

During the recent years, the important role of MMPs in various stages of cancer progression has been extensively documented. Inhibition of the activity of MMPs by synthetic inhibitors has been a recent approach in the treatment of cancer. Small molecule MMP inhibitors (MMPI) BMS-275291, marimastat (BB-2516), neovastat, prinomastat (AG3340), and metastat have been studied in advanced phase clinical trials for the treatment of various types of cancers (1–3).
some encouraging results from trials with synthetic MMPIs have been reported. In a randomized trial of 369 patients with advanced gastric cancer, patients who received marimastat gained some survival benefit when compared with patients who received placebo (33). Furthermore, treatment of patients (n = 44) with recurrent and progressive glioblastoma multiforme with a combination of marimastat and an alkylating agent temozolomide resulted in increased progression-free survival at the time point of 6 months (34). In a small group of patients with advanced renal cell carcinoma, treatment with a higher dose of neovastat was associated with longer survival than treatment with a lower dose (35).

The early clinical trials that failed to show any benefit from MMPI treatment have been criticized because patients with advanced cancers were studied without information on the specific expression profiles of MMPs in these cancer types (1). For example, it has been shown that the expression of MMP-11 and MMP-14 are indicators of poor prognosis in small cell lung cancer, whereas the expression of MMP-2 is undetectable in this tumor type (36). This information could have guided the selection of a more appropriate tumor type for testing of the synthetic MMPI tanomastat (BAY 12-9566), which is specifically targeted against MMP-2 and has minimal inhibitory effect on MMP-11. Furthermore, different MMPs are expressed in different stages of cancer progression. MMP-2, MMP-9, and MMP-14 have been shown to be involved in regulation of angiogenesis by different mechanisms (1). Thus, inhibition of their activity to prevent the formation of new blood circulation into the tumor should take place before development of an independent blood vessel network. Therefore, more information is required about the expression of different MMPs in various tumor types and in different stages of the tumors. The recent promising results of clinical trials with MMPIs suggest that through careful patient selection and study design, the MMPIs could ultimately be potential agents in the therapy of cancer.

In the present work, we have studied the levels of MMP-1, MMP-9, and MMP-13 and the proportions of their active and inactive forms in sera of patients with metastatic melanoma. Elevated serum MMP-9 levels were noted in melanoma patients compared with healthy control subjects. In addition, high serum levels of MMP-9 were found to be associated with poor overall survival. After standardizing for the age and gender, MMP-9 turned out to be an independent prognostic factor for OS in metastatic melanoma. Elevated MMP-9 levels were found to correlate with multiple tumor sites, liver metastases, and elevated levels of serum alkaline phosphatase, all suggesting that MMP-9 an important role in rapid progression of melanoma. These observations suggest that measurement of serum MMP-9 of melanoma patients could aid in detection of melanoma progression. The purpose of this study was also to find out clinically meaningful cutoff points for MMP-9 expression levels to frame further prospective studies. In addition, these results suggest MMP-9 as a therapeutic target in melanoma patients.

MMP-13 is characterized by wide substrate specificity and restricted expression. In our previous studies, we have found that it was expressed tumor cells in human melanoma metastases (17, 18). In the present study, we detected MMP-13 in the serum of patients with metastatic melanoma but also in the serum of healthy controls. The levels of MMP-13 in patient serum samples were low when compared with those of MMP-1 or MMP-9, and they did not differ from those in the control group. However, the MMP-13 in patient sera was found almost exclusively in the active 48-kDa form, whereas the sera of control subjects contained inactive MMP-13 as well.

In general, in previous studies, higher levels of MMPs have been detected in sera of patients than in controls. However, reduced levels have been reported in acute viral hepatitis (22) and in postoperative biliary atresia (37). In the present study,
we found that the MMP-1 levels were generally lower in melanoma patients. In addition, lower levels of MMP-1 were associated with an elevated serum alkaline phosphatase level. On the other hand, the disease of patients with elevated levels of MMP-1 progressed more rapidly after therapy, and MMP-1 turned out to be an independent prognostic indicator for TTP in multivariate analysis. The role of MMP-1 in melanoma progression seems controversial. It has been shown to be associated with advanced stages of malignant melanoma (9) and to correlate with shorter disease-free survival (17), but on the other hand, to be associated with favorable chemoinmunotherapy response (18) in stage IV melanoma. The results of the current work indicate that MMP-1 could have an important role in the maintenance of normal tissues; but on the other hand, its abundant expression can facilitate rapid disease progression in metastasized melanoma.

Several studies have been done to identify different easily assayed quantitative markers that could serve as prognostic indicators for patients with malignant melanoma. One of the most established is the level of the enzyme lactic dehydrogenase assessed as a marker for metastatic disease (38). Serum LHD levels have since been included in the new American Joint Committee on Cancer melanoma staging system, where stage IV melanoma patients with elevated serum lactate dehydrogenase levels are classified into the M1c group with dismal prognosis (8). Another well-documented marker for prognosis is the serum polypeptide S-100β. Patients positive for S-100β have been shown to have significantly shorter disease-free survival than patients negative for S-100β (38). However, there is a need for new easily measurable markers, which could predict disease progression or therapeutic response. In the present study, we have assessed the prognostic value of serum MMP-1, MMP-9, and MMP-13 in stage IV melanoma patients and identified MMP-9 as an independent prognostic factor for OS in metastatic melanoma. Due to the limited number of controls and patients in this study, further studies are obviously required. In addition, studies with serum samples obtained at different phases of melanoma progression could further elucidate the role of MMPs in growth and metastasis of melanoma. In conclusion, our findings provide evidence, that MMP-1, MMP-9, and MMP-13 have important roles at different phases of metastatic spread and that measurement of serum MMP-9, in particular, could be of clinical value when identifying patients at high risk for progression.

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