MIA (melanoma inhibitory activity): a potential serum marker for rheumatoid arthritis

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Summary

Objective. MIA (melanoma inhibitory activity) is correlated with metastasis in patients with malignant melanoma. As MIA is not only produced by melanoma cells, but also by differentiated chondrocytes, we examined whether serum levels of MIA are correlated with inflammation and/or joint destruction in rheumatic diseases.

Methods. MIA serum concentrations of patients with different rheumatic diseases were examined and compared with healthy individuals and malignant melanoma patients. In addition, MIA concentrations were correlated to inflammatory parameters and joint destruction.

Results. Increased MIA serum concentrations were found only in patients with rheumatic diseases associated with joint destruction, such as rheumatoid arthritis (RA), osteoarthritis, HLA B27-associated oligoarthritis, and psoriatic arthritis. Of these rheumatic diseases, a significant increase in MIA serum concentrations was seen only in patients with RA, associated with rheumatoid factor (RF) positivity and joint destruction.

Conclusions. In addition to RF, MIA might therefore be useful in the differential diagnosis of RA vs non-destructive rheumatic diseases, and the presence of elevated levels of MIA in serum very likely reflects joint destruction in RA.

Key words: MIA, Rheumatoid arthritis, Rheumatoid factor, Joint destruction, Cartilage.

Rheumatoid arthritis (RA) is characterized by progressive destruction of affected joints, reflected by an invasive growth of aggressive synovial tissue into adjacent cartilage and bone. Although advanced laboratory and radiological technologies exist, it is difficult to evaluate joint destruction due to the lack of markers indicating the presence and extent of joint destruction. In addition, in early disease, the differential diagnosis of RA vs other joint-destructive rheumatic diseases such as psoriatic arthritis, HLA B27-associated arthritides and gout, and numerous non-joint-destructive rheumatic diseases such as systemic lupus erythematosus (SLE), frequently appears difficult.

Similar to RA, invasive growth of tumours is associated with the destruction of tissue matrix. Recently, a novel protein, named MIA (melanoma inhibitory activity), derived from metastatic melanoma cell lines, has been characterized. The secreted form is a small 11 kDa protein of 131 amino acids encoded by a single gene on chromo-

some 19 with no known homology to other proteins [1–4]. In non-malignant tissues, MIA expression is predominantly seen in developing and mature cartilage [5, 6], whereas in malignancies, elevated serum levels are predominantly found in metastatic melanoma, and to a lesser extent in ovarian, pancreatic and breast cancer [7].

In addition, there is evidence that enhanced serum concentrations of MIA indicate the degree of metastasis in melanoma patients [7, 8]. Thus, we examined whether MIA might also be a marker for cartilage and bone degradation in rheumatic diseases, supported by the fact that MIA is produced by chondrocytes [5]. We further examined whether enhanced concentrations of MIA are specific for joint-destructive vs non-destructive rheumatic diseases, and evaluated correlations for clinical and laboratory parameters and MIA.

Patients and methods

Patients

Serum was obtained from 46 patients with RA, 31 patients with SLE, 29 patients with psoriatic arthritis
(PsA), 12 patients with HLA B27-associated oligoarthritis, 12 patients with gout, 10 patients with systemic sclerosis (SSc) and 10 patients with relapsing polychondritis (RP) visiting the out-patient clinic of the Division of Rheumatology and Clinical Immunology of the Department of Internal Medicine I of the University of Regensburg. None of the patients suffered from malignant melanoma. Controls consisted of 100 patients with metastatic melanoma and 120 healthy individuals. Serum samples were aliquoted and stored immediately at −20°C. In addition, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor (RF), titre of antinuclear antibodies, and red and white blood cell counts were evaluated on the day of the visit. X-rays were taken at the time of blood sampling and examined by two experienced rheumatologists and one radiologist. X-rays were scored destruction vs no destruction according to the presence of multiple cysts, erosions or severe joint destruction.

**Enzyme-linked immunosorbent assay (ELISA) for the detection of MIA**

Serum concentrations of MIA were measured using a recently developed quantitative ELISA, which is now commercially available ([7, 8] and Boehringer Mannheim, Germany). In brief, two monoclonal antibodies directed against 14-meric NH2-terminal and COOH-terminal peptides (monoclonal antibodies 1A12 and 2F7, provided by Boehringer Mannheim) were raised and conjugated to horseradish peroxidase and biotin, respectively. Ten microlitres of serum or standard were incubated with 200 μl of reagent containing biotinylated 2F7 antibody and horseradish peroxidase–1A12 antibody in a streptavidin-coated 96-well plate for 45 min with continuous shaking. After washing three times in phosphate-buffered saline (PBS), 200 μl of 2,2′-azino-di-(3)-ethylbenz-thiazoline sulphonate (Boehringer Mannheim) were added to the wells and measured colorimetrically at 405 nm. Standardization was performed using solutions of purified MIA derived from stably MIA-transfected Chinese hamster ovary cells with a range from 0.1 to 50 ng/ml. The standard curve was calculated in a linear fashion. All serum samples and standards were measured in duplicate and results never varied more than 5%. MIA serum concentrations of the different patient groups were compared with the serum concentrations of healthy individuals and patients with metastatic melanoma.

To evaluate the specificity of the ELISA system with regard to interfering factors in the sera of patients with RA, e.g. the presence of RF, we performed titration experiments using increasing amounts of non-labelled second antibody 2F7 (Fig. 1).

**Cell culture**

As fibroblasts are known to produce MIA following stimulation with mitogens [3, 5], we established cell cultures to exclude that enhanced MIA serum values are due to production by synovial fibroblasts. Synovial fibroblasts were obtained from biopsies of synovial tissue of patients with RA who met the criteria of the American College of Rheumatology, and from patients with long-term OA. After enzymatic digestion, fibroblasts were grown in culture flasks in DMEM–Cellgro (Mediatech, Washington, DC, USA) containing 10% fetal calf serum (Gibco Life Technologies, Grand Island, NY, USA). Cells were cultured for 3–5 passages, stained for fibroblast markers by immunocytochemistry (>95% could be stained positively for the fibroblast enzyme procollagenase and none were positive for the macrophage marker CD68 or the neutrophil marker cathepsin G; data not shown), and tested for mycoplasmas. At 70–80% confluency, supernatant from different RA and five different OA cultures was examined for the presence of MIA.

**Statistics**

For comparison of MIA serum concentrations, the Mann–Whitney test for non-paired parameters was applied. For evaluation of the association between MIA in sera and RF, ESR, CRP and joint destruction, Fisher’s exact test was performed. P values of <0.05 were regarded as significant.

**Results**

Using the ELISA test system, healthy individuals showed MIA serum concentrations ranging from 1.8 to
7.6 ng/ml. MIA values of healthy individuals followed a Gaussian distribution, and were age independent [7]. Applying 3 s.d. to baseline of healthy individuals, MIA values of at least 6.5 ng/ml (ELISA cut-off) were regarded as elevated. As compared to healthy individuals, patients with metastatic melanoma showed elevated MIA serum concentrations ranging from 7.1 to 82 ng/ml with an average of 18.8 ng/ml (Table 1).

In contrast, MIA serum concentrations of patients with rheumatic diseases varied considerably, and increased serum concentrations were found only in patients with joint-destructive rheumatic diseases.

A total of 24/46 of the patients with RA, 8/29 of the patients with PsA, 6/12 of the patients with HLA B27-associated oligoarthritis and 3/11 of the patients with OA showed elevated MIA serum concentrations. Interestingly, the highest values were found in patients with RA, ranging from 2.9 to 42.8 ng/ml. In addition, one patient with highly active RA and severe joint destruction showed an MIA serum concentration of 100 ng/ml. Except for one patient with PsA showing an MIA serum concentration of 14.8 ng/ml and one patient with HLA B27-associated oligoarthritis with an MIA serum concentration of 11.7 ng/ml, patients of all other groups did not have MIA serum concentrations >10 ng/ml. In the titration experiments using increasing amounts of the second antibody 2F7, detection of MIA could be completely inhibited, therefore indicating no interference of serum proteins, e.g. RF, with measurement of MIA concentrations (data not shown).

Of the potentially joint-destructive rheumatic diseases, the mean MIA serum concentration was 10.3 ± 10.2 ng/ml (range 2.9–100 ng/ml) for RA patients, 6.6 ± 3.0 ng/ml (range 2.1–11.7 ng/ml) for patients with HLA B27-associated oligoarthritis, 5.2 ± 1.4 ng/ml (range 3.3–7.8 ng/ml) for OA patients and 4.8 ± 3.2 (range 0–14.8 ng/ml) for patients with PsA. However, as compared to serum concentrations of healthy blood donors, only patients with RA had significantly elevated MIA serum concentrations (P < 0.05). Interestingly, although having an active flare of the disease at the time of presentation, none of the 11 patients with gout showed elevated MIA serum concentrations (mean 2.6 ± 1.0 ng/ml; range 1.0–3.8 ng/ml).

In contrast, none of the patients with non-joint-destructive rheumatic diseases, such as SLE, SSC and RP, showed increased MIA serum concentrations (one patient with SLE had a borderline MIA serum concentration of 6.6 ng/ml). MIA serum concentrations of SLE patients ranged from 1.2 to 6.6 ng/ml with a mean of 4.4 ± 1.3 ng/ml. SSC patients ranged from 1.1 to 4.5 ng/ml with a mean of 3.5 ± 2.8 ng/ml, and RP patients ranged from 1.4 to 5.5 ng/ml with a mean of 3.4 ± 2.7 ng/ml. All data are summarized in Table 1 and Fig. 2.

In patients with RA, 19/24 patients (79%) who had elevated serum MIA concentrations were also positive for RF, whereas only 5/24 patients (21%) showing elevated MIA serum concentrations were RF negative. Conversely, 14/22 patients (64%) having normal MIA serum concentrations were also RF negative, and only 8/22 patients (32%) with normal MIA serum concentrations had a positive RF. Thus, statistical analysis using Fisher’s exact test revealed that enhanced MIA serum concentrations were associated significantly with RF positivity.

In addition, 18/24 patients (75%) who had enhanced MIA serum concentrations also showed radiographic signs of joint destruction at the time of presentation independent of the duration of the disease, whereas only 6/24 (25%) patients with normal MIA serum concentrations showed no radiographic signs of joint destruction. On the other hand, 15/22 patients (68%) having normal MIA serum concentrations showed no radiographic signs of joint destruction, and only 7/22 patients (32%) with normal MIA serum concentrations showed radiographic signs of joint destruction (Table 2). Statistical analysis using Fisher’s exact test revealed, similar to the association with RF outlined above, that enhanced MIA serum concentrations were associated significantly with radiographic signs of joint destruction.

Of interest, both of the non-RA patients with MIA serum concentrations >10 ng/ml showed signs of radiographic joint destruction. In contrast to the associations with RF and joint destruction, enhanced MIA serum concentrations were not associated with ESR, CRP, leucocyte counts and antinuclear antibodies in these patients. In addition, cultured RA as well as OA synovial fibroblasts did not produce MIA (data not shown).

Discussion

The results of our experiments indicate that increased serum concentrations of MIA are not limited to patients with malignant disease, but can also be measured in patients with rheumatic diseases, especially in patients with RA.

In malignant melanoma, most recently MIA has been established as a reliable serum parameter for metastasis [7, 8]. In these patients, MIA is produced by malignant melanoma cells, and serum concentrations correlate with the occurrence of metastasis. Conversely, in this study, none of the patients showing normal MIA concentrations developed metastasis in the follow-up period of 1 yr. Although the pathophysiological function of MIA in melanoma patients still remains to be elucidated, there is preliminary evidence that MIA supports metastasis by cell rounding and decreased adhesion of melanoma cells (A. K. Bosserhoff, personal communication).

In healthy organisms, MIA expression is limited to cartilaginous tissue. Both in developing as well as in mature cartilage, MIA mRNA and protein can be detected [5, 6]. In chondrogenesis, MIA is expressed in the axial and peripheral skeleton, and in mature cartilage chondrocytes are known to be the only source for MIA synthesis. This was supported by the fact that, in the present study, cultured RA (and OA) synovial fibroblasts, which are known to be involved in joint destruction, in particular at the sites of invasion of the proliferating synovial tissue into adjacent cartilage [9,
### Table 1. Frequency and degree of enhanced concentrations of MIA in sera of patients with different rheumatic diseases. ELISA cut-off 6.5 ng/ml

<table>
<thead>
<tr>
<th></th>
<th>RA</th>
<th>PsA</th>
<th>HLA B27</th>
<th>OA</th>
<th>Gout</th>
<th>SLE</th>
<th>SSc</th>
<th>RP</th>
<th>MM</th>
<th>NP</th>
</tr>
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<tbody>
<tr>
<td>Patients examined</td>
<td>46</td>
<td>29</td>
<td>6/12</td>
<td>12</td>
<td>12</td>
<td>39</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>120</td>
</tr>
<tr>
<td>Number of patients with elevated MIA serum concentration</td>
<td>24/46</td>
<td>7/29</td>
<td>6/12</td>
<td>3/12</td>
<td>0/12</td>
<td>(1)/39</td>
<td>0/10</td>
<td>0/10</td>
<td>100/100</td>
<td>1/120</td>
</tr>
<tr>
<td>MIA serum concentration, mean ± s.d. (ng/ml)</td>
<td>10.3 ± 10.2*</td>
<td>4.8 ± 3.2</td>
<td>6.6 ± 3.0</td>
<td>5.2 ± 1.4</td>
<td>2.6 ± 1.0</td>
<td>4.4 ± 2.3</td>
<td>3.5 ± 2.8</td>
<td>3.4 ± 2.7</td>
<td>18.8 ± 12.5</td>
<td>3.6 ± 2.8</td>
</tr>
<tr>
<td>MIA serum concentration, range (ng/ml)</td>
<td>2.9–100</td>
<td>0–14.8</td>
<td>2.1–11.7</td>
<td>3.3–7.8</td>
<td>1.0–3.8</td>
<td>1.2–6.6</td>
<td>1.1–4.5</td>
<td>1.5–5.5</td>
<td>7.1–82</td>
<td>1.8–7.6</td>
</tr>
</tbody>
</table>

*P < 0.05 as compared to healthy individuals (NP).
MM = malignant melanoma; NP = healthy individuals. For other disease abbreviations, see text.
Fig. 2. Concentrations of MIA in sera of patients with different rheumatic diseases (values are the mean ± s.d. in ng/ml). Values in parentheses indicate the number of patients.

Table 2. Association between elevated MIA serum concentrations (MIA+) and RF, and between elevated MIA concentrations and the presence of radiographic joint destruction in RA patients (n = 46; MIA+, n = 24; MIA−, n = 22)

<table>
<thead>
<tr>
<th>Frequency of combination</th>
<th>Percentage of combination</th>
</tr>
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<tbody>
<tr>
<td>MIA+/RF+</td>
<td>19/24</td>
</tr>
<tr>
<td>MIA+/RF−</td>
<td>5/24</td>
</tr>
<tr>
<td>MIA−/RF+</td>
<td>8/22</td>
</tr>
<tr>
<td>MIA−/RF−</td>
<td>14/22</td>
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</tbody>
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Significance for association of MIA with RF: P = 0.0063 (Fisher’s exact test).

<table>
<thead>
<tr>
<th>Frequency of combination</th>
<th>Percentage of combination</th>
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<tbody>
<tr>
<td>MIA+/joint destruction +</td>
<td>18/24</td>
</tr>
<tr>
<td>MIA+/joint destruction −</td>
<td>6/24</td>
</tr>
<tr>
<td>MIA−/joint destruction +</td>
<td>7/22</td>
</tr>
<tr>
<td>MIA−/joint destruction −</td>
<td>15/22</td>
</tr>
</tbody>
</table>

Significance for association of MIA with joint destruction: P = 0.007 (Fisher’s exact test).

10] did not produce enhanced amounts of MIA. This is in contrast to cartilage oligomeric matrix protein (COMP), which has been considered as a marker for cartilage destruction, but is produced by both chondrocytes and synovial fibroblasts [11].

Little is known about the regulation of MIA synthesis and the physiological properties of MIA in cartilage metabolism, but there are indications that transcription of MIA might be dependent in part on nuclear transcription factors such as NFκB [3, 5]. As nuclear transcription factors including NFκB are also activated in various pathways leading to joint destruction in RA [12–14], we examined the presence of MIA in serum of patients with different rheumatic diseases and its association with laboratory parameters and radiological joint destruction.

Interestingly, only patients with RA showed serum levels of MIA as elevated as the majority of the melanoma patients, whereas in all patients with other rheumatic diseases, only slightly elevated MIA serum levels were detected. Therefore, it can be speculated that MIA is released actively from chondrocytes during the process of joint destruction in these patients, and is only in part due to a passive release of MIA from necrotic or apoptotic chondrocytes. For example, enhanced MIA serum concentrations might reflect an enhanced chondrocyte activation, e.g. the upregulation of interleukin (IL)-1-dependent collagenase synthesis in articular chondrocytes. This hypothesis that MIA reflects chondrocyte activation, but not disease activity per se, is not only supported by our data showing no association of elevated MIA levels with inflammation parameters, but also by recent findings showing progressive radiological joint destruction without inflammation in numerous RA patients [15]. Again, this is different to the findings addressing COMP. While MIA may be a marker for activated chondrocytes during cartilage destruction, COMP is also found in the cartilaginous matrix and is probably more passively released.

Numerous similarities of melanoma cells and chondrocytes exist in the metabolic pattern and the interaction with matrix. Both are known to produce numerous growth regulating factors such as transforming growth factors (TGFs), basic fibroblast growth factor (bFGF) and insulin-like growth factor (IGF) [16–18]. Growth factors enhance both catabolic as well as anabolic pathways in articular chondrocytes [19]. IGF and bFGF preferentially enhance matrix synthesis [18, 19], whereas TGF can also act as a catabolic stimulus [19]. Both melanoma cells and chondrocytes are regulated by or synthesize various cytokines, which may also trigger the production of matrix-degrading enzymes [16, 17, 20, 21]. Chondrocytes [21, 22] as well as melanocytes are susceptible to stimulation with IL-1, and melanoma cells may support metastasis by IL-1 production [23]. In articular and transformed human chondrocytes, as well as in human breast tumour tissue, collagenase-3 has been found, which could be upregulated by IL-1β and tumour necrosis factor alpha [24].

Of interest, distinct mechanisms of matrix degradation are similar in metastasis of melanoma and rheumatoid joint destruction. Matrix metalloproteinases and cysteine proteases (e.g. collagenases and cathepsins B, D, H and L), as well as the proteases plasmin and cathepsin D, which are found in considerable amounts at the sites of articular degradation in rheumatoid joints [10, 25, 26], are also key molecules in metastatic pathways in melanoma patients [27–32]. Similar to RA, degradation of type IV collagen is dependent on the presence of collagenase [33], whereas matrix metalloproteinase-2 contributed to early tumour progression in melanoma lesions [34]. Of interest, fibroblast-dependent matrix degradation by cathepsin B, which is a major pathway in rheumatoid joint destruction [9, 10, 35], can also be detected in fibroblasts derived from melanoma tissue [29]. Although angiogenesis is not only crucial for malignant diseases, but also plays a considerable role in the initiation and perpetuation of inflammation in rheumatoid synovium, current knowledge does not support the hypothesis that angiogenesis contributes to elevated MIA serum concentrations in RA synovium, as previous experiments revealed that endothelial cells...
are not a major source for MIA synthesis (A. K. Bosserhoff, unpublished data).

In addition to RF, with which elevated MIA serum concentrations are associated, an enhanced serum concentration of MIA might be a valuable laboratory parameter for earlier diagnosis, addressing the problem of differential diagnosis of oligoarthritic early RA vs non-joint-destructive diseases such as SLE. These hypotheses are further strengthened by the fact that in none of the patients with non-joint destructive rheumatic diseases, such as SLE, SSc and RP, could elevated MIA serum levels be found. Finally, the association of MIA with joint destruction, and not with laboratory parameters for inflammation in RA, supports the importance of non-T-cell-dependent pathways in joint destruction of RA synovial pathophysiology.

In summary, the results of the study indicate that an enhanced concentration of MIA is a novel, easily quantifiable serum marker for RA and might therefore be useful in the diagnosis and differential diagnosis of RA. In addition, the presence of elevated levels of MIA in serum very likely reflects chondrocyte activation in conjugation with joint destruction in RA.

Acknowledgements

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