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Melanoma inhibitory activity in melanoma diagnostics and therapy – a small protein is looming large

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Abstract: Malignant melanoma is a highly aggressive cancer with a very poor prognosis after the onset of metastasis. We have previously demonstrated that the protein melanoma inhibitory activity (MIA) is involved in the metastasis of and immunosuppression in malignant melanoma. Recently, we further established MIA as a therapeutic target to inhibit metastatic spread in malignant melanoma. We could show that an inhibition of MIA by a synthetic peptide decreased both the number of metastases as well as immunosuppression in a murine model of malignant melanoma. To control recurrence after surgical resection of a primary lesion, it is paramount to have diagnostic tools available that can detect a relapse due to the strong metastatic potential of melanoma. This follow-up is maintained with periodic re-examinations. Due to high cost and the associated radiation exposure, radiology examinations are avoided if possible. The analysis of prognostic markers in patient serum is therefore attractive. In this review, we focus on the quantitative analysis of the MIA protein as a prognostic tool because it has proven to be a useful serum marker for documenting disease progression of malignant melanoma. The MIA quantification assay itself is readily performed using an ELISA kit and common laboratory equipment. Because analysing MIA serum levels in combination with other established markers such as S100B improves their prognostic value, we feel that the quantification of MIA in the serum, among other markers, should be performed as a general standard of care in patients at risk of developing metastatic melanoma.

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Molecular background and therapeutic implications

Malignant melanoma is the most aggressive neoplasm of the skin. It is notable for its aggressive local growth and very early onset of metastasis. Melanoma inhibitory activity (MIA) protein is an 11 kDa protein that is strongly expressed and subsequently exocytosed by melanoma cells but not benign melanocytes (1). In an attempt to identify autocrine growth inhibitors of melanoma, MIA was purified from tissue culture supernatant of the human melanoma cell line HTZ-19 (1). However, subsequent studies found that MIA contributes significantly to melanoma development, cellular invasion and formation of metastases (2,3). The metastatic spread of melanoma cells in syngeneic animals was found to be directly linked to the MIA expression level (4,5).

Melanoma inhibitory activity is translated as a 131 amino acid precursor protein and processed into the mature protein consisting of 107 amino acids after shedding the secretion signal sequence (1). Subsequently, MIA is intra-cellularly transported to the cell rear (6,7). Following its locally directed exocytotic secretion, MIA binds to the cellular adhesion factors integrin $\alpha_4\beta_1$ and integrin $\alpha_5\beta_1$. MIA also has affinity for the integrin binding sites of molecules present in the extracellular matrix such as fibronectin, laminin and tenascin (8). Via this two-pronged mechanism, cell adhesion contacts to the extracellular matrix are selectively weakened at the basal pole of melanoma cells. This localized cell detachment at the cell rear enables melanoma cells to migrate in a defined direction and invade surrounding tissues, which consequently supports metastatic spread (Fig. 1). MIA also supports melanoma-induced immunosuppression, a process mediated by binding to integrin $\alpha_4\beta_1$, which is expressed on leucocytes. Furthermore, it has been shown that MIA reduces proliferation and cellular attachment to extracellular matrix components of IL2-stimulated peripheral blood mononuclear cells. Inhibition of the cytotoxicity of lymphokine-activated killer cells by MIA was demonstrated in the same study (9).

The three-dimensional structure of MIA protein has already been elucidated by nuclear magnetic resonance spectroscopy (10,11) and X-ray crystallography (12). These studies suggest that MIA defines a novel class of secreted proteins, the MIA protein family, consisting of MIA and its homologues OTOR, MIA-2 and TANGO (MIA-3). The MIA protein family is the first and to date only described family of secreted proteins presenting an SH3 domain-like fold (13).

In line with other proposed targets for melanoma treatment (14,15), subsequent studies by our group provided insight into the mode of action of MIA, which as a result of this understanding at the molecular level, also led to an inhibition strategy. MIA is functionally active only as a homodimer, and interference in the MIA–MIA interaction by the treatment of melanoma-bearing mice with an appropriately selected peptide was shown to decrease metastatic spread significantly (16). In a murine model for hepatic metastasis using B16 melanoma cells, not only the metastatic spread into the liver significantly reduced after i.v. treatment with the MIA dimerization-inhibiting peptide AR71, but also MIA-induced immunosuppression was inhibited as evidenced by the increased presence

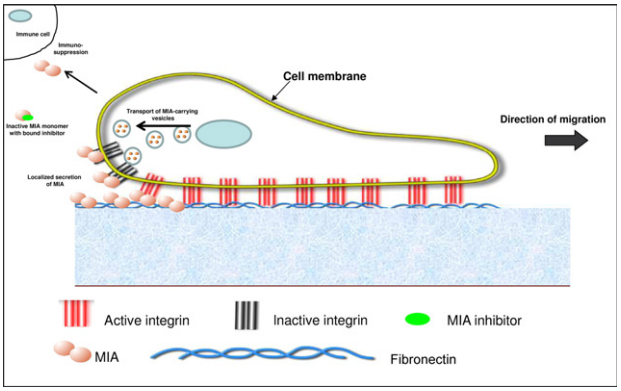

Figure 1. Schematic illustration of the molecular mode of action of melanoma inhibitory activity (MIA). Homodimeric MIA is secreted at the cell rear and binds to cell surface integrins as well as to the extracellular matrix, thereby facilitating localized detachment and directed migration of the melanoma cell. As a secondary function, MIA also mediates tumor-induced immunosuppression. Both effects can be impeded by a MIA dimerization inhibitor. Adapted reproduction with permission from (30).

of CD3-positive lymphocytes in the metastases and an increase in caspase3-induced apoptosis. We believe this to be a highly attractive inhibition strategy because it is based on the inhibition of an extracellular protein and therefore circumvents the many potential adverse effects of a drug requiring cellular permeability.

A more detailed summary of its molecular mechanisms, including its potential as a therapeutic target, has recently been published (17). In the following section, we wish to discuss the implications of MIA as a diagnostic marker of malignant melanoma.

MIA as a serum melanoma marker today

Because malignant melanoma has a grim prognosis after the onset of metastasis, it appears desirable to have a diagnostic marker for the progression of the disease. Similar to sentinel lymph node dissection or resection of the entire lymphatic basin, the determination of MIA serum levels is a prognostic procedure only, with no curative aspect.

However, routine follow-up is dependent on a reliable diagnostic procedure to provide a robust prognosis, especially in the light of newly approved therapies such as vemurafenib or ipilimumab. In our view, the serum level of MIA constitutes a substantive indicator of melanoma progression or recurrence, although the use of a serum marker alone is unlikely to entirely replace radiologic examinations. MIA was described as a diagnostic serum marker of melanoma progression in 1997, when it was shown that the MIA ELISA could classify 100% of the investigated sera of stage III and stage IV patients as positive for pathological MIA concentrations (18). The correlation of a high MIA serum level with a poor clinical prognosis was also confirmed by later studies for melanoma including uveal melanoma (19–21).

While the current AJCC standard serum marker for melanoma progression is lactate dehydrogenase (LDH), a study by Diaz-Lagares et al. (22) has found that both S100B and MIA have significantly higher prognostic values than LDH and YKL40. With a confidence interval of 0.95, the area under the curve (AUC) for the receiver operating characteristic (ROC) curve was 0.76 for S100B and 0.755 for MIA, which indicated a better prognostic value for both compared with LDH (AUC = 0.609). The authors concluded that the combination of MIA and S100B comprised the best prognostic indicator. In two older studies directly comparing S100B and MIA, a better correlation with disease progression was observed with S100B (23,24).

Another study by Hofmann et al. investigated the correlation between lymph node metastases and serum MIA levels. The authors found a correlation between the serum MIA level and the status of lymph nodes in the affected basin (25). This study confirms previous work by Vucetic et al. These authors also found a correlation between serum MIA levels and lymph node status. The correlation was characterized by an ROC with an AUC of 0.912 (with no confidence interval given), indicating that MIA has a high prognostic value for lymph node status (26).

Work by Henry et al. (27) found that the best overall prognostic accuracy was achieved with the combination of MIA and plasma proteasome level.

Essler et al. found that when comparing the prognostic value of 18-FDG PET/CT with S100B or MIA, serum MIA levels had a similar accuracy to 18-FDG PET/CT but a lower sensitivity. However, it was noted that patients who were 18-FDG PET/CT-positive and MIA or S100B negative had a better overall survival than 18-FDG PET/CT-positive and MIA- or S100B-positive patients. This led to the notable conclusion that in 18-FDG PET/CT-positive patients, molecular serum markers were more indicative of malignant potential and therefore higher mortality rather than overall disease burden (28).

It is generally difficult to directly compare studies of melanoma serum markers because the exact time of serum collection and whether and to what extent the included patients underwent lymph node dissection are not often stated. Notably, there is no fixed cut-off serum MIA level in the literature over which a sample is classified as positive. Most laboratories employ a cut-off higher than the manufacturer's recommendation of 6.5 ng/ml (95th percentile).

We therefore conclude that MIA constitutes a reliable but occasionally overlooked marker for melanoma progression, lymph node status and overall survival. The diagnostic procedure of determining the serum MIA level is inexpensive and readily performed using an ELISA kit.

Outlook

It is difficult to predict future developments with regard to prognostic melanoma markers, but we feel that the parallel determination of several serum markers, such as MIA, will be used to increase the prognostic value of the analyses. We do not believe that the analysis of mRNA markers from circulating melanoma cells in peripheral blood by qRT-PCR is the optimal approach, as even the authors of some these studies conclude that these blood analyses are not a reliable prognostic tool (29).

Moreover, with new emerging therapies such as vemurafenib for melanoma patients with the BRAF V600E mutation or ipilimumab as immunotherapy, we expect a general increase in demand for monitoring melanoma markers during follow-up.

Finally, we expect the clinical relevance of MIA as a therapeutic target to reach its full potential when MIA dimerization inhibitors arrive in clinical trials.

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Author contributions

AR and AKB both contributed to writing the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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