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Review

Expression, function and clinical relevance of MIA (Melanoma Inhibitory Activity)

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Summary. Despite its ambiguous name the protein melanoma-inhibitory-activity (MIA) was identified as a key molecule involved in progression and metastasis of malignant melanomas. Therefore, in this review we intend to update the current knowledge on expression patterns, transcriptional regulation, function and clinical relevance of MIA. Furthermore, we will cover the recently discovered MIA homologous proteins OTOR/MIAL, MIA 2 and TANGO.

In order to identify autocrine growth-regulatory factors secreted by melanoma cells, MIA was purified and cloned. Subsequent analyses of non-neoplastic tissues revealed specific MIA expression patterns in cartilage. In neoplastic tissues MIA expression was detected in malignant melanomas, in chondrosarcomas and less frequently in a variety of different adenocarcinomas including breast and colon cancers. For melanoma cells and chondrocytes it was shown that regulation of expression pattern was controlled on the level of mRNA transcription by defined transcription factors.

Evidence obtained from *in vitro* and *in vivo* experiments indicated that MIA plays an important functional role in melanoma metastasis and invasion. A number of studies from different laboratories evaluated MIA as a highly specific and sensitive marker, clinically useful for follow-up and therapy-monitoring of patients with malignant melanomas. In addition, preliminary data suggests a further potential application as a surrogate marker for measuring cartilage damage in rheumatoid arthritis.

Recently, it has become evident that MIA belongs to a gene family of four homologous proteins, MIA, OTOR (FDP, MIAL), MIA 2 and TANGO. Determination of the three-dimensional structure in solution identified MIA as the first member of this novel family of secreted, extracellular proteins adopting an SH3 domain-like fold. The data suggest specific protein-protein interactions with components of the extracellular matrix and possibly epitopes on cellular surfaces and will certainly attract further interest and investigations.

Key words: Malignant melanoma, Invasion, Metastasis, Serum marker, Transcriptional regulation

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Introduction

Melanoma progression and tumor growth are regulated by a complex network of paracrine and autocrine positive and negative growth factors. In order to characterise autocrine growth-regulatory factors, the melanoma inhibitory activity (MIA) protein was identified within a growth-inhibiting activity purified from the tissue culture supernatant of the human melanoma cell line HTZ-19d (Bogdahn et al., 1989; Blesch et al., 1994). Using different chromatographic separation steps, MIA was purified to homogeneity. Purified MIA confers growth-inhibition to malignant melanoma cells *in vitro* and causes significant alteration of cell morphology as melanoma cells round up. MIA activity was shown to be heat-resistant when incubated for three minutes at 100 °C, stable during acid treatment and sensitive to digestion with trypsine. Based on partial peptide sequences obtained by Edman degradation, a partial cDNA was amplified by reverse transcriptase-PCR and used as a probe to isolate the fully encoding cDNA (Blesch et al., 1994).

Cloning of MIA and expression pattern

The first human MIA cDNA sequence was obtained from a λgt11 phage insert isolated by screening a cDNA library of the human melanoma cell line HTZ-19d (Fig. 1) and verified by cDNA and genomic clones obtained from different sources. Analyzing normal skin and skin-derived melanocytic tumors by semi-quantitative RT-PCR did not reveal significant MIA mRNA levels in normal skin and melanocytes but moderate levels in the majority of benign melanocytic nevi and very high levels in all primary and metastatic malignant melanomas (Blesch et al., 1994; Bosserhoff et al., 1996). Subsequent

studies confirmed specific expression patterns of MIA mRNA and protein in malignant tumors, foremost malignant melanomas and absence of MIA mRNA expression in benign melanocytes cultured from normal skin biopsies (van Groningen et al., 1995; Bosserhoff et al., 1999; Perez et al., 2000 (Fig. 2)). The expression pattern data obtained by RT-PCR studies were supported by in situ hybridizations and coincided with protein expression visualized by immunohistochemistry (Fig. 3).

MIA mRNA was identified independently by a differential display approach comparing differentiated and dedifferentiated chondrocytes *in vitro*. Since dedifferentiation was accelerated by retinoic acid in this study, MIA was also referred to as CD-RAP, cartilage-derived retinoic acid-sensitive protein (Dietz and Sandell, 1996). Subsequent studies of murine embryos and murine adult tissues demonstrated cartilage-specific mRNA expression patterns (Fig. 4; Bosserhoff et al., 1997a).

Expression was limited to cartilaginous tissues,

```
  1        CCAGCACCCCCTTGCTCACTCTCTTGCTCACAGTCCACGATGGCCCGGTCCCTGGTGTGC
  1                                                  M  A  R  S  L  V  C

 61        CTTGGTGTCATCATCTTGCTGTCTGCCTTCTCCGGACCTGGTGTCAGGGGTGGTCCTATG
  8         L  G  V  I  I  L  L  S  A  F  S  G  P  G  V  R  G  G  P  M

121        CCCAAGCTGGCTGACCGGAAGCTGTGTGCGGACCAGGAGTGCAGCCACCCTATCTCCATG
 28         P  K  L  A  D  R  K  L  C  A  D  Q  E  C  S  H  P  I  S  M

181        GCTGTGGCCCTTCAGGACTACATGGCCCCCGACTGCCGATTCCTGACCATTCACCGGGGC
 48         A  V  A  L  Q  D  Y  M  A  P  D  C  R  F  L  T  I  H  R  G

241        CAAGTGGTGTATGTCTTCTCCAAGCTGAAGGGCCGTGGGCGGCTCTTCTGGGGAGGCAGC
 68         Q  V  V  Y  V  F  S  K  L  K  G  R  G  R  L  F  W  G  G  S

301        GTTCAGGGAGATTACTATGGAGATCTGGCTGCTCGCCTGGGCTATTTCCCCAGTAGCATT
 88         V  Q  G  D  Y  Y  G  D  L  A  A  R  L  G  Y  F  P  S  S  I

361        GTCCGAGAGGACCAGACCCTGAAACCTGGCAAAGTCGATGTGAAGACAGACAAATGGGAT
108         V  R  E  D  Q  T  L  K  P  G  K  V  D  V  K  T  D  K  W  D

421        TTCTACTGCCAGTGAGCTCAGCCTACCGCTGGCCCTGCC
128         F  Y  C  Q  *
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Fig. 1. Human MIA cDNA and protein sequence. The signal sequence responsible for translocation into the ER and subsequent secretion is underlined. The stop codon is marked by an asterisk (*). MIA is translated as a 131 amino acid precursor molecule and processed into a mature 107 amino acid protein after cleavage of the N-terminal secretion signal (Blesch et al., 1994).

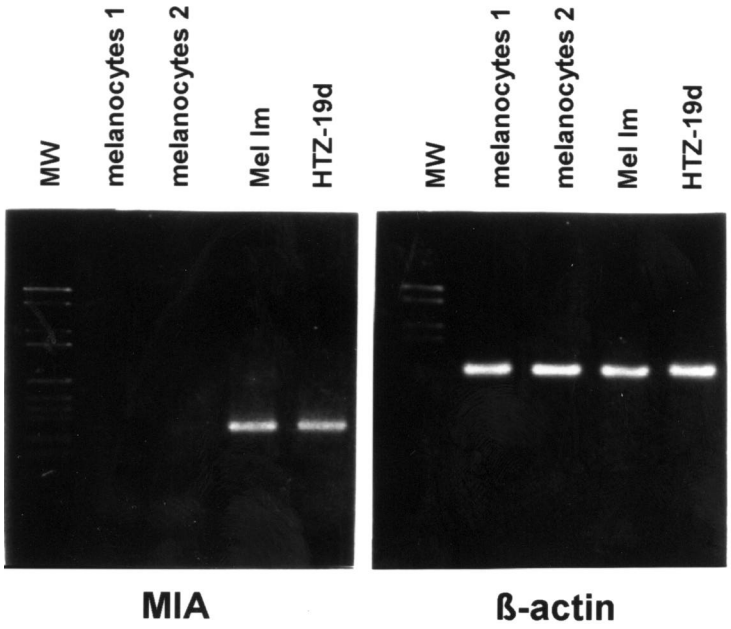

Fig. 2. MIA mRNA expression in melanocytes and melanoma cells. MIA cDNA was amplified by RT-PCR. Strong expression was detected in the melanoma cell lines (Mel Im, HTZ-19d), but not in human skin melanocytes. As a control ß-actin was amplified. The PCR products were separated on 1.5% agarose gels, stained with ethidiumbromide and visualized under UV light.

initiated with the advent of chondrogenesis and remained abundant throughout development of the cartilagenous skeletal system. Throughout fracture healing, MIA was detected in cartilage but not in bone or fibrous tissue. Thus MIA may be a molecular marker of cartilage formation during fracture healing (Sakano et al., 1999).

To determine whether MIA is associated with chondrocytic tumors, mRNAs from a variety of rodent tissues and cell lines were screened. Expression was detected in the Swarm rat chondrosarcoma and a chondrosarcoma cell line derived from it, but not in other tissues or tumors of non-cartilage origin except of melanomas. Immunolocalization revealed MIA protein in cartilage and confirmed the specific expression patterns determined by RT-PCR and *in situ* hybridizations (Fig. 5).

Xie et al. (2000) generated transgenic mice harboring the MIA promoter linked to the beta-galactosidase gene. Analysis of the transgene expression pattern by X-gal staining indicated beta-galactosidase activity in cartilage of embryos and adult animals. In addition, transient X-gal staining in mammary gland primordium from day 11.5 to 15.5 of gestation was detected. Histological examination revealed that the transgene is located in the epithelial cells of mammary buds. Previously, elevated levels of melanoma-inhibiting activity (MIA) were measured in the serum of a subgroup of patients with advanced-stage breast cancers. MIA expression in adenocarcinomas was therefore studied in a panel of 20 specimens obtained from 16 advanced-stage breast cancers and four metastases (Bosserhoff et al., 1999). Significant levels of mRNA were detected in 17 out of the 20 specimens and low levels in the other three tumours. Immunostaining visualised specific protein expression in the tumour cells of all 20 cancer specimens.

In summary, these results indicate that expression of MIA in non-neoplastic tissues is limited to cartilage and very few other tissues at specific developmental stages. However, under pathological circumstances high expression levels occur in melanomas, chondrosarcomas and less frequently in adenocarcinomas

Genomic structure

Both human and murine genomic MIA sequences were determined and deposited in the gene bank (X84707, X97965). The gene consist of 4 exons and is about 2kb in size (Fig. 6). The MIA locus was mapped as a single copy gene to human chromosome 19q13.32-13.33 (Koehler et al., 1996) and murine chromosome 7

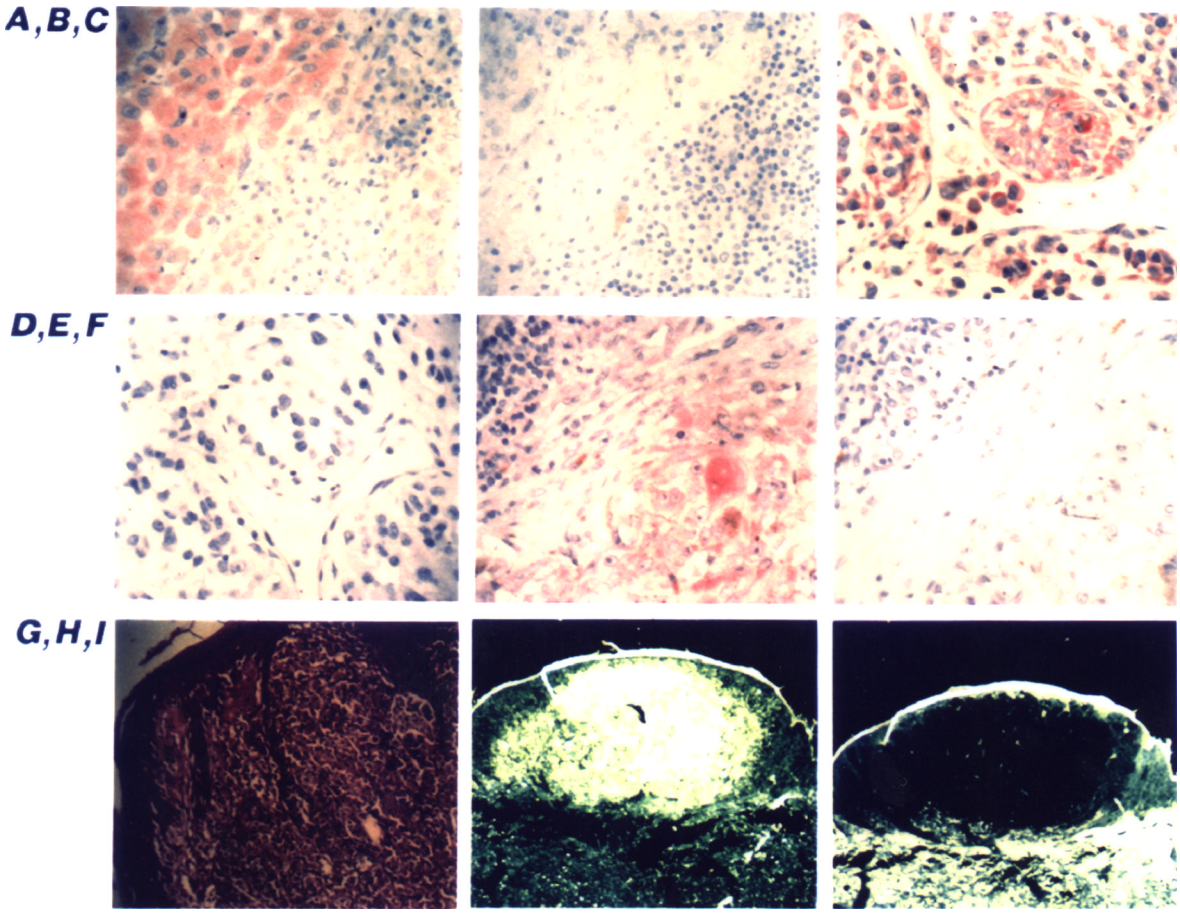

Fig. 3. Detection of MIA by immunohisto-chemistry and *in situ* hybridization. Primary cutaneous melanomas **(A-D, G-I)** and a melanoma metastasis **(E, F)** were immunostained **(A-F)** and analyzed by *in situ* hybridization **(G-I)**. **A, C and E** show immunostaining with a MIA-specific antibody; **B, D and F** are negative controls with unspecific primary antiserum; **G.** H&E staining. **H.** *in situ* hybridization with antisense, I with sense MIA riboprobes. x 100. (Bosserhoff et al., 1999).

(Bosserhoff et al., 1997a). Both chromosomes carry highly homologous gene regions, e.g. the gene for TGF-ß1 (transforming growth factor ß1) and part of the BMP family (bone morphogenic proteins). The exon-intron structure is highly conserved and was also found in all other species which have been analyzed so far, even in pufferfish (Tetraodon). Potential mouse mutants that mapped to the same region of chromosome 7 were identified. Two of the potential mutants with skeletal phenotypes were sequenced, *pudgy* (pu) and *extra toes with spotting* (XsJ) but revealed no mutations in the MIA coding sequence (Bosserhoff et al., 1997a).

Transcriptional regulation in malignant melanoma

The highly specific expression pattern of MIA in melanomas as compared to benign melanocytes stimulated us to investigate the mechanism of transcriptional regulation. We hypothesized that changes in the activity of the MIA promoter could possibly provide insight into gene regulatory mechanisms prototypic for malignant transformation of melanomas.

A fragment of approximately 1.4 kb 5´flanking DNA of the human gene conferred strong reporter gene expression in melanoma cells but not in melanocytes

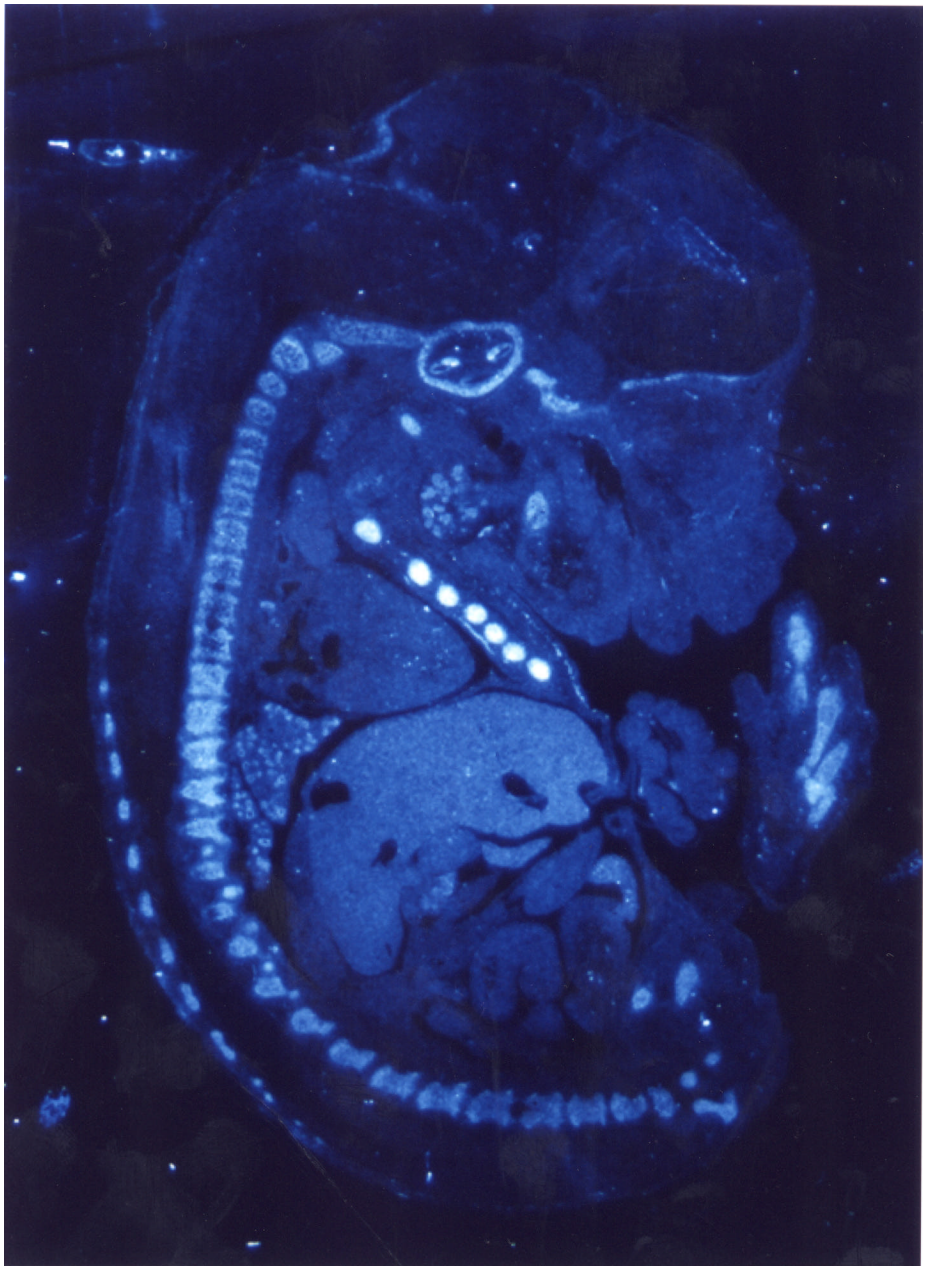

Fig. 4. MIA *in situ* hybridization of a mouse embryo day 14.5. For *in situ* hybridization an antisense MIA cRNA riboprobe was used. Strong signals were detected in cartilage. x 30. (Bosserhoff et al., 1997a).

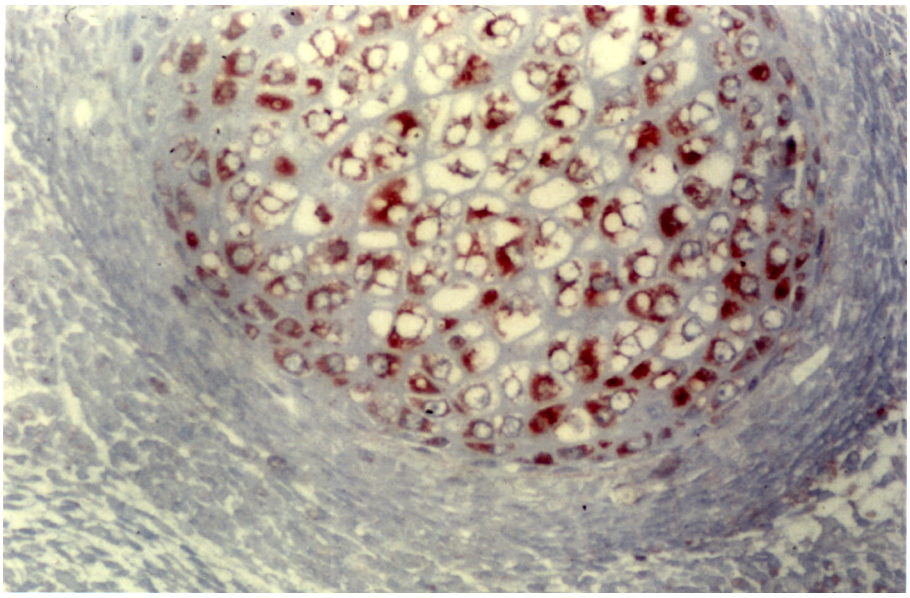

Fig. 5. Detection of MIA protein in cartilage. Immunohistochemical stainings of mouse cartilage were performed with an anti-MIA antibody and revealed strong immunosignals in and around chondrocytes. x 200. (Bosserhoff et al., 1997a).

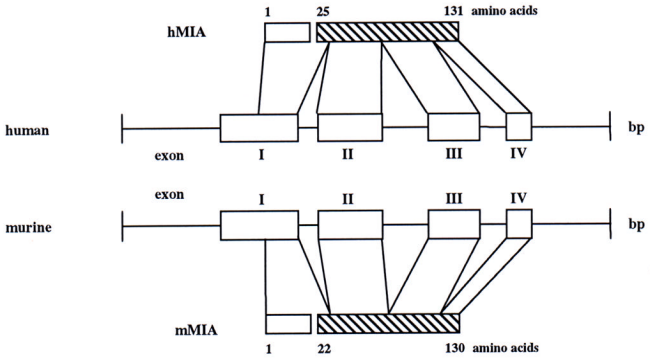

Fig. 6. Exon-intron structure of the human (h) and the murine (m) MIA genes.

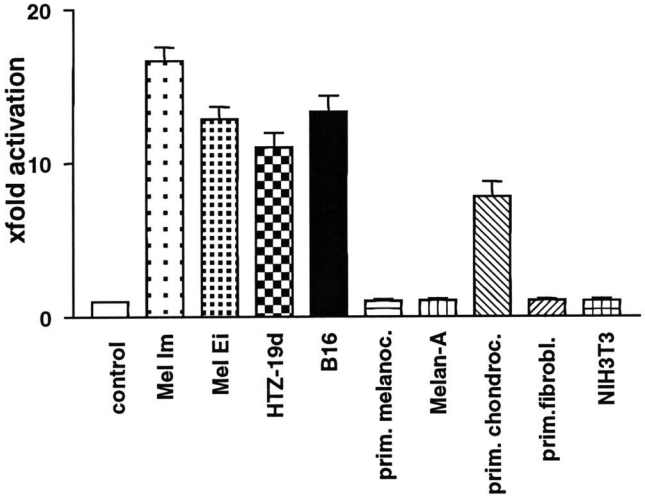

Fig. 7. Characterization of the MIA gene promoter. The MIA promoter was analyzed for transcriptional activity in melanocytes (human primary melanocytes, MelanA (murine melanocytes)), melanoma cells (Mel Im, Mel Ei, HTZ-19d (human), B16 (mouse)), primary human chondrocytes and fibroblasts (human primary fibroblasts, NIH3T3 (murine fibroblasts). Strong promoter activity was observed in melanoma cells and chondrocytes.

(Bosserhoff et al., 1996) (Fig. 7). Regulation of promoter activity usually results from binding of regulatory proteins (transcription factors) which specifically activate or repress the rate of transcription. To evaluate which transcription factors are important in regulating MIA expression, reporter constructs with a series of different truncated MIA promoter fragments were tested. The most active cis-regulatory element was identified between nucleic acids -230 and -130 (Golob et al., 2000). Promoter constructs with that particular region revealed melanoma-specific expression of the reporter gene, while constructs with deletions within that region completely lost transcriptional activity (Fig. 7). Cloning oligomeric fragments of this MIA promoter region in front of a minimal TK promoter identified a 30 bp enhancer element mediating expression of the reporter gene in melanoma cells but not in melanocytes or non-melanocytic cells. Gel mobility shift assays and southwestern blots led to the further identification of specific DNA-protein complexes in melanoma cells (Fig. 8). Fine mutational analysis of the cis-regulatory promoter elements revealed that two critical nucleic acid residues are essential for both transcriptional activity and formation of the band shift complexes. Mutagenesis of both base pairs abolished entirely promoter activity while mutation of only one of the base pairs caused only partial inactivation. By an initial small-scale affinity purification approach a protein of approximately 32 kDa in size was isolated from melanoma cells and referred to

as MATF ("melanoma-associated transcription factor") (Golob et al., 2000).

Transcriptional regulation in chondrocytes

Expression of MIA (CD-RAP) is initiated at the beginning of chondrogenesis and continues throughout cartilage development. To investigate transcriptional mechanisms mediating the cell type-specific expression in chondrocytes, deletion constructs of the mouse MIA promoter were cloned into reporter plasmids and assayed in chondrocytes. The results revealed a domain with high activity in chondrocytes (Xie et al., 1998) harboring a functional binding site of the transcription factor AP-2 (activating protein-2). Mutation of the AP-2 site caused decreased MIA promoter activity in C5.18 chondrocytes,

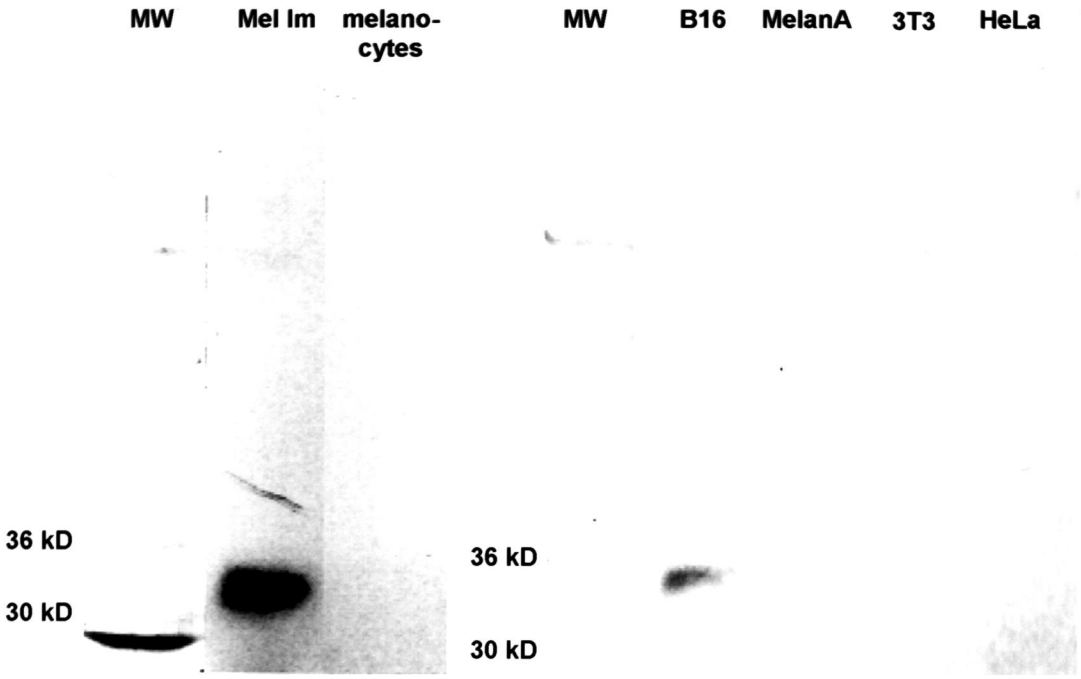

Fig. 8. Identification of a tumor- and cell type-specific DNA-protein complex in melanoma cells. Southwestern blotting identified a protein binding specifically to a cis-regulatory element of the MIA promoter. This protein is expressed in melanoma cells (Mel Im, B16), but not in melanocytes (primary human melanocytes, MelanA), fibroblasts (NIH3T3) and HeLa cells (cervical carcinoma cell line). The protein was designated MATF ("melanoma-associated transcription factor").

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MIA (Human)     MARSLVCLGVIILLSAFSGPGVRGGPMPKLADRKLCADQE    40
MIA (Murine)    MvwSpVlLGivvL.SvFSGPsradraMPKLADwKLCADeE    39
MIA (Bovine)    MAwSLVfLG.VvLLSAFpGPsagGrPMPKLADRKmCADeE    39
MIA (Rat)       MvcSpVlLGivIL.SvFSGlsradraMPKLADRKLCADeE    39

MIA (Human)     CSHPISMAVALQDYMAPDCRFLTIHRGQVVYVFSKLKGRG    80
MIA (Murine)    CSHPISMAVALQDYvAPDCRFLTIyRGQVVYVFSKLKGRG    79
MIA (Bovine)    CSHPISvAVALQDYvAPDCRFLTIHqGQVVYiFSKLKGRG    79
MIA (Rat)       CSHPISMAVALQDYvAPDCRFLTIyRGQVVYVFSKLKGRG    79

MIA (Human)     RLFWGGSVQGDYYGDLAARLGYFPSSIVREDQTLKPGKVD    120
MIA (Murine)    RLFWGGSVQGgYYGDLAARLGYFPSSIVREDlnsKPGKiD    119
MIA (Bovine)    RLFWGGSVQGDYYGDgAARLGYFPSSIVREDQTLKPaKtD    119
MIA (Rat)       RLFWGGSVQGDYYGDLAAhLGYFPSSIVREDlTLKPGKVD    119

MIA (Human)     VKTDKWDFYCQ                                 131
MIA (Murine)    mKTDqWDFYCQ                                 130
MIA (Bovine)    VKTDiWDFYCQ                                 130
MIA (Rat)       mKTDeWDFYCQ                                 130
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Fig. 9. Evolutionary conservation of the MIA gene. A comparison of human, murine, bovine and rat MIA peptide sequences reveals a high degree of homology.

indicating that this site functions as a cis-activating element. The results suggested that AP-2 is involved in regulation of MIA transcription in chondrocytes.

As the transcription factor Sox9 was identified to be important for chondrogenesis, the role of Sox9 in transcriptional regulation of MIA expression was analyzed (Xie et al., 1999). It was shown that Sox9 protein was able to bind to a SOX consensus sequence in the MIA promoter. Mutation of the SOX binding sequence diminished MIA promoter activity in chondrocytes while overexpression of SOX9 resulted in a dose-dependent increased activity in both chondrocytes and non-chondrogenic cells. Overexpression of SOX9 increased the level of endogenous MIA mRNA in chondrocytes, but was unable to induce endogenous gene expression in 10T1/2 mesenchymal cells or BALB/c-3T3 fibroblasts. In summary, these results suggested that both Sox9 and AP-2 play an important role in regulating MIA expression in chondrocytes but are not sufficient to initiate MIA expression on their own in non-chondrogenic cells.

MIA protein function

MIA is translated as a 131 amino acid precursor molecule and processed into a mature 107 amino acid protein after cleavage of a hydrophobic peptide signal directing the secretory pathway (Blesch et al., 1994). The amino acid sequence is highly conserved between species from fishes to mammals suggesting functional conservation (Fig. 9).

Based on monitoring biological activity during purification of the protein from tissue culture supernatant, MIA was initially believed to elicit antitumour activity by inhibiting proliferation of melanoma cell lines *in vitro* (Bogdahn et al., 1989; Blesch et al., 1994). However, further studies revealed expression patterns inconsistent with a tumor suppressor. Expression of MIA was not detected in normal skin and melanocytes but was associated with progression of melanocytic tumors (van Groningen et al., 1995; Bosserhoff et al., 1999). More recently, we observed that MIA specifically inhibits attachment of melanoma cells to fibronectin and laminin, thereby masking the binding site of integrins to these extracellular matrix components and promoting invasion and metastasis *in vivo* (Bosserhoff et al., 1998; Stoll et al., 2001). Thus, the growth-inhibitory activity *in vitro* may reflect the ability of the protein to interfere with the attachment of cell lines to culture dishes *in vitro* (Blesch et al., 1994).

Therefore, further experiments in hamster and mouse were performed to analyze the *in vivo* role of MIA during melanoma metastasis (Guba et al., 2000; Bosserhoff et al., in press). In a study of Guba et al. A-mel 3 hamster melanoma cells were transfected with sense- and antisense MIA cDNA and analyzed subsequently for changes in their tumorigenic and metastatic potential. Enforced expression of MIA in A-mel 3 cells significantly increased their metastatic potential as compared with control or antisense transfected cells but did not affect the growth rate of the primary tumor, cell proliferation or apoptosis. In addition, MIA overexpressing transfectants showed a higher rate of both tumor cell invasion and

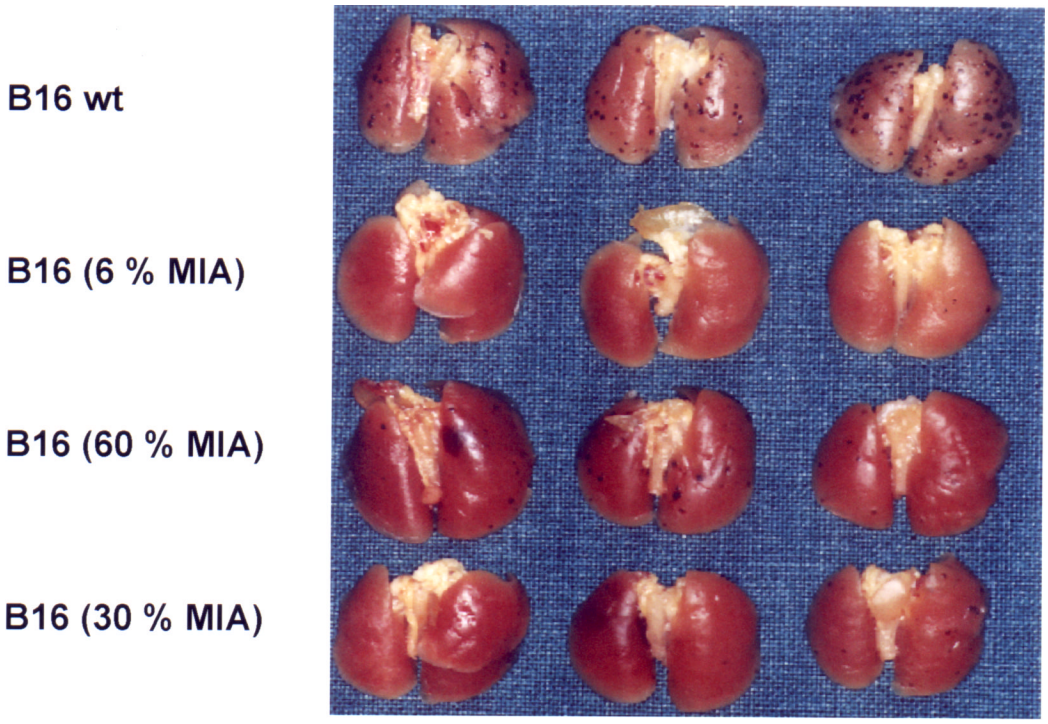

Fig. 10. Enhancement of metastasis in a murine melanoma model by MIA. In a murine model for *in vivo* metastasis using B16 mouse melanoma cells in C57Bl6 mice MIA function in metastasis was tested. B16 cell clones were stably transfected with antisense MIA cDNA constructs and expressed reduced amounts of MIA proteins (6, 30 and 60%). Dose-dependent reduction of metastasis was observed. Lung metastases are easily detected as black dots (Bosserhoff et al., Melanoma Res.).

extravasation. Consistently, cell clones transfected with an antisense MIA cDNA expression plasmid revealed significantly reduced metastatic potential. The changes in metastatic behaviour in correlation with the expression level of MIA provide evidence that upregulation of MIA during malignant transformation of melanocytic cells is causally involved in acquisition of the malignant cancer cell phenotype. These results were further confirmed by a second study using B16 mouse melanoma cells in C57Bl6 mice. Here, B16 mouse melanoma cells secreting different amounts of MIA were generated by stable transfection (Bosserhoff et al., in press). The capacity of these cell clones to form lung metastases in syngeneic C57Bl6 mice was strictly correlated to the level of MIA secretion (Fig. 10).

MIA protein structure

Recently it was shown by multidimensional NMR that recombinant human MIA adopts an SH3 domain-like fold in solution (Stoll et al., 2000, 2001), a structure with two perpendicular, antiparallel, three- and five-stranded beta-sheets (Fig. 11). Different from previously solved structures of proteins with SH3 domain folds, MIA is a single-domain protein and contains an additional antiparallel beta-sheet and two disulfide bonds. MIA is also the first prototypic member of a family of four extracellular proteins with an SH3 domain-like fold. NMR data were recently confirmed by x-ray crystallography (Lougheed et al., 2001). Furthermore, it was shown that MIA interacts with fibronectin and that MIA-interacting peptide ligands identified by phage display screening confirm with a consensus sequence of type III human fibronectin repeats, especially with FN14, which is close to an integrin alpha4beta1 binding site (Stoll et al., 2001).

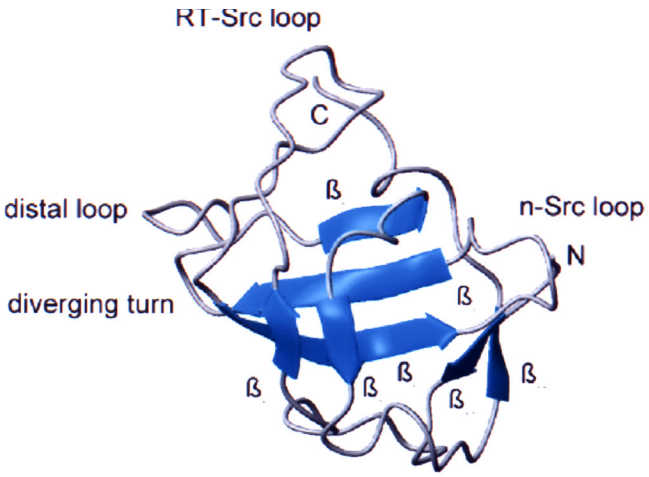

Fig. 11. Three-dimensional structure of MIA protein in solution. Multidimensional NMR studies revealed that MIA adopts an SH3 domain-like fold. The structure shows the typical two perpendicular, antiparallel, three- and five-stranded beta-sheets (Stoll et al., 2001; with permission of EMBO J.).

This may provide a mechanistic explanation for the role of MIA in metastasis *in vivo*, and supports a model in which the binding of human MIA to type III repeats of fibronectin competes with integrin binding, thus detaching cells from the extracellular matrix.

MIA as a serum marker in patients with malignant melanomas

Proteins strongly expressed by and released from tumor cells can be used as markers to monitor the development and systemic spread of the tumor disease. As studies of melanocytic tumours *in vitro* and *in vivo* indicated that MIA mRNA expression correlates with tumour progression, it was investigated whether MIA provides a clinically useful parameter in patients with metastatic melanoma stages III and IV. Therefore, a quantitative ELISA was designed and MIA serum levels were correlated with clinically determined melanoma stages (Bosserhoff et al., 1997b, 2000; Deichmann et al., 1999; Dréau et al., 1999; Schmitz et al., 2000; Stahlecker et al., 2000).

In the first study (Bosserhoff et al., 1997b) the cut-off for positive values (97th percentile) was set at 8.8 ng/ml based on measuring sera from a control group of healthy blood donors. 97% of sera obtained from patients with metastasized malignant melanomas in stage IV revealed enhanced MIA values and 81% of the sera from patients in stage III. After surgery MIA serum levels dropped significantly. Patients responding to chemotherapy showed decreasing MIA serum levels, while patients with progressive disease during or after chemotherapy had increasing MIA serum levels. Furthermore, slightly enhanced MIA serum levels in 13 and 23% of patients with stage I and II disease, respectively, were reported. Measuring repeatedly sera of 350 patients with a history of stage I or II melanoma during follow-up, 32 patients developing positive MIA values were detected. At the time of serum analysis, 15 of them had recurrent metastatic disease and one further patient presented with metastatic disease 6 months later. In contrast, none of the patients with normal MIA serum levels developed metastases during the follow-up period of 6-12 months. It was concluded that MIA represents a clinically useful, novel serum marker for systemic malignant melanomas revealing a very high sensitivity and specificity. Potential clinical applications included detection of occult metastases, detection of progression from localized to metastatic disease during follow-up and monitoring therapy of advanced melanomas (Bosserhoff et al., 1997b). Additional studies confirmed these results with minor differences in sensitivity and specificity (Dreau et al., 1999; Schmitz et al., 2000; Stahlecker et al., 2000; Bosserhoff et al., 2000).

Interestingly, MIA seropositivity was also reported for some patients with advanced gastrointestinal carcinomas and correlated with a very poor prognosis, suggesting that MIA serum levels correlates with progression to systemic metastasis in different types of

malignant tumors (Wagner et al., 2000).

Recently, S100 has been established as a second marker for monitoring patients with malignant melanomas. Initially, S100 was described as a serum marker for detection and quantitation of damage to the central nervous system, and was later measured in melanoma patients. S100 is a cytoplasmatic, calcium-binding protein with a molecular weight of 21 kDa and expressed in a wide variety of different cell types including astrocytes, Schwann cells and satellite cells in sympathic ganglia, and also in melanocytes and malignant melanomas. A number of studies investigated S100 as a marker for clinical staging and monitoring of metastatic malignant melanomas.

Several studies compared the significance of both markers, MIA and S100, for monitoring melanoma therapy and follow-up (Djukanovic et al., 2000; Schmitz et al., 2000; Juergensen et al., 2001). MIA and S100 were measured in sera from patients using an ELISA and a chemiluminescent assay (LIA), respectively. In the study of Juergensen et al. the response to chemotherapy in stage IV disease and relapse of melanoma during follow-up correlated with changes in MIA and S100 serum levels. In comparison, MIA revealed slightly higher specificity and sensitivity than S100. Djukanovic et al. observed in 81.5% (S100) and 73.8% (MIA) of the patients a direct correlation between serum marker values and clinical course. S100 beta levels were falsly positive in approximately 20% of the sera and falsely negative in 20%. For MIA measurements the respective values were 8% falsely positive and 32.5% falsely negative sera.

In conclusion, these data demonstrate that both markers are currently the most useful serum parameters to detect progression from localized to metastatic disease and for monitoring therapy of advanced melanomas. Minor differences in sensitivity and specificity are more likely to reflect subtle variations in technology than significant tumorbiological differences.

A further study investigated MIA as a potential serum marker for monitoring uveal melanomas and metastases thereof (Schaller et al., 2000). In patients with locally confined disease, MIA serum concentrations were below the cut-off in all cases, whereas in patients with overt metastatic disease the serum values were significantly elevated. These findings suggest an additional useful application of MIA as a serum marker for monitoring patients with uveal melanomas.

MIA as a serum marker in diseases other than malignant melanoma

High levels of MIA expression were observed in chondrocytes and therefore MIA was also investigated as a potential serum marker for rheumatoid arthritis and cartilage damage. MIA serum concentrations in patients with different rheumatic diseases were measured, correlated with inflammatory parameters and/or with the degree of joint destruction and compared with healthy individuals and melanoma patients (Muller-Ladner et al., 1999). Increased MIA serum concentrations were found only in patients with rheumatic diseases associated with joint destruction, such as rheumatoid arthritis, osteoarthritis, HLA B27-associated oligoarthritis, and psoriatic arthritis. Within these rheumatic diseases, the most significant increase in MIA serum concentrations was measured in patients with rheumatoid arthritis, associated with rheumatoid factor positivity and joint destruction. The authors concluded that in addition to rheumatoid factor, MIA might be useful to discriminate rheumatoid arthritis from non-destructive rheumatic diseases, and that the presence of elevated levels of MIA in serum was likely to reflect joint destruction in rheumatoid arthritis.

Neidhart et al. investigated the effect of pronounced physical exercise on MIA serum levels during and after Marathon running. Marathon runners have an increased risk of developing degenerative joint diseases. During running elevation of multiple cytokines in the blood can be measured and has been attributed to inflammatory processes in response to subtle tissue damage. Sera from eight endurance-trained runners were collected at different time points before, during and after the run. For controls, serum specimens were obtained from healthy blood donors and further from patients with articular knee injuries, rheumatoid arthritis or osteoarthritis. As compared with healthy controls, the runner's baseline serum levels of MIA were significantly increased. Elevation of MIA in the serum was comparable to measurements obtained from patients with rheumatoid arthritis. Only modest changes of MIA levels occurred during the run, while significant increases were observed 24 and 48 hours after running. The authors concluded from their data that elevated baseline levels of MIA might reflect increased joint matrix turnover and/or damage due to prior extreme physical training. Increased MIA levels after running were suggested to result from secondary inflammatory or reparative processes. These data suggest that MIA may be a marker for distinct aspects of articular metabolism and/or damage both under pathophysiological circumstances and after physical exercise.

Recently, MIA levels were measured in cerebrospinal fluid specimens obtained from patients with spinal diseases (Natsume et al., 2001). Cerebrospinal fluid samples were collected from patients with meningiomas, neurinomas, arachnoid cysts, cervical spondylotic myelopathy, lumbar disc herniation, lumbar canal stenosis and scoliosis. The concentrations of MIA in cervical myelopathy, lumbar canal stenosis, and lumbar disc herniation was significantly higher than in the control group. The authors concluded from their data that MIA was significantly elevated in spinal diseases that cause spinal stenosis. The study reports for the first time that enhanced MIA levels can be measured in cerebrospinal fluid as a result of damage or stress of structures in the central nervous system and could possibly be used as a marker for spinal diseases. Since

MIA in not expressed in the central nervous system under physiological circumstances, this study is potentially very interesting but awaits further confirmation.

A novel family of MIA-related genes

Recently, a novel gene, OTOR (other designations: FDP (fibrocyte-derived protein), MIAL (MIA-Like)), was cloned by two different experimental strategies and shown to be a close homologue of MIA (Cohen-Salmon et al., 2000; Robertson et al., 2000). Cohen-Salmon et al. (2000) identified OTOR via substractive cDNA screening in search for genes which are preferentially expressed in the inner ear. Robertson et al. (2000) performed cluster analysis of cochlear ESTs and identified OTOR to be uniquely expressed in the cochlea. OTOR encodes a small protein of 128 amino acids which is 43% identical and 67% similar to MIA. Both proteins have hydrophobic N-terminal regions with features characteristic for eukaryotic secretory signal peptides. After cleavage of the leader peptides, the mature MIA and OTOR proteins share 46% identical and 81.2% similar amino acids. All four cystein-residues, that are known to be essential for stabilizing the three-dimensional structure, are conserved. In addition all structural motifs important for the SH3 domain-like folding are highly conserved.

Subsequent studies of OTOR expression detected high levels of mRNA in cochlea and lower levels in the eyes and spinal cord (Robertson et al., 2000). In mouse embryos OTOR was expressed in the mesenchyme surrounding the otic epithelium starting at embryonic day 10.5. During development, these cells progressively aggregate, condense and differentiate into cartilaginous cells forming the otic capsule, which then no longer expresses OTOR. In contrast fibrocytes surrounding the epithelia continue to express significant OTOR mRNA levels but not MIA (Cohen-Salmon et al., 2000). There is preliminary evidence suggesting that OTOR may functionally be important for chondrogenesis as an *in vitro* antisense nucleotide approach using microdissected periotic mesenchyme cultures showed significant reduction in chondrogenesis (Cohen-Salmon et al., 2000).

OTOR was mapped to human chromosome 20p11.13 - p12.1 and was postulated to be a candidate gene for specific forms of deafness associated with malformations of the otic capsule (Cohen-Salmon et al., 2000). In addition, two novel members of the MIA gene family, MIA-2 and TANGO, are currently being investigated (Lougheed et al., 2001; own unpublished results). They share high homology with MIA and raise the possibility that function may be redundant in some tissues (Fig. 12).

Conclusion

Clinically, MIA has already been established as a serum marker in patients with cutaneous and uveal melanomas. The question of whether it is also a useful marker for other diseases such as rheumatoid arthritis or

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hMIA        MARSLVCLGVIILLSAFSGPGVRG.....GPMPKLADRKL     35
hOTOR       MARiLllflpglvavcavhg.........ifMdrLAskKL     31
hMIA-2      MAkfgVhrilllaiSltkc.........lestklLADlKk     31
hTANGO      MAaapglLvwllvLrlpwrvpg...qldpstgrrfsehKL     37
Consensus      SIGNALpeptid                    la  kl

hMIA        ADQEASHPISMAVALQDYMAPDCRFLTIHRGQVVYVFSK      75
hOTOR       ADdEavytISlAsAqeDYnAPDCRFinvkkGQqiYVySK      71
hMIA-2      gDlEaealInrvsAmrDYrgPDCRyLnftkGeeisVyvK      71
hTANGO      ADdEaSmlmyrgeALeDftgPDCRFvnfkkGdpVYVyyK      77
Consensus   cad ec   i    a  dy  pdcrf n  kg   yvy k

hMIA        LKGR..GRLFWGGSVQGDYYGDLAARLGYFPSSIVREDQT    113
hOTOR       LvkengageFWaGSVyGDg.qDemgvvGYFPrnlVkEqrv    110
hMIA-2      LaGe..redlWaGSkgke........fGYFPrdaVqieev    101
hTANGO      Larg..wpevWaGSVgrt........fGYFPkdliqvvhe    107
Consensus   l         wagsv            gyfp   v

hMIA        LKPGKVDVKTDKWDFYCQ                        131
hOTOR       yqeatkeVpTtdiDFfCe                        128
hMIA-2      fiseeiqmsTkesDFlCl                        119
hTANGO      ytkeelqVpTDetDFvCf                        125
Consensus          v t   df c
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Fig. 12. Identification of novel members of the MIA gene family. The MIA gene family consists of four members: MIA, OTOR, MIA-2 and TANGO, which show a high degree of homology and similarity to each other. The conserved cysteins are indicated by boxes.

lesions needs to be further investigated.

The solution of the three-dimensional structure identified MIA as the first extracellular protein adopting an SH3 domain-like fold and will have great future impact on basic research adressing protein interactions within extracellular matrices. Knock-out studies that are currently under way will contribute to elucidate the function of MIA and the recently discovered MIA gene family members. In addition, current studies investigating transcriptional regulation of the MIA gene will provide further molecular insights into changes of gene expression during malignant transformation.

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