Structure and Promoter Analysis of the Gene Encoding the Human Melanoma-inhibiting Protein MIA*

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We have recently described the isolation of a novel protein, MIA, which is secreted from malignant melanoma cells and elicits growth inhibition on melanoma cells in vitro (Blesch, A., Bosserhoff, A. K., Apfel, R., Behl, C., Hessdörfer, B., Schmitt, A., Jachimczak, P., Lottspeich, F., Schlingensiepen, H., Buettner, R., and Bogdahn, U. (1994) Cancer Res. 54, 5695–5701). Here, we report the structure of the human MIA gene locus, describe its expression pattern in melanocytic tumors in vivo, and provide an initial characterization of the MIA promoter. The MIA gene is encoded by four exons, and the mRNA initiation site was identified 70 base pairs upstream from the translation start codon. MIA mRNA expression in vivo correlated with progressive malignancy of melanocytic lesions and was inducible in other cells by phorbol esters. To investigate mechanisms mediating this melanoma-associated expression pattern, we analyzed the promoter activity of the 1.3-kilobase genomic sequences located 5′-upstream of the MIA gene. The MIA promoter conferred high levels of gene activation specifically in human and murine melanoma cells, and its activity was further enhanced by treatment with phorbol esters. Site-directed mutation of an NF-κB site within the MIA promoter did reduce the basal promoter activity in melanoma cells but did not change significantly enhancement by phorbol esters.

Growth and expansion of tumor cells including malignant melanomas are modulated by a complex network of growth factors, which regulate proliferation and cell-matrix interaction through a variety of different signal transduction pathways. Therefore, the net growth or regression of melanomas in vivo reflects integration of many different stimulatory or inhibitory factors produced both by the tumors cells and their environment. Well studied examples of growth regulatory proteins in melanoma cells include members of the tumor growth factor-β and platelet-derived growth factor families, transferrin, basic fibroblast growth factor, epidermal growth factor, and tumor growth factor-α (Herlyn and Malkowicz, 1991; Halaban et al., 1995). Rodeck et al., 1991; Shih and Herlyn, 1993).

We have recently isolated and cloned a novel protein that is secreted by malignant melanoma cell lines and exerts autologous growth inhibitory effects on melanoma cells in vitro (Bogdahn et al., 1989; Apfel et al., 1993; Blesch et al., 1994). Due to the growth inhibitory effect, which allowed purification by means of a bioassay, this protein was designated MIA (melanoma inhibitory activity). Isolation of fully encoding human and murine MIA cDNA clones revealed that MIA is translated as a 131-amino acid precursor protein and secreted into the tissue culture supernatant of melanoma cells after cleavage of a 24-amino acid signal peptide. MIA appears to constitute a unique protein since no significant sequence homology to any other known protein was detected.

Initial characterization of MIA expression by Northern blot analyses indicated that MIA is expressed in all melanoma cell lines that we tested and infrequently in glioma cell lines but not in fibroblast or epithelial cell lines. This interesting melanoma-associated expression pattern prompted us to examine in more detail skin biopsies along with benign and malignant melanocytic tumors in vivo for expression of MIA mRNA. We further aimed to isolate the entire genomic locus of the human MIA gene, to determine its exon-intron organization, and to provide an initial characterization of the melanoma cell type-specific function of the MIA gene promoter.

EXPERIMENTAL PROCEDURES

Isolation of the Human MIA Gene—A commercially available human placental genomic library in the phage λ FixI (Stratagene) was screened using the fully encoding human MIA cDNA (Blesch et al., 1994) as a probe. Two positive clones were obtained from 6 × 10⁸ phages, one of which was used for restriction analysis of the MIA gene locus (phage UB1 shown in Fig. 1). Two adjacent Xbal fragments (UB1-1 and UB1-3) hybridizing with the MIA cDNA probe were subcloned into the plasmid pBluescript.

Primer Extension Analysis and Rapid Amplification of 5′-cDNA Ends-Polymerase Chain Reaction—A primer extension experiment was performed following standard procedures (Sambrook et al., 1989) using a phoshpholabeled oligonucleotide (MIA extension, 5′-CAAGGCGGT-GCTGGGTCTCCAATTT-3′) complementary to nucleotides 26 to 51 of the MIA cDNA and 10 μg of total RNA isolated from the melanoma cell line Mel Im (Jacobi et al., 1995).

RACE-PCR was performed using the AmpliFinder RACE kit (Clontech) precisely as described previously (Bauer et al., 1994). Briefly, an antisense primer (5′-CAGCCATGGAGATAGGGT-3′) selected RNA isolated from Mel Im melanoma cells. After hydrolysis of the template mRNA, an anchor primer was ligated to the 3′-end of the cDNA, and then a PCR reaction was performed using the anchor primer and the same oligonucleotide that was used for the primer extension analysis as a nested MIA-specific primer.

Reverse Transcriptase-mediated Polymerase Chain Reaction (RT-PCR) was performed using the following primer pairs: 5′-CGCCTTCCCGCCTCAAATT-3′ and 5′-CAGCCATGGAGATAGGGT-3′ complementary to nucleotides 26 to 51 of the MIA cDNA and 10 μg of total RNA isolated from the melanoma cell line Mel Im (Jacobi et al., 1995).

The abbreviations used are: RACE-PCR, rapid amplification of 5′-cDNA ends-polymerase chain reaction; RT-PCR, reverse transcriptase-PCR; bp, base pair(s); kb, kilobase(s); CAT, chloramphenicol acetyltransferase; LTR, long terminal repeat; PMA, phorbol 12-myristate 13-acetate.

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PCR.—Primers MIA-reverse (5'-GATAAGCTTCTAGCGAGTCG-3') and MIA-sense (5'-CATGATGGGCTTCTATGGCCAA-GCGT-3') were employed for reverse transcription of total cellular RNA and PCR as described in detail previously (Buetter et al., 1993). 25 or 32 cycles of PCR were performed using the following profile: 45 s at 94 °C, 30 s at 55 °C and 60 s at 72 °C. PCR reaction products were fractionated on 1.8% agarose gels and subjected to Southern blot analysis.

Cell Lines, CAT Plasmids, and Transfections—Cell lines were used and cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Sources of cell lines were as follows: HeLa (ATCC CCL2), COS7 (ATCC CRL1651), HaCaT (Dr. Petra Boukamp, Heidelberg, Germany), melanoma cell lines Mel Im and HTZ-19 (J. Acab et al., 1995; Blesch et al., 1994), B16 (ATCC CRL6322), 1144, Mel Ei, Mel Wei, Mel Juso (Dr. J. udjohnJohnson, Munich), SK Mel-28 (ATCC HTB72), HepG2 (ATCC HB0065), PA-1 (ATCC CRL1572), DU145 (ATCC HTB81), B2 (ATCC HTB81), Soas (ATCC HTB85), and HL60 (ATCC CCL240). Melanocytes and fibroblasts were obtained from normal skin and monocytes from peripheral blood of healthy blood donors. PMA

The genomic region 5’ adjacent to the MIA gene from residue –1361 to –1 was amplified by PCR, inserted into the promoterless chloramphenical acetyltransferase plasmid pBLCAT3 (Ludcow and Schütz, 1987), resequenced, and then a series of 5’-deleted constructs was generated using nested deletion (Henikoff, 1984). 2 × 105 cells were seeded into 90-mm dishes and transiently transfected with 5 μg of plasmid DNA using DOTAP transfection reagent (Boehringer) the following day. To normalize transfection efficiency, 2.5 μg of an LTR-lacZ plasmid was cotransfected and an enzyme-linked immunosorbant assay (Boehringer) was used to quantify CAT activities.

Gel Mobility Shift Assays and Site-directed Mutagenesis—Two complementary synthetic oligonucleotides spanning the NF-kB site at position –207 to –198 in the MIA promoter (5’-ACCTAATCTGG-GAATTTCTTTGGGCTTAC-3’) were hybridized and phospho labeled. Nuclear extracts were prepared from B16 melanoma cells, and gel shifts were performed as described previously (Buetter et al., 1993). Competition experiments were performed using the same MIA/NF-kB binding site and further a 25-fold excess of an unrelated oligonucleotide (“170-digo” matching residues –195 to –160 of the MIA promoter), a mutated binding site (5’-ACCTAATCTGAACTTTCTTTGGGCTTAC-3’), or a perfect bona fide NF-kB site from the HIV-1 LTR (5’-ATCCGG-GACCTTCTCGGTGGCTTCCG-3’) (Wu et al., 1988). The same mutation of the MIA/NF-kB site that was generated for the gel shift competition experiment was also subcloned into the CAT3 plasmid containing the full 1361-bp MIA promoter using a site-directed mutagenesis kit (Clontech) (Deng and Nickoloff, 1992).

RESULTS

Structure of the Human MIA Gene—To isolate the genomic MIA locus, a λ FixII human placental DNA library was screened using the entire human cDNA (Blesch et al., 1994) as a probe. Two positive recombinant phages were identified, one of which was referred to as UB-1 and subjected to restriction and Southern blot analyses. The insert of phage UB-1 contained five different XbaI fragments spanning approximately 15 kb of genomic DNA. Two of these five XbaI fragments (UB1-1, 2.2-kb in size and UB1-3, 1.4 kb in size) hybridized to the MIA cDNA probe and were subcloned into the plasmid pBluescript and then fully sequenced on both strands. Comparison of the genomic and cDNA sequences revealed that the MIA gene consists of four small exons interrupted by three introns (Fig. 1) with consensus splice sequences at the intron-exon junctions. Further analysis by a PCR reaction confirmed that the two XbaI fragments were located adjacent in the genomic DNA. Thus, the entire locus encoding the MIA protein is encompassed within a small region of approximately 2 kb.

Determination of the Transcription Start Site—Three different methods were employed to identify the transcription initiation site. First, a series of MIA cDNA clones obtained from a HTZ-19 melanoma cell library was sequenced, and the one clone extending 5’ the most started at position –70 with respect to the ATG protein start codon (data not shown). Further, a primer extension assay was performed on RNA isolated from

Mel Im melanoma cells using an oligonucleotide complementary to MIA cDNA upstream of the protein start site. Analysis of the extension products on a sequencing gel (Fig. 2A) revealed a specific band of a size between 52 and 54 bp, suggesting that the 5’ terminus of the MIA mRNA is at residue –70. These results were then confirmed by RACE-PCR (Frohman et al., 1989), resulting in a specific product of approximately 70 bp (Fig. 2, B and C). The amplification product was subcloned into the plasmid pCRII Sequenced, and thereby shown to start at the translation start codon.

Expression Pattern of MIA in Melanoma Cells, Skin, and Melanocytic Tumors—We have recently described the molecular cloning of the MIA cDNA and have shown by Northern blot analyses that MIA mRNA is widely expressed in malignant melanoma cell lines in vitro, including HTZ-19 and Mel Im cells (Blesch et al., 1994). Therefore, we have used RNA isolated from these melanoma cell lines to establish conditions for RT-PCR to determine the MIA expression pattern in a series of cell lines and in benign and malignant melanocytic lesions in vivo.

As shown in Fig. 3A, specific MIA RT-PCR products were readily amplified from both melanoma cell lines but not from two different benign melanocyte cultures. To control the specificity of these reactions, we subcloned the PCR products into a plasmid vector and confirmed by sequencing that they represented MIA cDNA fragments (data not shown). In parallel β-actin mRNA was amplified to verify equal amounts and integrity of different RNA preparations.

We then used the same PCR conditions to perform PCR reactions on RNA samples from a series of different cell lines. As summarized in Table I and in good agreement with previously performed Northern blot analyses (Blesch et al., 1994), we detected high levels of MIA mRNA expression in every melanoma cell line we tested, including B16 murine melanoma cells. In contrast, we did not detect any significant expression in other skin-derived cells including normal fibroblasts and HaCaT keratinocytes (Fig. 3B). Also, other epithelial cell lines such as COS, HeLa, and HepG2 cells, DU145 (human prostate cancer) and J82 cells (human bladder cancer), or PA-1 teratocarcinoma cells did not express MIA. Interestingly, significant MIA expression was induced by treatment with phorbol esters in skin fibroblasts, HaCaT, COS, and HeLa cells.

These results prompted us to study the expression pattern of MIA mRNA in normal skin and skin-derived melanocytic tu-
mors. As shown in Table II and as examples in Fig. 4 from primary malignant melanomas (7 cases) and from lymph
and high levels were found in one case. In all specimens taken mRNA levels were detected in 8 of 15 benign melanocytic nevi, than 25 PCR cycles were performed. Low or moderate MIA
which minute mRNA levels were amplified when 32 rather not detect MIA mRNA in normal skin except for two cases, in
implying that MIA mRNA is elevated in malignant melanocytic nevi. The following cell lines were analyzed: melanoma
cells (Mel Im and a highly invasive subline Mel Im hi, HTZ-19, Mel Wei, Mel E1, B16), melanocytes (grown from human skin biopsies), fibroblasts (grown from human and murine skin biopsies), keratinocytes (HaCaT), HeLa COS (simian kidney epithelial cells), prostate cancer cells (DU 145), bladder cancer cells (J 82), HepG2 (hepatoma cells), and PA-3 (teratocarcinoma cells). + PMA indicates RT-PCR results from cell lines treated for 24 h with 10⁻⁹ M PMA.

![Figure 2](image)

**Fig. 2. Determination of the MIA mRNA initiation site.** A, primer extension assay. A 25-mer synthetic oligonucleotide was phosphor-labeled and used to extend the N-terminal end of the MIA mRNA. Extended products were size-fractionated on a 5% urea/polyacrylamide gel next to a sequencing reaction as a size marker. The largest extended product is 54 bp in length matching nucleic acid residue -70 relative to the ATG protein start codon. B, direct cloning of the N-terminal MIA cDNA end by RACE-PCR. First strand cDNA was synthesized from Mel Im poly(A⁻) RNA using an antisense primer in the first exon. An anchor primer was ligated to the 3'-cDNA end, and the resulting template was amplified by PCR using the anchor primer and a nested MIA primer. Shown is an ethidium-stained agarose gel of the PCR product next to a molecular size standard. C, graphic summary indicating relative location of RACE-PCR primers and the size of the expected PCR product.

![Figure 3](image)

**Fig. 3.** A, amplification of MIA cDNA by RT-PCR in melanocytes and melanoma cell lines Mel Im and HTZ-19 (left). Control PCRs were performed on β-actin mRNA in parallel (right). B, amplification of MIA cDNA in various cell lines indicated on top.

![Table I](image)

**Table I**

| MIA Expression in various cell lines | Cell line | 25 cycles | + PMA |
|-------------------------------------|-----------|-----------|-------|
|                                     | Mel Im    | +++       |       |
|                                     | Mel Im hi | +++       |       |
|                                     | HTZ-19    | +++       |       |
|                                     | SK-Mel 28 | +++       |       |
|                                     | Mel Wei   | +++       |       |
|                                     | Mel J uso | +++       |       |
|                                     | Mel E1    | +++       |       |
|                                     | B16       | ++        |       |
| Melanocytes                         | Neg       |           |       |
| Fibroblasts, human                  | Neg       | ++        |       |
| Fibroblasts, murine                 | Neg       | ++        |       |
| Keratinocytes                       | Neg       | ++        |       |
| COS                                 | Neg       | ++        |       |
| HeLa                                | Neg       | ++        |       |
| Hep G2                              | Neg       |           |       |
| PA-1                                | Neg       |           |       |
| DU145                               | Neg       |           |       |
| J82                                 | Neg       |           |       |

![Figure 4](image)

**Table II**

| MIA Promoter Analysis | Cell line |
|-----------------------|-----------|
| Mel Im                | 111       |
| Mel Im hi             | 111       |
| HTZ-19                | 111       |
| SK-Mel 28             | 111       |
| Mel Wei               | 111       |
| Mel J uso             | 111       |
| Mel E1                | 111       |
| B16                   | 111       |
| Melanocytes           | 111       |
| Fibroblasts, human    | 111       |
| Fibroblasts, murine   | 111       |
| Keratinocytes         | 111       |
| COS                   | 111       |
| HeLa                  | 111       |
| Hep G2                | 111       |
| PA-1                  | 111       |
| DU145                 | 111       |
| J82                   | 111       |

mors. As shown in Table II and as examples in Fig. 4A, we did not detect MIA mRNA in normal skin except for two cases, in which minute mRNA levels were amplified when 32 rather than 25 PCR cycles were performed. Low or moderate MIA mRNA levels were detected in 8 of 15 benign melanocytic nevi, and high levels were found in one case. In all specimens taken from primary malignant melanomas (7 cases) and from lymph node metastasis of malignant melanomas (3 cases), abundant MIA transcripts were amplified. From all of these specimens, β-actin cDNA was coamplified to control for equivalence and integrity of RNA preparations. In summary, we detected high levels of MIA mRNA in all malignant melanoma biopsies and cell lines, low or moderate MIA mRNA levels in most benign melanocytic nevi, and very low or no MIA mRNA in non-neoplastic skin biopsies, melanocytes, fibroblasts, and keratinocytes. In addition, we did not detect any MIA mRNA in a panel of normal mouse tissues including skin, spleen, brain, thymus, kidney, intestine, lung, and skeletal muscle (Fig. 4B).

**MIA Promoter Analysis**—A computer analysis of the genomic sequence located 5' adjacent to the MIA mRNA start revealed very few canonical sequences as putative binding sites for transcription factors. As annotated in Fig. 5, consensus SP-1 (Jones and Tjian, 1985), NF-κB (Lenardo and Baltimore, 1989), and CTF/NF-1 (Jones et al., 1989) binding sites are located 35, 130, and 555 bases upstream from the mRNA start site, respectively. No TATA box or any other known cis-regulatory motif was detected in the sequence.

To determine whether the MIA promoter is activated specifically in melanoma cells, we cloned the 1386-bp fragment shown in Fig. 5 in front of a promoterless CAT plasmid and tested its activity in several human melanoma cell lines in comparison to non-melanocytic cancer cells. We found that the MIA promoter confers high levels of gene expression specifically in human or murine melanoma cell lines but not in HeLa, HepG2, PA-1, and COS cells (Fig. 6, A and B). To map in more detail cis-regulatory elements mediating MIA mRNA expression in melanoma cells, we transfected a series of 5'-deleted CAT reporters both into B16 and COS cells. B16 cells were chosen for this experiment because they were transfected much more efficiently, and therefore small changes in promoter activity could be monitored reliably. Fig. 6B gives a summary of the promoter constructs and CAT activities obtained from transiently transfected B16 and COS cell cultures. Maximal CAT activity was observed when a promoter fragment ranging from -493 to -1 with respect to the ATG protein start codon was used. This promoter fragment conferred 14-fold activation to
the basal CAT plasmid in B16 cells in comparison to the Rous sarcoma virus-LTR that conferred 17-fold activation. Significant changes in CAT activities were observed when a series of fragments extending further 5' upstream was analyzed, indicating that silencer and enhancer elements are located between residues 2120 and 2761 and 2761 and 2493. Further deletion of the promoter to 2212 decreased significantly CAT activities, and a promoter fragment starting at 2170 was entirely inactive as compared with the promoterless pBLCAT3 plasmid. In summary, these CAT assays led to the conclusion that residues located between 2493 and 211 in the MIA promoter are necessary and sufficient to mediate high levels of cell type-specific gene expression.

Activation of the MIA Promoter Involves Binding of NF-κB—Since a consensus NF-κB binding site at residue 2207 was detected within the most active promoter fragment, we explored further the functional role of NF-κB in regulating MIA expression. Gel shift analyses were performed to investigate whether NF-κB binds to this site in melanoma cells. When we used a synthetic 30-mer binding site spanning residues 2216 to 2187 including the NF-κB site, one specific bandshift was observed (Fig. 7A). Binding was not competed when a similar binding site mutated at four critical residues in the NF-κB core sequence or an unrelated sequence of the MIA promoter (2170 oligonucleotide) were used in contrast to a synthetic binding site matching the NF-κB element in the HIV-1 LTR, which competed specifically the MIA-NF-κB bandshift activity.

NF-κB activity results from a gene family that is expressed in a large number of different cell types and tissues, and consequently the MIA-NF-κB site was also shifted when COS cell extracts were used (data not shown). To address whether binding of the NF-κB site is necessary for cell type-specific function of the MIA promoter, we introduced the same four bases that abolished binding of NF-κB in gel shift experiments by site-directed mutagenesis in the CAT reporter plasmid under the control of the full 1361-bp MIA promoter. This promoter construct mutated at the NF-κB site was transiently transfected into B16 melanoma cells in parallel with the wild-type promoter construct. As shown in Fig. 7B, we observed approximately 2-fold decreased activity in comparison to the wild-type promoter, whereas the mutation did not affect significantly the stimulating effect of PMA on CAT expression. These results indicate that the NF-κB contributes to the MIA promoter activity in melanoma cells but is dispensable for stimulation in response to PMA.

To test whether the induction by PMA represents a primary response or a late event, we determined the time course of mRNA induction and promoter activation. RT-PCR analyses of HeLa and COS cells revealed that the mRNA was first detected 8 h after the onset of PMA treatment. These results were in good agreement with CAT activities obtained from HeLa and

| Biopsy                | n   | -   | +   | ++  | +++ |
|-----------------------|-----|-----|-----|-----|-----|
| Skin                  | 8   | 6   | 2   | 5   | 3   |
| Benign nevus          | 15  | 6   | 5   | 3   | 1   |
| Melanoma              | 7   |     |     |     |     |
| MM metastasis         | 3   |     |     |     |     |

**TABLE II**

RT-PCR study of MIA expression in biopsies from skin and pigmented skin tumors

RT-PCR results were semi-quantified from ethidium bromide-stained agarose gels in parallel to control PCR reactions of β-actin mRNA. Four groups were defined: negative (−), weakly positive after 32 PCR cycles (+), weakly positive after 25 PCR cycles and strongly positive after 32 cycles (++), and strongly positive after 25 cycles (+++). The diagnosis of melanoma was based on the assessment of two independent pathologists, and immunohistochemical stainings were positive in 100% of all cases for S-100 and at least focally positive for HMB-45.
COS cells transfected transiently with the MIA promoter-CAT plasmid and treated with an inhibitor of RNA synthesis at various points after PMA induction. Stimulation of CAT activity was not observed when actinomycin D was added to the cell cultures earlier than 9 h after PMA, indicating that it does not represent a primary response (data not shown).

**DISCUSSION**

Here, we report the molecular cloning of the human genomic MIA locus, describe its organization, and provide an initial characterization of cis-regulatory elements within the 5′-genomic region mediating high levels of gene expression in melanoma cells. The complete exon-intron structure was determined by sequencing two adjacent genomic XbaI fragments that cover four small exons interrupted by three intervening introns. The coding nucleic acid residues matched perfectly to the cDNA sequence obtained recently from a malignant melanoma cDNA library (EMBL Library accession no. X75450). The 5′-mRNA start was mapped by a primer extension experiment and was further cloned by RACE-PCR using poly(A)+ RNA from Mel Im melanoma cells. These experiments revealed that the mRNA is initiated 70 bases upstream from the protein coding region downstream of a pyrimidine-rich sequence motif followed by the nucleic acid residues AC. As frequently observed with TATAA-less genes, the initiator sequence is flanked 5′ by a consensus SP-1 binding site. The polyadenylation signal AAATACAA is located 43 bases 3′ downstream from the protein stop codon. Assuming a tail of approximately 200–250 adениnes, the sizes of the predicted transcript and the mRNA (approximately 750 bases) observed on Northern blots (Blesch et al., 1994) are in good agreement.

Data summarized in Tables I and II and Figs. 3 and 4 indicate that MIA mRNA expression parallels closely the ma-
lignancy of pigmented skin tumors and is not expressed in normal tissues of adult mice. By means of RT-PCR results, we detected no or very little MIA mRNA in non-neoplastic skin biopsies, moderate levels in the majority of non-malignant melanocytic nevi, and very high levels in every biopsy from malignant melanomas or metastases from melanomas. Interestingly, the two skin biopsies that expressed very low levels of MIA mRNA were taken from sun-exposed facial skin, and therefore MIA expression might result from subtle activation of melanocytes not detectable on microscopic examination. In the small number of biopsies examined in this study, we were not able to correlate levels of MIA mRNA in benign melanocytic nevi with a certain histological type of nevi. Therefore, it will be necessary to explore in a larger study whether MIA expression provides a prognostic parameter to define nevi at risk for malignant progression. Analyses of other S100 positive tumors including astrocytomas, oligodendrogliomas, and glialoblastomas indicate that MIA expression is highly associated with melanocytic tumors and can be detected only occasionally in other neuroectodermally derived tumors (Blesch et al., 1994).2

The melanoma-associated expression pattern of MIA was further substantiated by RT-PCR amplifications and Northern blot analyses of cell cultures in vitro. Together with data published previously (Blesch et al., 1994), we have now tested 10 different malignant melanoma cell lines, every one of which expressed very high levels of MIA mRNA. In contrast, all cultures of non-neoplastic skin cells including fibroblasts, keratinocytes (HaCaT cells), or melanocytes did not express MIA mRNA. The close correlation between MIA expression and melanocytic tumors or tumor cell lines raises questions about the function of MIA in regulating growth and invasion of malignant melanomas. In vivo, there are a small number of biopsies examined in this study, we were not able to detect MIA expression on microscopic examination. In the melanoma cell lines, it was therefore MIA expression might result from subtle activation of melanocytes not detectable on microscopic examination.

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