Specific Expression and Regulation of the New Melanoma Inhibitory Activity-related Gene MIA2 in Hepatocytes

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The novel human gene MIA2 encoding a melanoma inhibitory activity (MIA) homologous protein was identified by a GenBank™ search. MIA2, together with MIA, OTOR, and TANGO, belongs to the novel MIA gene family sharing important structural features, significant homology at both the nucleotide and protein levels, and similar genomic organization. In situ hybridization, reverse transcriptase-PCR, and Northern blots presented a highly tissue-specific MIA2 expression pattern in the liver. Promoter studies analyzing transcriptional regulation of MIA2 revealed an HNF-1-binding site at position −236 controlling hepatocyte-specific expression. Mutation of the site led to a complete loss of promoter activity in HepG2 cell. Further sites detected in the MIA2 promoter were consensus binding sites for SMAD2/3 controlling hepatocyte-specific expression. Mutations of the site led to a complete loss of promoter activity in HepG2 cell. Further sites detected in the MIA2 promoter were consensus binding sites for SMAD2/3. Consistently, stimulation of MIA2 mRNA expression occurred by treatment with interleukin-6, transforming growth factor-β, and conditioned medium from activated hepatic stellate cells. In accordance with these results, MIA2 mRNA was found to be increased in liver tissue of patients with chronic hepatitis C infection compared with controls. MIA2 mRNA levels were significantly higher in patients with severe fibrosis or inflammation than in patients with less severe fibrosis or inflammation. In summary our data indicate that MIA2 represents a potential novel acute phase protein and MIA2 expression responds to liver damage. The increased transcription in more severe chronic liver disease suggests that MIA2 may serve as a marker of hepatic disease activity and severity.

Melanoma inhibitory activity (MIA) protein was identified previously within growth-inhibiting activities purified from the tissue culture supernatant of the human melanoma cell line HTZ-19 (1). MIA is translated as a 131-amino acid precursor molecule and is processed into a mature 107-amino acid protein after cleavage of a hydrophobic secretion signal. The position within the human genome was mapped to chromosome 19q13.32-13.33 (2). MIA mRNA was identified independently by differential display approaches comparing melanoma cell lines and also comparing differentiated and dedifferentiated cartilage cells in vitro. Therefore, MIA has also been referred to as cartilage-derived retinoic acid-sensitive protein (3). Subsequent studies of murine embryos and murine adult tissues demonstrated specific mRNA expression patterns in cartilage but not in any other non-neotopic tissue (4). Functionally, MIA was initially purified and described to exert antitumor activity by inhibiting proliferation of melanoma cell lines in vitro (5). However, further studies revealed expression patterns inconsistent with a tumor suppressor. Expression of the wild-type MIA protein gene was not detected in normal skin and melanocytes but was associated with progression of melanocytic tumors. More recently, it was suggested that the MIA protein specifically inhibits attachment of melanoma cells to fibronectin and laminin and thereby masks the binding site of integrins to these extracellular matrix components and promotes invasion in vitro (6). Additional in vivo studies revealed the importance of MIA for metastasis of malignant melanomas (7, 8). Furthermore, we and others (6, 9) have shown that MIA adopts an SH3 domain-like structure and interacts directly with fibronectin.

Recently, an MIA homologous protein, OTOR (FDP, MIAL), was identified and mapped to chromosome 20p11. For this protein a homology of 59% to MIA was determined. OTOR expression was reported to be highly tissue-specific and restricted to the cochlea and eye (10–12). The detection of OTOR stimulated us to search for further homologous proteins. Here we describe a novel MIA homologue, designated MIA2, and we define its expression pattern and regulation in hepatocytes.

Interestingly, we found specific expression in the liver and mechanisms of transcriptional regulation that identify MIA2 as a potential novel acute phase protein. Furthermore, our analysis of the expression levels of MIA2 in patients with chronic hepatitis C infection may suggest that MIA2 can serve as a marker of hepatic disease activity and severity.

EXPERIMENTAL PROCEDURES

Cell Lines and Tissue Culture—The following cell lines were used: Mel Im, Mel El, Mel Wei, SK-Mel 28, Mel Ho (DSMZ ACC 62), HTZ-19d, Mel Ju, Mel Juso (DSMZ ACC 74) (human melanoma (13)), human primary melanocytes NHEM (14), HeLa (human cervix carcinoma, ATCC CRL-7923), HepG2 (ATCC HB-8065), HCT 116 (ATCC CCL-247), CaCo-2 (ATCC HTB-37), SW48 (ATCC CCL-231), LoVo (ATCC CCL-229), SW480 (ATCC CCL-228), HT29 (ATCC HTB-38) (human colon carcinoma cell lines), U266 (B-lymphocytes, ATCC TIB-196), and Jurkat (T-lymphocytes, ATCC TIB-108).

Cells were grown at 37 °C in 5% CO₂ in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with penicillin (100 units/ml), streptomycin (10 μg/ml) (both Sigma), and 10% fetal calf serum (In-
vitro), and split 1:2 at confluence. Cells were detached by incubation with 0.05% trypsin, 0.04% EDTA (Sigma) in phosphate-buffered saline for 5 min at 37 °C. Primary human and murine hepatocytes, hepatic stellate cells (HSC), and Kupffer cells were isolated from human liver specimens obtained during resection of metastasis of non-hepatic tumors or rat livers, respectively. Exclusion criteria were known liver disease or history of liver fibrosis or inflammation in surrounding non-tumorous liver tissue. The cell isolation and subsequent culturing was performed as described before (15–17). HSC were activated by culturing on plastic for 14 days (17).

Primary human fibroblasts were isolated and cultured as described previously (18).

Stimulation of Cells—HepG2 cells were treated with the following cytokines: TNF-α (10 ng/ml), IL-1 (2 ng/ml), IL-6 (30 ng/ml), TGFB1 (2 ng/ml), LPS (10 μg/ml), PMA (10 nM), or with alcohol (0.1% ethanol) (all obtained from Sigma) for 6, 16, and 32 h.

To obtain conditioned medium of activated human HSC, the cells were seeded in T75 culture flasks (BD Biosciences) and cultured in serum-free medium for 24 h (12 ml/75 flask). Some cells were additionally treated with LPS (10 μg/ml) for the 24-h incubation period. 12 ml of pure HSC medium or medium supplemented with LPS (10 μg/ml) was filled into empty T75 flasks and incubated in parallel for 24 h to serve as controls. Supernatants and controls were collected and saved at −70 °C before stimulation of HepG2 cells. HepG2 cells were seeded in 6-well plates and incubated with 2 ml of the conditioned media for 6, 16, and 32 h.

Patients and Controls—Subjects included 11 patients (9 men and 2 women; age, 20–52 years, median age, 35.5) with chronic hepatitis C infection (positive for HCV-RNA and anti-HCV) and no prior interferon treatment. Exclusion criteria were co-infections with human immunodeficiency virus, hepatitis B virus, or other concomitant liver disease. Liver biopsies were taken, and histological staging and grading was performed according to the score proposed by Desmet et al. (19). Parts of the biopsies were used for RNA isolation.

Human liver specimens obtained during resection of metastasis of non-hepatic tumors served as controls. Exclusion criteria were known liver disease or histological evidence for liver fibrosis or inflammation in surrounding non-tumorous liver tissue. RNA was isolated from surrounding non-tumorous liver tissue.

RT-PCR Analysis—For RT-PCR total cellular RNA was isolated from cultured cells, from multiple tissues of C57BL/6 mice, or from human liver tissue using the RNeasy kit (Qiagen, Hilden, Germany). The integrity of the RNA preparations was controlled on an 1% agarose/formaldehyde gel, and subsequently cDNAs were generated by reverse transcription. First strand cDNA was synthesized using 2 μg of the isolated total RNA, 1 μM of random primer (Amersham Biosciences), 4 μl of 5× First Strand Buffer (Invitrogen), 2 μl of 10 mM dithiothreitol, 1 μl of 10 mM DNTPs, and 1 μl of Superscript Plus (Invitrogen) in a total volume of 20 μl. To screen for mRNA expression, semi-quantitative PCR was performed (PCT-300, Biozym) using the primer sequences MIA2 forward, ATG GCA AAA TCT TCC, with MIA2 forward, ATG GCA AAA TTT GGC GTT C, and MIA2 reverse, CCT GCC AGA AGG AAC AAG CG, and 2-MG reverse, TTG GTG GCA GTT TCA GGG ATA; 2-MG forward, CAG TGG AGA AGG AAC AAG CG, and 2-MG forward, CAG TGG AGA AGG AAC AAG CG, and MIA2 reverse, CCT GCC AGA AGG AAC AAG CG.

For RT-PCR studies of MIA2 mRNA expression in primary human hepatocytes, human liver specimens obtained during resection of metastasis of non-hepatic tumors served as controls. Exclusion criteria were known liver disease or histological evidence for liver fibrosis or inflammation in surrounding non-tumorous liver tissue. RNA was isolated from surrounding non-tumorous liver tissue.

Northern Blots—Multiple Tissue Expression Arrays (Clontech) were hybridized following the manufacturer's description. As probes cDNA fragments of hMIA2 were phosphorolabeled using a Klenow DNA polymerase-based random primer labeling kit (Bio-Rad).

In Situ Hybridization—In situ hybridization was performed as described previously (4). A 350-bp fragment of MIA2 was cloned into pBluescript (Clontech) for riboprobe synthesis. The riboprobes were synthesized by in vitro transcription using T7 and T3 RNA polymerase, respectively, and labeled by incorporation of [35S]UTP.

Promoter Constructs, Transfections, and Luciferase Assays—The 5′-flanking regions of the human MIA2 gene from residue −840 to −6, −707 to −6, and −218 to −6, respectively, were amplified by PCR, inserted into the plasmid pGL3-basic (Promega), and resequenced.

Site-directed Mutagenesis—Mutations in the 707-bp human MIA2 promoter region (707mut) destroying the HNF1-binding site were introduced using a site-directed mutagenesis kit (Clontech) as described by the manufacturer.

Gel Mobility Shift Assays—Complementary synthetic oligonucleotides corresponding to the HNF1 site in the MIA2 promoter region and to the HNF1 consensus binding site (Geneva, Montreal, Canada) were hybridized and phosphorolabeled (MIA2-HNF1 forward, ATC TGT AGT TAA ACC CTT AGG, and MIA2-HNF1 reverse, CCT AAG GGT TTA ATA ATT AAC AAG GAT; HNF1_cons forward, CCA GTT CAA CCA CTG GC, and HNF1_cons reverse, CCC AGT GGT TAA TCA TTA ACT GG). Nuclear extracts were prepared from HepG2, primary fibroblasts, Mel Im and Mel Ju cells, and gel shifts were performed as described previously (20). Competition experiments were performed using a 50-fold excess of the same binding site, a mutated binding site (HNF1_mut forward, CCA GCC GAG CAG CAA CCA CTG GC, HNF1_mut reverse, GCC AGT GGT TAA TCA TTA ACT GG) or an unrelated binding site. Supershifting was performed using an anti-HNF1 antibody (Geneva).

Statistical Analysis—Statistical numbers and results are expressed as mean ± S.D. Statistical significance between two groups was determined by using the Student's t test. A p value <0.05 was considered statistically significant.

RESULTS

We identified the MIA2 gene as a homologue of MIA by a gene search. MIA2, together with MIA, OTOR, and TANGO, belongs to a novel MIA gene family sharing important structural features, including an SH3 fold domain and two intramolecular disulfide bonds, and similar genomic organization. The four members share 34–45% amino acid identity and 47–59% cDNA sequence identity (Fig. 1A). Surprisingly, MIA2 contains an additional C-terminal region of 422 amino acids having no homology to known proteins. MIA2 therefore encodes a mature protein of 522 amino acids in addition to the hydrophobic secretory signal sequence (Fig. 1B). Structure modeling and protein folding recognition studies confirm the highly conserved SH3 structure in the N terminus present also in MIA and OTOR (which are both known to be secreted proteins).

To analyze expression patterns of MIA2, RT-PCR studies of adult human and murine tissues and in situ hybridization of murine embryo sections were performed. In general, RT-PCR studies with primers specific for MIA2 reveal a consistent expression pattern for both human and murine tissues. In contrast to MIA, which is exclusively expressed in cartilage but
not in any other non-neoplastic tissue, and OTOR, which shows a highly restricted expression pattern in cochlea, eye, and cartilage, the novel MIA related gene, MIA2, is expressed specifically in liver (Table I) and at very low levels in testis. The RT-PCR analysis was verified by in situ hybridization to mouse embryo sections of different embryonic stages between E12.5 and E14.5 using radiolabeled cRNA probes. Consistent with the RT-PCR, strong MIA2 expression was found in the developing liver (Fig. 2). Comparison between adult and fetal (gestational day 20) murine livers revealed a 5-fold higher MIA2 mRNA expression levels in adult liver (data not shown).

Next we analyzed parenchymal and non-parenchymal human liver cells, and we found strong MIA2 expression in primary hepatocytes but not in Kupffer cells or quiescent or activated hepatic stellate cells (Fig. 3A). Identical results were obtained with murine hepatic cells (data not shown). To estimate MIA2 mRNA expression relative to several known acute phase proteins, semiquantitative PCR analysis was performed using first strand cDNA from primary human hepatocytes. MIA2 mRNA expression was revealed to be lower than α1-antichymotrypsin mRNA expression but similar to the mRNA expression of α1-antitrypsin, α2-macroglobulin, and α1-acid glycoprotein (data not shown).

Furthermore, several tumor cell lines were studied, and expression was detected only in the hepatoma cell line HepG2 (Fig. 3B). These data indicated highly specific expression of MIA2 in hepatocytes and prompted us to further analyze the underlying gene regulatory mechanism.

Preliminary insight into putative cis-regulatory elements of the MIA2 promoter were obtained by data bank searches of the genomic 5'-flanking region. Analysis of 1500 bp indicated several consensus binding sites for transcription factors (Fig. 4A). Most importantly, an HNF1 site was found at 253 to 240 (relative to the adenine of the start codon). Furthermore, STAT, SMAD, GATA-1, GATA-2, cAMP-response element-binding protein, and AP-1 binding sites were localized. GenBank™ search revealed EST clones identical to MIA2 with a 5'-untranslated region of ~120 indicating that these sequences are indeed being transcribed. Consistently, a consensus TATA box sequence is located 29 bases further upstream suggesting

| Tissue   | Human | Murine | Human | Murine |
|----------|-------|--------|-------|--------|
| Lung     | -     | -      | -     | -      |
| Liver    | -     | -      | ++    | ++     |
| Kidney   | -     | -      | -     | -      |
| Colon    | -     | -      | -     | -      |
| Skin     | -     | -      | -     | -      |
| Bone     | -     | -      | -     | -      |
| Cartilage| +     | +      | -     | -      |
| Uterus   | -     | -      | -     | -      |
| Fat      | -     | -      | -     | ND     |
| Heart    | -     | -      | -     | -      |
| Brain    | -     | -      | -     | -      |
| Spleen   | -     | -      | -     | -      |
| Thymus   | -     | -      | -     | -      |
| Muscle   | -     | -      | +     | +      |
| Testis   | -     | -      | -     | -      |
| Cochrals | -     | -      | ND    | ND     |
| Trachea  | +     | ND     | -     | ND     |
| Prostate | -     | ND     | -     | ND     |
| Small intestine | -     | -      | -     | -      |

![Fig. 1. Comparison of human MIA, OTOR, MIA2, and TANGO cDNA sequences and MIA2 protein sequence. A, homology between the MIA gene family members on the cDNA level shown as a phylogenetic tree. The tree was constructed using the program DNAman based on the alignment of the complete cDNA sequence. B, sequence of MIA2 protein in comparison to MIA. Conserved cysteine residues are labeled with a box. Residues marked with a * are important for the hydrophobic core of the SH3 domain. The hydrophobic signal peptide is indicated by italics.](image1)

![Fig. 2. In situ hybridization of mouse embryos.](image2)
that transcription initiation occurs at residue −120 or very close.

707 and 218 bp of the promoter region were subcloned into pGL3-basic, a promoterless luciferase reporter construct. Reporter gene assays revealed strong activity of the promoter construct −707 in HepG2 cells but not in primary human fibroblasts or melanoma cells (Mel Im) (Fig. 4B). Activity of the promoter could be further enhanced in HepG2 by transfection of an HNF1 expression plasmid. Interestingly, transfection of HNF1 into Mel Im melanoma cells was sufficient to significantly activate the MIA2 promoter. The 218-bp promoter construct (lacking the HNF1 site) was neither active in HepG2 cells nor inducible by HNF1 (Fig. 4B). Furthermore, mutation of the HNF1 site in the 707 reporter construct by site-directed mutagenesis entirely inactivated promoter activity in HepG2 cells.

To confirm site-specific binding of HNF1 to the putative binding site at −253 to −240 in the MIA2 promoter, gel shift assays were performed. Binding of HNF1 present in HepG2 nuclear extracts to a consensus HNF1 site was competed by an anti-HNF1 antibody confirmed binding of HNF1 to the MIA2 promoter in HepG2 but not in Mel Im melanoma cells (Fig. 4D).

We next investigated whether cytokines, known to be elevated during liver diseases (interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNFα), transforming growth factor-β1 (TGF-β1)), lipopolysaccharide (LPS), alcohol, and phorbol myristyl acetate (PMA) were also involved in regulating hepatic MIA2 mRNA expression. Only IL-6 and TGF-β increased the MIA2-specific RT-PCR signal in isolated human hepatocytes and in HepG2 cells. As measured by quantitative RT-PCR analysis, both IL-6 and TGF-β induced MIA2-specific mRNA −8.5-fold. Combined treatment with both cytokines even further induced MIA2 mRNA expression by −12.3-fold (Fig. 5A). Induction of MIA2 mRNA expression already occurred 6 h after stimulation, and maximal stimulation was observed after 16 h (data not shown).

Regulation of MIA2 mRNA expression by the cytokines IL-6 and TGF-β1 is in accordance with the STAT3 (IL-6 regulation) and SMAD (TGF-β regulation) consensus binding sites in the MIA2 promoter. In chronic liver diseases both cytokines are mainly expressed by activated hepatic stellate cells or activated myofibroblasts. These cells play a major role in the pathophysiology of chronic liver disease. Therefore, we investigated the effect of serum-free medium conditioned by activated human HSC. MIA2 expression in HepG2 cells was strongly stimulated by cell culture supernatant of activated HSC pretreated with endotoxin and weakly, but still significantly, by the supernatant from unstimulated HSC. Quantitative RT-PCR results showed an 8.5-fold increase (2.2-fold, respectively) (Fig. 5B). Similarly as in cytokine experiments, maximum of MIA2-mRNA expression was seen after 16 h, starting at 6 h after stimulation with conditioned medium (data not shown).

Hepatic IL-6 and TGF-β levels are known to be elevated in chronic liver diseases. It is known that trans-differentiation of HSC into myofibroblastic cells is occurring in chronic liver diseases, leading to proliferation of the cells, to migration into the sites of liver damage, and increased expression of profibrogenic and proinflammatory genes, including TGF-β and IL-6. Therefore, we analyzed the expression of MIA2 mRNA in liver tissue of patients with chronic hepatitis C infection and various stages of fibrosis and grading of intrahepatic inflammation. Liver tissue without histological signs of fibrosis or inflammation from human donors without liver disease served as control. Intrahepatic MIA2 mRNA expression was found to be significantly elevated in hepatitis C patients compared with controls (0.14 ± 0.13 versus 1.62 ± 1.46, respectively; p = 0.043) (Fig. 6). Furthermore, hepatitis C patients with only mild, perportal fibrosis (staging 1; n = 6) had significantly lower intrahepatic MIA2 mRNA expression than patients with more advanced fibrosis (staging >1; n = 5) (0.85 ± 0.38 versus 2.55 ± 1.78, respectively; p = 0.016), as summarized in Fig. 6A. MIA2-mRNA expression was also lower in hepatitis C patients with little intrahepatic inflammation (grading 1 or 2), compared with patients with severe inflammation (grading >2); however, these differences did not reach statistical significance (1.04 ± 0.62 versus 2.64 ± 2.04, respectively; p = 0.027) (Fig. 6B).

**DISCUSSION**

We identified the novel human gene MIA2 as a new member of the MIA gene family. Furthermore, we analyzed the expression pattern and gained first insight into transcriptional regulatory mechanisms during liver diseases.

MIA2, together with MIA, OTOR, and TANGO, belongs to the novel MIA gene family sharing important structural features, significant homology at both the nucleotide and protein level, and similar genomic organization. MIA/OTOR and MIA2/TANGO are more closely related to each other than to the other MIA gene family members. Many residues important for structural folding are conserved between the four proteins (e.g., cysteine residues, amino acids in the hydrophobic core). Therefore, it can be speculated that all proteins belonging to the MIA gene family form an SH3 domain-like structure that was re-

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**Fig. 3. Analysis of MIA2 expression by RT-PCR.** A, RT-PCR specific for MIA2 revealed strong expression in primary human hepatocytes (ph Hepatocytes) of two different donors (I and II). B, (U206) or T-lymphocytes (Jurkat), quiescent (Q-HSC), and activated hepatic stellate cells (A-HSC) were MIA2-negative. Stimulation of HSC cells with TNFα did not induce MIA2 expression. B, RT-PCR analysis for MIA2 expression in a panel of different human tumor cells showed strong expression in HepG2 cells but not in melanoma (Mel Ho, HTZ19d, Mel Juso, Mel Ju, SK Mel 28, SK Mel 3, Mel Im, Mel Wei, Mel Ei) or colon carcinoma cell lines (CaCo-2, HCT116, SW48, LoVo, SW480, HT29). Further human primary melanocytes (NHEM) were tested and revealed to be negative. MW, molecular weight; neg, negative.
cently identified by NMR and x-ray crystallography of MIA (6, 9). The N terminus coding for the signal sequence is quite divergent, but analysis by Kyte-Doolittle blots revealed conservation of the hydrophobic character that is functionally important.

In contrast to MIA, which is exclusively expressed in cartilage but not in any other non-neoplastic tissue, and OTOR, which shows a highly restricted expression pattern in cochlea, eye, and cartilage, the novel MIA-related gene MIA2 is expressed specifically in hepatocytes. The RT-PCR analysis was verified by in situ hybridization to mouse embryo sections of different embryonic stages between E12.5 and E14.5 using radiolabeled cRNA probes. Consistent with the RT-PCR data, MIA2 expression is restricted to hepatocytes also during development. Furthermore, no mRNA expression of MIA2 was detected in cartilage indicating that the four members of the MIA gene family differ entirely with respect to their expression patterns.

To analyze transcriptional regulation, luciferase reporter gene constructs containing portions of the 5′-flanking region including an HNF1 consensus binding site (HNF1_cons) and by the HNF1 site present in the MIA2 promoter (HNF1_MIA2) but not by an oligonucleotide with a mutated HNF1-binding site (HNF1_mut). D, supershift experiment with an anti-HNF1 antibody. The antibody supershifted an HNF1 bandshift in HepG2 nuclear extract but not in Mel Im extract.
clearly show the importance of the HNF-1-binding site for the exclusive expression of MIA2 mRNA in hepatocytes.

HNF-1 is a transcription factor that is expressed in liver, digestive tract, pancreas, and kidney (21) and is involved in the regulation of a large number of hepatic genes including albumin, fibrinogen, or α-antitrypsin. It has been reported previously (22–24) that liver injury, chronic liver disease, or conditions associated with liver damage as elevated endotoxin levels lead to a down-regulation of HNF-1 activity. It is further interesting to note that several genes that contain a functional HNF-1 binding sequence are down-regulated during host response to infection or inflammation (25–27). However, hepatic MIA2 mRNA was found to be increased in patients with chronic hepatitis C infection. Furthermore, MIA2 transcription was even further elevated in liver tissue with severe fibrosis or inflammation. Our findings indicate that HNF-1 expression is required for basal expression but may be less important for enhanced transcriptional regulation in liver diseases.

Here, in accordance with STAT3 and SMAD consensus binding sites in the MIA2 promoter, IL-6 and TGF-β were identified as activators of MIA2 transcription, whereas other cytokines did not lead to enhancement of MIA2 expression.

The cytokine IL-6 is required for liver regeneration and repair and up-regulates transcriptionally a vast array of genes during liver regeneration and repair (28–30). IL-6 induces DNA binding of STAT transcription factors on regulatory elements of target genes. Although MIA2 is expressed constitutively in hepatocytes, its transcription was significantly increased during IL-6 stimulation.

TGF-β has pleiotropic functions including fibrinogenic action leading to trans-differentiation of HSC and negative regulation of proliferation and induction of apoptosis (31).

Interestingly, we found a synergistic effect of IL-6 and TGF-β stimulation on MIA expression in HepG2 cells. The cross-talk between IL-6 and TGF-β signaling pathways in a human hepatoma cell line was elucidated in a recent study (32), demonstrating that IL-6-induced activation of STAT3 activity and STAT3-mediated gene expression was augmented by TGF-β. These activities were due to physical interactions between STAT3 and MAD- and MAD-related protein-3, bridged by p300. As the consensus binding sites for STAT3 and SMAD are in direct proximity in the MIA2 promoter, we speculate that a similar mechanism for induction of MIA2 expression may be involved.

Our results indicate that different classes of transcription factors, tissue-specific (HNF-1) and growth- or stress-induced (STAT3 and SMAD), may interact during acute phase reaction or as an adaptive response to liver injury to amplify expression of the MIA2 gene, as demonstrated for other genes such as insulin-like growth factor-binding protein 1 (33).

STAT and SMAD pathways are known to be involved in the pathogenesis of liver fibrosis and inflammation, and elevated systemic and intrahepatic levels of IL-6 and TGF-β were found in acute and chronic liver diseases (30, 31). The activation process of HSC, causing trans-differentiation of the physiologically quiescent cells to an activated myofibroblast-like cell type, is one of the key events of hepatic fibrosis. In healthy liver or acute liver damage, Kupffer cells, the resident hepatic macrophages, are the main modulators of inflammation, secreting mainly TNF or IL-1. However, during liver disease activation of HSC occurs early, leading to participation in the regulation of the hepatic inflammatory response (31). Both
cytokines, IL-6, and TGF-β are expressed by activated, but not quiescent, HSC. Consequently, we found strong induction of MIA2 expression in HepG2 cells incubated with conditioned medium of activated HSC, particularly when HSC had been challenged with endotoxin. These results strongly suggest that activated HSC may also participate in vivo at least in part in the regulation of intrahepatic MIA2 expression. It is tempting to speculate that MIA2 expression may correlate with the activation process of these cells, allowing the use of MIA2 as a marker for fibrosis. Furthermore, transcriptional regulation and mRNA expression data, indicating that MIA2 is an abundantly expressed gene, render MIA2 to be a relevant acute phase protein.

In summary, our data identify MIA2 as a potential novel acute phase protein, secreted specifically from hepatocytes. Transcriptional regulation by IL-6, TGF-β, and conditioned medium from activated HSC and increased expression in fibrotic or inflamed liver tissue indicate that MIA2 may play a role in the pathophysiology of liver diseases and may serve as a marker of liver damage. Here it is tempting to speculate that in analogy to MIA, MIA-2 regulates attachment to extracellular matrix molecules in liver similar to MIA in cartilage. Further experiments need to address the question of whether MIA2 interacts with the same peptide epitopes as MIA and exerts the same functions in the regulation of cell attachment.

Acknowledgments—We are indebted to Jacqueline Schlegel, Astrid Hamm, Sandra Dahmen, Janine Mayers, Nicole Krott, and Claudia Abschlag for technical assistance. We thank Wolfgang Thasler for providing human hepatocytes and human liver tissue, Matthias Froh for providing Kupffer cells, and Gerd Kullak-Ublick for providing the HNF1 construct.

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*J. Biol. Chem. 2003, 278:15225-15231.*

doi: 10.1074/jbc.M212639200 originally published online February 13, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M212639200

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