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Tumor Metastasis-Associated Human *MTA1* Gene: Its Deduced Protein Sequence, Localization, and Association With Breast Cancer Cell Proliferation Using Antisense Phosphorothioate Oligonucleotides

Akihiro Nawa,[1] Katsuhiko Nishimori,[2] Paul Lin,[3] Yoshiyuki Maki,[4] Kennsuke Moue,[2] Hidetomo Sawada,[5] Yasushi Toh,[6] Kikkawa Fumitaka,[5] and Garth L. Nicolson[7]*

[1]Department of Tumor Biology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas
[2]Division of Molecular Biology, Department of Agriculture, Tohoku University, Sendai, Japan
[3]Gynecological Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas
[4]Science Tanaka Co., Ltd., Ishikari, Japan
[5]Department of Obstetrics and Gynecology, Nagoya University School of Medicine, Nagoya, Japan
[6]2nd Department of Surgery, Faculty of Medicine, Kyushu University, Fukuoka, Japan
[7]The Institute for Molecular Medicine, Huntington Beach, California

Abstract Using differential cDNA library screening techniques based on metastatic and nonmetastatic rat mammary adenocarcinoma cell lines we previously cloned and sequenced the metastasis-associated gene *mta1*. Using homology to the rat *mta1* gene we cloned the human *MTA1* gene and found it to be overexpressed in a variety of human cell lines. We found a close similarity between the human *MTA1* and rat *mta1* genes, as shown by 88% and 96% identities of the nucleotide and predicted amino acid sequences, respectively. Both genes encode novel proteins that contain a proline-rich region (SH3 binding motif), a putative zinc finger motif, a leucine zipper motif, and five copies of the SPXX motif often found in gene regulatory proteins. Using Southern blot analysis, the *MTA1* gene was found to be highly conserved among all species examined; and using Northern blot analysis, *MTA1* transcripts were found in virtually all cell lines of human origin that were analyzed, including melanoma and breast, cervix and ovarian carcinoma cells and normal breast epithelial cells. However, the expression level of the *MTA1* gene in a normal breast epithelial cell was approximately 50% of that found in rapidly growing breast adenocarcinoma cell lines and an atypical mammary cell line. Experimental inhibition of MTA1 protein expression using antisense phosphorothioate oligonucleotides resulted in growth inhibition of human MDA-MB-231 breast cancer cells with relatively high expression of the *MTA1* gene. Furthermore, the MTA1 protein was localized in the nuclei of cells transfected using a mammalian expression vector containing the full-length *MTA1* gene. The results suggest that the MTA1 protein may function in cellular signaling processes important in the progression and growth of cancer cells, possibly as a nuclear regulatory factor. J. Cell. Biochem. 79:202–212, 2000.

Key words: nuclear regulatory protein; gene expression; antisense oligonucleotides; cell proliferation; nucleosome remodeling histone deacetylase complex; gene structure; cancer cells

Several genes have been identified as metastasis-associated genes [Moustafa and Nicolson, 1998]. For example, it has been reported that *mst1*, *nm23*, *WDNM1*, *WDNM2*, *pGM21*, *stromelysin-3* and *KAI-1* genes [Dear et al., 1988, 1989; Ebralidze et al., 1989; Bisset et al., 1990; Phillips et al., 1990; Dong et al., 1995; Steeg et al., 1988] are associated with progression or metastasis of carcinoma cells, but direct evidence for the roles of these specific genes and their encoded products in particular steps of the metastatic process for the most part is not available. In previous studies we cloned a novel candidate metastasis-associated gene, *mta1* [Toh et al., 1994], which

Abbreviations used: FBS, fetal bovine serum; GADPH, glyceraldehyde-3-phosphate dehydrogenase; HA, hemagglutinin A; *MTA1*, metastasis-associated gene-1; NuRD, nucleosome remodeling histone deaceylase complex; nt, nucleotide; PONs, phosphorothioate oligonucleotides; SDS, sodium dodecylsulfate

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*Correspondence to: Garth L. Nicolson, The Institute for Molecular Medicine, 15162 Triton Lane, Huntington Beach, California 92649. E-mail: gnicimm@ix.netcom.com

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was isolated by differential cDNA library screening using the 13762NF rat mammary adenocarcinoma metastatic system [Pencil et al., 1993]. We found that *mta1* mRNA was differentially expressed in highly metastatic rat mammary adenocarcinoma cell lines [Pencil et al., 1993; Toh et al., 1994, 1995]; however, the function of the *mta1* gene product was not determined. We have now cloned the human homologue *MTA1* gene, characterized this gene and investigated the putative function of its encoded product.

Recently, two groups reported that nucleosome remodeling histone deacetylase complex (NuRD complex), which is involved in chromatin remodeling, contains MTA1 protein or a MTA1-related protein (MTA2); thus a possible function for the MTA1 protein has been reported [Zhang et al., 1998; Xue et al., 1998]. Using antisense phosphorothioate oligonucleotides (PONs), we have decreased *MTA1* gene and MTA1 protein expression in breast carcinoma cells, and this resulted in inhibition of breast cancer cell proliferation. The results suggest but do not prove that the MTA1 protein may play a role in regulating the growth of carcinoma cells.

MATERIALS AND METHODS

Isolation of Human *MTA1* cDNA Clones and Sequence Analysis

Two partial clones (1.1 and 2.6.1) of the human *MTA1* gene were obtained by screening an oligo-dT primed human melanoma A2058 cDNA library (Clontech Laboratories, Palo Alto, CA) using the rat *mta1* cDNA *Sac*II-*Bst*EII fragment. To obtain the 5′ end of the human *MTA1* gene we used the rapid amplification of 5′-cDNA ends (5′-RACE) method with a Marathon cDNA amplification kit (Clontech). After isolation of poly(A)$^+$ RNA from A2058 melanoma cells, amplification was performed using RT1 (5′-GACGCTGATTTGGTTCGGATTTGGCTTGTT-3′ corresponding to nt +1139/+1130 of human *MTA1* cDNA) and RT2 (5′-TTCCTCTTCTATTTCCCCTTCCTCGCC-3′ corresponding to nt +290/+264 of human *MTA1* cDNA). We obtained three independent clones containing the ATG codon of the human *MTA1* gene. Sequencing was performed with the USB sequence 2.0 kit (U. S. Biochemical Corp.) using plasmid-specific and *MTA1*-specific primers, and both strands of the double-stranded DNA templates were sequenced. Sequence analyses and alignments were performed with the program package from the Genetics Computer Group. The amino acid alignment of the predicted human and rat protein sequences was performed by the BESTFIT program; identical amino acids were aligned, and well-conserved amino acid replacements that scored better than 0.5 in the PAM-250 matrix and replacements scoring better than 0.1 were determined. The 2640 nt human *MTA1* sequence is available through accession number U35113 from the NCBI sequence data bank.

Cell Lines, and RNA and DNA Analyses

All cell lines were obtained from American Type Tissue Collection unless noted otherwise. Northern blot and Southern blot analyses were performed using standard procedures. RNA isolation from the different cell lines was done with TRI Reagent^{TM} (Molecular Research Center, Cincinnati, OH). Ten micrograms of total RNA was transferred after gel electrophoresis to Nytran-N filters (Amersham International, Buckinghamshire, U.K.) and hybridized in rapid hybridization buffer according to the manufacturer's instructions (Amersham). *MTA1* transcripts were detected with the ^{32}P-labeled clone 2.6.1. of human *MTA1* fragment (nt +926/+2640). All Northern filters were hybridized with ^{32}P-labeled GAPDH probe to control for the amounts of RNA in the lanes. The ZOO-BLOT membrane was purchased from Clontech. The filter was hybridized with the ^{32}P-labeled clone full-length human *MTA1* cDNA overnight in rapid hybridization buffer according to the manufacturer's instructions (Amersham) and then washed for 1 h at 50°C in 2 × SSC, 0.1% sodium dodecyl sulfate (SDS).

MTA1 Protein, Antibodies, and Immunoblotting

A 14-mer synthetic oligopeptide (EEALEKYGKDFTDI) from the predicted MTA1 protein sequence was conjugated to keyhole limpet hemocyanin (Sigma, St. Louis, MO) and injected into mice with Freund's adjuvant according to standard protocols. To evaluate the quality of polyclonal anti-human MTA1 protein antibodies, a GST-MTA1 fusion protein was generated. A human *MTA1* gene fragment (nt +264/+1008), containing the oligopeptide sequence antigen for generating anti-MTA1 antibodies, was inserted into the prokaryotic expression vector pGEX-2T (Pharmacia Biotech,

Upssala, Sweden), expressed in *Escherichia coli* strain BL21 and purified as described. Cell lysates were generated and immunoblotting assays were performed as described [Toh et al., 1994, 1995] employing MDA-MB-231 breast cancer cells. For immunoblotting, anti-human MTA1 antibody was diluted 1:1,000. Blocking experiments were performed at a peptide concentration of 30 mg/ml for 1 h at 4°C.

Antisense Oligodeoxynucleotides

PONs were synthesized on a autosynthesizer (Expedite 8900, Nihon PerSeptive, Tokyo, Japan) at Science Tanaka Co., Ltd., Ishikari, Japan. The crude dimethoxtritylated phosphorothioate sequences were purified on a TSK gel Oligo DNA-RP reverse-phase, high-performance liquid chromatography column (Tosho, Tokyo, Japan) before being detritylated and desalted. Following detritylation with 80% acetic acid, the oligonucleotides were dried, dissolved in 0.5 M NaCl, and the oligonucleotides desalted on a Nap-10 column (Pharmacia Biotec). The PONs used in this study were 24-mer in length and directed to the region of translation initiation on the corresponding human *MTA1* mRNA. Their sequences were as follows: sense *MTA1*, 5′-TTCCCAACTATAACAAGCCAAATC-3′; antisense *MTA1*, 5′-GATTTGGCTTGTTATAGTTGGGAA-3′. These oligonucleotides were designed based on the theory of PASTM (Toagoseico, Ltd., Tukuba, Japan). The antisense oligonucleotides sequences were searched against GeneBank and no significant human homologies were identified to genes except *MTA1*.

Construction of *MTA1-HA* Expression Vector

A *MTA1-HA* expression vector was constructed according to the method described previously [Martin and Orkin, 1990]. First, *MTA1* was inserted into pcDNA3 mammalian expression vector (Invitrogen) at *Bam*HI site. To link the HA sequence at the C terminal end of the *MTA1*, the fragment of *MTA1* gene was amplified by PCR using Pfu DNA polymerase (Stratagene) and the following primer sets (sense: 5′-CGAGAGCTGTTACACCACAC-3′; antisense: 5′-GTCCTCGATGACGATGGGCTC-3′). Next, double-strand oligonucleotides that code HA and have a *Xba*I site at the 3′-end were ligated to the amplified *MTA1* fragment, then this fragment was inserted into the *MTA1*-pcDNA3 vector at the *Msc*I and *Xba*I sites.

Transient Transfection and Immunofluorescence Studies

Using 5–8 μg of the appropriate mammalian expression construct 293T cells were transiently transfected by the calcium phosphate method. At 48 h after transfection, the cells were prepared for immunofluorescence as described elsewhere [Borden et al., 1995]. Briefly, cells were washed twice in phosphate-buffered saline (PBS), followed by fixation in methanol for 10 min at −20°C prior to a application of antibodies. Mouse anti-HA monoclonal antibody (Boehringer Mannheim) was used at a dilution of 1:100. Antibodies were detected with a 1:500 dilution of goat anti-mouse IgG (H+L) FITC antiserum (Cappel). Nuclei were stained with PBS containing 0.5 μg/ml propidium iodide (Sigma). Fluorescence was observed with a confocal laser microscope (MRC1024, BioRad) that recorded green (488 nm excitation) fluorescence.

Cell Growth Assays and MTA1 Protein Detection

Cells used for this study were maintained in monolayer culture in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), nonessential amino acids, sodium pyruvate, and antibiotics at 37°C , 95% air, 5% CO_2. For analysis of the effect of antisense-*MTA1* PONs on cell growth, MDA-MB-231 and MDA-MB-435 cells were plated in six-well dishes. When the cells were 80% confluent, they were washed with prewarmed PBS and treated with *MTA1*-PONs in Lipofectamine Plus Reagent (Life Technologies) containing DMEM with a fixed ratio of oligo versus Lipofectamine Plus Reagent (4 μl of Lipofectamine Plus Reagent per 500 nM oligo) for 3 h. Thereafter, the oligo-containing medium was replaced with DMEM, and cell numbers were determined with a hemocytometer. MDA-MB-231 and MDA-MB-435 cells at densities of 2,500 and 3,000 cells/well, respectively, in 96-well plates were incubated with transfected oligos in 100 μl DMEM plus 10% FBS. Cell growth was assessed after an additional six days (MDA-MB-435) or four days (MDA-MB 231) using a crystal violet assay [Cavanaugh and Nicolson, 1989]. Cells were washed with PBS, and were fixed at 25°C for 30 min with PBS containing 5 % (v/v) glutaraldehyde. Fixed cells were washed with water, allowed to dry, and stained for 30 min at 25°C with 50 μl of

0.1% crystal violet (Sigma), pH 9.5, 50 mM cyclohexylaminopropane sulfonic acid (ICN Biomedicals, Costa Mesa, CA). Stained cells were washed with water and solubilized with 50 μl of 10% acetic acid, and cell numbers were determined by reading absorbance at 570 nm on a Dynatec MR5000 plate reader. All assays were performed in triplicate per experiment, and the results were repeated at least three times. To determine the effect of antisense-*MTA1*-PONs on the expression of MTA1 protein, MDA-MB-231 cells were treated with PONs as described above, and the oligo-containing medium was replaced with DMEM containing 10% fetal calf serum. At 24 and 48 h after treatment, the cells were lysed and immunoblot analysis was performed as described above.

RESULTS

Isolation and Sequence Analysis of Human *MTA1* cDNA

The nucleotide sequence of the human *MTA1* gene (accession number U35113) was 88% identical to the rat *mta1* sequence. The human *MTA1* gene encoded a putative protein of 715 amino acid residues with a predicted molecular weight of ~82 kDa (Fig. 1). The amino acid sequences of the rat and human proteins were 96% identical and 98% similar (Fig. 1). Similar to the rat Mta1 protein, the human MTA1 protein contained a proline-rich stretch (LPPRPPPPAP) at the carboxy-terminal end of the molecule at residues 696–705. This sequence completely matched the consensus sequence for the src homology 3 domain-binding site, XPXXPPPFXP [Ren et al., 1993] or XpFPpXP [Yu et al., 1994] (where X stands for nonconserved residues, P for proline, p for residues that tend to proline, and F for hydrophobic residues) (Fig. 1). In this analysis of the human MTA1 protein, we also found a putative zinc finger DNA binding motif Cys-X2-Cys-X17-Cys-X2-Cys [Martin and Orkin, 1990] beginning at residues 393, and a leucine zipper motif [Vinson et al., 1989] beginning at residue 251. These sequences were also conserved in the rat Mta1 protein (Fig. 1). The human MTA1 protein was rich in SPXX motifs, and these are known to occur frequently in gene regulatory and DNA-binding proteins [Suzuki, 1989]. The human MTA1 protein contained five SPXX sequences (Fig. 1), corresponding to frequencies of 7.09×10^{-3} which is ~2.5 times the average protein frequency (2.89×10^{-3}). Furthermore, the MTA1 protein encoded three putative nuclear localization sequences (using the PSORT prediction software) (Fig. 1).

Evolutionary Conservation of *MTA1* Genes

To assess the extent of evolutionary conservation of the *MTA1* gene, we analyzed genomic DNA of several species by Southern blot analysis. Strong genomic signals were detected in monkey and yeast; moderate signals in human, rat, mouse, dog, cow, and rabbit; and weak signals were detected in chicken (Fig. 2A). Therefore, the *MTA1* gene was conserved in all species examined.

Expression of *MTA1* Gene in Human Cell Lines

To determine the expression of the *MTA1* gene in nontumorigenic and tumorigenic cells, we examined 14 cell lines of human origin. *MTA1* transcripts were found in virtually all cell lines analyzed. Figure 2B shows representative examples of the relative expression of the *MTA1* gene. Interestingly, MDA-MB-231 cells of high metastatic potential strongly expressed the *MTA1* gene, whereas MDA-MB-435 cells of poor metastatic potential (Price et al., 1990) expressed the *MTA1* gene at very low levels. The expression level of the *MTA1* gene in a normal breast epithelial cell line (Hs578Bst) with slow growth rate was from one-third to one-half that seen in breast adenocarcinoma cells and atypical breast cells (HBL-100) with a rapid growth rate (Fig. 2B). The relative expression (normalized with respect to GADPH expression) of the *MTA1* gene in various human cell lines from highest to lowest was as follows: MDA-MB-231, HeLa > SKOV-3, ZR-75-1, HBL-100, A2058 > OVCA-433, OVCA-432, Ovcar-3, HT-29, KM-12C, Hs578Bst > MBA-MD-435, OVCA-429 (data not shown). Thus the *MTA1* gene was expressed at various levels among different cell lines.

Localization of MTA1 Protein

Using indirect immunofluorescence we found that the MTA1 protein accumulated in the nucleus (Fig. 3 a, c, g, i). This nuclear immunoreactivity was present in many large, intense foci that were not detected near the nuclear membrane. In addition, the nucleolus region was negative for fluorescence (Fig. 3g, i).

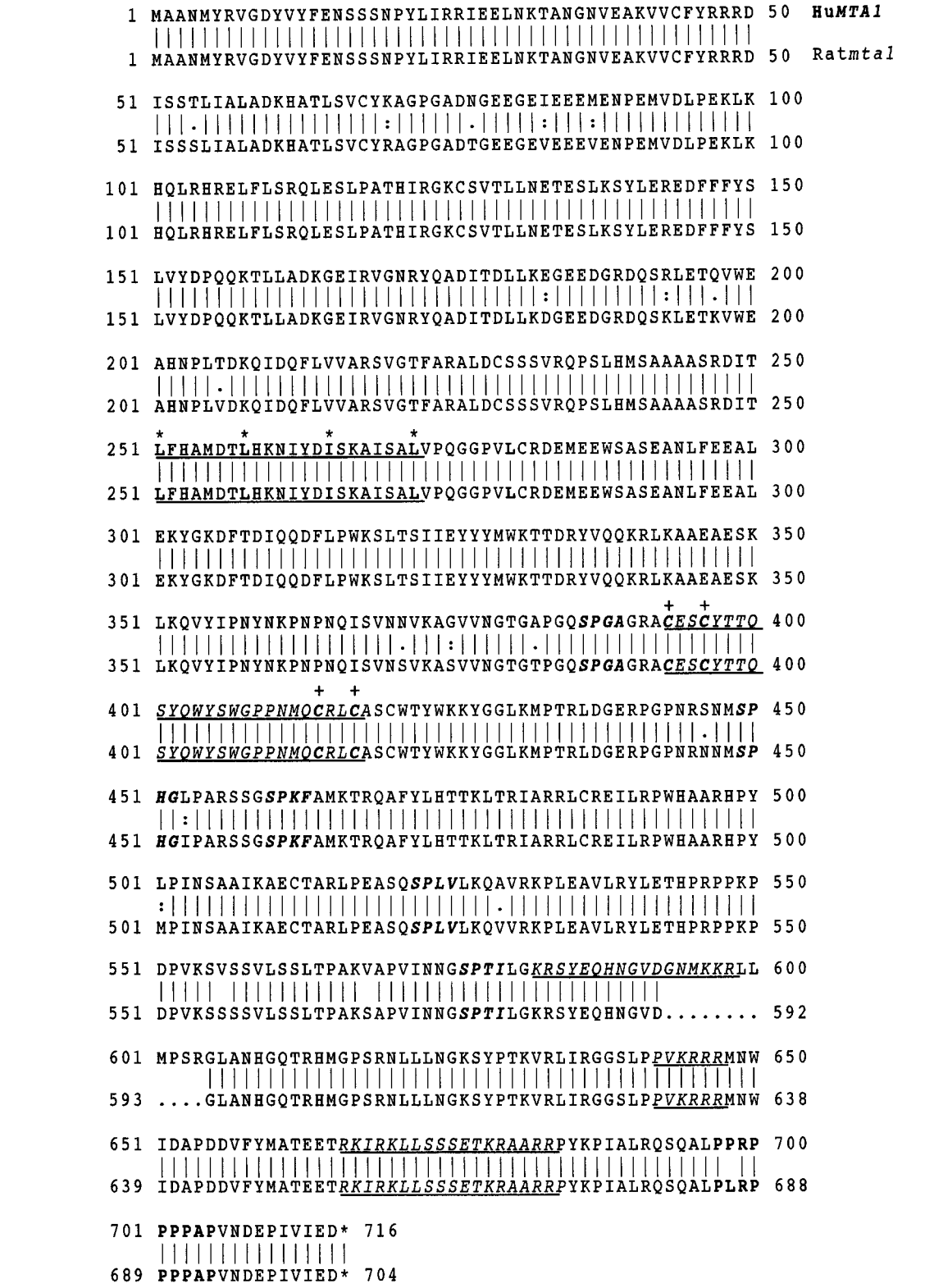

Fig. 1. Alignment of the predicted amino acid sequences of the human MTA1 and rat mta1 proteins. The identical amino acid residues (96%) between human MTA1 and rat Mta1 proteins are indicated by (|), well-conserved replacements by (:) and less conserved (.) (see Materials and Methods). The underlined polypeptide sequences 251–273 are characteristic of a lucine zipper motif. The underlined and italic polypeptide sequences 393–417 are characteristic of a GATA-type zinc finger motif. Five SPXX motifs are also present and conserved in both human MTA1 and rat Mta1 proteins. Three and two putative nuclear localization sequences (shown in underlined and italic figures) are in human MTA1 and rat Mta1, respectively. The C-terminal proline rich region found previously (10) starts at amino acid residue 696 of the human MTA1 protein.

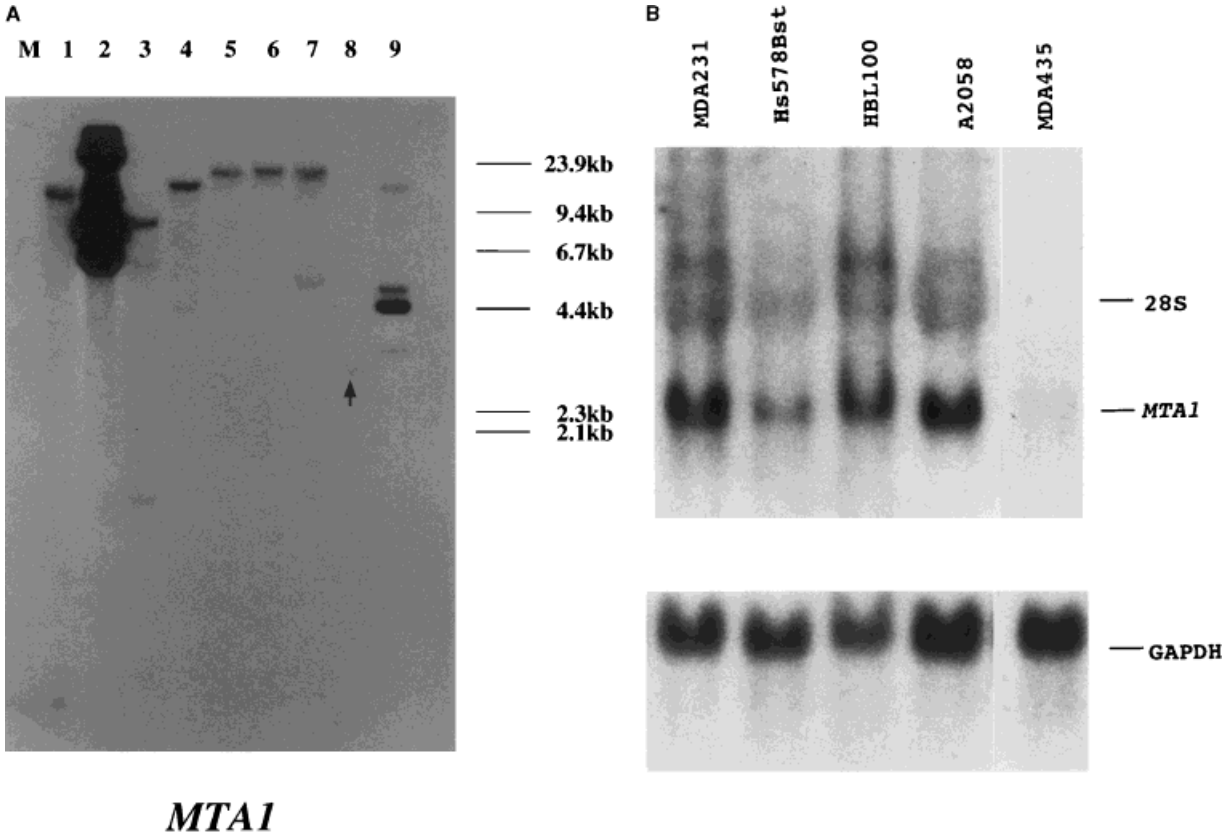

Fig. 2. **(A)** Evolutionary conservation of the genomic *MTA1* gene sequences. The ZOO-BLOT sheet (Clontech) was used for Southern blot analysis with the full-length human *MTA1* c-DNA. Each lane contains 4 μg of *Eco*RI digested genomic DNA isolated from the following species: Lane M: *Hin*dIII DNA size markers; Lane 1, human; Lane 2, monkey; Lane 3, rat; Lane 4, mouse; Lane 5, dog; Lane 6, cow; Lane 7, rabbit; Lane 8, chicken; Lane 9, yeast. Strong genomic signals are detected in monkey and yeast; a weak signal is detected in chicken. The *MTA1* gene is well conserved in all species we examined. **(B)** Examples of *MTA1* gene expression in human cell lines. A northern blot containing RNA isolated from a normal breast cell line (Hs578Bst), atypical breast cell line (HBL100), breast carcinoma cell lines (MDA-MB-231, MDA-MB-435) and a melanoma cell line (A2058) were hybridized with a *MTA1* probe (top) or a *GAPDH* probe (bottom). Note the differences in *MTA1* gene expression levels among the various mammary cell lines.

Effect of *MTA1* Antisense Oligonucleotides on Breast Cancer Cell Growth

To directly demonstrate a role for the *MTA1* gene in breast cancer cells, PONs treatment was continued for 4 h, after which the proliferation of MDA-MB-231 and MDA-MB-435 cells were monitored for several days. Antisense PONs markedly inhibited the cell growth of MDA-MB-231 cells to 22% of mock-treated cells and to 28% of sense PONs-treated cells at 72 h, respectively (Fig. 4A). In contrast, antisense PONs did not affect the growth of MDA-MB-435 cells (Fig. 4B).

Effect of *MTA1* Antisense Oligonucleotides on MTA1 Protein Levels

To confirm that the inhibition of cell proliferation by antisense PONs was involved in the suppression of the MTA1 protein, the MTA1 protein levels of MDA-MB-231 cells treated with antisense PONs and sense control were examined by Western blot analysis using anti-MTA1 protein polyclonal antibodies as described in Materials and Methods (Fig. 4C). As shown in Fig. 4D, the anti-MTA1 protein recognized an 83-kDa protein band that was identified as identical in migration to the MTA1 protein. This band disappeared after preincubation of anti-MTA1 protein with the immunogen oligopeptides (Fig. 4D). Since a lower molecular weight band at ~73 kDa remained after this preincubation treatment that was also was detected by preimmune mouse serum (Fig. 4D), we concluded that this 73-kDa band was a nonspecific band. To verify that the antisense effects were due to inhibition of target gene ex-

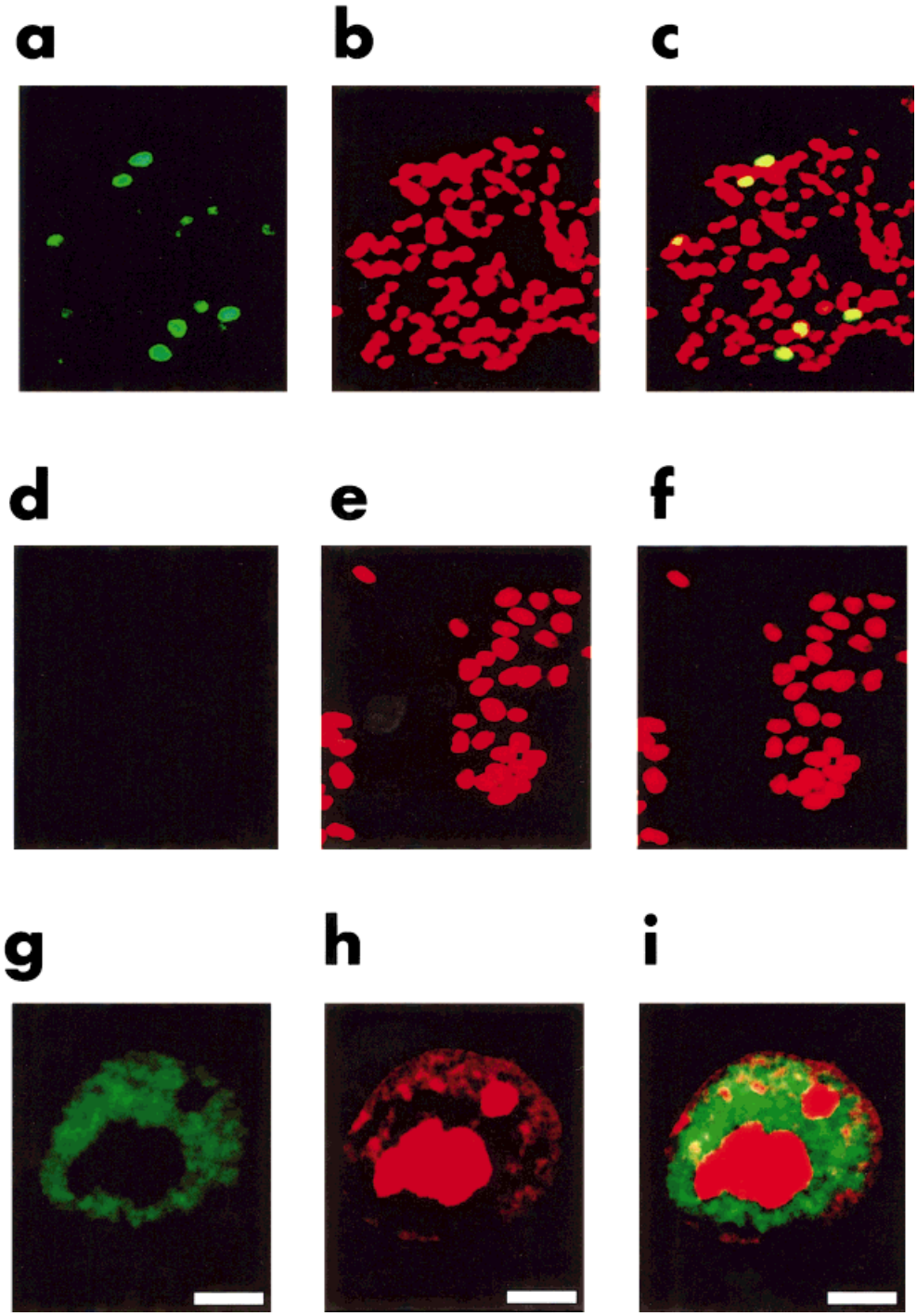

Fig. 3. Localization studies of the MTA1 protein. Cells were transfected as described in Materials and Methods. pcDNA3 containing human *MTA1 -HA* mammalian expression vectors were transfected (**a**, **b**, **g**) or only pcDNA3 mammalian expression vectors were transfected (**d**, **e**, **h**). Cells stained with mouse affinity-purified anti-HA antibody with FITC-labeled goat anti-mouse IgG as a secondary antibody (a, d, g). Excitation at 488 nm. Cells were stained with 0.5 μg/ml propidium iodide (b, e, h), and overlayed (**c**, **f**, **i**; co-localization is in yellow). Magnification X40, except for g, h, i, X400. Bar equals 10 μm (g, h, i).

pression, we quantitated MTA1 protein expression in MDA-MB-231 cells treated with antisense PONs or sense control PONs. We detected significant suppression of MTA1 protein by antisense PONs at 48 h (Fig. 4E), and antisense inhibition of the *MTA1* gene resulted in approximately 70% reduction of MTA1 protein levels in the antisense PONs-treated cells

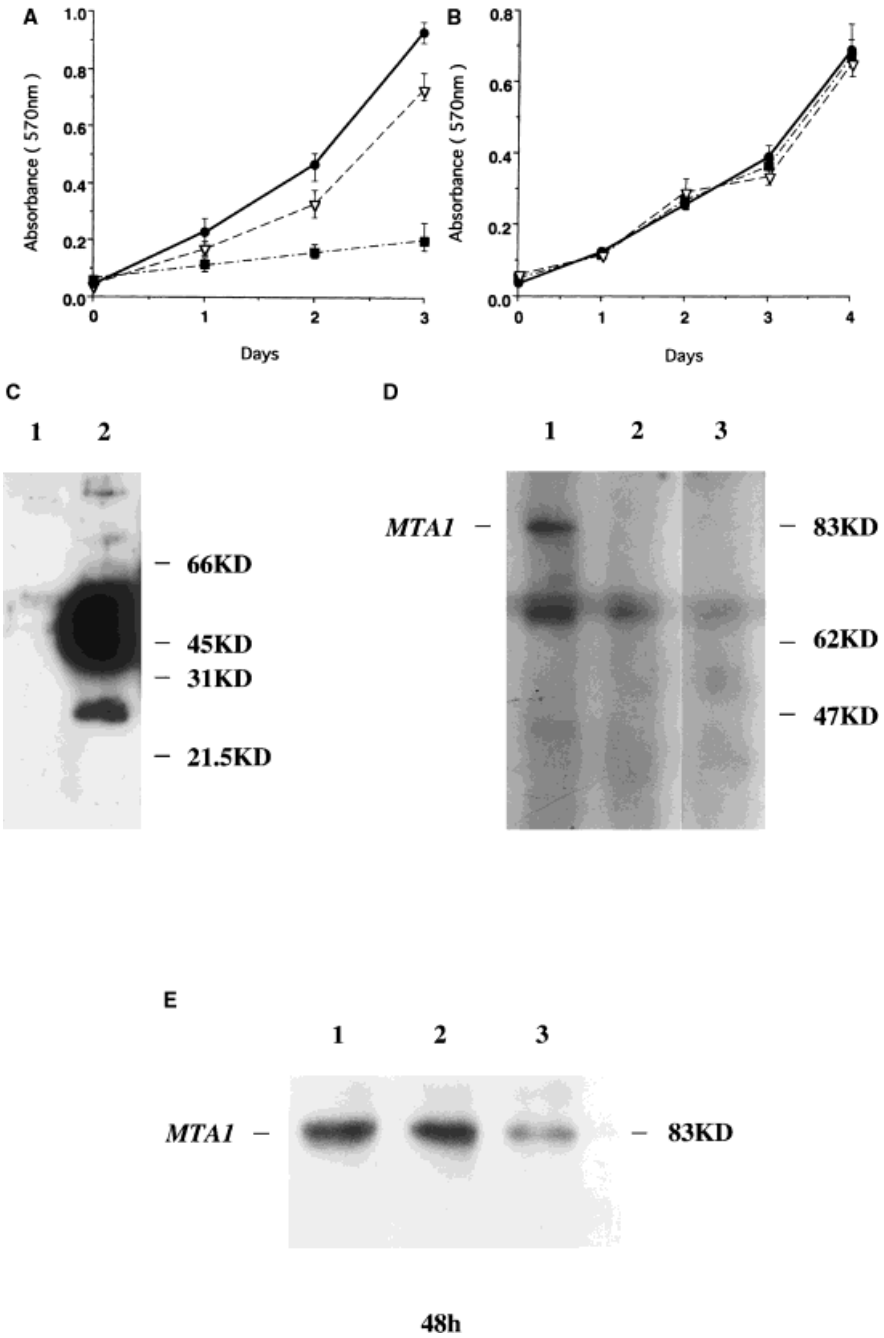
A
Absorbance (570nm)
Days
B
Absorbance (570nm)
Days
C
1 2
66KD
45KD
31KD
21.5KD
D
1 2 3
MTA1
83KD
62KD
47KD
E
1 2 3
MTA1
83KD
48h

Figure 4.

at 48 h compared with cells treated with sense control PONs (Fig. 4E).

DISCUSSION

The metastasis-associated *MTA1* gene is a novel, highly conserved gene that encodes a nuclear protein product of unknown function. The human MTA1 protein appears to be well conserved with only a 4% divergence at the amino acid sequence level between the human and rat genes [Toh et al., 1994]. The putative functional domains like the SH3-binding motif [Ren et al., 1993; Yu et al., 1994], GATA-type zinc finger motif [Martin and Orkin, 1990; Kudla et al., 1990], leucine zipper motif [Vinson et al., 1989], and the SPXX motifs [Suzuki, 1989] were highly conserved between the predicted human and rat protein sequences. The *MTA1* gene was expressed in all tumor cell lines analyzed thus far, but similar to the rat *mta1* gene we found different quantities of *MTA1* transcripts in various cells. With the exception of the human breast cancer cell line MDA-MB-435, we found that the expression level of the *MTA1* gene in untransformed breast epithelial cells was 28%–50% of that found in breast cancer or atypical mammary cell lines. In general, the more progressed mammary cells with higher amounts of MTA1 protein grow at faster rates, suggesting that the *MTA1* gene might be involved in the process of cellular proliferation.

We previously found that the rat *mta1* gene was expressed at low levels in normal tissues, with the exception of the testis [Toh et al., 1994]. In the testis, spermatogenesis occurs as a highly controlled and complex process typified by a high rate of cell proliferation that is tightly regulated by a number of growth factors and cytokines. This is consistent with the notion that the MTA1 protein might be involved in normal cellular functions, such as cell proliferation.

To determine if the *MTA1* gene is involved in the regulation of cell proliferation, we used antisense oligonucleotide treatment of breast cancer cell lines that show different levels of expression of the *MTA1* gene. Antisense PONs against the *MTA1* gene inhibited the cell growth of MDA-MB-231 breast cancer cells. Specific inhibition of gene expression by the use of antisense PONs has been extensively used as an experimental strategy [Higgins et al., 1993; Takeuchi et al., 1994; Thompson et al., 1994]; however, antisense PON methods may occasionally produce artifactual results that are unrelated to the inhibition of protein synthesis of a target gene [Yaswen et al., 1993; Stein, 1995]. Moreover, addition of PONs directly to cells sometimes results in their localization at the cell surface where they can cause nonspecific effects [Bennett et al., 1992]. Thus using the lipofectamine method to transfect specific and control PONs, we monitored MTA1 protein levels at various times. We found that after transfection of antisense PONs MTA1 protein decreased to 20%–30% of that found in sense-treated cells at 48 h. We failed to find significant changes in the amounts of MTA1 protein with the sense sequence PONs. This indicates that the growth inhibition by antisense PONs in MDA-MB-231 cells was a

Fig. 4. Effect of *MTA1* antisense PONs on the expression of the MTA1 gene in MDA-MB-231 cells and evaluation of mouse antibody to human MTA1 protein. **(A)** Time-course of MDA-MB-231 cell growth inhibition using *MTA1* antisense with PONs with MDA-MB-231 cells; **(B)** Same as A, except that MDA-MB-435 cells were used. Cells were cultured as described in Materials and Methods. Points and bars represent the mean±SE from triplicate cultures. •, No PONs; ▽, *MTA1* sense-PONs; ■, *MTA1* antisense-PONs. **(C)** Western blot analysis of GST-MTA1 fusion protein. Truncated human *MTA1* gene (+264/+1008 containing a sequence that encodes an oligopeptide antigen) was inserted into the prokaryotic expression vector pGEX-2T and expressed in *E. coli* (BL21); Lane 1, GST; Lane 2, GST-MTA1 fusion protein. **(D)** Western blot analysis and competition study of human MTA1 protein. Thirty μg of MDA231 cell lysate was loaded onto each lane, and after electrophoresis, the proteins were transferred to a Nylon membrane. The human MTA1 protein was detected with anti-peptide antibodies as a 83 kDa band (Lane 1). After the antibodies were pre-incubated with 30 μg/ml of the specific peptide, the human the MTA1 protein was not detectable (Lane 2). Preimmune mouse serum recognized a nonspecific 73 kDa protein (Lane 3). **(E)** Effect of *MTA1* antisense PONs on the expression of the MTA1 gene in MDA-MB-231 cells. Cells were cultured for 24 or 48 h after transfected *MTA1* antisense PONs as described in Materials and Methods. The cells were harvested, lysed, proteins separated by SDS-PAGE, and the gel was immunoblotted with anti-human MTA1 antibody as described in Materials and Methods. Ten μg of cell protein extract was loaded for each sample. Lanes 1 and 4, no PONs; Lanes 2 and 5, *MTA1* sense PON; Lanes 3 and 6, *MTA1* and antisense PON. In this figure, the level of MTA1 protein in cells treated with *MTA1* antisense decreased to 30% of the level without PON or using *MTA1* sense PON at 48 h.

sequence-specific effect. Interestingly, we could not demonstrate an effect of antisense PONs on the growth inhibition of MDA-MB-435 cells with a low level of MTA1 protein expression. Thus MDA-MB-435 cells might use different signaling pathways for regulating cellular growth that are unrelated to or are less dependent on the MTA1 protein. In support of this, the proliferation of MDA-MB-231 cells that expressed MTA1 protein at higher concentrations appeared to be much more growth-dependent on the concentration of MTA1 protein than MDA-MB-435 cells.

The MTA1 protein may be related to remodeling events in the nucleus. When the *MTA1* gene was transfected and expressed in 293Tcells, the MTA1 protein localized within the nuclear matrix. Recently, two groups reported that nucleosome remodeling histone deacetylase complex (NuRD complex), which is involved in chromatin remodeling, contains the MTA1 protein or an MTA1-related protein (MTA2) [Zhang et al., 1998; Xue et al., 1998]. The nuclear localization of MTA1 protein that we found is consistent with these reports. It is well known that trichostatin A, which is a potent specific inhibitor of histone deacetylase (HDAC), causes G1/G2 arrest in fibroblasts [Yoshida and Beppu, 1998]. Moreover, HDAC1, which is a component of the NuRD complex, has been shown to interact with Rb to repress transcription [Brehm et al., 1998; Luo et al., 1998; Magnaghi-Jaulin et al., 1998]. Acetylation of the C-terminal of p53 changed its ability to bind to DNA [Gu and Roeder, 1997]. Therefore, the MTA1 protein might interact with the histone deacetylase and act as a coactivator. In support of a nuclear function for the MTA1 protein, we found that it has a unique protein primary structure that suggests that it might function in signal transduction and DNA-binding. The MTA1 protein is the first mammalian protein found that contains the motif Cys-X2-Cys-X17-Cys-X2-Cys, which is a zinc-finger domain that also appears in GATA transcription factors. The same zinc-finger domain configuration has been found in *GLN3*, *areA* and *nit-2*, major regulatory factors for nitrogen metabolism in *Saccharomyces cerevisiae*, *Aspergillus* and *Neurospora*, respectively [Fu and Marzluf, 1990; Kudla et al., 1990; Minehart and Magasanik, 1991]. The nit-2 protein recognizes an identical core sequence of TATCTA, and a recent study has also shown that the GLN3 protein binds the nitrogen upstream activation sequence of *GLN1*, the gene that encodes glutamine synthetase [Minehart and Magasanik, 1991]. Considering these findings, it is plausible that the MTA1 protein could bind to a specific sequence of DNA and be involved in gene regulation.

Although we have yet to demonstrate that the MTA1 protein interacts with specific genes involved in cellular regulation, we have found [unpublished data] that the expression of the *MTA1* gene was increased four-times in *c-erbB2/neu* stable transfectants of MDA-MB-435 cells compared to untransfected cells. We have also found recently using a double-labeling procedure that the MTA1 protein is physically associated with histone deacetylase 1 (HDAC1) in a protein complex (NURD or nucleosome remodeling and histone deacetylation) complex [Toh et al., 2000]. Demonstration of the function and activities of the MTA1 protein and its role in nuclear protein complexes will be necessary to confirm our hypothesis that the *MTA1* gene is involved in gene and cell growth regulation. With this information we should be better able to understand the role of the *MTA1* gene in the progression of cancer and cancer cell growth.

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