Repression of 92-kDa Type IV Collagenase Expression by MTA1 Is Mediated through Direct Interactions with the Promoter via a Mechanism, Which Is Both Dependent on and Independent of Histone Deacetylation*

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Although the expression of the metastases-associated gene MTA1 correlates with tumor metastases, its role in regulating type IV collagenase expression is unknown. Enforced MTA1 expression in HT1080 cells reduced basal and 12-myristate 13-acetate-induced 92-kDa type IV collagenase (MMP-9) protein/mRNA levels. DNase I hypersensitivity and PstI accessibility assays revealed multiple regions of the MMP-9 promoter (−650/−450 and −120/+1), showing reduced hypersensitivity in the MTA1-expressing cells. Chromatin immunoprecipitation assays demonstrated MTA1 binding to the distal region, which spans several regulatory cis elements. Co-immunoprecipitation and chromatim immunoprecipitation assay experiments revealed histone deacetylase 2 (HDAC2)-MTA1 protein-protein interactions and the MTA1-dependent recruitment of HDAC2 to the distal MMP-9 promoter region, yielding diminished histone H3/H4 acetylation. However, HDAC2 binding and H3/H4 acetylation at the proximal MMP-9 region were unaffected by MTA1 expression. Furthermore, trichostatin treatment only partially relieved MTA1-repressed MMP-9 expression, indicating a HDAC-insensitive component possibly involving the nucleosome-remodeling Mi2 activity, which was recruited to the promoter by MTA1. In summary, (a) MMP-9 adds to a short list of MTA1-regulated genes, which so far only includes c-myc and p52, and (b) MTA1 binds to the MMP-9 promoter, thereby repressing expression of this type IV collagenase via histone-dependent and independent mechanisms.

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Additionally, MMP-9 is required for human bronchial epithelial cell migration and spreading following injury (8). In cancer, there is strong evidence implicating this type IV collagenase in the spread of the disease. Thus, Bernhard et al. (9) reported that the overexpression of this metalloproteinase in rat embryo cells conferred a metastatic phenotype, whereas the inhibition of MMP-9 expression by a ribozyme blocked metastasis of rat sarcoma cells (10).

The MMP-9 gene located on chromosome 20 (11) covers 13 exons spanning 7.7 kilobases, (12) and its transcription yields a 2.5-kilobase mRNA (13). The regulation of MMP-9 protein levels has been ascribed to transcriptional activation of the gene (14, 15), reduced mRNA turnover (16), and altered translational efficiency (17). The 5′-flanking sequence contains binding sites for AP-1, NFκB, Sp1, and Ets transcription factors within the first 670 base pairs, and these have been implicated in the regulation of MMP-9 gene expression by a variety of cytokines and contact inhibition (14, 18, 19). In addition, studies with transgenic mice have demonstrated the requirement of regions −522/+19 and −2722/−7745 for developmental regulation in mice and for tissue-specific expression in osteoclasts and migrating keratinocytes, respectively (20, 21).

Although MMP-9 has been implicated in both physiological and pathological processes, how its expression is regulated is still not fully understood. In the last 5 years, a great deal of interest has accrued in a group of genes collectively referred to as metastases-associated genes, which modulate cancer metastases but not tumorigenesis (22–24). One of these is the MTA1 gene, which is overexpressed in metastatic mammary adenocarcinoma and prostate cancers (25, 26) and subsequently determined to enhance the migration and invasion of immortalized human keratinocytes (27). The MTA1 gene resides on chromosome 14q32.3 (28) and encodes a protein, which binds histone deacetylases 1 and 2 (HDAC1 and HDAC2) (29). Furthermore, the MTA1 protein is one of the subunits of the human nucleosome-remodeling and deacetylation complex shown previously to regulate in vitro transcription of at least artificial reporter constructs via ATP-dependent nucleosome disruption and changes in histone acetylation (30).

Because MMP-9 has a well established role in tumor cell invasion and metastases and considering that MTA1 expression is associated with the metastatic phenotype, we undertook the present study to determine whether the expression of this collagenase is targeted by this metastases-associated gene.

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The abbreviations used are: HDAC, histone deacetylase; IP, immunoprecipitation; TSA, trichostatin A; ChIP, chromatin immunoprecipitation; CREB, cAMP-response-element-binding protein; PMA, phorbol 12-myristate 13-acetate.
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EXPERIMENTAL PROCEDURES

Plasmids and Antibodies—A plasmid construct expressing a myc-tagged human MTA1 (pB3-myc/MTA1) and its vector pB3-Myc were as described previously (31). To construct the MTA1-expressing vector in the Flp-In System (Invitrogen), the pB3-myc/MTA1 was digested with XhoI and BamHI. A 2.2-kilobase fragment corresponding to the MTA1 cDNA was purified and ligated into the pcDNA5/FRT vector. The resulting MTA1-expressing plasmid was designated as pcDNA5/FRT/MTA1.

Antibodies—Two polyclonal antibodies were purchased from Upstate Biotechnology (Lake Placid, NY), whereas the rabbit antibody directed against HADCA2 (H54) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibodies to HDAC1 (H11), HADCA2 (C-8), and a rabbit antibody to Mi2 (H-242) were purchased from Santa Cruz Biotechnology.

Cell Culture and Transfections—Human fibrosarcoma cells HT1080 were routinely maintained in McCoy 5A medium supplemented with 10% fetal bovine serum and antibiotics. For transfections, cells were transfected with poly-L-ornithine as described previously (15, 32). Clones were selected with the appropriate antibiotic: 600 μg/ml G418, 300 μg/ml zeocin, or 300 μg/ml hygromycin B.

Establishment of Flp-In MTA1-expressing Cells—This was performed as described in the two-step protocol provided by the manufacturer. Initially, a Flp-In host cell line containing a single copy integrated FRT sequence was established from HT1080 cells. For this purpose, HT1080 cells were transfected with pFRtlacZeo and selected in zeocin-containing medium. The resistant clones were expanded and analyzed by Southern blotting (33). To identify single integrants of the FRT sequence, DNA from the clones digested with HinIII was resolved for varying cell numbers were subjected to zymography as described (31).

Preparation of total RNA and reverse transcription were as described previously (15). cDNA was then subjected to multiplex PCR with 5 nM MMP-9 primers (5′-CCGGCATCTGAGGACTCGTCATACT-3′ and 5′-AGGGGCCGGACTCGTCATACT-3′) and 5′-GAGACAG-3′ modified PCP reaction cycle parameters: denaturation at 94 °C for 30 s; annealings at 56 °C for 30 s, and extension at 72 °C for 30 s. The number of PCR cycles is indicated in the figure legends. Alternatively, real-time PCR was done as described above to quantify the immunoprecipitated DNA. Two primer sets were used for amplifying the − 657/− 848 and − 868/− 354 regions, respectively: PN1, 5′-TGGCTCTATCTGCGGACTGACCC-3′ and 5′-ACTCCAGGCTGTCTGCTTCTT-3′; and P3, 5′-TGACCCCT-GAGTCACAGTTT-3′ and 5′-CTGCCAGAGGCTATGTTA-3′. A ΔCt value was calculated by subtracting the Ct value for the 2% input sample from the Ct value for the immunoprecipitated (IP) sample, i.e. ΔCt = Ctinput − CtIP. The percentage of the total input amount for the sample was calculated as described previously (35).

Restriction Enzyme Accessibility Assays—Restriction enzyme accessibility assays were performed essentially as described elsewhere (36, 37). 5 × 10⁶ cells were resuspended in hypotonic buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 2 mM MgCl₂, 0.15 mM spermidine, and 0.5 mM spermine) and incubated on ice for 5 min. Nonidet P-40 was then added (final concentration = 0.5%), and the cells were vortexed and incubated on ice for another 5 min. Nuclei were harvested by centrifugation, washed twice with RE buffer (10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 10 mM MgCl₂, 0.2 mM EDTA, 0.2 mM EGTA, 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride) on ice for 30 min. Nuclei were resuspended in hypotonic buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 5 mM MgCl₂, 150 mM NaF, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 1× protease inhibitor (Roche Molecular Biochemicals) on ice for 30 min. Cell lysates were centrifuged, and 60 μg of protein were resolved by SDS-polyacrylamide gel electrophoresis. Proteins were transferred to a nitrocellulose membrane, blocked with 5% milk, and incubated with a Myc monoclonal antibody and visualized by ECL. Western Blotting—Cells were washed with cold phosphate-buffered saline and lysed in modified radioimmune precipitation assay buffer (50 mM Tris-HCl, pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na₃VO₄, 1 mM NaF, and 1× protease inhibitor (Roche Molecular Biochemicals)) on ice for 30 min. Cell lysates were centrifuged, and 60 μg of protein were resolved by SDS-polyacrylamide gel electrophoresis. Proteins were transferred to a nitrocellulose membrane, blocked with 5% milk, and incubated with a Myc monoclonal antibody (9E10) or a goat anti-MTA1 antibody (A-18) (Santa Cruz Biotechnology) at 4 °C overnight. After extensive washing, the membrane was incubated with a horseradish peroxidase-conjugated secondary antibody and visualized by ECL.
RESULTS

Generation of MTA1-expressing HT1080 Cells—To answer the question as to whether MTA1 expression regulates metalloproteinase expression, we first generated HT1080 cells expressing the MTA1 coding sequence. To eliminate the confounding issue of clonal variation due to a heterogeneous cell population and random integration of the expression construct, we used the Flp-In system (Invitrogen). First, a host clone was generated by stably transfecting HT1080 cells with an expression construct (pFRT/ LacZeo) bearing a zeocin-resistant coding sequence and the Flp-In system recombination target site (FRT). Zeocin-resistant clones were subjected to Southern blotting (Fig. 1A) to select a clone bearing a single copy of the integrated plasmid. Clone F8 was determined to bear a single copy as indicated by the several bands hybridizing with the lacZ probe. Subsequently, clone F8 was co-transfected with a Flp recombinase-encoding plasmid (pOG44) and the pcDNA5/FRT/MTA1 plasmid when compared with the cells bearing the empty vector (designated FRT) or the F8 host clone (Fig. 1B).

Diminished MMP-9 Activity/mRNA Levels in MTA1-expressing HT1080 Cells—To determine whether MTA1 expression altered metalloproteinase expression, conditioned medium was analyzed by zymography. An enzymatic activity indistinguishable in size (92 kDa) from MMP-9 was detected in the control (FRT) cells. The intensity of this band was greatly induced by PMA (Fig. 2A), a known stimulant of MMP-9 expression (38). In contrast, the MTA1-expressing cells showed diminished basal MMP-9 activity and demonstrated an attenuated response to the phorbol ester. Equally important, a gelatinolytic activity migrating to the phorbol ester. Equally important, a gelatinolytic activity migrating at the 72-kDa position, which is encoded by a separate type IV collagenase (MMP-2) (11, 12, 39), was unaffected by MTA1 expression arguing against a generalized repressive effect of this metastases-associated gene. To corroborate these data, total RNA was extracted from cells treated under identical conditions and analyzed for steady-state MMP-9 transcript (Fig. 2B). Again, when compared with the FRT cells, the MTA1-expressing cells contained a lower basal level of MMP-9 mRNA and was induced to a lesser extent by the phorbol ester. In contrast, both basal and PMA-induced MMP-2 mRNA levels were unaffected by MTA1 expression (Fig. 2C). Thus, attenuated MMP-9 activity is due to a less abundant MMP-9 mRNA in the MTA1-expressing cells.

Reduced DNase I/Endonuclease Hypersensitivity at the in Vivo MMP-9 Promoter in the MTA1-expressing Cells—The ability of MTA1 to repress MMP-9 expression might be direct or indirect via the modulated expression of other genes. If the effect of MTA1 is direct, we predict that MTA1 would be bound to the endogenous MMP-9 promoter. As a first step in this direction, we identified the regions of the in vivo MMP-9 pro-
Chromatin was prepared and sheared by sonication, and proteins were cross-linked in the presence of formaldehyde. DNA was immunoprecipitated with the anti-c-Myc antibody (Fig. 5A), whereas no detectable signal was apparent with control clone P. Furthermore, the MMP-9 promoter sequence was not

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**MTA1 Is Bound to the MMP-9 Promoter in Vivo**—We then determined whether the distal DNase I hypersensitive region, which contains multiple regulatory cis elements, was bound with MTA1. Because the cloning strategy to generate the cells bearing the MTA1 sequence integrated at the FRT site had removed the myc tag, we stably transfected parental HT1080 cells with a myc-tagged MTA1 sequence. After selection of a clone (M10) with G418, MTA1 protein expression was confirmed by Western blotting using an anti-c-Myc antibody (Fig. 5A). In contrast, MTA1 protein was undetectable in clone P derived from HT1080 cells transfected with the empty vector. Subsequently DNA proteins were cross-linked in the myc-tagged MTA1-expressing clone M10 and the control clone P. Chromatin was prepared and sheared by sonication, and protein-bound DNA was immunoprecipitated with the anti-myc antibody. Precipitated DNA spanning the −634/−484 MMP-9 promoter region was amplified by PCR. The anti-myc antibody efficiently precipitated (Fig. 5C) the endogenous MMP-9 promoter fragment from the MTA1-expressing clone M10, whereas no detectable signal was apparent with control clone P. Furthermore, the MMP-9 promoter sequence was not

**Fig. 3.** Reduced DNase I hypersensitivity of the MMP-9 promoter in MTA1-expressing cells. Nuclei from FRT and MTA1 cells were digested with varying amounts of DNase I followed by DNA extraction. Equal DNA (50 μg) was digested overnight with ApaLI and subjected to Southern blotting using a 32P-labeled MMP-9 probe as indicated.

**Fig. 4.** Reduced restriction enzyme accessibility within the proximal MMP-9 promoter in MTA1-expressing cells. A, schematic description of the proximal MMP-9 promoter indicating the PstI cleavage site and the region amplified by PCR. B, nuclei from FRT and MTA1 cells were digested with 100 units PstI (37 °C, 15 min) followed by DNA extraction and purification. DNA (150 ng) was subjected to real-time PCR using the primer set P3, and the absolute amount of uncut DNA was determined by plotting Ct values against a standard curve. Data are expressed as mean values ± S.D. of three separate experiments.
Data are shown as mean values ± S.D. of six independent determinations.

HDAC2 Interacts with MTA1 and Is Bound to the −657/−484 MMP-9 Promoter Region in MTA1-expressing HT1080 Cells—Since MTA1 has previously been shown to bind HDAC1 and HDAC2, we considered the possibility that the more compact MMP-9 promoter regions as shown above was due to the recruitment of these deacetylases. To address this possibility, M10 and P cell lysates were immunoprecipitated with the anti-myc antibody, and the immunoprecipitate was subjected to Western blotting for the HDACs. HDAC2 was co-immunoprecipitated from lysates of the MTA1 clone (Fig. 6), whereas this deacetylase was undetectable with control clone P. In contrast, HDAC1 protein was undetectable in these immunoprecipitation assays using either clone (data not shown). These data reveal MTA1-HDAC2 protein-protein interactions in MTA1-expressing cells.

Using ChIP assays, we then determined whether MTA1 targeted HDAC2 to the regulatory regions of the MMP-9 promoter identified by DNase I hypersensitivity assays. Primer sets PN1 and P3 (Fig. 7A) corresponding to the proximal and distal hypersensitive regions were employed. Chromatin was immunoprecipitated with the anti-HDAC2 antibody, and the immunoprecipitated DNA was quantitated by real-time PCR (Fig. 7, B and C). For the MTA1-expressing clone M10, the anti-HDAC2 antibody precipitated the distal MMP-9 promoter fragment as evident using the PN1 primer set (Fig. 7B). In contrast, the amount of MMP-9 promoter precipitated by the HDAC2 antibody in the MTA1-deficient control clone P was barely above background (No Ab). The difference between the M10 and P clones in the amount of MMP-9 promoter fragment precipitated was statistically significant (p = 0.003). Although the anti-HDAC2 antibody precipitated the proximal MMP-9 promoter region as evident using primer set P3, the amount of DNA precipitated from the MTA1 clone M10 and the control clone P was essentially the same (p > 0.05) (Fig. 7C). These data suggest that MTA1 targets HDAC2 to the more distal regulatory region of the MMP-9 promoter.

Reduced Histone Acetylation at the −657/−484 MMP-9 Promoter Region in MTA1-expressing Cells—Since the data above indicated that targeting of HDAC2 to the −657/−484 MMP-9 promoter region was MTA1-dependent, reduced DNase I hypersensitivity might very well reflect diminished histone acetylation at this region, thereby creating a more compact structure resistant to digestion. To address this possibility, ChIP assays were repeated using antibodies directed at acetylated histones H3 and H4. Using the primer pair P3 spanning the −657/−484 MMP-9 promoter region, we found that either one of these antibodies precipitated diminished the amounts of the MMP-9 promoter from the MTA1 clones (p = 0.0011 and 0.0035 for acetylated histone H3 and acetylated histone H4, respectively) (Fig. 8A). In contrast, primer set P3, which amplifies the −86/+34 MMP-9 promoter region, indicated little change (p = 0.4062 and 0.0595, respectively) between the MTA1 and the FRT clones in either histone H3 or H4 acetylation (Fig. 8B). These data corroborate the previous experiments showing MTA1-dependent targeting of HDAC2 to the more distant MMP-9 promoter region.

Trichostatin A Only Partially Relieves MTA1-repressed MMP-9 Expression—The observations that HDAC2 binding (and histone deacetylation) to the proximal MMP-9 promoter region was unaffected by MTA1 could not explain the reduced DNase I hypersensitivity of this region evident in the MTA1-expressing clones (Fig. 9). Therefore, we speculated that this metastases-associated gene might regulate MMP-9 expression via a mechanism that was both dependent on and independent of histone deacetylation. To answer this question, MTA1 and FRT clones were treated with trichostatin A (TSA), a broad

Fig. 6. HDAC2-MTA1 protein-protein interactions. Proteins from the indicated clones were immunoprecipitated with the anti-myc-agarose beads and subjected to Western blotting for either the HDAC1 or MTA1 protein. The data are representative of two separate experiments.

Fig. 7. Binding of HDAC2 to the distal MMP-9 promoter region is MTA1-dependent. A, schematic of the MMP-9 promoter indicating the distal and proximal regions that the primer sets (PN1, P3) amplify. B and C, the MTA1-expressing clones (M10) and empty vector clone (P) were subjected to chromatin immunoprecipitation assays using a rabbit antibody against HDAC2. Precipitated DNA was subjected to real-time PCR for quantifying DNA within the distal (B) or proximal (C) MMP-9 promoter region using the primer sets PN1 and P3, respectively. Data are shown as mean values ± S.D. of six independent determinations.

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HDAC inhibitor, and analyzed for MMP-9 activity. TSA increased constitutive and PMA-inducible MMP-9 mRNA levels in both FRT and MTA1-expressing clones (Fig. 9A). However, TSA was unable to restore either the basal or the PMA-induced MMP-9 mRNA levels in the MTA1-expressing cells to that achieved in the FRT control cells. Furthermore, TSA failed to increase chromatin accessibility at the proximal MMP-9 promoter region in the MTA1-expressing cells over and above that achieved with the FRT cells (Fig. 9B). These data suggest that a more compact chromatin conformation at the proximal MMP-9 promoter region occurs independently of changes in histone deacetylation.

Since changes in histone acetylation could not entirely account for the MTA1-repressed MMP-9 expression, we considered the possibility that chromatin remodeling of the proximal MMP-9 promoter might occur via an additional mechanism. Previous studies have demonstrated that the Mi2 protein, which confers ATP-dependent chromatin-remodeling activity (30), is present in the nucleosome remodeling and deacetylase complex. Thus, we determined whether Mi2 was bound by the MMP-9 promoter. Interestingly, although the proximal primer set yielded negative data (data not shown), the distal primer set amplifying the −657/−484 sequence revealed that this chromatin-remodeling protein

Fig. 8. MTA1-dependent hypoacetylation of histones H3 and H4 at the distal but not proximal MMP-9 promoter region. Protein-DNA complexes in FRT and MTA1 cells were cross-linked and subjected to chromatin immunoprecipitation assays with antibodies against acetylated histones H3 and H4. Precipitated DNA was subjected to real-time PCR assays for quantifying DNA within the distal and proximal MMP-9 region using the primer sets PN1 (A) and primer set P3 (B), respectively. Data are shown as average values ± S.D. of six separate determinations.

Fig. 9. Repression of MMP-9 expression by MTA1 occurs via a histone acetylation-dependent and independent mechanism. A, the indicated cells were treated with 100 nM TSA for 2 days. Constitutive or PMA-induced MMP-9 expression was determined by reverse transcriptase-PCR as described in Fig. 2B. B, nuclei from the indicated cells treated with or without TSA was digested with PstI followed by real-time PCR assays as described in Fig. 4. Data are representative of duplicate experiments (A) and are shown as average values ± S.D. for six separate determinations (B).

Fig. 10. MTA1 recruits the chromatin-remodeling enzyme Mi2 to the MMP-9 promoter. A, protein from the M10 and empty vector P clones was immunoprecipitated with anti-c-Myc-agarose beads. Immunocomplexes were analyzed by Western blotting for the Mi2 and MTA1 proteins. B, DNA-protein complexes from the indicated cells were cross-linked with formaldehyde and subjected to chromatin immunoprecipitation assays with a rabbit anti-Mi2 antibody. The MMP-9 promoter fragment was amplified by PCR (35 cycles) using the primer set P1. The experiment was performed twice.
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was indeed bound to the MMP-9 promoter in the M10 clone. In contrast, the MMP-9 promoter was not immunoprecipitated with this antibody in the MTA1-deficient control clone P. Thus, the ability of MTA1 to repress MMP-9 expression may reflect, in part, the recruitment of Mi2 to the promoter. Presumably, like the p300/CREB-binding protein-associated factor (41), Mi2 acts at long range on the proximal region to achieve chromatin remodeling.

**DISCUSSION**

We report herein the repressive effect of the metastases-associated gene MTA1, on MMP-9 expression mediated via its direct interaction with the collagenase promoter. These findings are the first to demonstrate regulation of a protease by a chromatin-remodeling activity. Furthermore, with the exception of the estrogen-regulated genes c-myc and pS2 (29), this is the first endocrine-independent gene shown to be targeted by MTA1. Our findings that a chromatin-remodeling activity regulates MMP-9 expression indicates an additional level of control of expression for this gene previously only alluded to by transgenic approaches in which MMP-9 promoter constructs were stably integrated into the genome (20, 21).

It does not appear that MTA1 has a pleiotropic effect on gene expression because the expression of the 72-kDa type IV collagenase encoded by a separate gene (MMP-2) was unaffected. Additionally, preliminary expression array studies indicate few other genes regulated by MTA1. How the specificity for the MMP-9 promoter is conferred remains to be determined. In this regard, it is noteworthy that another chromatin-remodeling activity (Swi/Snf complex) in NIH3T3 cells and yeast also demonstrates selectivity toward subsets of genes (42, 43), although like MTA1, the mechanism by which this specificity is conferred is presently unknown.

Our study bears an important difference to that of Mazumdar et al. (29) who investigated the regulation of estrogen-responsive genes by MTA1. Thus, although the repressive effect of MTA1 on an estrogen response element-driven promoter was entirely abrogated by the HDAC inhibitor TSA, this was not the case for MMP-9 expression. This difference might suggest that estrogen-responsive genes are repressed entirely via changes in histone acetylation, whereas MTA1 regulates MMP-9 expression through a mechanism that is both dependent and independent upon histone deacetylation. Indeed, our observation that the Mi2 protein, which confers chromatin-remodeling activity independent of histone deacetylation (30), co-immunoprecipitated with MTA1 and was targeted to the MMP-9 promoter in vivo might explain the inability of TSA to fully restore MMP-9 expression in the MTA1-expressing cells.

How might MTA1 repress MMP-9 expression? We initially hypothesized that a more compact chromatin structure achieved by increased histone deacetylation (44) might reduce the binding of transcription factors to previously characterized cis elements (14, 18, 40) as we reported for the KiSS-1 metastases suppressor gene (15). However, our analysis using ChIP (data not shown) failed to show altered occupancy of the AP-1, NFκB, and Sp1 motifs in the MMP-9 promoter, which we and others (14, 18, 40) had previously reported to be regulatory for the expression of this collagenase. Furthermore, our DNase I hypersensitivity experiments did not reveal novel MMP-9 regulatory elements in the MTA1-expressing cells. These data, although not exhaustive, would argue against the possibility that MTA1 suppresses MMP-9 synthesis by way of reducing the access of transcription factor(s) to their cognate sequences in the MMP-9 promoter. A second possibility is that the trans-acting activity of the MMP-9 promoter-bound transcription factors is modulated by MTA1 or the chromatin structure. Such a control mechanism has been demonstrated for the Gcn4 and Hap4 transcription factors in yeast (45) and for p53 and Fos/Jun dimers in mammalian cells (46, 47), albeit in response to the Swi/Snf chromatin-remodeling complex. A third possibility is that a MTA1-mediated change in chromatin conformation culminates in decreased accessibility to the basal transcriptional machinery. Indeed, our data showing reduced PstI endonuclease accessibility proximal to the TATA box would be consistent with this latter scenario.

We were initially surprised by the observation that MTA1 repressed MMP-9 synthesis since expression of either gene correlates with the metastatic phenotype (25, 48, 49). Several reasons could explain this unexpected finding. First, it is now well accepted that MMP-9 expression in many human malignancies occurs in the neighboring stromal cells rather than the tumor cells themselves (50–54). Certainly, in situ hybridization experiments support this contention in breast cancer (55), a tissue in which increased MTA1 expression was first described (25). It is tempting to speculate that the absence of MMP-9 expression in these tumor cells reflects the increased expression of MTA1 (25). Alternatively, studies reporting that MTA1 expression correlates positively with tumor metastases are based on immunohistochemical and in situ hybridization analyses, and it is unclear whether the antibodies or RNA/cDNA probes employed in those investigations were specific for MTA1 or recognized other related proteins such as MTA1-L1 (56) or the recently identified naturally occurring short form of MTA1 (57).

In conclusion, we have provided bona fide evidence for a direct role of the MTA1 corepressor in the regulation of MMP-9 expression through a mechanism that is both dependent on and independent of histone deacetylation. MMP-9 is the first protease to be shown to be regulated by a chromatin-remodeling activity and to add to a short list of MTAT1-regulated genes, which at present only includes the estrogen-responsive genes pS2 and c-myc (29).

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REFERENCES

1. Bergers, G., Brekken, R., McMahon, G., Vu, T. H., Itoh, T., Tamaki, K., Tannawa, K., Thorpe, P., Ichihara, S., Werb, Z., and Hanahan, D. (2000) *Nat. Cell Biol.* 2, 737–744
2. Yu, T. H., Shieh, J. M., Bergers, G., Berger, J. B., Helms, J. A., Hanahan, D., Shapiro, S. D., Senior, B. R., and Werb, Z. (1998) *Cell* 98, 411–422
3. Yu, T. H., and Werb, Z. (2000) *Genes Dev.* 14, 2123–2133
4. Anderson, I. C., Shipp, M. A., Docherty, A. J. P., and Teicher, B. A. (1996) *Cancer Res.* 56, 715–718
5. Naglich, J. G., Jure-Kunkel, M., Gupta, E., Fargnoli, J., Henderson, A. J., Lewin, A. C., Talbott, R., Baxter, A., Bird, J., Savopoulos, R., Wills, R., Kramer, R. A., and Trail, F. P. (2001) *Cancer Res.* 61, 8460–8465
6. Murphy, G., Cockett, M. I., Ward, R. V., and Docherty, A. J. P. (1999) *Biochem. J.* 277, 277–279
7. Yu, Q., and Stamenkovic, I. (2000) *Genes Dev.* 14, 163–176
8. Legrand, C., Gilles, C., Zahm, J. M., Polette, M., Buissin, A. C., Kaplan, H., Birembaut, P., and Tournier, J. M. (1999) *J. Cell Biol.* 146, 517–529
9. Bernard, R. J., Gruber, S. B., and Muschel, R. J. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 4293–4297
10. Hua, J., and Muschel, R. J. (1996) *Cancer Res.* 56, 5279–5284
11. Linn, R., Dupont, B. R., Knight, C. B., Plasque, R., and Leach, J. R. (1996) *Cytogenet. Cell Genet.* 72, 159–161
12. Collier, I. E., Bruns, G. A. P., Goldberg, G. I., and Gerhard, D. S. (1991) *Genomics* 9, 429–434
13. Huhtala, P., Tuuttila, A., Chow, L., Lotfi, J., Keski-Oja, J., and Tryggvason, K. (1991) *J. Biol. Chem.* 266, 14645–14650
14. Sato, H., and Seiki, M. (1993) *Oncogene* 8, 395–405
15. Yan, C., Wang, H., and Boyd, D. D. (2001) *J. Biol. Chem.* 276, 1164–1172
16. Sebag, I., and Thompson, T. C. (1999) *Mol. Cell Biol.* 19, 407–416
17. Jiang, Y., and Muschel, R. J. (2002) *Cancer Res.* 62, 1910–1914
18. Sato, H., Kita, M., and Seiki, M. (1993) *J. Biol. Chem.* 268, 23460–23468
19. Himelestein, B. P., Lee, E. J., Sato, H., Seiki, M., and Muschel, R. J. (1998) *Clin. Exp. Metastasis* 16, 169–177
20. Mohan, R., Rinehart, W. B., Bargagna-Mohan, P., and Fini, M. E. (1998) *J. Biol. Chem.* 273, 23509–23514
21. Marnauti, C., Salunnumi, T., Kontusaari, S., Reponen, P., Monta, T., Foudart, J., and Tryggvason, K. (1999) *J. Biol. Chem.* 274, 5588–5596
22. Lee, J.-H., and Welch, D. R. (1997) *Cancer Res.* 57, 2384–2387
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23. Gao, A. C., Lou, W., Dong, J. T., and Isaacs, J. T. (1997) Cancer Res. 57, 846–849
24. White, A., Lamb, P. W., and Barrett, C. (1998) Oncogene 16, 3143–3149
25. Toh, Y., Pencil, S. D., and Nicolson, G. L. (1994) J. Biol. Chem. 269, 22958–22963
26. La Tulippe, E., Santagopan, J., Smith, A., Scher, H., Scardino, P., Reuter, V., and Gerald, W. L. (2002) Cancer Res. 62, 4499–4506
27. Mahoney, M. G., Simpson, A. J., Mest, M., Nee, M., Kari, C., Pepe, D., Choi, Y. W., Uitto, J., and Rodeck, U. (2002) Oncogene 21, 2161–2170
28. Cui, Q., Takiguchi, S., Matsusue, K., Toh, Y., and Yoshida, M. A. (2001) Cytogenet. Cell Genet. 93, 139–140
29. Mazumdar, A., Wang, R. A., Mishra, S. K., Adam, L., Bagheri-Yarmand, R., Mandal, M., Vadlamudi, R. K., and Kumar, R. (2001) Nat. Cell Biol. 3, 30–37
30. Xue, Y., Wong, J., Moreno, G. T., Young, M. K., Cote, J., and Wang, W. (1998) Mol. Cell 2, 851–861
31. Toh, Y., Kunisaka, S., Endo, K., Oshiro, T., Ikeda, Y., Nakashima, H., Baba, H., Kohnoe, S., Okamura, T., and Nicolson, G. L. (2000) J. Exp. Clin. Cancer Res. 19, 105–111
32. Nead, M. A., and McCance, D. J. (1995) J. Invest. Dermatol. 105, 668–671
33. Wang, H., Skibber, J., Juarez, J., and Boyd, D. (1994) Int. J. Cancer 58, 650–657
34. Yan, C., Wang, H., and Boyd, D. D. (2002) J. Biol. Chem. 277, 10804–10812
35. Frank, S., Schreuder, M., Fernandez, P., Taubert, S., and Amati, B. (2001) Genes Dev. 15, 2069–2082
36. Weismann, A. S., Plevy, S. E., and Smale, S. T. (1999) Immunity 11, 665–675
37. Rao, S., Procko, E., and Shannon, M. F. (2001) J. Immunol. 167, 4484–4503
38. Davia, G. E., and Martino, B. M. (1996) Cancer Res. 56, 1113–1120
39. Huhtala, P., Chow, L., and Tryggvason, K. (1990) J. Biol. Chem. 265, 11077–11082
40. Gum, R., Lengyel, E., Juarez, J., Chen, J.-H., Sato, H., Seiki, M., and Boyd, D. (1996) J. Biol. Chem. 271, 10672–10682
41. Krumm, A., Madisen, L., Yang, X.-J., Goodman, R., Nakatani, Y., and Geudtner, M. (1999) Proc. Natl. Acad. Sci. U. S. A. 95, 13501–13506
42. de la Serna, I. L., Carlson, K. A., Hill, D. A., Guido, C. J., Stephenson, R. O., Sif, S., Kingston, R. E., and Imbalzano, A. N. (2000) Mol. Cell Biol. 20, 2839–2851
43. Biggar, R. R., and Crabtree, G. R. (1999) EMBO J. 18, 2254–2264
44. Armstrong, J. A., and Emerson, B. M. (1998) Curr. Opin. Genet. Dev. 8, 165–172
45. Neely, K. E., Hassan, A. H., Brown, C. E., Howe, L., and Workman, J. L. (2002) Mol. Cell Biol. 22, 1615–1625
46. Lee, D., Kim, J. W., See, T., Hwang, S. G., Choi, E.-J., and Chee, J. (2002) J. Biol. Chem. 277, 22330–22337
47. Ito, T., Yamamura, M., Nishina, H., Yamamichi, N., Murakami, M. S., and Iba, H. (2001) J. Biol. Chem. 276, 2852–2857
48. Bernhard, E. J., Muschel, R. J., and Hughes, E. N. (1990) Cancer Res. 50, 3872–3877
49. Nakajima, M., Welch, D. R., Wynn, D. M., Tsuruo, T., and Nicolson, G. L. (1993) Cancer Res. 53, 5802–5807
50. Stahle-Backdahl, M., and Park, W. C. (1993) Am. J. Pathol. 142, 995–1000
51. Pyke, C., Ralkiaer, E., Huhtala, P., Hurskainen, T., Dano, K., and Tryggvason, K. (1992) Cancer Res. 52, 1336–1341
52. Pyke, C., Ralkiaer, E., Tryggvason, K., and Dano, K. (1993) Am. J. Pathol. 142, 359–365
53. Nielsen, B. S., Timshel, S., Kjeldsen, L., Sehested, M., Pyke, C., Borregaard, N., and Dano, K. (1996) Int. J. Cancer 65, 57–62
54. Coussens, L. L., Tinkle, C. L., Hanahan, D., and Werb, Z. (2000) Cell 103, 481–490
55. Nielsen, B. S., Sehested, M., Kjeldsen, L., Borregaard, N., Rygaard, J., and Dano, K. (1997) Lab. Invest. 77, 343–355
56. Futamura, M., Nishimori, H., Shiratsuchi, T., Saji, S., Nakamura, Y., and Tokino, T. (1999) J. Hum. Genet. 44, 52–56
57. Kumar, R., Wang, R. A., Mazumdar, A., Talalder, A. H., Mandal, M., Yang, Z. B., Bagheri-Yarmand, R., Sahin, A., Hortobagyi, G., Adams, L., Banres, C. J., and Vadlamudi, R. K. (2002) Nature 41, 654–657
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