Endogenous Inhibition of Histone Deacetylase 1 by Tumor-Suppressive Maspin

Xiaohua Li, Shuping Yin, Yonghong Meng, Wael Sakr, and Shijie Sheng

Abstract

Maspin, a noninhibitory serine protease inhibitor, exerts multifaceted tumor-suppressive effects. Maspin expression is associated with better differentiated phenotypes, better cancer prognosis, and better drug sensitivity. Consistently, maspin also correlates with increased expression of Bax and p21WAF1/CIP1. Interestingly, histone deacetylase 1 (HDAC1), a major HDAC responsible for histone deacetylation, was shown to interact with maspin in a yeast two-hybrid screening. In this study, we confirmed the maspin/HDAC1 interaction in human prostate tissues, in prostate cancer cell lines, and with purified maspin. We produced several lines of evidence that support an inhibitory effect of maspin on HDAC1 through direct molecular interaction, which was detected in both the nucleus and the cytoplasm. Both endogenously expressed maspin and purified maspin inhibited HDAC1. In contrast, small interfering RNA (siRNA) silencing of maspin in PC3 cells increased HDAC activity. Accordingly, maspin-transfected DU145 cells exhibited increased expression of HDAC1 target genes Bax, cytokeratin 18 (CK18), and p21WAF1/CIP1, whereas maspin siRNA decreased CK18 expression in PC3 cells. The maspin effect on HDAC1 correlated with increased sensitivity to cytotoxic HDAC inhibitor M344. Interestingly, glutathione S-transferase (GST, another maspin partner) was detected in the maspin/HDAC1 complex. Furthermore, a COOH-terminally truncated maspin mutant, which bound to HDAC1 but not GST, did not increase histone acetylation. Although HDACs, especially the highly expressed HDAC1, are promising therapeutic targets in cancer intervention, our data raise a novel hypothesis that the endogenous inhibitory effect of maspin on HDAC1 is coupled with glutathione-based protein modification, and provide new leads toward future developments of specific HDAC1-targeting strategies.

Introduction

Maspin is a 42 kDa protein in the serine protease inhibitor (serpin) family with an Arg residue at the p1 position of its reactive site loop (1). Experimental evidence shows a suppressive role of maspin in tumor growth, tumor-induced extracellular matrix remodeling, tumor angiogenesis, and tumor metastasis. In addition, maspin expression induced partial redifferentiation and tumor sensitivity to apoptosis (reviewed in ref. 2). Thus, a better understanding of the underlying molecular mechanisms of maspin is of high clinical significance.

Maspin is more closely related to clade B serpins, thus also named as serpin B5. To date, 13 human clade B serpins, mostly clustered on chromosome 18q21, have been identified (reviewed in ref. 3). Most of these serpins, with the exception of maspin, are inhibitory against serine proteases (4–9) and share high homologies at 60 conserved positions distributed throughout their linear sequences. Clearly, maspin is the most deviant clade B serpin. Based on the phylogenetic model of Benarafa and Remold-O’Donnell (3), maspin is also one of the most lonely and ancient clade B serpins. It is important to note that maspin is an epithelial-specific gene (10), and is the only clade B serpin whose deficiency is lethal in embryogenesis (11). These data and the tumor-suppressive effects of maspin raise the question about the significance of the ancestral sequence code in maspin.

A major breakthrough in translating the sequence code of maspin came from X-ray crystallographic analyses of the maspin structure (12, 13). Based on its high-resolution crystal structure, maspin is indeed a noninhibitory serpin against active serine proteases. Nonetheless, maspin retains the basic serpin conformation with an exposed reactive site loop (12, 13). Thus, maspin may interact with serine protease-like partners or other proteins. The binding of maspin to a serine protease–like molecule and other proteins may be uniquely accommodated by its conformational heterogeneity of the G-helix and its novel unstable shutter region salt bridge (13). In the meantime, breakthroughs in decoding the maspin mechanisms came from advances in the identification of diverse molecular partners/targets of maspin, including tissue-type plasminogen activator (14), pro–urokinase type plasminogen activator (pro-uPA; ref. 15), IFN responsive factor 6 (16), collagen type I (17), and glutathione S-transferase (GST; ref. 18). It is highly plausible that the unique functional significance of maspin in health and disease lies precisely in its ability to engage in these diverse molecular interactions, a task that cannot be simultaneously fulfilled by a serpin that has evolved to have more specific targets.

The evidence that maspin correlates with better differentiated phenotypes and better survival, and that maspin induces the expression of Bax (19) and p21WAF1/CIP1 (20), suggests that maspin may participate in specific transcriptional regulation. To this end, an earlier yeast two-hybrid screening suggests that maspin may bind to histone deacetylase 1 (HDAC1; ref. 18), a major class I HDAC that hydrolyzes the N-acetyl lysine residues in histone and other proteins (reviewed in ref. 21). HDAC1-mediated histone deacetylation leads to chromatin condensation, preventing the access of transcription factors to DNA and repressing gene expression. HDAC1 target genes include Bax, p21WAF1/CIP1, p27KIP1, and cytokeratin 18 (CK18; ref. 22–24).

The HDAC1-controlled epigenetics play a critical role in development (25) and tumor progression (26). It is reasonable to...
speculate that HDAC1 needs to be tightly regulated by a proactive mechanism in a cell type–specific manner. To date, no other polypeptide inhibitors of HDAC1 have been identified. The success in developing synthetic HDAC inhibitors, such as SAHA (27) and M344 (28), suggests that the natural HDAC1 inhibitors need an epitope that docks into the catalytic site of HDAC1. Interestingly, X-ray structural analysis of SIRT2, the yeast homologue of human class I HDACs, suggests that the HDAC catalytic site has a conformation similar to that of serine proteases or metalloproteinas (29). Thus, a serpin with sufficient structural flexibility to bind to a protease-like catalytic domain may act as an endogenous HDAC1 inhibitor.

In this study, we confirmed the maspin/HDAC1 interaction and provide the first evidence for an inhibitory maspin effect on HDAC1. Our data also revealed a novel dependence of maspin on GST in inhibiting HDAC1. Although HDACs, especially the highly expressed HDAC1, are promising therapeutic targets in cancer intervention, our data raise a novel hypothesis that the endogenous inhibitory effect of maspin on HDAC1 is coupled with glutathione-based protein modification, and provide new leads toward future developments of specific HDAC1-targeting strategies.

Materials and Methods

Chemicals and reagents. Recombinant maspin (rMas), GST-maspin fusion protein (G-Mas), and GST were purified as described (30). Polyclonal antibody against maspin reactive site loop (Abs4A) was custom-made and purified as described (1). Antibodies from commercial sources include monoclonal (mAb) and polyclonal antibodies against maspin from BD Transduction Laboratories (BTL), human prostate cancer cell line PC3 from American Type Culture Collection (Manassas, VA) was cultured at 37 °C in RPMI 1640 (Invitrogen, Carlsbad, CA) containing 5% fetal bovine serum (Hyclone, Logan, UT). Prostate cancer cells DU145-derived maspin transfected clones (Invitrogen, Carlsbad, CA) containing 5% fetal bovine serum (Hyclone, Logan, UT), Neo were cultured as described (28). The COOH terminus of maspin and mock-transfected clone (Neo) were cultured as described (28). The primers for p21WAF1/CIP1 were 5′-GGTACGATTCGGTCCGG-3′ and 5′-CGATAGCCTGGCAGACGCTGG-3′. The cDNA sequence encoding maspin mutant Mas334-380 was sequence confirmed and subcloned into the pAdenovector-CMV5 vector (Q-Bio Gene, Carlsbad, CA). The resulting plasmid was purified and used to generate the recombinant adenoviral DNA and the adenovirus (Ad-Mas334-380) using an established procedure (32).

In this study, we confirmed the maspin/HDAC1 interaction and provide the first evidence for an inhibitory maspin effect on HDAC1. Our data also revealed a novel dependence of maspin on GST in inhibiting HDAC1. Although HDACs, especially the highly expressed HDAC1, are promising therapeutic targets in cancer intervention, our data raise a novel hypothesis that the endogenous inhibitory effect of maspin on HDAC1 is coupled with glutathione-based protein modification, and provide new leads toward future developments of specific HDAC1-targeting strategies.

Human tissues and cell cultures. Extracts of histologically confirmed human prostate tumor tissues and the matching normal tissues from radical prostatectomy patients were made as described (18). Human prostate cancer cell line PC3 from American Type Culture Collection (Manassas, VA) was cultured at 37 °C with 6.5% CO2 in RPMI 1640 (Invitrogen, Carlsbad, CA) containing 5% fetal bovine serum (HyClone, Logan, UT). Prostate cancer cells DU145-derived maspin transfected clones (M3, M7, and M10) and mock-transfected clone (Neo) were cultured as described (28).

Transient transfection. Cells cultured in six-well plates were transfected using the siLentFect Lipid Reagent (Bio-Rad). In each transfection reaction, 10 μmol/L vehicle, Mas-siRNA, or Scr-siRNA was used. Cells were continuously cultured for 48 hours before further analyses.

Adenoviral expression of Mas334-380. The COOH terminus of maspin Arg340 in the pVL1393/mas vector (30) was fused in frame to a translation-stop codon using the Exsite PCR-Based Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA) and the following PCR primers: 5′-CCATA-GAGITGCACAGGACCTAATCTGCAGACCAGAAG-3′ and 5′-CCATTGTG-GCTTACCTTACGTGCCTGACACACCTTGCACCG-3′. The cDNA sequence encoding maspin mutant Mas334-380 was sequence confirmed and subcloned into the pAdenovector-CMV5 vector (Q-Bio Gene, Carlsbad, CA). The resulting plasmid was purified and used to generate the recombinant adenoviral DNA and the adenovirus (Ad-Mas334-380) using an established procedure (32). For routine in vitro infection, adenovirus was added to 24-hour-old cell culture.

Immunofluorescence staining. Cells at 70% confluence were fixed, permeabilized, blocked as previously described (18), and incubated with a mixture of anti-maspin mAb (1/400) and anti-HDAC1 polyclonal antibody (1/200) at 4 °C for overnight. Cells were washed and incubated for 45 minutes at room temperature with Alexa Fluor 488 FluoroNanogold anti-rabbit antibody (1/500), Alexa Fluor 594 FluoroNanogold anti-mouse antibody (1/100), or the mixture of the above two antibodies. After the nuclei were counterstained with 0.1 μg/mL Hoechst 33342, cells were mounted for confocal microscopic viewing.

Subcellular fractionation. Cells lysed with a Dounce homogenizer in a sucrose-supplemented extraction buffer (19) were centrifuged at 3,000 × g for 5 minutes. The supernatants were incubated with NP40 (to 1% v/v) for 20 minutes, and spun at 14,000 × g for 20 minutes. The resulting supernatants were collected as cytosolic fractions. Pellets from the first centrifugation of total cell lysates were incubated for 20 minutes in 50 μL nuclear extraction buffer (20) and centrifuged at 14,000 × g for 20 minutes. The resulting supernatants were collected as the nuclear extracts. All steps were done at 4 °C.

GSH-affinity pull-down. One microgram G-Mas or GST and 50 μL GSH-Sepharose 4B beads (50 μL) were incubated in PBS (to 500 μL) at room temperature for 1 hour. For background control, GSH-Sepharose 4B beads alone were incubated PBS. The beads were washed with PBS/0.5% Tween 20 (PBST). Twenty-five-microliter aliquots of the beads thus obtained were mixed with 250 μg cell lysates in PBS (to 500 μL), and incubated at 4 °C overnight. After washing with PBST, the bound proteins were eluted with 25 μL GSH as previously described (19).

Immunoprecipitation. Cell lysates in radioimmunoprecipitation assay (RIPA) buffer (18) were precleared with isotypic preimmune IgG plus Protein A/G beads (5% v/v), followed by a brief centrifugation at 10,000 × g. Aliquots of the supernatants were incubated with the indicated antibodies at 4 °C overnight, then mixed with protein A/G beads (2.5% v/v) at room temperature for 1 hour. Pellets harvested by brief centrifugation at 10,000 × g were washed with RIPA buffer with descending Triton X-100 strength (1% to 0.1%), and then washed with PBS.

HDAC activity assay. HDAC activity was measured using the HDAC Fluorescent Activity assay/Drug Discovery kit AK-500 according to the instructions from the manufacturer (Biomolecular Research Laboratory). Briefly, 15 μL nuclear fraction or 5 μL HDAC1 immunoprecipitate preparation was diluted to 25 μL with the assay buffer. Following the addition of fluorogenic HDAC substrate, the reaction mixtures were incubated for 30 minutes at 37 °C, and stopped by 50 μL ”developer” cocktail. The fluorescence of the reaction mixture was monitored at excitation/emission 560 nm/600 nm using a SPECTRAMax Gemini plate reader (Molecular Devices, Sunnyvale, CA).

Quantitative real-time PCR. Total RNA was extracted as described (18). One microgram of each RNA sample was reverse transcribed using BIO-RAD iScript cDNA Synthesis kit. Quantitative real-time PCR (QPCR) reactions using Stratagene SYBR Green master mix were done as described (18). The primers for Bax and GAPDH were as described (19). The primers for CK18 were 5′-ATCTGGTGTACCTTGGAC-3′ and 5′-ACCTGGCATC-CACTGCCTGG-3′. The primers for p21WAF1/CIP1 were 5′-AAGACACATGTT-GACCTGT-3′ and 5′-GGATGGAAATCTGTCACTGG-3′.

Miscellaneous. Cell viability was determined using the WST-1 kit (Roche Diagnostics, Indianapolis, IN). SDS-PAGE (34) and Western blotting with Bio-Rad polyvinylidine difluoride membrane (30) were done as previously described. Protein concentration determination using Bio-Rad protein concentration dye was done as described (31).
done based on the instructions from the manufacturer. The quality of RNA was determined based on the integrity of 18S and 28S rRNAs on agarose gel electrophoresis, and by the ratio (close to 2) of UV-visible light absorbance at 260 nm to UV absorbance at 280 nm. For statistical analyses, one-tailed matched pair Student’s t tests were done. *P < 0.01 was considered significant.

Results

Maspin specifically interacts with HDAC1. We first examined the maspin/HDAC1 interaction in human prostate tissues using GSH-affinity pull-down assay. This method had been successfully used to specifically pull-down maspin (18). As shown in Fig. 1A, we detected maspin, HDAC1, and GST in the affinity pull-down fractions. The level of HDAC1 in the pull-down fractions correlated with that of coeluted maspin but not GST. Because HDAC1 was up-regulated, whereas maspin was down-regulated in prostate tumor specimens, the molecular association between maspin and HDAC1 seemed to depend primarily on maspin.

Because HDAC1 is reported as a mostly nuclear protein, we tested the subcellular localization of the maspin/HDAC1 interaction in prostate cancer cell lines. As shown in Fig. 1B(a-b), PC3 cells that produce a moderate level of endogenous maspin (35) featured more maspin in the cytosolic fraction, but more abundant HDAC1 in the nuclear fraction. Maspin immunoprecipitation followed by Western blotting showed maspin/HDAC1 association both in the nucleus and cytoplasm. This result is supported by the confocal microscopic imaging of HDAC1 and maspin fluorescent stains in PC3 cells [Fig. 1B(c)]. The maspin/HDAC1 interaction was also detected in maspin-transfected prostate tumor cells DU145 by immunoprecipitation/Western blotting (data not shown) and by GSH-affinity pull-down assay (Fig. 1C). Maspin expressed by transfected clonal cell lines was present in both nuclear and cytosolic fractions, albeit the ratio of nuclear to cytosolic maspin was smaller than that in PC3 cells.

Taking advantage of the undetectable level of endogenous maspin in mock-transfected DU145 (Neo) cells, we tested whether purified maspin could specifically bind to endogenously expressed HDAC1. As shown in Fig. 1C(b), when Neo cell lysate was preincubated with G-Mas, endogenous HDAC1 was specifically pulled down by GSH beads. In parallel, GST immobilized to GSH beads or GSH beads alone did not pull down HDAC1. Interestingly, however, GST alone pulled down a significant amount of HDAC1 from the lysate of maspin-transfected clonal cell line M7. Because both maspin and HDAC1 were coeluted with GST from human prostate tissue extracts (Fig. 1A), these data further support a specific association of GST with the maspin/HDAC1 complex.

Maspin negatively regulates the HDAC1 activity. HDAC1 removes the acetyl groups from histone and other molecules. In our hands, maspin expression in stably transfected DU145 cells led to higher levels of AcH3 and AcH4 (Fig. 2A, and B, p21WAF1/CIP1 (37) and Bax (38) are critical regulators of cellular commitment to apoptosis. Thus, our data are consistent with the functional evidence that maspin expression partially restored epithelial differentiation (39, 40) and cell sensitivity to apoptosis (19).

Maspin did not block synthetic HDAC inhibitor–induced gene expression because the expression of Bax and CK18 in both Neo- and maspin-transfected DU145 cells was further induced by M344 (P < 0.001; Fig. 3A and B). It was noted that M344 treatment dose-dependently decreased the cell viability [Fig. 3D(a)]. The combination of M344 and maspin led to a further increase of apoptosis as compared to single treatment.
judged by PARP cleavage [Fig. 3(a)] and by cell viability assay [Fig. 3D(a)]. The IC_{50} values of M344 were 5.7 μmol/L against Neo cells and 1 μmol/L against two maspin-transfected clonal lines M3 and M7. Interestingly, we noted that HDAC inhibition by M344 also increased maspin expression both in DU145 cells and in PC3 cells [Fig. 3D(b)]. The increased maspin expression in both cell lines correlated with increased maspin secretion. These data suggest that the inhibitory effect of maspin or synthetic compounds on HDAC1 may positively feedback on maspin expression, which consequently further contributes to tumor suppression.

The maspin effect on HDAC1 depends on GST. We noticed a maspin-dependent association of GST with HDAC1 in human tissues and cell lines (Fig. 1). It has been shown that HDAC activation requires the binding of a divalent cation (e.g., Zn^{2+}) to the sulfide groups in the catalytic site (41). Protons transferred from sulfhydral groups rather than water molecules may reduce the sulfide and inactivate HDAC. Indeed, thiols and glutathione conjugates of depsipeptide inhibit HDAC (42). Earlier, we showed that maspin/GST interaction enhances GST activity and inhibits oxidative stress–induced generation of reactive oxygen species (ROS; ref. 18). As investigations are under way to examine whether maspin directly participates in the GST-dependent ROS reduction/detoxification, it is remains equally possible that maspin recruits GST to hydrogenate the sulfides at HDAC1 catalytic site. To test the latter possibility, we used site-directed mutagenesis approach and examined whether the maspin/GST interaction was essential for the inhibition of HDAC1 activity. Earlier, we produced an R340A mutation of maspin, MasR340A, as a purified protein (18), and an adenovirus-encoded protein (32). MasR340A has been shown to bind to GST with a significant lower affinity compared with wild-type maspin.
(Mas; ref. 18). In the current study, we also generated a COOH-terminally truncated maspin mutant Mas\(^{1-340}\). Mas, Mas\(^{R380A}\), and Mas\(^{1-340}\) were expressed in adenovirus-infected DU145 cells and analyzed by GSH pull-down assay. As expected, Mas was specifically coeluted with GST (Fig. 4A). In comparison, the level of GST-associated Mas\(^{R380A}\) was significantly lower, whereas no Mas\(^{1-340}\) was detected in the GSH pull-down fractions. To test the affinities of Mas and Mas\(^{1-340}\) for HDAC1, the lysates of adenovirus-infected cells were subjected to HDAC1 immunoprecipitation followed by Western blotting. As shown in Fig. 4B, GST and maspin were both coprecipitated with HDAC1 from Ad-Mas–infected cells. However, only Mas\(^{1-340}\) but not GST, was coprecipitated with HDAC1 from Ad-Mas\(^{1-340}\)–infected cells. These data suggest that GST was recruited to the HDAC1 complex by maspin. These data also suggest that maspin COOH-terminal domain may play a critical role for interaction with GST, whereas maspin NH\(_2\)-terminal domain (amino acids 1-340) may be essential for the interaction with HDAC1.

To test the role of GST in the maspin effect on HDAC1, we examined the levels of AcH3 and AcH4 in adenovirus-infected DU145 cells. As shown in Fig. 4C, reexpression of Mas, but not Mas\(^{1-340}\), increased the levels of AcH3 and AcH4. The functional loss of Mas\(^{1-340}\) in inhibiting HDAC-dependent histone deacetylation seemed to result from a qualitative change of maspin because it was not rescued by increasing the level of Mas\(^{1-340}\) expression [up to 5 plaque-forming unit (pfu) in Fig. 4C].

**Discussion**

Following the suggestion from our earlier yeast two-hybrid reactions, we confirmed the maspin/HDAC1 interaction and provided the first evidence for a novel GST-dependent inhibitory effect of maspin on HDAC1 activity. The specific antagonistic interaction between maspin and HDAC1 may underlie the emerging consensus that maspin, especially nuclear maspin, correlates with increased differentiation, cell cycle arrest, increased apoptotic sensitivity, and better prognosis of cancer (reviewed in ref. 2).

The molecular mode of maspin action represents a significant deviation from a current paradigm established for inhibitory serpins (43). However, X-ray crystallographic analyses of maspin revealed that maspin has a general serpin conformation with an exposed reactive site loop and an novel G-helix that may allow a significant amount of flexibility for induced conformational changes (13). This suboptimal serpin conformation may confer a distinct preference for partners, such as pro-uPA (32), that are not entirely committed to a serine protease conformation. HDAC1 catalyzes the hydrolysis of the N-acetyl lysine residues of proteins. The catalytic triad of HDAC1 (His, His, and Asn) may be folded in a conformation similar to the active sites of metalloproteinases and/or serine proteases (41). Thus, the unexpected maspin/HDAC1 interaction turned out not entirely surprising. Indeed, Kurtev et al. (44) recently reported that HDAC1 may bind to chick ovalbumin, a classic noninhibitory serpin homologue of maspin. Interestingly, maspin was not associated with other class I HDACs (e.g., HDAC2 and HDAC3, and data not shown) despite their sequence homologies with HDAC1 (45, 46).

The execution of the maspin effect on HDAC1 seemed to depend on GST (EC 2.5.1.18), an enzyme catalyzing hydrogenation of sulfide groups or disulfide bridges using GSH. GST-mediated protein modification may have a profound effect because numerous proteins, including transcription factors p53 (47) and HDAC1 (42) and signaling molecules such as c-Jun NH\(_2\)-terminal kinase (48), are regulated by sulfide hydrogenation or disulfide bond formation. In the case of HDAC1, an active conformation may be induced by a divalent cation, preferably Zn\(^{2+}\), that binds to the sulfide groups of oxidized cysteine residue residing in catalytic site (41). Thus, as illustrated in Fig. 5, GST-mediated sulfide reduction may displace the metal ion and inactivate HDAC1. Maspin, on the other hand, for having dual affinities for HDAC1 and GST, acts as a matchmaker. Based on our data, maspin may use its reactive site loop as well as its NH\(_2\)-terminal domain to bind activated HDAC1. The HDAC1-associated maspin may simultaneously induce conformational changes of HDAC1 to expose the sulfo-metal bond as a GST substrate and recruit GST through its COOH-terminal domain (including the sC1 strand).

It is important to note that *maspin* is an epithelial specific gene (10). Considering the essential roles of both HDAC1 and maspin in development and tumor progression, it is reasonable to speculate that maspin is the epithelial-specific inhibitor of HDAC1. Others
have shown that HDAC1 may be inhibited by synthetic compounds [e.g., SAHA (27) and M344 (28)] or metabolic by-products (e.g., 9-HSA; ref. 49) that have similar structures as the HDAC reaction transition-state intermediate. It is important to note that binding of maspin to HDAC1 did not block the action of pharmacologic HDAC inhibitor M344 (Fig. 3). It is possible that maspin distorts the HDAC1 catalytic site only to block the entry of biological substrate (e.g., N-acetyl group of histone lysine residues) but not small molecular weight inhibitors. Alternatively, by locking HDAC1 in an inactive state, maspin may enhance the accessibility of other HDACs by pharmacologic inhibitors, thus lowering the apparent IC_{50} values.

Our hypothetical model that links maspin and GST to endogenous HDAC1 regulation provides a basic framework for further investigation. Based on this model, several additional factors may affect the maspin effect on HDAC1. First, the molecular interaction between maspin and HDAC1 may not be mutually exclusive. HDAC1 may deacetylate molecules other than histones such as androgen receptor (50) and p53 (51). Maspin, on the other hand, may interact with pro-uPA (32), GST (18), and IRF6 (16) in a HDAC1-independent manner. The dominant maspin partnership may be cell type specific or differentiation status specific. We and others have shown that reexpression of maspin in DU145 cells was tumor suppressive (39, 52). Yet, a moderate level of maspin expression in PC3 cells clearly was not sufficient to block the tumorigenic and metastatic phenotypes, albeit maspin silencing inhibited HDAC1 activity in this cell line (Figs. 2 and 3). Second, the maspin/HDAC1 interaction may not be restricted to the nucleus. Consistent with the findings by others (53), we detected cytosolic HDAC1 and cytosolic maspin/HDAC1 interaction in PC3 cells (Fig. 1B). HDAC1 recruitment to the nucleus in PC3 cells may be associated with specific phases of cell cycle (54). In our study, when PC3 cells were forced into quiescence by serum starvation, more maspin was detected in the nucleus (data not shown). Finally, the involvement of GST suggests a sensitivity of the maspin effect on HDAC1 to oxidative stress. Oxidative stress in tissue microenvironment may underlie the etiology of cancer and other aging-related diseases (55, 56). As investigations are under way to examine whether maspin participates in GST-mediated ROS reduction/detoxification, it is important to investigate whether the maspin effect of HDAC1 is regulated by oxidative stress stimuli and/or by differential expression of GST.

Consistent with earlier clinical findings (57, 58), we found that HDAC1 was up-regulated as maspin was down-regulated in prostate tumor tissues (Fig. 1). Based on our new data, this inverse correlation between maspin and HDAC1 in tumor progression may lead to epigenetic changes in favor of dedifferentiation and apoptosis resistance, thus representing a gain of function. Consistently, it has been shown that cancer patients who retained higher levels of nuclear maspin responded better to chemotheraplay (59). Although HDACs, especially the highly expressed HDAC1, are promising therapeutic targets in cancer intervention (60), data from the current study provided not only novel insights into the endogenous regulation of HDAC1 activity by maspin and GST, but also leads toward future developments of novel HDAC1-targeting strategies.

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