Pleiotropic Inhibition of Pericellular Urokinase-type Plasminogen Activator System by Endogenous Tumor Suppressive Maspin

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ABSTRACT

Maspin is a novel serine protease inhibitor with tumor suppressive activity, inhibiting tumor invasion and metastasis. To date, the underlying molecular mechanism of maspin remains elusive. Recombinant maspin has been shown to specifically inhibit cell surface-associated urokinase-type plasminogen activator (uPA) and fibrinogen-bound tissue-type plasminogen activator. However, the biological role of endogenous maspin in plasminogen activation is totally unknown. To address this issue, we generated stable maspin-expressing transfectants using prostate carcinoma cells DU145 as the parental cell line. We report here that endogenous maspin exerts pleiotropic inhibitory effects on the pericellular uPA system. Maspin expression led to a significantly reduced level of cell-surface-bound uPA and uPA receptor proteins without altering the steady-state levels of the respective mRNAs. Treatment with receptor-associated protein (RAP), a specific inhibitor of low-density lipoprotein receptor-related protein, lead to a significantly increased level of secreted uPA and cell surface uPAR in maspin transfectants but not in the mock control cells. A combination of enzymatic and molecular analyses revealed that maspin inhibits the cell-surface-mediated plasminogen activation by forming an SDS-resistant complex with cell-surface-bound uPA. In addition, maspin expression led to a dramatic reduction in the release of active uPA, both high molecular weight and the low molecular weight, into the conditioned medium of maspin transfectant clones. Consistently, the conditioned medium of maspin transfectant clones had a significantly reduced activity in converting plasminogen to plasmin. The inhibitory effect of maspin on pericellular uPA correlates with significantly decreased cell invasion potential and motility in vitro. The maspin-neutralizing antibody (Abs4A) reversed the subdued invasive potential of maspin transfectant cells in a dose-dependent manner. In summary, this study provides the first evidence that endogenous maspin is a potent inhibitor of pericellular uPA. Furthermore, our results support a current hypothesis that maspin blocks tumor invasion and motility by inhibiting localized pericellular proteolysis.

INTRODUCTION

Maspin is a 42,000 Da novel tumor suppressive serpin (1). Accumulative evidence demonstrates that maspin inhibits the progression of prostate and breast tumor at the steps of invasion and metastasis (1–6). However, the underlying molecular mechanism of maspin remains elusive.

Maspin has a sequence homology with members in the serpin family including PAI-1 and PAI-2 (1). In 1998, Sheng et al. (7) reported that purified recombinant maspin specifically inhibits tPA that is associated with fibrinogen or poly-lysinse. However, purified maspin does not act as a classical serpin in cell-free solutions, i.e., it does not inhibit a series of serine proteases including tPA and uPA (7, 8). On the basis of the specific interaction between maspin and fibrinogen-associated tPA, it was speculated that maspin may target plasminogen activators that are bound to a biological surface such as the plasma membrane. Recently, McGowen et al. (9) described first evidence that the tumor cell surface-associated uPA is inhibited by recombinant maspin. The inhibitory effect of maspin on DU145 cell-mediated uPA activity was similar to that of uPA-neutralizing antibody, and was reversed by the polyclonal antibody made against the RSL sequence of maspin. Furthermore, the proteolytic inhibitory effect of recombinant maspin was quantitatively consistent with the inhibitory effect of maspin in cell migration.

It is intriguing to speculate that endogenous maspin may exert its tumor suppressive activity by targeting the cell surface-associated plasminogen activator(s). It is worth noting that, to date, the role of tPA in cell surface-mediated plasminogen activation remains unclear. However, uPA and its cell surface receptor uPAR have been shown to promote tumor metastasis by mediating pericellular plasminogen activation (10, 11). We report here that expression of endogenous maspin in prostate carcinoma cells DU145 resulted in a significant inhibition of uPA-mediated pericellular plasminogen activation. A maspin/uPA complex was detected both on the cell surface and in the conditioned medium of maspin transfectant clones. Consistently, the two active forms of uPA, HMW and LMW were significantly reduced in the conditioned medium. Interestingly, although the endogenous expression of maspin did not alter the transcription of the endogenous uPA and uPAR, it lead to a significantly reduced level of cell-associated uPAR protein. The maspin-induced reduction of secreted uPA and cell surface uPAR appears to result from the LRP-mediated internalization. Taken together, our data demonstrates a pleiotropic inhibitory effect of maspin on pericellular uPA system, thus supporting the hypothesis that maspin exerts its tumor suppressive function by inhibiting pericellular proteolysis.

MATERIALS AND METHODS

Chemicals and Reagents. Cell culture components were purchased from Life Technologies, Inc. (Gaithersburg, MD) or Hyclone (Logan, UT). Polyclonal antibodies against the β-sheet S3A sequence of maspin, AbS3A, were produced and purified as described previously (1). Recombinant HMW uPA, Glu-type plasminogen, chromogenic plasmin substrate Spectrozyme PL, polyclonal antibody against uPAR, and monoclonal antibodies against human uPA (α chain and β-chain) were obtained from American Diagnostica (Greenwich, CT). The purified glutathione S-transferase-receptor associated protein fusion protein was kindly provided by Dr. Steven Goniais (University of Virginia Health Sciences Center, Charlottesville, VA). Reagents for protein concentration analysis and protein gel electrophoresis were obtained from Bio-Rad (Hercules, CA). Sulforhodamine B and other chemicals and reagents of the highest purity, unless otherwise specified, were purchased from Sigma (St. Louis, MO).

Cell Lines and Cell Culture. Multiple stable maspin transfectant clones were generated by an established method (1) using human prostate carcinoma cells DU145 (American Type Culture Collection) as the parental cell line. The transcription of maspin in these transfectant cells is under the control of the
cytomegalovirus promoter. In parallel, mock transfectants were generated using the empty plasmid. Transfected cells were first selected based on their resistance to geneticin (G418) at 600 μg/ml and subsequently maintained at 300 μg/ml G418. The transfected cells were additionally screened for the expression of maspin using Western blot analysis and RT-PCR.

Both DU145 cells and the DU145-derived transfecant clones were maintained in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 5% fetal bovine serum. An additional 300 mg/ml of G418 was added to the culture medium for the stable transfected clones. The immortalized normal human prostate epithelial cells MLC8891 (9) were cultured in serum-free KGM medium (Life Technologies, Inc.). All of the cell cultures were kept in a humidifier incubator at 37°C with 6.5% CO2.

To investigate whether the LRP regulates the secretion of uPA and the internalization and catabolism of uPAR in maspin expressing transfecants, equal number of maspin-expressing transfecant clones and a mock control clone were incubated for 24 h in KGM-SF in the presence or absence of 200 nM of glutathione S-transferase-receptor associated protein, a specific LRP inhibitor (12, 13). The resulting conditioned medium was collected and concentrated, whereas the cells were lysed in a low salt, protease inhibitor-rich buffer [4 mM NaHCO3, 100 mM NaF, 20 mM KH2PO4, 2 mM sodium orthovanadate, 5 mM EDTA, 5 mM diisopropylfluorophosphate, 2 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin (pH 7.2); Ref. 14] containing 1% Triton X-100. The resulting protein samples were used for Western analyses.

**Protein Fractionation and Western Blotting.** Western analyses were performed as described (1) using the cell surface eluates, total cell lysate protein, and SF-CM. The protein fraction is briefly as follows. When the cells in culture reached approximately 70–80% confluence, the maintenance culture medium was replaced with KGM-SF. After 24 h of continued incubation, the conditioned medium was collected and subsequently concentrated using the Centricon-10 filter units (Amicon, Bedford, MA). In the meantime, the cells were detached with trypsin/EDTA followed by neutralization with trypsin inhibitor. The cell lysate was prepared by DOUNCE homogenization in a low salt, protease inhibitor-rich lysis buffer [4 mM NaHCO3, 100 mM NaF, 20 mM KH2PO4, 2 mM sodium orthovanadate, 5 mM EDTA, 5 mM diisopropylfluorophosphate, 2 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin (pH 7.2); Ref. 14] containing 1% Triton X-100, followed by centrifugation. To elute the cell surface-bound uPA, confluent cultures were treated with 50 mM glycine-HCl (pH 3.0) containing 0.1 M NaCl for 3 min at 37°C.

**In Vitro Motility and Invasion Assays Using MICS.** Briefly, the serum-starved cells (24 h) from different clonal lines were seeded onto the 8-μm polycarbonate insert (precoated with 50 μg/ml Matrigel for motility and 4 mg/ml for invasion). To verify the specificity of the maspin effect in inhibiting tumor cell invasion, cells in suspension were preincubated with the neutralizing polyclonal antibody directed against maspin RSL sequence (Abs4A) at 0, 10, and 20 μg/ml, respectively. The MICS chamber was incubated for 6 h at 37°C with 6.5% CO2 to allow the cells to migrate. On the other hand, the MICS chamber was incubated for 48 h at 37°C with 6.5% CO2 to assess the invasive potential of the different clonal lines. Cells attached to the bottom side of the polycarbonate membrane insert were fixed, stained, and counted under the microscope as described previously (5).

**RESULTS**

**Endogenous Expression of Maspin in Prostate Carcinoma Cells DU145.** To examine the effect of endogenous maspin on cell surface-associated uPA, prostate carcinoma cell line DU145 was chosen for generating stable maspin-expressing transfecants for the following reasons. First, DU145 cells do not express detectable endogenous maspin. Second, DU145 cells have been shown to respond to the inhibition of recombiant maspin in *in vitro* invasion and motility assays (5), Third, DU145 cells secrete and rely primarily on uPA to initiate the pericellular plasminogen activation (9). In addition, McGowen et al. (9) have shown that recombinant maspin specifically inhibits the DU145 cell surface-associated uPA.

As shown in Fig. 1A, maspin was detected in both the total cell lysates and the conditioned medium of multiple maspin transfecant clones. The expression of maspin appeared to be less than that in normal immortalized prostate epithelial cells (MLC8891) and varied slightly among different maspin transfecant clones. The mock transfecnt clone and the parental cell line DU145 expressed no detectable amount of maspin protein in either fraction. The endogenously expressed maspin had a molecular mass of 42 kDa, identical to that
found in normal prostate epithelial cells MLC8891. Subsequent quantitative RT-PCR (Fig. 1B) showed a similar maspin expression pattern as shown by Western blot. The stable DU145 cell-derived maspin transfectants as well as the mock transfectant cells had similar growth rate and similar morphological features as the parental cell line (data not shown).

To test whether endogenous maspin inhibits cell invasion and motility, we performed the MICS motility and invasion assays (5). As shown in Fig. 1C, as compared with parental cells DU145, all three of the maspin transfectant clones tested were inhibited by \( \sim 60\% \) in the motility assay. In contrast, the mock transfectant clone exhibited a similar migratory potential as the parental cells. Similarly, a significant reduction of about 40 – 50\% in the invasive potential was noted in maspin-expressing transfectants compared with that of the neo control (Fig. 1D). As shown in Fig. 1E, the inhibitory effect of endogenous maspin on tumor cell invasion appeared to be specific. The maspin-neutralizing antibody Abs4A had no significant effect on the invasive potential of the mock transfectant clone. In parallel, however, Abs4A reversed the subdued invasive potential of a maspin transfectant clone that of either parental or a mock transfectant clone. The cell surface-mediated plasminogen activation in each case was effectively blocked by a uPA-neutralizing antibody but not by a tPA neutralizing antibody. These data are consistent with the earlier evidence that DU145 cell-mediated plasminogen activation depends primarily on uPA (9).

A plasminogen-dependent zymogram was subsequently performed using the cell surface eluates to examine the maspin effect on uPA presentation on the cell surface. Compared with the uPA standard, the HMW uPA (activity) in the eluate derived from maspin transfectants was significantly diminished compared with that of the parental and neo control (Fig. 2B). Thus, the inhibition of the cell surface-mediated plasminogen activation correlates with a reduced level of cell-associated uPA in maspin-expressing transfectants.

To investigate whether endogenous maspin directly interacts with the cell surface-bound uPA, Western blotting of uPA using the cell surface-associated protein fraction was performed first under a non-reducing condition. It is known that the active HMW-uPA, a heterodimer derived from the single-chain pro-uPA, has the same molecular mass as pro-uPA (19). As shown in Fig. 3A, a uPA \( \beta \)-chain specific antibody detected a 54 kDa band, possibly a mixture of pro-uPA and the active HMW-uPA. This 54 kDa was greatly reduced in maspin transfectants as compared with both the parental and the neo control. The uPA antibody also detected an upper band of \( \sim 100 \) kDa, which coincides with the combined molecular weight of maspin (42 kDa) and uPA (54 kDa). Importantly, this upper band was only detected in the eluate of maspin transfectant clones. In parallel, the monoclonal antibody to the \( \alpha \) chain of uPA similarly detected an upper band in the eluate derived from maspin transfectants but not in
that of the parental or neo control (data not shown). Because others have shown that uPA forms an SDS-resistant 1:1 complex with inhibitory serpins PAI-1 and PAI-2 (20–22), it is likely that the uPA containing upper band detected in maspin transfectants represents the stable complex of maspin and uPA.

Because the 54 kDa uPA species detected by Western blotting as shown in Fig. 3A may be a mixture of pro-uPA and HMW-uPA, the decreased level of 54 kDa uPA in maspin transfectants as detected under the nonreducing condition may result from the loss of active HMW-uPA because of the formation of a stable maspin/uPA complex. However, the possibility exists that endogenous maspin expression also led to a reduced level of pro-uPA. To address this possibility, Western blotting under a reducing condition was performed using the uPA β-chain-specific antibody. It is known that the two subunits (24 kDa α chain and 33 kDa β-chain) in the active HMW-uPA are linked by disulfide bonds and can be separated on reduction (19). As shown in Fig. 3B, the 33 kDa uPA β-chain was detected in the active HMW-uPA standard, indicating that the two-chain active HMW-uPA had been successfully dissociated into two subunits under our reducing condition. The level of uPA β-chain was significantly decreased in the eluate of maspin transfectant clones compared with that of the parental and neo control, indicating that less active HMW-uPA was associated with maspin transfectants. In addition, the level of pro-uPA (54 kDa) decreased significantly in the cell surface-associated protein fraction of maspin transfectant clones as compared with that of parental cells or mock control. These results indicate that maspin expression leads to the reduction of both pro-uPA and active HMW-uPA that are associated with the cell surface.

Decreased Level of Cell-associated uPAR in Maspin Transfectant Clones. It has been well documented that the proteolytic activation of uPA (from pro- to active HMW-uPA) is mediated by its cell membrane-anchored uPAR (23, 24). To test the possible involvement of uPAR in the inhibitory effect of maspin on the cell surface-bound
ENDOGENOUS MASPIN INHIBITS PERICELLULAR uPA

Fig. 5. The plasminogen activation is reduced in the conditioned medium of DU145/M cells. The SF-CM was conditioned by the same number of cells of each cell line for 24 h. The coupled plasminogen activation assay was performed with 25 μg of CM protein from DU145 cells (○), DU145/Neo1 cells (○), DU145/M3 cells (○), DU145/M7 (●), and DU145/M10 (●). The data represent an average of triplicate repeats. SEs are not shown for clarity.

Western blottings were performed to examine the maspin effect on uPA profiles in the conditioned culture medium. Under the nonreducing condition, the uPA β-chain specific antibody detected two bands of ~54 kDa and 36 kDa, respectively (Fig. 6A), in the SF-CM of DU145 and neo control cells. The 54 kDa band represents a mixture of both the secreted pro-uPA and HMW-uPA, whereas the 36 kDa band represents the LMW-uPA in which β-chain is linked to a small fragment of α chain through disulfide bond (26, 27). Both the 54 kDa band and the 36 kDa band were significantly reduced in the SF-CM of the maspin transfectant clones as compared with that in the SF-CM of DU145 cells and the mock transfectant control cells. In fact, the LMW-uPA species was barely detectable in the SF-CM of the maspin transfectant clones. In addition, an upper band was detected in the SF-CM of all three of the maspin transfectant clones but not of DU145 cells or the mock transfectant clone. Similarly, under nonreducing condition, a monoclonal antibody to maspin detected this upper band in the conditioned medium derived from maspin transfectants but not from the mock control cell line (Fig. 6C). The molecular weight of this upper band was estimated as 100 kDa, approximately the combined molecular weight of maspin and uPA.

Under the reducing conditions, the uPA β-chain-specific antibody detected two major bands in the SF-CM of DU145 and DU145/Neo cells at ~54 kDa and ~33 kDa, respectively (Fig. 6B). The 54 kDa band represents the single chain pro-uPA, whereas the polypeptide 33 kDa corresponds to the β-chain of uPA derived from both the active HMW-uPA and the active LMW-uPA. Both the pro-uPA and the dissociated uPA β-chain were significantly reduced in the SF-CM of maspin transfectant clones as compared with that of DU145 cells or mock control cells. It is worth noting that the upper band of 100 kDa detected in the SF-CM of maspin transfectant clones under nonreducing condition was not detected under the reducing condition. Consistent results were obtained from Western analyses using uPA α-chain-specific antibody (data not shown).

To examine whether the 36 kDa LMW-uPA detected by Western blotting has the expected enzyme activity, plasminogen-dependent gelatinolytic zymogram was performed. As shown in Fig. 6D, in

Fig. 6. Concomitant reduction of pro-uPA, HMW-uPA, and LMW-uPA in the SF-CM of maspin transfectant clones. Western blotting of uPA under nonreducing (A) and reducing (B) conditions. In both A and B, uPA was detected by monoclonal antibody to β-chain of uPA. Used as a positive control was purified HMW-uPA (0.5 μg, shown in Lane 1 of each blot). Lanes 2–6 are concentrated conditioned medium protein (75 μg/lane) of DU145, DU145/Neo1, DU145/M3, DU145/M7, and DU145/M10 cells, respectively. C, Western blotting of maspin under nonreducing condition by a specific monoclonal antibody to maspin. Lanes 1–4 are concentrated conditioned medium protein (75 μg/lane) of DU145/Neo1, DU145/M3, DU145/M7, and DU145/M10 cells, respectively. D, the plasminogen-dependent gelatinolytic zymogram. A total of 5 μg of serum-free medium conditioned by DU145/Neo1 (Lane 3), DU145/M7 (Lane 4), and DU145/M10 (Lane 5) respectively, was loaded. The purified single-chain IPA (Lane 1) and HMW-uPA (Lane 2) were used as standards.
addition to a clear zone corresponding to the 54 kDa HMW-uPA, the clear zone of the 36 kDa LMW-uPA was detected in the SF-CM of the mock transfec tant control cells. Both HMW-uPA and LMW-uPA decreased dramatically in the SF-CM of maspin transfec tant clones. In parallel, gelatinolytic zymogram of metalloproteinase showed a similar level of pro-matrix metalloprotease-2 and pro-matrix metalloprotease-9, respectively, in all of the SF-CM samples tested (data not shown), additionally confirming an equal sample loading.

Previous studies have shown that the LRP promotes the endocytosis of uPA/uPAR complex on the interaction of uPA with its inhibitor such as PAI-1 (12, 13). As shown in Fig. 7, treatment with receptor-associated protein (RAP), a specific inhibitor of LRP, significantly increased the steady-state level of secreted uPA in the conditioned medium as well as cell surface uPAR protein in maspin transfec tangs. In contrast, no significant increase in secreted uPA and cell surface uPAR expression by the LRP inhibitor RAP was observed in the mock transfec tant control cell line. This data suggests that the reduction of secreted uPA and cell surface-associated uPAR protein in maspin transfec tands is in part attributable to the increased LRP-mediated clearance.

DISCUSSION

The goal of the current study was to investigate the effect of endogenous maspin on the pericellular uPA system. Using stable maspin transfec tands derived from prostate carcinoma cells DU145 we showed that endogenous maspin inhibited pericellular uPA activity, and inhibited cell motility and invasion in vitro.

The current paradigm of the inhibitory interaction between a serpin and its target protease states that the catalytic unit of the protease binds to the serpin RSL, forming a stable serpin:protease complex. Our previous data showed that the tumor suppressive and biochemical activity of maspin is dependent on its intact RSL, supporting the notion that maspin acts as an inhibitory serpin (5, 7). In the current study, a maspin/uPA complex was detected on the cell surface and in the conditioned medium of maspin transfec tands by immunoblotting only under nonreducing conditions but not under a reducing condition. Our data indicate that maspin can complex only with the intact heterodimeric HMW-uPA. Thus, the inhibitory effect of maspin on cell-associated uPA may involve not only the interaction between the RSL of maspin with the catalytic site containing ß-chain of uPA but may also require an association between another segregated functional domain of maspin with the ß-chain subunit of uPA. Consistently, Sheng et al. (7) have shown that recombinant maspin inhibited the sctPA associated with poly-lysine or fibrinogen through its RSL but stimulated the free sctPA via its NH$_2$-terminal 38 kDa fragment. This biphasic effect of recombinant maspin on sctPA derives from its two segregated domains interacting with the catalytic and the regulatory domains of sctPA, respectively.

Two other potent biological inhibitors of uPA, PAI-1 and PAI-2, have been shown to form a stable complex with uPA (20–22). The detection of an uPA/maspin complex on the cell surface in this study indicates that endogenous maspin indeed acted as an inhibitory serpin similar to PAI-1 and PAI-2 (28, 29). However, unlike PAI-1 and PAI-2 that also inhibit soluble uPA, maspin does not inhibit free uPA in solution (7, 8) or in conditioned cell culture medium (9). The current study, along with previous in vitro evidence (9), supports a hypothesis that direct interaction between endogenous maspin and uPA on the cell surface causes pleiotropic changes in the molecular profile of the pericellular uPA system and leads to a dramatic decrease of uPA-mediated plasminogen activation both on the cell surface and in the conditioned medium.

A body of literature demonstrates that the molecular interactions among factors in the pericellular uPA system are dynamically regulated (30, 31). The proteolytic activation of pro-uPA to two-chain active uPA on the cell surface is mediated by uPAR (32, 33). Maspin forms a stable complex with uPA on the cell surface and inhibits the uPA-mediated cell surface plasminogen activation. In addition, we noticed that maspin expression led to a significant and simultaneous reduction of cell surface-bound uPA and cell-associated uPAR. These data suggest an involvement of uPAR in the interaction between maspin with uPA. Our results showed that the expression of neither uPA nor uPAR at the mRNA level was altered by the constitutive expression of maspin. In addition, the presence of the LRP inhibitor RAP resulted in a significant increase of both secreted uPA and cell surface uPAR protein in maspin transfec tands but not in the mock control clone. Thus, it is likely that the reduced level of secreted uPA and cell surface uPAR in maspin transfec tands may in part be attributable to the increased LRP-dependent internalization of a maspin/uPA/uPAR complex. Our data are additionally in line with the earlier evidence with recombinant PAI-1 that the binding of PAI-1 to the uPAR-bound uPA triggers LRP-dependent endocytosis and enhances the catabolism of uPA and uPAR (12, 13, 34–38).

The previous study by McGowen et al. (9) showed that purified maspin does not form a complex with secreted uPA in the conditioned culture medium. Thus, it is likely that the maspin/uPAR complex detected in the SF-CM of maspin transfec tands resulted from the shedding from the cell surface. In the case of maspin transfec tands, as the stable maspin/uPA complex is continuously shed from the cell surface and accumulates in the conditioned medium (Fig. 6), a smaller amount of active HMW-uPA would be secreted to the conditioned medium (Fig. 6). Because active HMW-uPA is derived from the secreted pro-uPA, as more maspin/uPA complex is formed, more pro-uPA would be depleted from the conditioned medium (as compared with the parental and mock transfec tant control, Fig. 6). Moreover, it has been shown that the active HMW-uPA undergoes additional cleavage, producing an active LMW-uPA (26, 27). Our Western blotting and zymographic analysis revealed that endogenous maspin expression correlates with a dramatically reduced level of the LMW-uPA in the conditioned medium. It is possible that the complex formation between maspin and uPA on the cell surface may, by reducing the availability of the active HMW-uPA, also limit the formation and shedding of the LMW-uPA.

 Whereas our new evidence addresses the biochemical characteristics of maspin as a novel inhibitory serpin against pericellular uPA system, the changes of molecular profile in this system provoked by endogenous maspin expression may lead to additional indirect changes in other cellular pathways. For example, because uPAR has been shown to directly interact with and regulate the function of integrins, the transmembrane receptors of extracellular matrix proteins (39, 40), the reduction of cell-associated uPAR in the presence of maspin may help explain an earlier observation that maspin treatment
changed the integrin profile on breast carcinoma cell surface and altered cell adhesion to specific extracellular substrate in favor of a normal epithelial phenotype (6).

Expression of uPA by malignant cells correlates with an aggressive phenotype including increased tumor cell invasion and metastasis, presumably through the activation of plasminogen and the resulting pericellular matrix degradation (10, 11). Consistent with our earlier evidence with purified recombinant maspin, data from the current study demonstrated an important role of epithelial cell surface in mediating the inhibitory interaction between endogenous maspin and pericellular uPA. The proteolytic inhibitory effect of endogenous maspin coincided with a significant inhibition of cell motility and invasion potential in vitro. Furthermore, the anti-invasive effect of maspin appeared to be localized on the cell surface and dependent on its RSL sequence. Whereas extensive studies are under way to investigate whether uPA produced by other types of carcinoma cells is inhibited by maspin, it is intriguing to hypothesize that novel maspin-based therapeutic strategies may prove useful to specifically target human malignancies that are associated with markedly elevated uPA. To this end, it is important to point out, however, that different plasminogen activator inhibitors may play distinct roles in tumor progression. For example, PAI-1 along with uPA and uPAR, is causively involved in the progression of breast cancer. In contrast, maspin, which is down-regulated in several types of carcinomas, has a tumor suppressive activity.

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