The Human Myoepithelial Cell Is a Natural Tumor Suppressor

Mark D. Sternlicht, Paul Kedeshian, Zhi-Ming Shao, Suzi Safarians, and Sanford H. Barsky

Department of Pathology, UCLA School of Medicine, Los Angeles, California 90024

Abstract

Myoepithelial cells, which surround ducts and acini of glandular organs, form a natural border separating proliferating epithelial cells from basement membrane and underlying stroma. Myoepithelial cells in situ and in vitro constitutionally express high amounts of proteinase inhibitors that include tissue inhibitor of metalloproteinase 1, protease nexin-II, α-1 antitrypsin, and maspin. Human myoepithelial xenografts (HMS-X, HMS-3X, and HMS-4X), which our laboratory has established, accumulate an abundant extracellular matrix containing sequestered proteinase inhibitors. Humatrix, a gel that we have derived from HMS-X, inhibits tumor cell invasion (down to 25% ± 10% of Matrigel control; \( P < 0.01 \)), and our recently established human myoepithelial cell lines, HMS-1, HMS-3, and HMS-4, inhibit tumor cell invasion in cellular invasion (down to 42% ± 7% of control; \( P < 0.05 \)) and in conditioned media assays (down to 30% ± 8% of control; \( P < 0.01 \)). The anti-invasive effects of HMS-1, HMS-3, and HMS-4 can be enhanced by phorbol 12-myristate 13-acetate (down to 2% ± 1% of control) by a maspin-dependent mechanism and abolished by dexamethasone (up to 95% ± 5% of control) by a maspin-independent mechanism (\( P < 0.01 \)). HMS-X, HMS-3X, HMS-4X, and Humatrix inhibit tumor invasion and metastasis in severe combined immunodeficient mice (\( P < 0.001 \)). The cumulative data suggest that myoepithelial cells are natural paracrine suppressors of invasion and metastasis and may specifically inhibit the progression of precancerous disease states to invasive cancer in vivo.

Introduction

It has become clear that cancer cells come under the influence of important paracrine regulation from the host microenvironment (1). Such host regulation may be as great a determinant of tumor cell behavior in vivo as the specific oncogenic or tumor suppressor alterations occurring within the malignant cells themselves and may be mediated by specific extracellular matrix molecules, matrix-associated growth factors, or host cells themselves (2, 3). Both positive (fibroblast, myofibroblast, and endothelial cell) and negative (tumor-infiltrating lymphocyte and cytotoxic macrophage) cellular regulators exist that profoundly affect tumor cell behavior in vivo (4-5). One host cell, however, the myoepithelial cell, has escaped the paracrine onlooker’s attention. The myoepithelial cell, which lies on the epithelial side of the basement membrane, is thought to contribute largely to both the synthesis and remodeling of this structure. This cell lies in juxtaposition to normally proliferating and differentiating epithelial cells in health and to abnormally proliferating and differentiating epithelial cells in precancerous disease states, such as dysplasia and carcinoma in situ. This anatomical relationship suggests that myoepithelial cells exert important paracrine effects on normal glandular epithelium and may regulate the progression of carcinoma in situ to invasive carcinoma. Circumstantial evidence suggests that the myoepithelial cell naturally exhibits a tumor suppressor phenotype. Myoepithelial cells rarely transform, and when they do, they generally give rise to benign neoplasms that accumulate rather than degrade extracellular matrix (6). Myoepithelial cells directly or indirectly through their production of extracellular matrix are thought to regulate branching morphogenesis that occurs in the developing breast and salivary gland during embryological development (7).

The cell biology of the human myoepithelial cell has not been well investigated, and only a few isolated studies exist in the literature despite the near ubiquitous presence of this cell surrounding epithelial ducts and acini in numerous glandular organs. Because potentially important paracrine relationships might exist between myoepithelial cells and epithelial cells in both developmental biology and cancer, our laboratory has been very much interested in this cell and has established immortalized myoepithelial cell lines and transplantable xenografts from benign human myoepitheliomas of the salivary gland (HMS-1, HMS-X; HMS-3, HMS-3X) and breast (HMS-4, HMS-4X; Refs. 8 and 9). These cell lines and xenografts expressed identical myoepithelial markers as their in situ counterparts and displayed an essentially normal diploid karyotype. Unlike the vast majority of human tumor cell lines and xenografts that exhibited matrix-degrading properties, these myoepithelial lines/xenografts, like their myoepithelial counterparts in situ, retained the ability to secrete and accumulate an abundant extracellular matrix composed of both basement membrane

1 The abbreviations used are: HMS, human matrix secreting; CM, conditioned medium; TIMP, tissue inhibitor of metalloproteinase; PAI, plasminogen activator inhibitor; α1-AT, α1-antitrypsin; PN-IIIAPP, protease nexin II/β amyloid precursor protein; MMP, matrix metalloproteinase; PMA, phorbol 12-myristate 13-acetate; CHX, cyclohexamide; DCIS, ductal carcinoma in situ; HMEC, human mammary epithelial cell.

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and non-basement membrane components. When grown as a monolayer, the myoepithelial cell lines exerted profound and specific effects on normal epithelial and primary carcinoma morphogenesis (8). These studies support our position that our established myoepithelial lines/xenografts recapitulate a normal differentiated myoepithelial phenotype and can, therefore, be used experimentally as a primary myoepithelial surrogate. Prompted by these studies and by the conspicuous absence of studies examining the role of the myoepithelial cell in tumor progression, we decided to examine the myoepithelial cell from this perspective. Experiments with these cell lines/xenografts, together with relevant in situ observations, form the cornerstone of the present study, which observes that the human myoepithelial cell is a normal tumor suppressor.

Materials and Methods

Cell Lines and Xenografts. Human myoepithelial cell lines and xenografts including HMS-1, HMS-X, HMS-3, HMS-3X (salivary gland); and HMS-4, HMS-4X (breast) were established recently in our laboratory (8, 9). The murine EHS tumor was grown as an allograft in severe combined immunodeficient mice. Other cell lines used in this study included human melanoma lines C8161 (Dr. Mary J. C. Hendrix, University of Arizona Cancer Center, Tucson, AZ), which was transfected by us with pSV2neo in a previous study (3), M15 (Dr. Don Morton, John Wayne Cancer Center, Santa Monica, CA), and A375 (American Type Culture Collection, Rockville, MD); normal human mammary epithelial cells (HMEC; Clonetix, San Diego, CA); Hs578Bst human breast myofibroblasts (10); HIG-82 rabbit synoviocytes; human breast carcinoma lines MCF-7, T47D, BT-549, MDA-MB-157, MDA-MB-231, and Hs578T; human squamous cell carcinoma lines of salivary gland and vulva, A253 and A431 (American Type Culture Collection, Rockville, MD); and a human mucoepidermoid carcinoma cell line (M/E; Dr. Adi Gazdar, University of Texas Southwestern Medical School, Dallas, TX). Serum-free CM was collected from many of these lines over 24 h and concentrated 10–1000-fold using Centriprep-10 concentrators (Amicon, Beverly, MA).

Antibodies and Probes. TIMP-1 and maspin cDNA probes were kindly provided (Dr. Judith C. Gasson, UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA; Dr. Ruth Sager, Dana-Farber Cancer Institute, Boston, MA). Five μg of oligo(dT)-selected mRNA were separated on denaturing agarose gels, transferred to nylon membranes, and probed with [α-32P]dCTP-labeled probes using standard Northern blot protocols (8). Primary antibodies used to known proteinase inhibitors included rabbit anti-human maspin (PharMingen, San Diego, CA); rabbit anti-human α1-AT (DAKO, Carpinteria, CA); mouse monoclonal anti-human PAI types 1, 2, and 3 (American Diagnostica, Greenwich, CT); mouse monoclonal anti-human PN-II/APP (Boehringer Mannheim, Indianapolis, IN); rabbit anti-human α2-antiplasmin (Alexis, San Diego, CA); rabbit anti-rTIMP-1 (Dr. Judith C. Gasson, UCLA Jonsson Comprehensive Cancer Center, Los Angeles, CA); and mouse monoclonal anti-human protease nexin I (Dr. Dennis Cunningham, University of California, Irvine, CA). Concentrated CM was resolved on nonreducing 10% SDS-PAGE mini-gels and transferred to nitrocellulose membranes for Western blotting using standard protocols (9); in some instances, cellular lysates instead of CM were analyzed. Standardized immunoprecipitation protocols using Sepharose-protein A (11) were used in the evaluation of maspin activities.

Immunocytochemical Studies. Formalin-fixed paraffin-embedded human tissues of normal breast, DCIS, and the human myoepithelial xenografts, HMS-X, HMS-3X and HMS-4X, were incubated with rabbit anti-cow S-100 (1:1000; DAKO), smooth muscle actin (1:2000; DAKO), and the previously mentioned antibodies to maspin (1:500), α1-AT (1:2000); PN-II/APP (1:50); TIMP-1 (1:100), and PAI-1 (1:20). Peroxidase-conjugated sheep anti-mouse IgG and goat anti-rabbit IgG were used as secondary antibodies at 1:200 and 1:25 dilutions, respectively. Colorimetric detection of peroxidase-conjugated secondary antibody was with diaminobenzidine.

Zymography and Related Studies. Proteinases and their inhibitors were profiled by standard zymographic and reverse zymographic methods (12–15). To visualize MMPs, renatured gelatin- and casein-containing gels were equilibrated for 30 min in enzyme buffer [50 mM Tris-HCl (pH 7.6), 0.2 mM NaCl, 5 mM CaCl2, and 0.02% Brij-35] and incubated overnight in fresh buffer at 37°C. To detect MMP inhibitors, serum-free medium containing 25 ng/ml PMA was conditioned over 24 h by HIG-82 cells and activated with 1 μM para-aminophenylmercuric acetate. To visualize inhibitors of trypsin-like serine proteinases, renatured gelatin gels were equilibrated in 10 mM Tris-HCl (pH 7.5) for 30 min at room temperature and incubated for 4 h at 37°C in fresh buffer containing 0.2–1.0 μg/ml trypsin. Plasminogen activators were detected by adding 10 μg/ml plasminogen to casein-containing gels. Plasminogen activators and PAI-1 were also analyzed by fibrin direct and reverse zymography (16). PAI-2 could not be detected by this method, because unlike PAI-1, it could not be reactivated following SDS denaturation (17). Net antiplasmin activity in the various CM was determined using chromogenic substrate 2221 (H-D-Val-Leu-Lys-p-nitroaniline 2HCl) according to the acid-stopped method described by the supplier (Pharmacia Hepar, Franklin, OH).

In Vitro Invasion Assays of Invasion. Invasion experiments were conducted using a invasion chamber containing either Matrigel control (18) (Collaborative Biomedical Products, Bedford, MA) or Humatrix, a gel extracted from HMS-X according to previous methods (8). In a subsequent set of Matrigel invasion experiments, the invasion set up was slightly modified by us to test the effects of HMS-1, HMS-3, and HMS-4 CM (concentrated up to 100-fold) and HMS cells on invasion. Each well insert was layered with 60 μl of a 1:3 mixture Matrigel: basal keratinocyte serum-free medium (700 μg Matrigel/cm²) or Matrigel:CM and allowed to gel. On top of this cell-free Matrigel layer, a second 60 μl of the 1:3 Matrigel mixture (700 μg Matrigel/cm²) was added with or without suspended 106 HMS-1 or control cells. Highly invasive C8161 or MDA-MB-231 cells (105) were added on top of this second layer. Six hundred μl of
complete MEM with 10% FCS were added to the bottom well beneath the filter. The plates were incubated at 37°C for 72 h. Invasion was assessed by counting the cells that traveled across the filter, attached to the bottom side of the filter, and reached and colonized the bottom well. Pretreatment of HMS cells was carried out with the following agents: CHX (40 μg/ml) for 24 h; PMA (5 μM) for 2 min, 20 min, 2 h, 6 h, and 24 h; Dex (1.25 μM) for 24 h; N6,2′-O-dibutyryladenosine 3′,5′-cyclic monophosphate (5 mm) for 24 h; 5-azacytidine (10 μM) for 6 days; all trans-retinoic acid (1 μM) for 24 h; and sodium butyrate (5 mm; all agents from Sigma) for 24 h.

In Vivo Assays of Invasion and Spontaneous Metastasis.
Matrigel, Humatrix, the EHS allograft, and the human xenografts of HMS-X, HMS-3X, HMS-4X, M/E, MDA-MB-231, and A375 (1.0 cm) were established in groups of 10 severe combined immunodeficient mice each. These gels and xenografts from each group were immediately extirpated to insure that the injections had been correctly targeted to within their matrices. After 8 weeks, animals were sacrificed, and primary tumors and lungs were subjected to detailed histopathological examination, collagenase digestion, and culturing of the liberated cells in MEM EpiCells Matrix (Life Technologies, Inc., Gaithersburg, MD). The number of recovered neoC816l clones from each site was determined by phase-contrast image analysis of the cultured dishes.

Results

In Situ Observations
Ducts and acini were surrounded by a circumferential layer of myoepithelial cells exhibiting strong immunoreactivity for S-100, smooth muscle actin, and diverse proteinase inhibitors including maspin, α1-AT, PN-II/APP, and TIMP-1 (Table 1; Fig. 1A). PAI-1 immunoreactivity, although present, was less discriminating. In DCIS, the myoepithelial layer appeared either intact or focally disrupted, but the myoepithelial cells themselves exhibited the same pattern of immunoreactivity (Fig. 1B). The human tumoral-nude mouse xenografts (Fig. 1C) derived from the human myoepitheliomas of the salivary gland (HMS-X and HMS-3X) and breast (HMS-4X) demonstrated an immunocytochemical profile identical to each other and similar to that exhibited by the myoepithelial cells surrounding normal ducts and DCIS (Table 1). Not only was strong proteinase inhibitor immunoreactivity for certain inhibitors such as maspin present within the myoepithelial cells of these xenografts (Fig. 1D), but strong immunoreactivity for other proteinase inhibitors such as α1-AT and PN-II/APP was present within the extracellular matrix of these xenografts (Fig. 1E).

Experimental Studies

Studies of in Vitro Proteinase Inhibitor/Proteinase Factor Expression. Detailed studies conducted with the myoepithelial line HMS-1 revealed a constitutively high proteinase inhibitor to proteinase ratio in strong contrast to the high proteinase to proteinase inhibitor ratio observed in a number of non-myoeipithelial human cell lines (Figs. 2 and 3). Direct gelatin zymography of CM revealed only low levels of the Mr 92,000 and Mr 72,000 type IV collagenases (MMP-9 and MMP-2, respectively) in HMS-1; the Mr 72,000 collagenase was reduced 6-fold in HMS-1 compared to the levels in the majority of the malignant lines (Fig. 3A); direct fibrin zymography revealed visibly lower levels of the Mr 54,000 urokinase plasminogen activator in HMS-1 (Fig. 3B). This was also observed in casein/plasminogen gels. Tissue-type plasminogen activator was not detected in any cell line, nor was plasmin detected in control gels lacking plasminogen. Stromelysin-1 (MMP-3) was also not detected in HMS-1 (Fig. 2). The proteinase inhibitor expression profile of HMS-1, in contrast, was characterized by high constitutive expression in CM of several proteinase inhibitors including TIMP-1 (Fig. 3, C and D); PAI-1 (Fig. 3E); three trypsin inhibitors: α1-AT, PN-II/APP, and an unidentified Mr 31,000–33,000 inhibitor (Fig. 3, F–H) detected initially on reverse zymography; and the tumor suppressor maspin (Fig. 3, I and J). TIMP-2 and TIMP-3 were not detected. With respect to the trypsin serine proteinase inhibitors, the conspicuous doublet at Mr 116,000, consistently greater in HMS-1 than in any of the other lines examined, was confirmed on Western blot to be PN-II/APP (Fig. 3G). These bands represented the 770- and 751-amino acid isoforms of PN-II/APP, which possessed a Kunitz-type serine proteinase inhibitor domain. Interestingly in 2 mm urea extracts of HMS-X, HMS-3X, and HMS-4X, a novel MR 95,000 band of trypsin inhibition was detected by reverse zymography and confirmed by Western blot to represent an active breakdown product of PN-II (data not shown). This Mr 95,000 PN-II breakdown product was completely absent from HMS-1 CM and urea extracts of HMS-1 cells, suggesting that it was produced in situ within the myoepi-
Fig. 1  In situ immunocytochemistry profile of myoepithelial cells and their derived cell lines/xenografts. A, differential maspin immunoreactivity of myoepithelial cells surrounding breast ducts and acini; B, differential maspin immunoreactivity of myoepithelial cells in DCIS; C, appearance of human myoepithelial xenografts showing abundant extracellular matrix; D, cytoplasmic maspin immunoreactivity within myoepithelial xenograft, HMS-X; E, extracellular matrix immunoreactivity of PN-II/APP within HMS-X (α1-AT gave similar results). Anti-maspin, immunoperoxidase, ×250 (A) and ×400 (B and D); periodic acid Schiff, ×400 (C); anti-PN-II/APP, immunoperoxidase, ×400 (E).

ithelial extracellular matrix to which it bound. The retention of proteinase inhibitor activity by this breakdown product indicated that it retained the Kunitz-type serine proteinase inhibitor domain responsible for its ability to inhibit trypsin. In contrast to PN-II/APP, protease nexin I was not detected. The second trypsin serine proteinase inhibitor was present at Mr 54,000 and was α1-AT. This inhibitor appeared nearly equivalent in HMS-1 compared to the malignant lines examined on reverse zymography (Fig. 3F), but by Western blot its signal was markedly stronger and slightly more mobile in HMS-1 than in the malignant lines (Fig. 3H). These data were reconciled with the fact that α1-AT was probably less glycosylated in HMS-1. This relative underglycosylation caused α1-AT from HMS-1 to migrate slightly further into the gel and accounted for its poorer
reactivation following SDS denaturation on reverse zymography as compared to the more highly glycosylated isoforms present in the malignant lines. The third trypsin serine proteinase inhibitor detected at $M_r$ 31,000–33,000 was clearly not a degradation product of either PN-II or α1-AT, as demonstrated by negative Western blot. The $M_r$ 31,000–33,000 inhibitor was strongly expressed in HMS-1 and either absent or nearly absent in all of the malignant lines examined. Whether this unidentified inhibitor is a novel inhibitor is being determined. In contrast to the above inhibitors, PAI-1 was expressed only slightly greater in HMS-1 compared to the majority of the malignant lines by both reverse zymographic (Fig. 3E) and Western blot analysis. Neither PAI-2, PAI-3, or α2-antiplasmin was detected by Western blot analysis in any of the cell lines. Antiplasmin activity as determined by photometric assay was completely absent as well. The most striking difference, however, between the strong proteinase inhibitor profile of HMS-1 and the profile of the malignant cell lines examined was in the expression of maspin. Intense maspin transcripts (3.0 and 1.6 kb; Fig. 3J) and protein ($M_r$ 42,000; Fig. 3J) were identified in HMS-1 and HMS-1 CM, respectively, but were completely absent in all of the malignant lines examined (Fig. 2). With its proteinase inhibitor profile of increased maspin, TIMP-1, PN-II, α1-AT, and the $M_r$ 31,000–33,000 inhibitor, HMS-1 bore strong resemblance to normal HMECs (Fig. 2), except that the expression of all of these proteinase inhibitors including maspin was even more enhanced in HMS-1. Because HMEC cultures are derived from normal ducts and acini of the human breast, they likely contain myoepithelial as well as epithelial cells. Thus, the resemblance of HMS-1 to HMECs further supported our contention that HMS-1, although immortal, expressed a well-differentiated myoepithelial phenotype. In addition, because HMS-1 was a clonal line expressing a pure myoepithelial phenotype, it would be predicted to express certain myoepithelial-associated proteins such as maspin, α1-AT, PN-II/APP, and TIMP-1 to a greater degree than HMECs. Predictably, the myofibroblast line, Hs578Bst, was strongly expressive of TIMP-1 but did not express maspin, PN-II, or the $M_r$ 31,000–33,000 inhibitor (Fig. 2). The strong proteinase inhibitor profile exhibited by HMS-1 was shared by the other myoepithelial cell lines, HMS-3 and HMS-4, and the myoepithelial xenografts, HMS-X, HMS-3X, and HMS-4X.

**Studies of in Vitro Invasion.** In Matrigel invasion experiments, both the C8161 human melanoma line and the MDA-MB-231 line exhibited high degrees of Matrigel invasion. Their baseline invasion in Matrigel was expressed as 100% control. Fig. 2. Relative constitutive expression of diverse proteinase inhibitors and proteinases in HMS-1 compared to various nonmyoepithelial cell lines. Z, direct or reverse zymography; W, Western blot; N, Northern blot; C, chromogenic substrate assay.

![Enzymes/Inhibitors Table](image)
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Fig. 3 Pattern of expression of prototype myoepithelial line. HMS-1 (Lane 1) compared to a prototype malignant cell line, C8161 (Lane 2): direct gelatin zymography depicting the Mr 92,000 and Mr 72,000 type IV collagenases (A); direct fibrin zymography revealing the Mr 54,000 urokinase-type plasminogen activator (B); TIMP-1 expression demonstrated by Northern blot (C) and reverse zymography (D); PAI-1 expression demonstrated by reverse fibrin zymography as a Mr 52,000 lysis-resistant band (E); trypsin-like serine proteinase inhibitor expression demonstrated by reverse trypsin zymography as resistant bands of Mr 116,000, Mr 54,000, and Mr 31,000 (F); equivalently loaded Western blots using PN-IIJAPP and α1-AT antibodies identifying two of the α1-AT inhibitors (G); and maspin expression demonstrated by Northern blot (H) as 3.0- and 1.6-kb mRNA transcripts and Western blot as a Mr 42,000 protein (J).

When Humatrix (similarly concentrated to 3 mg/ml) was compared to Matrigel, the degree of invasion exhibited by these lines decreased to 35% ± 15% and 25% ± 10% of their baseline control (P < 0.01). When the Matrigel control was further modified by the addition of HMS-1 cells, the invasion of the two highly invasive human cell lines decreased to 42% ± 7% and 54% ± 8% of control (P < 0.05), respectively (Fig. 4A). The HMS-1 line was itself noninvasive in Matrigel. The M15 human melanoma line, which was itself also noninvasive in this chamber, did not inhibit invasion, despite being composed of cells twice as large as HMS-1. This suggested that the mechanism of invasion inhibition did not involve physical or mechanical obstruction. Predictably, the anti-invasive effects of HMS-1 could be abolished by CHX (40 μg/ml) 24-h pretreatment. When the Matrigel invasion chamber was further modified by the addition of HMS-1 CM, invasion was inhibited in a dose-response fashion up to 30% ± 8% of control (P < 0.01; Fig. 4B). Control (unconditioned) media similarly concentrated exerted no inhibition of C8161 or MDA-MB-231 invasion. Pretreatment of HMS-1 with DEX (25 μM) produced a complete invasion-permissive phenotype (95% ± 5% of control), whereas pretreatment with PMA (5 μM) produced an essentially nonpermissive phenotype (2% ± 1% of control; P < 0.05; Fig. 5A). The effects of DEX and PMA were quite dramatic. The effects of other agents including all trans-retinoic acid, N6,2′-O-dibutyryladenosine 3′,5′-cyclic monophosphate, sodium butyrate, and 5-azacytidine showed either permissive or nonpermissive trends but were less dramatic. The induction of PMA of the nonpermissive phenotype began after 20 min of pretreatment, was almost complete after 2 h, and maximized after 24 h (P < 0.05; Fig. 5B). The induction of this nonpermissive phenotype correlated with the induction of a dramatic 5-fold increase in maspin secretion measured in HMS-1 CM (Fig. 6, A and B). As a result of PMA treatment, both an immediate release (within 2 mm) of maspin from HMS-1 cells occurred (Fig. 6A) as well as a more sustained secretion for at least 24 h following PMA pretreatment.
Fig. 5  
A. effects of pharmacological treatment of HMS-1 cells with various agents inducing permissive and nonpermissive phenotypes. B. time course of PMA induction of nonpermissive phenotype. Bars, SD.

(Fig. 6B). The increased maspin secretion was not on the basis of an increase in steady-state maspin transcripts (Fig. 6C). PMA also resulted in a less dramatic 2-fold increase in both MMP-9 and TIMP-1 secretion. Immunoprecipitation experiments of PMA-treated HMS-1 CM with anti-maspin decreased maspin levels by 85% and resulted in a significant increase in C8161 and MDA-MB-231 invasion (up to 70% ± 10% and 65% ± 8% of controls, respectively), suggesting that the mechanism of the nonpermissive phenotype of HMS-1 was at least partially maspin dependent. The induction of DEX of an invasion-permissive phenotype in HMS-1 was not associated with a change in either maspin transcription or secretion. Similar anti-invasive effects with similar augmentation by PMA and reduction by DEX were observed in HMS-3 and HMS-4. Control nonmyoepithelial cell lines including normal fibroblasts exerted no anti-invasive effects and no augmentation/reduction with PMA/DEX.

Fig. 6  
A. immediate effects of PMA treatment on maspin secretion measured by Western blot of CM at designated times of PMA exposure. B. delayed effects of PMA on maspin secretion measured by Western blot of CM 72 h after PMA pretreatment for indicated time periods followed by removal of PMA. C. Northern blot of maspin expression following exposure to PMA for indicated time periods.

Studies of in Vivo Invasion and Metastasis. Highly metastatic C8161 cells injected into the matrices (Fig. 7A) of the primary myoepithelial xenografts HMS-X, HMS-3X, HMS-4X, and Humatrix (Fig. 7B) could be recovered in significant numbers from their primary injection sites, although the number of clones recovered were less than those recovered from Matrigel, the primary EHS allograft, and the nonmyoepithelial primary tumor xenografts (Fig. 7C), including breast (MDA-MB-231), mucoepidermoid carcinoma (M/E), and melanoma (A375) (Fig. 8). Histological and immunocytochemical analysis of the extirpated xenografts and matrices revealed C8161 cells actively invading through Matrigel, the EHS allograft, and all of the nonmatrix-producing primary human tumor xenografts (Fig. 7C) in contrast to the appearance of C8161 within the myoepithelial matrices (HMS-X, HMS-3X, HMS-4X, and Humatrix), where the C8161 cells were more confined to the immediate areas around the injection site (Fig. 7B). Pulmonary metastases of C8161 were completely absent in the myoepithelial xenografts and Humatrix-injected group, whereas they were quite numerous in the nonmyoepithelial matrix primary tumor xenograft groups, the EHS allograft, and Matrigel (P < 0.001; Fig. 8).

Discussion

The effects of host bystander cells (19) on tumor progression remains an understudied area of tumor biology. Of the different host cells studied in this regard, certainly the myoepithelial cell has been one of the least studied. This study has
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...myoepithelial matrices with ease. They grew but were contained within the myoepithelial matrices and could be extended to any organ or tissue where myoepithelial cells surround proliferating epithelial cells in precancerous disease states.

Tumor invasion of the extracellular matrix is thought to be determined, in part, by the balance of natural proteinases and proteinase inhibitors that occurs within the tumor's microenvironment (2). No one factor or factor(s) can be implicated universally to explain tumor invasion and metastasis, and in fact, certain specific factors, e.g., PAI-1, which would be expected to correlate negatively with the malignant phenotype, sometimes correlate positively. Still, situations that shift this balance to an excess of proteinase inhibitors would be situations that favor tumor suppression. The host bystander myoepithelial cells that were studied both in situ and in vitro clearly exhibited a proteinase inhibitor-dominated phenotype. The major inhibitors found to be elevated and differentially expressed within myoepithelial cells included PN-II, α1-AT, an uniden-tified Mr 31,000–33,000 inhibitor, TIMP-1, and maspin. PN-II has been shown to correlate directly with the degree of histological differentiation and inversely with the invasive and metastatic potential of various human carcinoma cell lines (20). This inhibitor may also act as a cell adhesion factor and has been shown to bind basement membrane heparan sulfate proteoglycan and laminin with high affinity (21, 22). Indeed, immunocytochemical studies revealed strong PN-II immunoreactivity within the matrices of HMS-X, HMS-3X, and HMS-4X, suggesting that myoepithelial matrix bound and accumulated this inhibitor. The second serine proteinase inhibitor identified that was also differentially expressed in myoepithelial cells and found within the myoepithelial matrix was the Mr 54,000 α1-AT. Interestingly, Takahashi et al. (23) found that the extracellular matrix of salivary gland basal cell adenoma, another type of myoepithelial tumor similar to that from which HMS-X was derived, was strongly immunoreactive for α1-AT. The third serine proteinase inhibitor differentially expressed by myoepithelial cells was an unidentified Mr 31,000–33,000 trypsin inhibitor. A family of extracellular matrix-associated trypsin inhibitors with molecular masses of Mr 27,000–33,000 has recently been identified in SV40 transformed human skin fibroblasts (24). Whether the Mr 31,000–33,000 inhibitor present in HMS-1 is one of these inhibitors or a new member of this family is being investigated presently. High levels of TIMP-1 were also found to be a characteristic of myoepithelial cells and their derived cell lines/xenografts. TIMP-2 and TIMP-3, however, were not detected. This family of inhibitors has been clearly shown to suppress tumor cell invasion and metastasis (25). Finally, the proteinase inhibitor most dramatically up-regulated in the myoepithelial cells was the tumor suppressor maspin. Although maspin belongs to the serpin superfamily of serine proteinase inhibitors (26), it probably functions as a ligand-binding serpin rather than as a proteinase inhibitor (27, 28). Still, the expression of maspin has been inversely correlated with malignant behavior, and it has been shown to inhibit tumor growth, invasion, and metastases.
tasis (26, 29). Recently, maspin has been observed to exert negative effects on tumor cell motility (30), and this mechanism could certainly be one such mechanism by which myoepithelial cells exert their tumor-suppressing activities. When one notes in addition to maspin the many diverse proteinase inhibitors constitutively expressed at high levels in the HMS lines, one must consider that the myoepithelial phenotype most likely is regulated by a high level pleiotropic pathway that orchestrates the concerted expression of these many different effector molecules. The anti-invasive phenotype of myoepithelial cells was not only in evidence by their high constitutive proteinase inhibitor profile present in situ but by the ability of Humatrix, HMS-1, HMS-3, HMS-4, and their CM to inhibit invasion of invasive breast carcinoma and melanoma lines in vitro. What was even more exciting was the strong potentiation of this anti-invasion effect by PMA, an agent that might have been predicted to promote the tumorigenic properties of the myoepithelial cell lines rather than their suppressing properties. The synchronous induction by PMA of a nonpermissive phenotype in the HMS lines and increased maspin secretion, which were both subsequently neutralized by maspin immunoprecipitation, implicated maspin mechanistically. Interestingly, the increased maspin secretion was not transcriptionally regulated, implicating either a translational or posttranslational mechanism mediated by PMA. Although maspin has been shown previously to be an autocrine tumor suppressor in experiments involving its cDNA transfected into breast carcinoma cell lines (26), in the present study we have shown that maspin can be a paracrine tumor suppressor mediating the natural suppressor effects of myoepithelial cells on tumor invasion.

Tumor metastasis is thought to be regulated by complex extracellular matrix interactions that may exert both stimulatory as well as inhibitory effects on the processes of tumor invasion and metastasis. In fact, previous coculture experiments with Matrigel have shown that this matrix stimulates tumor invasion and metastasis in severe combined immunodeficient mice (31, 32). In our study, however, the matrices of the three myoepithelial xenografts, HMS-X, HMS-3X, HMS-4X, and Humatrix were selectively inhibitory to tumor invasion and metastasis. Possible mechanisms of this effect include a blockade of tumor invasion by the proteinase inhibitors bound to the myoepithelial matrix and/or inhibitory effects of these bound proteinase inhibitors on angiogenesis. We have demonstrated previously that the proteinase inhibitor, PN-II, undergoes extracellular in vivo processing within the matrices of HMS-X, HMS-3X, and HMS-4X to a novel Mr 95,000 fragment retaining full proteinase inhibitor activity and found bound to the myoepithelial matrix (9). In any case, the inability of a highly metastatic cell line to invade and metastasize through specific myoepithelial-derived matrices is additional proof of the natural paracrine suppressor effects of myoepithelial cells.

References
The human myoepithelial cell is a natural tumor suppressor.

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