Membrane Type 1 Matrix Metalloproteinase Induces Epithelial-to-Mesenchymal Transition in Prostate Cancer*

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By mining DNA microarray data bases at GenBank™, we identified up-regulation of membrane type 1 matrix metalloproteinase (MT1-MMP) in human primary and metastatic prostate cancer specimens as compared with nonmalignant prostate tissues. To explore the role of up-regulated MT1-MMP in early stage cancer progression, we have employed a three-dimensional cell culture model. Minimally invasive human prostate cancer cells (LNCaP) were transfected with MT1-1 green fluorescent protein (GFP) chimeric cDNA as compared with GFP cDNA, and morphologic and phenotypic changes were characterized. GFP-expressing LNCaP cells formed multicellular spheroids with cuboidal-like epithelial morphology, whereas MT1-GFP-expressing cells displayed a fibroblast-like morphology and a scattered growth pattern in type I collagen gels. Cell morphologic changes were accompanied by decreased epithelial markers and enhanced mesenchymal markers, consistent with epithelial-to-mesenchymal transition. MT1-MMP-induced morphologic change and cell scattering were abrogated by target inhibition of either the catalytic domain or the hemopexin domain. We further demonstrated that MT1-MMP-induced phenotypic changes were dependent upon up-regulation of Wnt5a, which has been implicated in epithelial-to-mesenchymal transition. We conclude that MT1-MMP plays an important role in early cancer dissemination by converting epithelial cells to migratory mesenchymal-like cells.

Most human cancers are epithelial in origin. Carcinoma progression is often accompanied by the loss of an epithelial phenotype and the acquisition of a fibroblastic or mesenchymal phenotype (epithelial-to-mesenchymal transition (EMT)²). This transition has emerged as a critical step in the conversion of early stage cancer to invasive and metastatic cancer (1, 2). Turning an epithelial cell into a mesenchymal cell requires alterations in morphology, cellular architecture, adhesion, and migration. A molecular hallmark of EMT is the decrease or loss of expression of the adherens junction protein, E-cadherin, resulting in loss of cell-cell association and change of cell morphology. Decreased levels of E-cadherin and cytotokeratins and acquisition of mesenchymal proteins like fibronectin and vimentin are indicative of a switch toward a mesenchymal dedifferentiated phenotype; these phenotypic changes result in enhanced cell motility and invasiveness (3).

Enhanced production and activation of matrix metalloproteinases (MMPs), especially membrane type 1 MMP (MT1-MMP), have been described in most types of carcinoma, including commonly occurring prostate and breast cancer (4). High levels of MMPs in cancer tissues have been correlated with poor prognosis. MMPs have been linked with EMT through both autocrine and/or paracrine pathways (5). Secreted MMPs (e.g. MMP-2, -3, -9, and -28) have been associated with cancer cell EMT through various mechanisms (6–8). Although MT1-MMP is capable of cleaving E-cadherin in transfected breast cancer cells (9), the effect of this cleavage on EMT has not been characterized.

The multiplicity of distinct pathways and molecules, each of which can lead independently to EMT-like events is astonishing. The Wnt pathway is exceptional in its complexity (10). Three distinct intracellular pathways appear to transduce Wnt signals: the canonical Wnt/β-catenin pathway, the Wnt/Ca²⁺ pathway, and the Wnt/polarity pathway. It is unclear what factors are involved in determining which of the three intracellular pathways are activated. Wnt5a (wingless-type MMTV integration site family, member 5A) appears to initiate the Wnt/Ca²⁺ cascade. Up-regulation of Wnt5a has been associated with many types of human cancers (11). Wnt5a overexpression enhances tumor cell proliferation, cell motility, and invasion (12, 13). Wnt5a also augments repopulating capacity and primitive hematopoietic development of human blood stem cells in vivo (14). Wnt5a has recently been postulated to play a critical role in cancer cell EMT (15, 16). However, the mechanism that drives up-regulation of Wnt5a remains to be characterized.

There has been considerable debate recently concerning the requirement of MT1-MMP enzymatic activity in cancer cell migration in three-dimensional culture models (17–19). In the current study, we employed a three-dimensional ECM culture system in which cancer cells were embedded within a native
type I collagen gel to better characterize the mechanism by which MT1-MMP enhances cancer dissemination.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Type I collagen (acetic acid-extracted native type I collagen from rat tail tendon) was purchased from BD Biosciences Discovery Labware (Bedford, MA). EZ-Link Sulfo-NHS-SS-Biotin was purchased from Pierce. Recombinant TIMP-2 was purchased from CHEMICON International, Inc. (Temecula, CA). RNAi-Ready pSIREN-RetroQ and pIRES2/GFP vectors were purchased from Clontech. Cells were selected with 4 mg/ml of drug-treatments renewed every other day.

**Cell Lines**—To permit visualization of protein trafficking and cell migration/invasion using an enhanced green fluorescent protein (pEGFP from Clontech), we previously generated a fusion protein between MT1-MMP and EGFP by fusing EGFP cDNA to the C terminus of MT1-MMP cDNA (MT1-GFP) (21). This chimera has been demonstrated to have similar features to wild-type MT1-MMP (22). MT1-GFP and EGFP control cDNAs were stably transfected into less aggressive human prostate cancer LNCaP cells (ATCC, Manassas, VA), as described previously (21). Cells were maintained in RPMI 1640 medium with 10% fetal calf serum (Invitrogen).

**Construction of Small Interference RNA Vectors and Retroviral Infection**—Small interfering oligonucleotides specific for MT1-MMP and a control to express short hairpin RNA (shRNA) were designed using a Worldwide Web-based online software system (Block-it RNAi Designer; Invitrogen) for mammalian RNA interference. Three specific 21-nucleotide sequences spanning positions 1461–1481 (MT1-MMP-shRNA-1), 472–492 (MT1-MMP-shRNA-2), and 1643–1663 (MT1-MMP-shRNA-3) of the human MT1-MMP gene (GenBank accession number NM_004995) and three specific 21-nucleotide sequences spanning positions 64–85 (wnt5α-shRNA1), 307–328 (wnt5α-shRNA2), and 403–424 (wnt5α-shRNA3) of the human wnt5α gene (GenBank accession number NM_003392) were cloned as inverted repeats into the RNAi-Ready pSIREN-RetroQ vector. As a control, we used the RNAi-Ready pSIREN-Retro Q vector. Alexa 568-conjugated goat anti-mouse IgG was purchased from Invitrogen. Recombinant pro-MMP-2 was produced by COS-1 cells transfected with pro-MMP-2 cDNA as previously described (20).

**Cell Scattering Assay**—Collagen gel cultures in 24-well plates were performed as previously described (23). In brief, 4 × 10^4 cells/ml were resuspended in neutralized acid-extracted type I collagen (2.5 mg/ml, final concentration). After collagen gelation, 300 μl of complete medium were added above the gels. For inhibition studies, MMP inhibitors, TIMP-2, or anti-functional antibodies against MT1-MMP hemopexin domain were added. Cells were cultured for the indicated times with media and drug-treatments renewed every other day.

**HE Staining of Frozen Sections**—For histological sections, type I collagen gels were fixed in 4% paraformaldehyde. Sections were cut at 6 μm and stained with hematoxylin and eosin.

**Immunofluorescent Staining and Laser-scanning Confocal Microscopy**—Cultured cells were fixed with 4% paraformaldehyde, phosphate-buffered saline followed by blocking with 3% bovine serum albumin/phosphate-buffered saline. E-cadherin at cell-cell adherens junctions was detected by an antibody against the extracellular portion of E-cadherin (Zymed Laboratories Inc.) followed by secondary antibodies conjugated with Alexa 568 (Invitrogen). The immunostained cells were examined, and a z section series was obtained using a Leica TCS SP2 confocal microscope.

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FIGURE 1. Data mining DNA microarray for MT1-MMP. By analysis of Oncomine data bases, normalized MT1-MMP expression level in normal prostate, localized prostate cancer (PCa), and metastatic prostate cancer examined by DNA microarray was analyzed. MT1-MMP expression showed significant increase in primary and metastatic prostate cancer as compared with normal prostate ($p = 3.2E^{-4}$). n = samples.

RESULTS

By mining DNA microarray data bases at Gene Expression Omnibus (GEO/NCBI) and Oncomine (Cancer Profiling Database) for MT1-MMP expression in human prostate cancer specimens employing a cut-off of $p < 0.01$, we identified up-regulation of MT1-MMP in cancer tissues as compared with adjacent normal prostate tissue in two microarray data sets (26, 27). In the microdissected primary tumor data set of Yu et al. (26), MT1-MMP levels were significantly increased in primary tumor samples from patients with metastasis as compared with nonmetastatic prostate cancer (Fig. 1). Correlation of up-regulated MT1-MMP with prostate cancer metastasis has been confirmed using a surgical orthotopic implantation prostate cancer animal model (21). To study the mechanism underlying MT1-MMP-enhanced prostate cancer dissemination, we carried out a series of experiments using cell lines stably expressing MT1-MMP.

MT1-MMP Induces Cell Morphologic Changes and Cell Scattering in Three-dimensional Culture—Human LNCaP prostate cancer cells are well differentiated with high expression of E-cadherin, prostate-specific antigen, and cytokeratin 18 and a minimal level of vimentin expression (28). To study the role of MT1-MMP in early prostate cancer dissemination, stably transfected LNCaP cells expressing high and low levels of MT1-MMP and enhanced green fluorescent protein chimera (MT1-GFP) were selected using a quantitative real time RT-PCR approach. Gene expression levels of MT1-GFP chimera in high expressing LNCaP cells were roughly equivalent to nontransfected DU145 prostate cancer cells and HT1080 fibrosarcoma cells (supplemental Fig. 1). As assessed by immunoblotting, protein levels of MT1-GFP chimera in stable high expressing LNCaP cells were 9-fold less than transiently transfected LNCaP cells. β-actin was employed to equalize protein loading.

FIGURE 2. MT1-MMP induces morphologic changes in cancer cells cultivated in type I collagen gels. A, establishment of stable LNCaP cell lines expressing MT1-GFP chimera. Stable LNCaP cells expressing MT1-GFP chimera or GFP control were established and characterized by Western blotting using an anti-MT1-MMP antibody. Protein levels of MT1-GFP in high expressing LNCaP cells were ~9-fold less than transiently transfected LNCaP cells. β-actin was employed to equalize protein loading. B, no morphologic differences were observed between LNCaP cells, LNCaP cells stably expressing GFP, and LNCaP cells expressing MT1-GFP under two-dimensional culture conditions. Parental LNCaP cells, LNCaP cells stably expressing GFP or MT1-GFP chimeric cDNAs, were cultured on tissue culture dishes for 3 days, followed by phase-contrast and fluorescent microscopic (Nikon TE-2000S) examination. GFP is diffusely distributed throughout the GFP-transfected LNCaP cells, whereas fluorescence is focalized on the cell surface of MT1-GFP-transfected cells. Bar, 20 μm. C, induction of cell morphologic change and cell scattering by expression of MT1-MMP in LNCaP cells under three-dimensional culture conditions. LNCaP cells (4 × 10⁴/ml) stably expressing GFP or MT1-GFP chimeric cDNA were mixed within neutralized type I collagen gels (2.5 mg/ml). The cells were examined daily for 9 days under fluorescent microscopy. At day 6, a set of gels were fixed, and frozen sections were prepared for hematoxylin/eosin staining. Bar, 20 μm.
Although MT1-MMP-expressing LNCaP cells are capable of activating exogenous pro-MMP-2 (21), the morphology of GFP- and MT1-GFP-expressing cells in two-dimensional cultures was almost identical to that of nontransfected LNCaP cells (Fig. 2B). In contrast, when these cells were embedded in type I collagen gels (three-dimensional culture model), which more closely mimics physiological conditions, distinctive phenotypic differences were observed. Beginning on day 2, MT1-GFP-expressing LNCaP cells displayed an elongated, fibroblast-like morphology with distribution of the MT1-GFP fusion protein concentrated at the leading edge (Fig. 2C and supplemental Fig. 2). MT1-GFP/LNCaP cells gradually displayed a scattering growth pattern in three-dimensional collagen gels. In contrast, LNCaP cells expressing GFP formed multicellular spheroids with round or cuboidal-like morphology in three-dimensional type I collagen gels (Fig. 2C), recapitulating the epithelial phenotype of carcinoma cells (3). Hematoxylin/eosin staining of three-dimensional collagen gel sections confirmed these observations.

To further explore the specific function of MT1-MMP in LNCaP cell scattering, we generated three MT1-MMP-specific shRNA constructs using a retroviral vector. Two of the MT1-MMP-shRNA constructs (MT1-MMP-shRNA-1 and -3 with 87 and 77% inhibition of MT1-MMP mRNA, respectively) efficiently inhibited functional MT1-MMP in terms of pericellular pro-MMP-2 activation (Fig. 3A). Following the growth of MT1-GFP/LNCaP cells expressing MT1-MMP-shRNA-1 in three-dimensional type I collagen gels, the cells grew as multicellular spheroids with diminished GFP expression as compared with the disseminated cell phenotype described above (Fig. 3B). The defect of MT1-MMP-induced morphology change and scattering pattern correlated with reduced MT1-GFP expression as evaluated by microscopic examination of GFP fluorescence (Fig. 3B) and immunoblotting using anti-MT1-MMP antibody (data not shown). Together, these data highlight the role of MT1-MMP in conversion of epithelial cell shape to fibroblast-like morphology.

**Cancer Cell Expression of MT1-MMP Results in Shedding of E-cadherin**—Cell morphologic change from epithelial to mesenchymal appearance is accompanied by loss of cell-cell contact and represents a hallmark of EMT (29). Degradation or loss of E-cadherin expression is an important indicator of EMT (3). To determine if epithelial cell morphology change induced by expression of MT1-MMP is accompanied by loss of E-cadherin, co-localization of MT1-MMP with E-cadherin in cells was examined by laser-scanning confocal microscopy. In agreement with previous reports (30), MT1-MMP was uniformly localized at the cell surface in isolated cells (Fig. 2B). Interestingly, MT1-MMP distribution was reorganized from uniform cell surface localization in isolated cells to redistribution at both the apical surface and the lateral junction areas of adjacent cells. As visualized in confocal x-z sections, dominant distribution was limited to the cell-cell junction area following partial confluency of MT1-GFP-expressing cells (Fig. 4A). Endogenous E-cadherin, primarily enriched along the lateral membrane, was co-localized with MT1-MMP in LNCaP cells and displayed decreased intensity in MT1-MMP-expressing as compared with GFP-expressing LNCaP cells (Fig. 4A).

We next examined whether MT1-MMP sheds E-cadherin at cell-cell adherens junctions. E-cadherin was examined by immunoblotting of cell conditioned media from LNCaP cells and stably transfected LNCaP cells. Overexpression of MT1-GFP in LNCaP cells was associated with a 5-fold increase in 80-kDa soluble E-cadherin (Fig. 4B); no discernable difference of E-cadherin was noted in the cell lysates containing cytosolic as well as membrane proteins (data not shown). To further examine whether shedding of E-cadherin at cell-cell adherens junctions correlated with loss of cell surface E-cadherin, biotinylation of cell surface proteins followed by streptavidin precipitation and immunoblotting using anti-E-cadherin antibody was performed (25). Decreased biotinylated surface E-cadherin (120 kDa) was observed in MT1-GFP-expressing LNCaP cells as compared with wild-type LNCaP cells and LNCaP cells transfected with GFP cDNA (Fig. 4B), confirming the role of MT1-MMP in E-cadherin cleavage.

**Expression of MT1-MMP in LNCaP Cells Alters the Phenotype**—Cell morphologic change from a cuboid epithelial shape to a spindle-like, fibroblastic appearance and degradation...
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of E-cadherin is often accompanied by a decrease or loss of epithelial markers and gain of mesenchymal markers (3). To determine if expression of MT1-MMP results in loss/decrease of the epithelial phenotype of LNCaP cells and increase of mesenchymal markers, real time RT-PCR was performed. Down-regulation of epithelial markers (cytokeratin-8 and -18) and increased mesenchymal markers (vimentin and fibronectin) were found in MT1-GFP-expressing LNCaP cells cultivated in three-dimensional type I collagen gels (Fig. 5A). These phenotypic changes were further evaluated by immunoblotting using corresponding antibodies. Decreased cytokeratin-8 (1.8-fold) and -18 (4.4-fold) and increased vimentin (2.7-fold) and fibronectin (4.6-fold) proteins were detected in MT1-GFP/LNCaP cells cultured in three-dimensional type I collagen gels for 6 days followed by protein extraction with radioimmune precipitation buffer. 20 μg of total cell lysates were used for immunoblotting using anti-cytokeratin-8, -cytokeratin-18, -fibronectin, or -vimentin antibodies. β-Actin was employed to equalize protein loading.

Both Proteolytic Activity and Cell Migration Are Required for MT1-MMP-induced Cell Scattering/Invasion; the Cytoplasmic Tail of MT1-MMP Is Not Required—We previously demonstrated in two-dimensional cultures that the catalytic and hemopexin (PEX) domains of MT1-MMP play independent roles in ECM degradation and cell migration, respectively (24). To explore the role of the catalytic and PEX domains of MT1-MMP in cell scattering/invasion in a three-dimensional model, natural MMP inhibitors and anti-PEX domain of MT1-MMP antibodies were employed. As shown in Fig. 6A, the three-dimensional invasive ability of MT1-GFP expressing LNCaP cells (scattered pattern) was abolished by the addition of recombinant TIMP-2 (Fig. 6, A (c)) but not vehicle control (Fig. 6A (a)). When cells were treated with anti-PEX domain antibodies, the cell scattered growth pattern was also replaced by spheroid cell aggregates (Fig. 6A (d)). No effect on MT1-MMP-induced cell scattering was noted in the normal IgG control. To dissect the role of the anti-PEX antibody in MT1-MMP-induced cell scattering, the fluorescein isothiocyanate-labeled substrate (fibronectin) degradation-migration assay was employed (24). As demonstrated in Fig. 6B, this antibody inhibited MT1-MMP-induced cell migration but did not alter MT1-MMP proteolytic activity. In contrast, substrate degradation was blocked in the presence of recombinant TIMP-2. These data demonstrate that both the catalytic and hemopexin domains of MT1-MMP are required for LNCaP cell scattering/invasion.

The cytoplasmic domain of MT1-MMP has been reported to play an important role in endocytosis (31, 32), but its role in cell
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To determine if interference with MT1-MMP-induced cell scattering correlated with change in cell phenotype, quantitative real time RT-PCR was performed employing epithelial and mesenchymal cell markers. Similar to LNCaP cells expressing GFP, inactive MT1-GFP chimera cells (MT1-MMP E240-A-GFP) (24) prominently displayed epithelial markers (cytokeratin-8 and -18) but not the mesenchymal markers displayed by MT1-GFP/LNCaP cells and MT-GPIuPAR/GFP/LNCaP cells (Fig. 6D).

MT1-MMP Induces EMT through Up-regulation of Wnt5a—To identify potential key regulators of MT1-MMP-induced EMT, gene expression profiles of three-dimensional cultured GFP/LNCaP and MT1-GFP/LNCaP cells were analyzed using a DNA microarray approach. Initial analysis reveals that a panel of genes related to EMT is up-regulated by expression of MT1-GFP.3 The most prominent of these up-regulated genes was wnt5a. Three independent real time RT-PCR experiments confirmed the ~10-fold increase of wnt5a gene expression in MT1-GFP/LNCaP cells (Fig. 7A). Immunoblotting using an anti-Wnt5a antibody (R&D Systems) displayed markedly enhanced Wnt5a expression in the conditioned medium of three-dimensional cultured cells expressing MT1-MMP compared with parental LNCaP cells and GFP-expressing LNCaP cells (Fig. 7B).

By mining DNA microarray data bases at GEO/NCBI and Oncomine, we identified up-regulation of Wnt5a in human primary prostate cancer tissues as compared with adjacent normal prostate tissue (26) (Fig. 7C); the highest levels in the primary tumor were noted in patients with metastasis. Up-regulation of Wnt5a has been previously demonstrated to induce cancer cell EMT, leading to enhanced cell migration and invasion (13, 16).

To examine the effect of Wnt5a on MT1-MMP-mediated cell phenotypic changes, we generated three shRNA constructs

3 days followed by microscopic examination. As shown, cell scattering occurs in MT1-GFP-anchored cells but not in cells lacking functional MT1-MMP activity. D, no phenotypic changes were observed in LNCaP cells expressing constitutively inactive MT1-GFP. Total RNA was extracted from three-dimensional cultured GFP/LNCaP cells, MT1-GFP/LNCaP cells, MT1-A-GFP/LNCaP cells, and MT-GPIuPAR/GFP/LNCaP cells. Phenotypic change was examined by real time RT-PCR using specific primers for cytokeratin-8, -18, vimentin, and fibronectin. β-Actin and GAPDH were employed to normalize the corresponding samples. The relative levels of genes were determined using the ΔΔCT method. Each bar represents the mean ± S.E.
against human Wnt5a. After selection of infected MT1-GFP/LNCaP cells with puromycin, the resistant cells were pooled, and total RNA was extracted, followed by real-time RT-PCR. Decreased Wnt5a mRNA expression by 78.8% (Wnt5a-shRNA1), 26.2% (Wnt5a-shRNA2), and 61.7% (Wnt5a-shRNA3) was obtained with three separate Wnt5a shRNAs as compared with luciferase shRNA control (Fig. 7D). Expression of epithelial (cytokeratin-8 and -18) and mesenchymal (vimentin and fibronectin) markers in Wnt5a shRNA expressing MT1-GFP/LNCaP cells was examined by real-time RT-PCR. Supressing Wnt5a expression abrogated MT1-GFP/LNCaP-induced phenotypic changes (data not shown).

To examine the effect of Wnt5a on MT1-MMP-mediated cell invasion, these three Wnt5a siRNAs expressed in MT1-GFP/LNCaP cell lines were evaluated using our three-dimensional collagen invasion assay. Cell invasive ability induced by the expression of MT1-MMP in LNCaP cells was inhibited by 98, 65, and 96% by shRNA1, -2, and -3, respectively (Fig. 7, E and F), corresponding to the expression level of Wnt5a (Fig. 7D). These data suggested that Wnt5a is a downstream effector for MT1-MMP-induced cell migration/invasion.

**DISCUSSION**

Experimental and clinical evidence has highlighted a key role for MMPs in cancer invasion and metastasis (4). Although targeting MMPs with synthetic inhibitors was successful in interfering with cancer growth and dissemination in preclinical models (4, 36), the use of MMP inhibitors in randomized clinical trials of patients with advanced cancers failed to demonstrate efficacy (36, 37). The reason for these negative results may be related to the selection of patients with late stage cancer and employment of broad spectrum inhibitors (36, 37). It has been proposed that specific inhibitors of MMPs employed in early stage cancer need to be explored further before surrendering this avenue of treatment.

EMT has emerged as a critical step in the conversion of early stage to invasive cancer (1, 38). A hallmark of EMT is the loss or degradation of E-cadherin and gain of mesenchymal function (1, 3). MMPs have been linked with cancer cell EMT through different mechanisms that remain to be more completely elucidated (6–8). Our data demonstrate that expression of MT1-MMP in well differentiated prostate cancer cells induces morphologic changes from cuboidal epithelial-appearing to spindle-shaped cells with fibroblast-like morphology accompanied by decreased epithelial markers and increased mesenchymal markers.
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MMP-dependent E-cadherin shedding has been previously implicated in EMT-like phenotypic changes (6, 39–42). However, it remains to be understood how soluble MMPs reach cell-cell adherens junction for ectodomain shedding of E-cadherin. We demonstrate for the first time that MT1-MMP is not only anchored at the apical surface of transfected cells but also localized at lateral cell-cell junctions, where it co-localizes with E-cadherin. MT1-MMP is dynamically shifted upon cell-cell contact and sheds E-cadherin, leading to dissociated cell-cell contact and enhanced cell migration. It is unclear what signal drives MT1-MMP redistribution.

The field of gene expression data analysis has grown in the past few years from being purely data-centric to integrative (43). Although a large volume of gene expression profile data is publicly available through GEO/NCBI or other data bases, data mining is just beginning to be incorporated into the mainstream of cancer research. By setting at a threshold value of \( p < 0.01 \), we found that expression of MT1-MMP is significantly increased not only in prostate cancer tissue (Fig. 1) but also in breast, ovarian, colon, lung, and pancreatic cancers (data not shown) as compared with corresponding adjacent or normal tissues. We also identified up-regulation of Wnt5a expression in microarray data sets from prostate cancer tissues as compared with adjacent normal prostate tissue (Fig. 7C). Wnt5a has been previously implicated in enhanced cell migration and a mesenchymal-like phenotype (15, 44–46). Using a shRNA knockdown approach in MT1-GFP/LNCaP cells, we demonstrate that Wnt5a is a downstream effector of MT1-MMP-induced EMT change, resulting in enhanced cell invasion in three-dimensional collagen gels. The inhibition of tumor invasion by MT1-MMP transfected LNCaP cells expressing Wnt5a shRNA corresponds to the expression level of Wnt5a. Based on these data, we propose that MT1-MMP expression up-regulates Wnt5a, which then leads to the EMT-like phenotype.

The structure-function relationship of MT1-MMP-induced EMT-like change was also evaluated in the current study. LNCaP cells transfected with MT1-MMP cDNA mutated with a GPI anchor replacing the transmembrane, and cytoplasmic domains displayed scattered growth in three-dimensional gels. These data raise the question of how MT1-MMP signals the cell to migrate. Since MT1-MMP was previously demonstrated to cross-talk with plasma membrane protein CD44 (47), a cell surface glycoprotein involved in cell-cell and cell-matrix interactions, CD44 interactions need to be further studied. Cell signalling through phosphatidylinositol 3-kinase and mitogen-activated protein kinase pathways (48, 49), receptor tyrosine kinases (RTKs) (50), Ras (51), and Rac1 (24) are also worthy of consideration.

In the current study in three-dimensional type I collagen matrices, as opposed to two-dimensional studies (24), both the catalytic and hemopexin domains of MT1-MMP were required for LNCaP cell scattering and morphologic changes resembling EMT. These data offer potential therapeutic applications for interference with early cancer dissemination by targeting the PEX domain of MT1-MMP. Indeed, polyclonal antibody targeting the hemopexin domain of MT1-MMP blocked MT1-MMP-mediated cell migration and scattering without interfering with the proteolytic activity of MT1-MMP.

The protease requirement (33) for tumor invasion has been challenged (19, 52). Protease-independent cancer cell invasion has been described to involve 1) a plasticity mechanism by amoeboid transitioned cancer cells (19), 2) Rho-induced contractility generating protrusions to facilitate motility (18), and 3) alternating stationary and migratory events with MMP-independent pseudopod formation (53). Additional studies will be required to clarify the subject.

The mechanism by which epithelial-derived cancer cells engage gene programs necessary to promote invasion and metastasis is not well defined (33, 54, 55). There are numerous examples of advanced carcinomas that adopt some mesenchymal features yet retain characteristics of well differentiated epithelial cells (56). Morphologic and molecular heterogeneity, including incomplete EMT, are well described (57). Several developmentally important genes that induce EMT have been shown to act as E-cadherin repressors. Included in this list are Snail, Slug, the zinc finger protein SIP1 (ZEB 2), E12/E47 (3), and Twist (58). Based on the complexity and controversy concerning the role of EMT in three-dimensional cell migration, it appears that the functions of numerous factors differ, depending on the origin of cells, developmental stages, and architecture of the organ. The “one size fits all” approach to cancer invasion is outmoded.

In conclusion, we have demonstrated that MT1-MMP induces less aggressive cancer cell phenotypic changes resembling EMT. Coordination of extracellular matrix degradation and cell migration induced by MT1-MMP is a prerequisite for this EMT-like change. Targeting either the catalytic domain or hemopexin domain of MT1-MMP will interfere with cell inva-
sion. Since EMT often occurs at the early stage of cancer invasion and metastasis, our data provide evidence for directing MMP inhibitors in treatment of cancer dissemination.

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