Matriptase-2 Inhibits Breast Tumor Growth and Invasion and Correlates with Favorable Prognosis for Breast Cancer Patients

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Abstract

**Purpose:** The type II transmembrane serine proteases are cell surface proteolytic enzymes that mediate a diverse range of cellular functions, including tumor invasion and metastasis. Matriptase (matriptase-1) and matriptase-2 belong to the type II transmembrane serine protease family. Matriptase-1 is known to play a role in breast cancer progression, and elevated levels of matriptase-1 correlate with poor patient outcome. The role of matriptase-2 and its cellular function in cancer is unknown. This study aimed to provide new insights into the significance of matriptase-2 in cancer.

**Experimental Design:** Matriptase-2 expression levels were assessed in a cohort of human breast cancer specimens (normal, n = 34; cancer, n = 95), in association with patient clinical variables, using both quantitative and qualitative analysis of the matriptase-2 transcript along with immunohistochemical techniques. Matriptase-2 was also experimentally overexpressed in the MDA-MB-231 human breast cancer cell line. The effects of matriptase-2 overexpression were examined through a series of *in vitro* and *in vivo* studies.

**Results:** Here, we show that reduced matriptase-2 levels in breast cancer tissues correlate with an overall poor prognosis for the breast cancer patient. This study also reveals that matriptase-2 overexpression in breast cancer cells significantly suppressed tumorigenesis in CD1 athymic mice (*P* = 0.000003). Furthermore, we report that matriptase-2 overexpression dramatically reduced the invasive (*P* = 0.0001) and migratory properties (*P* = 0.01) of the breast cancer cells.

**Conclusions:** Matriptase-2 suppresses breast tumor development *in vivo*, displays prognostic value for breast cancer patients, inhibits both breast cancer cell invasion and motility *in vitro*, and may play a contrasting role to matriptase-1 in breast cancer.

Proteases, including metalloproteinases, are key to the destruction of the matrix barriers during the metastatic spread of cancer cells. In recent years, a new class of serine proteases, termed type II transmembrane serine proteases (TTSP), has emerged as another group of enzymes associated with tumor metastasis. TTSPs are characterized by a short NH2-terminal cytoplasmic tail, a membrane-spanning region, potential ligand binding domains, and a COOH-terminal serine protease domain (1). These proteolytic enzymes are ideally positioned to interact with other cell surface and soluble proteins and extracellular matrix components and have also been implicated in tumor progression. Members of the TTSP family include enteropeptidase/enterokinase, matriptase-1/membrane-type serine protease-1/TADG-15, hepsin/TMPRSS1, TMPRSS3/TADG-12, corin, and DESC1 (2–9). Matriptase-2 (also known as TMPRSS6) is a recently identified membrane-bound enzyme that belongs to the TTSP family (10).

Studies suggest that TTSP expression is widely dysregulated during tumor growth and progression (11). Presently, the TTSP generating the most interest is matriptase-1. Matriptase-1 was originally identified in breast cancer cells (3) and seems to play an important role in tumor invasion and metastasis. This protease possesses the ability to activate growth and angiogenic factors, such as hepatocyte growth factor, urokinase-type plasminogen activator, and protease-activated receptor 2, and is also involved in extracellular matrix degradation (12, 13). Matriptase-1 has been generating attention in cancer-based studies as it is highly expressed in a variety of epithelial-derived cancers, including breast, colorectal, prostate, cervical, and ovarian (4, 14–18), and elevated levels are associated with poor prognosis in breast cancer patients (15). Inhibition of matriptase-1 led to suppression of both prostate and ovarian primary tumor growth and metastasis in murine models (3, 4, 19, 20), whereas overexpression of matriptase-1 was found to initiate multistage squamous cell carcinogenesis and enhance epidermal
tumor formation in transgenic mice (21). However, there is very little information available on matriptase-2, one of the newest members of the TTSP family. Matriptase-2 is closely related to matriptase-1 (35% homology); however, there are also distinct structural and enzymatic differences between the two proteases (10). Matriptase-1 is located at 11q25, whereas the gene encoding matriptase-2 maps to chromosome 22q13.1 (10). To date, the biological and biochemical functions of matriptase-2 are unknown, and it is unclear whether matriptase-2 plays any part in the neoplastic process. Our report provides new insights into the biological functions of matriptase-2 and its role in breast cancer.

In the present study, we assessed the functions of matriptase-2 through a series of in vivo and in vitro tests and further examined matriptase-2 expression in a cohort of human breast cancer patient specimens (normal, n = 34; cancer, n = 95) in association with patient clinical variables. Our findings strongly suggest that matriptase-2 plays a contrasting role to matriptase-1 in breast cancer. These studies are the first to show that matriptase-2 inhibits breast tumor growth in vivo, suppresses breast cancer cell motility and invasion in vitro, and may offer prognostic value in breast cancer patients.

Materials and Methods

Cell lines and culture. All cell lines used in this study were obtained from the American Type Culture Collection. This study used human breast cancer cells (MDA-MB-157, MDA-MB-231, MDA-MB-435, MDA-MB-436, MDA-MB-453, MCF7, BT474, BT549, and ZR-75-1), human prostate cancer cells (DU-145, PC-3, and CA-HPV-10), human colorectal cancer cells (HRT-18 and HT-115), human melanoma cells (A-549), human hepatocellular carcinoma cells (PLC-PRF-5), human fibroblast cells (MRC-5 and BR.3.G), human epithelial cell line (EJ-138 and T-24), human melanoma cells (G-361), human lung carcinoma cells (A-549), human hepatocellular carcinoma cells (PLC-PRF-5), human fibroblast cells (MRC-5 and 1BR.3.G), human epithelial cell line (ECV-304), and human endothelial cell line (HECV).

Cell lines were cultured with DMEM supplemented with 10% FCS, penicillin, and streptomycin (Life Technologies).

Human breast specimens. A total of 129 breast samples was obtained from breast cancer patients (34 were background normal breast tissue and 95 were breast cancer tissue). These tissues were collected immediately after mastectomy and snap frozen in liquid nitrogen, with approval of the local ethical committee. Background normal mammary tissues were removed from the same patients. The pathologist verified normal background and cancer specimens, and it was confirmed that the background samples were free from tumor deposits. The median follow-up for the cohort was 6 years (May 2001).

For patient clinical data, see Table 1. Nominal tumor heterogeneity within the tumors was taken into account by normalizing matriptase-2 against cytokeratin 19 (all primers used during quantitative PCR are described in Table 2).

Western blot analysis of matriptase-2 expression in human cancer cell lines. Matriptase-2 protein expression was assessed in a variety of human cancer cell line lysates through standard Western blot analysis. This recently developed matriptase-2 antibody is currently the only one commercially available (Triple Point Biologics). Protein expression was assessed using Uvitech analysis software. Matriptase-1 (Triple Point Biologics) and β-actin (Santa Cruz Biotechnology) antibodies were also employed in this study. Matriptase-1 (Triple Point Biologics) and β-actin (Santa Cruz Biotechnology) antibodies were also used in this study.

Immunohistochemical staining of breast specimens. Frozen sections of breast tumor (n = 32) and background tissue (n = 32) were cut at a thickness of 6 μm using a cryostat. The sections were mounted on super frost plus microscope slides, air dried, and then fixed in a mixture of 50% acetone and 50% methanol. The sections were then placed in “Optimax” wash buffer for 5 to 10 min to rehydrate. Sections were incubated for 20 min in a 0.6% bovine serum albumin blocking solution and probed with the matriptase-2 antibody (Triple Point Biologics) and also without primary antibody to act as a negative control. Following extensive washings, sections were incubated for 30 min in the secondary biotinylated antibody (MultiLink Swine anti-goat/mouse/rabbit immunoglobulin; Dako, Inc.). Following washings, the avidin-biotin complex (Vector Laboratories) was then applied to the sections. Following extensive washing steps, Diaminobenzidine chromogen (Vector Laboratories) was then added to the sections and incubated in the dark for 5 min. Sections were then counterstained in Gill’s hematoxylin and dehydrated in ascending grades of methanol before clearing in xylene and mounting under a coverslip. Staining was independently assessed by the authors.

Generation of a matriptase-2–expressing breast cancer cell line. The full sequence of matriptase-2 was amplified using the standard PCR procedure described above and the primer pairs described in Table 2.

| Table 1. Breast cancer patient clinical data details |
| --- | --- |
| Clinical data | Sample no. |
| Tissue sample | Background 34 |
| Tumor | 95 |
| NPI | 1 (<3.4) 53 |
| 2 (3.4-5.4) 32 |
| 3 (>5.4) 10 |
| Tumor grade | 18 |
| 2 | 32 |
| 3 | 45 |
| TNM staging | 52 |
| I | 34 |
| II | 7 |
| III | 2 |
This matriptase-2 sequence was then cloned into pEF6/V5-His-TOPO vector (Invitrogen) and then electroporated into the MDA-MB-231 breast cancer cell line with the aim of inducing the expression of matriptase-2 into a cell line that does not normally express it [note: multiple clones were used and assessed, all details of these procedures have been adapted from reports described previously (24, 25)]. The MDA-MB-231 cells expressing matriptase-2 were called MDA-Mat-2 EXP, whereas the control group of cells contained the same plasmid vector (minus the matriptase-2 sequence) and was termed MDA-pEF-Control.

**Tumor growth in an athymic nude mouse model.** Female athymic nude mice (4-8-weeks old; CD1; Charles River Laboratories) were s.c. injected with the MDA-MB-231 breast cancer cells (1 × 10⁶) in Matrigel (2.5 mg/mL). MDA-MB-231 breast cancer cells had been genetically modified to express matriptase-2 (MDA-Mat-2 EXP) or contained the empty expression vector to act as the control group (MDA-pEF-Control). MDA-Mat-2 EXP cells were grown in the absence of the selective antibiotic for the 28-day period. Mice were weighed twice weekly in accordance with Home Office regulations and measured once weekly to verify and measure tumor development. Tumor size was measured using digital calipers and calculated as mm³ = 0.512 × width² × length. These experimental procedures were done over a 4-week period (experimental end point), and mice were kept in sterilized, filtered cages in 12-h dark/12-h light standardized environmental conditions approved by the local ethical committee.

**Wounding/migration assay.** The migratory properties of MDA-MB-231 breast cancer cells were assessed to determine the effect of the forced expression of matriptase-2 in the invasive breast cancer cells. This technique to measure cell motility has been described in a previous study (24). The cells were seeded at a density of 50,000 per well into a 24-well plate and allowed to reach confluence. The layer of cells was then scraped with a fine gauge needle to create a wound of ~200 μm. The movement of cells to close the wound was recorded and analyzed as described previously using a time-lapse video system (24). After the addition of a treatment, the motile qualities of the cells were monitored and recorded on video for 100 min. Wound closure/cell migration was evaluated with motion analysis and line morphometry software (Optimus 6). Results were exported to a spreadsheet (Excel) for further evaluation and interpretation.

**Tumor cell invasion assay.** We quantified the invasive nature of the matriptase-2–modified cancer cells using the standard invasion assay procedure as described previously (26). Transwell chambers, equipped with a 6.5-mm-diameter polycarbonate filter insert (8-μm pore size; Becton Dickinson Labware), were precoated with 50 μg/insert of solubilized tissue basement membrane, Matrigel (Collaborative Research Products). MDA-pEF-Control and MDA-Mat-2 EXP breast cancer cells were seeded at a density of 15,000 per insert and allowed to invade for 3 days. Following incubation, cells that had invaded through the basement membrane were fixed (4% formaldehyde) and then stained with crystal violet. For analysis, the cells were counted in 10 fields per insert (×40 magnification) to determine the mean number of invaded cancer cells.

**Statistical analysis.** The results were assessed using nonpaired (two sided) Student’s t test and one-way ANOVA test. Matriptase-2 transcript values obtained in the study are given as mean copy number ± SD. A P value of <0.05 was defined as statistically significant.

### Results

**Matriptase mRNA expression in cell lines and human breast tissues.** A variety of 24 human normal and cancer cell lines were examined for the presence of matriptase-1 or matriptase-2 through reverse transcription-PCR (RT-PCR; see Fig. 1A). Matriptase-1 was strongly expressed in all the prostate (PC-3, DU-145, and CA-HPV-10) and colon (HT-115 and HRT-18) cancer cell lines examined and was also strongly expressed in some of the breast cancer cell lines (BT474, MCF7, and ZR-75-1) and the lung cancer cell line (A-549). The presence of matriptase-2 transcripts was only observed in 4 of the 24 cell lines examined. Matriptase-2 was strongly expressed in the liver cancer cell line (PLC-PRF-5) and was also present in the three

### Table 2. Primer sequences

<table>
<thead>
<tr>
<th>Molecule/accession no.</th>
<th>Sense primers (5’-3’)</th>
<th>Antisense primers (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin/NM_001101</td>
<td>ATGATATC GCGCTGCCTGTC</td>
<td>CGCTCGGTGAGGATCTTCA</td>
</tr>
<tr>
<td>Matriptase-1/AF133086</td>
<td>CTTTGAGGCCACCTTCTT</td>
<td>GGTAGGCGCTGGGTA</td>
</tr>
<tr>
<td>Matriptase-2/NM_153609</td>
<td>TGAAGACATAGCTGCAATG</td>
<td>GTAGTACCTGGAAGTACCG</td>
</tr>
<tr>
<td>Matriptase-2 (Q-PCR)</td>
<td>CCGAGTGACTAAGGGAGAC</td>
<td>ACTGAAACCTGACGTAACATCTT</td>
</tr>
<tr>
<td>Cytokeratin 13 (Q-PCR)</td>
<td>CAGGTGAGGTACCATGAC</td>
<td>AGCTCACATTTGCGATGTCCT</td>
</tr>
<tr>
<td>Matriptase-2 (full sequence)</td>
<td>ATGCCCGTGGCGCCAGGG</td>
<td>GGTGACGACCTGTGGATCA</td>
</tr>
</tbody>
</table>

Abbreviation: Q-PCR, quantitative PCR.

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**Fig. 1.** A, RT-PCR expression of matriptase-1 and matriptase-2 in a variety of 24 human cell lines. The expression of matriptase-2 mRNA was assessed (and reassessed) in a panel of human cell lines. Matriptase-2 was only expressed in a few breast cancer cells and in the liver cancer cell line. B, matriptase-2 protein expression in human cell lines. Western blotting was used to confirm the results observed through RT-PCR. The matriptase-2 protein was found to be expressed in the MCF7 and ZR-751 breast cancer cell lines, which had a low invasive nature. In addition, matriptase-2 was also expressed by the PLC-PRF-5 liver cancer cell line and the HRT-18 colorectal cancer cell line.
breast cancer cell lines that strongly expressed matriptase-1 (BT474, MCF7, and ZR-75-1). In addition, matriptase-2 was not expressed in human endothelial cells (HECV) or stromal fibroblasts (MRC-5 and IBR.3.G).

**Matriptase-2 protein expression in human cell lines.** Matriptase-2 protein expression was assessed in a variety of normal and cancer cell lines (see Fig. 1B). A specific band at 90 kDa was identified, which corresponded to the mature matriptase-2 protein. Matriptase-2 was only found to be expressed (to varying degrees) within the breast cancer cell lines of a general low/noninvasive nature (MCF7 and ZR-751) as described in previous studies (26). Matriptase-2 was absent in the highly invasive MDA-MB-231 breast cancer cell line. The matriptase-2 protein was also found to be abundantly produced by the liver cancer cell line (PLC-PRF-5) as expected due to the fact that the liver is the main source of matriptase-2 in the body. Matriptase-2 was not detected in the stromal fibroblasts (MRC-5), the endothelial cells (HECV), or the prostate cancer cells (DU-145 and CA-HPV-10). Therefore, our Western blot analysis confirmed the data we report with the initial RT-PCR studies.

**Immunohistochemical staining of human breast specimens.** Matriptase-2 immunostaining was observed in the human breast tissue sections (n = 32 pairs). Matriptase-2 was expressed in the normal mammary tissue (Fig. 2, left), and its distribution was mainly confined to the intensely stained epithelial cells. It was noted that stromal cells were negative for matriptase-2 immunoreactivity. We report that matriptase-2 protein levels were dramatically reduced/absent in the breast cancer cells from the tumor tissue specimens (Fig. 2, right). Stromal cells in tumor tissues were also negative for matriptase-2 expression. Finally, no obvious endothelial cell staining was observed in either normal or tumor tissues.

**Real-time quantitative PCR analysis of matriptase-2 expression in human breast tissues.** We quantified the mean transcript expression in the breast specimens (tumor, n = 95; background, n = 34) using real-time quantitative PCR (all values are displayed as mean matriptase-2 transcript copies/μL of RNA from 50 ng total RNA). We report that comparison of normal breast tissue against breast cancer tissue showed significantly elevated levels (P = 0.024) in the tumor specimens (see Fig. 3A). Matriptase-2 mRNA was observed at lower levels in
background tissue (18.37 ± 9.9 copies/μL) in relation to the breast cancer tissues (263 ± 107 copies/μL).

Matriptase-2 expression in relation to patient prognosis. The quantity of matriptase-2 expressed by each patient sample was assessed by quantitative PCR. We analyzed matriptase-2 levels against survival status of the breast cancer patients as determined by the Nottingham Prognostic Index (NPI; see Fig. 3B). The NPI 1 group (NPI score <3.4; n = 53) represented patients with a good prognosis, the NPI 2 group (NPI score 3.4-5.4; n = 32) represented patients with a moderate prognosis, whereas the NPI 3 patients (NPI score >5.4; n = 10) had a poor prognosis.

A one-way ANOVA-based analysis of the quantity of matriptase-2 transcript revealed a highly significant correlation between the levels of matriptase-2 and NPI staging (P = 0.001). Subanalysis of individual groups has shown that patients with a moderate and poor prognosis (NPI 2 and NPI 3, respectively) had significantly reduced levels of matriptase-2 when compared with the patients of the good prognosis group (NPI 1). In the NPI 2 (27.7 ± 16.1) and NPI 3 groups (5.61 ± 2.04), P = 0.027 and P = 0.020, respectively, compared with the NPI 1 group (461 ± 191).

Breast tumor grade and matriptase-2 expression. Matriptase-2 levels were assessed in relation to breast tumor grade (grade 1, n = 18; grade 2, n = 32; grade 3, n = 45). The poorly differentiated grade 2 (156.3 ± 89.6 copies/μL) and grade 3 (131 ± 119 copies/μL) tumors had significantly reduced levels of matriptase-2 compared with well-differentiated grade 1 tumors (726 ± 460 copies/μL) through one-way ANOVA-based analysis (P < 0.001), although these values did not reach statistical significance through individual group subanalysis (grade 1 versus grade 2, P = 0.23; grade 1 versus grade 3, P = 0.22; see Fig. 3C).

The relationship between the levels of the matriptase-2 transcripts and histologic types was also analyzed (see Fig. 3D). There was almost a significant difference between matriptase-2 expression in ductal tumors (294 ± 133; n = 78) compared with lobular tumors (58.3 ± 56; n = 8; P = 0.065). With a larger sample number of lobular tumors, this value may reach significance. Sample number of other tumor types was too small for a convincing analysis (mucinous, n = 0; medullary, n = 2; tubular, n = 2).

Tumor-node-metastasis classification of patients. We also analyzed matriptase-2 expression in relation with patient outlook through tumor-node-metastasis (TNM) grouping

Fig. 3. A to D, quantitative PCR analysis of matriptase-2 expression in human breast cancer tissues. A, matriptase-2 expression is significantly elevated in human breast cancer tissues compared with normal background breast tissue. B, NPI of patient prognosis. Patients with a poor prognosis have significantly reduced levels of matriptase-2 expressed in their tumors compared with the tumors of patients with a good prognosis. C, tumor grading revealed that the levels of matriptase-2 seemed to inversely correlate with poor differentiation. The lowest levels of matriptase-2 were observed in the grade 3 tumors. The patients with well-differentiated grade 1 tumors had significantly higher levels of matriptase-2 compared with the patients in the groups with poorer differentiated tumors. D, comparison between matriptase-2 transcript expression and tumor histologic subtype. Matriptase-2 expression was high in ductal tumors compared with lobular tumors, although these results did not reach significance with this small sample number. Sample number of other tumor types was too small for a convincing analysis. E, comparison of matriptase-2 expression between the TNM classification groups. These results show that matriptase-2 expression is inversely correlated with prognosis. Patients with an overall poor outcome (TNM 3) expressed significantly lower levels of matriptase-2 compared with those with good outcome (TNM 1).
(TNM 1, \( n = 52 \); TNM 2, \( n = 34 \); TNM 3, \( n = 7 \); see Fig. 3E). TNM group 4 was excluded from the analysis due to low sample number in the group (\( n = 2 \)). Again, we show that matriptase-2 levels are dramatically reduced in patients with an overall poor outcome. Patient TNM grouping revealed that TNM 2 (63.3 ± 38.7 copies/\( \mu L \)) and TNM 3 (9.46 ± 9.23 copies/\( \mu L \)) patients had decreased levels of matriptase-2 (\( P = 0.058 \) and 0.028, respectively) compared with the TNM 1 group (439 ± 190 copies/\( \mu L \)). TNM group 3 identified the breast cancer patients with an overall poor outcome; therefore, matriptase-2 expression was inversely correlated with tumor spread.

**Experimental overexpression of matriptase-2 within human breast cancer cells.** We confirmed that the pEF6/V5-TOPO vector expression system had successfully induced the expression of matriptase-2 within the MDA-MB-231 breast cancer cell line (see Fig. 4A and B). This highly invasive breast cancer cell line was a good candidate for matriptase-2 expression, as we show that the wild-type MDA-MB-231 cell line did not express the matriptase-2 gene. RT-PCR was used to show that matriptase-2 was absent in the MDA-MB-231 wild-type (MDA-wild-type) and empty vector control (MDA-pEF-Control) breast cancer cells and present within the cells forced to express it (MDA-Mat-2 EXP; see Fig. 4A). Real-time quantitative PCR was also done to confirm that matriptase-2 was expressed within the MDA-Mat-2 EXP cells. These experiments were done in triplicate. The quantity of matriptase-2 transcripts in wild-type MDA-MB-231 cells was found to be 183 ± 78 copies/\( \mu L \), MDA-pEF-Control expressed 578 ± 106 copies/\( \mu L \), whereas the MDA-Mat-2 EXP cells expressed matriptase-2 at the significantly (\( P < 0.001 \)) elevated level of 6,679,654 ± 3,845,515 copies/\( \mu L \) (see Fig. 4B). To further confirm the presence of matriptase-2 in the transfected cells, matriptase-1 and matriptase-2 protein levels were assessed within the wild-type, empty vector–transfected, matriptase-1–transfected, and matriptase-2–transfected breast cancer cells. We also used the PLC-PRF-5 liver cancer cell line as a positive control (see Fig. 4C). Matriptase-2 protein levels were found to be high within the MDA-Mat-2 EXP breast cancer cells. These levels were then quantified using the latest Uvitech analysis software (see Fig. 4D). The properties of these new matriptase-2–expressing breast cancer cells were ready for analysis through a series of in vitro and in vivo studies.

**Matriptase-2 suppresses tumor cell invasion.** The MDA-MB-231 cell line is a highly invasive cell line that does not normally express matriptase-2 (see Fig. 1). On forced expression of matriptase-2, there was a change in the aggressive nature of these breast cancer cells (see Fig. 5A). The forced expression of matriptase-2 in this cell line resulted in a dramatic reduction in the degree of invasion (\( P < 0.0001 \) versus control). This is in contrast to the role of matriptase-1 in tumor spread, as matriptase-1 is recognized as proinvasive factor that...
dramatically enhances human cancer cell invasion. Importantly, our data suggest that the presence of matriptase-2 may suppress limit the invasive nature of cancer cells.

Matriptase-2 reduces tumor cell motility/wound closure. The presence of matriptase-2 also significantly reduced the motile nature of breast cancer cell migration. This wound closure migration assay assessed the influence of matriptase-2 on cancer cell motility. The motile properties of the control group MDA-MB-231 breast cancer cells (MDA-pEF-Control) and MDA-MB-231 cells forced to express matriptase-2 (MDA-Mat-2 EXP) were analyzed over a 100-min period. We reveal that the presence of matriptase-2 significantly suppressed (P = 0.01) the migratory nature of these breast cancer cells.

Discussion
The most deadly aspect of cancer is its ability to metastasize. The metastatic cascade consists of a series of sequential, interrelated steps that are not as yet completely understood. An emerging class of serine proteases, known as TTSPs, has been generating interest in recent years for their roles in the spread of cancer. However, for the majority of TTSPs, the physiologic role and mechanism of action are still unclear. One of the most studied members of this family is matriptase/MT-SP1 (matriptase-1). Matriptase-1 is expressed in a wide variety of human epithelial cancers and enhances breast cancer invasion, and elevated levels are associated with poor patient outcome (4, 13, 16, 17, 21, 27). Matriptase-2 was recently identified as a member of the TTSP family (10), and to date, there is very little information available on matriptase-2. We sought to examine the importance of matriptase-2 in breast cancer.

Our initial studies examined matriptase-1 and matriptase-2 expression within a variety of human normal and cancer cell lines and breast tissue from breast cancer patients. We report that matriptase-2 was responsible for suppression of tumor growth within the nude mice.

Matriptase-2 expression suppressed tumor development in vivo. Athymic nude mice were s.c. injected with MDA-pEF-Control or MDA-Mat-2 EXP breast cancer cells and tumors were allowed to develop over a 4-week period. Our study showed that tumor growth was retarded in mice injected with MDA-Mat-2 EXP breast cancer cells when compared against the control group (P = 0.000003). We report that the presence of matriptase-2 was responsible for the suppression of tumor growth within the mice.
that matriptase-2 displays a limited distribution pattern, as matriptase-2 was only expressed in small number of breast cancer cell lines and in a liver cancer cell line. Consistent with our findings, previous reports show that the majority of TTSPs generally display highly restricted distribution, such as with corin and enteropeptidase, which are predominantly expressed by cardiac myocytes and intestinal enterocytes (2, 8). Moreover, our findings also suggest that matriptase-1 and matriptase-2 do not share the same expression patterns. Matriptase-1 was strongly expressed by the prostate and colon cancer cell lines, whereas matriptase-2 expression was lacking within these cell lines. Therefore, matriptase-2 may show tissue-specific functions in liver and breast tissues. Importantly, our immunohistochemical studies of normal mammary tissue show that matriptase-2 was strongly expressed by the normal breast epithelia from breast cancer patients. We also report that matriptase-2 immunoreactivity was low/absent within stromal and endothelial cells of mammary tissue. Taken together with our cell line data, our findings strongly suggest that matriptase-2 is primarily of epithelial origin in tissues.

Matriptase-1 is reported to correlate with breast cancer patient prognosis (15). Therefore, our study also assessed matriptase-2 expression in relation to breast cancer patient clinical data in a cohort of human breast cancer specimens through quantitative PCR and immunohistochemical analysis. We report that aberrant matriptase levels are expressed within the breast tissues. Immunohistochemical staining of the patient tissues strongly suggests that matriptase-2 levels were reduced within the tumor tissue of patients. We reveal high levels of matriptase-2 immunoreactivity within the normal breast epithelia compared with the low levels observed within breast cancer cells.

It seems that matriptase-2 may represent a survival factor in human breast cancer, as patients with a good prognosis expressed elevated levels of matriptase-2. Matriptase-2 mRNA levels were dramatically reduced in patients with a moderate to poor prognosis as defined by the NPI. This trend was also observed through the TNM classification and tumor grading of breast cancer patients. Indeed, patients classed as having a good outlook expressed high levels of matriptase-2; however, levels were significantly reduced as the outlook for the patient worsened. Taken together, these results suggest that matriptase-2 expression is inversely correlated to breast cancer patient prognosis. Interestingly, the overall matriptase-2 transcript level in tumors from patients with good prognosis was dramatically elevated when compared with normal breast tissues. This suggests that matriptase-2 may only exercise its role at specific fragments of the cancer progression pathway, such as in the early stages of tumor progression, as these elevated levels of matriptase-2 in the early stages of progression may aid tumor development into the later stages. In summary of our quantitative PCR-based studies, our findings suggest that matriptase-2 acts as a prognostic indicator for breast cancer patients, as we show that patients expressing high/ elevated levels of matriptase-2 have a favorable prognosis in contrast to those patients with low/reduced levels of matriptase-2 and a poor prognosis. Another TTSP, known as hepsin, is reported to share a similar expression pattern to matriptase-2 and, similarly to matriptase-1, has recently been implicated in the biological activation of hepatocyte growth factor (1, 28). Matriptase-2 seems to show similar properties in breast cancer as hepsin reveals in prostate cancer. Hepsin expression is elevated in prostate and ovarian carcinomas and correlates inversely with measures of patient prognosis (29–31). Interestingly, hepsin also possesses ability to inhibit prostate cancer cell growth and invasion (32), also a feature of matriptase-2 action that we report in the current study.

We sought to elucidate the functional importance of matriptase-2 in breast cancer; therefore, our studies have led to the generation of a matriptase-2–expressing human breast cancer cell line (MDA-MB-231). These studies used Transwell invasion assays and cell motion analysis software to provide a reliable measure of in vitro cellular invasiveness and motility. Our approaches have proved that the forced expression of matriptase-2 within these breast cancer cells greatly reduced the aggressive invasive properties of these tumor cells and also significantly suppressed the migratory nature of these breast cancer cells. In the next stage of our research, we examined whether the presence of matriptase-2 could inhibit breast tumor development in vivo. Athymic mice were s.c. injected with matriptase-2–expressing breast cancer cells, and tumors were allowed to develop over a 28-day period. The overexpression of matriptase-2 dramatically suppressed breast tumor growth within the mice. These results show that the presence of matriptase-2 within the breast cancer cells was responsible for suppression of tumor growth within the mice. Therefore, our findings suggest that matriptase-2 may play a role in limiting the motile and invasive nature of breast cancer cells and development of breast tumors.

In view of our data, we suggest that matriptase-1 and matriptase-2 may have contrasting roles in breast cancer. Matriptase-1 is reported to enhance cancer migration and invasion, as this protease activates such prometastatic factors as urokinase-type plasminogen activator, protease-activated receptor 2, and hepatocyte growth factor (12, 13); however, our findings show that the presence of matriptase-2 significantly suppresses breast cancer invasion and migration. Furthermore, a recent study reports that matriptase-1 overexpression was found to initiate multistage squamous cell carcinogenesis and enhance epidermal tumor formation in vivo (21), whereas our results suggest that matriptase-2 actually inhibits breast tumor formation in vivo. Moreover, our findings show that matriptase-2 plays an opposing role to matriptase-1 regarding patient prognosis. Matriptase-1 levels are elevated in tumor tissues and high levels are associated with poor patient prognosis (14–18), whereas in the current study we show that matriptase-2 levels are reduced in tumor tissues and a high level correlates to a favorable prognosis.

To date, a physiologic function or substrate has been defined for very few of the TTSPs. It is known that matriptase-1 activates hepatocyte growth factor, protease-activated receptor 2, and urokinase-type plasminogen activator, yet whether matriptase-2 is able to interact with these factors remains to be determined. Most membrane-anchored proteases can be shed, and so it is likely that matriptase-2 also functions as a secreted enzyme in addition to its cell autonomous behavior on the tumor. It is crucial to identify the mechanisms and functional consequences of matriptase-2 action before we can fully appreciate the direct implications of matriptase-2 expression in breast cancer. A new study has suggested that the matriptase-2 gene is a candidate for a breast cancer risk factor in the Eastern Finnish population and reveals that there is a possibility of matriptase-2...
involvement in cancer progression and suggests that a more specific study into the association of matriptase-2 and breast cancer is necessary (33). Thus, we believe our results will shed light onto the role of matriptase-2 in cancer. In an ongoing research program of our group, we have recently obtained tentative evidence that suggests that the involvement of matriptase-2, in the control of cancer invasion and growth, acts via mechanisms beyond the traditional enzymatic action and that regulation of the formation of focal adhesion complexes in cancer cells is pivotal to matriptase-2 action. This lead is currently under intensive investigation.

In conclusion, our study indicates that matriptase-2 may play a contrasting role to matriptase-1 in breast cancer. For the first time, we reveal that matriptase-2 suppresses breast tumor development in vivo, displays prognostic value for breast cancer patients, and inhibits breast cancer cell invasion and motility in vitro.

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References

Matriptase-2 Inhibits Breast Tumor Growth and Invasion and Correlates with Favorable Prognosis for Breast Cancer Patients

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