Expression of the Serine Protease Matriptase and Its Inhibitor HAI-1 in Epithelial Ovarian Cancer: Correlation with Clinical Outcome and Tumor Clinicopathological Parameters[1]

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ABSTRACT

***Purpose*: Matriptase is a type II transmembrane serine protease expressed by cells of surface epithelial origin, including epithelial ovarian tumor cells. Matriptase cleaves and activates proteins implicated in the progression of ovarian cancer and represents a potential prognostic and therapeutic target. The aim of this study was to examine the expression of matriptase, and its inhibitor, hepatocyte growth factor activator inhibitor-1 (HAI-1), in epithelial ovarian cancer and to assign clinicopathological correlations.**

***Experimental Design*: We have determined by immunohistochemistry the expression of matriptase and HAI-1 in 54 epithelial ovarian cancers. Statistical analyses of immunohistochemistry expression data with clinical outcome and clinicopathological parameters were then performed.**

***Results*: Of 54 tumors tested, 39 (72%) and 11 (20%) were positive for matriptase and for HAI-1, respectively. All HAI-1-positive tumors were also matriptase positive. Analysis of clinicopathological parameters demonstrated a loss of matriptase associated with stage III/IV tumors as compared with stage I/II tumors ($P = 0.030$). There was also a loss of HAI-1 expression associated with stage III/IV tumors ($P = 0.039$). Of 34 stage I/II tumors, 28 (82%) stained positive for matriptase, and 10 (29%) stained positive for HAI-1; 10 (29%) tumors showed coexpression. Of 20 stage III/IV tumors, however, 11 stained positive for matriptase (55%), only 1 of which coexpressed HAI-1 ($P = 0.039$).**

***Conclusions*: Advanced-stage ovarian tumors that express matriptase are more likely to do so in the absence of its inhibitor, HAI-1, indicating that an imbalance in the matriptase:HAI-1 ratio could be important in the development of advanced disease. Such an imbalance could promote the proteolytic activity of matriptase and, consequently, a more invasive phenotype.**

INTRODUCTION

Ovarian cancer is the leading cause of death from gynecological malignancy. Unfortunately, the majority of ovarian cancer patients have advanced-stage disease with locoregional dissemination within the peritoneal cavity at the time of clinical presentation. Patients with early-stage disease (stage I and stage II tumors) have a much better prognosis than those patients with tumors that are detected at a later stage (stage III and stage IV tumors). Although generally a chemosensitive disease, especially to platinum-based compounds, resistance to these agents develops in most cases, contributing significantly to the high mortality rate. Better therapies are clearly needed, and the search for potential therapeutic targets, as well as predictive and prognostic markers, is of great importance.

Matriptase was first isolated as a type II transmembrane serine protease expressed in human breast milk and in breast carcinoma (1–3) and subsequently cloned (4). Other groups have also independently identified this serine protease. The gene sequence was initially identified as *ST14/SNC19* through subtractive hybridization gene expression difference analysis (5). Reverse biochemical studies led to the isolation from the prostate cancer cell line PC-3 of the membrane-type serine protease, MT-SP1, which is identical to matriptase (6). Matriptase has also been cloned as *TADG-15*, a member of a series of tumor-associated, differentially expressed genes (7). Its mouse homologue, epithin, has been cloned from thymic stroma (8). Matriptase is expressed in a variety of carcinomas, including ovarian carcinomas, and is overexpressed in ovarian tumors relative to normal OSE[3] (7, 9).

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[3] The abbreviations used are: OSE, ovarian surface epithelium; uPA, urokinase plasminogen activator; HGF, hepatocyte growth factor; HGFA, HGF activator; LPA, lysophosphatidic acid; S-1-P, sphingosine-1-phosphate; HAI-1, hepatocyte growth factor activator inhibitor-1; FIGO, Fédération Internationale des Gynaecologistes et Obstetristes;

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Latent uPA and pro-HGF are substrates for matriptase (10, 11). Both substrates participate in neoplastic progression, most notably in the plasmin-mediated remodeling of the extracellular matrix surrounding tumors (uPA), the activation of latent growth factors such as HGF (uPA), and in the stimulation of cancer cell growth and motility (uPA and HGF). The binding of uPA, itself a serine protease, to its receptor increases the proliferation of human ovarian cancer cells *in vitro* (12). Antisense inhibition of uPA significantly reduces the i.p. spread of ovarian cancer when grown as xenografts in mice (13). Both uPA and its inhibitor PAI-1 predict the survival of patients with advanced ovarian cancer and are associated with the malignant progression of the disease (14–16). HGF stimulates the *in vitro* motility, chemotaxis, and proliferation of ovarian carcinoma cells (17). HGF is found in ascitic fluid of both benign and malignant ovarian tumors and stimulates the migration of ovarian carcinoma cells (18).

The lipid metabolites LPA and S-1-P induce the activation of matriptase in immortalized breast epithelial cells.[4] In ovarian carcinoma cells, LPA increases cellular proliferation and cell survival, the synthesis of proteins such as vascular endothelial growth factor, interleukin 8, and uPA, and promotes resistance to cytotoxic agents such as cisplatin (19). Ascites from ovarian cancer patients contain LPA at concentrations that activate ovarian cancer cells and are significantly higher than that found in ascites from nonmalignant conditions (20, 21). LPA and S-1-P may therefore promote the progression of ovarian carcinoma through the activation of matriptase.

The activity of matriptase is modulated by an inhibitor of the enzyme, termed HAI-1. HAI-1 also binds and inhibits the activity of a serine protease termed HGFA, also capable of cleaving and activating latent HGF (22, 23), in addition to binding and inhibiting matriptase (3). HAI-1 is found predominantly in the columnar epithelium of many tissue types, including breast, stomach, lung, kidney, prostate, and uterus, and is up-regulated in injured or regenerative tissues (24). HAI-1 may suppress the growth and motility of carcinoma cells by inhibiting the generation of active uPA by matriptase, active HGF by matriptase or HGFA, or by inhibiting the activity of additional unidentified serine proteases. Recently, HAI-1 immunoreactivity in human primary colorectal carcinomas was found to be decreased significantly in cells within colon carcinomas relative to adjacent normal mucosa or adenomas (25). In contrast, the immunoreactivity of HGFA in these same tumors was comparable with that of adenomas but modestly increased relative to normal mucosa, indicating a change in the HGFA:HAI-1 ratio within these colon tumors that favors HGFA.

Taken together, these lines of evidence support a role for matriptase in the growth, invasion, and metastasis of numerous carcinomas and in particular, ovarian carcinomas. To test the hypothesis that the expression of matriptase, HAI-1, or the matriptase:HAI-1 ratio may correlate with the clinical stage, histological grade, histological type, or clinical outcome of patients with ovarian cancer, we have stained by IHC 54 epithelial ovarian tumors for matriptase and for HAI-1. We show significant associations of low matriptase and HAI-1 expression with advanced FIGO stage and of low frequency of matriptase and HAI-1 coexpression in advanced-stage tumors that favor matriptase protease activity, as compared with early-stage tumors.

PATIENTS AND METHODS

Expression of Matriptase in Normal Human Ovarian Surface Epithelium. First-strand cDNA was prepared using the 1st Strand cDNA Synthesis kit (Roche, Lewes, United Kingdom) from 1 μg of DNaseI-treated total RNA extracted from a culture of normal human ovarian surface epithelial cells (Prof. S. Hillier, University of Edinburgh, Edinburgh, United Kingdom). Aliquots of 2 μl of first-strand cDNA were used in 20-μl PCR reactions with primers specific for matriptase [5′-GAATACCTCTCCTACGACTC-3′ (sense) and 5′-ACACTGAAGTCCACCCTGGG-3′ (antisense)] and for γ-actin [5′-ATGGCATCGTCACCAACTGG-3′ (sense) and 5′-ATGACAATGCCAGTGGTGCG-3′ (antisense)]. Matriptase RT-PCR amplification conditions used a touchdown protocol ranging from 67°C to 55°C: 94°C for 5 min; 94°C for 30 s, 67°C-58°C (decreasing 3°C/cycle) for 30 s, 72°C for 2 min at one cycle each; 94°C for 30 s, 55°C for 30 s, 72°C for 45 s for 30 cycles; and 72°C for 5 min. For γ-actin, a protocol incorporating a single annealing temperature of 55°C was used for 35 cycles. PCR products were separated on agarose gels and visualized after ethidium bromide staining.

Patients and Tissues. Primary ovarian cancer specimens were obtained from 54 patients with ovarian cancer at the time of surgery at the Western General Hospital and Edinburgh Royal Infirmary (Edinburgh, United Kingdom) from 1988 to 1993. Optimal debulking surgery was attempted in all patients. Those with residual disease received chemotherapy according to local best practice guidelines at the time of presentation. Tumor specimens were fixed in 4% formaldehyde in PBS and rinsed in PBS before embedding in paraffin. Five-μm sections of tumor were cut from each block for immunohistochemical analysis for matriptase or HAI-1. Tumor histology was examined by two specialist gynecological histopathologists to determine their types and grades. Survival was defined as the time between histological diagnosis of ovarian cancer and ovarian cancer-specific death (30 deaths). Patients who died of other causes (2 patients) or who were still alive at the cutoff date for this study (5/29/01) were treated as censored (22 patients). The median survival time for all patients was 6 years and 1 month. The median follow-up time for censored patients was 8 years and 2 months (range, 23 months to 11 years). The histology of tumors fell into six categories: serous (18 tumors; 33%); mucinous (8 tumors; 15%); endometroid (17 tumors; 31%); clear cell (9 tumors; 17%); mixed endometrioid/clear cell (1 tumor; 2%); and unclassified carcinoma (1 tumor; 2%). Tumor stage was classified according to FIGO guidelines. Stage I tumors represented 57% of the sample; stage II, 6%; stage III, 35%; and stage IV, 2%. In this cohort of samples, the demographics are such that stage I tumors are overrepresented relative to the expected distribution of ovarian cancers (57% in this sample compared with 23% in our current clinical practice). Similarly, stage III

IHC, immunohistochemistry; ISH, *in situ* hybridization; LOH, loss of heterozygosity; RT-PCR, reverse transcription-PCR.

[4] R. B. Dickson and C-Y. Lin, unpublished data.

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tumors are underrepresented (35% in our sample compared with 51% in our current clinical practice). Tumor histological grade was classified as poorly differentiated (22 tumors of 49 classified; 45%), moderately differentiated (16 of 49; 33%), or well differentiated (11 of 49; 22%) according to standard histopathological grading. Histological grade was not available for 5 patients.

Immunohistochemistry for Matriptase and HAI-1. Immunohistochemistry was performed using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) with minor modifications to the manufacturer's protocol. Briefly, 5-μm tumor sections were heated in an oven to 56°C for 1 h and then dewaxed in xylene. Slides were then rehydrated by immersion in a decreasing gradient of ethanol in water. Endogenous peroxidase activity was quenched by immersion in 1.5% H_2O_2/methanol for 20 min, followed by washed in PBS. Sections were blocked for 30 min in blocking buffer (2% rabbit serum, 0.1% BSA in PBS) before incubation with the primary antibody. Sections were incubated in the presence of the matriptase-specific monoclonal antibody clone S5 (IgG1; Ref. 9) at a concentration of 1 μg/ml or the HAI-1-specific monoclonal antibody clone M58 (IgG1; Ref. 3) at a concentration of 5 μg/ml for 1 h at room temperature. Ovarian cancer sections known to show positive staining for matriptase and HAI-1 were used as positive controls, and mouse IgG at a concentration of 5 μg/ml was used with a duplicate of these same sections as a negative control. After incubation in the primary antibody, sections were washed in PBS to remove unbound antibody and then were incubated with a biotinylated rabbit antimouse secondary antibody. After washes in PBS, the staining was completed by incubation with streptavidin-horseradish peroxidase and 3,3′-diaminobenzidine colorimetric reagents from the Vectastain kit according to the manufacturer's protocol. The colorimetric reaction for the negative control slides was developed for the same amount of time as the experimental slides and did not show any development of the color reagent. After verification of positive staining of the positive control slides, all experimental slides were scored independently for matriptase (S5) or HAI-1 (M58) staining by two specialist gynecological histopathologists.

***In Situ* Hybridization.** Probes for use in ISH were prepared by generating digoxigenin-labeled sense and antisense RNA riboprobes using the Dig-RNA labeling kit (Boehringer-Mannheim, Mannheim, Germany) according to a modified manufacturer's protocol. Briefly, a 650-bp *Bam*HI-*Sac*II fragment of the matriptase sequence corresponding to the 5′ end of the matriptase cDNA was cloned into the pBluescript SK− vector (Stratagene, La Jolla, CA). This vector was subsequently linearized with *Sac*II or *Bam*HI and used as a template for the synthesis of sense and antisense digoxigenin-labeled riboprobes, respectively, with T7 or T3 RNA polymerase (Life Technologies, Inc., Rockville, MD), according to the manufacturer's protocol, using digoxigenin-11-UTP. Synthesized probes were purified by G50 column chromatography to remove unincorporated nucleotides, including unincorporated digoxigenin-11-UTP. The concentration of the labeled riboprobes was determined spectrophotometrically. The accuracy of the concentration assignment was confirmed by analysis of the riboprobes by 1% agarose/2.2 M formaldehyde gel electrophoresis, followed by ethidium bromide staining. The equal efficiency of digoxigenin incorporation into sense and antisense probes was confirmed by dot blotting of equal amounts of probe onto Hybond-N nylon membranes (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom), followed by detection of labeled riboprobe with an alkaline phosphatase-conjugated anti-digoxigenin antibody and colorimetric substrate (data not shown). In addition, the efficiency of digoxigenin incorporation was confirmed by dot blotting equal amounts of denatured double-stranded vector containing the full-length sequence of matriptase and probing these blots with digoxigenin-labeled sense or antisense probes for matriptase. Equal signals were observed for equal amounts of sense or antisense probe used in the hybridization to membrane-bound plasmid (data not shown). For detection of matriptase mRNA in paraffin-embedded ovarian tumor sections, 20 ng of labeled sense or antisense riboprobe were used in a standard protocol provided by Boehringer-Mannheim (Mannheim, Germany). Briefly, 5-μm paraffin-embedded ovarian tumor sections were deparaffinized, rehydrated, treated with 0.2 M HCl, permeabilized with proteinase K, and postfixed with 4% paraformaldehyde before prehybridization in 50% formamide/1× SSC at 65°C and hybridization at 65°C in hybridization buffer for 12 h in a humidified chamber. After hybridization, unbound probe was removed by two washes in 2× SSC, two washes in 1× SSC, and two washes in 0.1× SSC at 42°C. Bound probe was detected by use of an alkaline phosphatase-conjugated anti-digoxigenin antibody that produces an insoluble blue precipitate in the presence of a nitrotetrazolium blue/X-phosphate color solution. Sense and antisense probes were hybridized and washed under identical conditions, and the colorimetric reactions were stopped at the same time for sense and antisense hybridized sections.

Statistical Analysis. The expression profiles of matriptase and HAI-1 were each analyzed in relation to the patient clinicopathological data using the χ^2 test or Fisher's exact test as appropriate. Survival curves were constructed by the method of Kaplan-Meier, and the impact of matriptase and HAI-1 on survival was assessed using the log-rank test and Cox proportional hazards regression. All analyses were performed with the STATA (version 6.0) software package.

RESULTS

Immunostaining for Matriptase and HAI-1. Fifty-four ovarian tumors of various histological types were stained by immunohistochemistry for either matriptase or HAI-1, using monoclonal antibodies that are specific for matriptase (S5) or HAI-1 (M58). These monoclonal antibodies have been used previously to demonstrate the expression of matriptase and HAI-1 in epithelial-derived tissues, including ovarian cancers of an epithelial origin (9). In addition, we have shown that these monoclonal antibodies successfully stain fixed, paraffin-embedded, cultured breast cancer cells that express matriptase and HAI-1 (MCF-7 breast cancer cells) but do not stain fixed, paraffin-embedded breast cancer cells that do not express these antigens (MDA-MB-231 breast cancer cells; data not shown).

The histological type, histological grade, clinical staging (classified according to FIGO guidelines), size of residual disease after initial surgery, survival status at the time of the

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Table 1 Summary of clinicopathological parameters and immunohistochemical staining patterns of 54 ovarian tumors[a]

Clinicopathological parameter	No. (%)	Positive Expression by IHC no. (%)	
		Matriptase	HAI-1
FIGO stage			
I	31 (57%)	25 (81%)	10 (32%)
II	3 (6%)	3 (100%)	0 (0%)
III	19 (35%)	11 (58%)	1 (5%)
IV	1 (2%)	0 (0%)	0 (0%)
Histological grade			
Well differentiated (grade I)	11 (20%)	10 (91%)	5 (45%)
Moderate (grade II)	16 (30%)	11 (69%)	2 (12%)
Poor (grade III)	22 (41%)	14 (64%)	3 (14%)
Unknown	5 (9%)	4 (80%)	1 (20%)
Patient status			
Death from ovarian cancer	30 (55%)	20 (67%)	5 (17%)
Death other than ovarian cancer	2 (6%)	1 (50%)	1 (50%)
Alive at time of study	22 (39%)	18 (82%)	5 (23%)
Residual tumor after surgery			
<2 cm	46 (85%)	31 (67%)	11 (24%)
2–5 cm	4 (7.5%)	4 (100%)	0 (0%)
>5 cm	4 (7.5%)	4 (100%)	0 (0%)
Tumor histology			
Serous	18 (33%)	11 (61%)	1 (5%)
Mucinous	8 (15%)	6 (75%)	3 (33%)
Endometrioid	17 (31%)	14 (82%)	6 (35%)
Clear cell	9 (17%)	6 (67%)	0 (0%)
Mixed endometrioid/clear cell	1 (2%)	1 (100%)	0 (0%)
Unclassified carcinoma	1 (2%)	1 (100%)	1 (100%)

[a] Patient and tumor clinicopathological summary profile. A summary of the clinical and pathological data relating to the panel of ovarian tumors used in this study and the scorings obtained from the immunohistochemical analysis of matriptase (S5 monoclonal antibody) and HAI-1 (M58 monoclonal antibody) expression is shown.

writing of this report, and matriptase and HAI-1 immunohistochemical staining for the tumors are presented in Table 1.

Of the 54 ovarian tumors, 39 (72%) and 11 (20%) were positive for matriptase and HAI-1, respectively. All HAI-1-positive tumors were also matriptase positive; conversely, 28 of 39 (72%) tumors that scored positive for matriptase were negative for HAI-1. Representative examples of matriptase and HAI-1 tumor staining from the various histological subtypes are shown in Fig. 1, *A-F*. An example of positive matriptase (Fig. 1*G*) and positive HAI-1 (Fig. 1*H*) immunohistochemical staining of normal OSE is also presented. The OSE in Fig. 1*G* is lined by active Mullerian epithelium. In addition, we have demonstrated matriptase expression in normal OSE by ISH and by RT-PCR (data not shown).

Relationship between Matriptase/HAI-1 Immunostaining and Clinicopathological Parameters. Statistical analyses were performed comparing the detection of matriptase and also HAI-1 immunohistochemical staining with respect to a number of clinicopathological parameters. No relationship was found between the expression of either matriptase or HAI-1 with histological subtype, tumor grade, residual size of tumor after surgery, or overall survival of patients. Hazard ratios with 95% confidence intervals are given in Table 2. It should be noted that with only 30 deaths in this cohort of patients, there is little power to detect any potential survival differences. The high proportion of stage I patients may have compounded this limitation in the analysis.

Relationship between Matriptase/HAI-1 Immunostaining and Stage of Disease. A statistically significant relationship was found, however, between the expression of both matriptase ($P = 0.030$) and of HAI-1 ($P = 0.039$) with the stage of disease comparing tumors of stages I and II with those of stages III and IV (Table 3). From a total of 54 ovarian tumors tested, 28 of 34 stages I and II tumors (82%), and 11 of 20 stages III and IV tumors (55%) scored positive for matriptase. For HAI-1, 10 of 34 stages I and II tumors (29%), and 1 of 20 stages III and IV tumors scored positive (5%). Therefore, stage I/II ovarian tumors are statistically more likely to express matriptase than are the more advanced disease stage III/IV tumors. Correspondingly, loss of HAI-1 expression is statistically significantly more likely to be associated with stage III/IV tumors than stage I/II tumors. Taken together, these data indicate that early-stage tumors are more likely to coexpress matriptase and HAI-1 ($P = 0.039$), whereas advanced-stage tumors that express matriptase are significantly more likely to do so in the absence of HAI-1. Such an imbalance could promote the proteolytic activity of matriptase and, consequently, a more invasive phenotype in the advanced tumors.

Matriptase *In Situ* Hybridization of Ovarian Tumors *In situ* hybridization with sense and antisense digoxigenin-labeled riboprobes specific for matriptase was performed on 11 ovarian tumor sections (4 endometrioid, 3 serous papillary, 2 mucinous, 1 clear cell, and 1 unclassified carcinoma) to correlate mRNA expression with detection of protein by immunohistochemistry. Of these 11 sections, 6 were scored positive for matriptase by immunohistochemistry, and 5 were scored as negative. Of the 6 IHC-positive tumors, 4 showed strong reactivity with a matriptase-specific RNA probe after ISH, and 2 showed moderate reactivity (data not shown). Of the 5 IHC-negative tumors, all 5 showed no significant reactivity after ISH for matriptase (data not shown). This suggests that regulation of matriptase protein expression may be at the level of transcription.

DISCUSSION

The human gene for matriptase is located within chromosome 11q24–q25 (5). LOH of chromosome 11 in this region has been shown in ovarian cancer (26–30), and LOH of 11q24–q25 has been associated with poor survival of patients with ovarian cancer (27, 28). This suggests that this region harbors an ovarian tumor suppressor gene or a suppressor of tumor aggressiveness. Although frequent LOH of 11q24–q25 has also been identified in colorectal carcinoma, no correlation with adverse patient survival was found (31). LOH in the 11q22–11qter region has also been observed in breast cancers and of 11q22–24 in cervical carcinomas, suggesting that this region may harbor a tumor suppressor gene common to many types of human tumors (32–34). Loss of 11q24.1–11q25, similar to that found in ovarian cancers, may also be associated with poor survival in breast cancer (35).

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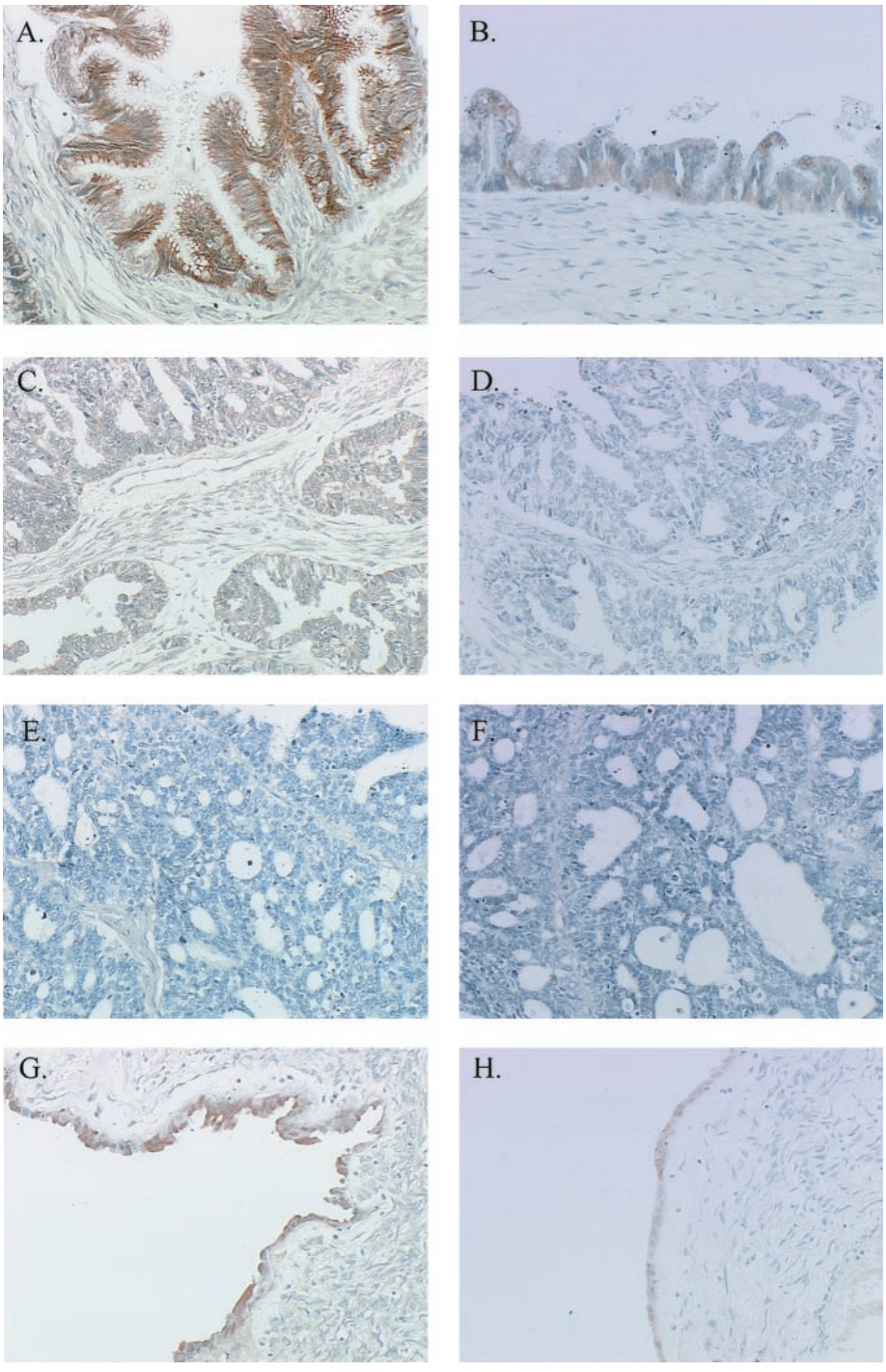

Fig. 1 Immunohistochemical staining of ovarian tumors for matriptase and HAI-1. The protein expression of matriptase and HAI-1 in paraffin-embedded tumor sections was determined by immunohistochemical staining of tumors using monoclonal antibodies that recognize either matriptase (S5) or HAI-1 (M58). A representative sample of the staining patterns observed is presented (×10 objective; final magnification, ×100). Some tumor sections showed positive staining for matriptase (*A*) and HAI-1 (*B*). Some tumor sections displayed positive staining for matriptase (*C*) and negative staining for HAI-1 (*D*). Other tumor sections displayed negative staining for both matriptase (*E*) and HAI-1 (*F*). Positive matriptase (*G*) and HAI-1 (*H*) staining in normal OSE is shown. The OSE (*G*) is lined by active Mullerian epithelium.

Despite the frequent LOH in the region of the matriptase gene and the original cloning of the gene as a putative tumor suppressor gene (*ST14*, alias *SNC19*) by subtractive hybridization methods using normal and cancerous colon tissue (5), little biochemical evidence exists that matriptase may be a tumor suppressor or negative modifier of tumor aggressiveness. In fact, because of its ability to cleave and activate growth factors and other serine proteases thought to be involved in cancer progression, matriptase has been implicated as a potential pro-invasion and metastasis factor. In support of this hypothesis, matriptase (TADG-15) mRNA expression was found by RT-PCR and by IHC using a rabbit polyclonal anti-matriptase antibody to be present in ovarian tumors but absent in the normal OSE (7). In this study, we have demonstrated by IHC and by ISH that matriptase is indeed expressed in normal OSE. This difference may reflect methodological differences or differences in the antibody reagents used. In addition, we have detected

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Table 2 Summary of Cox proportional hazards regression analysis[a]

	Hazard ratio (95% Confidence interval)	*P*
Matriptase	0.73 (0.34–1.57)	0.42
HAI-1	0.77 (0.29–2.02)	0.60

[a] Summary of Cox proportional hazards regression analysis. The expression profile of matriptase and HAI-1 were each analyzed in relation to the patient clinicopathological data using the χ^2 test or Fisher's exact test as appropriate. Survival curves were constructed by the method of Kaplan-Meier. The impact of matriptase and HAI-1 expression on patient survival was assessed using the log-rank test and Cox proportional hazards regression. Hazard ratios, with 95% confidence intervals, and corresponding *P*s are shown.

Table 3 Summary of matriptase and HAI-1-positive staining by IHC and clinical stage in 54 ovarian tumors[a]

	No. positive by IHC	
FIGO stage	Matriptase (%)	HAI-1 (%)
I/II	28/34 (82)	10/34 (29)
III/IV	11/20 (55)	1/20 (5)

[a] Summary of the association between the number and percentage of tumors staining positive by immunohistochemistry for matriptase and for HAI-1 expression with FIGO clinical stage. Analysis of clinicopathological parameters demonstrated a loss of matriptase associated with stage III/IV tumors as compared with stage I/II tumors ($P = 0.030$). There was also a loss of HAI-1 expression associated with stage III/IV tumors ($P = 0.039$). Of 34 stage I/II tumors tested, 28 of 34 (82%) stained positive for matriptase, and 10 of 34 (29%) stained positive for HAI-1, with 10 (29%) tumors showing coexpression. Of 20 stage III/IV tumors, however, 11 stained positive for matriptase (55%), only 1 tumor of which coexpressed HAI-1 ($P = 0.039$).

matriptase expression in cultured human OSE cells by RT-PCR (data not shown).

To further elucidate the potential role of matriptase in neoplasia, we examined the levels of matriptase immunoreactivity and also those of its inhibitor HAI-1 in a cohort of 54 primary ovarian tumor samples using immunohistochemistry. In a correlative study, we have also examined by mRNA ISH the expression of matriptase in a subset of these ovarian tumors (data not shown). We have shown by IHC that expression of matriptase is statistically more likely to be associated with ovarian tumors of stages I/II, as compared with tumors of stages III/IV. Correspondingly, the expression of its inhibitor, HAI-1, is also more likely to be associated with stage I/II tumors rather than stage III/IV tumors. Significantly, however, stage III/IV tumors that do express matriptase are more likely to do so in the absence of HAI-1, a ratio that favors matriptase proteolytic activity. Furthermore, expression of matriptase mRNA was shown by ISH to correlate with immunohistochemical detection of the protein.

Our data are consistent with the hypothesis that the matriptase:HAI-1 ratio is important in the development or progression of ovarian cancer. Matriptase is present as a latent, one-chain enzyme that is cleaved to an active, two-chain enzyme held together by disulfide bonds. The activated enzyme binds the inhibitor HAI-1, which effectively eliminates the proteolytic activity of matriptase (36). In a previous study, we observed the loss of HAI-1 protein expression in some tumors of OSE origin that maintain matriptase protein expression as determined by Western blot analysis (9). Our present study suggests that the matriptase:HAI-1 ratio is increased in tumors of more advanced stage, thereby favoring matriptase expression with unopposed proteolytic activity. Matriptase proteolytic activity may therefore activate pro-invasive/metastatic factors such as uPA and HGF, or other as yet uncharacterized substrates. Active HGF may then promote tumor growth and motility, and uPA may promote the proteolytic degradation of the basement membrane and extracellular matrix and the activation of latent growth factors, consequently leading to an invasive phenotype. This cascade of events could be promoted by activators of matriptase (S-1-P and LPA) that have been found to be elevated in ovarian cancer.

This study suggests that larger scale studies of matriptase, HAI-1, and the matriptase:HAI-1 ratio, perhaps by IHC or ISH using microtissue arrays or tumor cytosols in an ELISA-based format are indicated to determine the full potential of these proteins as predictors of response to therapy or as prognostic markers in ovarian cancer. Furthermore, the determination of matriptase activity, in addition to its expression, may be important in this regard. Monoclonal antibodies that recognize only the activated form of the enzyme and not both the latent and active forms as in this study (S5) may be useful in this regard once they have been optimized for use in IHC or in ELISA-based methods (36). In the future, identification of a high matriptase:HAI-1 ratio or matriptase activity in an advanced-stage ovarian cancer may indicate the use of an anti-matriptase-based therapy for the individualization of patient treatment. The definition of tumors as positive for matriptase, or more importantly the activated form of matriptase, opens up potential avenues for therapeutics based on small molecule inhibitors. Indeed, a structure-based database screening approach identified a novel analogue of hexamidine as a potent inhibitor of matriptase activity, while showing only weak inhibitory effects toward thrombin or uPA (37). Studies of this nature may pave the way to the identification of even more potent and selective matriptase inhibitors for testing in clinical trials as new ovarian cancer therapeutics.

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REFERENCES

1. Shi, Y. E., Torri, J., Yieh, L., Wellstein, A., Lippman, M. E., and Dickson, R. B. Identification and characterization of a novel matrix-degrading protease from hormone-dependent human breast cancer cells. Cancer Res., *53:* 1409–1415, 1993.

2. Lin, C. Y., Wang, J. K., Torri, J., Dou, L., Sang, Q. A., and Dickson, R. B. Characterization of a novel, membrane-bound, 80-kDa matrix-degrading protease from human breast cancer cells. Monoclonal antibody production, isolation, and localization. J. Biol. Chem., *272:* 9147–9152, 1997.

3. Lin, C. Y., Anders, J., Johnson, M., and Dickson, R. B. Purification and characterization of a complex containing matriptase and a Kunitz-type serine protease inhibitor from human milk. J. Biol. Chem., *274:* 18237–18242, 1999.

4. Lin, C. Y., Anders, J., Johnson, M., Sang, Q. A., and Dickson, R. B. Molecular cloning of cDNA for matriptase, a matrix-degrading serine protease with trypsin-like activity. J. Biol. Chem., *274:* 18231–18236, 1999.

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5. Zhang, Y., Cai, X., Schlegelberger, B., and Zheng, S. Assignment of human putative tumor suppressor genes *ST13* (alias *SNC6*) and *ST14* (alias *SNC19*) to human chromosome bands 22q13 and 11q24→q25 by *in situ* hybridization. Cytogenet. Cell Genet., *83:* 56–57, 1998.

6. Takeuchi, T., Shuman, M. A., and Craik, C. S. Reverse biochemistry: use of macromolecular protease inhibitors to dissect complex biological processes and identify a membrane-type serine protease in epithelial cancer and normal tissue. Proc. Natl. Acad. Sci. USA, *96:* 11054–11061, 1999.

7. Tanimoto, H., Underwood, L. J., Wang, Y., Shigemasa, K., Parmley, T. H., and O'Brien, T. J. Ovarian tumor cells express a transmembrane serine protease: a potential candidate for early diagnosis and therapeutic intervention. Tumour Biol., *22:* 104–114, 2001.

8. Kim, M. G., Chen, C., Lyu, M. S., Cho, E. G., Park, D., Kozak, C., and Schwartz, R. H. Cloning and chromosomal mapping of a gene isolated from thymic stromal cells encoding a new mouse type II membrane serine protease, epithin, containing four LDL receptor modules and two CUB domains. Immunogenetics, *49:* 420–428, 1999.

9. Oberst, M., Anders, J., Xie, B., Singh, B., Ossandon, M., Johnson, M., Dickson, R. B., and Lin, C. Y. Matriptase and HAI-1 are expressed by normal and malignant epithelial cells *in vitro* and *in vivo*. Am. J. Pathol., *158:* 1301–1311, 2001.

10. Takeuchi, T., Harris, J. L., Huang, W., Yan, K. W., Coughlin, S. R., and Craik, C. S. Cellular localization of membrane-type serine protease 1 and identification of protease-activated receptor-2 and single-chain urokinase-type plasminogen activator as substrates. J. Biol. Chem., *275:* 26333–26342, 2000.

11. Lee, S. L., Dickson, R. B., and Lin, C. Y. Activation of hepatocyte growth factor and urokinase/plasminogen activator by matriptase, an epithelial membrane serine protease. J. Biol. Chem., *275:* 36720–36725, 2000.

12. Fischer, K., Lutz, V., Wilhelm, O., Schmitt, M., Graeff, H., Heiss, P., Nishiguchi, T., Harbeck, N., Kessler, H., Luther, T., Magdolen, V., and Reuning, U. Urokinase induces proliferation of human ovarian cancer cells: characterization of structural elements required for growth factor function. FEBS Lett., *438:* 101–105, 1998.

13. Wilhelm, O., Schmitt, M., Hohl, S., Senekowitsch, R., and Graeff, H. Antisense inhibition of urokinase reduces spread of human ovarian cancer in mice. Clin. Exp. Metastasis, *13:* 296–302, 1995.

14. Kuhn, W., Schmalfeldt, B., Reuning, U., Pache, L., Berger, U., Ulm, K., Harbeck, N., Spathe, K., Dettmar, P., Hofler, H., Janicke, F., Schmitt, M., and Graeff, H. Prognostic significance of urokinase (uPA) and its inhibitor PAI-1 for survival in advanced ovarian carcinoma stage FIGO IIIc. Br. J. Cancer, *79:* 1746–1751, 1999.

15. Chambers, S. K., Ivins, C. M., and Carcangiu, M. L. Plasminogen activator inhibitor-1 is an independent poor prognostic factor for survival in advanced stage epithelial ovarian cancer patients. Int. J. Cancer, *79:* 449–454, 1998.

16. Schmalfeldt, B., Kuhn, W., Reuning, U., Pache, L., Dettmar, P., Schmitt, M., Janicke, F., Hofler, H., and Graeff, H. Primary tumor and metastasis in ovarian cancer differ in their content of urokinase-type plasminogen activator, its receptor, and inhibitors types 1 and 2. Cancer Res., *55:* 3958–3963, 1995.

17. Corps, A. N., Sowter, H. M., and Smith, S. K. Hepatocyte growth factor stimulates motility, chemotaxis and mitogenesis in ovarian carcinoma cells expressing high levels of c-met. Int. J. Cancer, *73:* 151–155, 1997.

18. Sowter, H. M., Corps, A. N., and Smith, S. K. Hepatocyte growth factor (HGF) in ovarian epithelial tumour fluids stimulates the migration of ovarian carcinoma cells. Int. J. Cancer, *83:* 476–480, 1999.

19. Fang, X., Gaudette, D., Furui, T., Mao, M., Estrella, V., Eder, A., Pustilnik, T., Sasagawa, T., Lapushin, R., Yu, S., Jaffe, R. B., Wiener, J. R., Erickson, J. R., and Mills, G. B. Lysophospholipid growth factors in the initiation, progression, metastases, and management of ovarian cancer. Ann. NY Acad. Sci., *905:* 188–208, 2000.

20. Xu, Y., Gaudette, D. C., Boynton, J. D., Frankel, A., Fang, X. J., Sharma, A., Hurteau, J., Casey, G., Goodbody, A., Mellors, A., *et al*. Characterization of an ovarian cancer activating factor in ascites from ovarian cancer patients. Clin. Cancer Res., *1:* 1223–1232, 1995.

21. Xiao, Y. J., Schwartz, B., Washington, M., Kennedy, A., Webster, K., Belinson, J., and Xu, Y. Electrospray ionization mass spectrometry analysis of lysophospholipids in human ascitic fluids: comparison of the lysophospholipid contents in malignant *vs* nonmalignant ascitic fluids. Anal. Biochem., *290:* 302–313, 2001.

22. Shimomura, T., Denda, K., Kitamura, A., Kawaguchi, T., Kito, M., Kondo, J., Kagaya, S., Qin, L., Takata, H., Miyazawa, K., and Kitamura, N. Hepatocyte growth factor activator inhibitor, a novel Kunitz-type serine protease inhibitor. J. Biol. Chem., *272:* 6370–6376, 1997.

23. Kataoka, H., Shimomura, T., Kawaguchi, T., Hamasuna, R., Itoh, H., Kitamura, N., Miyazawa, K., and Koono, M. Hepatocyte growth factor activator inhibitor type 1 is a specific cell surface binding protein of hepatocyte growth factor activator (HGFA) and regulates HGFA activity in the pericellular microenvironment. J. Biol. Chem., *275:* 40453–40462, 2000.

24. Kataoka, H., Suganuma, T., Shimomura, T., Itoh, H., Kitamura, N., Nabeshima, K., and Koono, M. Distribution of hepatocyte growth factor activator inhibitor type 1 (HAI-1) in human tissues. Cellular surface localization of HAI-1 in simple columnar epithelium and its modulated expression in injured and regenerative tissues. J. Histochem. Cytochem., *47:* 673–682, 1999.

25. Kataoka, H., Hamasuna, R., Itoh, H., Kitamura, N., and Koono, M. Activation of hepatocyte growth factor/scatter factor in colorectal carcinoma. Cancer Res., *60:* 6148–6159, 2000.

26. Foulkes, W. D., Campbell, I. G., Stamp, G. W., and Trowsdale, J. Loss of heterozygosity and amplification on chromosome 11q in human ovarian cancer. Br. J. Cancer, *67:* 268–273, 1993.

27. Gabra, H., Taylor, L., Cohen, B. B., Lessels, A., Eccles, D. M., Leonard, R. C., Smyth, J. F., and Steel, C. M. Chromosome 11 allele imbalance and clinicopathological correlates in ovarian tumours. Br. J. Cancer, *72:* 367–375, 1995.

28. Gabra, H., Watson, J. E., Taylor, K. J., Mackay, J., Leonard, R. C., Steel, C. M., Porteous, D. J., and Smyth, J. F. Definition and refinement of a region of loss of heterozygosity at 11q23.3-q24.3 in epithelial ovarian cancer associated with poor prognosis. Cancer Res., *56:* 950–954, 1996.

29. Davis, M., Hitchcock, A., Foulkes, W. D., and Campbell, I. G. Refinement of two chromosome 11q regions of loss of heterozygosity in ovarian cancer. Cancer Res., *56:* 741–744, 1996.

30. Launonen, V., Stenback, F., Puistola, U., Bloigu, R., Huusko, P., Kytola, S., Kauppila, A., and Winqvist, R. Chromosome 11q22.3–q25 LOH in ovarian cancer: association with a more aggressive disease course and involved subregions. Gynecol. Oncol., *71:* 299–304, 1998.

31. Connolly, K. C., Gabra, H., Millwater, C. J., Taylor, K. J., Rabiasz, G. J., Watson, J. E., Smyth, J. F., Wyllie, A. H., and Jodrell, D. I. Identification of a region of frequent loss of heterozygosity at 11q24 in colorectal cancer. Cancer Res., *59:* 2806–2809, 1999.

32. Gudmundsson, J., Barkardottir, R. B., Eiriksdottir, G., Baldursson, T., Arason, A., Egilsson, V., and Ingvarsson, S. Loss of heterozygosity at chromosome 11 in breast cancer: association of prognostic factors with genetic alterations. Br. J. Cancer, *72:* 696–701, 1995.

33. Gentile, M., Olsen, K., Dufmats, M., and Wingren, S. Frequent allelic losses at 11q24.1–q25 in young women with breast cancer: association with poor survival. Br. J. Cancer, *80:* 843–849, 1999.

34. Hampton, G. M., Penny, L. A., Baergen, R. N., Larson, A., Brewer, C., Liao, S., Busby-Earle, R. M., Williams, A. W., Steel, C. M., Bird, C. C., *et al*. Loss of heterozygosity in cervical carcinoma: subchromosomal localization of a putative tumor-suppressor gene to chromosome 11q22–q24. Proc. Natl. Acad. Sci. USA, *91:* 6953–6957, 1994.

35. Gentile, M., Wiman, A., Thorstenson, S., Loman, N., Borg, A., and Wingren, S. Deletion mapping of chromosome segment 11q24–q25, exhibiting extensive allelic loss in early onset breast cancer. Int. J. Cancer, *92:* 208–213, 2001.

36. Benaud, C., Dickson, R. B., and Lin, C. Y. Regulation of the activity of matriptase on epithelial cell surfaces by a blood-derived factor. Eur. J. Biochem., *268:* 1439–1447, 2001.

37. Enyedy, I. J., Lee, S. L., Kuo, A. H., Dickson, R. B., Lin, C. Y., and Wang, S. Structure-based approach for the discovery of bis-benzamidines as novel inhibitors of matriptase. J. Med. Chem., *44:* 1349–1355, 2001.

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