

Expression of Matriptase and Clinical Outcome of Human Endometrial Cancer

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Abstract. *Background:* Matriptase, a type II transmembrane serine protease is involved in angiogenesis, degradation of extracellular matrix and in progression of some epithelial cancers. The purpose of the present study was to examine matriptase expression and evaluate its clinicopathological significance in endometrial cancer. *Patients and Methods:* Matriptase expression was examined in normal endometrium ($n=20$), endometrial hyperplasia ($n=11$) and endometrial cancer ($n=65$) samples. The distribution of cases that scored positive for each of the biological parameters examined was correlated with matriptase expression status obtained by immunohistochemistry. *Results:* Matriptase was found to be significantly overexpressed in endometrial cancer as compared with normal endometrium and endometrial hyperplasia. Interestingly, matriptase expression is associated with stage ($p=0.010$), grade ($p=0.021$), depth of myometrial invasion ($p=0.004$), cervical involvement ($p=0.021$), lymph node metastasis ($p=0.009$), LVS involvement ($p=0.041$) and peritoneal cytology ($p=0.045$). The high matriptase expression was a significant predictor for poor prognosis when compared with low matriptase expression (Disease-free survival rate; $p=0.032$, Overall survival rate; $p=0.011$). *Conclusion:* High matriptase expression in endometrial cancer may be associated with poor prognosis.

Endometrial cancer is the most frequent gynecologic malignancy in the world (1). Asian nations such as Japan, China and Korea have an incidence that is 4-5 times lower than in western industrialized nations (2). In Japan, endometrial cancer is currently the fourth most common

gynecologic malignancy in women, with an estimated incidence of 4,381 new cases in 2006 (3). The incidence of endometrial cancer in Asian countries, however, has been increasing markedly in recent years. Clinical parameters such as stage of the disease, nuclear grade, histologic subtypes and tumor size correlate with the outcome of the disease. Although it is a relatively common cancer, the molecular genetic factors related to the development of endometrial cancer and its prognosis have only recently begun to be investigated. It is hoped that, through a better understanding of the molecular genetic alterations implicated in endometrial cancer, a more complete profile of risk factors can be developed.

The metastatic process involves degradation of the extracellular matrix (ECM) including interstitial basement membrane by proteases that facilitate cells detachment, local and systemic spreading. To accomplish local invasion, tumor cells use extracellular and cell surface proteolytic enzymes to degrade the basement membrane proteins (4, 5). Several studies have demonstrated a critical role of matrix metalloproteinases (MMPs), which can degrade the extracellular matrix and basement membrane proteins and facilitate the initial invasion events (4). MMPs and serine proteases have been implicated in degradation of the extracellular matrix and modulation of cell-substratum adhesion in tumor cells. The type II transmembrane serine proteinases family is a newly identified superfamily of membrane-associated serine proteinases, including matriptase subfamily (6). Matriptase contains a transmembrane domain, two CUB domains, four low-density lipoprotein-receptor domains and a serine protease domain (6, 7). It is expressed in a wide range of epithelial tissues, such as the epidermis, gastrointestinal tract and respiratory tract, and also in endothelial cells, neural cells and white blood cells. Matriptase activates not only sc-hepatocyte growth factor (sc-HGF) but also pro-urokinase plasminogen activator (pro-uPA) and protease-activated receptor-2 (PAR-2) (8, 9). The intimate association of matriptase with human carcinoma was subsequently confirmed in a large number of studies that showed overexpression of protease in a wide variety of benign and malignant tumors. Matriptase was found to be significantly over-expressed in

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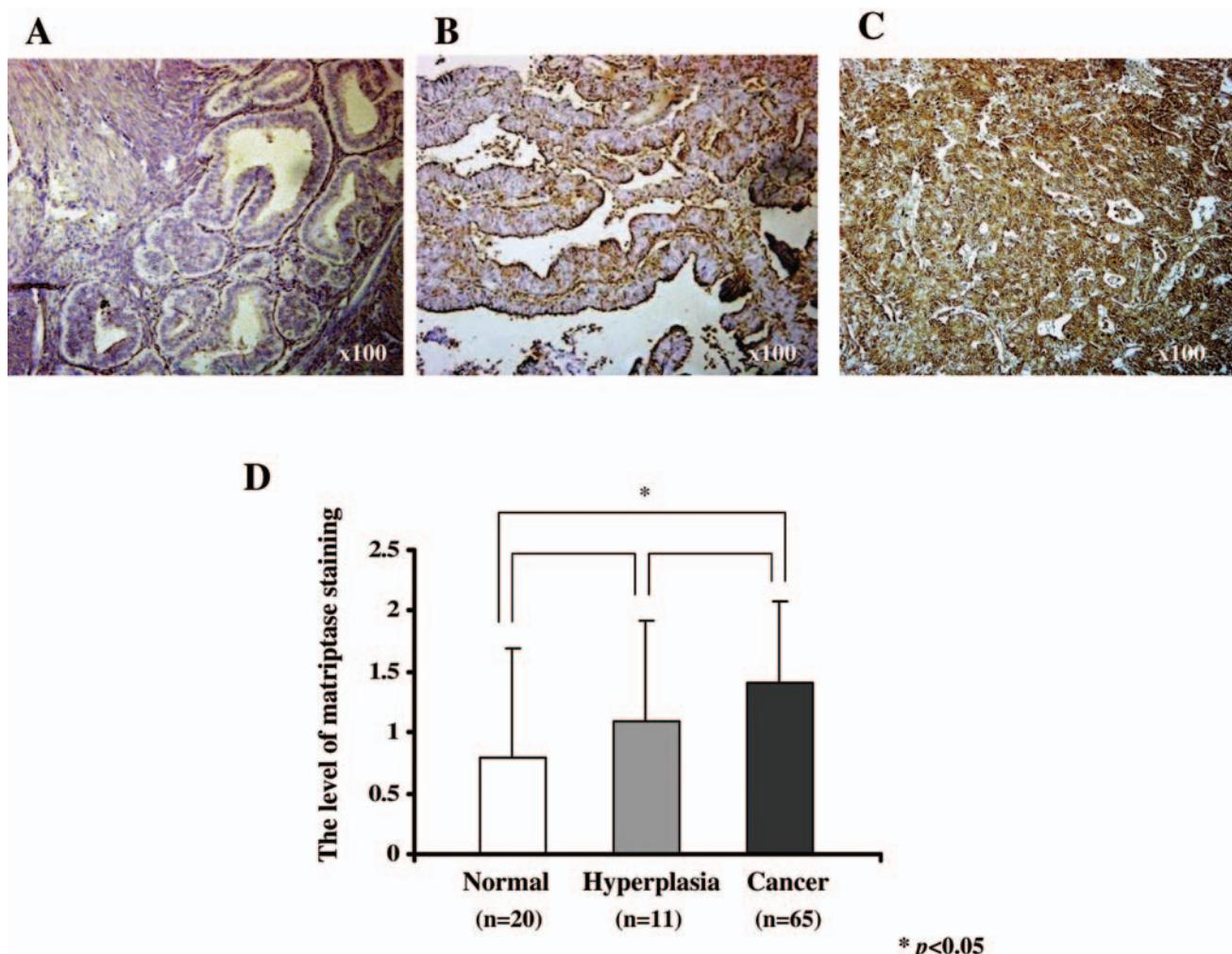


Figure 1. Illustrates representative immunostaining patterns of matriptase (A) Weak epithelial cell staining. (B) Moderate epithelial cell staining. (C) Strong epithelial cell staining (original magnification $\times 100$). (D) Histogram of matriptase expression by tissue type (normal endometrium, endometrial hyperplasia, endometrial cancer). The relative strength of matriptase immunohistochemical staining was assessed qualitatively.

cancer samples compared with matched normal tissues in breast, cervical, ovarian and prostate carcinoma (10-14). In the current study, the levels of matriptase protein expression are investigated and correlated with clinicopathological characteristics in patients suffering of endometrial cancer.

Patients and Methods

Patients and tissues samples. Patients with normal endometrium ($n=20$) (Proliferative phase ($n=7$), Secretory phase ($n=6$), Menopause ($n=7$)), endometrial hyperplasia ($n=11$) (simple endometrial hyperplasia ($n=2$), complex endometrial hyperplasia ($n=2$), atypical endometrial hyperplasia ($n=7$)) and adenocarcinoma ($n=65$) were treated at Okayama University Hospital between January 1996 and November 2004. Patients with distant metastasis were excluded from this study. Tumor specimens were obtained at the time of surgery and immediately fixed in 10% neutral-buffered

formalin and embedded in paraffin. Histological cell types were diagnosed according to the WHO classification: 65 were classified as endometrioid adenocarcinomas. Histological grades according to the International Federation of Gynecology and Obstetrics (FIGO) staging classification were as follows: 19 were grade 1, 32 were grade 2 and 14 were grade 3. Surgical staging was reviewed based on the FIGO staging system: 28 were allocated to stage I, 4 to stage II, 25 to stage III, and 8 to stage IV. The median age at the time of surgery was 56 years (range 33-81). Disease-free and overall survival rates were defined as the interval between the initial operation and either clinically or radiologically proven recurrence or death, respectively.

Immunohistochemistry and staining evaluation. Formalin-fixed, paraffin-embedded sections, 4 μ m thick were deparaffinized with xylene and rehydrated in ethanol. Endogenous peroxidase activity was quenched by methanol containing 0.3% hydrogen peroxidase for 15 min. Then, sections were incubated at room temperature

with a matriptase (Santa Cruz Biotechnology, Santa Cruz, CA, USA) followed by staining using a streptavidin-biotin-peroxidase kit (Nichirei, Tokyo, Japan). The sections were counterstained with hematoxylin. The level of matriptase staining in epithelial cells was classified into three groups by scoring the percentage of positive cells: strong (2); >50% of cells stained, moderate (1); 10–50% of cells stained, and weak (0); <10% of cells stained. Microscopic analyses were independently conducted by two independent examiners with no prior knowledge of the clinical data. Final decisions in controversial cases were made using a conference microscope.

Statistical analyses. The Mann–Whitney *U*-test was used to examine the association between clinicopathological factors and matriptase expression. Survival rates were calculated by the Kaplan–Meier method and differences were examined by the log-rank test. Factors found to be significant were then chosen for stepwise Cox's multivariate proportional hazard model to determine their prognostic values. These analyses were performed utilizing StatView 5.0 software (Abacus Concepts, Berkeley, CA, USA). *P*-values <0.05 were considered statistically significant.

Results

Matriptase expression in human endometrial tissues. Expression of matriptase was examined in human endometrial tissues by immunostaining; Figures 1A–C illustrates representative immunostaining patterns of matriptase. Weak epithelial matriptase staining was observed in 20 cases (20.8%), moderate matriptase staining in 33 cases (34.4%) and strong matriptase staining in 43 cases (44.8%), respectively. The mean score of epithelial staining in matriptase was 0.8 for normal human endometrium, 1.09 for hyperplasia and 1.40 for cancer samples. Interestingly, endometrial cancer had the strongest expression of matriptase compared with normal endometrium and endometrial hyperplasia as shown in Figure 1D (Mann–Whitney *U*-test, *p*<0.05).

Clinicopathological parameters. Table I shows the distribution of cases scored as positive for each of the biological parameters examined, according to clinicopathological characteristics in the overall population. As expected, the level of matriptase expression positivity showed a statistically significant association with clinicopathological parameters such as advanced stage (*p*=0.010), high grade (*p*=0.021), depth of myometrial invasion (*p*=0.004), cervical involvement (*p*=0.021), lymph node metastasis (*p*=0.009), lymph vascular space (LVS) involvement (*p*=0.041) and peritoneal cytology (*p*=0.045).

Univariate survival and multivariate analysis. Figure 2 shows the disease-free and overall survival curves of 65 patients with endometrial cancer, according to matriptase expression status. The disease-free and overall survival rates of patients exhibiting high matriptase expression (score 2) were

Table I. Association between matriptase and clinicopathological factors in endometrial cancer.

| Variable | No. of cases | Matriptase score (mean±SE) | <i>p</i> -value |
|------------------------------|--------------|----------------------------|-----------------|
| Age (years) | | | 0.912 |
| <60 | 42 | 1.45±0.71 | |
| ≥60 | 23 | 1.47±0.67 | |
| FIGO stage | | | 0.010* |
| I+II | 32 | 1.25±0.76 | |
| III+IV | 33 | 1.67±0.53 | |
| FIGO grade | | | 0.019* |
| 1 | 19 | 1.10±0.81 | |
| 2+3 | 46 | 1.61±0.57 | |
| Depth of myometrial invasion | | | 0.004* |
| <1/2 | 39 | 1.28±0.76 | |
| ≥1/2 | 26 | 1.73±0.45 | |
| Cervical involvement | | | 0.021* |
| Negative | 49 | 1.37±0.73 | |
| Positive | 16 | 1.75±0.44 | |
| Lymph node metastasis | | | 0.009* |
| Negative | 51 | 1.37±0.72 | |
| Positive | 14 | 1.79±0.43 | |
| LVS involvement | | | 0.041* |
| Negative | 40 | 1.33±0.73 | |
| Positive | 25 | 1.68±0.56 | |
| Ovarian metastasis | | | 0.398 |
| Negative | 53 | 1.43±0.69 | |
| Positive | 12 | 1.58±0.67 | |
| Peritoneal cytology | | | 0.045* |
| Negative | 46 | 1.36±0.74 | |
| Positive | 19 | 1.68±0.48 | |

*Mann–Whitney *U*-test. FIGO, International Federation of Gynecology and Obstetrics; LVS, lymph vascular space.

significantly lower than those of patients exhibiting low matriptase expression (score 0–1) (*p*=0.032 and 0.011, respectively). The results of the univariate survival analyses of other variables are shown in Table II.

Discussion

This is the first study to examine the status of matriptase expression and its possible roles in conjunction with clinical outcome in patients with endometrial cancer. In endometrial carcinoma, conventional clinicopathological factors such as FIGO grade and stage, histological type, LVS invasion and depth of myometrial invasion are well-known prognostic factors. The proposed underlying molecular mechanisms implicated in the progression of endometrial cancer include overexpression of oncogenes such as HER-2/neu, myc and loss of tumor suppressor genes such as p53 (15–17). This study evaluated the prognostic significance of matriptase overexpression in relation to endometrial cancer.

The basement membrane is a specialized extracellular matrix structure that separates the epithelial and stromal cell

Table II. Disease-free and overall survival analysis of prognostic factor using the log-rank test in endometrial cancer

| Variable | No. of cases | Estimated 5-year DFS(%) | p-value | Estimated 5-year OS(%) | p-value |
|------------------------------|--------------|-------------------------|---------|------------------------|---------|
| Age (years) | | | 0.104 | | 0.844 |
| <60 | 42 | 76.2 | | 76.2 | |
| ≥60 | 23 | 65.2 | | 73.9 | |
| FIGO stage | | | <0.001* | | <0.001* |
| I+II | 32 | 93.8 | | 93.9 | |
| III+IV | 33 | 51.5 | | 54.5 | |
| FIGO grade | | | 0.047* | | 0.020* |
| 1 | 19 | 89.4 | | 94.7 | |
| 2+3 | 46 | 65.2 | | 67.4 | |
| Depth of myometrial invasion | | | 0.001* | | 0.007* |
| <1/2 | 39 | 87.2 | | 82.1 | |
| ≥1/2 | 26 | 50 | | 65.4 | |
| Cervical involvement | | | <0.001* | | <0.001* |
| Negative | 49 | 83.7 | | 85.7 | |
| Positive | 16 | 37.5 | | 43.8 | |
| Lymph node metastasis | | | <0.001* | | <0.001* |
| Negative | 51 | 86.3 | | 88.2 | |
| Positive | 14 | 21.4 | | 28.6 | |
| LVS involvement | | | <0.001* | | <0.001* |
| Negative | 40 | 90 | | 90 | |
| Positive | 25 | 44 | | 52 | |
| Ovarian metastasis | | | <0.001* | | <0.001* |
| Negative | 53 | 83 | | 86.8 | |
| Positive | 12 | 25 | | 75 | |
| Peritoneal cytology | | | 0.094 | | 0.042* |
| Negative | 46 | 78.3 | | 82.6 | |
| Positive | 19 | 57.9 | | 57.9 | |
| Matriptase | | | 0.007* | | 0.005* |
| Low (0-1) | 32 | 87.5 | | 90.6 | |
| High (2) | 33 | 57.6 | | 60.6 | |

*Mann-Whitney U-test. FIGO, International Federation of Gynecology and Obstetrics; LVS, lymph vascular space.

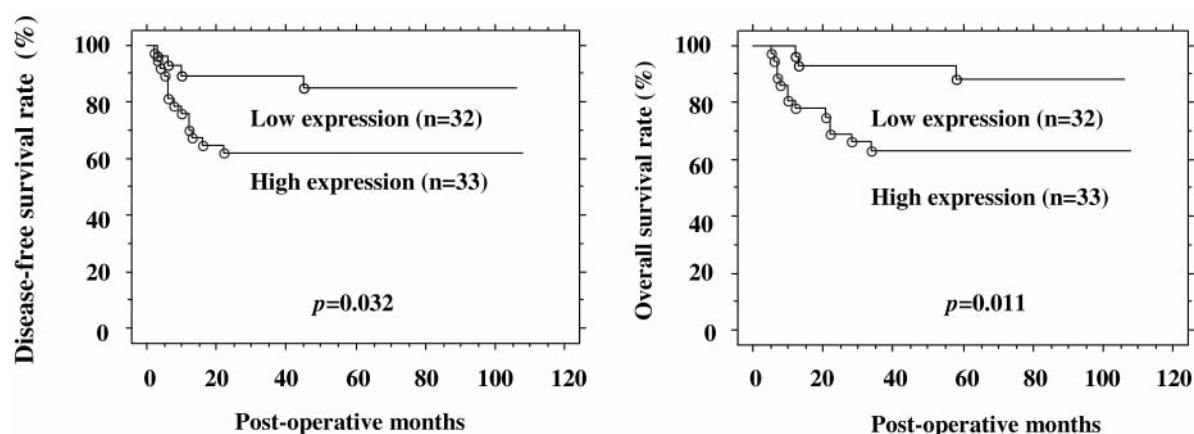


Figure 2. Kaplan-Meier plots for disease-free and overall survival of the 65 patients with endometrial cancer according to their epithelial matriptase expression status. Low epithelial expression, score 0-1; high epithelial expression, score 2.

compartments. To accomplish local invasion, tumor cells use extracellular and cell surface proteolytic enzymes to degrade the basement membrane proteins (4, 5). Several studies have demonstrated a critical role of serine protease, which can degrade the extracellular matrix and basement membrane proteins, and facilitate the initial invasion events. Serine proteases have been implicated in degradation of the extracellular matrix and modulation of cell-substratum adhesion in tumor cells. Type II transmembrane serine proteases are a specialized group of cell surface proteolytic enzymes. Matriptase also known as MT-SP1, epithin and TAGD-15 is the prototypic member of the recently identified matriptase subfamily of type II transmembrane serine protease. Matriptase is a strictly epithelial protease with a fairly widespread, but not ubiquitous, expression in human and mouse tissues. Expression has been documented in epidermis, cornea, salivary gland, oral and nasal cavities, thyroid, thymus, esophagus, trachea, bronchioles, alveoli, stomach, pancreas, gallbladder, duodenum, small intestine, colon, rectum, kidney, adrenals, urinary bladder, ureter, seminal vesicles, epididymis, prostate, ovaries, uterus and vagina (18, 19). Several reports have assessed the level of expression of matriptase during malignant progression and the potential value of matriptase as a prognostic marker in various human cancers. In prostate and cervical cancer, matriptase mRNA and protein are up-regulated in cancerous lesions compared with normal tissue and there is a positive correlation between matriptase expression and histopathological grade of the tumor (11, 14). In contrast, in the gastrointestinal tract, a significant down-regulation of matriptase mRNA compared with normal tissue, as well as a decrease of matriptase mRNA levels increasing tumor grade, has been reported (20). Although initial studies concluded that matriptase mRNA was not significantly increased in tumors compared with normal breast tissue (21), other studies reported that high matriptase expression is predictive of poor survival as assessed by immunohistochemistry (22). In the current study, matriptase protein expression was examined and correlated with clinicopathological characteristics in patients suffering from endometrium tissues by immunohistochemistry. There was an increase in the level of matriptase expression in endometrial cancer compared with normal endometrium and endometrial hyperplasia on patient tissues (Figure 1D). Furthermore, a significant association between matriptase expression and some of the traditional prognostic factors for endometrial carcinoma such as advanced stage, poorly differentiated tumor of histology, depth of myometrial invasion, cervical involvement, lymph node metastasis, LVS involvement, ovarian metastasis and peritoneal cytology was demonstrated (Table I). Interestingly, a strong matriptase immunostaining pattern was significantly associated with poor prognosis in endometrial cancer (Figure 2 and Table II). These results suggest that expression of matriptase in endometrial cancer may be associated with aggressive biological

characteristics, and may play an important role in prognosis and/or recurrence.

These findings all indicate that matriptase protein determined by immunohistochemistry could be an important tool for identifying patients with poor prognosis of endometrial cancer.

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