

Expression of the serine protease, matriptase, in breast ductal carcinoma of Chinese women: Correlation with clinicopathological parameters

J.-S. Jin^{1*}, T.-F. Cheng^{2*}, W.C. Tsai¹, L.-F. Sheu¹, H. Chiang³ and C.-P. Yu¹

¹Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, ²Department of General Surgery, Shin-Kong Wu Ho-Su Memorial Hospital and ³Taipei Institute of Pathology, Taipei, Taiwan, R.O.C.

Summary. Matriptase is a serine protease expressed by cells of surface epithelial origin, including epithelial breast tumor cells. Matriptase cleaves and activates proteins implicated in the progression of cancer and represents a potential prognostic and therapeutic target. The aim of this study was to examine matriptase expression in breast tumors of Chinese women and to identify its clinicopathological correlations. Immunohistochemical analysis of matriptase was performed in tissue microarrays of 251 breast tumors including 30 fibroadenomas, 59 ductal carcinomas *in situ* (DCIS), 38 grade I invasive ductal carcinomas (IDC), 79 grade II IDC, and 45 grade III IDC. The matriptase scores were significantly higher in the tumors than their non-tumor counterparts (178±12 for fibroadenoma; 275±11 for DCIS; 299±10 for grade I IDC; 251±10 for grade II IDC; and 314±11 for grade III IDC). In cases of IDC, matriptase scores were significantly correlated with tumor staging and nodal staging. Our findings demonstrate that matriptase is over-expressed in breast ductal carcinoma of Chinese women. It therefore may be a good biomarker for diagnosis and treatment of malignant breast tumors.

Key words: Serine protease matriptase, Invasive ductal carcinoma, Ductal carcinoma *in situ*, Fibroadenoma

Introduction

Tumor metastasis to distal organs and local invasion are multistep processes (Tryggvason et al., 1987; Liotta et al., 1991; Duffy, 1992). The break down of the major component (type IV collagen) of basement membrane

and invasion by tumor cells is an important reason for treatment failure in patients with breast malignancies (Anagnostopoulos et al., 2006; Jaaback et al., 2006; Kuroi et al., 2006; Yamashita et al., 2006). Identification of target molecules promoting tumor cell invasion may result in new therapies that arrest local invasion and metastatic spread of breast cancer.

During the last few years, several novel transmembrane serine proteases, including matriptase, have been cloned and identified in humans (Tanimoto et al., 2001). Matriptase was first isolated as a transmembrane serine protease from breast carcinoma (Shi et al., 1993; Lin et al., 1997, 1999) and its gene sequence was identified (Zhang et al., 1998). Our previous studies have demonstrated that matriptase expression was significantly increased in ovary adenocarcinomas and renal cell carcinomas (Jin et al., 2006a,b) and that these increases correlated significantly with clinicopathological parameters (Jin et al., 2006a,b). However, the expression profiles of matriptase in Chinese women remain unclear.

Previous quantitative analyses of immunohistochemical staining intensity in individual slides were limited because the signal generated by chemical reaction varied under different environmental conditions and also in different slides (Lam et al., 2004). Our laboratory has successfully developed an immunostaining system using tissue microarrays for evaluating different specimens simultaneously (Jin et al., 2006a-d). In the present study, the expression of matriptase in 251 breast tumors of Chinese women was evaluated using this tissue microarray system. We compared matriptase immunostaining scores in a series of patients with fibroadenoma, ductal carcinoma *in situ* (DCIS), or different grades of invasive ductal carcinoma (IDC). Our findings demonstrate that matriptase is overexpressed in breast cancer.

Materials and methods

Paraffin-embedded tumor tissues were retrieved

Offprint requests to: Cheng-Ping Yu, M.D., Ph.D., Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, No. 325, Sec. 2, Cheng-Gong Road, Taipei, Taiwan, R.O.C. e-mail: jsjin@ndmctsg.edu.tw

*Both authors have contributed equally and qualify as the first author

from the archives of the Department of Pathology, Tri-Service General Hospital, and three tissue microarray slides were constructed. The three tissue microarray slides consisted of 251 breast tumors including 30 fibroadenomas, 59 ductal carcinomas in situ (DCIS), 38 grade I invasive ductal carcinomas (IDC), 79 grade II IDC, and 45 grade III IDC.

One tissue core (2 mm in diameter) was taken from selected area (one per area) of each paraffin-embedded block, and tissue microarray slides were constructed according to previously published methods (Jin et al., 2006a-d). The slides were reviewed and pathological diagnosis was made by at least two experienced pathologists.

All tumors were pathologically staged according to the 1997 TNM system and assigned a histopathological grade. Stage T1 was defined as a tumor less than 2 cm in greatest dimension. Stage T2 was defined as a tumor greater than 2 cm but not more than 5 cm in greatest dimension. Stage T3 was defined as a tumor more than 5 cm in greatest dimension. Stage T4 was defined as tumor of any size with direct extension to chest wall or skin. The histological grade of IDC was based on an assessment of tubule formation, nuclear pleomorphism, and mitotic count according to the study of Ellis (Ellis et al., 1993).

Immunohistochemistry

Tissue microarray sections were dewaxed in xylene, rehydrated in alcohol, and immersed in 3% hydrogen peroxide for 10 min to suppress endogenous peroxidase activity. Antigen retrieval was performed by heating each section in a steam cooker for 30 min. After 3 rinses (each for 5 min) in phosphate buffered saline (PBS), sections were incubated for one hour at room temperature with a rabbit anti-human matriptase/ST14 antibody (1:100; Bethyl Laboratories, Montgomery, TX, USA) diluted in PBS. After 3 washes (each for 5 min) in PBS, sections were incubated with horseradish peroxidase-labeled mouse anti-rabbit immunoglobulin (DAKO, Glostrup, Denmark) for one hour at room temperature. After 3 additional washes, peroxidase activity was developed with diaminobenzidine (DAB) at room temperature.

For evaluation of immunoreactivity and histological appearance, all tissue microarray slides were examined and scored by two authors concurrently. The intensity of cytoplasmic and membranous immunostaining of tumor cells was scored on a scale of 0 (no staining) to 4 (strongest intensity), and the percentage of tumor cells with cytoplasmic or membranous staining at each intensity was estimated from 0 to 100. Matriptase expression in fibroadenomas was evaluated in the epithelial components of this tumor. The absolute value of the proportion of cells at each intensity level was multiplied by the corresponding intensity value, and these products were added to obtain an immunostaining score ranging from 0 to 400 (Jin et al., 2006a-d).

Statistical analysis

All results are expressed as mean \pm standard error of the mean (SEM). The immunostaining score of matriptase for breast tumors was compared with the score of normal breast epithelial tissue. Statistical analysis was performed using the Student t-test between groups. A P value less than 0.05 was considered to be statistically significant. SigmaState software (Jandel Scientific, San Rafael, CA, USA) was used to perform linear regression testing to analyze the relationship between matriptase immunostaining scores and clinicopathological parameters.

Results

Clinicopathological characteristics

The study sample included 251 Chinese women (median age, 48 years; range, 36-78). Table 1 shows the histopathological differentiation, TNM category, and tumor staging distribution.

Matriptase expression in breast tumors

Diagnosis in each of the 251 cases was confirmed by two pathologists who evaluated hematoxylin and eosin sections (Fig. 1). In all malignant breast tumors, matriptase immunoreactivity was seen on the cell surface and in the cytoplasm of tumor cells but not in the surrounding stromal cells or extracellular matrix (Fig. 1).

Table 1. Clinicopathologic characteristics of tumors in 162 cases of infiltrating ductal carcinoma.

VARIABLE	n
Histopathological differentiation	
Grade I	38
Grade II	79
Grade III	45
TNM category ^a	
T1	55
T2	70
T3	17
T4	20
N0	86
N1	57
N2	19
M0	142
M1	20
Tumor stage ^a	
I	39
II	84
III	28
IV	11

^aAJCC: the American Joint Committee on Cancer

Matriptase in breast cancer

In table 2, matriptase score was significantly higher in all 30 fibroadenoma specimens (178 ± 12) than in normal ductal epithelia (145 ± 16). All IDC and DCIS specimens were strongly (intensity score >3) positive for matriptase. The matriptase scores were significantly

higher in the DCIS and IDC than in their non-tumor counterparts (275 ± 11 for DCIS; 299 ± 10 for grade I IDC; 251 ± 10 for grade II IDC; and 314 ± 11 for grade III IDC) (Fig. 1, Table 2). In addition, the immunostaining scores for matriptase were significantly higher in DCIS and

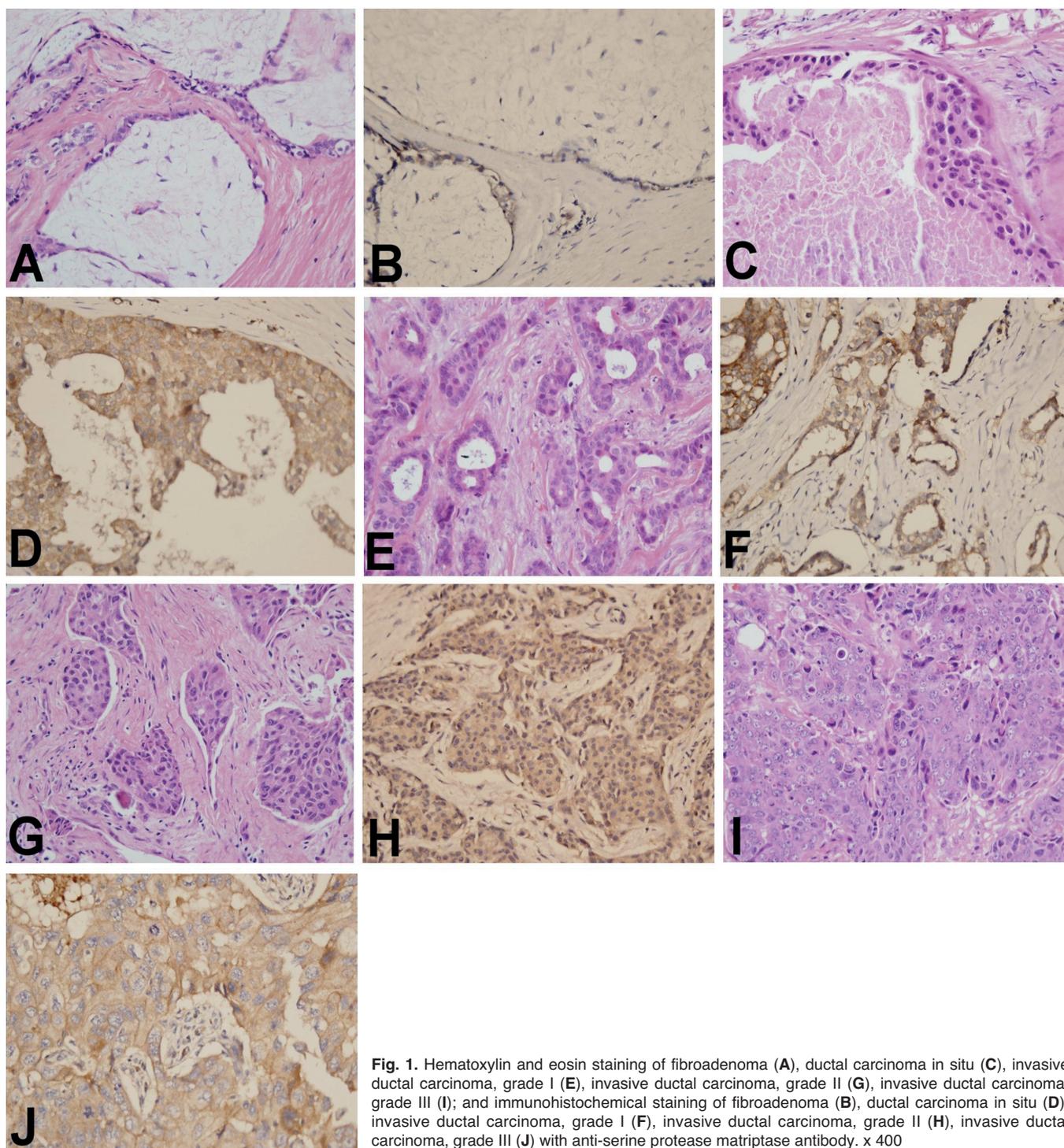


Fig. 1. Hematoxylin and eosin staining of fibroadenoma (A), ductal carcinoma in situ (C), invasive ductal carcinoma, grade I (E), invasive ductal carcinoma, grade II (G), invasive ductal carcinoma, grade III (I); and immunohistochemical staining of fibroadenoma (B), ductal carcinoma in situ (D), invasive ductal carcinoma, grade I (F), invasive ductal carcinoma, grade II (H), invasive ductal carcinoma, grade III (J) with anti-serine protease matriptase antibody. x 400

Matriptase in breast cancer

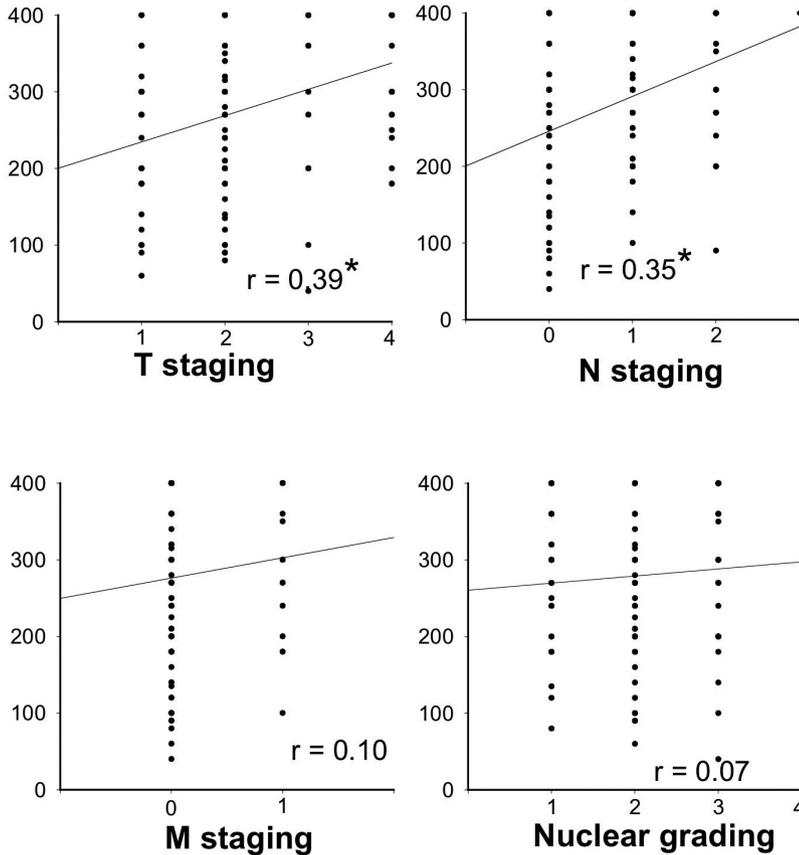


Fig. 2. Clinicopathological correlation of TNM staging and nuclear grading with matriptase immunostaining scores in breast cancer. *: indicates statistical significance of linear regression testing ($p < 0.05$).

Table 2. Immunostaining scores for matriptase in breast tumors.

Tumor	% staining	Intensity	Total score	P value
Fibroadenoma (n = 30)	64±4	2.8±0.3	178±12 *	0.032
DCIS (n = 59)	32±4	3.0±0.3	275±11 *+	<0.001
IDC, grade I (n = 38)	83±4	3.6±0.4	299±10 *+	<0.001
IDC, grade II (n = 79)	78±6	3.2±0.4	251±10 *+	<0.001
IDC, grade III (n = 45)	86±4	3.7±0.3	314±11* +	<0.001
Normal epithelia (n = 30)	52±5	2.6±0.3	145±16	

Data are the means ± standard error of the mean (SEM) of immunostaining score for matriptase in breast tumors and normal ductal epithelia. *: $p < 0.05$ vs. normal ductal epithelia. +: indicates significant difference comparing to fibroadenoma. DCIS: ductal carcinoma in situ; IDC: invasive ductal carcinoma.

IDC than in fibroadenoma.

Correlation of matriptase scores with clinicopathological features

Of the 162 cases of IDC, there were 55 stage T1, 70 stage T2, 17 stage T3, 20 stage T4, 86 stage N0, 57 stage N1, 19 stage N2, 142 stage M0, and 20 stage M1 cases (Fig. 2, Table 1). Fig. 2 shows that matriptase score

correlated with TNM staging in IDC cases. The immunostaining scores for matriptase in IDC show significant correlation with T staging and nodal (N) staging. However, there was no significant relationship between matriptase score and M stage or histological grade.

Discussion

Our studies demonstrate that matriptase is overexpressed in breast DCIS and IDC, and high-level expression of matriptase associates with more aggressive breast tumors in Chinese women. Using pharmacological agents to inhibit matriptase activity may improve the treatment of breast ductal carcinoma.

Matriptase has been identified as a novel cell surface protease by several investigators because it is consistently overexpressed in human cancers. These include ovary, renal, prostate, uterine, colon, cervical, head and neck squamous cell carcinoma, and breast cancers (Benaud et al., 2002; Bhatt et al., 2003; Jin et al., 2006a,b; Oberst et al., 2001, 2002; Tanimoto et al., 2001). The expression of matriptase was detected exclusively in tumors of epithelial and not of mesenchymal origin, and not in the surrounding tumor stroma (Benaud et al., 2002; Bhatt et al., 2003; Oberst et

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al., 2001, 2002; Tanimoto et al., 2001). This observation is confirmed by our finding of negative staining for matriptase in the fibrovascular stroma of IDC specimens and positive staining for matriptase in DCIS and IDC specimens.

In fact, because of matriptase's 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, our results demonstrated that matriptase protein is overexpressed in all IDC and DCIS. Our current study may lead to the identification and clinical trials of even more potent and selective matriptase inhibitors to treat breast cancer.

In conclusion, we demonstrated that matriptase is expressed in IDC and DCIS, and that increased expression of matriptase in IDC has a significant clinicopathological relationship to this tumor's aggressiveness. Our results strongly support the hypothesis that matriptase is a novel biomarker for diagnosis and treatment of IDC.

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