

# Matriptase and survivin expression associated with tumor progression and malignant potential in breast cancer of Chinese women: Tissue microarray analysis of immunostaining scores with clinicopathological parameters

Wen-Chiuan Tsai<sup>a</sup>, Chi-Hong Chu<sup>b</sup>, Cheng-Pin Yu<sup>a</sup>, Lai-Fa Sheu<sup>a</sup>, Ann-Chen<sup>a</sup>, Hung Chiang<sup>c</sup> and Jong-Shiaw Jin<sup>a,\*</sup>

<sup>a</sup>Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan

<sup>b</sup>Division of General Surgery, Department of Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan

<sup>c</sup>Taipei Institute of Pathology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan

**Abstract.** *Objective:* The aim of this study was to examine the expression of matriptase and survivin in breast carcinoma and correlate with clinicopathological parameters.

*Methods:* Immunohistochemical analysis of matriptase and survivin were performed in tissue microarray slides of 290 cases, including 11 normal breast tissue; 27 fibrocystic disease; 17 fibroadenoma; 6 atypical ductal hyperplasia; 39 ductal carcinoma in situ, low grade (DCIS, low grade); 39 ductal carcinoma in situ, high grade (DCIS, high grade); 27 invasive ductal carcinoma, grade I (IDC, grade I); 78 invasive ductal carcinoma, grade II (IDC, grade II); and 46 invasive ductal carcinoma, grade III (IDC, grade III).

*Results:* The average immunostaining scores of matriptase were 44.1 in normal breast tissue, 52.7 in fibrocystic disease, 76.5 in fibroadenoma, 81.7 in atypical ductal hyperplasia, 133.7 in low-grade DCIS, and 155.8 in high-grade DCIS. Among 151 breast IDC cases, the average immunostaining scores of matriptase were 172.7 in grade I, 211.7 in grade II, and 221.2 in grade III. Additionally, the average immunostaining scores of survivin also correlate with tumor grades and stages.

*Conclusions:* Higher expressions of matriptase and survivin correlate significantly with clinicopathological parameters in breast cancer and the malignant potential in premalignant lesions. In addition, higher survivin expression had poorer prognosis of breast IDC cases.

**Keywords:** Atypical ductal hyperplasia, ductal carcinoma in situ, invasive ductal carcinoma, matriptase, survivin

## 1. Introduction

Invasive breast cancer is one the most common carcinomas in women, especially in the West. Etiologies of breast cancer include family history, diet, and hormonal imbalance [1]. Multiple factors, such as tumor histopathological grades and subtypes, clinical stages, and lymph node metastasis have been proved to play

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\*Corresponding author: Jong-Shiaw Jin, M.D., Ph.D., Department of Pathology, Tri-Service General Hospital, National Defense Medical Center, No. 325, Sec. 2, Cheng-Kung Road, Neihu 114, Taipei, Taiwan. Tel.: +886 2 87927155; Fax: +886 2 26913324; E-mail: jsjin@ndmctsgh.edu.tw.

important roles in predicting prognosis and determining adjuvant treatment [1]. New prognostic factors include distinct breast cancer subgroups with different biological features [2]. Premalignant breast disease, such as atypical ductal hyperplasia, is an important predictor of later malignancy [3]. Several immunohistochemical markers, such as p53, Her2/neu, EGFR, estrogen receptor, E-cadherin, have been evaluated for discrimination of breast intraepithelial proliferative lesions, but only cytological evidence has been effective [3]. The development of a novel biomarker to prevent subjective error may be necessary to assist pathologists in making accurate diagnoses.

Matriptase is a multi-domain serine protease that has been detected in several normal-surface epithelia [4–6]. Matriptase was first isolated as a type II transmembrane serine protease expressed in normal breast tissue and carcinoma cells [7–9]. Activation of hepatocyte growth factor (HGF), urokinase plasminogen activator (uPA), and PAR-2 by matriptase cleaves the extracellular matrix and can induce tumor invasiveness and metastasis [10–12]. Higher expression of matriptase has been proved to be associated with tumor progression in uterine cervical and ovarian cancer [13,14].

Survivin is a 16.3 kD protein consisting of 142 amino acids and belongs to the protein family that inhibits cellular apoptosis [17]. Abundant embryonic tissue and most malignant cells significantly increase levels of survivin expression [18]. Survivin has multifunctional effects on cell proliferation [19], angiogenesis [20–22] and inhibition of apoptosis [18], but detailed mechanisms are still vague [23]. In a recent study, expression of survivin was revealed to be associated with Her2/neu, vascular endothelial growth factor (VEGF), uPA and PAI-1 elevation [23]. Although several cell-culture studies have been performed, correlation between survivin and the clinicopathological parameters of breast cancer are still paradoxical [23–27].

In this study, we tested the hypothesis that higher expression of matriptase and survivin have an effect on prognostic significance in breast carcinoma and malignant potential in breast hyperplastic lesions. In addition, we compared the expression of these two biomarkers in breast neoplasms to investigate whether or not co-upregulation effects exist.

## 2. Materials and methods

Paraffin-embedded tumor tissues were obtained from the Department of Pathology, Tri-Service General Hos-

pital, and tissue microarray slides were constructed. Two hundred and ninety cases were involved in this study, including 11 cases with normal breast tissue, 27 with fibrocystic disease, 17 fibroadenoma, 6 atypical ductal hyperplasia, 39 low-grade DCIS, 39 high-grade DCIS, 27 grade I IDC, 78 grade II IDC, and 46 grade III IDC. Histopathological gradings of ductal carcinoma in situ and invasive carcinoma were determined according to criteria of the WHO grading system [1].

One core was taken from a selected area of each paraffin-embedded tumor tissue and tissue microarray slides were constructed. Each representative core in the tissue microarray slide was 2 mm in diameter and the pathological diagnosis in these cases was reviewed by at least two experienced pathologists. Tissue microarray slides showed uniform staining representative of the original paraffin-embedded specimens. All tumors were pathologically staged according to the 1997 American Joint Committee on Cancer (AJCC/TNM system). None of these cases had ever received radiation or chemotherapy before surgery.

### 2.1. Immunohistochemistry

Tissue microarray sections were de-waxed in xylene, rehydrated in alcohol, and immersed in 3% hydrogen peroxide for 5 minutes to suppress endogenous peroxidase activity. Antigen retrieval was performed by heating (100°C) each section for 30 minutes in 0.01 mol/L sodium citrate buffer (pH 6.0). After 3 rinses (each for 5 minutes in phosphate buffered saline [PBS]), sections were incubated for 1 hour at room temperature with a rabbit anti-human matriptase/ST14 antibody (1:100, BETHYL Laboratories, Montgomery, TX, USA) and a rabbit anti-human survivin antibody (1:200, R&D System, Wiesbaden, Germany) diluted in PBS. After 3 washes (each for 5 minutes in PBS), sections were incubated with biotin-labeled secondary immunoglobulin (1:100, DAKO, Glostrup, Denmark) for 1 hour at room temperature. After 3 additional washes, peroxidase activity was developed with AEC+ substrate chromogen (DAKO, Glostrup, Denmark) at room temperature. Sections of normal stromal tissue of breast were used as negative controls.

For evaluation of immunoreactivity and histological appearance, all tissue microarray experiments were repeated two times and the slides were examined and scored by two authors concurrently. For assessment of matriptase and survivin immunostaining scores, the intensity of cytoplasmic and membranous immunostaining was scored on a scale of 0 (no staining) to 4

Table 1  
Matriptase and survivin immunostaining scores in breast disease

	% staining	Intensity	Total score
<i>Matriptase expression</i>			
FCD ( <i>n</i> = 27)	43.8 ± 5.9	1.0 ± 0.1	52.7 ± 7.7
Fibroadenoma ( <i>n</i> = 17)	66.5 ± 6.6	1.1 ± 0.1	76.5 ± 10.9
ADH ( <i>n</i> = 6)	66.8 ± 10.7	1.2 ± 0.2	81.7 ± 13.4*
DCIS, low grade ( <i>n</i> = 39)	80.8 ± 4.9*	1.6 ± 0.1*	133.7 ± 14.7* <sup>+</sup>
DCIS, high grade ( <i>n</i> = 39)	81.1 ± 3.5*	2.0 ± 0.2*	155.8 ± 15.7* <sup>+</sup>
IDC, grade I ( <i>n</i> = 27)	82.3 ± 4.6*	2.1 ± 0.2*	172.7 ± 14.8* <sup>+</sup>
IDC, grade II ( <i>n</i> = 78)	87.4 ± 1.9*	2.4 ± 0.1*	211.7 ± 11.3* <sup>+</sup>
IDC, grade III ( <i>n</i> = 46)	90.7 ± 1.8*	2.4 ± 0.2*	221.2 ± 16.2* <sup>+</sup>
Normal breast tissue ( <i>n</i> = 11)	35.9 ± 9.9	0.9 ± 0.2	44.1 ± 15.4
<i>Survivin expression</i>			
FCD ( <i>n</i> = 27)	69.4 ± 4.0	1.6 ± 0.1	113.1 ± 8.6*
Fibroadenoma ( <i>n</i> = 17)	75.6 ± 5.2	1.9 ± 0.2	150.6 ± 19.2*
ADH ( <i>n</i> = 6)	76.7 ± 7.6	2.0 ± 0.3	161.7 ± 19.9*
DCIS, low grade ( <i>n</i> = 39)	85.7 ± 2.8*	2.3 ± 0.1*	198.4 ± 12.0* <sup>+</sup>
DCIS, high grade ( <i>n</i> = 39)	86.4 ± 2.6*	2.5 ± 0.2*	217.8 ± 18.4* <sup>+</sup>
IDC, grade I ( <i>n</i> = 27)	87.3 ± 3.4*	2.7 ± 0.2*	240.7 ± 21.0* <sup>+</sup>
IDC, grade II ( <i>n</i> = 78)	88.6 ± 1.7*	2.8 ± 0.1	247.7 ± 12.5* <sup>+</sup>
IDC, grade III ( <i>n</i> = 46)	90.4 ± 1.9*	3.0 ± 0.4*	268.0 ± 13.7* <sup>+</sup>
Normal breast tissue ( <i>n</i> = 11)	55.5 ± 6.2	1.3 ± 0.2	64.5 ± 4.7

Data are means ± standard error of the mean (SEM) of the matriptase and survivin immunostaining scores in normal breast tissue and neoplasm. \**p* < 0.05 vs. normal breast tissue. <sup>+</sup>*p* < 0.05 vs. atypical ductal hyperplasia.

Abbreviations: fibrocystic disease, FCD; atypical ductal hyperplasia, ADH; ductal carcinoma in situ, DCIS; invasive duct carcinoma, IDC.

(strongest intensity), and the percentage of cells with stained cytoplasm or plasma membrane was estimated at each intensity. The percentage of cells (from 0 to 100) was multiplied by the corresponding immunostaining intensity (from 0 to 4) to obtain an immunostaining score ranging from 0 to 400.

## 2.2. Statistical analysis

All results are expressed as mean ± standard error of the mean (S.E.M.). The immunostaining scores of matriptase and survivin in breast diseases were compared with the score in normal breast epithelia. Statistical analysis was performed using the ANOVA test between groups. A *p*-value of less than 0.05 was considered to be statistically significant. SigmaStat software (Jandel Scientific, San Rafael, CA, USA) was used to perform linear regression testing and Pearson or Spearman correlation test to analyze the relationship between expression of these two biomarkers and clinicopathological parameters in breast IDC cases.

In addition, survival time of subjects was calculated from the date of surgery to the date of death. 121 of 151 IDC patients in the study received 5-year follow up; subjects were divided into 2 groups in order to compare survival times with matriptase and survivin immunostaining scores. Statistical analysis of survival time was done using the Kaplan-Meier survival test.

## 3. Results

### 3.1. Clinicopathological characteristics

Among all 151 breast IDC cases, age distribution ranged from 27 to 89 years and mean age was 54.3. The average tumor size was 3.5 cm in diameter. Other information, including histopathological grading and staging distribution, are shown in Tables 1, 2 and 3.

### 3.2. Matriptase expression in breast diseases

Matriptase immunoreactivity was seen on the cell membrane and cytoplasm of all 290 cases, with varying intensities and percentages (Table 1). The average matriptase immunostaining score was 44.1 in normal breast epithelium (Fig. 1A–1B); 52.7 in fibrocystic disease (Fig. 1D–1E); 76.5 in fibroadenoma (Fig. 1G–1H); 81.7 in atypical ductal hyperplasia (Fig. 1J–1K); 133.7 in DCIS, low grade (Fig. 1M–1N); and 155.8 in DCIS, high grade (Fig. 1P–1Q). In addition, among 151 IDC cases, the average immunostaining scores of matriptase were 172.7 for grade I (Fig. 2A–2B), 211.7 for grade II (Fig. 2D–2E), and 221.2 for grade III (Fig. 2G–2H). Through a series of statistical analyses, significant positive correlation of matriptase staining scores with his-

Table 2  
The immunostaining patterns of matriptase and clinicopathological parameters of breast invasive ductal carcinoma

TNM stage	No. of cases	Average intensity	Average % tumor	Average score	Correlation
T stage					
T1	39	2.0	80.9	168.6	Positive correlation ( $P = 0.009$ )
T2	49	2.2	88.4	196.8	
T3	37	2.5	88.7	223.1	
T4	26	2.7	89.0	244.5	
N stage					
N0	47	2.0	83.1	171.9	Positive correlation ( $P = 0.011$ )
N1	46	2.2	86.7	194.6	
N2	32	2.6	87.3	232.1	
N3	26	2.6	91.7	245.6	
M stage					
M0	107	2.2	85.4	198.0	No correlation ( $P = 0.30$ )
M1	44	2.4	86.3	218.0	
AJCC stage					
Stage I	36	1.9	81.1	161.1	Positive correlation ( $P = 0.038$ )
Stage II	49	2.0	87.1	176.3	
Stage III	22	2.4	89.3	215.7	
Stage IV	44	2.4	89.5	218.0	

Table 3  
The immunostaining patterns of survivin and clinicopathological parameters of breast invasive ductal carcinoma

TNM stage	No. of cases	Average intensity	Average % tumor	Average score	Correlation
T stage					
T1	39	1.6	82.0	132.2	Positive correlation ( $P = 0.005$ )
T2	49	2.0	86.9	175.8	
T3	37	2.4	93.3	224.0	
T4	26	3.3	95.2	317.5	
N stage					
N0	47	1.8	84.4	150.8	Positive correlation ( $P = 0.028$ )
N1	46	2.0	87.4	173.7	
N2	32	2.7	91.4	245.9	
N3	26	3.3	93.5	298.8	
M stage					
M0	107	2.8	87.0	210.4	Positive correlation ( $P = 0.047$ )
M1	44	3.1	94.8	295.6	
AJCC stage					
Stage I	36	1.6	82.4	133.0	Positive correlation ( $P = 0.017$ )
Stage II	49	2.0	88.3	172.0	
Stage III	22	2.7	92.4	248.6	
Stage IV	44	3.1	94.8	295.6	

tological grading system of breast IDC was confirmed (Table 1,  $P < 0.05$ ).

Among all breast IDC cases, 39 cases of stage T1 were found, 49 stage T2 cases, 37 stage T3 cases, and 26 stage T4 cases. In this study, more advanced stages of breast IDC had higher matriptase intensity and immunostaining scores. The average immunostaining score of matriptase was 168.6 in stage T1, 196.8 in stage T2, 223.1 in stage T3, and 244.5 in stage T4 cases of breast IDC. Positive correlation of matriptase staining scores with T stages was validated based on P values of less than 0.05. In addition, more advanced

N (nodal) stages of breast IDC are also associated with higher intensity, greater percentage of tumor staining and immunostaining scores of matriptase expression. Finally, we divided breast IDC cases from stages I to IV based on the clinical staging system. The immunostaining scores of various clinical stages of breast IDC are as follows: 161.1 for stage I, 176.2 for stage II, 215.7 for stage III, and 218.0 for stage IV. The immunostaining scores of matriptase positively correlated with the clinical staging system ( $P < 0.05$ , Table 2 and Fig. 3).

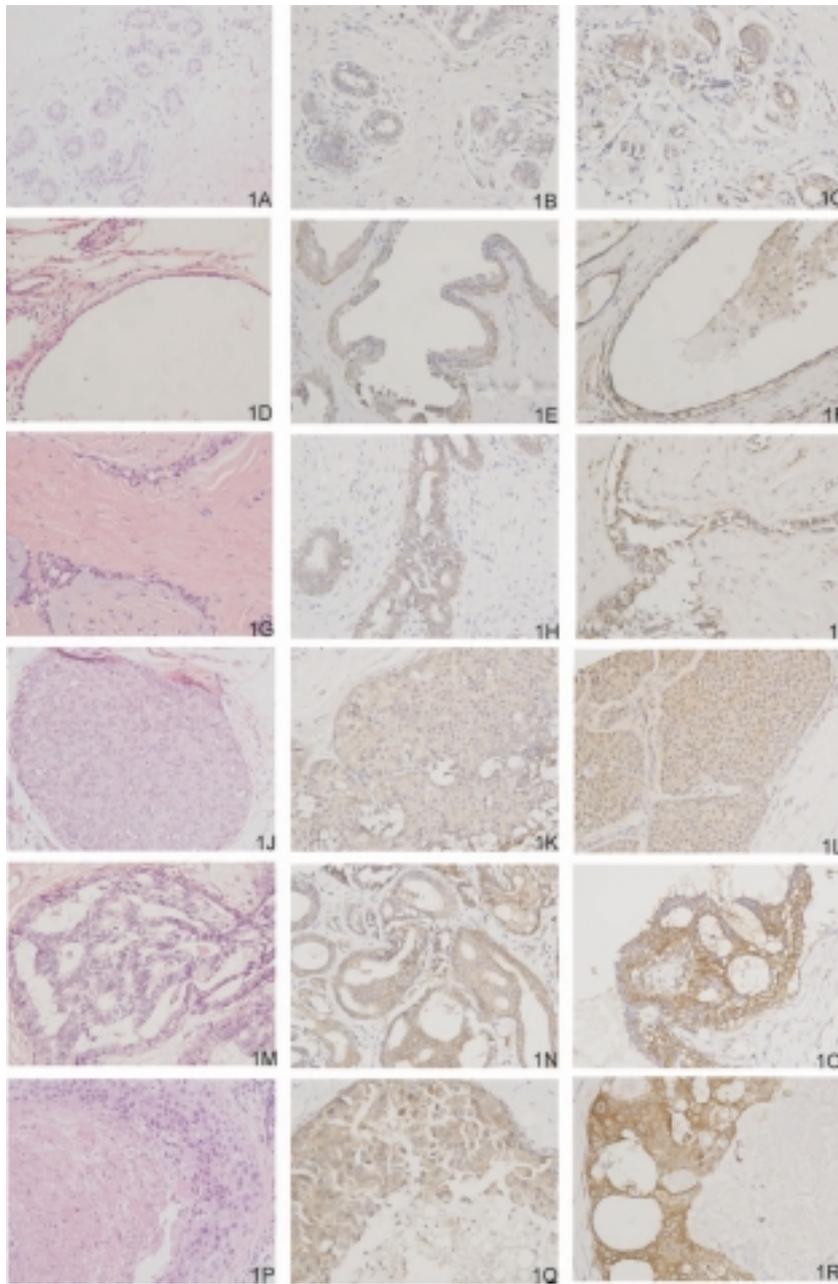


Fig. 1. Hematoxylin and eosin staining of normal breast tissue (1A), fibrocystic disease (1D), fibroadenoma (1G), atypical ductal hyperplasia (1J), low-grade DCIS (1M), and high-grade DCIS (1P). Immunohistochemical analysis of matriptase in normal breast tissue (1B), fibrocystic disease (1E), fibroadenoma (1H), atypical ductal hyperplasia (1K), low-grade DCIS (1N), and high-grade DCIS (1Q). Immunoreexpression of survivin in normal breast tissue (1C), fibrocystic disease (1F), fibroadenoma (1I), atypical ductal hyperplasia (1L), low-grade DCIS (1O), and high-grade DCIS (1R). (Original magnification X 400).

### 3.3. *Survivin expression in breast diseases*

The immunostaining scores of survivin in normal breast tissue and various diseases are shown in Tables 1 and 3. Survivin immunoreactivities were present

on the cell membrane and cytoplasm of all 290 cases, with varying intensities and percentages of tumor cells. Immunostaining scores of survivin were 64.5 in normal mammary glands (Fig. 1C), 113.1 in fibrocystic disease (Fig. 1F), 150.6 in fibroadenoma (Fig. 1I),

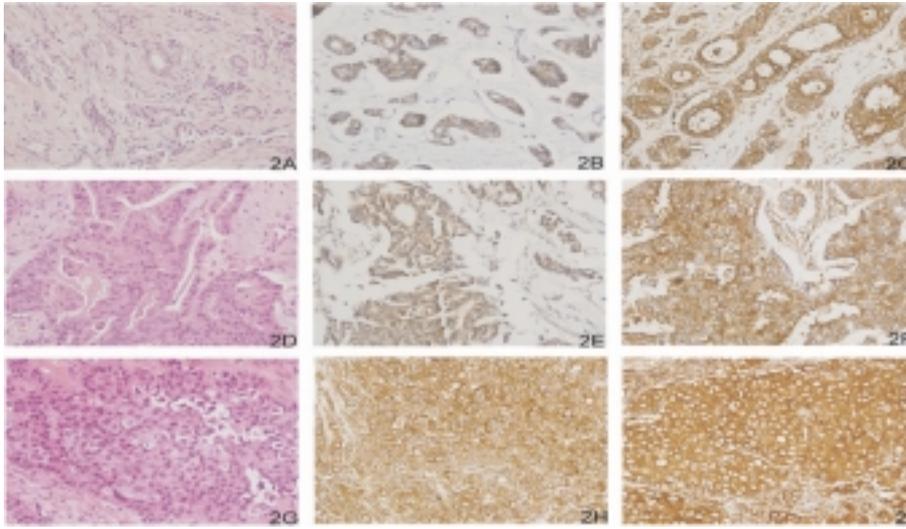


Fig. 2. Hematoxylin and eosin staining of IDC, grade I (2A); IDC, grade II (2D); and IDC, grade III (2G). Immunohistochemical analysis of matriptase in IDC, grade I (2B), IDC, grade II (2E), and IDC, grade III (2H). Immunoexpression of survivin in IDC, grade I (2C), IDC, grade II (2F), and IDC, grade III (2I). (Original magnification X 400).

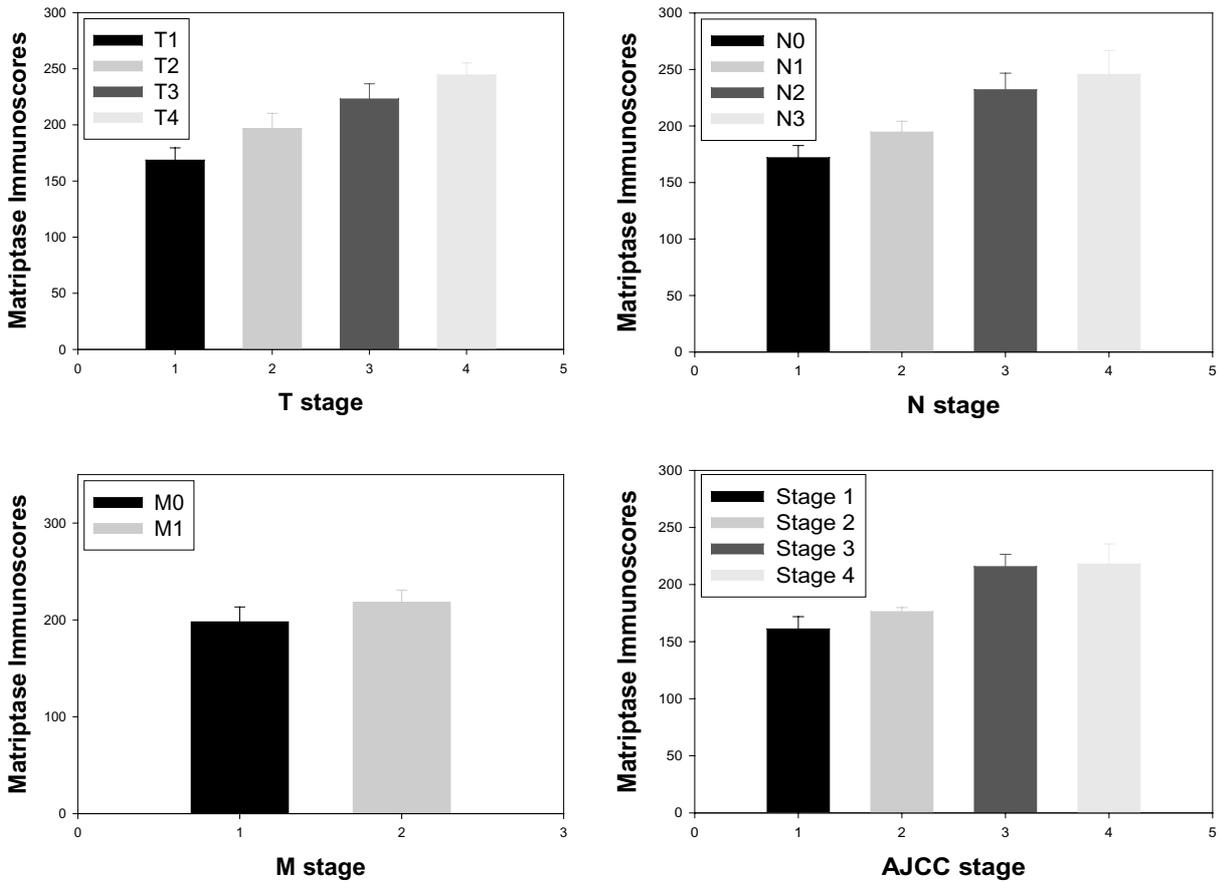


Fig. 3. Clinicopathological correlations with matriptase immunostaining scores in breast IDC.

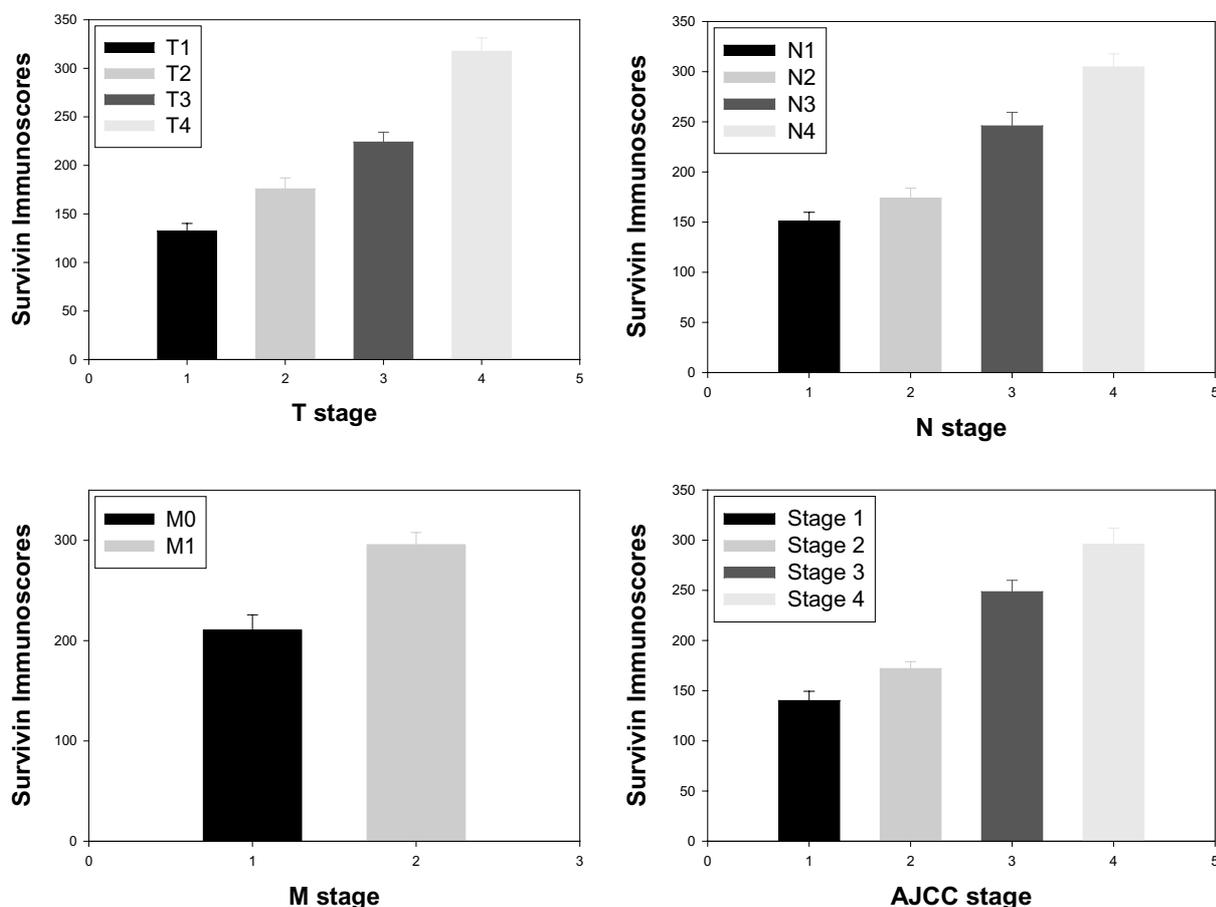


Fig. 4. Clinicopathological correlations with survivin immunostaining scores in breast IDC.

161.7 in atypical ductal hyperplasia (Fig. 1L), 198.4 in low-grade DCIS (Fig. 1O), and 217.8 in high-grade DCIS (Fig. 1R). Among 151 breast cancer cases, average immunostaining scores were 240.7 in IDC, grade I (Fig. 2C); 247.7 in IDC, grade II (Fig. 2F); and 268.0 in IDC, grade III (Fig. 2I). Statistical analysis showed positive correlation of survivin immunostaining scores with histological grading of breast cancer (Table 1,  $P < 0.05$ ).

Average immunostaining scores of survivin were 132.2 for stage T1, 175.8 for stage T2, 224.0 for stage T3, and 317.5 for stage T4 cases of breast IDC. T (tumor size) stages correlated significantly with higher survivin immunostaining scores ( $P < 0.05$ ). In addition, more advanced N and M stages of breast IDC cases had significantly higher immunostaining scores of survivin expression. Immunostaining scores of survivin in various clinical stages of breast cancer cases are as follows: 133.0 for stage I, 172.0 for stage II, 248.6 for stage III, and 295.6 for stage IV. Immunostaining

scores of survivin also correlated significantly with the clinical staging system ( $P < 0.05$ , Table 3 and Fig. 4).

#### 3.4. Relationship of matriptase and survivin expression with survival time

We divided 121 RCC cases that had received 5-year follow up into two groups based on matriptase and survivin immunostaining scores, respectively. According to matriptase immunostaining scores, 65 cases had higher expression (immunostaining score  $\geq 210$ ) in one group and, in the other group, the remaining 56 cases had lower immunoreactivity (immunostaining score  $< 210$ ). Similarly, these included cases after follow up were also divided into two groups based on survivin immunostaining scores. There were 58 cases of higher survivin expression (immunostaining score  $\geq 250$ ) and 63 cases of lower survivin immunoreactivity (immunostaining score  $< 250$ ). Using matriptase and survivin immunostaining

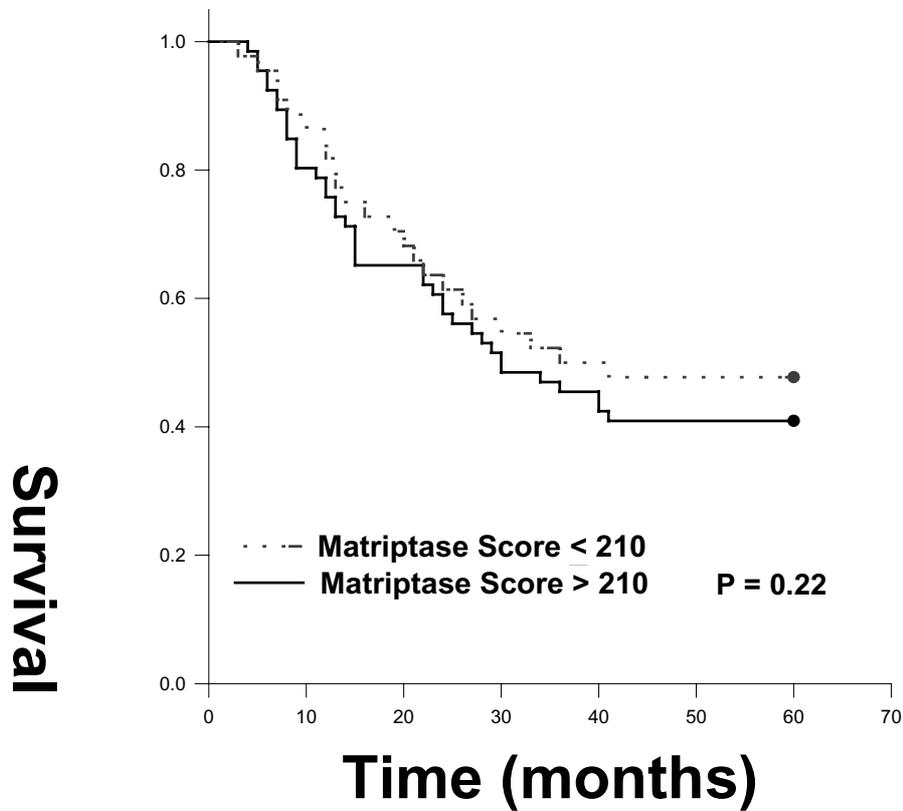


Fig. 5. Overall survival of 121 patients with breast IDC. Higher matriptase immunostaining scores were associated with poorer survival, but did not reach statistical significance. Survival rates were analyzed using the Kaplan-Meier survival test.

as variable parameters, higher scores in these biomarkers were associated with higher mortality, and survivin immunoscores reached statistical significance (Figs 5 and 6).

### 3.5. Correlation between matriptase and survivin in breast IDC

Significantly higher matriptase immunostaining scores correlated positively with survivin immunostaining scores in breast IDC cases. Correlation between matriptase and survivin immunostaining scores for all 151 breast IDC cases is shown in Fig. 7.

## 4. Discussion

Breast cancer is one of the most common cancers, representing more than 25% of all malignancies in Chinese women [28]. In clinical follow up study, intraductal proliferative lesions of breast may be associated with cancer development. Risks of breast IDC

development were 1.5-fold in usual ductal hyperplasia, 4- to 5-fold in atypical ductal hyperplasia, and 8- to 10-fold in DCIS [30]. However, many similarities are still found in ductal hyperplasia and DCIS based on histopathologic features. In several studies, erbB-2 has been proved to be associated with high histological grades of breast cancer, but was shown to have little effect on the distinction between these premalignant lesions [30].

Matriptase is an epithelial-derived, integral serine protease that has been associated with cancer cell invasion and metastasis [31]. Expression of matriptase had been detected in normal human tissue, especially in glandular and squamous epithelia [32]. Latent uPA and pro-HGF are substrates for matriptase. These two substrates both participate in tumor progression through reactions with the extracellular matrix [10,11]. Matriptase is highly expressed by various carcinomas, such as cancer of the breast, ovary, colon, endometrium, prostate and stomach [14,35,36]. However, even though we see a relationship between matriptase expression and clinicopathological parameters in breast

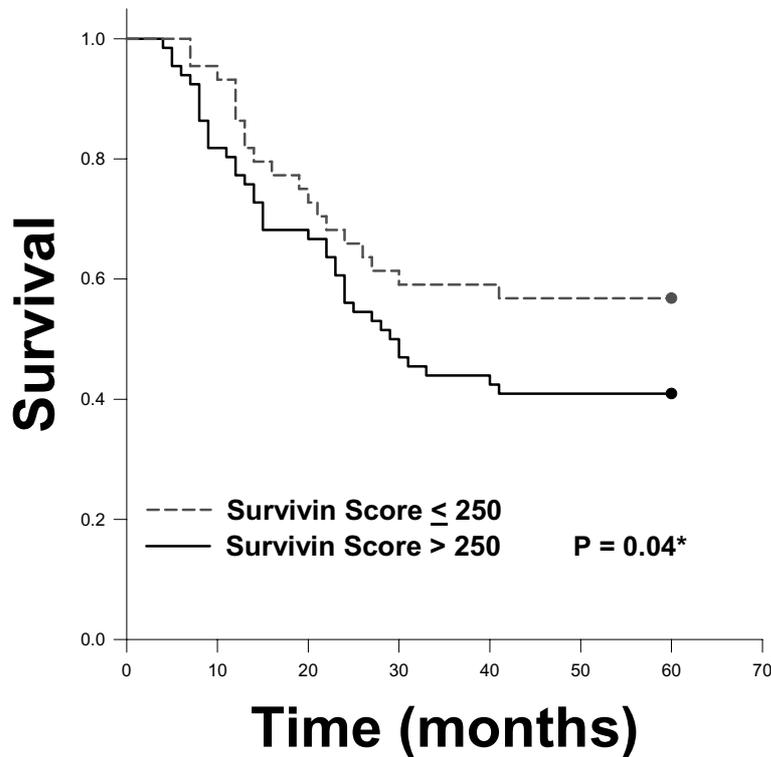


Fig. 6. Overall survival of 121 patients with breast IDC. Higher survivin immunostaining scores were associated with poorer survival, and reach statistical significance. Survival rates were analyzed using the Kaplan-Meier survival test.

cancer, discrimination of intraepithelial proliferative lesions is still vague.

Survivin, an anti-apoptotic factor, is associated with increased tumor aggressiveness and a poorer prognosis in several malignancies, including nasopharyngeal, esophageal, liver, pancreatic, colorectal, renal, urinary bladder, and hematological malignancies [37–44]. In some *in vivo* studies, survivin expression has been proved to be a prognostic factor [23,24], and the up-regulation of survivin may be associated with p53 gene expression [26]. However, the detailed mechanism of survivin expression is not clear in breast cancer. Direct evidence still lacks to show correlation between survivin expression and clinicopathological staging of the breast cancer in large clinical studies.

In the current study, we first demonstrated that matriptase and survivin immunostaining scores indeed correlated with histological grading and clinical staging in Chinese women with breast cancer. The immunostaining scores of atypical ductal hyperplasia in matriptase were  $81.7 \pm 13.4$ . It represented that the range of immunostaining scores in 95% atypical ductal hyperplasia cases was from 68 to 94. The suggestive cut-off value of immunostaining scores of matriptase

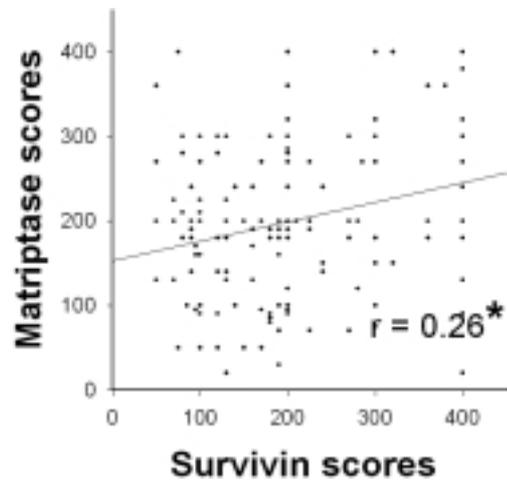


Fig. 7. Correlation of matriptase immunostaining scores with survivin immunostaining scores in breast IDC cases. \* indicates statistical significance of linear regression testing ( $p < 0.05$ ).

tase between atypical ductal hyperplasia and low grade DCIS was 95. Similarly, the suggestive cut-off value of immunostaining scores of survivin between atypical ductal hyperplasia and low grade DCIS was 180.

In previous studies, both matriptase and survivin have been proved to be associated with uPA activation, but the relationship between the two was still not clear [14,23]. Our study, on the other hand, demonstrated positive correlation between matriptase and survivin immunohistochemical scores in Chinese women with breast IDC. The up-regulation of matriptase associated with survivin elevation in breast cancer may also have adjuvant effects on tumor progression in breast cancer.

In our study, all tumor tissues were placed in a single tissue array slide. The tissue microarray technique is a powerful tool for simultaneous histological and immunohistochemical evaluation of tumors [45]. Previous studies measuring immunohistochemical intensity of individual cases were limited because of the variability of the chemical signal generated under different environmental conditions [45]. However, the advantage of tissue microarray is that all tissues are under the same conditions and evaluated in one slide [45]. In this study, the clear cut difference seen between epithelial and stromal components in breast tissue by using matriptase or survivin immunostaining revealed the credibility of using tissue microarray slides. Therefore, immunostaining scores used in our study could reflect the relative protein expression levels of matriptase and survivin in breast tissue.

## 5. Conclusion

In conclusion, higher matriptase and survivin immunoscores were correlated significantly with more advanced TNM stages of breast IDC cases. Although there are still unknown mechanisms in tumor progression, we demonstrated that matriptase and survivin were applicable biomarkers to predict clinicopathological parameters. Therefore, the development of pharmacological agents to target the survivin pathway may prolong survival time and slow tumor progression in patients with breast IDC.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contribution

WCT and JSJ designed the study, counted the immunohistochemical scores of these two biomarkers, and reviewed the histopathology in breast cancer. CPY and LFS diagnosed all included cases in this study and graded breast cancer. JCY provided tissue blocks and clinical data. AC performed statistical analysis and HC performed immunohistochemical stains for these cases.

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