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ORIGINAL PAPER

Prognostic value of TMPRSS4 expression in patients with breast cancer

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Abstract Transmembrane protease, serine 4 (TMPRSS4), is a novel type II transmembrane serine protease that is highly expressed on the cell surface in pancreatic, thyroid, lung, and other cancer tissues, although its oncogenic significance and molecular mechanisms are unknown. In a series of 109 primary breast cancer patients, we performed a comprehensive analysis of TMPRSS4 expression using immunohistochemistry. The relationship between TMPRSS4 expression and the clinicopathological characteristics or prognosis was evaluated. Results showed that breast cancer tissues exhibited higher levels of TMPRSS4 expression compared with benign tissues (65.1 versus 17.5 %, $P < 0.001$). High expression of TMPRSS4 was significantly correlated with lymph node metastasis ($P < 0.001$), high pathological grade ($P = 0.001$), and tumor size >2 cm ($P = 0.006$), but not correlated with other clinicopathological parameters, including the patient's age ($P = 0.289$), menopausal status ($P = 0.300$), histological subtype ($P = 0.418$), and status of estrogen receptor (ER) ($P = 0.913$), progesterone receptor (PR) ($P = 0.247$), and HER-2 ($P = 0.882$). Patients with high expression of TMPRSS4 had shorter OS and DFS than those with low expression ($P = 0.0009$ and $P = 0.0044$, respectively). TMPRSS4 expression and lymph node metastasis were independent prognostic factors for both OS and DFS by multivariate analysis. Based on our results, we propose TMPRSS4 as a putative biological marker for breast cancer and as an indicator of poor prognosis.

Keywords Type II transmembrane serine protease · Breast cancer · Prognosis · Immunohistochemistry

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Introduction

Breast cancer is one of the most common malignancies in women and has become the second leading cause of death for women worldwide [1]. Despite advances in breast cancer prevention, diagnosis, and therapy, the prognosis and survival for most patients have not dramatically changed [2]. To date, adjuvant systemic therapy in women with early-stage disease is guided by prognostic and predictive factors, including stage, grade, estrogen receptor (ER) and progesterone receptor (PR) status, and HER2 amplification [3]. Due to the heterogeneity within specific subgroups of breast cancer and the inter-observer variability with detection frequencies, not all breast cancers can be successfully classified into specific risk groups based on the expression profile of these traditional markers alone. Therefore, new prognostic and predictive factors are still required to optimize treatments among these patients.

In normal tissues, cell surface proteases are involved in regulating cellular activities, such as cell–cell interaction, adherence to matrix components, motility, and homeostasis. Overexpression of these proteases is linked to cancer progression [4], because malignant cells require a range of proteolytic activities to enable growth, survival, motility, invasion, and digestion of the extracellular matrix. The transmembrane protease, serine 4 (TMPRSS4) gene, initially referred to as TMPRSS3, is located on chromosome

11.q23.3 and encodes a member of the type II transmembrane serine proteases (TTSPs) family [4]. It has been reported that TMPRSS4 is upregulated in pancreatic cancer [5] and has been suggested as a diagnostic marker for the malignant thyroid neoplasms [6]. Jung et al. [7] reported that TMPRSS4 controls the invasive and metastatic potential of human cancer cells by facilitating an epithelial–mesenchymal transition (EMT). Kim et al. [8] also demonstrated that TMPRSS4 induced integrin alpha5 expression and its signal transduction in human colorectal cancer tissues, leading to invasiveness and EMT accompanied by downregulation of E-cadherin. Recent qPCR studies showed that high levels of TMPRSS4 message in non-small-cell lung cancer (NSCLC) patients were associated with a poor prognosis [9]. In addition, siRNA knockdown of TMPRSS4 in cancer cell lines and in metastatic potential mouse model reduced cell invasion and migration, thus implying a role for TMPRSS4 in metastasis [10].

In view of the evidence for the expression of TMPRSS4 at the transcriptional level in pancreatic, colorectal, and thyroid cancers, and NSCLC via Northern blot analyses, microarray gene-chips, and RT-PCR [6, 11–13], endogenous protein expression of TMPRSS4 in normal breast and breast cancer cells has not been examined. The objectives of the present investigation were to study the connection between TMPRSS4 expression and breast cancer progression and evaluate their potential relation to the clinical outcome.

Materials and methods

Patients

This study used archival material from the Department of Pathology, No. 202 Hospital in Shenyang, including the tissues from 109 consecutive patients with histologically confirmed breast cancer and 40 benign tissue samples between January 2005 and August 2007, which was harvested from the patients treated by surgical resection. All the patients included in present study did not receive any chemotherapy and radiation therapy before, and their complete clinical data, including age, menopausal status, histological type, lymph nodes status, tumor size, grade, ER status, PR status, and HER2 status, were available and reviewed. Males were excluded and all patients were females.

Outcome data include survival status, overall survival (OS) time, disease-free survival (DFS) time. DFS and OS times were defined as the time interval from the date of surgery to the date of first recurrence or death, which were the two assessments used for prognostic analyses.

Informed consent was obtained from all patients, and all healthy controls for the use of their samples to detect TMPRSS4 expression. The present study conformed to the ethical standards of the World Medical Association Declaration of Helsinki and was approved by the Ethics Committee of No. 202 Hospital.

Immunohistochemical staining

Immunohistochemical analysis of breast tissue was performed as described previously before. Briefly, paraffin sections were cut at 4 μm thickness, mounted on silane coated slides, and incubated overnight at 37 °C. Sections were washed with distilled water after two changes of xylene and three changes of ethanol. Antigen retrieval was performed using citrate buffer (pH 6.0), and sections were held in Tris buffered saline (TBS). Endogenous peroxidase activity was blocked by incubation in 3 % hydrogen peroxide. The sections were incubated overnight in primary antibody (Proteintech Group, Inc., China) diluted with 1/50 in 1 % BSA in Tris buffer (100 mM, pH 7.6) at room temperature. Antibody binding was amplified using horseradish peroxidase-conjugated goat anti-rabbit IgG for 15 min each, and the complex was visualized using DAB Horseradish Peroxidase Color Development Kit ((Maixin Co., Fuzhou, China).

Sections were assessed microscopically for positive DAB staining. Two observers independently evaluated the immunostaining results. Semiquantitative expression levels were based on staining intensity and distribution. The percentage of positive-staining tumor cells was scored as follows: 0 (no positive tumor cells), 1 (<15 % positive tumor cells), 2 (15–50 %positive tumor cells), and 3 (>50 % positive tumor cells). In cytoplasm, staining intensity was graded as follows: 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The staining intensity score plus the percentage of positive staining was used to define the TMPRSS4 expression levels: 0–2, low expression and 3–6, high expression, which classified breast cancer patients into two groups.

Statistical analysis

All data were analyzed using SPSS 13.0 software (SPSS, Chicago, IL, USA). The two-sided Pearson's $\chi 2$ test and the Fisher's exact test were used to compare the clinicopathological parameters between low- and high-expression groups. For analysis of the follow-up data, DFS and OS were evaluated using the Kaplan–Meier method and log-rank tests. Univariate and multivariate Cox proportional hazards regression analyses were performed to evaluate the impact of expression of TMPRSS4 and other categorical factors on DFS and OS, respectively.

Results

Expression of TMPRSS4 in breast cancer tissue

Patients and tumor characteristics were summarized in Table 1. All patients were women with a mean age of 48.5 years (range 28–69 years). Expression of TMPRSS4 was examined in the breast cancer and benign tissues. Immunohistochemical examination showed that TMPRSS4 was located in the cytoplasm and cell membrane in breast cancer tissue (Fig. 1). The high expression of TMPRSS4 was 65.1 % (71/109) in 109 breast cancer specimens and 17.5 (7/40) in 40 benign tissue ($P < 0.001$). The different intensities of the staining were shown in Fig. 1.

Analysis of correlation of TMPRSS4 with clinicopathologic parameters

We analyzed the associations between the levels of TMPRSS4 expression and a series of clinicopathological characteristics, including age, histological type, tumor size, lymph node stage, grade, menopausal status, and status of ER, PR, and HER-2 in breast cancer patients (Table 2). High expression of TMPRSS4 was significantly correlated with, lymph node metastasis ($P < 0.001$), high pathological grade ($P = 0.001$), and tumor size >2 cm ($P = 0.006$), but not correlated with other clinicopathological parameters, including the patient's age ($P = 0.289$), menopausal status ($P = 0.300$), histological subtype ($P = 0.418$), and status of ER ($P = 0.913$), PR ($P = 0.247$), and HER-2 ($P = 0.882$).

Table 1 Clinicopathological characteristics of breast cancer patients

	Numbers	%
Age (years)		
≤50	62	56.9
>50	47	43.1
Menopausal status		
Premenopausal	59	54.1
Postmenopausal	50	45.9
Histological subtype		
Ductal	81	74.3
Lobular	28	25.7
LN metastasis		
Negative	69	63.3
Positive	40	36.7
Tumor size		
≤2 cm	31	28.4
>2 cm	78	71.6
Grade		
I, II	70	64.2
III	39	35.8
ER status		
Negative	61	56.0
Positive	48	44.0
PR status		
Negative	57	52.2
Positive	52	47.8
Her-2 status		
Negative	42	38.5
Positive	67	61.5

Association of TMPRSS4 expression with overall survival and disease-free survival in patients with breast cancer

The Kaplan–Meier 5-year survival curves stratified for TMPRSS4 expression were shown in Fig. 2a, b. Patients with high expression of TMPRSS4 had shorter OS and DFS than those with low expression ($P = 0.0009$ and $P = 0.0044$, respectively). Moreover, these data revealed that 5-year OS and DFS were 65.2 and 59.7 % in patients with high TMPRSS4 expression level, and 90.0 and 85.0 % in patients with low TMPRSS4 expression level, respectively. Univariate and multivariate analyses were carried out using Cox proportional hazard model to evaluate the impact of TMPRSS4 expression and pathological factors on the prognosis of breast cancer patients (Table 3). Univariate analysis of OS rate showed four statistically significant variables: lymph node metastasis ($P = 0.003$), tumor size ($P = 0.029$), grade ($P = 0.036$), and TMPRSS4 expression ($P = 0.006$). Univariate also showed that DFS rate was consistent with OS rate in terms of statistically significant variables: lymph node metastasis ($P = 0.002$), tumor size ($P = 0.037$), grade ($P = 0.031$), and TMPRSS4 expression ($P = 0.011$). In multivariate analyses, both TMPRSS4 expression ($P = 0.007$ and $P = 0.026$, respectively) and lymph node metastasis ($P = 0.004$ and $P = 0.016$, respectively) were associated with poor OS and DFS.

Discussion

Breast cancer is a major public health problem and the most common malignant tumor for women worldwide [14, 15]. Although the incidence of breast cancer is lower in China compared to that in western countries, it has increased by 80 % in young women in the past two decades, with a total increase in 50–100 % [16]. To date, established prognostic indicators for breast cancer include parameters such as tumor size, lymph node status, vascular invasion, ER status, PR status, Her2 gene amplification, and Ki67 status,

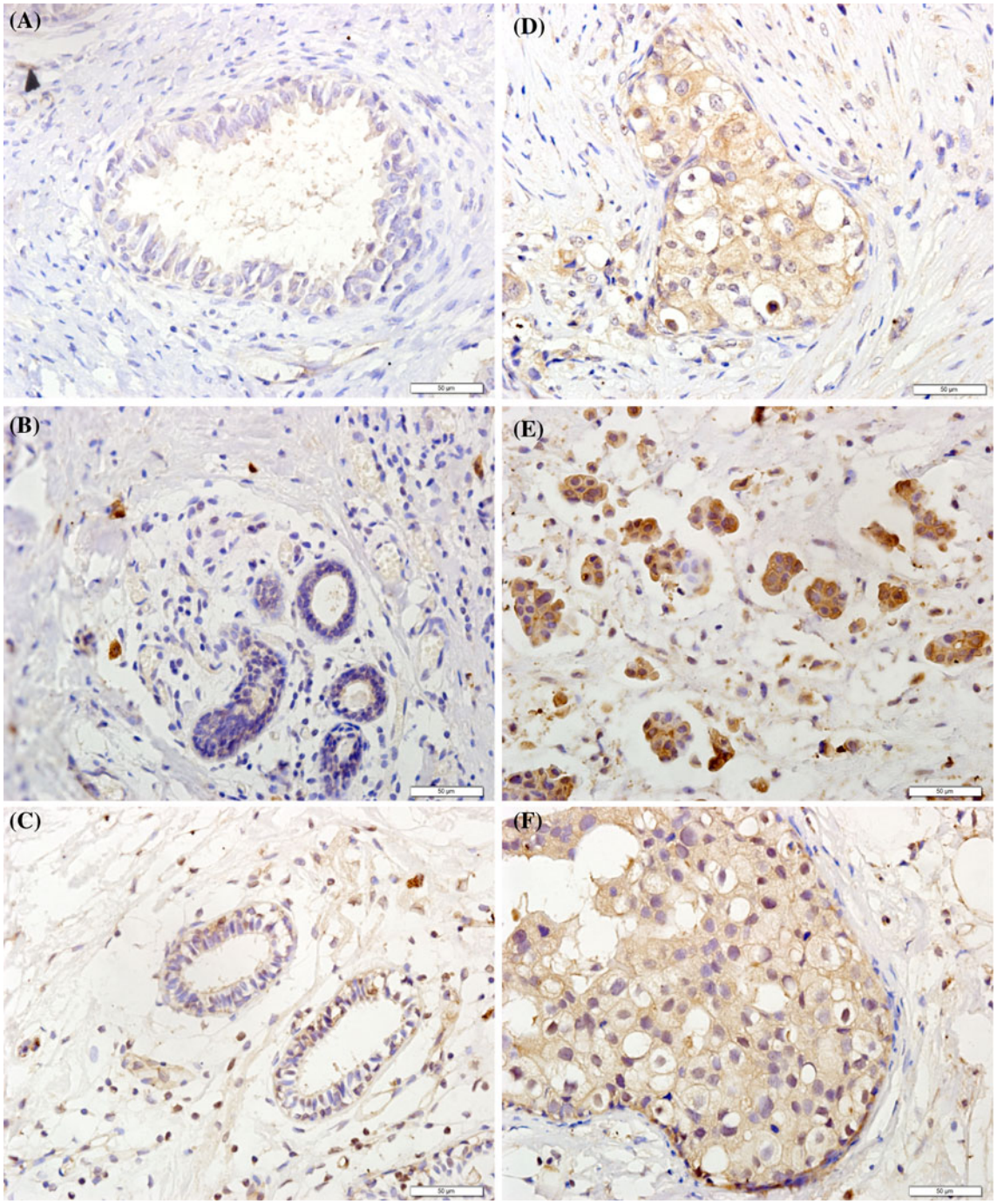

Fig. 1 Immunohistochemistry staining for TMPRSS4 in representative specimens. **a–c** Benign breast specimen; **d–f** breast cancer specimens. Positive TMPRSS4 staining in breast cancer tissues appeared as *brown* particles, which were mainly localized within the cytoplasm and cell membrane

which are derived from careful histological analysis of primary breast cancer samples [17]. These parameters help physicians to select adjuvant systemic therapy. However, these remain imperfect tools, in that some patients receive systemic chemotherapy even though they can be cured by surgery alone. In contrast, those who were categorized in low-risk group had short disease-free survival without receiving adjuvant chemotherapy. Therefore, it is necessary to identify some new prognostic and predictive factors for a more definitive insight into tumor biology and to substantiate the importance of the existing biomarkers.

Table 2 Correlation between TMPRSS4 expression and clinicopathological parameters

	Low TMPRSS4 expression	High TMPRSS4 expression	χ^2 value	*P* value
Age (years)				
≤50	19 (30.6 %)	43 (69.4 %)	1.126	0.289
>50	19 (40.4 %)	28 (59.6)		
Menopausal status				
Premenopausal	18 (30.5 %)	41 (69.5 %)	1.074	0.300
Postmenopausal	20 (40.0 %)	30 (60.0 %)		
Histological subtype				
Ductal	30 (37.0 %)	51 (73.0 %)	0.657	0.418
Lobular	8 (28.6 %)	20 (71.4 %)		
LN metastasis				
Negative	33 (47.8 %)	36 (52.2 %)	13.92	<0.001
Positive	5 (12.5 %)	35 (87.5 %)		
Tumor size				
≤2 cm	17 (54.8 %)	14 (45.2 %)	7.61	0.006
>2 cm	21 (26.9 %)	57 (73.1 %)		
Grade				
I, II	32 (45.7 %)	38 (54.3 %)	10.15	0.001
III	6 (15.4 %)	33 (84.6 %)		
ER status				
Negative	21 (34.4 %)	40 (65.6 %)	0.012	0.913
Positive	17 (35.4 %)	31 (64.6 %)		
PR status				
Negative	17 (29.8 %)	40 (70.2 %)	1.34	0.247
Positive	21 (40.4 %)	31 (59.6 %)		
Her-2 status				
Negative	15 (35.7 %)	27 (64.3 %)	0.022	0.882
Positive	23 (34.3 %)	44 (65.7 %)		

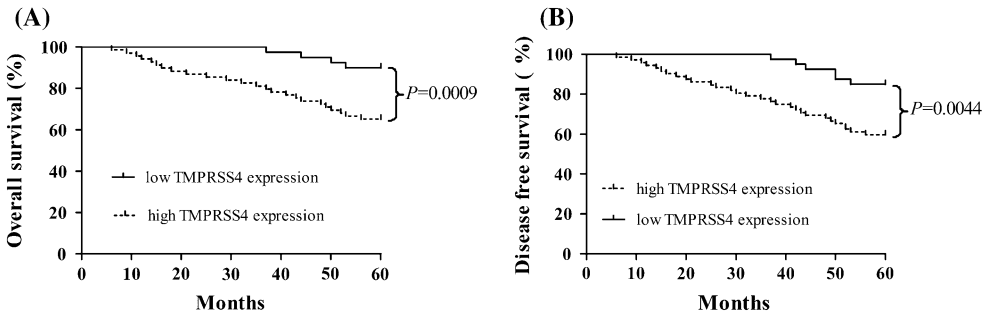

Fig. 2 Kaplan–Meier curves for overall survival (*OS*) and disease-free survival (*DFS*) of patients with breast cancer stratified by TMPRSS4 expression. **a** OS curves of breast cancer patients according to TMPRSS4 immunostaining; **b** DFS curves of breast cancer patients according to TMPRSS4 immunostaining. *P* values were obtained by log-rank test

The key observations obtained in the present study were (1) TMPRSS4 expression in breast cancer tissue was higher than benign breast tissue. (2) TMPRSS4 expression was associated with tumor size, lymph node metastasis, and grade, which reflects tumor progression. (3) DFS and OS time were significantly shorter for patients with increased TMPRSS4 expression. These findings may imply that the increased expression of TMPRSS4 plays an essential role in tumor progression and metastasis. Furthermore, high expression of TMPRSS4 may also predict the long-term outcome after surgery in breast cancer.

TMPRSS4, as a member of TTSPs family, was identified through its strong upregulation in pancreatic cancer, and its deduced sequence of 437 amino acids contains a

Table 3 Prognostic factors in the Cox proportional hazards model

Variables	Univariate			Multivariate		
	HR	95 % CI	*P*	HR	95 % CI	*P*
OS						
Age (≤50 versus >50 years)	1.236	0.821–2.350	0.633			
Menopausal status (pre versus post)	1.241	0.703–2.287	0.621			
Histological subtype (ductal versus lobular)	0.899	0.556–1.773	0.710			
Lymph node metastasis (negative versus positive)	3.313	1.571–7.081	0.003	3.168	1.897–6.711	0.004
Tumor size (≤2 cm versus >2 cm)	2.861	1.214–6.131	0.029	2.620	1.148–4.023	0.066
Grade (I, II versus III)	2.794	1.036–5.243	0.036	2.484	1.027–4.855	0.071
ER status (negative versus positive)	1.353	0.622–2.881	0.380			
PR status (negative versus positive)	1.485	0.741–3.073	0.296			
Her-2 status (negative versus positive)	1.372	0.626–2.963	0.349			
TMPRSS4 expression (low versus high)	3.237	1.346–6.954	0.006	3.415	1.514–6.559	0.007
DFS						
Age (≤50 versus >50 years)	1.278	0.735–2.220	0.623			
Menopausal status (pre versus post)	1.376	0.841–2.257	0.589			
Histological subtype (ductal versus lobular)	0.901	0.623–1.292	0.557			
Lymph node metastasis (negative versus positive)	3.839	1.618–7.352	0.002	3.001	1.814–6.502	0.016
Tumor size (≤2 cm versus >2 cm)	2.701	1.512–6.851	0.037	2.446	1.112–3.814	0.084
Grade (I, II versus III)	2.912	1.111–5.109	0.031	2.331	1.019–4.120	0.091
ER status (negative versus positive)	1.414	0.755–2.719	0.375			
PR status (negative versus positive)	1.512	0.781–3.114	0.229			
Her-2 status (negative versus positive)	1.386	0.631–2.988	0.391			
TMPRSS4 expression (low versus high)	3.146	1.301–6.625	0.011	3.137	1.321–6.125	0.026

OS overall survival, *DFS* disease-free survival, *HR* hazard ratio, *CI* confidence interval

serine protease domain with putative trypsin-like activity and a transmembrane domain [5]. TMPRSS4 is highly expressed in pancreatic, thyroid, lung, and colorectal cancers, but the biological functions of TMPRSS4 and its underlying mechanisms are not, however, well understood. Kim semi et al. reported that TMPRSS4 induces invasion, migration, and metastasis of cancer cells by facilitating the epithelial–mesenchymal transition (EMT) events, which EMT is a process implicated in the progression of early-stage noninvasive tumors to invasive malignancies [18, 19]. Jung et al. [7] found that depleting TMPRSS4 in cell lines established from lung and colon cancers using siRNA affected cell proliferation via regulation of cell cycle progression, invasion, and adhesion in vitro, which may be a result of downregulation of ERK1/2 and p38 MAPK activation. Further analysis of TMPRSS4-mediated signaling in cancer cells suggested that multiple downstream signaling pathways are activated including focal adhesion kinase (FAK) and extracellular signal regulated kinase (ERK) resulting in the downregulation of E-cadherin and induced expression of integrin α5, a critical molecule implicated in tumor cell invasion, migration, and tumor progression. Analyzing the relationship between TMPRSS4 expression and clinicopathological parameters of breast cancer, our study also showed that TMPRSS4 was highly expressed in breast cancer tissues compared with benign tissues and was significantly correlated with tumor size, tumor grade, and lymph node metastasis, suggesting that TMPRSS4 maybe participated in the progression of breast cancer and involved in EMT of breast cancer.

The next question we addressed was whether the expression of TMPRSS4 was associated with the clinical outcome in breast cancer. In our study, high TMPRSS4 expression was associated with poor DFS and OS in breast cancer, which is consistent with the evidence provided by Larzabal et al. [9], indicating that TMPRSS4 protein expression is involved in the formation and proliferation of breast cancer. Univariate and multivariate Cox regression analysis revealed that TMPRSS4 could serve as an independent prognostic factor for breast cancer. These results show for the first time (to the best of our knowledge) an association between high TMPRSS4 and poor prognosis.

A limitation of the present study is that it involves only correlative observations between TMPRSS4 expression and clinicopathological parameters in breast cancer, without direct evidence of the function and mechanism of

TMPRSS4. Furthermore, validation of the predictive significance of TMPRSS4 requires large-scale studies on homogenous populations. It is unlikely that clinicopathological subgroups of adequate size could be pooled within a single institution.

Conclusion

In conclusion, our study provides evidence that TMPRSS4 has an important role in the progression of breast cancer. Based on our results in patients, we propose TMPRSS4 as a putative biological marker for breast cancer and as an indicator of poor prognosis. Further in-depth studies are still needed to expand samples and elucidate the molecular mechanisms of TMPRSS4 in breast cancer.

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Conflict of interest The authors declare that they have no conflict of interest relating to the publication of this manuscript.

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