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Moesin is an independent prognostic marker for ER-positive breast cancer

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Abstract. Moesin, a cytoskeletal protein belonging to the ezrin-radixin-moesin family serves important roles in cell motility, invasion and metastasis. Moesin has been demonstrated to be of prognostic significance in tumor progression, due to its role in the metastatic process; however, its role in breast cancer is not well characterized. In the present study, the moesin expression was determined using immunohistochemistry in 404 and 46 patients with breast cancer and fibroadenoma, respectively, and the associations between moesin expression and the clinical parameters and prognostic values were analyzed. The positive rate of moesin protein expression was 47.8% (193/404) in breast cancer tissues, which was significantly higher than in fibroadenoma tissues (15.2%, 14/46). Overexpression of moesin was significantly associated with advanced clinical stage (P=0.002), positive lymph node metastasis (P<0.0001), and estrogen receptor (ER; P=0.008) and progesterone receptor (P=0.026) status. Patients with high moesin expression had significantly lower recurrence-free survival time, compared with patient with low moesin expression. Notably, overexpression of moesin was significantly associated with poor prognosis in patients with ER-positive breast cancer, and in patients treated with tamoxifen. Using a Cox proportional hazard regression model, further analysis was conducted, which demonstrated that moesin overexpression was a predictive prognostic factor for reduced overall survival time in patients with ER-positive breast cancer, and in patients treated with tamoxifen. These results indicated that moesin may be a potential marker for poor prognosis in patients with ER-positive breast cancer treated with tamoxifen. In conclusion, moesin serves an important role in the progression of breast cancer, and may be a valuable marker of breast cancer prognosis.

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Introduction

In females, breast cancer is the most common malignancy type, and is the second leading cause of cancer-associated mortalities globally in 2012 globally (1). The incidence of breast cancer is notably lower in China, compared with more developed regions, namely North America and Western Europe (2). The molecular and clinical heterogeneity of breast cancer renders it necessary to identify biomarkers of clinical outcomes, in order for patients to be treated with the most appropriate chemotherapeutic protocols; therefore, identification of biomarkers that will predict breast cancer prognosis is important for future development of individualized treatment for patients with breast cancer.

Moesin is an ezrin-radixin-moesin (ERM) family protein and connects the actin cytoskeleton to transmembrane receptors (3,4). It belongs to the band 4.1 superfamily, which share a 300-amino-acid domain termed the 4.1 ERM domain (5). ERM members serve an important role in regulating cell adhesion, migration and morphogenesis, by regulating actin cytoskeleton remodeling (6,7). Previously, ERM proteins were demonstrated to be correlated with endothelial cell migration, permeability, leukocyte diapedesis and conjugation between T cells and antigen-presenting cells (8-10). It also had been indicated that overexpression of moesin protein expression was associated with cancer progression in malignant cancer types, such as papillary thyroid carcinomas (11), glioblastoma tumors (12) and pancreatic cancers (13,14). Moesin protein expression was associated with increased tumor size, lymph node metastasis and invasion in oral squamous cell carcinoma (15). Additionally, in tumor cells, moesin delocalizes from the plasma membrane to the cytoplasm and has a higher incidence of lymph node metastasis (16-18). These studies indicated that moesin may be a potential molecular target for cancer therapy.

The role of moesin in breast cancer remains unclear. To date, a limited number of studies have demonstrated an association between moesin expression and clinicopathological parameters, as well as its prognostic role in breast cancer;

thus, in the present study, immunohistochemistry (IHC) was performed to investigate the expression of moesin in 404 patients with sporadic breast cancer and 46 patients with breast fibroadenoma, and its prognostic role, based on clinicopathological features, survival data and therapeutic responses, was analyzed.

Materials and methods

Ethics statement. The Medical Ethics Committee of China Medical University (Shenyang, China) approved the present study. Due to the retrospective nature of the study, the ethics committee waived the requirement of written informed consent by the patients. All of the samples were anonymous.

Patients and tissue specimens. A total of 404 breast cancer tissue specimens and 46 breast fibroadenoma tissue specimens were obtained from patients who underwent surgery at the Department of Surgical Oncology, First Hospital of China Medical University (Shenyang, China) between January 2005 and December 2009. Table I summarizes the clinicopathological data of the 404 patients with breast cancer. The average age of the breast cancer patients was 51 years (range, 20-82 years). Breast cancer was staged according to the Tumor-Node-Metastasis (TNM) staging system (19), as follows: Stage I (n=106); stage II (n=247); and stage III (n=51). The histological grading was performed according to the World Health Organization (WHO) grading system (20), as follows: Grade 1 (n=77); grade 2 (n=301); and grade 3 (n=26). All clinicopathological data were retrospectively retrieved from medical records. None of the patients received any chemotherapy, radiation therapy or hormonal therapy prior to surgery.

IHC staining. All breast tumor samples were fixed in 4% paraformaldehyde for 24 h at 4˚C prior to being dehydrated and embedded in paraffin. Serial coronal 4 μm sections from breast tumors were sliced and subjected to IHC staining. Briefly, the sections were dewaxed in xylene and rehydrated through graded absolute ethanol (75, 85, 95 and 100%) at room temperaure for 5 min each. Antigen retrieval was accomplished through boiling (100˚C) sections in 10 mM citrate buffer (pH 6.0; cat. no. MVS-0066; Fuzhou Maixin Biotech Co., Ltd., Fuzhou, China) for 15 min using a microwave. Sections were treated with 3% H_2O_2 in 0.1 M PBS to block endogenous peroxidase activity for 30 min at room temperature. The non-specific blocking was performed by incubating sections with 10% normal goat serum (cat. no. KIT-9710; Fuzhou Maixin Biotech Co., Ltd.) at 37˚C for 30 min. The sections were incubated with mouse moesin monoclonal antibody (1:200 dilution; 38/87 clone; cat. no. sc-58806; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) overnight at 4˚C. Biotinylated goat anti-mouse secondary antibodies (cat. no. KIT-9710; Fuzhou Maixin Biotech Co., Ltd.) were applied to sections at 1:200 and incubated for 30 min at 37˚C, and then the streptavidin-peroxidase conjugate (1:200; Fuzhou Maixin Biotech Co., Ltd.) was added and incubated at 37˚C for 30 min. Finally, the immunoreactions of sections were visualized by staining with 3'-diaminobenzidine (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 1-3 min and counterstained with hematoxylin for 30 sec at room temperature. Finally, the IHC-stained sections were observed with a light microscope (Nikon Corporation, Tokyo, Japan) in x400 and x1,000 magnifications.

Evaluation of IHC. The expression was scored on the basis of percentage of positive cells and intensity of the staining by two independent investigators blinded to the clinicopathological features. Each slide was assigned an intensity score from 0-3 (I0, I1-3: I1, weak staining; I2, moderate staining; and I3, strong staining) and the proportion of positively stained tumor cells over all the tumor cells was recorded from a range of 0-100% (P0, 0; P1, percentage of cells staining weakly; P2, percentage of cells staining moderately; and P3, percentage of cells staining strongly) using 5% increments, as previously reported (21,22). A final H score (range 0-300), which was used to assess the cutoff point for moesin high or low expression using receiver operating characteristic (ROC) curves, was achieved by adding the sum of scores obtained for each intensity and proportion of stained area [H score=(I1xP1)+(I2xP2)+(I3xP3)].

Selection of cutoff score. The ROC curves were used to assess optimal cutoff scores for increased moesin expression. The sensitivity and specificity for each outcome of the study was plotted to generate ROC curves. For ROC analysis, the clinicopathological parameters were dichotomized as follows: Node metastasis (yes/no); TNM stage (I-II/III-IV); tumor size ($\leq$2.0 cm/2.0<n<5.0 cm/$\geq$5.0 cm, measured by the longest length of the tumor); histological grade (G1 and G2/G3); estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor-2 (HER-2) status (negative/positive); and overall survival (OS) and recurrence-free survival (RFS) time. The scores were selected as the cutoff score, which was closest to the point with both maximum sensitivity and specificity; thus, tumors were defined as having a 'low expression' of moesin when the score was below the threshold, however, when score was above the threshold, tumors were defined as having a 'high expression' of moesin.

Statistical analysis. All statistical analyses were performed using SPSS 11.5 software package (SPSS Inc., Chicago, IL, USA). Data are presented as means ± standard error of the mean. Pearson's χ^2 test or Fisher's exact probability test was used to compare categorical data. The time from the first day of diagnosis to the occurrence of local recurrence or distant metastasis was calculated as the RFS time. The time from the first day of diagnosis to the disease-associated mortality, or last known follow-up was calculated as the OS time. Survival curves were plotted using the Kaplan-Meier method and assessed by the log-rank test. The association between potential confounding variables and prognosis (OS or RFS time) was evaluated by univariate and multivariate Cox proportional hazards regression models. $P<0.05$ was considered to indicate a statistically significant difference.

Results

IHC expression of moesin in breast cancer. IHC was conducted in order to detect the expression pattern of moesin in 404 breast cancer and 46 breast fibroadenoma samples. Immunoreactivity was observed primarily in the cytoplasm of tumor cells, and IHC staining for moesin in representative samples of breast

Table I. Characteristics of patients with breast cancer.

Features	Total no.	n (%)
Age at diagnosis, years	404	
≤51		223 (55.2)
>51		181 (44.8)
Menopause state	404	
Pre-menopause		221 (54.7)
Post-menopause		183 (45.3)
Tumor size, cm	404	
≤2.0		159 (39.4)
>2.0, <5.0		188 (46.5)
≥5.0		57 (14.1)
Nodes metastasis	404	
0		264 (65.3)
1-3		105 (26.0)
>4		35 (8.7)
TNM stage	404	
I		106 (26.2)
II		247 (61.1)
III		51 (12.6)
Histological grade	404	
I		77 (19.1)
II		301 (74.5)
III		26 (6.4)
Histological subtype	404	
Ductal infiltration		321 (6.2)
Intraductal carcinoma		25 (79.5)
Other		58 (14.4)

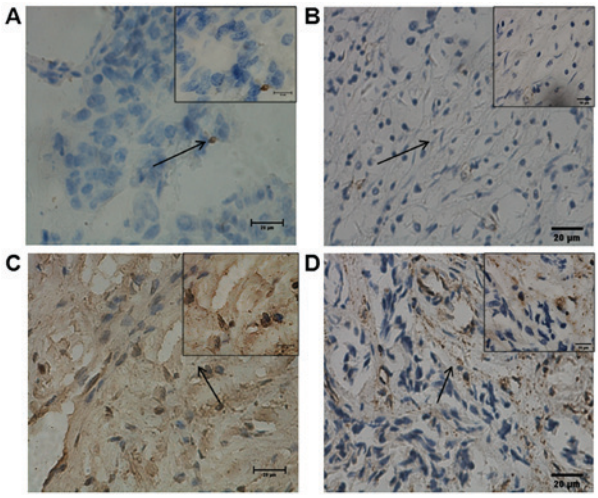

Figure 1. Immunohistochemical staining of moesin proteins. Representative micrographs of immunostaining for moesin. (A) Low immunostaining for moesin in fibroadenoma tissues. (B) Low immunostaining for moesin in breast cancer tissues. (C) High immunostaining for moesin in fibroadenoma tissues. (D) High immunostaining for moesin in breast cancer tissues. Arrows indicate the field enlarged. Magnification, x400, scale bar, 20 μm. Arrows indicate the magnified regions in the insert, x1,000, scale bar, 10 μm.

tumor and fibroadenoma tissues were depicted in Fig. 1. Moesin-immunoreactivity staining was observed in 70.5% (285/404) breast cancer samples and 15.2% (7/46) breast fibroadenoma samples. Moesin immunoreactivity was significantly higher in breast cancer samples, compared with fibroadenoma samples (P<0.001).

Cutoff value selection for moesin expression. To improve the assessment of moesin expression in breast cancer, ROC curve analysis was performed to define an optimal cutoff value for moesin expression, based on the IHC evaluation results. The ROC curves for each clinicopathological features were depicted in Fig. 2. ROC curve analysis for TNM stage demonstrated the greatest area; thus, the cutoff value was selected according to the TNM stage and the cutoff score for moesin expression was defined as 15.0, IHC scores >15.0 and ≤15.0 of tumors were defined as 'high' and 'low' moesin expression, respectively. On the basis of cut-off score, low moesin expression was detected in 211 (52.2%) breast tumor samples, and high moesin expression was observed in 193 (47.8%) breast tumor samples.

Association of moesin expression with clinicopathological parameters. Subsequently, the association of the moesin expression with the clinicopathological parameters of patients with breast cancer was analyzed (Table II). The high expression of moesin was significantly associated with patients with an age ≤51 years at diagnosis, compared with patients aged >51 years (P=0.058). Tumors with a high expression of moesin were significantly associated with the lymph node metastasis, compared with tumors with low expression of moesin (P<0.0001). Furthermore, the expression of moesin was significantly increased in patients with stage III breast cancer, compared with patients with stage I or II breast cancer (P=0.002); however, there was no significant association between the expression of moesin with other features, such as menopausal status, tumor size, histological subtype and histological grade (P>0.05).

Subsequently, the association between moesin expression and ER, PR and HER-2 status in patients with breast cancer was analyzed (Table II). Tumors that were ER-negative or PR-negative exhibited a significantly higher moesin expression, compared with ER-positive or PR-positive tissues (P=0.008 and P=0.026, respectively). No significant association was observed between moesin expression and HER-2 status (Table II). Patients with triple-negative breast cancer had a significantly higher expression of moesin, compared with non-triple negative breast cancer (P=0.010).

Characteristics stratified by ER status. Due to moesin demonstrating a significant association with ER status, analysis of the association between moesin expression levels and clinicopathological parameters was performed in patients with ER-positive and ER-negative breast cancer, separately. When the patients were stratified by ER status, 279 patients had ER-positive breast cancer and 125 patients had ER-negative breast cancer. In patients with ER-positive breast cancer, a high moesin expression was significantly associated with node metastasis, TNM stage and histological subtype. The patients with a higher histological grade (grade III) of tumor and an age at diagnosis ≤51 years were significantly associated with

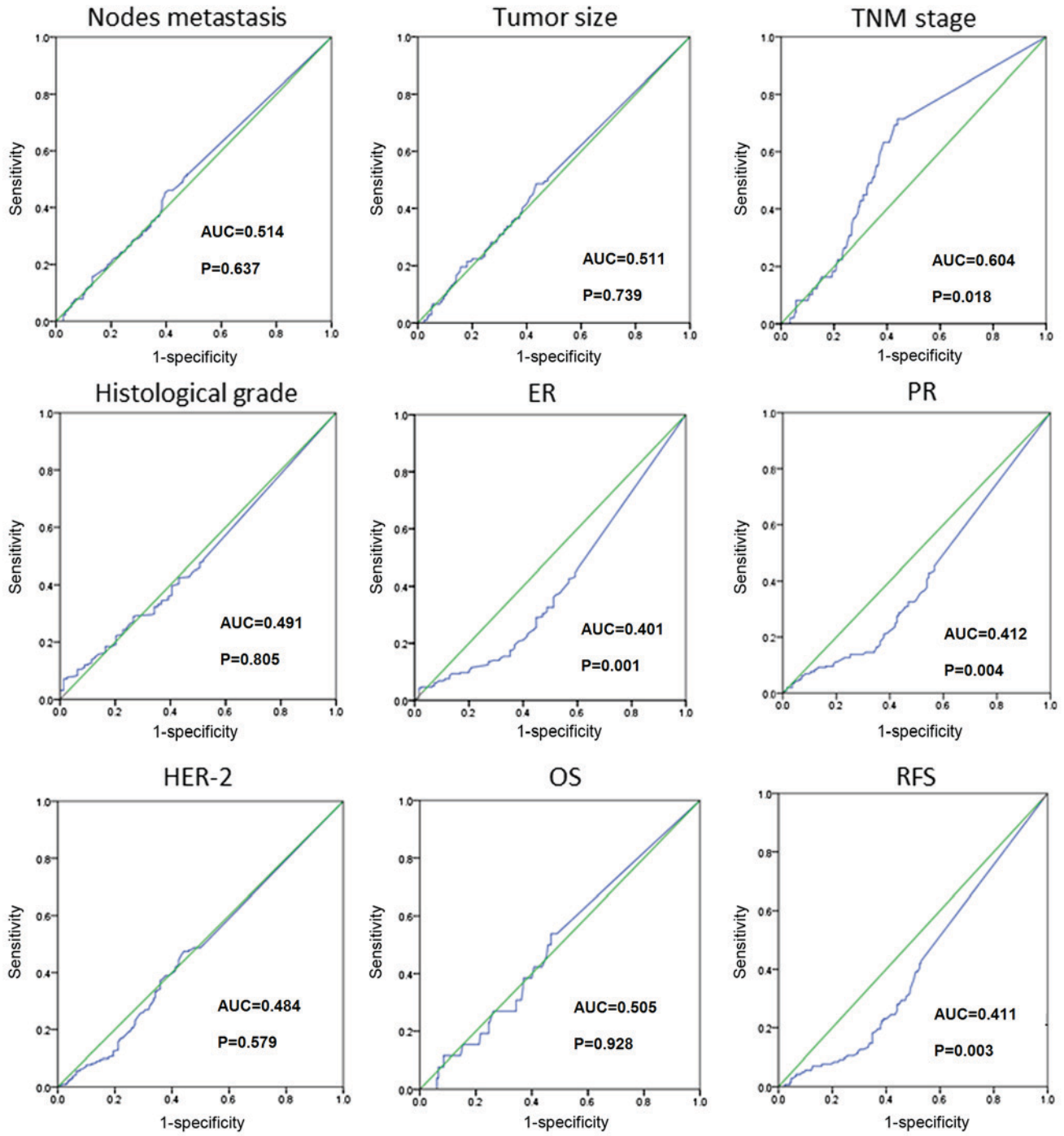

Figure 2. ROC curves were produced to determine the cutoff score for the overexpression of moesin in breast cancer. ROC curves were plotted by the sensitivity and specificity for each clinical outcome, and the AUCs and P-values were indicated. ROC, receiver operating characteristic; AUC, areas under curve; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor-2; OS, overall survival; RFS, recurrence-free survival.

a higher moesin expression, compared with grade I-II tumors in patents aged >51 years (P=0.075 and P=0.063, respectively; Table III). In contrast, among the ER-negative samples, the expression of moesin was increased in node metastasis and PR negative tumors, compared with non-node metastasis PR positive tumors (P=0.026 and P=0.042, respectively; Table III).

Association of moesin expression with the outcome of patients with breast cancer. Kaplan-Meier analysis was conducted to evaluate the association of moesin expression with the OS and RFS time of patients with breast cancer. There was no significant association between moesin expression and OS time (P=0.452; Fig. 3A). High expression of moesin in patients with breast cancer indicated a reduced RFS time (P=0.032; Fig. 3B), compared with patients with low expression of moesin. The association of moesin expression with the RFS time in ER-status subgroups of patients with breast cancer were further analyzed. The expression levels of moesin were not significantly associated with RFS time in patients with ER-negative breast cancer (P=0.807; Fig. 3C). The low expression of moesin was associated with increased RFS time in patients with ER-positive breast cancer (P=0.025, Fig. 3D).

Table II. Association of moesin expression with clinicopathological features of patients with breast cancer.

Features	Total cases	Moesin expression, n (%)		χ^2	P-value[a]
		Low	High		
Age at diagnosis	404	211	193	3.596	0.058
≤51	223	107 (48.0)	116 (52.0)		
>51	181	104 (57.5)	77 (42.5)		
Menopause state	404			1.309	0.252
Pre-menopause	221	110 (49.8)	111 (50.2)		
Post-menopause	183	102 (55.7)	81 (44.3)		
Tumor size, cm	404			0.002	0.859
≤2.0	159	83 (52.2)	76 (47.8)		
>2.0, ≤5.0	188	98 (52.1)	90 (47.9)		
>5.0	57	28 (49.1)	29 (50.9)		
Nodes metastasis	404			17.84	<0.001[b]
0	264	140 (53.0)	124 (47.0)		
1-3	105	64 (61.0)	41 (39.0)		
>4	35	7 (20.0)	28 (80.0)		
Histological subtype	404			4.375	0.224
Infiltrating ductal carcinoma	321	170 (53.0)	151 (47.0)		
Infiltrating lobular carcinoma	10	2 (20.0)	8 (80.0)		
Other	73	39 (53.4)	34 (46.6)		
TNM stage	404			12.619	0.002[b]
I	106	56 (52.8)	50 (47.2)		
II	247	140 (56.7)	107 (43.3)		
III	51	15 (29.4)	36 (70.6)		
Histological grade	404			4.013	0.129
I	77	38 (49.4)	39 (50.6)		
II	301	164 (54.5)	137 (45.5)		
III	26	9 (34.6)	17 (65.4)		
ER status				7.007	0.008[b]
Negative	125	53 (42.4)	72 (57.6)		
Positive	279	158 (56.6)	121 (43.4)		
PR status				4.954	0.026[b]
Negative	143	64 (44.8)	79 (55.2)		
Positive	261	147 (56.3)	114 (43.7)		
HER-2 status				0.060	0.807
Negative	170	90 (52.9)	80 (47.1)		
Positive	234	121 (51.7)	113 (48.3)		
Triple negative status	404			6.644	0.010[b]
Non-triple negative	369	200 (54.2)	169 (45.8)		
Triple negative	35	11 (31.4)	24 (68.6)		

[a]P-values were obtained from Pearson's χ^2 or Fisher's exact test; [b]P-values of statistical significance (P<0.05). TNM, Tumor-Node-Metastasis; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth receptor 2.

Subsequently, the association of each clinicopathological variable with OS and RFS time was evaluated using the univariate Cox regression model (Table IV). The univariate analysis demonstrated that a large tumor size, advanced TNM stage, lymph node metastasis, poor histological grade and HER-2-positive tumors were significantly associated with reduced OS and RFS time in patients with breast cancer. Additionally, a high moesin expression in patients with breast cancer was associated with a reduced RFS time. Following this, multivariate Cox regression models using

Table III. Patient demographics stratified by ER expression.

	ER positive (n=279)			ER negative (n=125)		
Features	Low moesin expression (n=158)	High moesin expression (n=121)	P-value[a]	Low moesin expression (n=53)	High moesin expression (n=72)	P-value[a]
Age at diagnosis			0.063			0.540
≤51	79 (51.6)	74 (48.4)		28 (40.0)	42 (60.0)	
>51	79 (62.7)	47 (37.3)		25 (45.5)	30 (54.5)	
Menopause state			0.356			0.540
Pre-menopause	82 (54.3)	69 (45.7)		28 (40.0)	42 (60.0)	
Post-menopause	76 (59.8)	52 (40.6)		25 (45.5)	30 (54.5)	
Tumor size, cm			0.823			0.992
≤2.0	64 (55.7)	51 (44.3)		19 (43.2)	25 (56.8)	
2.0<n≤5.0	74 (56.1)	58 (43.9)		24 (42.9)	32 (57.1)	
>5.0	20 (62.5)	12 (37.5)		10 (40.0)	15 (60.0)	
Nodes metastasis			0.002			0.026[b]
0	102 (57.0)	77 (43.0)		38 (44.7)	47 (55.3)	
1-3	49 (66.2)	25 (33.8)		15 (48.4)	16 (51.6)	
>4	7 (26.9)	19 (73.1)		0 (0.00)	9 (100.0)	
Histological subtype			0.021			0.384
Infiltrating ductal carcinoma	126 (56.5)	97 (43.5)		44 (44.9)	54 (55.1)	
Infiltrating lobular carcinoma	1 (11.1)	8 (88.9)		1 (57.8)	0 (57.8)	
Other	31 (64.6)	16 (35.4)		8 (30.7)	18 (69.2)	
TNM stage			0.003			0.139
I	40 (52.6)	36 (47.4)		16 (53.3)	14 (46.7)	
II	107 (63.3)	62 (36.7)		33 (42.3)	45 (57.7)	
III/IV	11 (32.4)	23 (67.6)		4 (23.5)	13 (76.5)	
Histological grade			0.075			0.191
I	26 (45.6)	31 (56.4)		12 (60.0)	8 (40.0)	
II	129 (60.3)	85 (39.7)		35 (40.2)	52 (59.8)	
III	3 (37.5)	5 (62.5)		6 (33.3)	12 (66.7)	
PR status			0.691			0.042[b]
Negative	29 (59.2)	20 (40.8)		35 (37.2)	59 (62.8)	
Positive	129 (56.1)	101 (43.9)		18 (58.1)	13 (41.9)	
HER-2 status			0.758			0.355
Negative	65 (55.6)	52 (44.4)		25 (47.2)	28 (52.8)	
Positive	93 (57.4)	69 (42.6)		28 (38.9)	44 (61.1)	

[a]P-value obtained from Pearson's χ^2 or Fisher's exact test; [b]P-values of statistical significance (P<0.05). TNM, Tumor-Node-Metastasis; HER-2, human epidermal growth receptor-2; ER, estrogen receptor; PR, progesterone receptor.

clinical stage, tumor size, nodes metastasis, histological grade, moesin expression, and ER, PR and HER-2 status identified TNM stage as the only independent prognostic factor (Table IV).

The Cox proportional hazards model was repeated for the ER-positive and ER-negative subgroups. In patients with ER-positive breast cancer, moesin overexpression was associated with a reduced RFS time (Table V). In patients with ER-negative breast cancer, the expression of moesin was not associated with OS or RFS time (Table VI).

Association of moesin expression with the therapeutic response. Subsequently, the association of moesin expression with therapeutic responses of patients receiving chemotherapy and endocrine therapy was investigated. No significant association was determined between moesin expression levels and OS time in patients treated with anthracycline alone or combined with paclitaxel chemotherapy (Fig. 4A) and patients treated with other chemotherapies (Fig. 4B). Moesin overexpression in patients treated with anthracycline alone or combined with paclitaxel chemotherapy demonstrated a significantly reduced

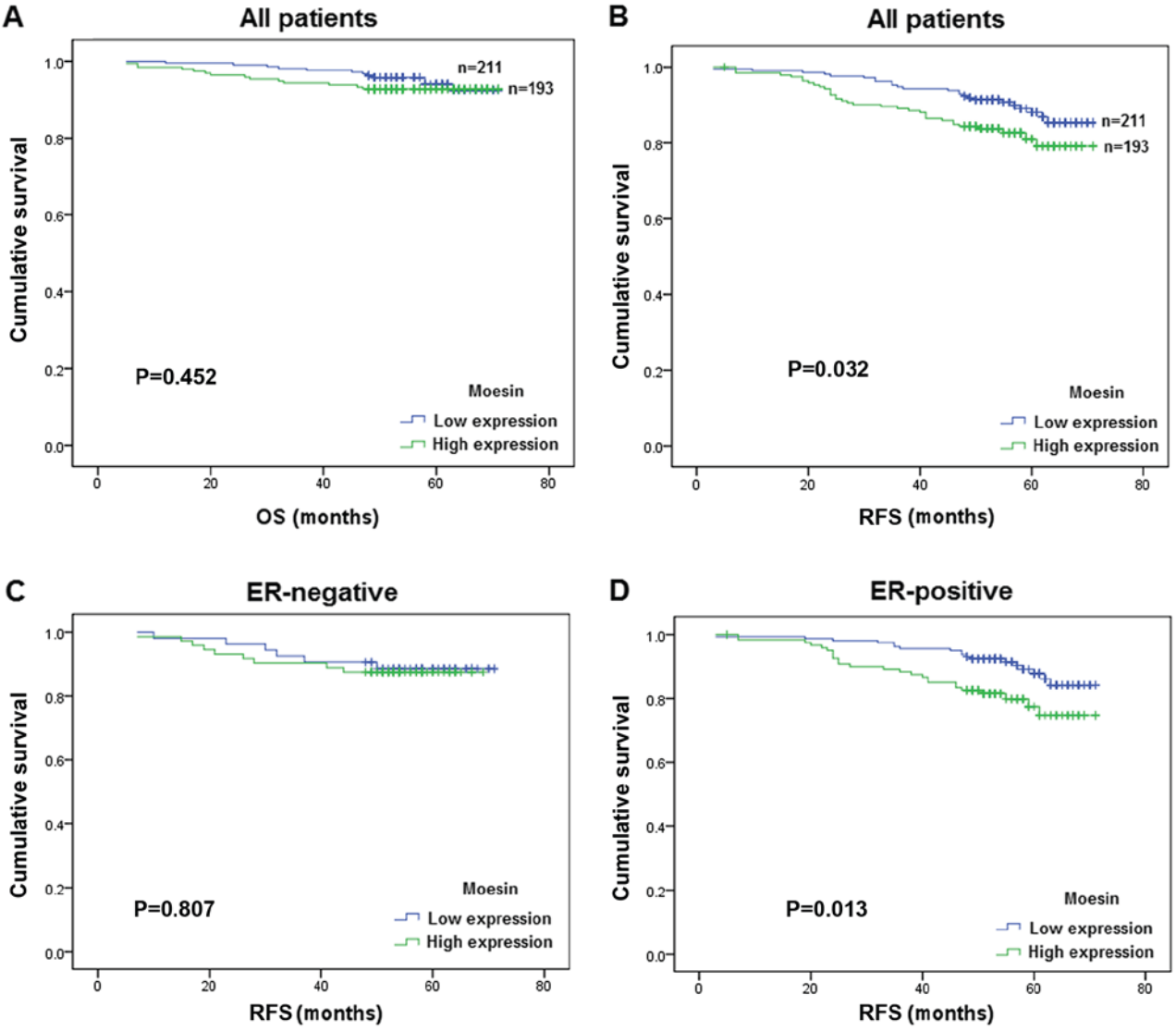

Figure 3. High and low moesin expression groups were compared with survival outcome in patients with breast cancer stratified by ER status. (A) OS time of all patients was analyzed by Kaplan-Meier survival analysis and the log-rank test. (B) RFS time of all patients was analyzed by Kaplan-Meier survival analysis and the log-rank test. (C) RFS time of patients with ER-negative breast cancer was analyzed by Kaplan-Meier survival analysis and the log-rank test. (D) RFS time of patients with ER-positive breast cancer was analyzed by Kaplan-Meier survival analysis and the log-rank test. ER, estrogen receptor; OS, overall survival; RFS, recurrence-free survival.

RFS time, compared with patients with low moesin expression (P=0.027; Fig. 4C). The expression of moesin was not significantly associated with RFS time in patients treated with other chemotherapies (Fig. 4D), and was not associated with OS time in patients not treated with endocrine therapy (Fig. 4E) or patients treated with endocrine therapy (Fig. 4F). Additionally, the expression of moesin was not associated with RFS time in patients not treated with endocrine therapy (Fig. 4G). Moesin overexpression in patients treated with tamoxifen demonstrated a significantly reduced RFS time, compared with patients with low moesin expression (P=0.005; Fig. 4H).

Discussion

In the present study, the expression of moesin in a large cohort of patients with breast carcinoma was determined. The results demonstrated that moesin expression was higher in breast cancer than in fibroadenoma samples. These results are consistent with other studies, which determined an upregulated expression of moesin in a variety of malignant tumors, including breast (18), prostate (14), laryngeal squamous cell carcinoma (23) and astrocytoma (4). These data indicated that moesin may serve an important role in tumorigenesis.

As nuclear steroid receptors, ER and PR regulate the transcriptional expression of breast cancer development-associated genes (24). Patients with ER- and/or PR-negative breast cancer had a higher risk of mortality following their diagnosis, compared with patients with ER- and/or PR-positive breast cancer (16,18). There are various patterns of gene expression characteristics in ER-positive and ER-negative breast cancer. In the present study, it was demonstrated that moesin expression was increased in PR-negative or ER-negative breast tumors, compared with PR-positive or ER-positive breast tumors, indicating that the ER and PR signaling pathways may be involved in moesin overexpression in breast cancer. The data regarding

Table IV. Cox regression analysis of clinicopathological data association with OS and RFS time of patients with breast cancer.

	OS				RFS			
	Univariate (n=404)		Multivariate (n=404)		Univariate (n=404)		Multivariate (n=404)	
Factor	RR (95% CI)[a]	P-value[a]	RR (95% CI)[b]	P-value[b]	RR (95% CI)[a]	P-value[a]	RR (95% CI)[b]	P-value[b]
Age, years (>51/≤51)	1.738 (1.040-2.903)	0.035[c]	2.088 (0.233-18.718)	0.511	1.177 (0.795-1.742)	0.415	1.141 (0.640-2.036)	0.654
Histological subtype (ductal/other)	1.951 (0.491-1.136)	0.173	6.962 (0.839-57.744)	0.162	0.958 (0.723-1.268)	0.763	0.924 (0.612-1.395)	0.708
Tumor size, cm (>5.0/>2.0, ≤5.0/≤2.0)	2.288 (1.341-3.903)	0.002[c]	0.675 (0.258-1.768)	0.424	1.709 (1.122-2.603)	0.013	0.845 (0.444-1.610)	0.609
Nodes metastasis (yes/no)	4.497 (2.529-7.998)	<0.001[c]	0.582 (0.153-2.207)	0.426	2.409 (1.613-3.600)	<0.001[c]	1.190 (0.548-2.584)	0.660
TNM stage (III-IV/I-II)	9.263 (5.225-16.423)	<0.001[c]	15.385 (3.723-63.574)	<0.001[c]	3.944 (2.662-5.844)	<0.001[c]	3.417 (1.370-8.522)	0.008[c]
Histological grade (III/II/I)	3.222 (1.385-7.494)	0.007[c]	6.962 (0.839-57.744)	0.072	1.197 (0.710-2.016)	0.500	0.653 (0.323-1.320)	0.235
Menopause state (post/pre)	1.846 (1.101-3.094)	0.020[c]	1.478 (0.171-12.801)	0.723	1.311 (0.886-1.941)	0.176	1.183 (0.382-3.664)	0.711
ER (positive/negative)	0.627 (0.374-1.051)	0.076	1.026 (0.346-3.047)	0.963	0.949 (0.621-1.450)	0.809	1.215 (0.560-2.639)	0.622
PR (positive/negative)	0.628 (0.377-1.047)	0.074	0.671 (0.241-1.872)	0.446	0.891 (0.590-1.344)	0.582	1.298 (0.606-2.781)	0.503
HER-2 (positive/negative)	1.994 (1.125-3.535)	0.018[c]	1.599 (0.597-4.281)	0.350	1.619 (1.059-2.474)	0.026[c]	1.729 (0.938-3.189)	0.079
Moesin (positive/negative)	1.343 (0.621-2.904)	0.452	0.725 (0.291-1.806)	0.490	1.762 (1.034-2.976)	0.032[c]	1.783 (0.970-3.276)	0.062

[a]P-value obtained from univariate Cox regression analysis; [b]P-value obtained from multivariate Cox regression analysis; [c]P-values of statistical significance (P<0.05). RR, relative risk; CI, confidence interval; TNM, Tumor-Node-Metastasis; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth receptor-2; OS, overall survival; RFS, recurrence-free survival.

Table V. Cox regression analysis of clinicopathological data association with OS and RFS time of patients with estrogen receptor-positive breast cancer.

	OS				RFS			
	Univariate (n=404)		Multivariate (n=404)		Univariate (n=404)		Multivariate (n=404)	
Factor	RR (95% CI)[a]	P-value[a]	RR (95% CI)[b]	P-value[b]	RR (95% CI)[a]	P-value[a]	RR (95% CI)[b]	P-value[b]
Age, years (>50/≤50)	1.481 (0.767-2.858)	0.242	1.765 (0.451-6.910)	0.415	1.195 (0.657-2.173)	0.559	1.820 (0.467-7.100)	0.389
Histological subtype (ductal/others)	1.951 (0.764-4.978)	0.162	0.148 (0.016-1.389)	0.094	0.987 (0.652-1. 495)	0.953	0.949 (0.571-1.577)	0.840
Tumor size, cm (>5.0/>2.0, ≤5.0/≤2.0)	3.304 (1.656-6.591)	0.001[c]	1.719 (0.535-5.525)	0.363	1.522 (0.775-2.989)	0.223	1.146 (0.550-2.385)	0.716
Nodes metastasis (yes/no)	4.798 (2.267-10.152)	<0.001[c]	0.286 (0.031-2.612)	0.268	1.763 (0.987-3.148)	0.050	1.101 (0.454-2.670)	0.831
TNM stage (III-IV/I-II)	9.755 (4.595-20.794)	<0.001[c]	3.222 (1.086-9.560)	0.002[c]	4.219 (2.193-8.117)	<0.001[c]	3.263 (1.099-9.689)	0.033[c]
Histological grade (III/II/I)	8.092 (1.108-59.073)	0.039[c]	0.753 (0.338-1.677)	0.488	0.978 (0.469-2.036)	0.953	0.725 (0.330-1.594)	0.424
Menopause state (post/pre)	1.868 (0.956-3.651)	0.068	0.736 (0.072-7.489)	0.796	1.268 (0.698-2.307)	0.436	0.751 (0.200-2.827)	0.672
PR (positive/negative)	0.801 (0.351-1.828)	0.598	1.302 (0.489-3.466)	0.598	1.060 (0.472-2.383)	0.888	1.337 (0.501-3.543)	0.559
HER-2 (positive/negative)	1.465 (0.732-2.930)	0.281	1.944 (0.932-4.056)	0.076	1.664 (0.867-3.192)	0.126	1.822 (0.893-3.718)	0.099
Moesin (positive/negative)	1.054 (0.258-4.299)	0.942	1.054 (0.258-4.299)	0.942	2.128 (1.159-3.908)	0.015	2.273 (1.102-4.687)	0.026[c]

[a]P-value obtained from univariate Cox regression analysis; [b]P-value obtained from multivariate Cox regression analysis; [c]P-values of statistical significance (P<0.05). RR, relative risk; CI, confidence interval; TNM, Tumor-Node-Metastasis; HER-2, human epidermal growth receptor-2; PR, progesterone receptor; OS, overall survival; RFS, recurrence-free survival.

Table VI. Cox regression analysis of clinicopathological data association with OS and RFS time of patients with estrogen receptor-negative breast cancer.

	OS				RFS			
	Univariate (n=404)		Multivariate (n=404)		Univariate (n=404)		Multivariate (n=404)	
Factor	RR (95% CI)[a]	P-value[a]	RR (95% CI)[b]	P-value[b]	RR (95% CI)[a]	P-value[a]	RR (95% CI)[b]	P-value[b]
Age, years (>50/≤50)	3.152 (0.815-12.191)	0.096	0.128 (0.001-152.9)	0.942	0.871 (0.310-2.448)	0.794	0.325 (0.036-2.971)	0.320
Histological subtype (ductal/other)	1.034 (0.462-2.315)	0.936	0.507 (0.056-4.612)	0.547	1.163 (0.625-2.163)	0.633	0.869 (0.371-2.031)	0.745
Tumor size, cm (>5.0/>2.0, ≤5.0/≤2.0)	0.517 (0.107-2.491)	0.411	0.100 (0.012-0.859)	0.036	0.805 (0.248-2.613)	0.718	0.354 (0.091-1.375)	0.133
Nodes metastasis (yes/no)	3.429 (0.967-12.151)	0.056	0.234 (0.043-1.279)	0.094	2.622 (0.951-7.232)	0.063	1.265 (0.263-6.078)	0.769
TNM stage (III-IV/I-II)	7.465 (2.158-25.818)	<0.001[c]	14.865 (1.762-125.417)	0.013[c]	5.188 (1.844-14.599)	0.002[c]	7.154 (1.272-40.230)	0.026[c]
Histological grade (III/II/I)	3.162 (0.504-19.845)	0.219	9.535 (0.113-802.983)	0.319	1.108 (0.305-4.024)	0.876	0.621 (0.110-3.499)	0.590
Menopause state (post/pre)	3.152 (0.815-12.191)	0.096	25.129 (0.002-255.685)	0.938	1.149 (0.417-3.170)	0.788	2.587 (0.285-23.458)	0.398
PR (positive/negative)	0.031 (0.000-12.891)	0.259	0.023 (0.015-125.157)	0.918	1.043 (0.332-3.275)	0.954	1.317 (0.315-5.503)	0.706
HER-2 (positive/negative)	2.987 (0.634-14.069)	0.166	1.108 (0.206-5.966)	0.905	2.100 (0.669-6.598)	0.204	1.633 (0.433-6.166)	0.469
Moesin (positive/negative)	1.321 (0.608-2.880)	0.644	0.916 (0.166-5.048)	0.920	1.137 (0.405-3.194)	0.808	1.054 (0.308-6.166)	3.608

[a]P-value obtained from univariate Cox regression analysis; [b]P-value obtained from multivariate Cox regression analysis; [c]P-values of statistical significance (P<0.05). RR, relative risk; CI, confidence interval; TNM, Tumor-Node-Metastasis; HER-2, human epidermal growth receptor-2; PR, progesterone receptor; OS, overall survival; RFS, recurrence-free survival.

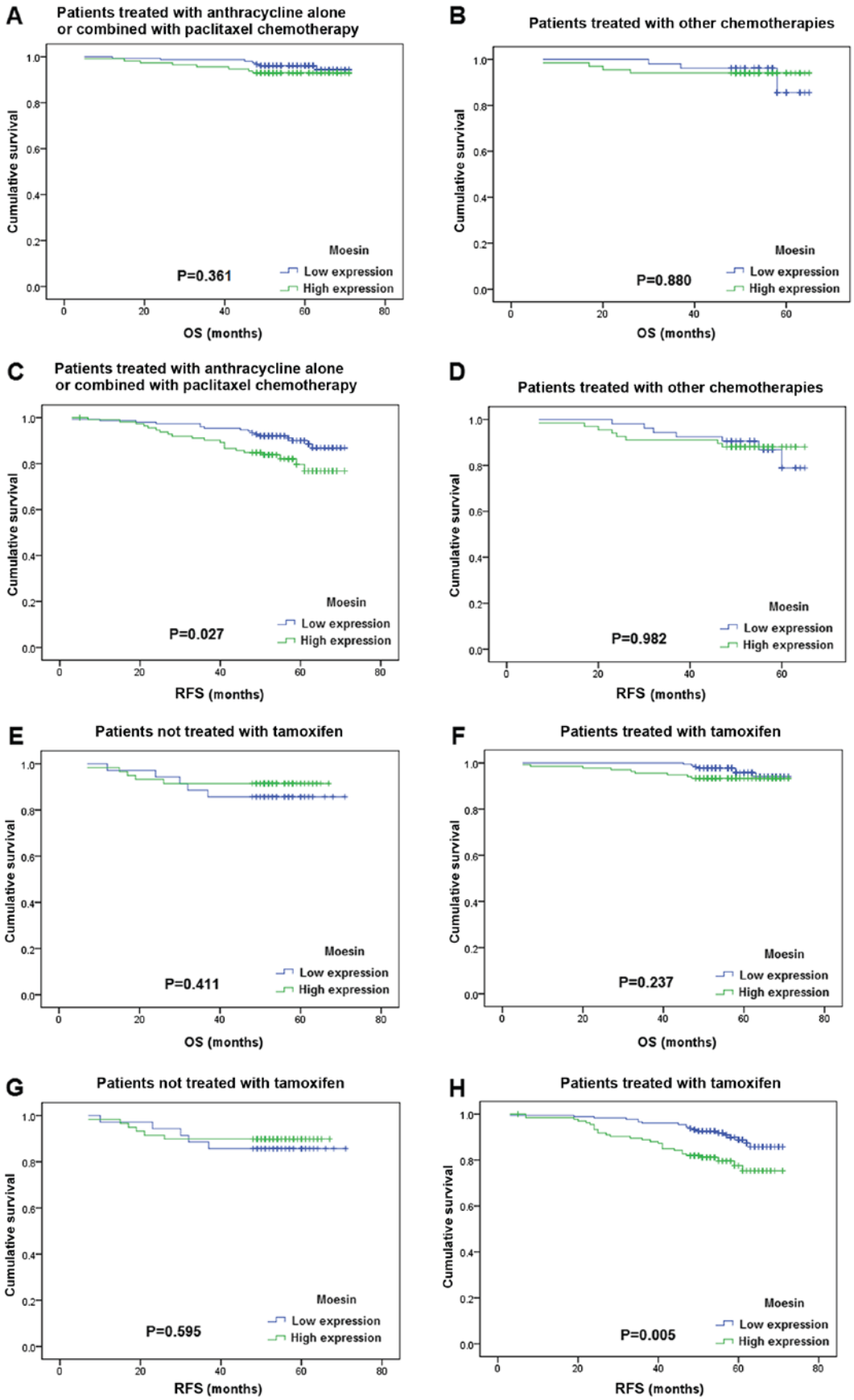

Figure 4. Kaplan-Meier survival analysis of moesin expression in patients with chemotherapy or tamoxifen treatment. Kaplan-Meier curves depicting the association between moesin expression and OS time in patients receiving (A) anthracycline alone or combined with paclitaxel, or (B) other chemotherapies. Kaplan-Meier curves depicting the association between moesin expression and RFS time in patients receiving (C) anthracycline alone or combined with paclitaxel, or (D) other chemotherapies. Kaplan-Meier curves depicting the association between moesin expression and OS time in patients (E) not receiving or (F) receiving tamoxifen treatment. Kaplan-Meier curves depicting the association between moesin expression and RFS time in patients (G) not receiving or (H) receiving tamoxifen treatment. RFS, recurrence-free survival; OS, overall survival.

moesin expression in ER-positive breast cancers are limited. Carmeci *et al* (25) investigated the expression of moesin in ER-negative and ER-positive tumors, and they determined that moesin expression decreased significantly in ER-positive tumors, compared with ER-negative tumors, which was consistent with the present study; however, in their *in vitro* study, moesin protein was overexpressed in ER-negative breast cancer cell lines but absent from ER-positive breast cancer cell lines (25).

There are limited studies that have demonstrated that moesin may affect cancer development or progression; therefore, the association of moesin protein expression with clinicopathological features from patients with breast cancer was investigated in the present study. The data indicated that the moesin expression was positively associated with the clinical stage and lymph node metastasis of breast cancer. The association of moesin expression with lymph node metastasis and clinical stage has also been determined in laryngeal squamous cell carcinoma (23), oral cancer (26) and gastric adenocarcinoma (27). In melanoma cells, moesin was demonstrated to contribute to cell invasion in a 3D matrix and early lung colonization (3). All of these results indicated that moesin may have an important role in promoting tumor metastasis and invasiveness. The mechanism of moesin contributing to tumor dissemination can be explained as follows: When moesin is activated, it interacts with the actin-cytoskeleton, which effects cell migration, invasion, adhesion and survival (13); however, Fernando *et al* (17) indicated that moesin is inversely correlated with breast cancer metastasis in patients, and this is discordant with the results of the present study. The different methods may be responsible for different roles of moesin in breast cancer cells. Additionally, Fernando *et al* (17) investigated the mRNA expression level of moesin, and although there is an overall positive correlation between the mRNA and protein expression levels, the moesin protein expression levels should more accurately reflect the role of moesin in breast cancer, compared with mRNA levels. Furthermore, moesin protein expression was not associated with the histological grades of breast cancer, which is consistent with the results reported by Ichikawa *et al* (28) regarding head and neck carcinoma, but disagree with the studies which determined an association between moesin expression with the grades of astrocytoma (29), breast cancer (18) and endometrial adenocarcinoma (30). A previous study indicated the prognostic role of moesin in patients with breast cancer (31). The IHC analysis in the present study demonstrated that the expression level of moesin had no impact on OS time, but indicated a notable association with RFS time in patients with breast cancer. Previous studies (32-34) indicated that estrogen can facilitate breast cancer progression, identifying moesin as a target of ER in breast cancer cells. For these reasons, the prognostic significance of moesin according to ER status was analyzed. In patients with ER-positive breast cancer, moesin expression levels were significantly associated with poor prognosis, as demonstrated by all of the patients in that group. Furthermore, in multivariate analyses, the prognostic significance of moesin was demonstrated for RFS time, but not for OS time, in patients with ER-positive breast cancer.

The present study determined that moesin protein overexpression was associated with poor prognosis in patients with breast cancer receiving tamoxifen treatment. Endocrine therapy is frequently effective in patients with ER-positive breast cancer; however, patients frequently develop resistance to endocrine therapy, and one of the important mechanisms of the acquired resistance is the loss of ER expression. In the present study, it was determined that high expression of moesin was associated with poor prognosis in patients receiving anthracycline alone or combined with paclitaxel chemotherapy, or endocrine therapy, but not in patients receiving other chemotherapy or no endocrine therapy. These data indicated that moesin may become an additional important biomarker for endocrine responsiveness in ER-positive cancer types, and further clinical studies are required to define the role of moesin in patients with ER-positive breast cancer.

Collectively, the present study extends previous publications and has a number of notable conclusions. It was demonstrated that combining ER-negative status and moesin scoring was a prognostic factor in ER-positive breast cancer. The present study determined that moesin protein overexpression was associated with poor prognosis in patients with breast cancer receiving tamoxifen treatment. These data indicated that moesin may be an important biomarker for endocrine responsiveness in ER-positive cancer types.

To conclude, it was determined that moesin expression is associated with clinicopathological characteristics and outcomes in ER-positive breast cancer. This indicates that moesin may be an additional predictor for endocrine responsiveness and a possible therapeutic target for the ER-positive tumors.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MW, FJ and LY assisted in the design and conception of the present study. LY, HZ, LZ, MH and ZY analyzed and interpreted the data of patients with breast cancer. HZ and LY performed the histological examination of the breast cancer tissues. LY and LZ, contributed in drafting the manuscript. LY, LZ and MW were major contributors in revising the manuscript. MH, HW and FJ collected the patients' clinical data and performed the follow-up study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Medical Ethics Committee of China Medical University (Shenyang, China) approved the present study. Due to the retrospective nature of the study, the ethics committee waived the requirement of written informed consent by the patients. All of the samples were anonymous.

Patient consent for publication

The patient, or parent, guardian or next of kin (if patient is deceased) provided verbal informed consent for the publication of any associated data and accompanying images.

Competing interests

The authors declare that they have no competing interests.

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