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Expression of ribosome-binding protein 1 correlates with shorter survival in Her-2 positive breast cancer

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Key words

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The aim of this study is to investigate the expression of ribosome-binding protein 1 (RRBP1) in invasive breast cancer and to analyze its relationship to clinical features and prognosis. RRBP1 expression was studied using real-time quantitative PCR and western blotting using pair-matched breast samples and immunohistochemical staining using a tissue microarray. Then the correlation between RRBP1 expression and clinicopathologic features was analyzed. RRBP1 mRNA and protein expression were significantly increased in breast cancer tissues compared with normal tissues. The protein level of RRBP1 is proved to be positively related to histological grade ($P = 0.02$), molecular subtype ($P = 0.048$) and status of Her-2 ($P = 0.026$) and P53 ($P = 0.015$). We performed a grade-stratified analysis of all patients according to the level of RRBP1 expression and found that RRBP1 overexpression highly affected overall survival in patients with early-stage (I and II) tumors ($P = 0.042$). Furthermore, Her-2 positive patients with negative RRBP1 expression had longer overall survival rates than those with positive RRBP1 expression ($P = 0.031$). Using multivariate analysis, it was determined that lymph node metastasis (LNM, $P = 0.002$) and RRBP1 expression ($P = 0.005$) were independent prognosis factors for overall survival. RRBP1 is a valuable prognostic factor in Her-2-positive breast cancer patients, indicating that RRBP1 is a potentially important target for the prediction of prognosis.

Breast cancer is the leading cancer in women and represents the second leading cause of cancer death among all women.[1] Her-2, one of the most well characterized breast cancer oncogenes, is found in approximately 25% of invasive breast cancers and is strongly associated with poor prognosis and a more aggressive phenotype.[2,3] Overexpression of Her-2 has been demonstrated to promote breast cancer invasion and metastasis.[4] In particular, tumors with Her-2 overexpression are known to be refractory to various types of chemotherapy and endocrine therapy and to be associated with shortened overall survival.[5,6] Although breast cancer patients with Her-2 overexpression can benefit from a targeted therapy that uses the humanized monoclonal antibody trastuzumab or the Her-2 kinase inhibitor lapatinib, the prognosis of Her-2-positive patients is hard to predict.[7,8] Thus, the identification of a new cancer biomarker, in addition to common clinicopathological risk factors to select Her-2-positive patients with good or bad outcomes, will assist the follow-up management of Her-2-positive breast cancer after surgery.

During the early stages of tumor development, cancer cells with high proliferation rates require the increased activity of endoplasmic reticulum protein folding, assembly, transport and subjected to endoplasmic reticulum stress (ERS). During tumorigenesis, an adaptive stress response promotes the unfolded protein response (UPR) and initiates the activation of survival cascade mechanisms. The activation of the UPR affects tumorigenesis and protects tumor cells from ERS.[9] Ribosome-binding protein 1 (RRBP1), a membrane protein, was originally identified as a ribosome-binding protein on the rough endoplasmic reticulum. It is essential for the transportation and secretion of intracellular proteins in mammalian cells.[10,11] RRBP1 has been studied in yeast, where it is a member of the ERS response and associated UPR.[12] It has also been shown to interact with KIF5B, a motor protein highly expressed in several cancer cell lines.[13] With regard to the possible role of RRBP1 in tumorigenesis and progression, RRBP1 overexpression has frequently occurred in colorectal cancer[14] and lung cancer.[15] However, thus far, there has been no evidence concerning the significance of RRBP1 mRNA and protein expression in breast cancer.

In the present study, we examine the expression of RRBP1 through real-time quantitative PCR (real-time qPCR) and western blotting using pair-matched breast samples and immunohistochemical staining using a tissue microarray (TMA), respectively. The correlations between RRBP1 expression and clinicopathologic features are analyzed.

Materials and Methods

Patients and clinical samples. This study used archival material from the Department of Pathology at Harbin Medical University Cancer Hospital, including tissue samples from 389 consecutive patients with histologically-confirmed breast cancer and 117 paired-normal tissue samples, all from 2006 (Table 1). The patients received a minimum of four courses of anthracycline-based and/or taxane-based chemotherapy after surgery. Hormone treatment with tamoxifen or aromatase inhibitors was given to patients with hormone receptor positive (ER or PR, or both). Her-2-positive patients who agreed to receive anti-Her-2 targeted therapy were treated with adjuvant trastuzumab for 1 year at Harbin Medical University Cancer Hospital. Fresh cancer tissues and matched normal tissues from 48 patients were collected and stored at −80°C immediately after resection to extract protein and RNA. Cancer tissues and their paired-normal tissues were examined diagnostically by two pathologists. All of the patients had invasive breast cancer. The patients who presented with recurrent tumors, metastatic disease at presentation, bilateral tumors or other previous tumors, and those who had previously received neoadjuvant treatment were excluded. The tumor size at the largest diameter of the invasive cancer was measured in millimeters by the pathologist. Four-micron tissue sections were prepared from a formalin-fixed and paraffin-embedded sample. All breast cancer patients were routinely tested for ER(estrogen receptor), PR(progesterone receptor), Her-2, P53 and Ki67, assayed in paraffin-embedded, formation-fixed tissue using antibodies against the proteins ER, PR, Her-2, P53 and Ki67 (Zhong shan-Bio, Beijing, China). Immunohistochemical staining for ER and PR was performed using a conventional detection method and was considered positive if 1% or more of the nuclei in the invasive component of the tumor was stained.[16] Positive staining for HER-2 was defined based on the percentage of tumor cells and the intensity of the membrane staining. A scored of 0 was given when no staining was observed or membrane staining of fewer than 10% of the tumor cells was observed. A faint or barely perceptible incomplete membrane staining detected in more than 10% of the tumor cells was scored as 1+. A weak to moderately complete membrane staining observed in more than 10% of the tumor cells was scored as 2+. A strong complete membrane staining observed in more than 10% of the tumor cells was scored as 3+. Scores of 0 to 1+ were regarded as negative, and 3+ were regarded as positive.[17] We selected a Ki67 index of 14% or more Ki67-positive tumor nuclei as the best cut-point for human visual assessment.[18] For P53, positive staining of more than 10% of the tumor cells was defined as positive tumor expression and staining of 10% or fewer of the cells as negative tumor expression.[19] The present study was approved by the Ethical Committee of Harbin Medical University in Harbin, China. Informed consent was obtained from all the patients.

Follow up. The clinical and pathological records of all patients in the study were reviewed periodically. Examinations were performed every 4–6 months for the first 5 years and every 12 months thereafter during the follow-up period. Patients were followed regularly for a minimum of 5 years of follow up at Harbin Medical University Cancer Hospital. The clinical records were obtained from the departments providing follow-up care. Survival was calculated in months from the date of diagnosis to whichever of the following occurred first: the date of death, the date last known to be alive, or 20 November 2012, which was the follow-up cut-off date used in our analysis. The median follow-up time was 72 months (range, 3–82 months).

Table 1. Clinicopathological composition of tumor patients

Characteristics	Number of cases
Age (years)	
Median	49
Range	28–78
Tumor size (cm)	
≤2	128
>2	261
LNM	
Negative	170
Positive	219
TNM stage	
I, II	259
III	130
Histological grade	
I, II	135
III	254
ER	
Negative	226
Positive	163
PR	
Negative	171
Positive	218
Her-2	
Negative	247
Positive	84
Ki67	
Negative	194
Positive	195
P53	
Negative	77
Positive	312
Subtype	
Luminal A	74
Luminal B	131
Her-2	47
Basal-like	79

ER, estrogen receptor; LNM, lymph node metastasis; PR, progesterone receptor.

RNA preparation and reverse transcription. Total RNA was extracted according to the protocol of TRIzol reagent (Invitrogen, Beijing, China) after ensuring that tumors contained more than 75% tumor cells and approved by pathologists in the pathology department. RNA quality and concentration were measured using a GeneQuant pro (GE Healthcare, Piscataway, NJ, USA). cDNA was synthesized from 1.0 μg of total RNA in a 20 μL reaction mixture using a PrimeScript RT reagent Kit with gDNA Eraser (Takara Bio, Otsu, Japan).

Real-time quantitative PCR. Real-time quantitative (qPCR) was performed using the ABI 7500 Fast Sequence Detection System (Applied Biosystems, Foster City, CA, USA) and Faststart Universal SYBR Green Master (ROX) reagent (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. The primers of RRBP1 were designed as follows: forward, 5′TGAATCCTCCAAAGACCACA3′; reverse, 5′CTTTCCCTCTCGCGTCTCT 3′. GAPDH was applied as the internal reference and its primers were as follows: 5′AACGACCCCTTCATTGAC3′; reverse, 5′TCCACGACATACTCAGCAC3′. Amplification was performed under the

following conditions: 95°C for 10 min, followed by 40 cycles of 95° for 15 s and of 60°C for 60 s. The results of the Real-time qPCR experiments were calculated using the $2^{-\Delta\Delta C_t}$ method. Experiments were performed in triplicate in the same reaction.

Western blotting analysis. Frozen tissue samples were homogenized in RIPA buffer consisting of 1% protease inhibitor mixture. The mixture was centrifuged at 14 000 *g* for 15 min at 4°C and the supernatant was obtained. Total proteins were quantified using the Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA), and 30 μg of protein per sample was separated onto a denaturing polyacrylamide gel containing SDS, and then transferred to a methanol-activated PVDF filter membrane (Bio-Rad, Hercules, CA, USA). Before immunodetection, membranes were blocked within 5% nonfat dry milk. Primary antibodies, anti-RRBP1 (1:1000; rabbit polyclonal; Abcam, Cambridge, MA, USA), were diluted in the buffer and incubated at 4°C overnight. After subsequent washing with TBST, membranes were incubated with secondary antibody (HRP-conjugated anti-rabbit) for 1 h at room temperature. The experiment was repeated in triplicate. The bands were detected by enhanced chemiluminescence detection reagents (Applygen Technologies, Beijing, China).

Tissue microarrays. Tissue microarrays (TMA) allowed the examination of a single biomarker in a high-throughput fashion to test a large number of normal and cancerous tissues simultaneously. TMA blocks were obtained by punching a tissue cylinder (core) with a diameter of 1.5 mm through a histological representative area of each "donor" tumor block, which was then inserted into an empty "recipient" TMA paraffin block using a manual tissue arrayer, as described previously.[20] After the construction of the array block, all the tissue blocks were cut with a microtome to 4 μm and affixed to the slide. Blocks from 389 invasive breast cancer patients and their matched normal breast samples were arrayed as triplicate spots of 1.5 mm diameter on slides.

Immunohistochemistry staining. The tissue sections were dried at 70°C for 3 h. After deparaffinization and hydration, sections were washed in PBS (3 min × 3). The washed sections were treated with 3% H_2O_2 in the dark for 5–20 min. After washing in distilled water, sections were washed in PBS (5 min × 3). Antigen retrieval was performed in citrate buffer (pH 6.0) at 100°C for 10 min. Each section was then treated with RRBP1 rabbit polyclonal antibodies (Abcam, Cambridge, MA; at a dilution of 1:200 solution) at 4°C overnight. After washing in PBS (5 min × 3), each section was incubated with secondary antibody at room temperature for 30 min. After washing in PBS (5 min × 3), each section was treated with diaminobenzadine working solution at room temperature for 3–10 min, and the slides were counterstained with hematoxylin. For negative controls, the primary antibody was substituted with PBS. The positive controls were lung cancer tumors with positive expression of RRBP1.[15]

Evaluation of ribosome-binding protein 1 protein expression by immunohistochemistry. Semiquantitative expression levels were based on the intensity of staining in a series of randomly selected ten high-power fields, which was considered as representative of the average in a 400 × magnification field. Staining intensity was classified into four groups: level 0 (no staining), level 1 (0–20% of tumor cells stained), level 2 (20–50% of tumor cells stained) and level 3 (>50% of tumor cells stained).[15] Overall expression was then graded as either negative expression (level 0) or positive expression (level 1–3).

Statistical analyses. All analyses were performed using statistical software (SPSS 17.0 for Windows; SPSS, Chicago, IL, USA). Associations between RRBP1 expression and patients' clinicopathological features, including age, tumor size, lymph node metastasis (LNM), TNM stage, histological grade, molecular subtype, and status of ER, PR, Her-2, Ki67 and P53 were assessed using the χ^2-test. The Kaplan–Meier method was used to estimate overall survival (OS). The influence of different variables on survival was assessed using Cox univariate and multivariate regression analyses. Risk ratios and their 95% confidence intervals (CI) were recorded for each marker. For continuous variables, student's *t*-test was performed. The level of significance was set at $P < 0.05$.

Results

Patients' characteristics. Analyses for the immunoreactivity of RRBP1 were performed using specimens from 389 untreated female invasive breast cancer patients. The clinical characteristics of the patients are listed in Table 1. The median age of the patients was 49 years old (range, 28–78). Of all the patients, LNM were present in 219 patients (56.3%), and absent in 170 patients (43.7%). A total of 259 (66.6%) patients were classified at stage I and II, and 130 (33.4%) were stage III. A total of 135 (34.7%) patients were classified as grade I and II, and the remaining patients were grade III (65.3%).

Ribosome-binding protein 1 mRNA and protein expression in breast cancer tissues. The analysis of real-time qPCR was used to confirm mRNA level. The mean expression value of RRBP1 mRNA in cancer tissue (24.10 ± 45.03, normalized by GAPDH gene expression) was significantly higher than the value in normal breast tissues (6.27 ± 12.18, $P = 0.001$). We then defined twofold changes as upregulation of RRBP1 mRNA between the cancer and corresponding normal tissues (Fig. 1a). The results revealed that 83.3% (40/48) of breast cancer tissues expressed RRBP1 compared to the matched normal tissues.

The difference in RRBP1 expression between cancer and normal tissues reflected at protein level was investigated using

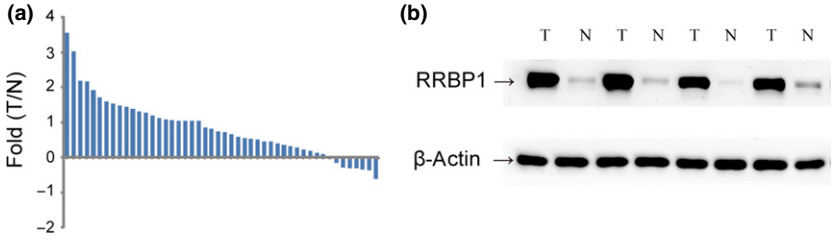

Fig. 1. Ribosome-binding protein 1 (RRBP1)-elevated expression in fresh breast cancer tissues. (a) Histogram of RRBP1 mRNA expression in breast cancer. The RRBP1 mRNA expression was calculated using the $2^{-\Delta\Delta C_t}$ method and the relative expression in each patient was presented as a ratio of T (tumor tissue)/N (normal tissue). (b) Representative western blot analysis of RRBP1 expression in breast tissues. The levels of β-actin were used as an internal control.

western blotting and immunohistochemistry. Overall, breast cancer tissues exhibited dramatically higher levels of RRBP1 protein expression compared with normal tissues. Significantly greater expression of RRBP1 protein was found in cancer tissues than in matched normal tissues during western blotting analysis (Fig. 1b). Immunoreactivity for RRBP1 protein is shown in brown color and presented diffusely in the cytosolic regions of invasive breast cancer cells (Fig. 2). In 17 of 117 paired-normal tissues, we demonstrated positive RRBP1 expression (i.e. 14.5% of the normal tissues). Of the 389 breast cancer specimens, we found positive expression in 164 (42.2%) samples ($P < 0.001$).

Relationship between immunoreactivity of ribosome-binding protein 1 and clinicopathological features. We analyzed the associations between levels of RRBP1 expression and a series of clinicopathological characteristics, including age, tumor size, LNM, TNM stage, histological grade molecular subtypes, and status of ER, PR, Her-2, Ki67 and P53 in breast cancer patients (Table 2). RRBP1 was significantly correlated with histological grade, Her-2, P53 and molecular subtypes. We detected RRBP1 expression in 73 of the 135 (49.7%) grade I and II patients, and in 91 of 254 (37.6%) grade III patients ($P = 0.020$). The data demonstrated that the RRBP1 expression was much greater in early-stage than in late-stage invasive breast cancer. A total of 45 of 84 patients (53.6%) who were Her-2 positive had significantly higher incidence of RRBP1 expression than those patients who were Her-2 negative (39.7%, 98 of 247, $P = 0.026$). RRBP1 was present in 29.9% (23 of 77) and 45.2% (141 of 312) of patients in the P53 negative group and P53 positive group, respectively ($P = 0.015$). Meanwhile, expression of RRBP1 protein was associated with subtypes of breast cancer; the positive rate was higher in the Her-2-positive subtype ($P = 0.048$). We found that there were no significant correlations between RRBP1 and clinicopathological parameters, including the patients' age ($P = 0.521$), tumor size ($P = 0.656$), LNM ($P = 0.630$), TNM stage ($P = 0.967$) and status of ER ($P = 0.272$), PR ($P = 0.860$) and Ki67 ($P = 0.325$).

Prognostic significance of ribosome-binding protein 1 expression in different grades of breast cancer patients. To eliminate the effect of histological grade on prognosis, we performed a grade-stratified analysis of all patients according to the level of RRBP1 expression and found that RRBP1 overexpression highly affected OS in patients with early-stage (I and II) tumors ($P = 0.042$). However, no such association was observed in the breast cancer patients with late-stage tumors ($P = 0.584$). The survival curves for the breast cancer patients in the two groups stratified by histological stage are illustrated in Figure 3.

Predictive significance of ribosome-binding protein 1 expression in Her-2 positive invasive breast cancer patients. The Kaplan–Meier 5-year survival curve stratified for RRBP1 expression in Her-2-positive patients is shown in Figure 4. Among the 84 Her-2-positive patients, RRBP1 expression showed significant effects on OS ($P = 0.031$). These data indicate that RRBP1 expression is associated with worse OS in Her-2-positive breast cancer.

Both univariate and multivariate survival analyses were used to evaluate the effects of RRBP1 expression and clinicopathological characteristics on prognosis in Her-2-positive patients. Univariate analyses of OS using Cox regression analysis identified LNM ($P = 0.012$), TNM stage ($P = 0.007$) and RRBP1 expression ($P = 0.039$) as significant prognostic predictors. Other features had no prognostic value. Using multivariate analysis, we found that LNM ($P = 0.002$) and RRBP1 expression ($P = 0.005$) were independent prognostic factors (Table 3).

Discussion

Ribosome-binding protein 1 is an endoplasmic reticulum membrane protein[10,21,22] that is essential for ribosome binding and for the translocation of nascent proteins across the membrane of the rough endoplasmic reticulum.[23] It has been shown to play an important role in procollagen biosynthesis in secretory tissues and in the terminal differentiation of secretory tissues.[10,21–26] Recent studies reveal that the RRBP1 level affects the development of tumorigenesis.[14,15] RRBP1 was highly expressed in lung cancer tissue compared with adjacent normal tissues, as assessed by immunohistochemistry using lung cancer TMA and real-time qPCR using paired normal cancer tissues.[15] Elevated levels of RRBP1 proteins have been detected in colorectal cancer tissues.[14] Although several studies on the expression of RRBP1 in cancers have supported the hypothesis that overexpression of RRBP1 is a stage in tumor development, until now there has been no report of RRBP1 being involved in breast cancer development. In the present study, we detected that the expression of RRBP1 is upregulated at both mRNA and protein levels in breast cancer. Our results concur with the data analysis from the UCSC cancer browser (https://genome-cancer.ucsc.edu, TCGA), which shows higher RRBP1 expression levels in breast cancer tissues than in normal breast tissues ($P < 0.001$). Our results are consistent with published data and associate higher RRBP1 expression in human breast cancer tissues with increasing propensity for tumor formation.

We first investigated the elevated expression of RRBP1 in human invasive breast cancer at the level of mRNA and protein. We then attempted to correlate RRBP1 expression with clinicopathological features and survival. Overall, positive correlations of RRBP1 with histological grade ($P = 0.020$), Her-2 status ($P = 0.026$), P53 status ($P = 0.015$) and molecular subtypes ($P = 0.048$) have been demonstrated in the current study.

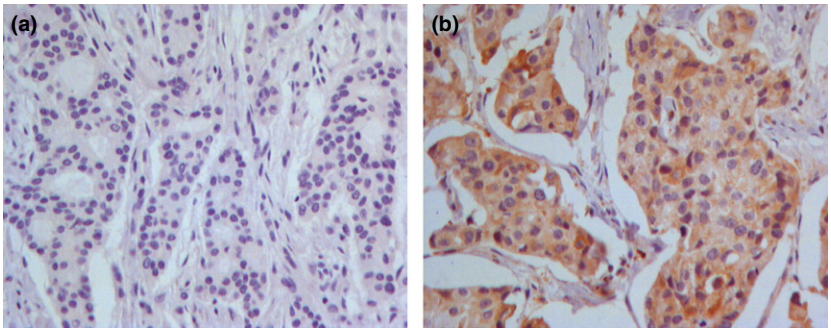

Fig. 2. Immunohistochemical staining of ribosome-binding protein 1 (RRBP1) in breast tissues. (a) Negative staining in normal breast tissue. (b) RRBP1 positive expression in breast cancer tissue.

Table 2. Correlation between ribosome-binding protein 1 expression and clinicopathological features

Characteristics	Cases	Negative	Positive	*P*-value
Age				
>50	209	124 (59.3)	85 (40.7)	0.521
≥50	180	101 (56.1)	79 (43.9)	
Tumor size				
≤2	128	72 (56.2)	56 (43.8)	0.656
>2	261	153 (58.6)	108 (41.4)	
LNM				
Negative	170	96 (56.5)	74 (43.5)	0.630
Positive	219	129 (58.9)	90 (41.1)	
TNM stage				
I, II	259	150 (57.9)	109 (42.1)	0.967
III	130	75 (57.7)	55 (42.3)	
Histological grade				
I, II	135	74 (50.3)	73 (49.7)	0.020
III	254	151 (62.4)	91 (37.6)	
ER status				
Negative	226	136 (60.2)	90 (39.8)	0.272
Positive	163	89 (54.6)	74 (45.4)	
PR status				
Negative	171	103 (60.2)	68 (39.8)	0.860
Positive	218	122 (56.0)	96 (44.0)	
Her-2 status				
Negative	247	149 (60.3)	98 (39.7)	0.026
Positive	84	39 (46.4)	45 (53.6)	
Ki67 status				
Negative	194	117 (60.3)	77 (39.7)	0.325
Positive	195	108 (55.4)	87 (44.6)	
P53 status				
Negative	77	54 (70.1)	23 (29.9)	0.015
Positive	312	171 (54.8)	141 (45.2)	
Subtype				
Luminal A	74	41 (55.4)	33 (44.6)	0.048
Luminal B	131	76 (58.0)	55 (42.0)	
Her-2	47	19 (40.4)	28 (59.6)	
Basal-like	79	52 (65.8)	27 (34.2)	

ER, estrogen receptor; LNM, lymph node metastasis; PR, progesterone receptor.

The relationships of many clinical, pathological and molecular factors to patient survival in invasive breast cancer have been investigated. Although prognosis in general correlates with clinical variables, such as tumor stage, it is currently difficult to predict the clinical outcome for individual patients with early-stage tumors. The endoplasmic reticulum is a key cellular organelle that is involved in protein homeostasis. When the normal mechanisms within the endoplasmic reticulum that ensure fidelity of protein folding are disturbed, unfolded proteins accumulate in the endoplasmic reticulum. Many known triggers for endoplasmic reticulum dysfunction lead to disturbances in protein folding in the endoplasmic reticulum.[27–30] The initial response is to correct this through inhibition of protein synthesis and increased degradation of unfolded protein by way of proteasomes. Simultaneously, adaptive genes are activated which improve protein folding and help the cell adapt to the trigger for the UPR.[31] During the early stage of tumor development, the activation of the UPR affects tumorigenesis and protects tumor cells from ERS.[32] In our research, a statistically significant correlation was found between earlier grade and positive RRBP1 expression ($P = 0.020$), which suggested that RRBP1 overexpression might be an early event in the progression of invasive breast cancer. Moreover, previous studies have shown that RRBP1 is associated with the UPR.[12,15] Therefore, RRBP1 could be a key player in the initial maintenance of breast cancer cell survival. Furthermore, by performing a

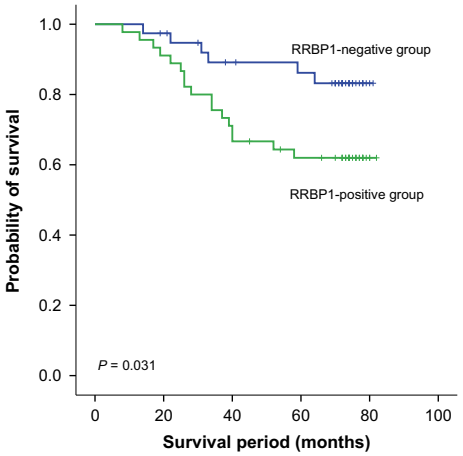

Fig. 4. Kaplan–Meier analysis for overall survival (OS) based on ribosome-binding protein 1 (RRBP1) expression in Her-2-positive breast cancer patients.

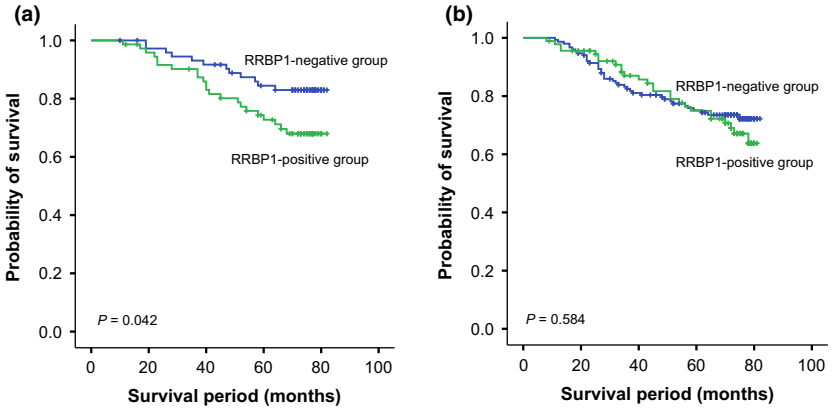

Fig. 3. A stage-stratified analysis of ribosome-binding protein 1 (RRBP1) expression in patients with breast cancers. Survival curves showed the correlation of RRBP1 with overall survival (OS) in breast cancer patients in different histological grades: (a) OS in histological grade I and II ($P = 0.042$); and (b) OS in histological grade III ($P = 0.584$).

Table 3. Prognostic factors in the Cox proportional hazards model

Variables	Risk ratio	Univariate 95% confidence interval	*P*-value	Risk ratio	Multivariate 95% confidence interval	*P*-value
Age (years)						
≥50/<50	1.339	0.587–3.055	0.487			
Tumor size (cm)						
≤2.0/>2.0	0.986	0.366–2.656	0.977			
LNM						
Positive/negative	4.004	1.359–11.795	0.012	5.581	1.859–16.751	0.002
TNM stage						
I and II/III	3.244	1.371–7.672	0.007			
Histological grade						
I and II/III	1.461	0.632–3.378	0.375			
ER status						
Positive/negative	0.244	0.057–1.041	0.057			
PR status						
Positive/negative	0.732	0.289–1.858	0.512			
Ki67 status						
Positive/negative	2.790	0.828–9.395	0.098			
P53 status						
Positive/negative	0.514	0.202–1.306	0.162			
Adjuvant trastuzumab						
Received/not received	0.414	0.176–0.978	0.044			
RRBP1 expression						
Positive/negative	2.667	1.051–6.769	0.039	3.874	1.501–9.997	0.005

ER, estrogen receptor; LNM, lymph node metastasis; PR, progesterone receptor.

grade-stratified analysis of all patients according to the level of RRBP1 expression, we found that RRBP1 overexpression highly affected OS in patients with early-grade (grade I and II) tumors ($P = 0.042$). We demonstrated that RRBP1 overexpression might be a prognostic indicator for early-stage invasive breast cancer.

Amplification of Her-2 gene is observed in approximately 25% of invasive breast cancers.[2,3] In addition, Her-2 is also overexpressed in a variety of other human tumors, including ovarian,[2] lung,[33] gastric[34,35] and oral[36] cancers. Breast cancer tumors overexpressing Her-2 oncoprotein are associated with a more aggressive clinical behavior.[37,38] The current standard of care for Her-2-positive breast cancer is therapy with the humanized monoclonal antibody tratuzumab,[39] which targets the C-terminal portion of Her-2.[40] Previous study has shown that RRBP1 is associated with the UPR.[12,15] In addition, experiments have revealed that the UPR is a possible means of overriding the effect of trastuzumab in Her-2-positive cancer cells.[41] In this study, correlation between Her-2 and RRBP1 was also observed ($P = 0.026$). Statistical analysis of the UCSC cancer browser database also revealed that RRBP1 expression is highly correlated with Her-2 expression in human breast cancer ($P = 0.016$). Moreover, high RRBP1 expression correlates with poor OS in Her-2-positive patients ($P = 0.031$). RRBP1 expression is an independent predictor for survival in Her-2 positive patients. Thus, we hypothesized that UPR-caused trastuzumab resistance might be the reason for poor prognosis in patients with RRBP1 expression. However, further research is required to pinpoint its molecular mechanisms.

In summary, we show a significant correlation between expression of RRBP1 and histological grade, Her-2, P53 and molecular subtypes in breast cancer. Our data also imply that RRBP1 is a valuable prognostic factor in Her-2-positive breast cancer patients, indicating that RRBP1 is a potentially important target for the prediction of prognosis. The roles of RRBP1 in breast cancer need to be tested *in vivo*. Therefore, further studies are required to identify its function and its role in the progression and poor prognosis of Her-2-positive patients. In addition, more clinical trails should be applied to confirm whether RRBP1 could be used as a novel prognostic indicator.

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Disclosure Statement

The authors have no conflict of interest to declare.

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