

Ubiquitin-like modifier-activating enzyme 7 as a marker for the diagnosis and prognosis of breast cancer

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Abstract. Ubiquitin-like modifier-activating enzyme 7 (UBA7) is a specific E1-like ubiquitin-activating enzyme involved in interferon-stimulated gene 15 (ISG15) conjugation. UBA7 expression has been reported to be notably decreased in lung cancer. The present study aimed to investigate the changes in UBA7 expression in breast cancer and the association between UBA7 expression and clinical characteristics, and to elucidate the diagnostic and prognostic significance of UBA7 in breast cancer. The clinical data and RNA-sequencing expression values of 1,104 patients with breast cancer were downloaded from The Cancer Genome Atlas database. The associations between UBA7 expression and clinical characteristics were determined using χ^2 and Fisher's exact tests. UBA7 expression values were divided into low and high groups using the optimal cut-off value, as determined by the overall survival (OS) value identified via a receiver operating characteristic (ROC) curve analysis, to further study the association between UBA7 expression and clinical characteristics. The diagnostic capability of UBA7 was assessed via ROC analysis, and Kaplan-Meier curve and Cox regression analyses were performed to determine the prognostic value of UBA7. The results demonstrated that UBA7 expression was decreased in breast cancer, and significant differences were observed between groups with regards to vital status, tumor classification, metastasis classification, histological type, sex, molecular subtype, and expression levels of progesterone receptor, estrogen receptor

(ER) and human epidermal growth factor receptor 2. Low and high UBA7 expression levels were associated with age, ER expression, menopause status, Tumor-Node-Metastasis classification stage, margin status, vital status, radiation therapy use, OS and relapse-free survival. Furthermore, patients with low UBA7 expression levels had a poor prognosis. UBA7 expression also demonstrated an ability to diagnose patients at all clinical stages. Taken together, the results indicated that UBA7 expression was significantly decreased in breast cancer, and was associated with clinical characteristics and prognosis. Thus, UBA7 can be deemed as a potential biomarker in breast cancer, and may serve as a target in treatment.

Introduction

Today, breast cancer is the second leading cause of cancer-associated mortality in women in the United States (1,2). It is reported that 1 in 8 women (13%) are expected to develop breast cancer in their lifetime in the United States (1,2). Breast cancer is commonly divided into two types: Infiltrating lobular carcinoma, which originates in the cells of the lobules, and infiltrating ductal carcinoma, which originates in the ducts (2). A few subtypes originate in the stromal tissues, such as the fatty and fibrous connective tissues of the breast. There are four molecular subtypes of breast cancer, according to the expression levels of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki-67, including luminal A, luminal B, HER2-enriched and basal-like (2). The pathogenesis of breast cancer remains unclear; however, it is considered to begin with alterations at the genomic level (2). Different types of breast cancer have a wide range of tumor growth rates and variable clinical courses, thus potential biomarkers are required to aid with diagnosis and prognosis, and may also function as novel targets in the treatment of breast cancer.

Interferon-stimulated gene 15 (ISG15) is the first ubiquitin-like protein to be discovered and reported as a negative regulator in IFN- α/β immunity, and to play a key role in anti-virus and antitumor defences (3,4). ISG15 is upregulated by IFNs (5) and has the ability to conjugate with ubiquitin-associated proteins (3,4). The conjugation process, known as

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interferon-stimulated gene 15 conjugation (ISGylation), includes three respective steps, namely activation, conjugation and ligation, and occurs in several cellular signaling pathways (6). A total of three classes of enzymes partake in this process, including an E1 activating enzyme [ubiquitin-like modifier-activating enzyme 7 (UBA7)], E2 conjugating enzymes, such as ubiquitin-carrier protein H8 (UBCH8) and ubiquitin-carrier protein H6 (UBCH6), and E3 ligases, such as estrogen-responsive finger protein (EFP), and HECT domain and RCC1-like domain-containing protein 5 (HERC5) (7). Currently, UBA7 is the only known E1-activating enzyme (8), and ISGylation has the ability to both activate and inhibit the activity of target proteins (4).

UBA7, also referred to as ubiquitin-activating enzyme E1-like protein (UBE1L), is the specific E1-like ubiquitin-activating enzyme that functions in ISGylation (9). Currently, the only known biological function of UBA7 is catalysing ISGylation. Both type I IFN and retinoic acid can induce UBA7 expression (10). Most previous studies on the UBA7/ISG15 signaling pathway in cancer have focused on lung cancer (8-12). UBA7 is located on chromosome 3p21.3 and is considered to be a candidate tumor suppressor gene. The loss of allelic heterozygosity (LOH) on 3p21.3 has been observed in 70-80% of non-small cell lung cancer (NSCLC) and 90-100% of SCLC cases (8). Furthermore, UBA7 expression was demonstrated to be notably decreased in several lung cancer cell lines (11). A number of studies have indicated that UBA7 suppresses tumor growth by inhibiting cyclin D1 expression, and that it can downregulate epidermal growth factor receptor (EGFR) expression in human bronchial epithelial cells (9,12). However, to the best of our knowledge, there are currently no studies on the change in UBA7 expression in breast cancer. Thus, the present study set out to determine whether UBA7 could function as a biomarker in breast cancer.

The present study evaluated UBA7 expression in breast cancer, while subtypes were also taken into consideration, and the potential association between UBA7 expression and clinical characteristics was analyzed. The efficiency of UBA7 expression in diagnosis was also assessed, along with its prognostic value using overall survival (OS) and relapse-free survival (RFS) as outcome measures.

Materials and methods

Data collection. The clinical data and RNA-sequencing (RNA-seq) expression values of patients with breast cancer were downloaded from The Cancer Genome Atlas (TCGA) database (<https://cancergenome.nih.gov/>), and the corresponding accession code was TCGA-BRCA. The RNA-seq by expectation-maximization expression values were used in the statistical analysis of RNA-seq data.

Statistical analysis. R software (version 3.5.2) and associated packages were used for data analysis (13,14). Wilcoxon test, Kruskal Wallis test and Dunn's post-hoc test were used in this study. mRNA expression differences between groups of discrete variables were depicted as boxplots generated by the ggplot2 package (version 3.2.1) in R software, and the association between clinical characteristics and UBA7 expression was

Table I. Clinical patient characteristics.

Characteristics	Number of cases (%)
Age, years	
<60	590 (53.44)
≥60	514 (46.56)
Sex	
N/A	2 (0.18)
Female	1090 (98.73)
Male	12 (1.09)
Histological type	
N/A	3 (0.27)
Infiltrating ductal carcinoma	790 (71.56)
Infiltrating lobular carcinoma	204 (18.48)
Other	107 (9.69)
Molecular subtype	
N/A	255 (23.04)
Basal	142 (12.86)
HER2-enriched	67 (6.07)
Luminal A	422 (38.22)
Luminal B	194 (17.57)
Normal	24 (2.17)
ER status	
N/A	50 (4.53)
Indeterminate	2 (0.18)
Negative	239 (21.65)
Positive	813 (73.64)
PR status	
N/A	51 (4.62)
Indeterminate	4 (0.36)
Negative	345 (31.25)
Positive	704 (63.77)
HER2 status	
N/A	183 (16.58)
Equivocal	180 (16.3)
Indeterminate	12 (1.09)
Negative	565 (51.18)
Positive	164 (14.86)
Menopause status	
N/A	93 (8.42)
Indeterminate	34 (3.08)
Peri	40 (3.62)
Post	706 (63.95)
Pre	231 (20.92)
T classification	
N/A	2 (0.18)
T1	281 (25.45)
T2	640 (57.97)
T3	138 (12.50)
T4	40 (3.62)
TX	3 (0.27)
N classification	
N/A	2 (0.18)
N0	516 (46.74)

Table I. Continued.

Characteristics	Number of cases (%)
N1	367 (33.24)
N2	120 (10.87)
N3	79 (7.16)
NX	20 (1.81)
M classification	
N/A	2 (0.18)
M0	917 (83.06)
M1	22 (1.99)
MX	163 (14.76)
Stage	
N/A	10 (0.91)
I	182 (16.49)
II	626 (56.70)
III	252 (22.83)
IV	20 (1.81)
X	14 (1.27)
Lymph node status	
N/A	379 (34.33)
No	28 (2.54)
Yes	697 (63.13)
Margin status	
N/A	72 (6.52)
Close	31 (2.81)
Negative	922 (83.51)
Positive	79 (7.16)
Vital status	
N/A	2 (0.18)
Deceased	155 (14.04)
Living	947 (85.78)
Radiation therapy	
N/A	102 (9.24)
No	445 (40.31)
Yes	557 (50.45)
Neoadjuvant treatment	
N/A	3 (0.27)
No	1088 (98.55)
Yes	13 (1.18)
Targeted molecular therapy	
N/A	525 (47.55)
No	46 (4.17)
Yes	533 (48.28)
Sample type	
Metastatic	7 (0.63)
Primary tumor	1097 (99.37)
Overall survival	
N/A	17 (1.53)
No	933 (84.51)
Yes	154 (13.95)
Relapse-free survival	
N/A	192 (17.39)
No	816 (73.91)
Yes	96 (8.70)

Table I. Continued.

Characteristics	Number of cases (%)
UBA7	
High	588 (53.26)
Low	516 (46.74)

N/A, not available; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; UBA7, ubiquitin-like modifier-activating enzyme 7.

assessed using χ^2 and Fisher's exact tests. A receiver operating characteristic (ROC) curve generated by the pROC package (version 1.15.3) in R software was used to determine the diagnostic capability of UBA7. UBA7 expression levels were divided into low and high groups, according to the optimal cut-off value, as determined by OS analysis using the ROC curve. Kaplan-Meier curves were generated to compare OS and RFS between the low and high expression groups, and P-values were calculated using the log-rank test. Univariate Cox analysis was performed to select the variables associated with OS and RFS, and multivariate Cox analysis was performed to assess the effect of UBA7 expression on survival and other clinical characteristics. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. Both the clinical data and RNA-seq expression data of 1,104 patients with breast cancer were downloaded from TCGA database. The detailed clinical characteristics, including molecular subtype, Tumor-Node-Metastasis stage (2), residual tumor status, survival status and therapy type, are presented in Table I.

Association between UBA7 expression and clinicopathological characteristics. Low UBA7 mRNA expression levels were observed in breast cancer tissues compared with those in normal tissues from the same patients ($P = 0.039$; Fig. 1A). Significant differences in UBA7 expression were also demonstrated according to: Vital status ($P = 2.1 \times 10^{-4}$), T classification ($P = 0.025$), M classification ($P = 0.0003$), histological type ($P = 1.6 \times 10^{-13}$) and sex ($P = 0.016$) (Fig. 1). Furthermore, UBA7 expression was indicated to be significantly associated with the expression of breast cancer-associated molecules, as determined by the molecular subtype of breast cancer ($P = 4.1 \times 10^{-11}$; Fig. 2A), and the expression of PR ($P = 8.3 \times 10^{-12}$; Fig. 2B), ER ($P = 1.2 \times 10^{-7}$; Fig. 2C) and HER2 ($P = 0.0089$; Fig. 2D). However, no significant associations were demonstrated between UBA7 expression and the use of radiation therapy ($P = 0.43$; Fig. 2E), neoadjuvant therapy ($P = 0.11$; Fig. 2F) or targeted molecular therapy ($P = 0.92$; Fig. 2G).

UBA7 expression was divided into high and low groups according to the cutoff point (9.542), and low UBA7 level was demonstrated to be associated with histological type ($P = 0.0005$), molecular subtype ($P = 0.0005$), and the expression of ER ($P = 0.0005$), PR ($P = 0.0005$) and HER2 ($P = 0.0185$),

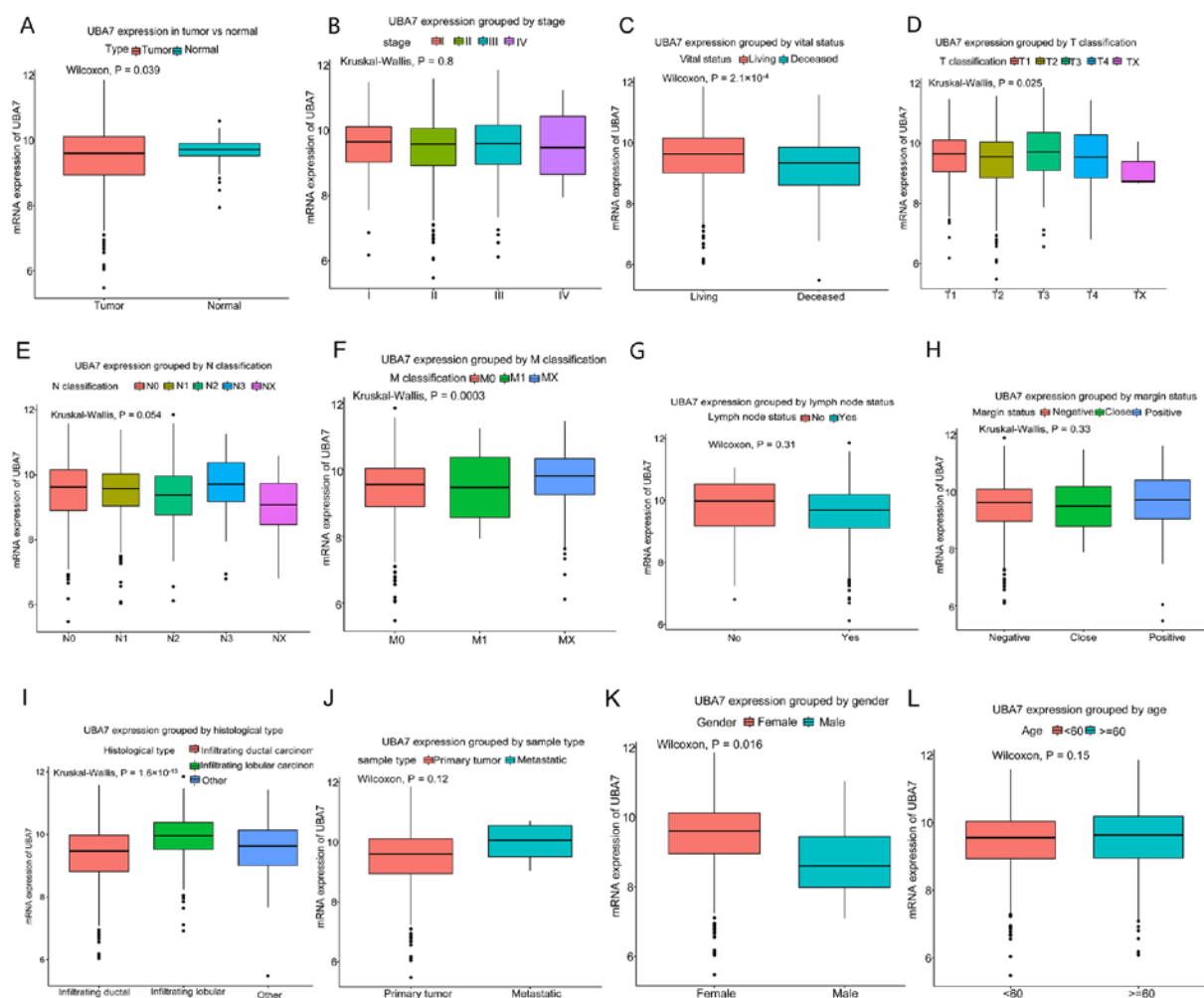


Figure 1. Differences in UBA7 expression according to clinicopathological characteristics. (A) UBA7 expression was lower in breast cancer tissues compared with that in normal tissues. Expression was significantly associated with (C) vital status, (D) tumor classification, (F) metastasis classification, (I) histological type and (K) gender. However, no significant association was observed with regard to (B) clinical stage, (E) N classification, (G) lymph node status, (H) margin status, (J) sample type and (L) age. UBA7, ubiquitin-like modifier-activating enzyme 7; T, tumor; M, metastasis.

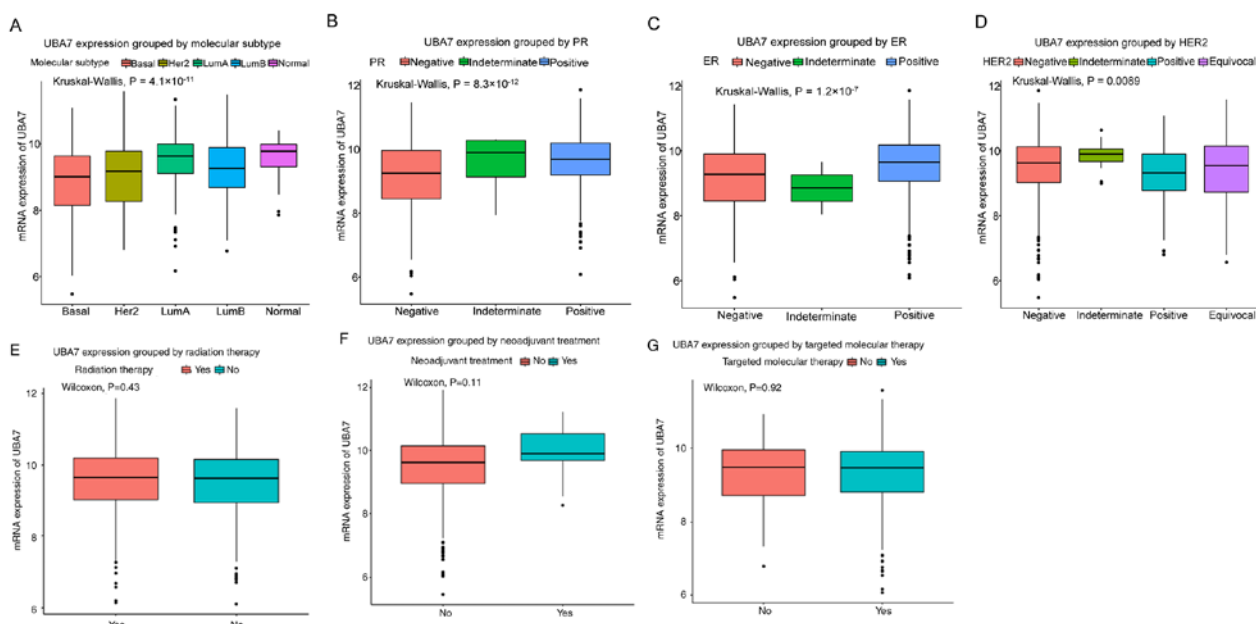


Figure 2. Differences in UBA7 expression according to clinicopathological characteristics. Significant differences were observed according to (A) molecular subtype of breast cancer, and (B) PR, (C) ER and (D) HER2 expression. However, no significant differences were observed according to (E) radiation therapy, (F) neoadjuvant treatment and (G) targeted molecular therapy. UBA7, ubiquitin-like modifier-activating enzyme 7; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

Table II. Association between UBA7 mRNA expression and clinicopathological characteristics in patients with breast cancer.

Characteristics	Patients, n	UBA7 mRNA expression		χ^2	P-value
		High, n (%)	Low, n (%)		
Age, years				2.9564	0.0830
<60	589	299 (51.02)	290 (56.2)		
≥60	513	287 (48.98)	226 (43.8)		
Sex				3.8682	0.0785
Female	1090	583 (99.49)	507 (98.26)		
Male	12	3 (0.51)	9 (1.74)		
Histological type				43.5504	0.0005 ^a
Infiltrating ductal carcinoma	790	377 (64.33)	413 (80.19)		
Infiltrating lobular carcinoma	204	150 (25.6)	54 (10.49)		
Other	107	59 (10.07)	48 (9.32)		
Molecular subtype				37.2883	0.0005 ^a
Basal	142	44 (11.11)	98 (21.63)		
HER2-enriched	67	26 (6.57)	41 (9.05)		
Luminal A	422	237 (59.85)	185 (40.84)		
Luminal B	194	75 (18.94)	119 (26.27)		
Normal	24	14 (3.54)	10 (2.21)		
ER status				17.7115	0.0005 ^a
Indeterminate	2	1 (0.18)	1 (0.2)		
Negative	239	98 (17.56)	141 (28.43)		
Positive	813	459 (82.26)	354 (71.37)		
PR status				30.3951	0.0005 ^a
Indeterminate	4	2 (0.36)	2 (0.4)		
Negative	345	141 (25.27)	204 (41.21)		
Positive	704	415 (74.37)	289 (58.38)		
HER2 status				10.4688	0.0185 ^b
Equivocal	180	90 (18.95)	90 (20.18)		
Indeterminate	12	9 (1.89)	3 (0.67)		
Negative	565	307 (64.63)	258 (57.85)		
Positive	164	69 (14.53)	95 (21.3)		
Menopause status				5.2218	0.1549
Indeterminate	34	14 (2.61)	20 (4.22)		
Peri	40	21 (3.91)	19 (4.01)		
Post	706	390 (72.63)	316 (66.67)		
Pre	231	112 (20.86)	119 (25.11)		
T classification				7.3029	0.1014
T1	281	163 (27.82)	118 (22.87)		
T2	640	321 (54.78)	319 (61.82)		
T3	138	81 (13.82)	57 (11.05)		
T4	40	20 (3.41)	20 (3.88)		
TX	3	1 (0.17)	2 (0.39)		
N classification				8.1021	0.0985
N0	516	284 (48.46)	232 (44.96)		
N1	367	196 (33.45)	171 (33.14)		
N2	120	53 (9.04)	67 (12.98)		
N3	79	46 (7.85)	33 (6.4)		
NX	20	7 (1.19)	13 (2.52)		
M classification				8.9899	0.0090 ^a
M0	917	472 (80.55)	445 (86.24)		
M1	22	10 (1.71)	12 (2.33)		
MX	163	104 (17.75)	59 (11.43)		

Table II. Continued.

Characteristics	Patients, n	UBA7 mRNA expression		χ^2	P-value
		High, n (%)	Low, n (%)		
Stage				6.3959	0.1714
I	182	106 (18.24)	76 (14.81)		
II	626	334 (57.49)	292 (56.92)		
III	252	128 (22.03)	124 (24.17)		
IV	20	9 (1.55)	11 (2.14)		
X	14	4 (0.69)	10 (1.95)		
Lymph node status				0.0215	1.0000
No	28	16 (3.77)	12 (3.99)		
Yes	697	408 (96.23)	289 (96.01)		
Margin status				0.4795	0.7816
Close	31	15 (2.7)	16 (3.35)		
Negative	922	496 (89.37)	426 (89.31)		
Positive	79	44 (7.93)	35 (7.34)		
Vital status				19.4879	0.0005 ^a
Deceased	155	57 (9.73)	98 (18.99)		
Living	947	529 (90.27)	418 (81.01)		
Radiation therapy				0.0058	0.9535
No	445	241 (44.3)	204 (44.54)		
Yes	557	303 (55.7)	254 (55.46)		
Neoadjuvant treatment				2.9898	0.0925
No	1088	575 (98.29)	513 (99.42)		
Yes	13	10 (1.71)	3 (0.58)		
Targeted therapy				0.1257	0.7571
No	46	23 (8.36)	23 (7.57)		
Yes	533	252 (91.64)	281 (92.43)		
Sample type				0.9341	0.4663
Metastatic	7	5 (0.85)	2 (0.39)		
Primary tumor	1097	583 (99.15)	514 (99.61)		
Overall survival				19.9101	0.0005 ^a
No	933	520 (90.28)	413 (80.82)		
Yes	154	56 (9.72)	98 (19.18)		
Relapse-free survival				10.6443	0.0005 ^a
No	816	474 (92.4)	342 (85.71)		
Yes	96	39 (7.6)	57 (14.29)		

^aP<0.01; ^bP<0.05. ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; UBA7, ubiquitin-like modifier-activating enzyme 7.

M classification (P=0.0090), vital status (P=0.0005), OS (P=0.0005) and RFS (P=0.0005; Table II).

Diagnostic capability of UBA7. ROC curves were generated to determine the diagnostic ability of UBA7 in breast cancer (Fig. 3). The area under the curve (AUC) value was 0.559 (Fig. 3A), which indicates a reasonable diagnostic ability. Subsequently, subgroup analysis of different stages was performed and all data presented an adequate diagnostic ability (0.540 for stage I, 0.562 for stage II, 0.557 for stage III and 0.551 for stage IV; Fig. 3B-E).

Low UBA7 expression is associated with poor survival. Kaplan-Meier curves were generated and a log-rank test was performed to evaluate the prognostic value of UBA7 in breast cancer, and OS and RFS were used as prognostic parameters. The results demonstrated that patients with low UBA7 expression levels had poorer OS (P<0.0001; Fig. 4A). Further molecular subtype analysis demonstrated that patients with luminal A breast cancer (P=0.0210; Fig. 4B), luminal B breast cancer (P=0.0054; Fig. 4C), infiltrating ductal carcinoma (P=1.4x10⁻⁴; Fig. 4F) or infiltrating lobular carcinoma

Table III. Univariate and multivariate analyses of overall survival in patients with breast cancer.

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age, years	1.91	1.39-2.63	<0.001 ^a	2.31	1.44-3.68	<0.001 ^a
Histological type	0.93	0.74-1.17	0.543			
Molecular subtype	1.01	0.88-1.16	0.901			
ER	0.85	0.71-1.02	0.074			
PR	0.87	0.73-1.03	0.096			
HER2	1.29	1.05-1.57	0.013 ^b	1.10	0.88-1.37	0.3840
Menopause status	1.16	0.94-1.43	0.165			
Stage	1.64	1.40-1.91	<0.001 ^a	2.12	1.60-2.80	<0.001 ^a
Lymph node status	1.10	0.93-1.30	0.274			
Margin status	1.42	1.11-1.81	0.005 ^a	0.98	0.70-1.38	0.9290
UBA7	2.09	1.50-2.90	<0.001 ^a	2.10	1.30-3.39	0.0020 ^a

^aP<0.01; ^bP<0.05. HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; UBA7, ubiquitin-like modifier-activating enzyme 7.

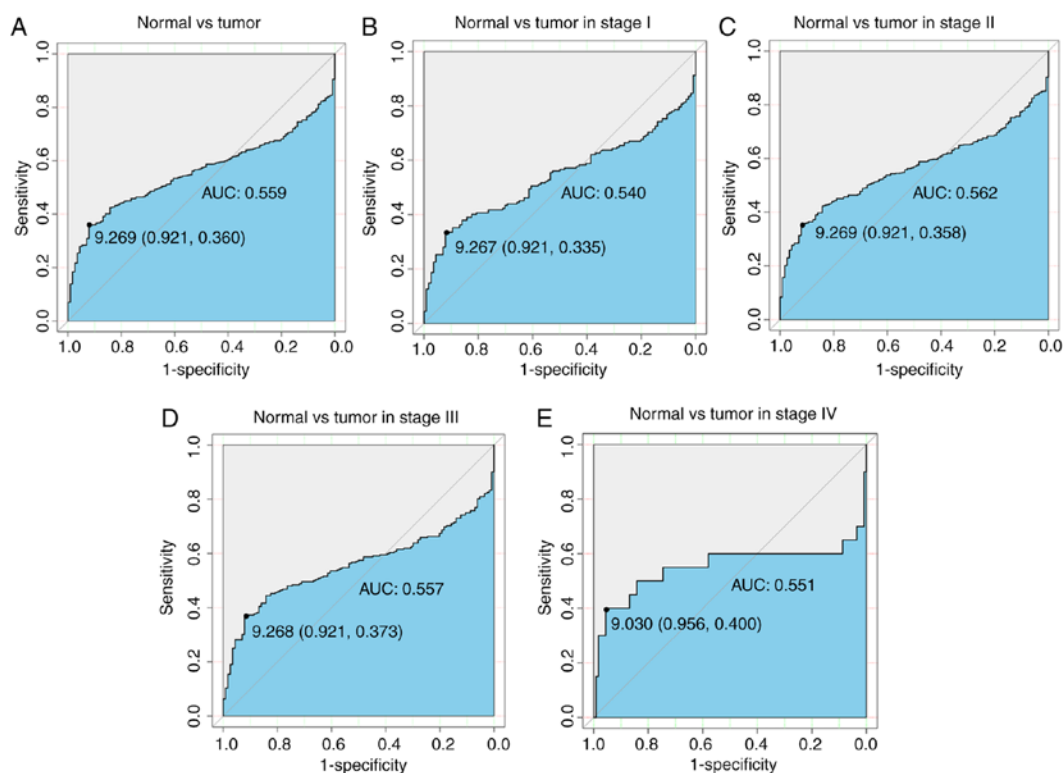


Figure 3. Receiver operating characteristic curves of UBA7 in different clinical stages. UBA7 exhibited mild diagnostic ability. (A) The AUC is 0.559, which represents a reasonable diagnostic ability. (B-E) Subgroup analysis of different stages also demonstrated a reasonable diagnostic ability. UBA7, ubiquitin-like modifier-activating enzyme 7; AUC, area under the curve.

($P=0.0033$; Fig. 4G) had poorer OS in the presence of low UBA7 expression. Univariate Cox analysis identified the critical variables associated with OS, including age ($P<0.001$), HER2 expression ($P=0.0130$), stage ($P<0.001$), margin status ($P=0.0050$) and UBA7 expression ($P<0.001$) (Table III). Multivariate Cox analysis indicated that age [hazard ratio (HR), 2.31; $P<0.001$], stage (HR, 2.12; $P<0.001$) and UBA7

expression (HR, 2.10; $P=0.0020$) may serve as independent prognostic factors, as presented in Table III.

Patients with low UBA7 expression levels also had a poor prognosis in terms of RFS ($P=0.0011$; Fig. 5A). Furthermore, low UBA7 expression was associated with poor RFS in patients with luminal B breast cancer ($P<0.0001$; Fig. 5C), infiltrating ductal carcinoma ($P=0.0031$; Fig. 5F)

Table IV. Univariate and multivariate analyses of relapse-free survival in patients with breast cancer.

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	P-value	HR	95% CI	P-value
Age, years	1.45	0.97-2.16	0.072			
Histological type	0.86	0.65-1.14	0.290			
Molecular subtype	0.99	0.82-1.20	0.945			
ER	0.78	0.63-0.97	0.026 ^a	0.88	0.63-1.22	0.441
PR	0.78	0.64-0.96	0.019 ^a	0.87	0.64-1.18	0.360
HER2	0.93	0.70-1.22	0.596			
Menopause status	0.95	0.74-1.22	0.713			
Stage	1.71	1.40-2.08	<0.001 ^b	1.55	1.24-1.92	<0.001 ^b
Lymph node status	0.86	0.70-1.06	0.159			
Margin status	1.59	1.23-2.06	<0.001 ^b	1.48	1.13-1.94	0.005 ^b
UBA7	1.95	1.30-2.93	0.001 ^b	1.59	1.03-2.45	0.037 ^a

^aP<0.05; ^bP<0.01. HR, hazard ratio; CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; UBA7, ubiquitin-like modifier-activating enzyme.

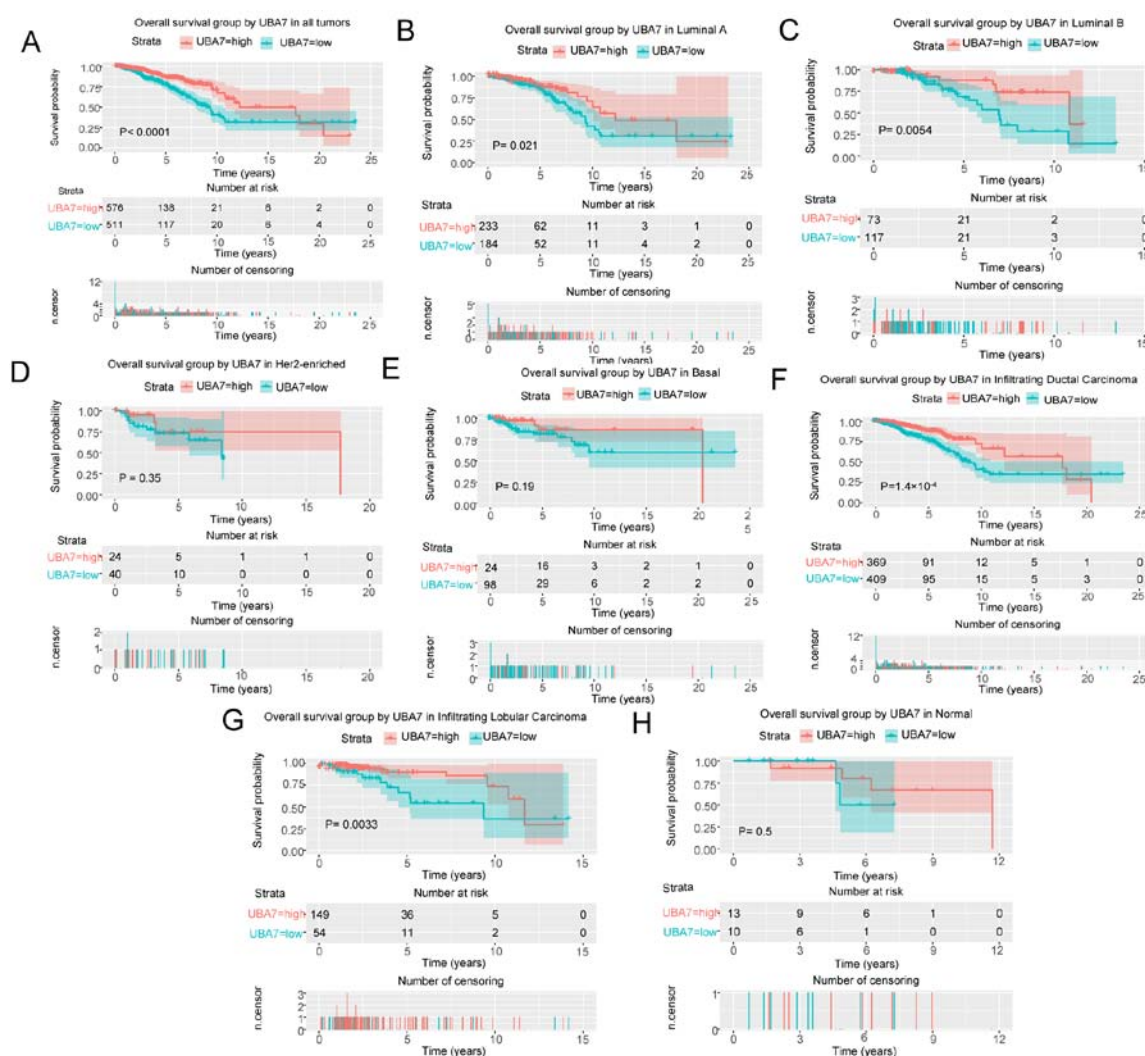


Figure 4. Analysis of the association between UBA7 expression and OS. (A) Patients with low UBA7 expression had poor OS. Patients with (B) Luminal A breast cancer, (C) Luminal B breast cancer, (F) infiltrating ductal carcinoma and (G) infiltrating lobular carcinoma had poor OS in the presence of low UBA7 expression. However, no significant associations were observed between UBA7 expression and OS in patients with (D) HER2-enriched breast cancer and (E) basal-like breast cancer, and in (H) normal cases. UBA7, ubiquitin-like modifier-activating enzyme 7; OS, overall survival; HER2, human epidermal growth factor receptor 2.

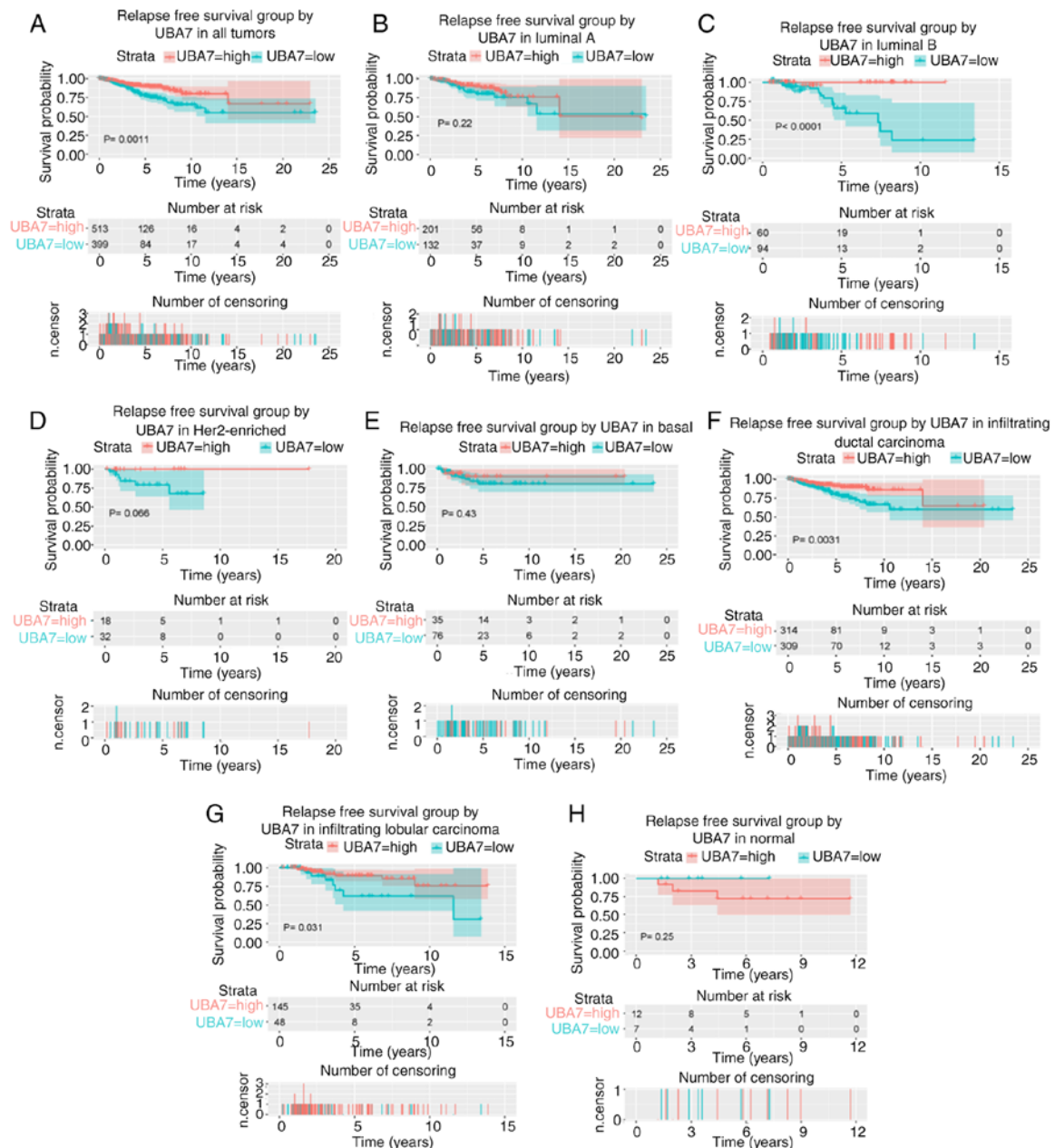


Figure 5. Survival analysis of UBA7 expression in terms of RFS. (A) Patients with low UBA7 expression had a poor prognosis in terms of RFS. Low UBA7 expression was significantly associated with poor RFS in patients with (C) Luminal B breast cancer, (F) infiltrating ductal carcinoma and (G) infiltrating lobular carcinoma. However, no significant associations were observed between UBA7 expression and RFS in patients with (B) Luminal A breast cancer, (D) HER2-enriched breast cancer and (E) basal-like breast cancer, and in (H) normal cases. UBA7, ubiquitin-like modifier-activating enzyme 7; RFS, relapse-free survival; HER2, human epidermal growth factor receptor 2.

and infiltrating lobular carcinoma ($P=0.0310$; Fig. 5G). Univariate Cox analysis and multivariate Cox analysis indicated that stage ($P<0.001$), margin status ($P=0.0050$) and UBA7 expression ($P=0.0370$) were independent prognostic factors for poor RFS (Table IV).

Kaplan-Meier curves were generated using the log-rank test to further investigate the association between UBA7 expression and the expression levels of ER, PR and HER2 in terms of OS (Fig. 6) and RFS (Fig. 7). The results demonstrated patients with ER-positive ($P=1.8 \times 10^{-4}$; Fig. 6B). PR-negative ($P=0.0180$; Fig. 6C), PR-positive ($P=0.0031$; Fig. 6D) and HER2-negative ($P=0.0180$; Fig. 6E) breast cancer had poor OS in the presence of low UBA7 expression. Furthermore,

patients with ER-positive ($P=0.0066$; Fig. 7B) and PR-negative ($P=0.0350$; Fig. 7C) breast cancer also had poor RFS in the presence of low UBA7 expression.

Discussion

Previous studies have set out to identify novel biomarkers for the diagnosis and prognosis of different diseases (15-24); however, to the best of our knowledge, the present study was the first to demonstrate that UBA7 may function as a biomarker in breast cancer. The results demonstrated that UBA7 expression was decreased in breast cancer compared with that in normal tissues. Furthermore, UBA7 expression differences were

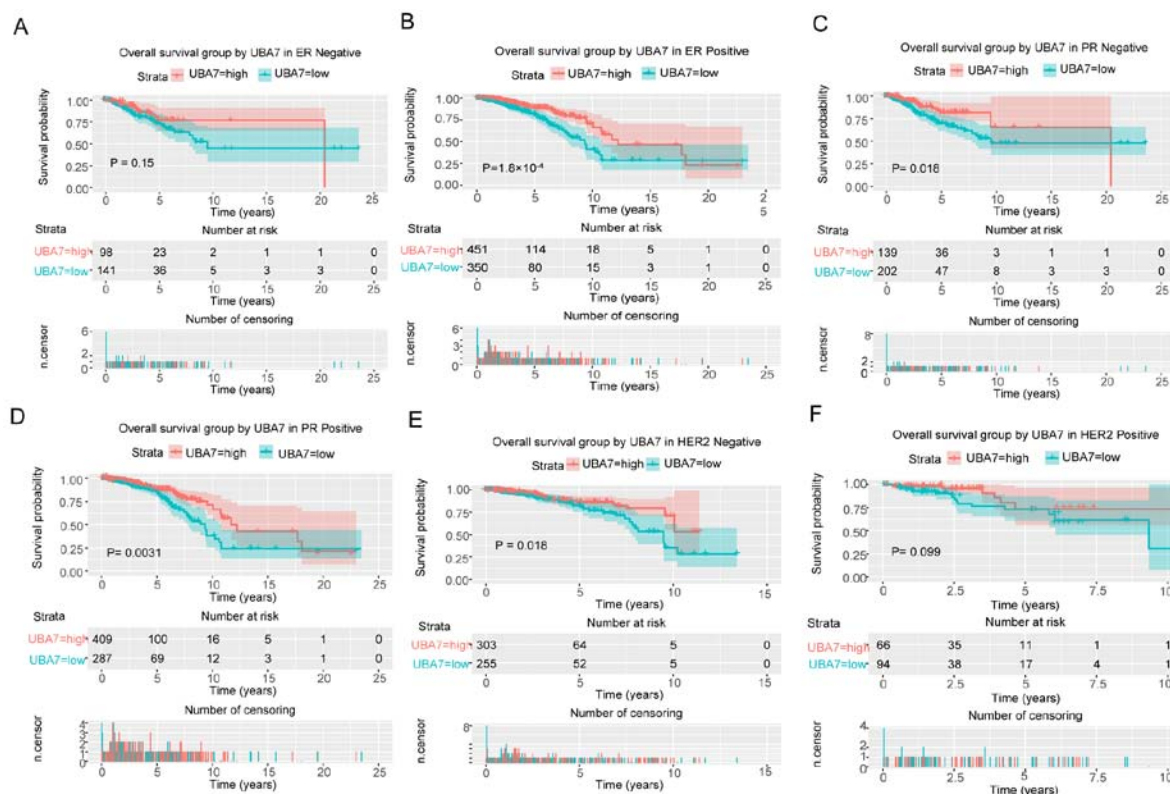


Figure 6. Survival analysis of UBA7 expression in terms of OS, according to the expression of ER, PR and HER2. Low UBA7 expression was significantly associated with poor OS in (B) ER-positive, PR (C) -negative and (D) -positive, and (E) HER2-negative patients. However, no significant associations were observed between low UBA7 expression and OS in (A) ER-negative and (F) HER2-positive patients. UBA7, ubiquitin-like modifier-activating enzyme 7; OS, overall survival; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

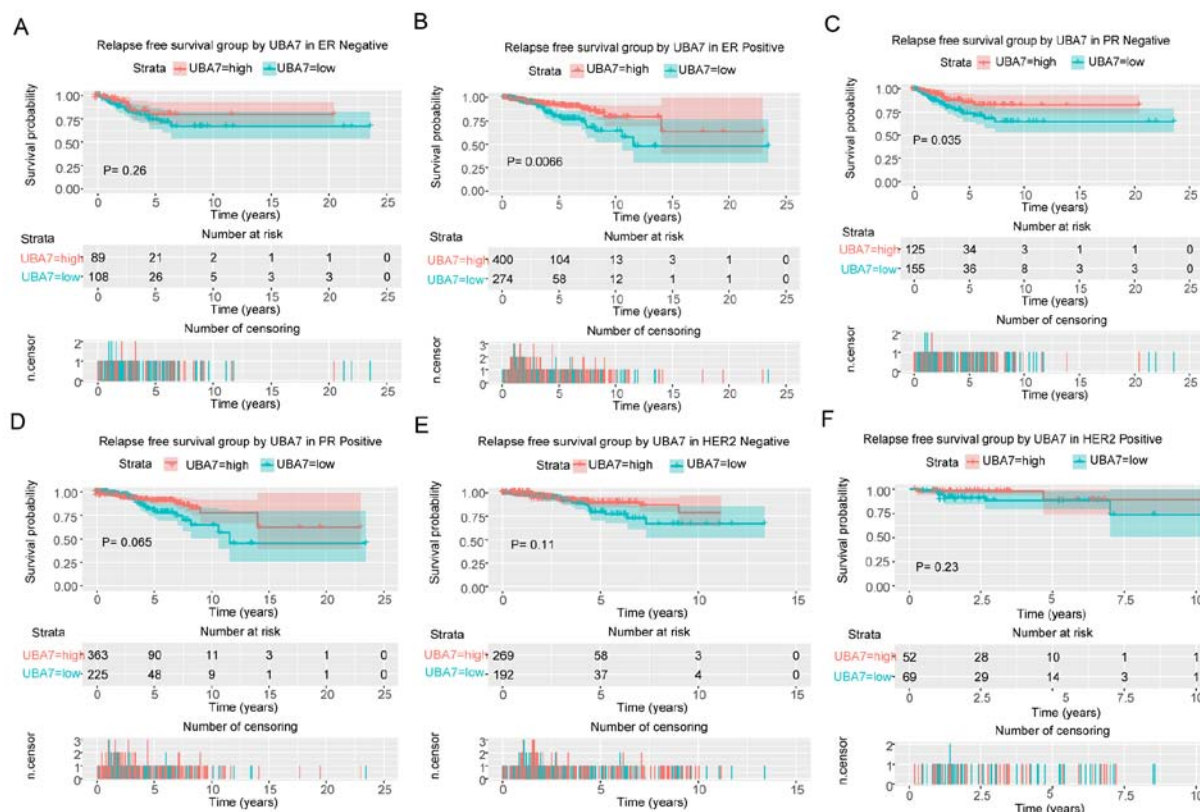


Figure 7. Survival analysis of UBA7 expression in terms of RFS, according to the expression of ER, PR and HER2. Low UBA7 expression was significantly associated with poor RFS in (B) ER-positive and (C) PR-negative patients. However, no significant associations were observed between low UBA7 expression and RFS in (A) ER-negative, (D) PR-positive, and HER2 (E) -negative and (F) -positive patients. UBA7, ubiquitin-like modifier-activating enzyme 7; RFS, relapse-free survival; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

associated with vital status, tumor classification, metastasis classification, histological type, sex, molecular subtype, and the expression of PR, ER and HER2. The low and high UBA7 expression groups also demonstrated differences in some clinical characteristics, including molecular subtype, histological subtype and expression of ER, PR and HER2, vital status, OS and RFS. Patients with lower UBA7 expression had a poor prognosis. Although UBA7 demonstrated a reasonable diagnostic capability, it may still be used in combination with other markers to diagnose breast cancer.

Currently, the only known biological function of UBA7 is catalysing ISG15 conjugation. Previous studies have reported that UBA7 has the potential to act as a tumor suppressor gene in human lung cancer (8-12). UBA7 is located on human chromosome region 3p21.3, and LOH on chromosome 3p21.3 has been observed in 70-80% of NSCLC and 90-100% of SCLC cases (8). The expression levels of UBA7, previously referred to as UBE1L, have been reported to be markedly decreased in several lung cancer cell lines (11,25,26). Notably, Kok *et al* (27) demonstrated that although one allele of UBA7 was absent in lung cancer cell lines, the remaining allele was still detectable and without mutation (27). The results of the present study demonstrated that UBA7 expression was significantly decreased in breast cancer and that stage IV tumors had the lowest UBA7 expression levels. Furthermore, UBA7 expression was also associated with clinical characteristics, and low UBA7 expression was demonstrated to be associated with poor prognosis, especially in patients with ER-positive and PR-positive breast cancer.

A number of studies have demonstrated that UBA7 can promote the formation of a complex between ISG15 and cyclin D1, and inhibit cyclin D1, which is associated with cancer cell proliferation (8-12). UBA7-knockdown has been reported to increase cyclin D1 expression; however, overexpression of UBA7 has been shown to reduce cyclin D1 expression and suppress lung cancer cell proliferation (9). Furthermore, it has been reported that UBA7 can suppress EGFR at the post-translational level in HBE-Beas-2B cells and suppress the subsequent AKT/NF- κ B signaling pathway (12). However, deficiency of UBA7 has no effect in K-ras (LA2)-mutant mice with lung cancer, thus UBA7 is not considered a tumor suppressor gene in the K-ras lung cancer mouse model (8). In the present study, the decreased UBA7 expression levels in breast cancer were not as obvious as it was demonstrated to be in lung cancer, thus it is suggested that UBA7 may not have the same effect on breast cancer cells. Furthermore, the results of the present study demonstrated that UBA7 was associated with the expression of PR, ER and HER2, and the molecular subtypes of breast cancer, thus UBA7 may have the potential to influence the proliferation of breast cancer cells through these molecules, which are closely associated with breast cancer. The association between UBA7 expression and cyclin D1, EGFR, ER, PR, HER2 and other tumor-associated molecules requires further experimental evidence; however, UBA7 may function as a target in treatment according to current data on lung cancer.

The molecular mechanism underlying UBA7 regulation remains unclear; however, a number of molecules have been reported to influence UBA7 expression. For example, IFN- α has the ability to induce ISGylation-associated protein expression, including UBA7 expression (28). Furthermore,

retinoic acid has been demonstrated to induce UBA7 expression, which in turn induces ISGylation of promyelocytic leukaemia/retinoic acid receptor- α protein and causes degradation in acute promyelocytic leukaemia (29). Previous studies have reported that chemopreventive polyphenols (10,30,31), such as curcumin, epigallocatechin gallate and resveratrol, have the ability to influence UBA7 expression by altering the intracellular reactive oxygen species status, which is regulated via the c-Jun N-terminal kinase/nuclear factor erythroid 2-related factor 2 signaling pathway; however, this association exhibits dose-dependent effects (10). IFNs have been used with the intent to cure cancer for several years, including breast cancer (30). IFNs have been reported to exert antitumor effects by regulating the immune system and affecting cell proliferation and apoptosis (31). A limitation of the present study was failure to assess the influence of IFNs on UBA7 expression. Thus, future studies will focus on the use of IFNs and other molecules, including chemopreventive polyphenols, which may influence UBA7 expression, while studying the change in UBA7 expression in breast cancer, and will aim to investigate whether IFNs suppress breast cancer through the UBA7 pathway.

Although UBA7 has been considered a potential tumor suppressor gene in lung cancer for decades, its underlying molecular mechanism still remains unclear. Furthermore, the role of UBA7 in different types of cancer still lacks sufficient evidence to support this hypothesis. To the best of our knowledge, the present study was the first to uncover UBA7 expression changes in breast cancer. The results demonstrated that UBA7 expression was decreased in breast cancer and was also associated with clinical characteristics. In addition, low UBA7 expression was associated with a poor prognosis. Taken together, these results suggest that UBA7 may function as a biomarker for the clinical diagnosis and prognosis of breast cancer, and even as a target in treatment.

The present study aimed to identify the changes in UBA7 expression in breast cancer using bioinformatic methods from a macro perspective. Further studies and evidence from the laboratory are required to support the stated speculations, and the potential molecular mechanisms also need to be investigated. The subsequent stages of research have already been designed, and will focus on laboratory work, including the collection of tissues from patients, analysis of UBA7 and other related molecules expression levels in tissues, and the exploration of associations between molecules and clinical features, and even potential molecular mechanisms using cells.

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Availability of data and material

The datasets generated and analyzed during the present study were obtained from The Cancer Genome Atlas (TCGA) database (<https://cancergenome.nih.gov/>).

Authors' contributions

ML and YL designed the study and wrote the manuscript. YJ and FH had a significant role in the study design, data collection and manuscript review. SQ and YJ acquired and analysed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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