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The clinicopathological and prognostic significances of LATS1 expression in breast cancer

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Summary. Aim. Large tumor suppressor gene 1 (LATS1) belongs to the PKA/PKG/PKC serine/threonine kinase subfamily of the Hippo signaling pathway and inactivates nuclear co-activators YAP1 and WWTR1 by phosphorylation. This study aimed to discern the clinicopathological and prognostic significances of LATS1 expression in breast cancer.

Methods. We examined LATS1 expression in breast carcinogenesis and compared it with clinicopathological parameters and survival information of breast cancer patients using immunohistochemistry, western blotting, RT-PCR, and bioinformatics analysis.

Results. LATS1 expression was downregulated in breast cancer at both mRNA and protein levels ($P<0.05$). LATS1 mRNA expression was negatively correlated with low ER and PR expression, aggressive subtypes (TNBC and HER2+ vs. luminal), and poor survival ($P<0.05$). Its protein expression was negatively linked to T stage, N stage, M stage, TNM stage, histological grade, PR status, and unfavorable prognosis ($P<0.05$). There was a positive correlationship between nuclar and cytoplasmic LATS1 expression in breast cancer ($P<0.05$).

Conclusions. The downregulation of LATS1 expression plays a vital role in the carcinogenesis and progression of breast cancer. Thus, LATS1 loss was employed to indicate the aggressive behaviors and poor prognosis of breast cancer.

Key words: LATS1, Breast cancer, Clinicopathological parameter, Prognosis

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Introduction

Breast cancer is the most common cancer and the second leading cause of cancer-related death in women. Although there has been great progress made in screening, diagnosis, and treatment strategies, it remains a major health problem for women; its incidence rate continues to rise (Torre et al., 2016; DeSantis et al., 2019; Miller et al., 2019). Therefore, it is necessary to find new diagnostic and therapeutic targets and prognostic markers.

LATS1 (large tumor suppressor 1) is a core member of the Hippo signaling pathway, belonging to the Ndr/LATS subfamily of PKA/PKG/PKC serine/threonine kinases. It inactivates the nuclear co-activators YAP1 and WWTR1 by phosphorylation (Hergovich and Hemmings, 2009; Zhao et al., 2010; Pfleger, 2017). LATS1 protein is localized to the mitotic apparatus where it interacts with CDC2, reduces H1 histone kinase activity and negatively regulates CDC2/cyclin A (Visser and Yang, 2010; Furth and Aylon, 2017). The RASSF1A-LATS1 axis stabilizes replication forks by suppressing CDK2-induced BRCA2 phosphorylation (Pefani et al., 2015). CHO1 phosphorylation by LATS1 activates centrosomal LIMK1 during cytokinesis (Okamoto et al., 2015); however, LATS1 restricts centrosome overduplication by stabilizing Cdc25B (Mukai et al., 2015). LATS1-induced YAP phosphorylation dissociates it from TEAD4, facilitates YAP-RUNX3 complex formation, and suppresses its translocation into the nucleus, thereby regulating genes essential for proliferation, cell death, and migration (Hergovich et al., 2006; Hao et al., 2008; Han et al., 2017; Jang et al., 2017; McNeill and Reginensi, 2017). LATS1 phosphorylates CDC26 to assemble the tetratricopeptide repeat subcomplex APC/C (Masuda et al., 2015), angiomotin to inhibit YAP transcription and cell growth (Adler et al., 2013), and FOXL2 to repress STAR mRNA expression (Pisarska et al., 2010). LATS1

is ubiquitinated by NEDD4, E3 ubiquitin ligase CRL4, and WWP1 E3 ligase, leading to its proteasomal degradation and nuclear localization of YAP and its transcriptomic activity (Salah et al., 2013; Yeung et al., 2013; Li et al., 2014). LATS1 also stabilizes Beclin-1 independent of its serine/threonine kinase activity by ubiquitinating Beclin 1 at lysines K27, K32, and K263 and suppressing autophagy by forming an inactive Beclin-1 dimer (Tang et al., 2019).

Downregulated LATS1 expression can result from genetic mutation, loss of heterozygosity (LOH), promoter hypermethylation, ubiquitination, and degradation in various human cancers (Takahashi et al., 2005; Jiang et al., 2006; Ji et al. 2012; Wierzbicki et al., 2013; Yabuta et al., 2013; Lin et al., 2014; Furth and Aylon, 2017). LATS1 is recruited to the plasma membrane for multi-site phosphorylation and activation by hMOB1 (Hergovich et al., 2006). The dissociation of LATS1 from WWC3 reduces LATS1 phosphorylation to suppress lung cancer invasion and metastasis, while its interaction with TNFAIP8 promotes aggressiveness of hepatocellular carcinoma (HCC) cells (Dong et al., 2017). LncRNA uc.134 reduces CUL4A-induced LATS1 ubiquitination and YAP phosphorylation to repress HCC progression (Ni et al., 2017). Zhang et al. (2017) demonstrated that circular RNA_LARP4 could inhibit both proliferation and invasion of gastric cancer cells by sponging miR-424-5p and inducing LATS1 overexpression. LATS1 ablation promotes the luminal and stem phenotypes by initiating ubiquitination and DCAF1-dependent proteasomal degradation of ER (estrogen receptor) α (Britschgi et al., 2017). Pan et al. (2019) found that LATS1/2 abrogation could inhibit the growth of murine MC38 colon cancer cells by uncontrolled activation of YAP and its targets (Wisp2 and Ccdc80), especially under detachment conditions. LATS1 knockout (KO) mice have a low neonate survival, retardation of growth and mammary gland development, infertility, pituitary dysfunction, increased incidence of soft-tissue sarcomas and ovarian stromal cell tumors, and high sensitivity to carcinogenic treatments (St John et al., 1999). LATS1/2 sustains intestinal cell stemness through Wnt activation, TEAD-dependent transcription, mesenchymal-to-epithelial transition (EMT), and differentiation into granulosa cells (Li et al., 2020). The current study analyzed the clinicopathological and prognostic significance of LATS1 mRNA and protein expression in breast cancer cells using pathological or bioinformatics analyses.

Materials and methods

UALCAN database

The UALCAN (http://ualcan.path.uab.edu) database is a comprehensive web portal that allows deep analyses of TCGA mRNA expression and CPTAC protein expression data (Chandrashekar et al., 2017). The LATS1 mRNA expression screening conditions were as follows: TCGA analysis; Enter gene symbol(s): LATS1; TCGA dataset: breast invasive carcinoma. The LATS1 protein expression screening conditions were as follows: CPTAC analysis; Enter gene name(s): LATS1; CPTAC dataset: breast cancer. The Xiantao platform (https://www.xiantao.love/) was also used to compare LATS1 and clinicopathological characteristics of breast cancer (e.g., estrogen and progesterone receptor expression).

Kaplan-Meier Plotter database

The effects of LATS1 expression on the survival of breast cancer patients with distinct subtypes were determined using the Kaplan-Meier plotter analysis. The y-axis represents the impact of LATS1 expression on overall (OS), post-progression (PPS), or relapse-free (RFS) survival, and the x-axis represents the observation time. The two groups of patients were compared by Kaplan-Meier curves, and the hazard ratios with their corresponding 95% confidence intervals and log-rank p-values were calculated. The screening conditions were as follows: Cancer: Breast Cancer; Gene symbol: LATS1; Affyid: 227772_at (Gyorffy et al., 2010).

Xiantao database

The relationship between LATS1 mRNA expression and clinicopathological parameters of breast cancer patients were determined using the Xiantao platform. The screening conditions were as follows: Cancer: Breast Cancer; Gene symbol: LATS1; The parameters: age, race, T stage, N stage, M stage, pathologic stage, histological type, PR status, ER status, HER2 status, and PAM50.

Table 1. The clinicopathological characteristics of breast cancer samples.

Clinicopathological features	Paraffin-embedded Samples (n=826)	Frozen Samples (n=137)
Age(years)	45 (23-82)	45(25-75)
T staging		
1	214	43
2	398	79
3	43	9
4	16	6
N staging		
0	368	107
1-3	300	30
M staging		
0	490	134
1	14	3
TNM staging		
1-2	490	78
3-4	181	59
Histological grade		
1	35	9
2-3	791	128

Samples and pathology

Normal breast tissue samples (n=69), adenomatosis (n=73), fibroadenoma (n=162), primary breast cancer (n=826), and metastatic breast cancer in the lymph nodes (n=108) were obtained from The Affiliated Hospital of Chengde Medical University. The samples were prepared in pathological blocks. The average patient age was 45 years (23-82 years) at the time of surgical operation. Among these brease cancer cases, there were 300 cases of lymph node metastasis and 14 cases of distant metastasis (Table 1). In addition, another 137 fresh breast cancer samples and paired normal breast tissues were collected by the Affiliated Hospital of Chengde Medical University between 2015 and 2020 and frozen in liquid nitrogen for protein and RNA extraction (Table 1). The average patient age was 45 years (25-75years). These breast cancer patients never received adjuvant treatment, radiotherapy, or chemotherapy before the operation. Patients provided signed informed consented, and the Ethics Committee of the Affiliated Hospital of Chengde Medical University approved the study.

Tissue microarray (TMA)

Tissue punches (2-mm diameter) were taken from representative areas of HE (hematoxylin-eosin)-stained breast cancer slides and added to recipient blocks (maximum 70 cores) using a tissue microarrayer. The TMA blocks were serially dissected into 4 μm-thick sections and mounted onto poly-lysine-coated glass slides.

RNA extraction and RT-PCR

Total RNA was extracted from the breast tissues using Trizol. cDNA was synthesized from the RNA (2 μg) using AMV reverse transcriptase and random primers. The primers were 5'-GGGTCCTCGG CAAAGTTTA-3' and 5'- TTTCTTGGCACAAACAC CAT-3' (130 bp) for LATS1 and 5'-CAATGACCC CTTCATTGACC-3' and 5'-TG GAAGATGGTGA TGGGATT-3' (135 bp) for GAPDH. Real-time PCR was carried out using the SYBR Premix Ex Taq II kit, with GAPDH as an internal control. LATS1 mRNA expression levels in the samples were calculated using the $2^{-\Delta\Delta CT}$ method and normalized with normal tissue.

Western blotting

The breast tissues were homogenized in RIPA lysis buffer, and protein concentration was determined using the Kuamas brilliant blue method. The protein samples (35 μg) were subjected to 10% SDS-PAGE electro-phoresis and transferred to PVDF membranes. The blots were blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline with Tween 20 (TBST) and then incubated with mouse anti-LATS1 (1:1000; Abcam) or rabbit anti-GAPDH (1:2000; CST) antibody for 1 h at room temperature respectively. After the incubation, the membranes were washed with TBST and incubated with anti-rabbit or anti-mouse HRP-conjugated secondary antibody (Dako). The stained bands were visualized with enhanced chemilumi-nescence using the Azure Biosystem C300.
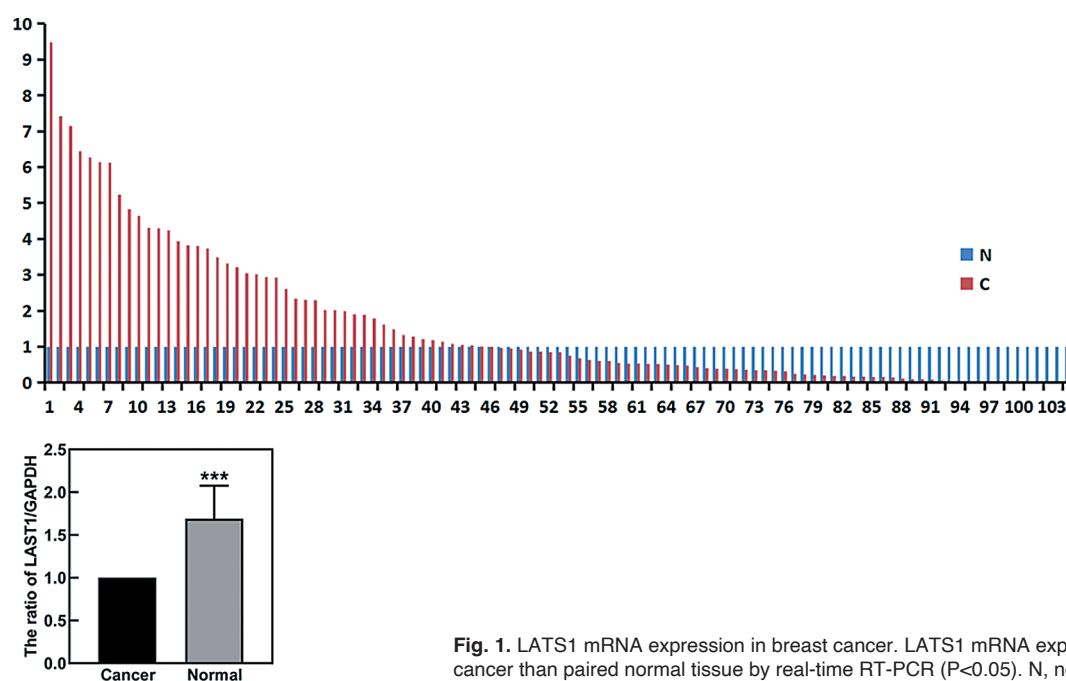

Fig. 1. LATS1 mRNA expression in breast cancer. LATS1 mRNA expression level was lower in breast cancer than paired normal tissue by real-time RT-PCR (P<0.05). N, normal tissue; C, cancer.

Immunohistochemistry (IHC)

Paraffin-embedded tissue sections were dewaxed, debenzylated, and rehydrated. Antigen retrieval was performed by microwaving the samples for 20 min in Target Retrieval Solution (Dako). Endogenous peroxidase was inactivated with hydrogen peroxide. The samples were blocked with 5% BSA for 30 min. The sections were incubated with mouse anti-human LATS1 antibody (1:100; Abcam) for 2 h and then HRP-conjugated anti-mouse secondary antibody (1:200; Dako) for 1 h. After each step, the sections were rinsed three times with PBS for 5 min. The sections were stained with DAB, counterstained with Mayer's hematoxylin, dehydrated, cleared, and mounted. Sections stained in the absence of primary antibody were used as negative controls. For the analysis, 100 cells were randomly chosen and counted from five representative fields by two independent researchers (ZHC and XLW). The positive rate classifications were as follows: 0=0%; 1=1-49%; 2=50-74%; 3≥75%. The positive intensity classifications were as follows: 1=weak; 2=medium; 3=strong. The LATS1 score was calculated as the intensity × positive rate, with the scores defined as follows: -=0; +=1-2; ++=3-5; +++=6-9. Moreover, any score above 0 was considered positive for LATS1 expression.

Statistical analysis

Statistical analysis was carried out using the Spearman test for the rank data and Mann-Whitney U for the mean comparison. Kaplan-Meier survival was used to analyze univariate survival data (low vs. high expression, according to the median). Multivariate survival analysis was performed using the Cox's proportional hazard model. All statistical analysis was performed using SPSS v. 26.0 software. A P-value <0.05 was statistically regarded as significant.

Results

The clinicopathological significance of LATS1 mRNA levels in breast cancer

RT-PCR analysis revealed that LATS1 mRNA expression was lower in breast cancer than in matched normal tissue (Fig. 1, P<0.001). Similar results were obtained from the UALCAN database (Fig. 2A, P<0.001). LATS1 mRNA expression was lower in breast cancer than normal mucosa even stratified by patient age (Fig. 2B, P<0.001), nodal metastasis status (Fig. 2C, P<0.05), and TNM staging (Fig. 2D, P<0.001). In comparison to normal breast tissue, LATS1 mRNA was underexpressed in several breast cancer subtypes, including luminal, HER2-positive, and triple-negative breast cancer (TNBC) (Fig. 2E, P<0.001). The levels in TNBC and HER2-positive breast cancer were lower than in luminal breast cancer (Fig. 2E, P<0.001). Furthermore, LATS1 mRNA levels were higher in normal breast tissue than in TP53-mutant and non-mutant breast cancer samples (Fig. 2F, P<0.001).
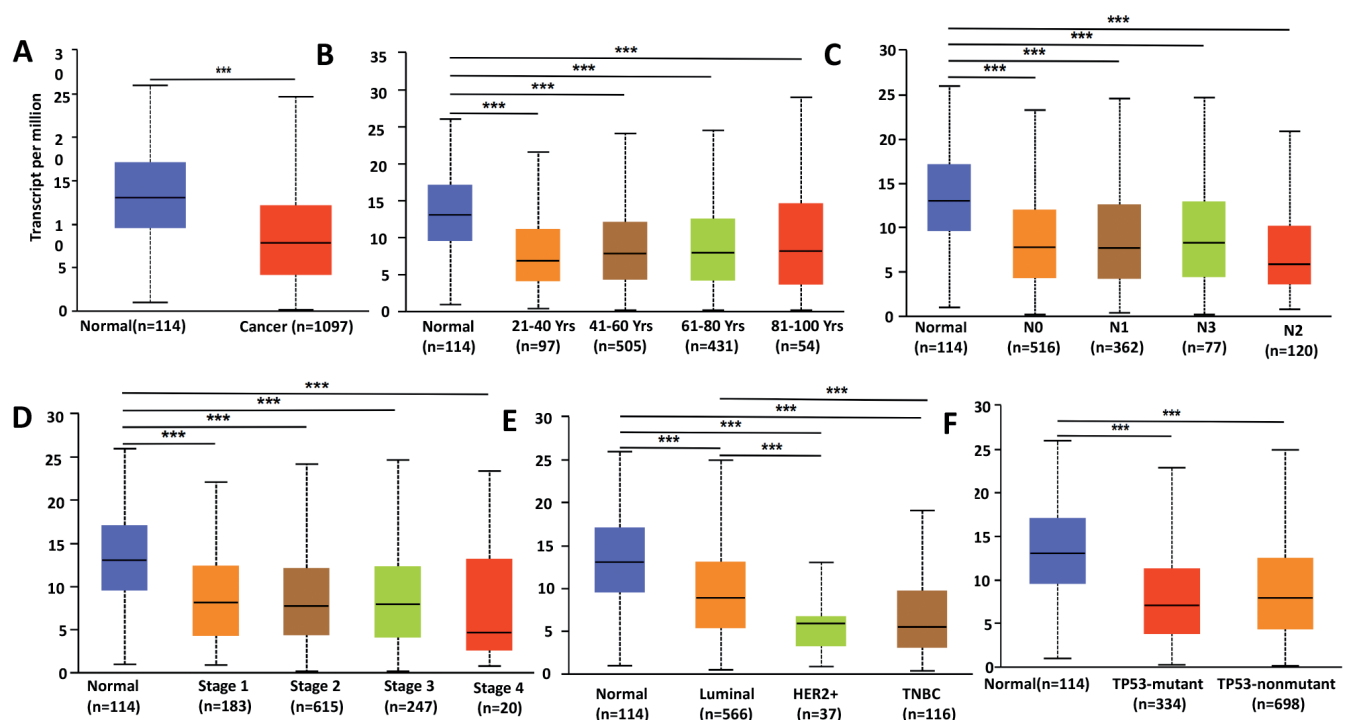

Fig. 2. The clinicopathological significance of LATS1 mRNA expression. UALCAN portal was used to evaluate the LATS1 mRNA expression in breast cancer **(A)**. It was compared with age **(B)**, N staging **(C)**, TNM staging **(D)**, molecular subtyping **(E)**, and TP53 mutation **(F)** of breast cancer patients.

Analysis of the Xiantao database (Table 2) showed that LATS1 mRNA expression was higher in White patients than those of other races (P<0.001). The expression levels were positively correlated with PR (progesterone receptor) and ER (estrogen receptor) mRNA expression levels in breast cancer (P<0.01). Moreover, the levels were higher in luminal breast cancer than in HER2-positive and Basal breast cancer (P<0.001).

The clinicopathological significance of LATS1 protein levels in breast cancer

LATS1 protein hypoexpression was observed in breast cancer compared to normal breast tissue (Fig. 3, P<0.001). LATS1 protein levels were negatively associated with young age (P=0.0092) and PR status (P=0.0416). According to the UALCAN database, LATS1 protein expression levels were lower in breast cancer than in the normal tissue (Fig. 4A, P=0.0088). Breast cancer patients ages 41-60 and 61-80 years had lower LATS1 protein expression levels than the normal group or the 80-100 yr-old breast cancer patient group (Fig. 4B, P<0.05). Its protein expression was also decreased in Stage II and III tumors compared to normal breast tissue (Fig. 4C, P<0.05). HER2-positive breast

cancer had lower LATS1 protein expression levels than the other subtypes (Fig. 4D, P<0.05).

Immunohistochemistry showed LATS1 nuclear staining in normal breast tissue and fibroadenoma; however, the staining was weak or negative in primary or metastatic breast cancer (Fig. 5). No cytoplasmic staining was observed in normal breast tissue; positive in fibroadenoma, primary and metastatic cancer. In particular, the positive nuclear LATS1 expression rates were 23.2% (16/69), 24.6% (18/73), 23.5% (38/162), 15.0% (124/826), and 14.8% (16/108) for normal breast tissue, adenomatosis, fibroadenoma, primary breast cancer, and metastatic breast cancer, respectively (Table 3). Moreover, nuclear LATS1 protein staining was weaker in primary and metastatic breast cancer than in normal tissue, fibroadenoma, and adenomatosis (Table 3, P<0.01). Cytoplasmic LATS1 was detected in normal breast tissue (92.8%, 64/69), adenomatosis (31.5%, 23/73), fibroadenoma (27.8%, 45/162), primary breast cancer (35.7%, 295/826), and metastatic breast cancer (39.8%, 43/108). The cytoplasmic LATS1 staining was weaker in adenomatosis, fibroadenoma, and primary and metastatic breast cancer than in normal tissue (Table 3, P<0.01). Nuclear LATS1 levels were negatively correlated with young age (Table 4, P=0.002), T stage (Table 4, P=0.019), N stage (Table 4, P=0.023),
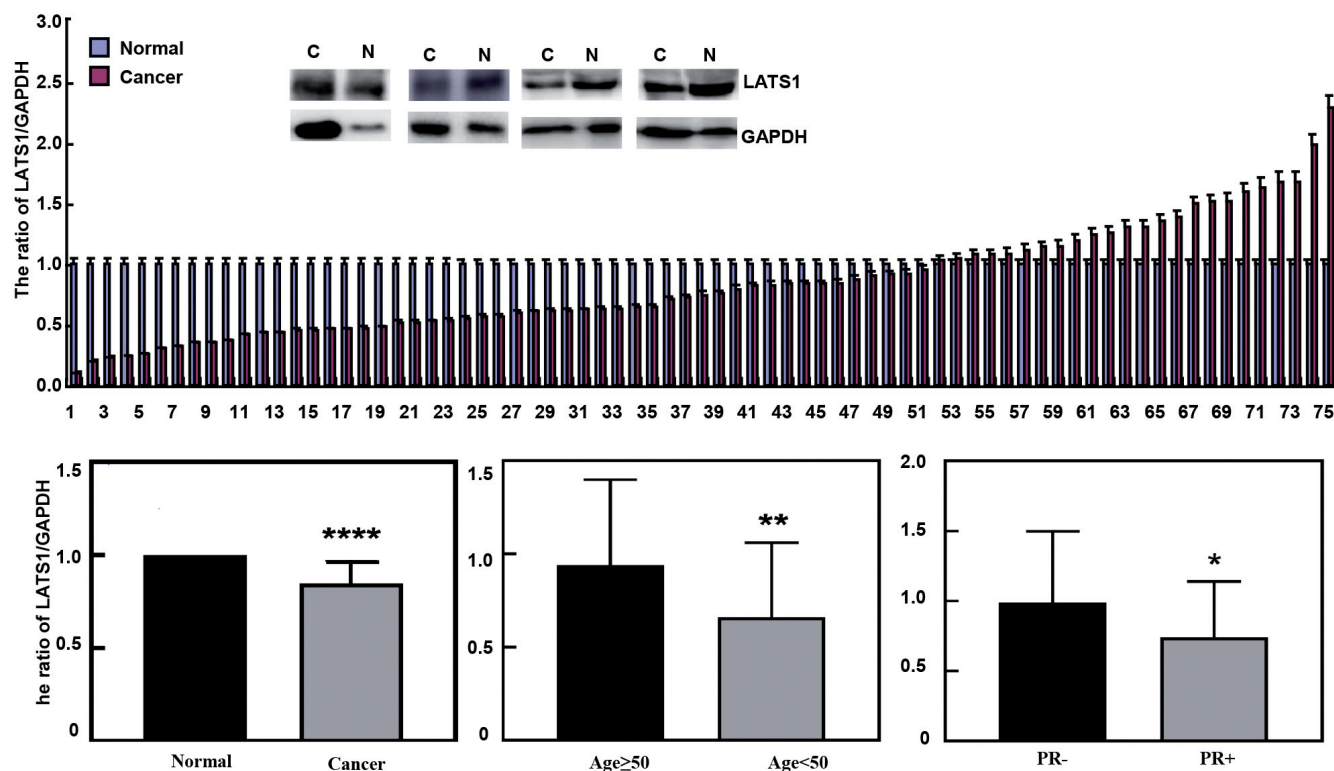

Fig. 3. LATS1 expression in breast cancer. Tissue lysate was loaded and probed with anti-LATS1 antibody (140kDa) with GAPDH (36kDa) as an internal control. LATS1 protein expression level was lower in breast cancer than paired normal tissue by Western blot (P<0.05). It was negatively correlated with age and progesterone receptor (PR) expression of breast cancer patients (P<0.05). N, normal tissue; C, cancer.

histological grade (Table 4, P<0.001), and HER2 status (Table 4, P=0.0003). Cytoplasmic LATS1 levels were negatively correlated with N stage (Table 4, P=0.040), M stage (Table 4, P=0.022) ,and histological grade (Table 4, P=0.004). Nuclear LATS1 expression was positively related to cytoplasmic LATS1 expression in the breast cancer samples (Table 5, P<0.001).

The associstion between LATS1 expression and breast cancer prognosis

According to the univariate analysis of the IHC results, LATS1 protein expression was not a prognostic factor for the overall survival (OS) or disease-free survival (DFS) of breast cancer patients (Table 6, P>0.05). In contrast, T stage (P=0.001), N stage (P<0.001), TNM stage (P<0.001), radiotherapy (P=0.018), and endocrine therapy (P =0.004) were closely related to OS (P<0.05), and T stage (P=0.002), N stage (P<0.001), M stage (P=0.021), TNM stage (P<0.001), PR expression (P=0.024) Ki-67 expression (P=0.044), radiotherapy (P=0.008), and endocrine therapy (P=0.003) were closely associated with DFS (P<0.05). Cox's analysis indicated that age (P=0.038) and endocrine therapy (P=0.017) were independent factors for the OS of breast cancer patients (Table 7, P<0.05), whereas age (P=0.028) and Ki-67 expression (P=0.028) were independent factors for DFS (Table 7, P<0.05).

Based on Kaplan-Meier plotter, LATS1 mRNA expression was positively correlated with the post-progression survival (Fig. 6A, P=0.026) and relapse-free survival (Fig. 6B, P<0.001) of breast cancer patients. It was the same for OS of the breast cancer patients with lymph node metastasis negativezhens (Fig. 6C, P=0.035) or ER expression negativity (Fig. 6D, P=0.011) and PPS of those with lymph node involvement, grade 3, or ER negativity (Fig. 6E-G, P=0.024, P=0.042, P=0.028,

Table 2. The relationship between LATS1 mRNA expression and clinicopathological characteristics of breast cancer.

Characteristic	Variables	Low expression	High expression	p
Age, n (%)	≤60	305 (28.2%)	296 (27.3%)	0.601
	>60	236 (21.8%)	246 (22.7%)	
Race, n (%)	Asian	34 (3.4%)	26 (2.6%)	<0.001
	Black or African American	128 (12.9%)	53 (5.3%)	
	White	345 (34.7%)	408 (41%)	
T stage, n (%)	T1	134 (12.4%)	143 (13.2%)	0.761
	T2	312 (28.9%)	317 (29.4%)	
	T3	74 (6.9%)	65 (6%)	
	T4	19 (1.8%)	16 (1.5%)	
N stage, n (%)	N0	253 (23.8%)	261 (24.5%)	0.499
	N1	181 (17%)	177 (16.6%)	
	N2	55 (5.2%)	61 (5.7%)	
	N3	44 (4.1%)	32 (3%)	
M stage, n (%)	M0	434 (47.1%)	468 (50.8%)	0.087
	M1	14 (1.5%)	6 (0.7%)	
Pathologic stage, n (%)	Stage I	89 (8.4%)	92 (8.7%)	0.295
	Stage II	308 (29.1%)	311 (29.3%)	
	Stage III	124 (11.7%)	118 (11.1%)	
	Stage IV	13 (1.2%)	5 (0.5%)	
Histological type, n (%)	IDC	382 (39.1%)	390 (39.9%)	0.908
	ILC	103 (10.5%)	102 (10.4%)	
PR status, n (%)	Negative	213 (20.6%)	129 (12.5%)	<0.001
	Indeterminate	0 (0%)	4 (0.4%)	
	Positive	299 (28.9%)	389 (37.6%)	
ER status, n (%)	Negative	160 (15.5%)	80 (7.7%)	<0.001
	Indeterminate	0 (0%)	2 (0.2%)	
	Positive	352 (34%)	441 (42.6%)	
HER2 status, n (%)	Negative	263 (36.2%)	295 (40.6%)	0.272
	Indeterminate	8 (1.1%)	4 (0.6%)	
	Positive	81 (11.1%)	76 (10.5%)	
PAM50, n (%)	Normal	25 (2.3%)	15 (1.4%)	<0.001
	LumA	239 (22.1%)	323 (29.8%)	
	LumB	94 (8.7%)	110 (10.2%)	
	Her2	55 (5.1%)	27 (2.5%)	
	Basal	128 (11.8%)	67 (6.2%)	

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor.

respectively). Moreover, LATS1 mRNA expression was positively associated with the RFS of luminal-A breast cancer patients (Fig. 6H, P=0.002). The protein expression was positively associated with OS of all the breast cancer patients (Fig. 6I, P=0.04).

Discussion

LATS1 may maintain ploidy by regulating mitosis and the G_1 tetraploid checkpoint and retard the G_2/M transition by inactivating CDK1 kinase activity. LATS1

Table 3. LATS1 protein expression during breast carcinogenesis and subsequeng progression.

Groups	n	Nuclear LATS1 expression					n	Cytoplasmic LATS1 expression				
		-	+	++	+++	%		-	+	++	+++	%
Normal breast tissues	69	53	11	5	0	23.2	69	5	49	13	2	92.8
Adenomatosis	73	55	3	9	6	24.6	73	50	6	5	12	31.5#
Fibroadenoma	162	124	8	12	18	23.5	162	117	9	10	26	27.8#
Primary cancer	826	702	81	20	23	15.0*	826	531	205	59	31	35.7#
Metastatic cancer	108	92	11	2	3	14.8*	108	65	34	4	5	39.8#

PR, positive rate. *P<0.01, compared with normal tissue, adenomatosis or fibroadenoma. #P<0.01, compared with normal tissue.

Table 4. Relationship between LATS1 expression and clinicopathological features of breast cancer.

Clinicopathological features		Nuclear LATS1 expression						Cytoplasmic LATS1 expression					
	n	-	+	++	+++	%	P	-	+	++	+++	%	P
Age(years)							0.002						0.699
<55	532	340	136	35	21	36.1		454	52	11	15	14.7	
≥55	294	191	70	23	10	53.9		249	29	8	8	15.3	
T staging							0.019						0.927
1	214	173	26	10	5	19.2		136	50	20	8	36.4	
2	398	360	28	4	6	9.5		256	113	19	10	35.7	
3	43	36	5	1	1	16.3		25	13	4	1	41.9	
4	16	13	3	0	0	18.7		8	7	1	0	50	
N staging							0.023						0.040
0	368	310	37	13	8	15.8		222	104	27	15	39.7	
1	150	136	10	1	3	9.3		104	36	7	3	30.7	
2	87	78	7	1	1	10.3		56	22	8	1	35.6	
3	63	57	6	0	0	8.5		43	19	1	0	31.7	
M staging							0.126						0.022
0	490	419	52	10	9	14.5		310	134	35	11	36.7	
1	14	14	0	0	0	0		13	1	0	0	9.1	
TNM staging							0.258						0.716
1-2	490	421	45	13	11	14.1		310	130	32	18	37.7	
3-4	181	161	17	2	1	11.0		115	53	12	1	37.5	
Histological grade							<0.001						0.004
1	35	22	4	2	7	37.1		17	6	4	8	51.4	
2-3	791	681	77	17	16	13.9		514	200	54	23	35.0	
ER expression							0.224						0.634
-	222	181	33	1	7	18.5		131	65	17	9	41.0	
+	461	395	38	14	14	14.3		282	126	33	20	38.8	
PR expression							0.937						0.884
-	298	251	36	2	9	15.8		180	86	20	12	39.6	
+	384	324	35	13	12	15.6		232	105	30	17	39.6	
HER2 expression							0.003						0.353
-	116	88	17	5	6	24.1		68	29	12	7	41.4	
+	564	488	53	9	14	13.5		345	162	37	20	38.8	
Ki-67 expression							0.377						0.801
-	29	22	4	1	2	24.1		17	8	2	2	41.4	
+	449	366	55	11	17	18.5		265	127	35	22	41.0	

ER, estrogen receptor; PR, progesterone receptor.

also influences cytokinesis by negatively regulating LIMK1 during actin polymerization (Hirota et al., 2000; Yang et al., 2001; Xia et al., 2002). The functional loss of LATS1 disrupts normal mitosis, resulting in chromosome instability and cell transformation (Hirota et al., 2000; Yang et al., 2001;Yabuta et al., 2013). Previous studies showed that LATS1 was downregulated in many cancer types, including gastric cancer (Xu et al., 2011; Zhang et al., 2017; Kim et al., 2019), cervical cancer (Deng et al., 2017), malignant peripheral nerve sheath tumors (Wu et al., 2018a,b), ovarian cancer (Yagi et al., 2019), renal cell carcinoma (Chen et al., 2014; Wang et al., 2020a,b), metastatic prostate cancer (Zhao et al., 2012), basal cell carcinoma (Pellegrini et al., 2017), astrocytoma (Jiang et al., 2006), head and neck squamous cell carcinoma (Seinmann et al., 2009), colorectal cancer (Wierzbicki et al., 2013), lung cancer (Lin et al., 2014; Han et al., 2017; Wang et al., 2017, Wang et al., 2020a,b), oral squamous carcinoma (Reddy et al., 2015), and glioma (Ji et al., 2012), possibly due to

Table 5. Relationship between cytoplasmic and nuclear LATS1 expression in breast cancer.

Nuclear LATS1 expression	n	Cytoplasmic LATS1 expression				
		-	+	++	+++	%
-	703	529	168	5	1	85.1
+	81	2	38	41	0	9.8
++	19	0	0	12	7	2.3
+++	23	0	0	0	23	2.8
Total	826	531	206	58	31	35.7

P<0.001.

Table 6. Univariable survival analyses for the patients with breast cancer.

Clinicopathological features	Overall survival		Disease-free survival	
	P	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)
Age (<55 vs + ≥55, years)	0.081	1.494(0.951-2.347)	0.078	1.420(0.962-2.095)
T staging (T1-2 vs T3-4)	0.001	2.635(1.475-4.706)	0.002	2.503(1.400-4.475)
N staging (N0-1 vs N2-3)	<0.001	8.330(5.158-13.451)	<0.001	8.530(5.270-13.805)
M staging (M0 vs M1)	0.289	2.152(0.522-8.870)	0.021	5.223(1.278-21.349)
TNM stage (I-II vs III-IV)	<0.001	6.805(4.194-11.043)	<0.001	7.415(4.560-12.059)
ER expression (- vs +)	0.487	1.494(0.951-2.347)	0.443	0.854(-0.571.279)
PR expression (- vs +)	0.061	0.847(0.531-1.353)	0.024	0.641(-0.4360.943)
HER2 expression (- vs +)	0.905	0.652(0.417-1.020)	0.991	1.004(-0.5231927)
Ki-67 expression (- vs +)	0.879	0.956(0.460-1.988)	0.044	0.502(0.257-0.982)
Chemotherapy (- vs +)	0.080	1.082(0.390-3.004)	0.219	1.572(0.764-3.238)
Radiotherapy (- vs +)	0.018	2.006(0.921-4.367)	0.008	1.694(1.149-2.498)
Endocrine therapy (- vs +)	0.004	1.729(1.099-2.720)	0.003	0.559(0.380-0.822)
Nuclear LATS1 expression (-/+ vs ++/+++)	0.925	0.946(0.298-3.001)	0.821	0.875(0.275-2.777)
Cytoplasmic LATS1 expression (-/+ vs ++/+++)	0.342	0.644(0.260-1.595)	0.222	0.569(0.230-1.408)

CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor.

Table 7. Multivariable survival analyses for overall survival and disease-free survival of the prognosis of patients in breast cancer.

Clinicopathological features	Overall survival		Disease-free survival	
	P	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)
Age (<55 vs + ≥55, years)	0.038	2.056(1.039-4.069)	0.028	1.914(1.072-3.416)
T staging (T1-2 vs T3-4)	0.993	1.006(0.279-3.622)	0.929	0.946(0.282-3.180)
N staging (N0-1 vs N2-3)	0.120	6.255(0.622-62.905)	0.234	2.704(0.526-13.895)
M staging (M0 vs M1)	0.289	3.021(0.391-23.313)	0.456	2.077(0.304-14.208)
TNM staging (I-II vs III-IV)	0.694	1.622(0.146-18.063)	0.308	2.421(0.443-13.232)
ER expression (- vs +)	0.756	1.173(0.430-3.201)	0.824	0.907(0.382-2.154)
PR expression (- vs +)	0.540	0.736(0.277-1.960)	0.318	0.657(0.288-1.499)
HER2 expression (- vs +)	0.833	0.888(0.293-2.690)	0.711	0.845(0.347-2.060)
Ki-67 expression (- vs +)	0.672	1.266(0.425-3.767)	0.028	0.426(0.199-0.912)
Chemotherapy (- vs +)	0.227	2.649(0.545-12.865)	0.349	1.851(0.510-6.721)
Radiotherapy (- vs +)	0.487	0.755(0.341-1.670)	0.657	1.166(0.591-2.302)
Endocrine therapy (- vs +)	0.017	0.319(0.125-0.816)	0.158	0.559(0.249-1.253)
Nuclear LATS1 expression (-/+ vs ++/+++)	0.982	0.000(0.000-0.100)	0.488	2.710(0.162-45.468)
Cytoplasmic LATS1 expression (-/+ vs ++/+++)	0.090	0.161(0.019-1.328)	0.051	0.131(0.017-1.011)

CI, confidence interval; ER, estrogen receptor; PR, progesterone receptor.
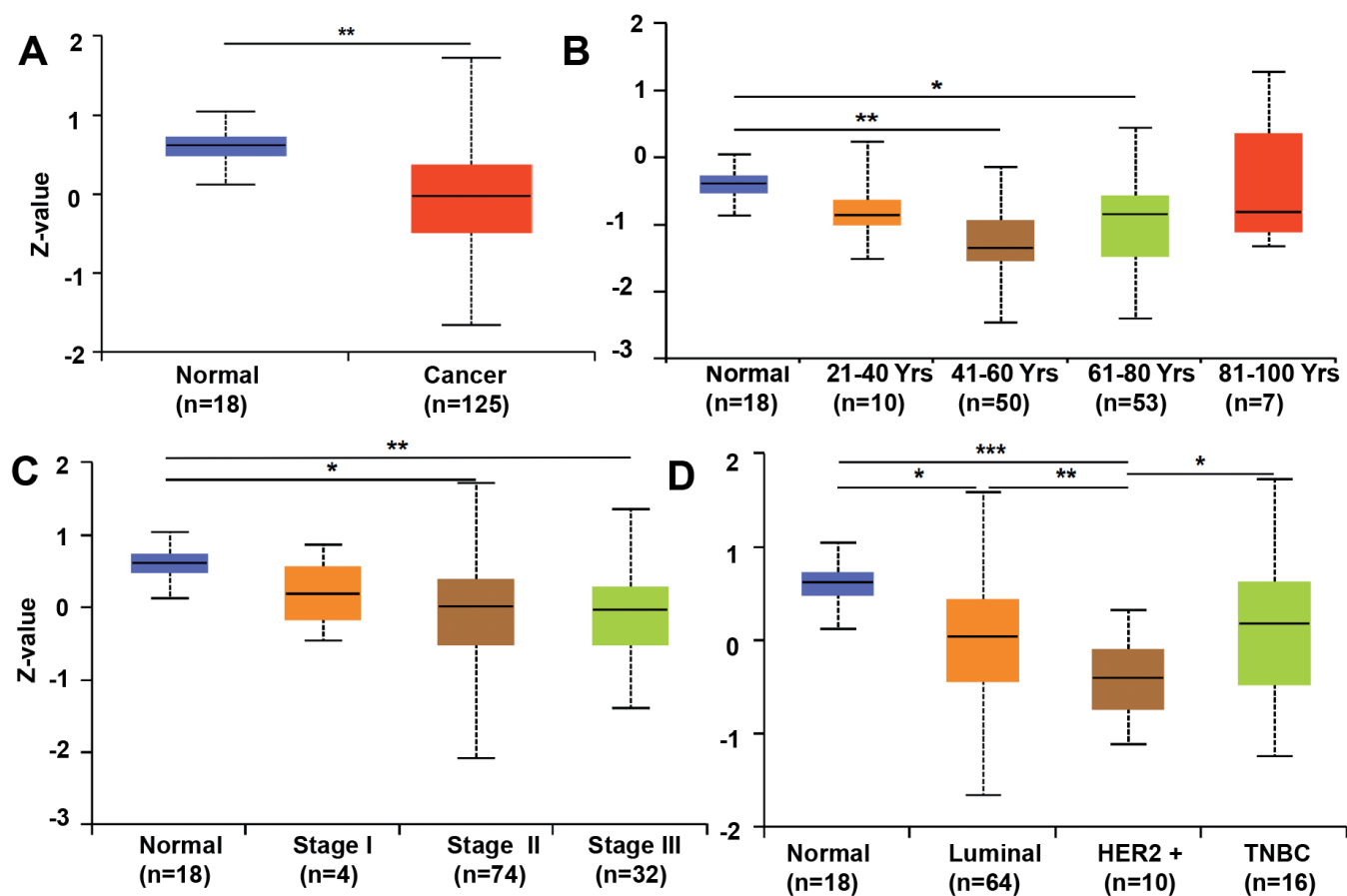
Fig. 4. The clinicopathological significance of LATS1 protein expression. UALCAN portal was used to evaluate the LATS1 protein expression in breast cancer **(A)**. It was compared with age **(B)**, TNM staging **(C)**, and molecular subtyping **(D)** of breast cancer patients. TNBC, triple-negative breast cancer.
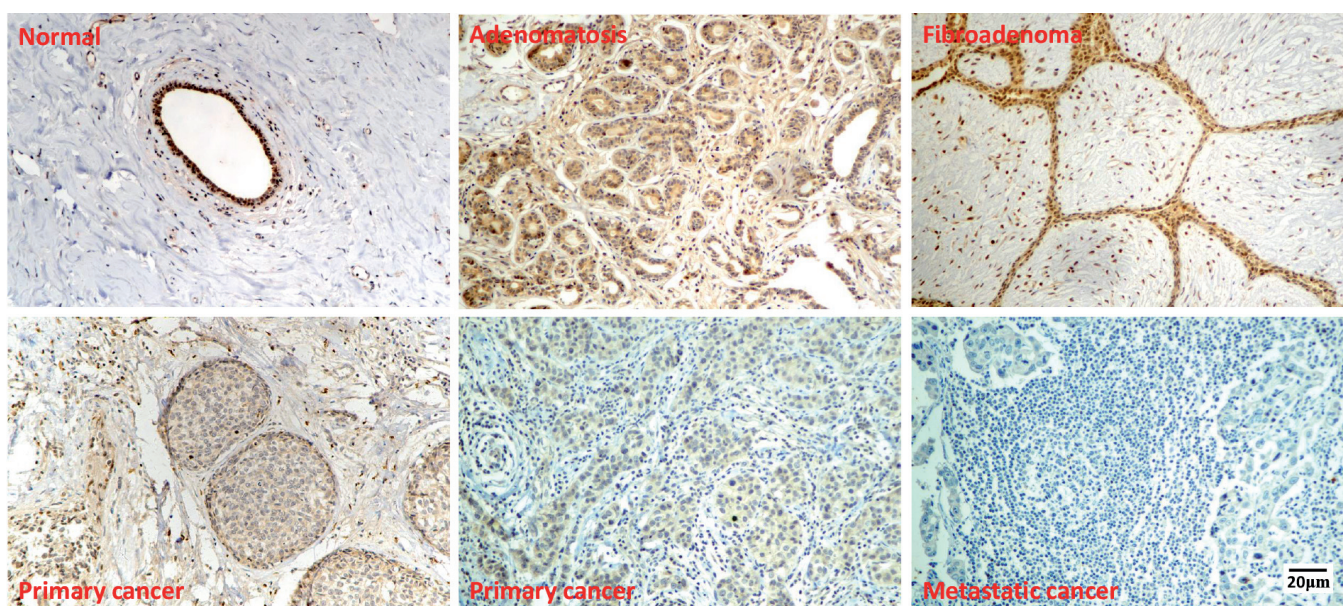

Fig. 5. LATS1 expression in breast carcinogenesis. Immunoreactivity to LATS1 protein was observed in normal tissue, adenomatosis, fibroadenoma, primary and metastatic cancers in lymph node of breast. x 200.

promoter hypermethylation. We found that LATS1 mRNA and protein expression levels were significantly lower in breast cancer tissue than normal breast tissue. Our previous study showed the nuclear-cytoplasmic translocation of LATS1 protein during head and neck squamous cell carcinogenesis (Wu et al., 2018a,b); however, LATS1 translocation was not observed during breast carcinogenesis. The downregulation of LATS1 expression during breast carcinogenesis suggests that

LATS1 normally functions as a tumor suppressor. Importantly, LATS1 mRNA and protein levels were the lowest in HER2-positive breast cancer patients, indicating that loss of LATS1 might represent a molecular marker for HER2-targeted therapy, and LATS1 could be a gene therapy target for HER2-positive breast cancer. These results are supported by a study that demonstrated the promoting effects of LATS1 abrogation on the luminal and stem phenotypes
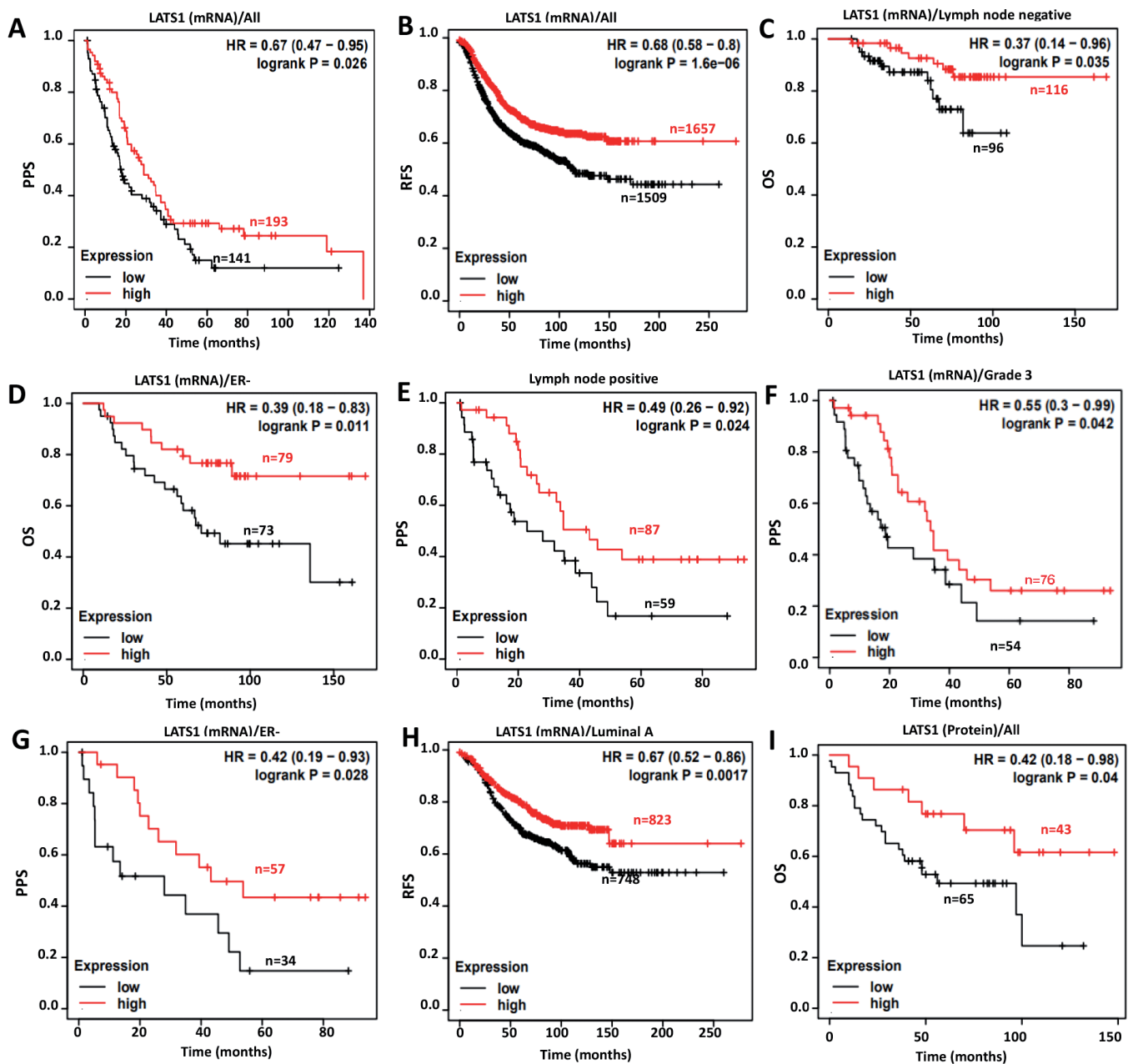
Fig. 6. The prognostic significance of LATS1 expression in breast cancer. According to the data from Kaplan-Meier plotter, LATS1 expression was positively related to either overall (OS), post-progression (PPS), and relapse-free(RFS) survival rate of the patients with breast cancer. HR, hazard ratio.

(Britschgi et al., 2017).

LATS1 phosphorylates angiomotin to inhibit cell migration and angiogenesis by negatively regulating F-actin polymerization via LIMK1 (Hergovich and Hemmings, 2009; Visser and Yang, 2010). LATS1 overexpression significantly suppresses cell growth, migration, and invasion in glioma U251 cells (Ji et al., 2012), renal cell carcinoma (RCC) cells (Chen et al., 2014), cervical cancer cells (Deng et al., 2017), and non-small cell lung cancer (NSCLC) cells (Lin et al., 2014). In addition, LATS1 overexpression can suppress proliferation, migration, metastasis, and the epithelial-mesenchymal transition of head and neck squamous cell carcinoma through p21 and Bax hyperexpression and the hypoexpression of XIAP, survivin, Cyclin B1, Cyclin D1, MMPs, and Twist (Wu et al., 2018a,b). LATS1 protein can associate with CDC2, downregulate Cyclin A protein levels, and reduce CDC2 kinase activity, leading to a G_2/M blockade (Xia et al., 2002; Xu et al., 2016). In this study, LATS1 expression was negatively associated with tumor size, lymph node metastasis, and the TNM stage of breast cancer. Takahashi et al. (2005) reported that tumor cells with lower LATS1 expression were more prone to invasion and metastasis than those with higher expression. Several studies have shown that LATS1 expression is negatively correlated with tumor size, lymph node metastasis, histological grade or TNM stage in breast cancer, glioma, and cervical cancer (Takahashi et al., 2005; Ji et al., 2012; Deng et al., 2017). In the current study, LATS1 expression was negatively correlated with TNM stage and histological grade, indicating that its loss or downregulation might be a useful biological marker of the aggressiveness of breast cancer and aging of cancer patients. In the future, it would be interesting to investigate the relationship between LATS1 expression and cell cycle markers (e.g., Cyclin A) in breast cancer cells.

High LATS1 expression was associated with a better prognosis in patients with ovarian serous carcinoma (Montavon et al., 2019), gastric cancer (Son et al., 2017), and NSCLC (Lin et al., 2014). In contrast, decreased LATS1 protein levels were associated with a worse prognosis and an independent prognostic factor in RCC (Godlewski et al., 2018) and glioma (Ji et al., 2012). In line with our previous study (Xu et al., 2016), there was no relationship between LATS1 protein expression and the OS or DFS of breast cancer patients, although TNM staging, radiotherapy, and endocrine therapy were independent factors for OS and DFS. However, the Kaplan-Meier plotter analysis showed that breast cancer patients with high LATS1 mRNA expression had better OS, RFS, PPS, and patients with high LATS1 protein expression have a better OS. Rybarczyk et al. (2017) demonstrated that LATS1 promoter hypermethylation and decreased LATS1 mRNA and protein levels in tumor samples were associated with higher TNM stages and Fuhrman's grades and patient survival in RCC. Takahashi et al. (2005) found that LATS1 mRNA hypoexpression was significantly associated with poor prognosis of breast cancer patients. These discrepancies may be attributable to the different subjects, sampling, methodologies, and clinical parameters used in the studies.

Conclusions

Downregulated LATS1 expression plays a vital role in breast cancer tumorigenesis and subsequent development. LATS1 represents a potential diagnostic and therapeutic target for breast cancer, particularly HER2-positive patients.

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