Comparison of the expression levels of lysine-specific demethylase 1 and survival outcomes between triple-negative and non-triple-negative breast cancer

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Received March 20, 2020; Accepted November 2, 2020

DOI: 10.3892/ol.2020.12363

Abstract. Lysine-specific demethylase 1 (LSD1) is a nuclear protein and the first histone demethylase to be identified. LSD1 is an evolutionarily conserved member of the FAD-dependent amine oxidase family and serves an important role in controlling gene expression. LSD1 has been implicated in the tumorigenesis and progression of several types of human cancer; however, to the best of our knowledge, the expression levels and clinical significance of LSD1 in triple-negative breast cancer (TNBC) and non-triple-negative breast cancer (NTNBC) have not been investigated in detail. Therefore, the present study aimed to compare the expression levels of LSD1 in TNBC and NTNBC to determine the prognostic significance of LSD1 in breast cancer. Previous studies have suggested that LSD1 may be involved in the carcinogenesis and progression of breast cancer; however, the findings of the present study indicated that LSD1 may not be a suitable molecular treatment target and auxiliary diagnostic indicator for TNBC and NTNBC.

Introduction

Breast cancer is a heterogeneous disease, that may be subclassified into triple-negative breast cancer (TNBC) and non-triple-negative breast cancer (NTNBC). Compared with NTNBC, TNBC has unique clinicopathological characteristics, such as higher risk of recurrence, larger tumor size, lymph node metastasis and poor prognosis, and represents a major health concern (1). TNBC accounts for 15-20% of breast cancers and is characterized by the lack of expression of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) (2). Compared with the other subtypes of breast cancer, TNBC has a relatively early onset and a higher degree of malignancy (3). In addition, patients with TNBC have a worse prognosis compared with other breast cancer subtypes, mainly because TNBC has no specific targets; therefore, the hormone receptors or HER2 cannot be targeted with therapy as in other subtypes (4). Although the molecular alterations in TNBC have been widely investigated, identifying the mechanisms that regulate the initiation and progression of TNBC may provide further insight into the development and progression of TNBC.

Lysine-specific demethylase 1 (LSD1), also referred to as KDM1A and AOF2, was the first histone demethylase to be discovered (5). LSD1 encodes a nuclear protein containing a SWIRM domain, a FAD-binding motif and an amine oxidase domain. The protein is a component of several histone deacetylase complexes and may silence genes by functioning as a histone demethylase. Notably, alternative splicing results in multiple transcript variants. The expression levels of LSD1 in certain subtypes of breast cancer have been investigated and LSD1 expression has been found to be frequently upregulated in several human malignancies, including breast (6), prostate (7), lung (8) and colon (9) cancer, neuroblastoma (10) and hepatocellular cancer (11). Notably, Lim *et al* (12) reported a significant positive association between LSD1 upregulation and a negative ER status. Another previous study identified an inverse correlation between high LSD1 expression levels and a low PR status (6). Recently, Cao *et al* (13) demonstrated that the overexpression of LSD1 promoted breast cancer cell proliferation, migration and invasion. In addition, it has been suggested that the ability of LSD1 to promote breast cancer growth and pulmonary metastasis may involve the resistance to immune checkpoint blockade (14). However, the expression and significance of LSD1 in breast cancer, particularly in the most aggressive subtype, TNBC, remain unclear.

The present study aimed to systematically investigate the expression levels of LSD1 in normal breast tissue, TNBC and NTNBC tissues using immunohistochemical staining, and analyze the potential association between LSD1 expression levels and clinicopathological characteristics of breast cancer.

Materials and methods

Bioinformatics analysis. The expression profile of LSD1 across various types of human cancer was examined through

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Key words: lysine-specific demethylase 1, survival analysis, breast cancer, triple-negative breast cancer, non-triple-negative breast cancer

the Broad Institute FireBrowse portal (http://firebrowse.org). On the homepage, 'LSD1' was typed into the search box and 'View Expression Profile' was selected. The boxplots produced plotted the expression levels of the target gene, with red bars representing tumor samples and blue bars representing normal samples.

The mRNA expression levels of LSD1 in breast cancer tissues were compared with their matched normal tissues using The Cancer Genome Atlas (TCGA) datasets in the Oncomine database (http://www.oncomine.org). The thresholds used to obtain the most significant probes of the queried gene for each microarray dataset included a 2-fold difference in expression levels between the cancer and normal tissues and $P<1x10^{-4}$. For each gene, the mRNA expression levels in three independent datasets were analyzed. The prognostic values of LSD1 in breast cancer were analyzed using the Kaplan-Meier plotter (http://kmplot.com/analysis) and the survival rates of patients with high and low expression levels of LSD1 were illustrated using a Kaplan-Meier survival plot.

Tissue specimens. The participants in the present study were all newly diagnosed patients with breast cancer who were treated at the Department of Breast Surgery of The Affiliated People's Hospital of Jiangsu University between December 2010 and October 2016. Samples were collected from 238 patients with breast cancer, including 112 TNBC and 126 NTNBC tissues. In addition, 80 normal tissues adjacent to the TNBC and 93 normal tissues adjacent to NTNBC were collected. The pathological data of all patients were complete and included tumor size, age, lymph node metastasis, clinical stage and histological type. All patients were regularly followed up; the follow-ups mainly occurred via phone or partly using an online platform. The patient's survival, living conditions and presence of any abnormal symptoms were assessed until the follow-up deadline, which was set as June 2019. No subjects were lost during the follow-up period. All the tissue specimens were collected after obtaining informed patient consent and the use of the breast cancer specimens was approved by The Affiliated People's Hospital of Jiangsu University Institutional Review Board.

Immunohistochemical analysis. Immunohistochemistry (IHC) was performed to determine LSD1 expression levels in the tissues. The anti-LSD1 antibody (1:400; cat. no. 2184S) was purchased from Cell Signaling Technology, Inc.; a rabbit two-step detection kit and DAB color development kit were purchased from Beijing Zhongshan Jinqiao Biotechnology, and hematoxylin and water-soluble mounting tablets were purchased from Boster Biological Technology. All tissue samples for the experiment were provided by The People's Hospital of Jiangsu University and tissue chips were prepared with the assistance of Shanghai Changzheng Hospital. Each experiment used a known positive tissue section as the positive control and PBS solution instead of the primary antibody as the negative control.

IHC scoring. The interpretation of the results was performed using a double-blind reading under the guidance of a pathology expert. The lower part that was brownish yellow referred to the immunoreactive score, which was based on the comprehensive evaluation of the degree of cellular staining and the percentage of positive cells. In total, ≥10 fields of view were visualized under a high magnification and 100 tumors were viewed in each field of view. The percentage of positive cells was counted and the following scoring system was used: 0 points (≤5%), 1 point (5-25%), 2 points (25-50%), 3 points (25-75%) and 4 points (≥75%). The staining intensity was scored as follows: 0 (no staining), 1 (weakly stained), 2 (moderately stained) or 3 (strongly stained). The LSD1 immunostaining score was calculated as (positive percentage score) x (staining intensity score). In this experiment, a score of ≥4 points was considered as positive.

Statistical analysis. Statistical analysis was performed using SPSS 25.0 software (IBM Corp.). The association between the expression levels of LSD1 and clinicopathological characteristics was analyzed using a χ^2 test or Fisher exact probability method. The log-rank test and Kaplan-Meier method were also used and the survival analysis was depicted graphically. Following the univariate analysis, variables with $P<0.05$ were used for subsequent multivariate analysis based on the Cox proportional hazards model. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Upregulated LSD1 expression is not associated with a poor prognosis in breast cancer. The gene expression levels of LSD1 were analyzed in 37 cases of human cancer using TCGA database. The columns in Fig. 1A represent the accurate quantification of the gene and isoform expression levels from the RNA-Seq data. The results revealed that LSD1 expression levels were upregulated in almost all cancer tissues compared with their respective matched normal tissues. The expression levels of LSD1 were the highest in testicular germ cell tumor and the lowest in cholangiocarcinoma. Notably, the LSD1 gene exhibited a similar expression pattern in breast cancer (Fig. 1A). The Oncomine database analysis comparing the cancer tissues with normal tissues also revealed that the mRNA expression levels of LSD1 were significantly upregulated in breast cancer tissues compared with the corresponding normal tissues in three independent analyses (Fig. 1B-D). The results of the Kaplan-Meier analysis revealed no significant association between the expression levels of LSD1 and the overall survival rate of patients with breast cancer (P=0.31; Fig. 1E).

LSD1 expression is upregulated in TNBC and NTNBC tissues. To further investigate the results obtained through the bioinformatics analysis, the protein expression levels of LSD1 in breast cancer tissues were also investigated. For this analysis, samples from 238 patients with breast cancer (including 112 TNBC and 126 NTNBC cases) who were diagnosed between 2010 and 2016 were used; >95% of the tumors were ≤5 cm, ~35% of the patients had lymph node metastasis and ~15% of the patients were diagnosed at an advanced TNM stage (stage III/IV). The protein expression levels of LSD1 in both the TNBC and NTNBC subtypes were significantly upregulated compared with those in adjacent normal tissues ($P<0.001$; Table I). Subsequently, the proportion of LSD1 expression in breast tumors was further determined. IHC staining revealed that

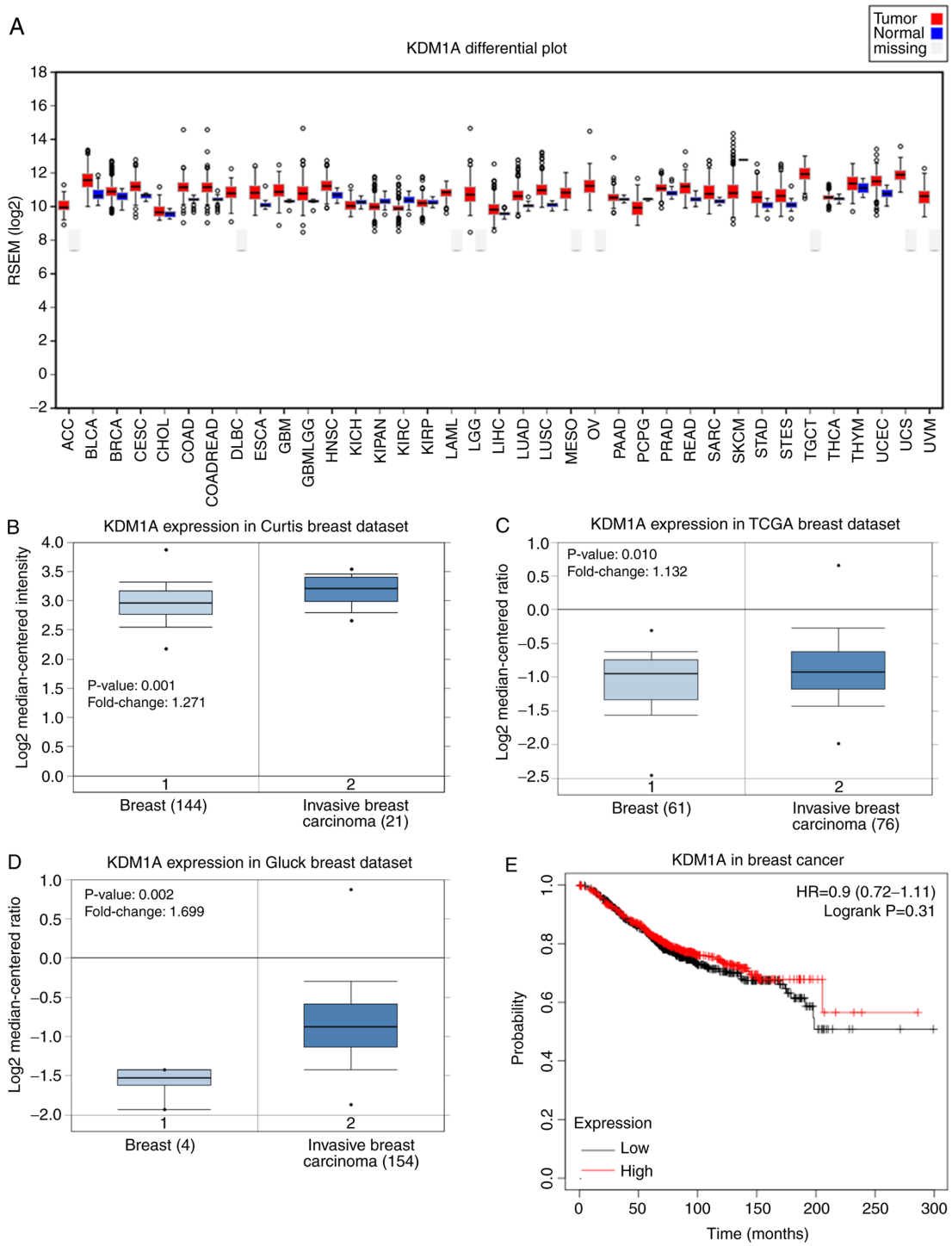

Figure 1. Expression levels and prognostic impact of LSD1 in breast cancer. (A) FIREHOSE analysis of LSD1 (KDM1A) expression profiles. The boxplots show the expression levels of LSD1; the red bars are for the tumor samples and the blue bars are for the normal tissue samples. Analysis of the mRNA expression levels of LSD1 in breast cancer was performed using the Oncomine database. The mRNA expression levels of LSD1 were analyzed in the (B) Curtis, (C) TCGA and (D) Gluck independent breast datasets. *P<0.05 vs. normal tissues. (E) Kaplan-Meier plotter analysis of LSD1 expression levels in breast cancer. The red line indicates the overall survival rate with high expression levels of LSD1, while the black line indicates the overall survival rate with low expression levels of LSD1 in breast cancer. KDM1A/KDM/LSD1, lysine-specific demethylase 1; TCGA, The Cancer Genome Atlas.

LSD1 was localized mainly to the cell nucleus in both breast cancer subtypes (Fig. 2). Positive staining for LSD1 expression was recorded in 90 (40%) of the 231 breast cancer samples; specifically, in 42 (40%) of the 105 TNBCs and 48 (38%) of the 126 NTNBCs (Fig. 2). There were no significant differences in the LSD1 expression levels between the TNBC and NTNBC samples (P>0.05; Table SI).

Association between LSD1 expression and clinicopathological characteristics. The present study further analyzed the association between LSD1 expression levels and the clinicopathological characteristics of breast tumors, including age, tumor size, lymph node metastasis status and clinical stage. The expression levels of LSD1 in breast cancer were not significantly associated with any of the

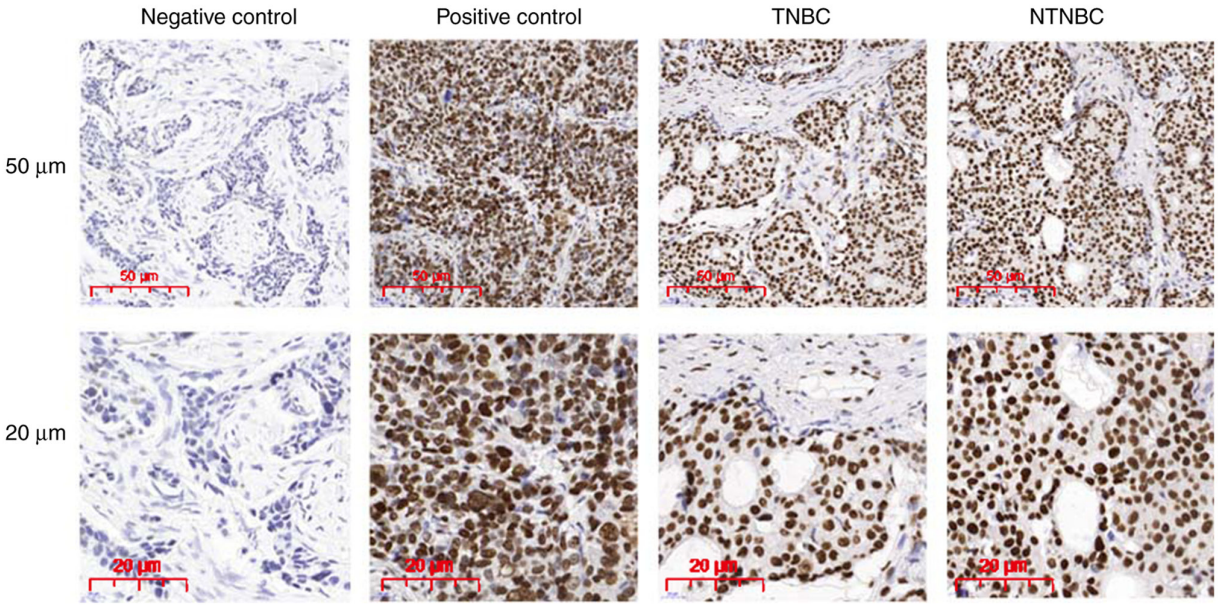

Figure 2. IHC staining of LSD1 expression levels with tissue arrays. IHC staining of LSD1 expression levels in breast cancer samples. Both LSD1 negative control and positive control samples are also shown, alongside the TNBC and NTNBC tumor samples. Two differentially stained LSD1-positive breast tumor samples were used to demonstrate the specific nuclear localization of LSD1 in the tumor cells. Scale, 20 or 50 μm. LSD1, lysine-specific demethylase 1; IHC, immunohistochemistry; TNBC, triple-negative breast cancer; NTNBC, non-triple negative breast cancer.

Table I. Expression of LSD1 in breast cancer.

	LSD1 protein expression			
Variables	All cases	Negative	Positive	P-value
Breast cancer				<0.001
Tumor	231	141	90	
Normal	173	163	10	
TNBC				<0.001
Tumor	105	63	42	
Normal	80	78	2	
NTNBC				<0.001
Tumor	126	78	48	
Normal	93	85	8	

LSD1, lysine-specific demethylase 1; TNBC, triple-negative breast cancer; NTNBC, non-triple-negative breast cancer.

individual clinical indicators (P>0.05; Table II). However, in TNBC, the LSD1 expression levels were significantly associated with age (P=0.019) and TNM stage (P=0.031), but not with tumor size or lymph node metastasis (P>0.05; Table II). Positive staining for LSD1 was detected in 48.9% of the patients aged >55 years and in 44.9% of patients with TNM stage I+II disease. In addition, positive staining for LSD1 was also detected in 33.8% of patients aged <55 years and in 12.5% of patients with TNM stages III+IV disease. In NTNBC, the expression levels of LSD1 were not significantly associated with any of the clinicopathological indicators (P>0.05; Table II). Taken together, these results suggest that LSD1 expression levels may be associated with patient age and TNM stage in TNBC.

Association between LSD1 expression and patient prognosis. To determine the role of LSD1 and the clinicopathological indicators in predicting breast cancer outcomes, the postoperative survival in patients with breast cancer was tracked and analyzed. Patients were classified into LSD1-positive and LSD1-negative expression groups according to the IHC results. For breast cancer, the mass size (4 cm), lymph node metastasis (N) and TNM stage were significantly inversely associated with patient survival. The survival period of the patients with a large tumor diameter, late clinical stage and lymph node metastasis was significantly decreased compared with that of patients with smaller tumors, diagnosed at an earlier clinical stage and without metastasis to the lymph nodes (Table III). The χ^2 value of the log-rank test revealed that the cumulative survival difference between tumor size (4 cm) was the most significant, followed by TNM stage and N stage. In TNBC, the tumor size (4 cm) and N stage were significantly inversely associated with patient survival (Table III). The cumulative survival rate difference in the N stage was the most significant, followed by tumor size. In NTNBC, the tumor size (4 cm) and TNM stage were significantly inversely associated with patient survival (Table III). The cumulative survival rate difference in the TNM stage was the most significant, followed by tumor size. Taken together, these data suggested that tumor metastasis, but not LSD1 expression levels, may be a major factor associated with mortality in patients with TNBC and NTNBC.

Cox regression analysis was subsequently used to calculate various prognostic parameters for the survival of patients with TNBC and NTNBC. Univariate analysis identified four prognostic factors: TNM stage (I+II vs. III+IV), N stage (N0 vs. N1-3), LSD1 expression levels (negative or positive) and tumor size (≤4 vs. >4 cm). However, upon performing multivariate Cox proportional hazard regression, increased LSD1 expression levels were not identified as a significant

Table II. Correlation of LSD1 expression with clinicopathological characteristics in breast cancer.

		LSD1 protein expression		
Variables	All cases	Negative	Positive	P-value
Breast cancer				
Age (years)				
≤40	18 (missing 2)	10	6	0.901
>40	220 (missing 5)	131	84	
≤50	93 (missing 2)	58	33	0.498
>50	145 (missing 5)	83	57	
≤55	133 (missing 3)	86	44	0.071
>55	105 (missing 4)	55	46	
Tumor size (cm)				
≤2	124 (missing 4)	77	43	0.311
>2	114 (missing 3)	64	47	
≤4	223 (missing 7)	130	86	0.313
>4	15	11	4	
≤5	231 (missing 7)	136	88	0.858
>5	7	5	2	
N stage				0.406
N0	154 (missing 5)	88	61	
N1-3	84 (missing 2)	53	29	
TNM stage				0.179
I+II	209 (missing 7)	120	82	
III+IV	29	21	8	
TNBC				
Age (years)				
≤40	10 (missing 2)	4	4	0.711
>40	102 (missing 5)	59	38	
≤50	38 (missing 2)	24	12	0.314
>50	74 (missing 5)	39	30	
≤55	65 (missing 3)	43	19	0.019
>55	47 (missing 4)	20	23	
Tumor size (cm)				
≤2	42 (missing 4)	24	14	0.619
>2	70 (missing 3)	39	28	
≤4	102 (missing 7)	57	38	1.000
>4	10	6	4	
≤5	107 (missing 7)	60	40	1.000
>5	5	3	2	
N stage				0.113
N0	73 (missing 5)	37	31	
N1-3	39 (missing 2)	26	11	
TNM stage				0.031
I+II	96 (missing 7)	49	40	
III+IV	16	14	2	
NTNBC				
Age (years)				
≤40	8	6	2	0.680
>40	118	72	46	
≤50	55	34	21	0.986
>50	71	44	27	
≤55	68	43	25	0.739
>55	58	35	23	
Tumor size (cm)				
≤2	82	53	29	0.389
>2	44	25	19	
≤4	121	73	48	0.156
>4	5	5	0	
≤5	124	76	48	0.525
>5	2	2	0	
N stage				0.743
N0	81	51	30	
N1-3	45	27	18	
TNM stage				0.528
I+II	113	71	42	
III+IV	13	7	6	

LSD1, Lysine-specific demethylase 1;TNBC, triple negative breast cancer, NTNBC, non-triple negative breast cancer.

independent predictor of poor survival in patients with breast cancer (P>0.05; Table IV), which was consistent with the results of the bioinformatics analysis. Taken together, these data suggested that LSD1 expression levels may not be inversely associated with a poor prognosis in breast cancer and, in fact, the best predictor of poor prognosis in TNBC may be the N stage (P<0.05; Table IV).

Discussion

LSD1 is a member of the monoaminoxidase enzyme family, which play an important role in controlling gene expression through histone modifications (15). Consistent with the perceived role of LSD1 in cell proliferation, overexpression of LSD1 has been reported in a diverse range of human tumors, including breast cancer (6). For example, Serce *et al* (6) reported upregulated expression levels of LSD1 in invasive ductal breast cancer. The expression levels of LSD1 were also found to increase with progression of ductal carcinoma *in situ* (DCIS) to invasive ductal carcinoma (6). Similarly, a study by Scoumanne and Chen (16), which analyzed the role of LSD1 in the human malignant breast cancer cell line MCF7, discovered that downregulation of LSD1 expression reduced the number of proliferating breast cancer cells. However, the expression levels of LSD1 in TNBC and NTNBC have not been analyzed to date. Therefore, to the best of our knowledge, the present study was the first to systematically analyze LSD1 expression levels in TNBC and NTNBC.

From the clinical data, it may be concluded that the survival of patients with TNBC is poor. The poor prognosis of TNBC may be due to its biological characteristics, such as younger age at onset, higher rate of breast cancer family history, larger tumor size, more advanced clinical stage at diagnosis, higher rate of lymph node metastasis, higher histological grade, earlier recurrence and metastasis, and resistance to endocrine

Table III. Univariate analysis of the association between LSD1 expression and clinicopathological variables in patients with breast cancer (log-rank test).

Variables	All cases	95% CI	P-value
Breast cancer			
Age (years)			
≤40	18	86.864 (70.122-103.606)	0.853
>40	220	87.143 (83.316-90.971)	
≤50	93	91.935 (85.641-98.229)	0.571
>50	145	86.157 (81.339-90.974)	
≤55	133	91.712 (86.403-97.020)	0.510
>55	105	85.889 (80.247-91.531)	
Tumor size (cm)			
≤2	124	92.546 (87.114-97.978)	0.333
>2	114	88.493 (82.501-94.484)	
≤4	223	92.274 (88.225-96.323)	<0.001
>4	15	65.188 (48.425-81.950)	
≤5	231	90.706 (86.569-94.842)	0.753
>5	7	88.750 (75.909-101.591)	
N stage			0.028
N0	154	93.754 (89.110-98.399)	
N1-3	84	81.177 (74.504-87.851)	
TNM stage			0.004
I+II	209	92.601 (88.459-96.743)	
III+IV	29	73.670 (61.913-85.426)	
LSD1 protein expression			0.486
Negative	141	88.697 (82.812-94.582□	
Positive	90	92.599 (86.766-98.432□	
TNBC			
Age (years)			
≤40	10	88.200 (66.128-110.272)	0.619
>40	102	84.273 (78.434-90.112)	
≤50	38	89.054 (78.581-99.526)	0.748
>50	74	84.362 (77.649-91.076)	
≤55	65	87.595 (79.387-95.803)	0.934
>55	47	85.777 (77.890-93.665)	
Tumor size (cm)			
≤2	42	87.073 (76.790-97.356)	0.866
>2	70	88.353 (80.870-95.836)	
≤4	102	89.952 (83.867-96.037)	0.027
>4	10	63.828 (41.666-85.989)	
≤5	107	87.576 (81.283-93.870)	0.712
>5	5	89.333 (72.263-106.404)	
N stage			0.016
N0	73	93.124 (86.437-99.812)	
N1-3	39	75.396 (65.027-85.766)	
TNM stage			0.278
I+II	96	89.218 (82.880-95.556)	
III+IV	16	74.643 (57.360-91.926)	
LSD1 protein expression			0.248
Negative	63	84.203 (75.189-93.217)	
Positive	42	92.302 (84.161-100.444)	
NTNBC			
Age (years)			
≤40	8	73.075 (52.867-93.283)	0.411
>40	118	86.088 (81.640-90.536)	
≤50	55	85.974 (79.409-92.539)	0.842
>50	71	85.361 (79.425-91.296)	
≤55	68	87.806 (82.385-93.226)	0.263
>55	58	83.084 (76.003-90.165)	
Tumor size (cm)			
≤2	82	87.075 (81.803-92.348)	0.268
>2	44	79.614 (72.530-86.698)	
≤4	121	86.586 (82.188-90.984)	0.004
>4	5	68.200 (43.333-93.067)	
≤5	124	85.868 (81.437-90.299)	0.248
>5	2	87.000 (87.000-87.000)	
N stage			0.541
N0	81	86.275 (80.815-91.735)	
N1-3	45	84.282 (76.821-91.743)	
TNM stage			0.002
I+II	113	87.472 (83.025-91.918)	
III+IV	13	70.019 (57.408-82.63)	
LSD1 protein expression			0.895
Negative	78	85.406 (79.533-91.278)	
Positive	48	85.399 (78.452-92.346)	

LSD1, lysine-specific demethylase 1; TNBC, triple-negative breast cancer, NTNBC, non-triple-negative breast cancer.

and targeted therapy. The present study revealed that LSD1 expression levels were upregulated in ~40% (90/231) of breast cancer cases. Importantly, LSD1 was found to be upregulated in both TNBC and NTNBC. The expression levels of LSD1 also appeared to be similar between older and younger patients with breast cancer. In addition, the expression levels of LSD1 were not significantly associated with poor prognosis or the cumulative survival rate of postoperative patients with breast cancer. Bioinformatics analysis also revealed that LSD1 expression levels were not significantly associated with the prognosis of breast cancer. However, Nagasawa *et al* (17) concluded that the upregulation of LSD1 was a poor prognostic factor in breast cancer, particularly the basal-like subtype of invasive breast cancer. This inconsistency may be due to the insufficient data on LSD1 expression levels obtained via IHC staining in the present study, which may lead to differences in the LSD1 prognostic impact. In the present study, LSD1 expression levels were found to be closely associated with the breast tumor size and distant metastasis (TNM stage) in TNBC. Lim *et al* (12) demonstrated that the overexpression of LSD1 in breast cancer was significantly positively correlated with the absence of the ER. In addition, the knockdown of LSD1 by small interfering RNA induced the regulation of multiple

Table IV. Cox multivariate analysis of prognostic factors of overall survival in breast cancer.

Variables	Hazards ratio	95% CI	P-value
Breast cancer			
LSD1 protein expression (negative vs. positive)	0.928	0.498-1.728	0.813
Tumor size, cm (≤4 vs. >4)	2.529	0.998-6.411	0.05
N stage (N0 vs. N1-3)	1.481	0.716-3.061	0.289
TNM stage (I+II vs. III+IV)	1.351	0.522-3.495	0.535
TNBC			
LSD1 protein expression (negative vs. positive)	0.699	0.301-1.624	0.405
Tumor size, cm (≤4 vs. >4)	2.479	0.785-7.826	0.122
N stage (N0 vs. N1-3)	2.714	1.112-6.622	0.028
TNM stage (I+II vs. III+IV)	0.517	0.151-1.769	0.293
NTNBC			
LSD1 protein expression (negative vs. positive)	1.145	0.415-3.163	0.794
Tumor size, cm (≤4 vs. >4)	2.663	0.556-12.763	0.221
N stage (N0 vs. N1-3)	0.483	0.107-2.185	0.344
TNM stage (I+II vs. III+IV)	5.362	0.948-30.348	0.058

LSD1, lysine-specific demethylase 1; TNBC, triple-negative breast cancer; NTNBC, non-triple-negative breast cancer; CI, confidence interval.

proliferation-related genes, including p21, ERBB2 and CNA2, thereby inhibiting the proliferation of breast cancer cells (12). However, the specific mechanisms underlying the association between LSD1 and cancer development have not been fully elucidated. Upregulated expression levels of LSD1 have been reported to be a hallmark of breast cancer cells (18). However, according to the data obtained in the present study, LSD1 was not found to play an important role in breast cancer progression or metastasis. Thus, LSD1 may be a secondary factor associated with breast cancer-related mortality.

LSD1 may be involved in the carcinogenesis and progression of breast cancer. It has been reported that the CtBP/LSD1/COREST complex interacts with ZNF516 to participate in the epithelial-to-mesenchymal transition (EMT) process, and inhibits the proliferative and invasive ability of breast cancer cells (19). LSD1 was also discovered in another study to regulate the EGFR signaling pathway and affect EMT (11), thereby inhibiting breast cancer cell invasion. LSD1 appears to serve a role in regulating the expression of oncogenic proteins in breast cancer cells (20), and the upregulation of LSD1 may be an early tumor-promoting event in breast cancer. Thus, LSD1 may be involved in the occurrence and development of TNBC, in addition to NTNBC, which may hold therapeutic promise. The combination of a LSD1 inhibitor (21), pargyline (22) and the HDAC inhibitor SAHA (vorinostat) (23) significantly inhibited the growth and apoptosis of TNBC cells. Therefore, to further elucidate the specific mechanism underlying the role of LSD1 in breast cancer *in vivo* and *in vitro*, future studies should explore the association between LSD1 and breast cancer cell proliferation, migration and invasion.

In conclusion, the findings of the present study indicated that LSD1 expression levels may be upregulated in breast cancer and the expression levels of LSD1 may be associated with clinical stage in TNBC. However, the detection of LSD1 was not found to be a marker for the early diagnosis of breast cancer or a potential target for early treatment strategies. The results of the present study may shed some light on the complex epigenetic regulatory mechanisms of breast cancer, which may help identify novel therapeutic targets.

Acknowledgements

The authors would like to thank Dr Miao Chen (Department of Pathology, The Affiliated People's Hospital of Jiangsu University, Zhenjiang), for reviewing the clinicopathological data in this manuscript.

Funding

The present study was supported by grants from the National Natural Science Foundation of China (grant no. 81170573) and the Key Research and Development Program of Jiangsu Province (grant no. BE2020678).

Availability of data and materials

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

Authors' contributions

GS and QL conceived and designed the study; KZ performed the experiments; KZ and YL wrote the manuscript; YL, TH, AS, ML and WB were involved in the conception of the study. All authors agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Human breast tissue specimens were preserved in the Breast Cancer Tissue Bank at The Affiliated People's Hospital of Jiangsu University (Zhenjiang, China). All the tissue specimens for this study were collected after obtaining informed patient consent and the use of the breast cancer specimens was approved by The Affiliated People's Hospital of Jiangsu University Institutional Review Board.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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