Lipocalin-1 Expression as a Prognosticator Marker of Survival in Breast Cancer Patients

Xueyan Zhang^a Yingnan Cui^b Miao He^c Yan Jiao^d Zhaoying Yang^b

^aSchool of Nursing, Jilin University, Changchun, China;

^bDepartment of Breast Surgery, China-Japan Union Hospital of Jilin University, Changchun, China;

^cDepartment of Anesthesia, The Second Hospital of Jilin University, Changchun, China;

^dDepartment of Hepatobiliary and Pancreatic Surgery, The First Hospital of Jilin University, Changchun, China

Keywords

Breast cancer · Diagnosis · LCN1 · Lipocalin-1 · Prognosis · Tear lipocalin

Abstract

Purpose: LCN1 (lipocalin-1), a gene that encodes tear lipocalin (or von Ebner's gland protein), is mainly expressed in secretory glands and tissues, such as the lacrimal and lingual gland, and nasal, mammary, and tracheobronchial mucosae. Analysis of the Cancer Genome Atlas (TCGA) Breast Carcinoma (BRCA) level 3 data revealed a relationship between LCN1 expression and survival in breast cancer patients. **Methods:** The χ^2 test and Fisher exact test were applied to analyze the clinical data and RNA sequencing expression data, and the association between LCN1 expression and clinicopathologic features was determined. The receiver-operating characteristic (ROC) curve of LCN1 was drawn to assess its ability as a diagnostic marker, and the optimal cutoff value was obtained from the ROC curve to distinguish groups with high and low LCN1 expression. Cox regression was used to compare both groups, and a log-rank test was applied to calculate *p* values and compare the Kaplan-Meier curves. Furthermore, GEO datasets were employed for external data validation. **Results:** Analysis of 1,104 breast cancer patients with a primary tumor revealed that LCN1 was overexpressed in breast cancer. High LCN1 expres-

sion was associated with clinicopathologic features and poor survival. Analyzing the area under the ROC curve (AUC) of LCN1, it was found that its diagnostic ability was limited. Multivariate analysis indicated that LCN1 expression is an independent predictor of survival in breast cancer patients. Through validation in GEO datasets, LCN1 expression was higher in tumor than normal tissue of the breast. High LCN1 expression was associated with poor survival in breast cancer patients. **Conclusions:** High LCN1 expression is an independent prognosticator of a poor prognosis in breast cancer.

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Introduction

Breast cancer is the most common cancer worldwide and the leading cause of cancer death in women [1, 2]. It can be divided into 5 molecular subtypes on the basis of its intrinsic molecular profiles, including luminal A, luminal B, HER2-enriched, basal-like, and normal-like breast cancer. Currently, there are well-established prognostic and/or therapeutic breast cancer markers like ER (estrogen receptor), PR (progesterone receptor), HER2 (human epidermal growth factor receptor 2), Ki67 antigen, tumor protein p53, and breast cancer susceptibility genes (BRCA1 and BRCA2) [3]. Exosomal biomarkers (such as CD47, Del-1, miR-1246, and miR-21) can be ap-

Y.J. and Z.Y. contributed equally to this work and should be regarded as co-corresponding authors.

Yan Jiao
Department of Hepatobiliary and Pancreatic Surgery
The First Hospital of Jilin University
71 Xinmin Street, Changchun, Jilin 130021 (China)
E-Mail lagelangri1@126.com

Zhaoying Yang
Department of Breast Surgery
China-Japan Union Hospital of Jilin University
126 Xiantai Street, Changchun, Jilin 130033 (China)
E-Mail zhaoyingyang@163.com

Table 1. Correlations of LCN1 expression in breast cancer tissue with clinicopathologic features

Clinical characteristics	Cases, <i>n</i>	LCN1 expression, <i>n</i> (%)		χ^2 test	<i>p</i> value
		high	low		
Age					
<60 years	589 (53.45)	102 (17.32)	487 (82.68)	0.0000	1.0000
≥60 years	513 (46.55)	88 (17.15)	425 (82.85)		
Gender					
Female	1,090 (98.73)	188 (17.25)	902 (82.75)	0.0000	1.0000
Male	12 (1.09)	2 (16.67)	10 (83.33)		
Histological type					
Infiltrating ductal carcinoma	790 (71.56)	147 (18.61)	643 (81.39)	5.3376	0.0693
Infiltrating lobular carcinoma	204 (18.48)	24 (11.76)	180 (88.24)		
Other	107 (9.69)	19 (17.76)	88 (82.24)		
Molecular subtype					
Basal	142 (12.86)	29 (20.42)	113 (79.58)	8.6955	0.0692
HER2	67 (6.07)	13 (19.4)	54 (80.6)		
Luminal A	422 (38.22)	58 (13.74)	364 (86.26)		
Luminal B	194 (17.57)	22 (11.34)	172 (88.66)		
Normal	24 (2.17)	6 (25)	18 (75)		
ER					
Indeterminate	2 (0.18)	0 (0)	2 (100)	6.2464	0.0440
Negative	239 (21.65)	54 (22.59)	185 (77.41)		
Positive	813 (73.64)	129 (15.87)	684 (84.13)		
PR					
Indeterminate	4 (0.36)	0 (0)	4 (100)	5.7837	0.0555
Negative	345 (31.25)	73 (21.16)	272 (78.84)		
Positive	704 (63.77)	110 (15.63)	594 (84.38)		
HER2					
Equivocal	180 (16.3)	31 (17.22)	149 (82.78)	0.9357	0.8168
Indeterminate	12 (1.09)	2 (16.67)	10 (83.33)		
Negative	565 (51.18)	100 (17.7)	465 (82.3)		
Positive	164 (14.86)	34 (20.73)	130 (79.27)		
Menopause status					
Indeterminate	34 (3.08)	3 (8.82)	31 (91.18)	16.3272	0.0010
Perimenopausal	40 (3.62)	13 (32.5)	27 (67.5)		
Postmenopausal	706 (63.95)	134 (18.98)	572 (81.02)		
Premenopausal	231 (20.92)	25 (10.82)	206 (89.18)		
T classification					
T1	281 (25.45)	48 (17.08)	233 (82.92)	0.8468	0.9321
T2	640 (57.97)	110 (17.19)	530 (82.81)		
T3	138 (12.5)	24 (17.39)	114 (82.61)		
T4	40 (3.62)	8 (20)	32 (80)		
Tx	3 (0.27)	0 (0)	3 (100)		
N classification					
N0	516 (46.74)	90 (17.44)	426 (82.56)	7.3576	0.1182
N1	367 (33.24)	56 (15.26)	311 (84.74)		
N2	120 (10.87)	30 (25)	90 (75)		
N3	79 (7.16)	10 (12.66)	69 (87.34)		
Nx	20 (1.81)	4 (20)	16 (80)		
M classification					
M0	917 (83.06)	152 (16.58)	765 (83.42)	2.4890	0.2881
M1	22 (1.99)	6 (27.27)	16 (72.73)		
Mx	163 (14.76)	32 (19.63)	131 (80.37)		
Stage					
I	182 (16.49)	28 (15.38)	154 (84.62)	4.0374	0.4010
II	626 (56.7)	108 (17.25)	518 (82.75)		
III	252 (22.83)	47 (18.65)	205 (81.35)		
IV	20 (1.81)	6 (30)	14 (70)		
X	14 (1.27)	1 (7.14)	13 (92.86)		
Lymph node involvement					
No	28 (2.54)	4 (14.29)	24 (85.71)	0.0165	0.8977
Yes	697 (63.13)	119 (17.07)	578 (82.93)		

Table 1 (continued)

Clinical characteristics	Cases, <i>n</i>	LCN1 expression, <i>n</i> (%)		χ^2 test	<i>p</i> value
		high	low		
Margin status					
Close	31 (2.81)	9 (29.03)	22 (70.97)	3.1951	0.2024
Negative	922 (83.51)	159 (17.25)	763 (82.75)		
Positive	79 (7.16)	12 (15.19)	67 (84.81)		
Vital status					
Deceased	155 (14.04)	35 (22.58)	120 (77.42)	3.1814	0.0745
Living	947 (85.78)	155 (16.37)	792 (83.63)		
Radiation therapy					
No	445 (40.31)	84 (18.88)	361 (81.12)	0.7950	0.3726
Yes	557 (50.45)	92 (16.52)	465 (83.48)		
Neoadjuvant treatment					
No	1,088 (98.55)	188 (17.28)	900 (82.72)	0.0000	1.0000
Yes	13 (1.18)	2 (15.38)	11 (84.62)		
Targeted molecular therapy					
No	46 (4.17)	5 (10.87)	41 (89.13)	0.2960	0.5864
Yes	533 (48.28)	80 (15.01)	453 (84.99)		
Sample type					
Metastatic	7 (0.63)	0 (0)	7 (100)	0.5011	0.4790
Primary tumor	1,097 (99.37)	190 (17.32)	907 (82.68)		

Bold values ($p < 0.05$) indicate statistically significant correlations. High and low LCN expression added up to 100% in each subgroup, e.g., age <60 years: high LCN1 expression 17.32% (102/589) + low LCN1 expression 82.68% (487/589) = 100% (589).

plied for the early diagnosis and prognosis of breast cancer [2]. Gene biomarkers also showed a certain degree of prognostic value in breast cancer, including uPA, PAI-1, cyclin D, cyclin E, p27, and p21 [4]. More effective prognostic factors for breast cancer will be discovered with the development of biological science and technology.

LCN1 (lipocalin-1), known as tear lipocalin, is the main lipid-binding protein in tears. It can be combined with a variety of hydrophobic molecules, including fatty acids, fatty alcohols, retinoic acid, retinol, cholesterol, phospholipids, glycolipids, and arachidonic acid and its peroxidation products [5]. LCN1 is a member of the lipocalin family, which has the ability to scavenge hydrophobic, potentially harmful molecules [6] and was associated with several diseases, including primary open angle glaucoma [7], keratoconus tears [8], and meibomian gland disease [9]. It was identified in a number of tissues and glands, including mammary, nasal, and testicular mucosae [10], the lachrymal, lingual, and sweat gland, the mucosal gland of the tracheobronchial tree [11], and the prostate [12]. Another member of the lipocalin superfamily, LCN2 (lipocalin-2), plays an important role in the progression of cancer [13]. It was reported to promote breast cancer progression and was highly associated with ER-negative breast tumors [14]. However, the relationship between LCN1 and breast cancer is still unknown. Therefore, we explored this relationship in an innovative way to make up for the gap of LCN1 in breast cancer.

The relationship between LCN1 expression and the prognosis of survival was investigated in breast cancer patients through analysis of the Cancer Genome Atlas (TCGA) breast cancer (BRCA) level 3 data. GEO datasets were used for external data validation. To our knowledge, this is the first time LCN1 has been linked to breast cancer.

Materials and Methods

Data Mining

The clinical data and RNA sequencing expression data of breast cancer patients were downloaded from TCGA (<https://cancer-genome.nih.gov/>). We applied RNA sequencing by expectation-maximization expression values to the analysis process. GEO datasets were downloaded from the GEO database (<https://www.ncbi.nlm.nih.gov/geo/>).

Statistical Analysis

Using the ggplot2 package in R, the expression difference of discrete variables was visualized into boxplots. The χ^2 test and the Fisher exact test were used to find the correlation between LCN1 expression and clinicopathologic features in R (version 3.5.2). To assess the diagnostic capability of LCN1, the pROC package was applied to draw its ROC curve, and the optimal cutoff value was obtained from the ROC curve to determine high and low LCN1 expression groups. Through the survival package of R, we used the Kaplan-Meier curves to compare differences in overall survival and relapse-free survival between both groups. *p* values were calculated by log-rank test. Univariate analysis was applied to screen significant clinicopathologic features, and multivariate analysis confirmed that LCN1 expression affected the survival of breast cancer patients.

Fig. 1. Differences in LCN1 expression were shown in boxplots according to clinical stage, molecular subtype, menopause status, TNM stage, tumor margins, and ER, PR, HER2, and vital status.

Results

Patient Characteristics

We analyzed the TCGA-BRCA level 3 data. The clinical characteristics included age, gender, menopause status, ER, PR, HER2, TNM stage, clinical stage, histological type, molecular subtype, lymph node status, margin status, vital status, neoadjuvant treatment, radiation therapy, targeted molecular therapy, and sample type. The corresponding patient characteristics are listed in Table 1.

LCN1 Expression and Association with Clinicopathologic Features in Breast Cancer

LCN1 expression was higher in tumor than in normal tissue ($p = 9.7e-11$). Moreover, differences in LCN1 expression were shown in boxplots according to clinical stage, molecular subtype, menopause status ($p = 0.004$), TNM stage, margin status ($p = 0.022$), ER, PR ($p = 0.025$), HER2, and vital status (Fig. 1). Then we validated it in

GEO datasets. LCN1 expression was also higher in breast tumor than in normal breast tissue in GSE5764, GSE40057, and GSE26910 (online suppl. Fig. S1; see www.karger.com/doi/10.1159/000503168 for all online suppl. material). The χ^2 and Fisher exact test were used. The results revealed that high LCN1 expression was associated with ER ($p = 0.0440$) and menopause status ($p = 0.0010$; Table 1). Besides, the results of LCN1 validation in GEO datasets also showed that breast tissue had a higher LCN1 expression than adipose fat in women at increased risk of breast cancer ($p = 3e-15$) based on GSE66159 (online suppl. Fig. S2A). We compared LCN1 expression in patients with primary breast tumor in different body mass index (BMI) groups ($p = 0.0088$): normal (BMI ≤ 24.9), overweight (BMI 25–29.9), and obese patients (BMI ≥ 30). LCN1 expression was significantly higher in the obese group than in the normal group (online suppl. Fig. S2B) and was positively correlated with BMI ($R^2 = 0.0429$) based on GSE24185 (online suppl. Fig. S2C).

Fig. 2. The receiver-operating characteristic curve of LCN1 in different clinical stages (normal vs. tumor). AUC, area under the curve.

Diagnostic Capability of LCN1 Expression

The AUC in the ROC curve of LCN1 was 0.654, which indicated its limited diagnostic capability. Subgroup analyses of the different stages were also shown. It revealed moderate diagnostic capability in stage IV (AUC: stage I, 0.634; stage II, 0.658; stage III, 0.652; stage IV, 0.752; Fig. 2).

LCN1 Expression and Breast Cancer Survival

The Kaplan-Meier curves were used to determine the relationship between LCN1 expression and breast cancer survival. Using log-rank tests, high LCN1 expression was associated with poor overall survival ($p = 0.00015$; Fig. 3) and poor relapse-free survival ($p = 0.0044$; Fig. 4). Subgroup analyses revealed that high LCN1 expression was correlated with poor overall survival in patients with ER-negative ($p = 0.0020$) and HER2-negative ($p = 0.0099$) tumors, PR tumors (negative: $p = 0.0071$; positive: $p = 0.0400$), and infiltrating ductal carcinoma ($p = 0.0062$; Fig. 3). The analyses also revealed that high LCN1 expression was related to poor relapse-free survival in patients with ER-negative tumors ($p = 0.0370$), HER2 tumors (negative: $p = 0.0300$; positive: $p = 0.0200$), luminal B tu-

mors ($p = 0.0100$), and infiltrating ductal carcinoma ($p = 0.0460$; Fig. 4). The prognostic role of LCN1 was validated in GEO datasets (GSE20711, GSE42568, and GSE88770), and the Kaplan-Meier curves showed that high LCN1 expression was associated with poor overall survival and poor relapse-free survival (online suppl. Fig. S3).

High LCN1 Expression as an Independent Predictor for Poor Survival in Breast Cancer

Univariate analysis was applied to screen significant clinicopathologic features, and multivariate analysis confirmed that LCN1 expression affected the survival of breast cancer patients. Univariate analysis showed that poor overall survival was related to age, HER2, clinical stage, margin status, and LCN1 expression (Table 2). Similarly, poor relapse-free survival was related to ER, PR, clinical stage, margin status, and LCN1 expression (Table 3). Multivariate analyses indicated that high LCN1 expression was an independent predictor of poor overall survival ($p = 0.002$; HR 2.24, 95% CI 1.33–3.78; Table 2) and poor relapse-free survival ($p = 0.016$; HR 1.85, 95% CI 1.12–3.06; Table 3).

Fig. 3. Kaplan-Meier curves of overall survival in breast cancer. Overall survival analysis and subgroup analyses of histological type, ER, PR, HER2, and molecular subtype.

Table 2. Univariate and multivariate analyses of overall survival in breast cancer patients

Parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	<i>p</i> value	HR	95% CI	<i>p</i> value
Age (≥60/<60 years)	1.91	1.39–2.63	0.000	2.35	1.47–3.75	0.000
Histological type (infiltrating ductal carcinoma/ infiltrating lobular carcinoma/other)	0.93	0.74–1.17	0.543			
Molecular subtype (basal/HER2/lum. B/lum. A/normal)	1.01	0.88–1.16	0.901			
ER (positive/indeterminate/negative)	0.85	0.71–1.02	0.074			
PR (positive/indeterminate/negative)	0.87	0.73–1.03	0.096			
HER2 (positive/indeterminate/negative/equivocal)	1.29	1.05–1.57	0.013	1.18	0.95–1.47	0.141
Menopause status (post-/peri-/premenopausal/indeterminate)	1.16	0.94–1.43	0.165			
Stage (IV/III/II/I)	1.64	1.4–1.91	0.000	2.13	1.62–2.81	0.000
Lymph node involvement (yes/no)	1.1	0.93–1.3	0.274			
Margin status (positive/close/negative)	1.42	1.11–1.81	0.005	1.01	0.72–1.42	0.950
LCN1 (high/low)	2.05	1.4–3	0.000	2.24	1.33–3.78	0.002

Bold values (*p* < 0.05) indicate statistically significant correlations. HR, hazard ratio; CI, confidence interval, lum., luminal.

Fig. 4. Kaplan-Meier curves of relapse-free survival in breast cancer. Relapse-free survival analysis and subgroup analyses of histological type, ER, PR, HER2, and molecular subtype.

Table 3. Univariate and multivariate analyses of relapse-free survival in breast cancer patients

Parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	<i>p</i> value	HR	95% CI	<i>p</i> value
Age (≥ 60 / < 60 years)	1.45	0.97–2.16	0.072			
Histological type (infiltrating ductal carcinoma/ infiltrating lobular carcinoma/other)	0.86	0.65–1.14	0.290			
Molecular subtype (basal/HER2/lum. B/lum. A/normal)	0.99	0.82–1.2	0.945			
ER (positive/indeterminate/negative)	0.78	0.63–0.97	0.026	0.86	0.62–1.19	0.351
PR (positive/indeterminate/negative)	0.78	0.64–0.96	0.019	0.85	0.62–1.15	0.281
HER2 (positive/indeterminate/negative/equivocal)	0.93	0.7–1.22	0.596			
Menopause status (post-/peri-/premenopausal/indeterminate)	0.95	0.74–1.22	0.713			
Stage (IV/III/II/I)	1.71	1.4–2.08	0.000	1.63	1.31–2.03	0.000
Lymph node status (yes/no)	0.86	0.7–1.06	0.159			
Margin status (positive/close/negative)	1.59	1.23–2.06	0.000	1.47	1.12–1.92	0.006
LCN1 (high/low)	1.97	1.22–3.18	0.005	1.85	1.12–3.06	0.016

Bold values ($p < 0.05$) indicate statistically significant correlations. HR, hazard ratio; CI, confidence interval; lum., luminal.

Discussion

Our team aimed to determine novel biomarkers for the diagnosis and prognosis of diseases in the field of oncology [15–21]. Analysis of TCGA-BRCA level 3 data demonstrated that LCN1 expression was obviously higher in tumor tissue than in normal tissue. LCN1 expression may be an independent factor for the prognosis of survival in breast cancer patients. Besides, we validated LCN1 expression and survival analyses by GEO datasets. To our knowledge, this is the first study which investigated the association between LCN1 expression and breast cancer.

LCN1, a gene that encodes tear lipocalin, is located on chromosome 9q34 [22]. Tear lipocalin is a member of the lipocalin family and synthesized by 3 genes: LCN1a, LCN1b, and LCN1c. LCN1a encodes tear lipocalin and 2 other genes are not expressed [10]. LCN1 can scavenge lipids from the corneal surface to keep it moist and intact [23]. Compared to control tears, the content of LCN1 was reduced in dry eye disease [24]. LCN1 expression was also decreased in tears of patients with the Sjögren's syndrome [25]. LCN1 expression has also been analyzed in human teratocarcinoma-derived NT2 precursor cells using reverse-transcriptase PCR [26]. However, no other studies have studied its expression levels in malignant tumors, especially breast cancer. In our study, we found that LCN1 expression was obviously higher in tumor than normal tissue based on both the TCGA database and GEO datasets.

LCN1 is a biomarker of many benign diseases, including dry eye disease [27], diabetic retinopathy [28], chronic obstructive pulmonary disease [29], and tobacco-related diseases [30]. However, the role of LCN1 in breast cancer is unclear. In our study, we found that high LCN1 expression was associated with clinicopathologic features, including ER status and menopause. LCN2 has also been reported to affect the steroid status of mammary epithelial cells and change ER status [31]. LCN1 levels were compared in different groups of patients with primary breast tumor, and the results showed that high LCN1 expression was associated with high BMI in breast cancer patients. It is known that obese people are more likely to have hyperlipidemia. LCN1 may be associated with hyperlipidemia, which may be associated with morbidity, tumor growth, and metastases in breast tumors [32]. In addition, high levels of NGAL (neutrophil gelatinase-associated lipocalin), also known as LCN2, in biological fluids may be used to prognosticate disease-free survival in breast cancer patients [33] and were associated with aggressive breast cancer types and a poor prognosis [31]. Our study also indicated that high LCN1 expression was an independent predictor of a poor prognosis in breast cancer patients, and it revealed moderate diagnostic ca-

pability in stage IV. LCN1 may be a new biomarker for the diagnosis and prognosis of breast cancer.

LCN1 was reported to bind with a variety of hydrophobic molecules and may act as a scavenger of potentially harmful, hydrophobic molecules [5, 6]. It was shown to be involved in inflammatory processes of oral and ocular tissue [30], cellular defense against oxidative stress [34], and immune defense against fungal infections [6]. Moreover, LCN1 also participated in the protection of human epithelial cells [35]. LCN2 has been reported as a potential therapeutic target for breast cancer metastases, and abnormal expression of LCN2 was associated with epithelial-to-mesenchymal transition, angiogenesis, cell migration, and invasion in breast cancer [36]. In our study, the results showed that high LCN1 expression was associated with poor survival in patients with infiltrating ductal carcinoma. It is known that invasive ductal carcinoma of the breast originates from the luminal epithelium [37]. High LCN1 expression might have a correlation with infiltrating ductal carcinoma through its effects on mammary epithelial cells. However, experimental confirmation is needed.

Due to the limited number of samples, further analysis of large sample data are required for validation. The results of LCN1 studies in other diseases foster further experiments or clinical studies to verify its significance as a prognostic marker for breast cancer.

In conclusion, we determined the association between LCN1 expression and breast cancer based on TCGA database analyses. Besides, we also validated LCN1 expression and survival analyses by GEO datasets. Thus, LCN1 expression may be an independent predictor of a poor prognosis in breast cancer patients.

Acknowledgments

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Statement of Ethics

This study did not require ethical approval as the data used have been published previously, and hence are already in the public domain.

Disclosure Statement

The authors declare that there are no conflicts of interest.

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