JBUON 2020; 25(6): 2552-2561
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

CXCL13/CXCR5 are potential biomarkers for diagnosis and prognosis for breast cancer

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Summary

Purpose: *Breast cancer is known as the second frequent cancer in the world, even as the most common cancer among women. This study aimed to explore the correlation of CXCL13/CXCR5 expression with clinical characteristics in breast cancer and evaluate their potential to be used as biomarkers in diagnosis and prognosis of this disease.*

Methods: *A total of 133 female patients diagnosed with breast cancer were collected. The expression of CXCL13 and CXCR5 mRNA was analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) and immunohistochemical staining.*

Results: *The expression of CXCL13 and CXCR5 was significantly higher in breast cancer tissue than in normal breast tissues, with a high correlation coefficient of 0.9973. Positive cell numbers and positive expression rates of CXCL13 and CXCR5 in cancer breast tissue were much higher than those in normal breast tissue, and raised with increase of cancer stage. The high expression of CXCL13 and CXCR5 in breast cancer tissue was notably associated with lymph node metastasis, distant metastasis, disease stage, but not with age, Her-2 status, histological type or tumor size. Immunohistochemistry analysis showed that the positive expression of CXCL13 and CXCR5 was related with cancer stage, Also, positive expression of CXCL13 was correlated with positive expression of CXCR5. Patient age, Her-2 status, tumor size, histological type, and lymph node metastasis were independent factors for the 5-year survival rate of breast cancer patients, whereas the 5-year survival was correlated with distant metastasis and expression of CXCL13 and CXCR5.*

Conclusions: *These results suggested that CXCL13 and CXCR5 expressions could act as potential biomarkers for breast cancer diagnosis and prognosis.*

Key words: *CXCL13, CXCR5, breast cancer, diagnosis*

Introduction

Breast cancer is known as the second frequent cancer in the world, even as the most common cancer among women. In 2012, approximately 1.7 million patients were newly diagnosed with breast cancer, which reached 25% of all kinds of cancers [1]. Although many advances have been made in the diagnosis and treatment of breast cancer, sometimes interventions are ineffective due to the high proliferative capacity of tumor cells and the inherent resistance of clinical therapies [2]. Breast cancer is the main cause of cancer-related mortality in women , accounting for 14% of cancer deaths worldwide [3].

It has been reported that chemokines expressed by tumor cells in the tumor microenvironment couldn't be regulated by the host autonomously

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Received: 03/10/2020; Accepted: 31/20/2020

[4]. Chemokines are essential coordinators of cell-cell adhesion and interaction and play a vital role in chemo-attraction and leukocyte recruitment by binding to the corresponding G-protein coupled receptors (GPCRs) of target cells [5,6]. CXCL13, C-X-C motif chemokine ligand 13, were initially recognized as a regulatory subgroup of T cells among the stromal cells in B cell follicle and directed homing of B cells [7]. Previous studies had that CXCL13 plays important roles in inflammatory diseases. High expression of CXCL13 was found in breast cancer patients, and CXCL13 was associated with cancer metastasis [8-10]. In addition, the expression of CXCL13 in early breast cancer was remarkably up-regulated and was closely related with prognostic factors, including lymph node metastasis [11]. These studies indicated that high expression of CXCL13 was a disadvantage in breast cancer.

The function of chemokines was induced by their interaction with a specific receptor [12,13], and CXCR5 is the main receptor of CXCL13 [14,15]. The expression of integrin and the entry of T cells into lymph nodes was promoted by CXCR5-CXCL13 interaction. As a result, the immune system of CXCR5-deficient mice was severely injured [15,16]. A study has shown that anti-CXCL13 or anti-CXCR5 antibody injected into the breast cancer could block the chemotaxis of cancer cells effectively, and CXCR5 expression in tumor tissues was dramatically up-regulated compared with that in normal breast tissues [10]. Also, it was observed that CXCL13 was up-regulated in lymph nodes at the most common metastatic sites of breast cancer [8]. All these studies demonstrated that CXCL13 and CXCR5 were closely associated with breast cancer.

To further investigate the enrollment of CXCL13 and CXCR5 in breast cancer, we tested CXCL13 and CXCR5 mRNA expressions in breast cancer and normal adjacent breast tissues. The correlation of their expression with breast cancer clinical characteristics was also examined. Thus, the potential of CXCL13 and CXCR5 used as biomarkers in breast cancer diagnosis and prognosis was clarified in our study.

Table 1. Patient characteristics at enrollment

Patient characteristics	*n*
Age (years)	
31-56	70
56-82	63
Clinical stages	
I	15
II	52
III	34
IV	32
Histologic type	
Non-invasive	2
Invasive	131
T stage	
Tx	6
T1	37
T2	72
T3	8
T4	10
N stage	
Nx	5
N0	37
N1	62
N2	25
N3	4
M stage	
Mx	1
M0	100
M1	32
Disease stage	
I	16
II	54
III	30
IV	33
Her-2	
-	37
+	96

Methods

Patient selection and tissue sample preparation

One hundred trirty-three female patients diagnosed with breast cancer patients who have been treated in 970 Hospital of the PLA Joint Logistic Support Force from August 30, 2009 to December 31, 2013 were involved in the study. These patients ranged in age from 31 to 82 years with an average of 56 years. The inclusion criteria were: 1) have breast cancer only on one side; 2) were treated with modified radical mastectomy or radical mastectomy; 3) were never treated by radiotherapy, chemotherapy or medical treatment; 4) have complete diagnosis and treatment records; and 5) have confirmed TNM stage and histological diagnosis, which are listed in Table 1. After surgery, all patients were given adju-

Table 2. Sequences of primers used in qRT-PCR

	Primer Sequence
CXCL13	Forward: 5'- GCTTGAGGTGTAGATGTGTCC -3'
	Reverse: 5'- CCCACGGGGCAAGATTTGAA -3'
CXCR5	Forward: 5'- GGTCACCCTACCACATCGTC -3'
	Reverse: 5'- GCCATTCAGCTTGCAGGTATTG -3'
β-actin	Forward: 5'- TAGGCCGTGGCTCAAGAAC -3'
	Reverse: 5'- TGCATCTCCAAGTTGCCTTTG -3'

vant therapies such as chemotherapy, radiotherapy and endocrine therapy according to each patient's condition.

The patients were followed up for at least 5 years (range 60 to 88 months, median 72 months). Follow-up results were recorded including 7 with local relapse, 8 with lung metastasis, 5 with bone metastasis, 3 with liver metastasis, 2 with contralateral breast metastasis and 34 death cases during our study. Her-2 status was determined by immunohistochemistry and positive status was defined when the percentage of stained cells was over 10% [17].

Sample tissues including primary cancer, metastatic cancer and, adjacent tissues were collected during surgery. Adjacent tissues of cancer were taken from the side of the tumor at least 5 cm away from the edge of the tumor. Tissues of 80 patients with breast hyperplasia were collected from August 30, 2009 to December 31, 2013 and used as a control while calculation of sensitivity and specificity of CXXL13/CXCR5 was used as biomarkers. The patient age ranged from 28 to 61 with an average age of 48.7. Postoperative pathological diagnosis was benign breast hyperplasia. Tissues were stored in the refrigerator at -80°C after liquid nitrogen freezing and a portion of samples were fixed in 4% formaldehyde for immunohistochemical (IHC) staining. Experimental plan and procedures have been approved by the Ethics Committee of 970 Hospital of the PLA Joint Logistic Support Force under the declaration of Helsinki (2013 version). All 133 breast cancer patients signed the informed consent to participate in this study.

Quantitative real-time polymerase chain reaction (qRT-PCR)

To measure mRNA level of CXCL13 and CXCR5, the total RNA samples were extracted from the breast cancer tissues and adjacent normal tissues by using TRIzol Kit (Invitrogen, Carlsbad, CA, USA), and then applied as template for reverse transcription reaction by the SuperScript III Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA, USA). Real-time PCR was performed by Mastercyclero ep realplex2 Real-Time PCR system (Eppendorf, Hamburg, Germany). The relative level of the mRNA was calculated with the $2^{-\Delta\Delta Ct}$ method [18]. The primers were obtained from Sangon Biotech (Shanghai, China), which were listed in Table 2.

Immunohistochemical (IHC) staining

Samples were mounted onto Superfrost microscope slides (Thermo Fisher Scientific, Waltham, MA, USA) and blocked with 3% hydrogen peroxide in 50% methanol for 20 min. Sections were treated with 10 mM sodium citrate (pH 6.0) for 10 min heating for epitope retrieval. After blocking with 5% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 20 min at room temperature, rabbit anti-human CXCL13 polyclonal antibody (1:1000, NSJ Bioreagents CA, USA) and rabbit anti-human CXCR5 polyclonal antibody (1:1000, Aachen, Germany) were added, and incubated overnight at 4°C. After that, horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:1000, Aachen, Germany) was added, and incubated for 40 min. 3, 3'-diaminobenzidine (DAB) (Solarbio, Beijing, China) was then applied for chromogenic reaction followed by counterstain hematoxylin for better visualization. Then, the slides were dehydrated with ethanol and cleared with xylene. The cells were counted using Aperio Imagescope software.

Slide evaluation was performed as described before [19,20]. The percentage of positive cells was determined by the following standard: 0 point: < 5%; 1 point: 6-25%; 2 point: 26-50%; 3 point: 51-75%; 4 point: > 75%. Staining intensity was classified as follows: 0 point: no staining; 1 point: light yellow; 2 point: yellow or dark yellow; 3 point: brown or dark brown using multiplication of intensity and percentage fractions to determine the result, which ranged from 0 to 12. A score over 5 points was defined as high expression. The immunostaining was considered 0 or negative when the score was 0; + or weak positive, 1-3; ++ or moderately positive, 4-7; +++ or strong positive, 8-12. Scoring was performed by two experienced pathologists according to the immunoreactive score (IRS) based on 5 fields of each slides at a magnification of 200×.

Calculation of the sensitivity and specificity

Sensitivity referred to the percentage of women diagnosed with cancer who were truly positive during the screening interval. Specificity referred to the true negative proportion of women who were not diagnosed with cancer during the screening interval [21]. The sensitivity and specificity were calculated as: sensitivity=TP/(TP+FN)×100%; specificity=TN/(TN+FP)×100%. True positives, TP; false negatives, FN; true negatives, TN; false positives, FP.

Statistics

Statistical analyses were performed using SPSS 19.0 software (IBM, Armonk, NY, USA). All data were expressed as mean ± standard deviation (SD) using two-tailed Student's t-test for comparison between two groups. Comparison between multiple groups was done using One-way ANOVA test followed by *post hoc* test (least significant difference). For correlation analysis, the Spearman's correlation coefficient method and chi-square test were used. Survival curves were plotted with Kaplan-Meier method accompanied with log-rank test. Factors influencing long-term survival were analyzed using univariate and multivariate Cox regression analyses. Statistically significant difference was considered at $p<0.05$, and very significant difference was assumed at $p<0.01$.

Results

The mRNA expression of CXCL13 and CXCR5 is increased in cancer breast tissues

The mRNA expression of CXCL13 and CXCR5 was examined by qRT-PCR and the results are presented in Figure 1. Compared with the normal breast tissues, the mRNA expression of both CXCL13 and CXCR5 was dramatically increased in the cancer breast tissues (Figure 1A and 1B). Correlation

analysis revealed that CXCL13 and CXCR5 mRNA expression were notably correlated with a correlation coefficient of 0.9973 (Figure 1C).

The expression of CXCL13 and CXCR5 is correlated with breast cancer clinical characteristics

The levels of CXCL13 and CXCR5 in the top third were defined as high expression [9,22]. Among the 133 breast cancer patients, 43 (32.3%) were observed with high expression of CXCL13 and high expression of CXCR5 was found in 46 (34.6%) tissues. Therefore, the high expression of CXCL13 was defined as the condition that the mRNA expressions of CXCL13 in 43 cancer breast tissues were over 7.51 times of that in normal breast tissues. Similarly, the high expression of CXCR5 was defined as the condition that the mRNA expressions of CXCR5 in 46 cancer breast tissues were over 8.08 times of that in the normal breast tissues.

We used Chi-square analysis to determine the relationship between the high expression of CXCL13 and CXCR5 with breast cancer characteristics. It was found that CXCL13 high expression was related with lymph node metastasis, distant metastasis, disease stage, and CXCR5 mRNA expression ($p<0.05$). However, the high expression of CXCL13 was independent on the clinical characteristics of the patients including age, Her-2 status,

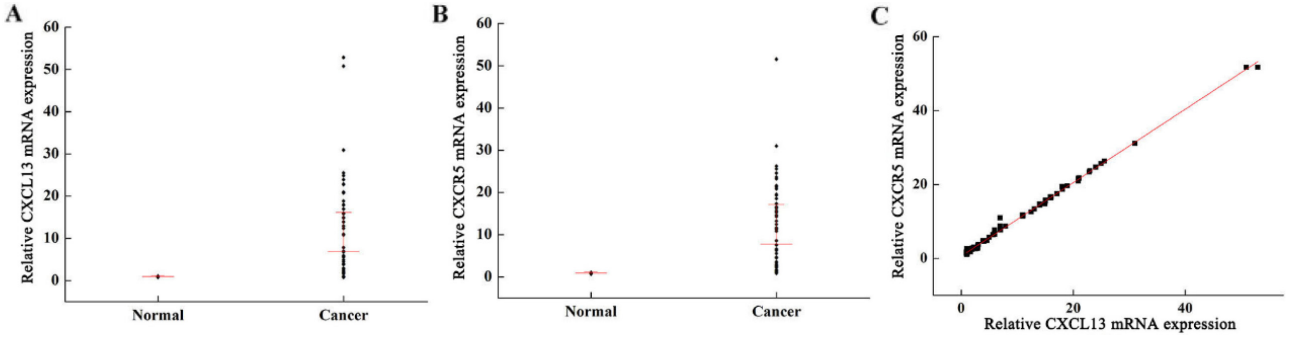

Figure 1. mRNA expression of CXCL13 and CXCR5 in breast cancer tissue and normal breast tissue. **A:** mRNA expression of CXCL13 in breast cancer tissue and normal breast tissue. **B:** CXCR5 mRNA in breast cancer tissue and normal breast tissue. **C:** Correlation between mRNA expression of CXCL13 and mRNA expression of CXCR5 (*$p<0.05$).

Table 3. CXCL13 and CXCR5 expression with clinical characteristics

Groups	*n*	*CXCL13 High Expression*			*CXCR5 High Expression*		
		n	x^2	*p value*	*n*	x^2	*p value*
Age (years)							
31-56	70	19	1.818	0.178	22	0.651	0.420
56-81	63	24			24		
Her-2							
-	37	14	0.711	0.399	15	0.803	0.370
+	96	29			31		
Histologic type							
Noninvasive	2	0	0.970	0.325	0	1.074	0.300
Invasive	131	43			46		
Tumor size							
T1+ T2	115	31	0.179	0.672	33	0.531	0.466
T3+ T4	18	6			7		
Lymph node metastasis							
-	42	6	4.990	0.025	7	4.557	0.033
+	91	33			35		
Distant metastasis							
-	101	19	14.233	<0.001	22	29.993	<0.001
+	32	17			24		
Disease stage							
I/II	70	11	18.650	<0.001	12	37.775	<0.001

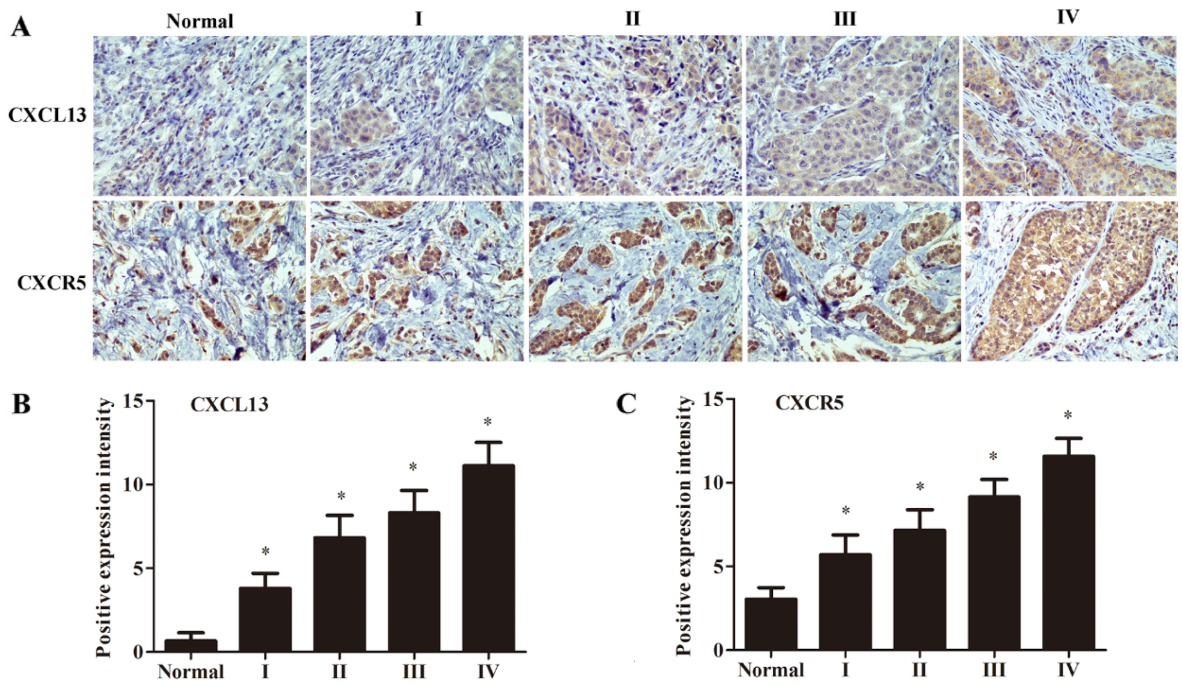

Figure 2. Expression status of CXCR5 and CXCL13 in various tissue samples. **A:** Immunohistochemistry analysis in normal breast tissue and cancer breast tissue at different disease stages. **B:** Positive cell numbers of CXCL13 in normal breast tissue and cancer breast tissues at different disease stages. **C:** Positive cell numbers of CXCR5 in normal breast tissue and cancer breast tissues at different disease stages (*p<0.05).

Table 4. Correlations of positive expression of CXCL13 and CXCR5 with clinical stages

Groups	*n (%)*	*CXCL13 ++/+++*	*r*	*p value*	*n (%)*	*CXCR5 ++/+++*	*r*	*p value*
Normal	5 (3.7)	0	0.750	<0.001	4 (3.0)	1	0.730	<0.001
Stage I	2 (13.3)	1			2 (13.3)	1		
Stage II	18 (34.6)	10			17 (32.7)	11		
Stage III	24 (70.5)	16			24 (70.5)	17		
Stage IV	30 (93.8)	23			28 (87.5)	25		

Normal: adjacent healthy tissues ; Stage I, T1N0M0; stage II, T0~1N1M0, T2N0~1M0; stage III, T0~2N2M0, T3N1~2M0, T4 N1~4M0, T1~4N3M0; stage IV, M1T1~4N1~4.

histological type and tumor size (p>0.05) (Table 3). In addition, the high expression of CXCR5 was also correlated with lymph node metastasis, distant metastasis and disease stages (p<0.05), but not with the clinical characteristics including age, histological type, Her-2 status or tumor size (p>0.05) (Table 3).

Table 5. Correlations of CXCL13 positive expression with positive expression of CXCR5

CXCR5	*CXCL13*				*r*	*p value*
	-	+	++	+++		
-	12	2	5	0	0.325	0.001
+	0	35	8	2		
++	0	14	10	11		
+++	0	6	11	17		

Positive expression of CXCL13 and CXCR5 determined by IHC was correlated with disease stages

To determine the correlation of CXCL13 and CXCR5 with disease stages, we performed the IHC analysis. Our results suggested that CXCL13 protein was observed mainly in the cancer breast tissue and the invasion interstitial between cancer and breast tissues (Figure 2A). CXCR5 protein displayed strong expression in cancer breast tissue, the invasion interstitial between cancer and breast tissues, the outer periphery of the cancer breast tissue and the cytoplasm and cell membranes of the epithelial cells (Figure 2A). The expression of CXCL13 and CXCR5 showed no or weak staining in the normal breast tissues and the stained color intensity displayed stronger with the cancer progression

from stage I to stage IV (Stage I, T1N0M0; stage II, T0~1N1M0, T2N0~1M0; stage III, T0~2N2M0, T3N1~2M0, T4 N1~4M0, T1~4N3M0; stage IV, M1T1~4N1~4). Thus, the expression of CXCL13 and CXCR5 was elevated in advanced tumor stages.

In the normal breast tissue, the positive expression rate of CXCL13 was 3.7%, and the positive expression rate of CXCR5 was 3.0%. In the cancer breast tissues, the positive expression rate of CXCL13 was 55.6%, and the positive expression rate of CXCR5 was 53.4%. In terms of positive cell numbers, compared to normal breast tissue, positive cell numbers of both CXCL13 and CXCR5 in the cancer breast tissue were significantly higher ($p<0.05$) (Figure 2B and 2C). Also, in the cancer breast tissues, positive cell numbers of both CXCL13 and CXCR5 increased with the progression of the tumor, and the differences in each disease stage was statistically significant ($p<0.05$) (Figure 2B and 2C). These data demonstrated that the positive expression rate of CXCL13 and CXCR5 in the cancer breast tissue was correlated with breast cancer clinical stages ($p<0.05$). The positive expression rate of CXCL13 and CXCR5 in breast cancer tissues was highly correlated ($p<0.05$) (Tables 4 and Table 5).

5-year survival of breast cancer patients is associated with distant metastasis, disease stage, expression of CXCL13 and CXCR5

The correlation between 5-year survival and CXLC13 and CXCR5 expression was examined. By the end of the follow-up period, the overall 5-year survival rate was 75.9%. Eleven (25.6%) out of the 43 patients with CXCL13 high expression survived after 5 years, and 14 (30.4%) out of the 46 patients with CXCR5 high expression survived after 5 years (Table 6). Chi-square test also showed that the 5-year survival was not associated with the features including age, Her-2 status, histologic type, tumor size, and lymph node metastasis, but was associated with the characteristics including distant metastasis, CXCL13 and CXCR5 high expression (Table 6). These results demonstrated those poor 5-year survival rates were frequently observed

Table 6. 5-year survival rate and disease characteristics

Group	*n*	*5-year survival n (%)*	x^2	*p value*	*95% CI*
Age (years)					
31-56	70	57 (81.4)	2.437	0.119	
56-81	63	44 (69.8)			
Her-2					
-	37	24 (64.9)	3.441	0.064	
+	96	77 (80.2)			
Histologic type					
Non-invasive	2	2 (100)	0.643	0.423	
Invasive	131	99 (75.6)			
Tumor size					
T1+T2	115	89 (81.7)	2.131	0.144	
T3+T4	18	12 (66.7)			
Lymph node metastasis					
-	42	32 (86.5)	2.13	0.145	
+	91	68 (74.7)			
Distant metastasis					
-	101	90 (90)	45.561	<0.001	0.554-0.883
+	32	10 (31.3)			
Disease stage					
I/II	70	64 (91.4)	19.403	<0.001	0.554-0.883
III/IV	63	37 (58.7)			
CXCL13 high expression					
-	90	90 (100)	88.197	<0.001	0.872-1.044
+	43	11 (25.6)			
CXCR5 high expression					
-	87	87 (100)	79.700	<0.001	0.872-1.044
+	46	14 (30.4)			

in breast cancer patients with high CXCL13 and CXCR5 expression.

Among the 133 patients, 33 (24.8%) with high expression of CXCL13 survived at least 5 years, and 37 (27.8%) with high expression of CXCR5 survived at least 5 years (Table 7). The correlation of high CXCL13 and CXCR5 expression with 5-year survival was highly correlated, with a correlation coefficient of 0.722 (CXCL13) or 0.641 (CXCR5).

When breast cancer patients were evaluated with the Kaplan-Meier analysis, high CXCL13 and CXCR5 expression, distant metastasis and advanced tumor stages were observed to be associated with poor breast cancer survival (Figure 3A-3D). Univariate analysis showed that the 5-year survival was independent of age, Her-2 status, histological type, tumor size and lymph node metastasis ($p>0.05$), but was dependent on distant metasta-

Table 7. 5-year survival rate and positive expression of CXCL13 and CXCR5

	Group	*n*	*5-year survival, n (%)*	*r*	*p value*
CXCL13	-	12	12 (100)	0.722	0.001
	+	57	56 (98.3)		
	++	34	19 (55.9)		
	+++	30	14 (46.7)		
CXCR5	-	19	19 (100)	0.641	0.001
	+	45	45 (100)		
	++	35	30 (85.7)		
	+++	34	7 (20.6)		

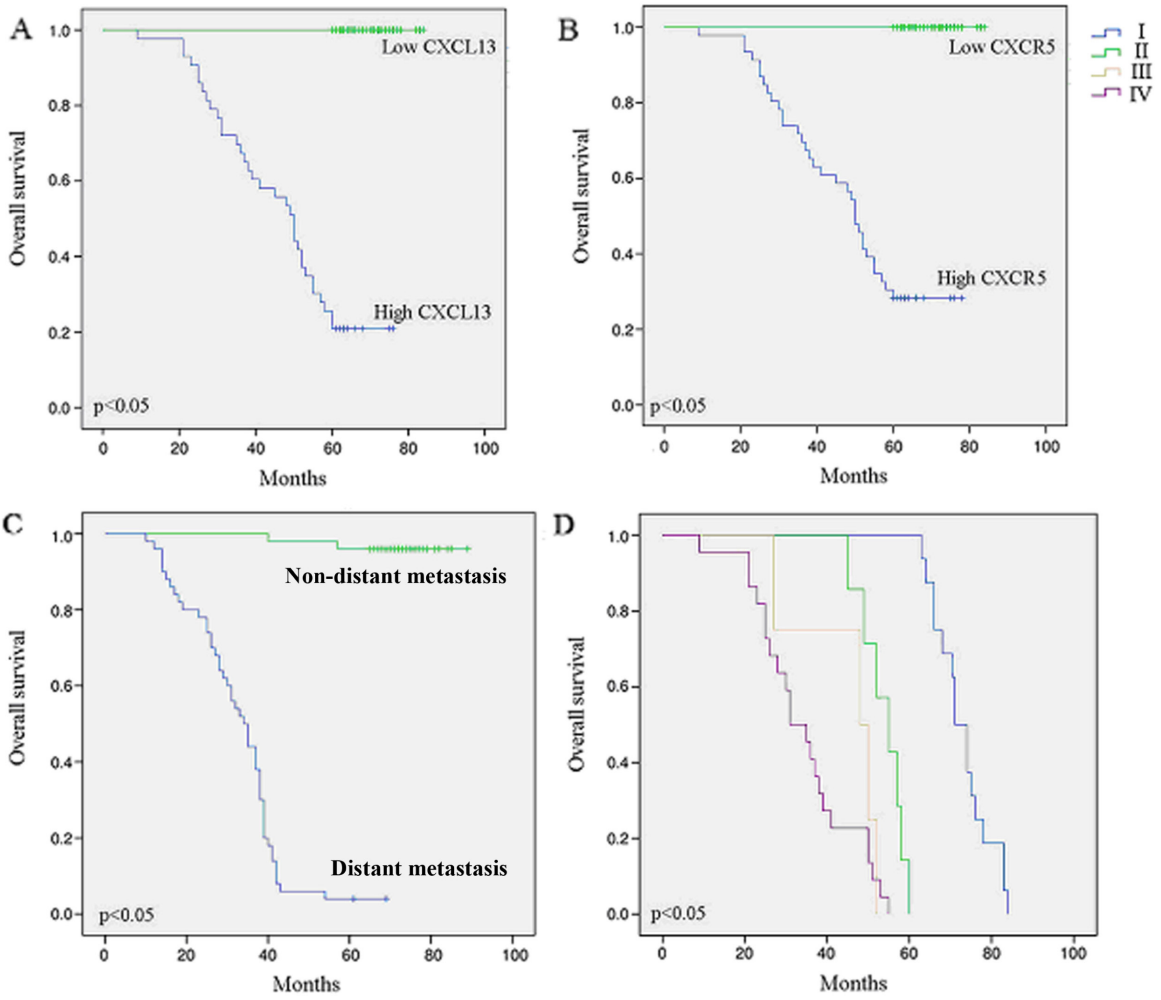

Figure 3. Survival analysis of breast cancer patients with various factors. Kaplan-Meier survival curves in breast cancer patients according to **A:** CXCL13 mRNA expression, **B:** CXCR5 mRNA expression, **C:** distant metastasis or **D:** clinical disease stage.

Table 8. Univariate Cox proportional hazard regression analysis of overall survival in breast cancer patients

Risk factor	*Hazard ratio*	*95% CI*	*p value*
Age (years)			
(31-56 *vs.* 56-81)	1.03	0.71-1.29	0.906
Her-2			
(- *vs.* +)	1.09	0.91-1.39	0.887
Histology type			
(Noninvasive vs invasive)	1.14	0.94-1.42	0.675
Tumor size			
(T1+T2 *vs.* T3+T4)	0.82	0.64-1.87	0.453
Lymph node metastasis			
(- *vs.* +)	1.05	0.85-1.31	0.894
Distant metastasis			
(- *vs.* +)	1.35	1.15-1.57	0.021
Disease stage			
(I/II *vs.* III/IV)	1.64	1.32-1.98	0.003
CXCL13 mRNA			
(- *vs.* +)	2.35	1.51-3.72	<0.001
CXCR5 mRNA			
(- *vs.* +)	1.96	1.47-2.96	<0.001
CXCL13 protein expression			
(-/+ *vs.* ++/+++)	2.51	1.59-2.85	<0.001
CXCR5 protein expression			
(-/+ *vs.* ++/+++)	2.14	1.21-3.05	<0.001

CI: confidence interval; +: positive; -: negative.

Table 9. Multivariate Cox proportional hazard regression analysis of overall survival in breast cancer patients

Risk factor	*Hazard ratio*	*95% CI*	*p value*
Distant metastasis			
(- *vs.* +)	1.57	1.18-2.11	0.001
Disease stage			
(I/II *vs.* III/IV)	2.01	1.52-2.54	0.004
CXCL13 mRNA			
(- *vs.* +)	2.59	1.56-3.41	<0.001
CXCR5 mRNA			
(- *vs.* +)	2.03	1.27-2.74	<0.001
CXCL13 protein expression			
(-/+ *vs.* ++/+++)	2.88	1.64-3.59	<0.001
CXCR5 protein expression			
(-/+ *vs.* ++/+++)	2.34	1.31-3.14	<0.001

CI: confidence interval; +: positive; -: negative.

Table 10. Sensitivity and specificity of CXXL13/CXCR5 used as biomarkers

	Control	*Cancer*	*Sensitivity (%)*	*Specificity (%)*
CXCL13	80	133	65.41 (97/133)	71.25 (57/80)
CXCR5	80	133	63.91 (85/133)	63.75 (51/80)

sis, disease stage, high expression of CXCL13 and CXCR5 ($p < 0.05$) (Table 8). Moreover, multivariate analysis also revealed that high CXCL13 and CXCR5 expression, distant metastasis and advanced tumor stages were related to reduction of 5-year survival (Table 9). Furthermore, the sensitivity and specificity of CXCL13 and CXCR5 to detect breast cancer from normal were examined. As shown in Table 10, CXCL13 and CXCR5 exhibited good sensitivity and specificity in breast cancer detection. Therefore, CXCL13 and CXCR5 can be potential biomarkers for diagnosis and prognosis of breast cancer.

Discussion

It was observed that CXCL13 and CXCR5 were highly expressed in breast cancer [8-10]. In the current study, we also found that the expression of CXCL13 and CXCR5 was extremely correlated with a correlation coefficient of 0.9973. In a recent study, it was reported that autocrine CXCL13-CXCR5 signaling potentially induces epithelial-mesenchymal transition of breast cancer cells via RANKL-Src-PI3Kp110α during lymph node metastasis [8]. Inhibition of CXCL13 could lead to a number of favorable changes in breast cancer, such as reduced tumor volume and down-regulated expression of related genes in the CXCR5/ERK signaling pathway [23]. Therefore, targeting CXCL13 with CXCL13 inhibitor can provide a novel therapeutic method for breast cancer.

In this study, our results showed that CXCL13 and CXCR5 expressions are highly correlated to the breast cancer clinical characteristics. The high expression of both CXCL13 and CXCR5 is associated with the clinical characteristics including lymph node metastasis, distant metastasis, disease stage, but without including age, Her-2 status, histological type and tumor size. IHC experiments revealed that both CXCL13 and CXCR5 expressions are highly correlated to breast cancer stages and that the higher disease stage, the stronger IHC staining. Also, higher disease stage of breast cancer tissue, is accompanied with higher positive cell numbers of both CXCL13 and CXCR5. Therefore, these results indicated that the expression pattern of CXCL13 and CXCR5 could be used to predict the clinical diagnosis of breast cancer.

There were some reports claiming that high expression of CXCL13 and CXCR5 was associated with adverse prognosis or lymph node metastasis in breast cancer [24,25]. However, emerging studies showed that CXCL13 and CXCR5 are associated with a favorable disease outcome [26,27]. Although CXCL13 expression is often lost in aggressive triple-negative breast cancers (TNBC), those patients that retained CXCL13 or those with high expression were found to have superior outcomes [26]. Moreover, studies found that, compared with low tumor CXCL13 mRNA expression levels, the high expression levels were strongly related to favorable 5-year distant metastasis-free survival and disease-free survival rate in aggressive TNBC. A four-gene signature (*HLF, CXCL13, SULT1E1*, and *GBP1*) was proved to be associated with favorable outcome in TNBC [27]. In our study, we observed that the 5-year survival rate of breast cancer patients was correlated with distant metastasis and expression of CXCL13 and CXCR5. Taken together, CXCL13/CXCR5 expressions could act as good biomarkers in breast cancer prognosis.

Both CXCL13 and CXCR5 are highly expressed in breast cancer tissue, and the expression pattern is highly related to breast cancer clinical characteristics. These results suggested that CXCL13/CXCR5 are potential biomarkers in clinical breast cancer diagnosis. The 5-year survival of breast cancer patients is correlated with distant metastasis and expression of CXCL13/CXCR5. Therefore, CXCL13/CXCR5 expressions could act as potential biomarkers for breast cancer prognosis.

Conflict of interests

The authors declare that they have no competing interests, and all authors confirm its accuracy.

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