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ORIGINAL ARTICLE

The expression of KI-67 and LEF-1 in patients after breast cancer resection and its effects on patients' prognosis

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Summary

Purpose: *To investigate the expression of KI-67 and LEF-1 in patients after breast cancer resection and its effects on patients' prognosis.*

Methods: *A total of 89 breast cancer patients admitted to the first affiliated Hospital of Shantou University Medical College from January 2010 to February 2013 were enrolled as the study group, and 76 healthy individuals were enrolled as the control group. Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect the expression of KI-67 and LEF-1 in the serum. The relationship of the two indexes and clinicopathological data of the breast cancer patients were analyzed. In addition, the diagnostic value of KI-67 and LEF-1 in breast cancer patients was analyzed by receiver operating characteristic (ROC) curves, and their diagnostic value in the postoperative 5-year recurrence was also analyzed. Furthermore, the expression of KI-67 and LEF-1 in patients with postoperative recurrent breast cancer within 5 years was evaluated.*

Results: *The expression of KI-67 and LEF-1 in the study group was higher than in the control group ($p<0.05$), and the expression of KI-67 and LEF-1 was significantly related to the tumor size and lymph node metastasis (both $p<0.05$). ROC curve showed that the area under the curve (AUC) of the diagnostic value of KI-67 and LEF-1 for breast cancer patients was 0.860 and 0.858 respectively, and that of the diagnostic value KI-67 combined with LEF-1 for breast cancer patients was 0.924. In addition, the AUC of the diagnostic value of KI-67 and LEF-1 for the recurrence of breast cancer within 5 years was 0.699 and 0.651, respectively, and that of diagnostic value of KI-67 combined with LEF-1 for the recurrence of breast cancer within 5 years was 0.758. The expression of KI-67 and LEF-1 in patients with recurrent disease within 5 years after operation was higher than in patients without recurrence.*

Conclusion: *The expression of KI-67 and LEF-1 in breast cancer patients is significantly higher than in healthy individuals, which has certain diagnostic value in breast cancer. The expression of the two indexes is related to tumor size and lymph node metastasis, and the survival of patients with high expression of KI-67 and LEF-1 is worse.*

Key words: *KI-67, LEF-1, breast cancer, diagnostic value*

Introduction

Breast cancer is the main type of cancer in women, accounting for 23% of invasive cancers [1,2], and it is a complex disease. According to statistics, it is estimated that there are 1,384,155 new breast cancer cases in the world, and nearly 459,000 deaths, and it is estimated that by 2050 there will be about 3.2 million new breast cancer cases in females in the world each year [3,4]. Although the mortality rate of breast cancer has decreased in recent years, their disease is difficult to be well controlled [5,6]. Some of the patients with early breast cancer have also developed metastatic disease at the time of diagnosis [7,8]. The causes of breast cancer development are not completely clear, so early

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diagnosis and treatment is an important means to improve the survival of such patients [9,10].

KI-67 is a nuclear protein that can induce tumor proliferation and is expressed in each cycle of cell division. With high activity, KI-67 is regarded as a marker of tumor proliferation. It is highly expressed in malignant cells, and cannot be detected in normal cells, so it is often used to predict cancer progression [11-13]. At present, some studies have reported the effect of KI-67 on the prognosis of patients with advanced breast cancer [14], but there are few studies on the expression and prognosis of KI-67 after early breast cancer resection.

LEF-1 is a lymphatic enhancement factor, belonging to the T-cell factor transcription family, and is also an important component of the Wnt/β-catenin signaling pathway [15]. Studies have shown that LEF-1 is abnormally expressed in many human malignant tumors, with high sensitivity and specificity, and is closely related to the occurrence and development of tumors [16]. One study by Mohindra et al [17] has shown that LEF-1 is a highly sensitive and specific marker of papillary thyroid carcinoma. However, there are few studies on the expression of LEF-1 in breast cancer.

Therefore, we aimed to study the expression of KI-67 and LEF-1 in breast cancer, and explore their relationship with clinicopathological features and their influence on prognosis, so as to provide references for clinic practice.

Methods

Collection of specimens

A total of 89 patients with breast cancer (average age 51.7±6.4 years) admitted to the first affiliated hospital of Shantou University Medical College from January 2010 to February 2013 were enrolled into a study group, and 76 healthy individuals (average age 52.3±6.7 years) were enrolled into a control group during the same period.

Inclusion of exclusion criteria

Inclusion criteria of the patients: Patients diagnosed with breast cancer by histopathology [16], patients with complete clinical data, patients willing to cooperate for the treatment and follow-up, patients without chemotherapy contraindications, patients with expected survival more than 3 months, and those with normal organ function. This study was approved by the ethics committee of the first affiliated Hospital of Shantou University Medical College, and all participants and their families were informed of the study and they signed informed consent forms.

Exclusion criteria of the patients: Pregnant or lactating women, patients suffering from congenital immunodeficiency, severe infection, inflammatory disease, or severe hematopoietic damage, patients with a history of other malignant tumors or cardiovascular and cerebrovascular diseases, and those with poor treatment compliance.

Treatment methods

Treatment plan: Breast cancer resection was performed for patients according to their condition, and radiotherapy and chemotherapy were carried out according to their condition after operation to prevent recurrence and metastasis.

Main instruments and reagents

PCR instrument (7500, ABI Company USA), UV spectrophotometer (6135000041, Eppendorf Company, Germany), total RNA extraction kit EasyPure miRNA Kit (ER601-01, TransGen Biotech Co., Beijing, China), reverse transcription + PCR kit TransScript miRNA First-Strand cDNA Synthesis SuperMix (AT351-01, TransGen Biotech Co., Beijing, China), TransScript Green Two-Step qRT-PCR SuperMix (AQ201-01, TransGen Biotech Co., Beijing, China). All primers were designed and synthesized by Shanghai Biotechnology Co., Ltd (Table 1).

PCR detection method

The tissue (3 mm) stored at -80°C was taken out and grounded in liquid nitrogen. The tissue suspension was extracted strictly according to the instruction of total RNA kit, and the concentration and purity of the extracted RNA were determined by ultraviolet spectrophotometer and protein electrophoresis. TransScript® miRNA RT Enzyme Mix and 2×TS miRNA Reaction Mix were used to reversely transcript the total RNA strictly in accordance with the manufacturer's instructions. Subsequently, PCR amplification experiments were carried out in 20 μL total volume consisting 1 μL cDNA, 0.4 μL upstream and downstream primers, 10 μL 2×TransTaq® Tip Green qPCR SuperMix, 0.4 μL Passive Reference Dye (50×), and ddH2O added to adjust the volume. The conditions of PCR reaction were as follows: Pre-denaturation at 94°C for 30s, followed by 40 cycles of denaturation at 94°C for 5s, and annealing at 60°C for 30s. Three rep-

Table 1. Primer sequences

	Upstream primer	*Downstream primer*
KI-67	5'-CTTTGGGTGCGACTTGACG-3'	5'-GTCGACCCCGCTCCTTTT-3'
LEF-1	5'-AGAACACCCCGATGACGGA -3'	5'-GAGGGTCCCTTGTTGTAGAGG -3'
β-Actin	5'-GGCATCGTGATGGACTCCG-3'	5'-GCTGGAAGGTGGACAGCGA-3'
GAPDH	5'-ATGGTGAAGGTCGGAGTGAA-3'	5'-TGGGTGGAATCATACTGGAAC-3'

licate wells were set for each sample, and the experiment was repeated three times. KI-67 was detected with β-Actin as an internal reference, LEF-1 was detected with GAPDH as an internal reference, and $2^{-\Delta ct}$ was used to analyze the obtained data.

Follow-up

A total of 89 patients and their families were followed up by telephone and visits. The patients were followed up every 3 months during the first 1-2 years after surgery and every 6 months from the 2nd to 5th year after surgery, 5 years in total. The follow-up deadline was February 2018. The overall survival was recorded as the time from the first day after surgery to the last follow-up time or death.

Observation indicators

Main observation indicators: The expression of KI-67 and LEF-1 in patients after breast cancer resection, the diagnostic value of KI-67 and LEF-1 in breast cancer patients and the diagnostic value of KI-67 and LEF-1 in recurrence of breast cancer patients within 5 years after operation.

Secondary observations indicators: The relationship between KI-67, LEF-1 and the clinicopathological characteristics of breast cancer patients, and the expression of KI-67 and LEF-1 in patients with recurrent breast cancer within 5 years after operation.

Statistics

In this study, the collected data were statistically analyzed using SPSS20.0 (Chicago SPSS Co., Ltsd) and visualized into required figures using GraphPad Prism 7 (San Diego Grapad Software Co., Ltd.). Counting data were expressed as percents and analyzed using the Chi-square (x^2) test. Measurement data were expressed as the mean±standard deviation (mean±SD), which were all in a normal distribution. Comparison between two groups was carried out using the independent sample t-test, and the log rank test was adopted for analysis, while ROC was adopted for evaluating the ability of KI-67 and LEF-1 in diagnosing breast cancer. P<0.05 indicated a significant difference.

Table 2. General clinical data of the study group and the control group

Group	*Study group (n=89)*	*Control group (n=76)*	*t/x^2 value*	*p value*
Age, years	51.7±6.4	52.3±6.7	0.874	0.558
BMI (kg/m^2)	23.15±1.82	23.04±1.97	0.373	0.710
Menstrual situation, n (%)			0.028	0.867
Menopause, n (%)	34(38.20)	30(39.47)		
Not menopause	55(61.80)	46(60.53)		
Tumor size (cm), n (%)			0.598	0.440
≤2	57 (64.04)	53 (69.74)		
>2	32 (35.96)	23 (30.26)		
Lymph node metastasis, n (%)			0.261	0.610
Yes	48 (73.03)	44 (57.89)		
No	41 (26.97)	32 (42.11)		
ER, n (%)			2.881	0.090
Positive	66 (74.16)	47 (61.84)		
Negative	23 (25.84)	29 (38.16)		
PR, n (%)			2.227	0.136
Positive	70 (78.65)	52 (68.42)		
Negative	19 (21.35)	24 (31.58)		
HER-2, n (%)			2.829	0.093
Positive	68 (76.40)	49 (64.47)		
Negative	21 (23.60)	27 (35.53)		
Allergic reaction, n (%)			0.310	0.757
Yes	39 (43.82)	40 (52.63)		
No	50 (56.18)	36 (47.37)		

ER: estrogen receptor, PR: progesterone receptor

Table 3. Expressions of KI-67 and LEF-1 in breast cancer resection

Group	*Study group (n=89)*	*Control group (n=76)*	*T value*	*p value*
KI-67	8.673±1.435	7.236±1.357	6.574	<0.001
LEF-1	12.367±2.276	8.275±2.214	11.660	<0.001

Results

General clinical data of the study and control groups

There was no significant difference between the study group and the control group in general clinical baseline data including age, body mass index (BMI), menstruation, tumor size, bone metastasis, lymph node metastasis, ER, PR, and HER-2 (all p>0.05) (Table 2).

Expression of KI-67 and LEF-1 in patients after breast cancer resection

The expression of KI-67 and LEF-1 in the serum of participants in the two groups was detected by double antibody sandwich enzyme-linked immunosorbent assay (ELISA) and it was found that the expression of KI-67 in the study group was 8.673 ± 1.435, significantly higher than in the control group (7.236 ± 1.357) (p<0.05), and the expression of LEF-1 in the study group was 12.367±2.276, also significantly higher than in the control group (8.275±2.214) (p<0 05) (Table 3).

Correlation of KI-67 and LEF-1 with clinicopathological features of breast cancer patients

We found that patients with different tumor size, lymph node metastasis and ER showed significantly different expression of KI-67 (all p<0.05), and patients with different tumor size, lymph node metastasis, and HER-2 showed significantly different expression of LEF-1 (p<0.05) (Table 4).

ROC curve analysis of the diagnostic value of KI-67 and LEF-1 in breast cancer patients

The ROC curves of KI-67 and LEF-1 in the diagnostic value for breast cancer were drawn according to the expression of the two indexes. The area under the curve (AUC) of KI-67 in the diagnostic value for breast cancer was 0.860, and the 95% CI was 0.805-0.916; the AUC of LEF-1 in the diagnostic value for breast cancer was 0.858, and the 95% CI was 0.803-0.913; and the AUC of KI-67 combined with LEF-1 in the diagnostic value for breast cancer was 0.924, while the 95% CI was 0.883-0.965 (Table 5 and Figure 1).

Table 4. Relationship between KI-67 and LEF-1 and clinicopathological features of breast cancer patients

Clinicopathological features	*n*	*KI-67(n=89)*	*T value*	*P value*	*LEF-1(n=89)*	*T value*	*P value*
Age, years			1.188	0.238		0.768	0.445
≤50	48	8.215±1.264			11.823±1.879		
>50	41	8.536±1.278			12.135±1.946		
Tumor size, cm			2.181	0.031		3.312	0.001
≤2	31	8.224±1.218			11.436±1.812		
>2	58	8.863±1.366			12.942±2.156		
Lymph node metastasis			2.630	<0.010		3.384	0.001
Yes	57	8.923±1.352			11.323±1.926		
No	32	8.174±1.167			12.821±2.137		
History of smoking			1.172	0.245		0.911	0.365
No	42	8.316±1.231			11.952±2.068		
Yes	47	8.627±1.267			12.357±2.115		
History of alcohol abuse			0.991	0.325		0.534	0.594
No	50	8.567±1.235			11.743±1.836		
Yes	39	8.832±1.274			11.958±1.942		
Menstrual situation			0.240	0.811		0.277	0.782
Menopause	49	8.612±1.283			11.963±1.972		
Not menopause	40	8.547±1.261			11.847±1.957		
ER			2.493	0.015		0.377	0.707
Positive	53	8.216±1.154			11.911±2.031		
Negative	36	8.862±1.265			12.079±2.105		
PR			0.798	0.427		0.251	0.803
Positive	58	8.517±1.224			12.069±2.063		
Negative	31	8.735±1.236			12.185±2.116		
HER-2			0.168	0.867		4.204	<0.001
Positive	60	8.926±1.365			12.975±2.137		
Negative	29	8.875±1.289			10.965±2.065		

The expression of KI-67 and LEF-1 in patients with recurrence within 5 years and their diagnostic value in the 5-year recurrence of breast cancer patients

We determined the expression of KI-67 and LEF-1 in patients with recurrent breast cancer and those without recurrence, and found that the expression of KI-67 in patients with recurrent breast cancer was higher than in patients without recurrence (8.973±1.214 vs. 8.207±1.126, p<0.05), and the expression of LEF-1 in patients with recurrent breast cancer was also higher than in patients without recurrence (12.998±1.836 vs. 12.013±1.434, p<0.05). According to the expression of KI-67 and LEF-1 in patients with and without recurrence within 5 years after treatment, ROC curve was drawn to analyze the diagnostic value of the two indexes in the recurrence of patients after breast cancer resection, and it was found that the AUC of KI-67 was 0.699 and 95% CI was 0.566-0.832; the AUC of LEF-1 was 0.651, and the 95% CI was 0.506-0.795; the AUC of KI-67 combined with LEF-1 was 0.758, and the 95% CI was 0.620-0.897 (Table 6 and Figures 2 and 3).

Discussion

Globally, breast cancer is the most commonly cancer among women, posing a serious threat to women's physical and mental health. Breast cancer is a highly heterogeneous disease [18,19], with high incidence, and its high recurrence rate seriously compromises the quality of life of patients. Although the emergence of various endocrine therapy drugs, chemotherapy drugs, targeted therapeutic drugs, and immunotherapy drugs has improved the prognosis of patients with various molecular breast cancers to some extent, the better improvement of the prognosis of breast cancer patients still depends on the study of prognostic factors [20-22]. Therefore, the treatment and prevention via prognostic targets can effectively improve the therapeutic effect in breast cancer.

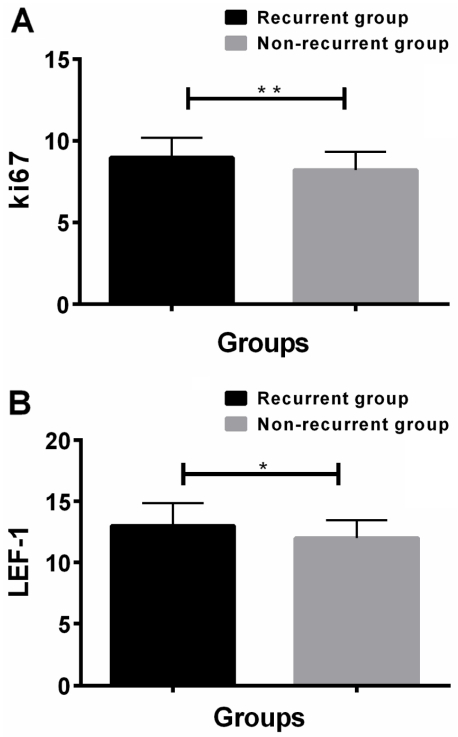

Figure 2. Expression of KI-67 and LEF-1 in patients with recurrence and those without recurrence. **A:** Expression of KI-67 in patients with recurrence was higher than in patients without recurrence (p=0.009, t=2.675). **B:** The expression of LEF-1 in patients with recurrence was higher than in patients without recurrence (p=0.012, t=2.569). *p<0.05, **p<0.01.

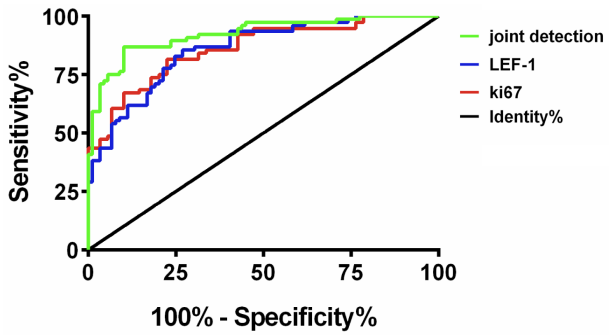

Figure 1. ROC curve analysis of the diagnostic value of KI-67 and LEF-1 in breast cancer patients. When the sensitivity and specificity of KI-67 were 81.58% and 76.40%, the optimal cut-off point was 8.113. When the sensitivity and specificity of LEF-1 were 85.53% and 71.91%, the optimal cut-off point was 10.760. When the sensitivity and specificity of the joint detection curves were 85.53% and 89.89%, the optimal cut-off point was 0.516.

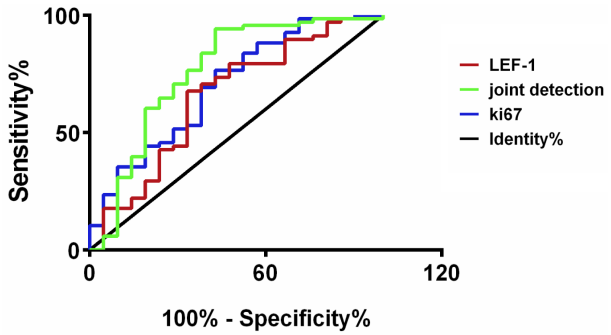

Figure 3. The diagnostic value of KI-67 and LEF-1 in the recurrence of breast cancer patients within 5 years after operation. When the sensitivity and specificity of KI-67 were 76.47% and 52.38%, the optimal cut-off point was 9.094. When the sensitivity and specificity of LEF-1 were 67.65% and 61.90%, the optimal cut-off point was 12.680. When the sensitivity and specificity of the joint detection curves were 92.65% and 57.14%, the optimal cut-off point was 0.616.

Table 5. Diagnostic value of KI-67 and LEF-1 in breast cancer patients

Indicators	*AUC*	*95%CI*	*Specificity %*	*Sensitivity %*	*Yoden index*	*Cut-off*
KI-67	0.860	0.805-0.916	76.40	81.58	57.98	<8.113
LEF-1	0.858	0.803-0.913	71.91	85.53	57.44	<10.760
joint detection	0.924	0.883-0.965	89.89	85.53	75.42	>0.516

AUC: area under the curve, Cut-off: cut off point

Table 6. Diagnostic value of KI-67 and LEF-1 in patients with breast cancer after 5 years of recurrence

Indicators	*AUC*	*95%CI*	*Specificity %*	*Sensitivity %*	*Yoden index*	*Cut-off*
KI-67	0.699	0.566-0.832	52.38	76.47	28.85	<9.094
LEF-1	0.651	0.506-0.795	61.90	67.65	29.55	<12.680
Joint detection	0.758	0.620-0.897	57.14	92.65	36.70	>0.616

AUC: area under the curve, Cut-off: cut-off point

In recent years, research on KI-67 in the process of tumor genesis has shown that KI-67 is a marker of tumor proliferation. In this study, the expression of KI-67 in breast cancer patients was significantly higher than in healthy individuals. Sun et al [23] have studied the expression of KI-67 in breast cancer patients and the clinicopathological characteristics and prognosis of the patients, finding that KI-67 is highly expressed in breast cancer patients, which is similar to our research results. However, different from our study, Sun et al have used immunohistochemical method to detect KI-67 in breast cancer and normal breast tissue, which requires extraction of cells and tissues by surgery before detection. We adopted the RT-PCR method that only requires blood sampling, which was simpler than the immunohistochemical method adopted by Sun et al and was also convenient to observe the preoperative and postoperative comparisons of patients. The immunohistochemical method cannot be used for preoperative and postoperative comparisons. Therefore, we believe that our research method has certain advantages compared with that adopted by Sun et al. In the study of Bucan et al [24], LEF-1 was also highly expressed in breast cancer patients, which is similar to our results. We speculated that LEF-1, as a lymphokine, may play a regulatory role in tumor growth. By observing the expression of KI-67 and LEF-1 in breast cancer patients, we found that the expression of KI-67 and LEF-1 in patients with different tumor size and lymph node metastasis was significantly different. Because KI-67 is a nuclear protein that could induce tumor proliferation, while LEF-1 is a lymphokine, we inferred that when breast cancer patients had tumor enlargement or increase of the number of lymphocytes, their KI-67 and LEF-1 factors would show specificity. ROC analysis on the diagnostic value of KI-67 and LEF-1 in breast cancer patients revealed that the optimal specificity and sensitivity of KI-67 were 76.40% and 81.58%, respectively, when the AUC of KI-67 was 0.860 and the cut-off point was less than 8.113, while the optimal specificity and sensitivity of LEF-1 were 71.91% and 85.53%, respectively, when the AUC of LEF-1 was 0.858 and cut-off point was less than 10.760. These results suggest that KI-67 and LEF-1 may be used as diagnostic indicators for breast cancer.

We followed up the patients for 5 years and found that the expression of KI-67 and LEF-1 in patients with recurrent breast cancer was higher than those without recurrence. The ROC curve was drawn to analyze the diagnostic value of KI-67 and LEF-1 in 5-year recurrence of breast cancer patients. What was found was that when the AUC of KI-67 curve was 0.699 and the cut-off point was less than 9.094, the optimal specificity and sensitivity can be obtained to be 52.38% and 76.47%, respectively, and when the AUC of LEF-1 curve was 0.651 and the cut-off point was less than 12.680, the optimal specificity and sensitivity can be obtained to be 61.90% and 67.65%, respectively. It was further inferred that KI-67 and LEF-1 could be used as diagnostic indicators for breast cancer.

However, this study still has some shortcomings. First, although we have found that the expression of KI-67 and LEF-1 is related to clinicopathological features, we have not deeply explored it, and the specific regulatory mechanism is still unclear, which needs to be further studied in the follow-up experiments. Secondly, we have not studied the interaction between KI-67 and LEF-1, and the mechanism is still unclear, which also needs further confirmation by subsequent experiments.

Finally, we have not studied the expression of KI-67 and LEF-1 in other tumors. Therefore, we need to do further research on KI-67 and LEF-1 in subsequent experiments.

In summary, the expression of KI-67 and LEF-1 in breast cancer patients is higher than in healthy individuals, and their expression is related to tumor size and lymph node metastasis. In addition, higher expression of KI-67 and LEF-1 indicates worse survival rate of patients.

Conflict of interests

The authors declare no conflict of interests.

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