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Correlation between expression of LRP16, Ki67 and EGFR and breast cancer clinical pathologic factors and prognosis

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Abstract. – OBJECTIVE: To investigate the expression of leukemia-related protein 16 (LRP16), proliferating cell nuclear antigen K-67 (Ki67) and epidermal growth factor receptor-1 (EGFR-1) in breast cancer tissue and to explore the correlation between the expression of those proteins and breast cancer clinical pathologic factors and prognosis.

PATIENTS AND METHODS: The expressions of LRP16, Ki67 and EGFR in breast cancer tissues of 86 cases were detected by immunohistochemical method and the correlations between the expression of LRP16, Ki67 and EGFR and clinical pathologic factors and prognosis were investigated.

RESULTS: Positive expression rates of LRP16, Ki67 and EGFR in breast cancer tissue were 52.3%, 70.9% and 16.3%, respectively. There was no statistical difference in the expression of RP16, Ki67 and EGFR between different age groups (p>0.05). The expression of LRP16 was correlated with clinical stage, histological grade, tumor size and lymphatic metastasis ($p<0.05$); the expression of Ki67 was correlated with clinical stage, histological grade, tumor size and lymphatic metastasis ($p<0.05$); the expression of EGFR was correlated with histological grade ($p<0.05$). Comparison of postoperative local recurrence and metastasis time between LRP 16 positive group and negative group showed statistically significant difference ($p<0.05$); comparison of postoperative local recurrence and metastasis time between Ki67 positive group and negative group also showed statistically significant difference ($p<0.05$); comparison of postoperative local recurrence and metastasis time between EGFR positive group and negative group showed no statistically significant difference ($p<0.05$).

CONCLUSIONS: Detection of expression levels of LPR16, Ki67 and EGFR in breast cancer tissue improves the understanding of biological behaviors of breast cancer, which in turn provide clinical guidance in diagnosis, treatment and prognosis assessment.

Key Words:
LRP 16, Ki67, EGFR, Breast cancer, Clinical pathologic factors, Prognosis.

Introduction

Breast cancer is one of the most common malignant tumors in women and ranks first among all the female malignant tumors in China. The incidence rate of breast cancer is increasing gradually and incidence age gradually gets lower[1]. LRP16 was a novel leukemia related gene identified in peripheral blood lymphocytes of a healthy adult by Yu et al[2] and Han et al[3] in 2000. Plenty of studies showed that LRP16 could be involved in proliferation, metastasis and invasion of breast cancer with the stimulation of estrogen. As a proliferating cell nuclear antigen, Ki67 is mainly used to judge cell proliferative activity, and it's also considered to be the most reliable indicator of proliferation activity of tumor cells. Most scholars believe that patients with Ki-67 positive expression show poor prognosis[4,5]. EGFR is a membrane receptor with tyrosine kinase activity and plays a pivotal role in promoting eukaryotic cell wound recovery, including mitosis of stimulating cells, cell migration and differentiation, cell apoptosis and angiogenesis[6]. According to relevant studies, overexpression of EGFR is usually correlated with poor prognosis, formation of tumor vessel and tumor metastasis in several kinds of solid tumor tissues[7]. In recent years, EGFR was treated as a target of oncotherapy. Therefore, this paper aimed to detect the expression of LRP16, Ki67 and EGFR in breast cancer tissue and investigate the correlation between expression of LRP16, Ki67 and EGFR and clinical pathological factors and

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prognosis, so as to provide guidance in clinical diagnosis, treatment and prognosis evaluation of breast cancer.

Patients and Methods

Sample Sources

Breast cancer tissue was collected from 86 patients who were diagnosed with breast cancer by histopathological examination were collected after tissue excision. The age of patients ranged from 30 to 82 with an average age of 49 and a median age of 50. TNM classification: 31 cases at Stage I, 46 cases at Stage II and 8 cases at Stage III. Histological grade: 11 cases at Stage I, 47 cases at Stage II and 28 cases at Stage III. All patients had complete clinical and pathological data and did not receive radiotherapy and chemical treatment before surgery. The study was approved by the Ethics Committee of The Second Affiliated Hospital of Mudanjiang Medical University (Mudanjiang, Heilongjiang Province, China). All the patients signed the informed consent.

Reagents and Instrument

Immunohistochemical kit, LRP16 rabbit anti-human polyclonal antibody, Ki67 mouse anti-human monoclonal antibody and EGFR mouse anti-human monoclonal antibody were purchased from Maixin Biotech Co., Ltd. (Fujian Province, China); microtome: Zhongwei Electronic Instruments Plant RM2015 (Changzhou, Jiangsu Province, China); dryer: China 101-0AB; tissue flotation workstation: Nuopu Technology NP-P; optical microscope: OlympusBX4 (Tokyo, Japan); microscopy digital camera: Olympus BX40F4 (Tokyo, Japan).

Experimental Methods

Samples were fixed in 4% formaldehyde solution and subjected to a series of routine operations, including paraffin embedding, tissue section, flotation, fishing and drying. The samples for immunohistochemical staining, were prepared according to the instructions of kit. Tissue sections with positive expression were used as positive control. In negative control, PBS solution instead of primary antibodies was used.

Determination of the Results

Results were determined according to the references[8-10]. Tiny pale yellow, brown or dark brown particles indicated the positive signal. In immunohistochemistry, the positive signal of LRP16 and Ki67 expression was in karyon positive while EGFR was only expressed in cytoplasm or cell membrane.

Statistical Analysis

The data were processed using SPSS13.0 statistical software (SPSS Inc., Chicago, IL, USA). X^2 and *t*-test were used to analyze data based on the experiment purpose. $p<0.05$ was considered to be statistically significant.

Results

Positive Expression Rate of LRP 16, Ki67 and EGFR in Breast Cancer Tissue

Among 86 breast cancer patients, positive signal of LRP16 expression was observed in 45 patients and the positive expression rate was 52.3%; positive signal of Ki67 expression was observed in 45 patients and the positive expression rate was 70.9%; positive signal of EGFR expression was observed in 45 patients and the positive expression rate was 16.3% (Table I).

Correlation Between Expression of LRP16, Ki67 and EGFR and Clinical Pathological Factors of Breast Cancer

As shown in Table II, no statistical differences were found in the expression levels of all the indicators between different age groups ($p>0.05$). Expression of LRP16 was correlated with clinical stage, histological grade, tumor size and lympha-

Table I. Positive expression rates of LRP16, Ki67 and EGFR in breast cancer tissue.

Indicator	Total Number	Number of patients with negative staining	Number of patients with positive staining	Positive expression rate (%)
LRP16	86	41	45	52.3
Ki67	86	25	61	70.9
EGFR	86	72	14	16.3

Table II. Correlation between expression of LRP16, Ki67 and EGFR and clinical pathologic factors in breast cancer.

Clinical pathological factors	No. Total number	Number of patients with positive LRP16	*p* χ^2	Number of patients with positive Ki67	*p* χ^2	Number of patients with positive EGFR	*p* χ^2
Age	86						
≥50	49	24 (49.9%)	>0.05	21(42.9%)	>0.05	8 (16.3%)	>0.05
<50	37	19 (51.4%)	1.572	20 (54.1%)	3.102	5 (13.6%)	1.03
Clinical stage							
Stage I	34	12 (29.4%)	<0.05	15 (44.1%)	<0.05	2 (5.9%)	>0.05
Stage II	41	23 (56.1%)	7.347	35 (85.4%)	18.869	4 (9.8%)	2.326
Stage III	11	7 (63.6%)		10 (91.0%)		3 (27.3%)	
Histological grade							
Stage I	25	17 (68.0%)	<0.05	12 (48.0%)	<0.05	1 (4.0%)	<0.05
Stage II	49	34 (69.4%)	14.623	36 (73.5%)	10.407	2 (4.1%)	6.483
Stage III	12	11 (91.7%)		12 (100%)		1 (8.3%)	
Tumor size (d/cm)							
<3	52	15 (28.8%)	<0.05	29 (55.8%)	<0.05	4 (7.70%)	>0.05
>3	34	29 (85.3%)	17.832	30 (88.2%)	5.107	4 (11.8%)	0.331
Lymphatic metastasis							
No	38	14 (36.8%)	<0.05	20 (52.6%)	<0.05	4 (10.5%)	>0.05
Yes	48	32 (66.7%)	6.019	41(85.4%)	7.185	5 (10.4%)	0.293

tic metastasis (χ^2=7.347, 14.623, 17.832, 6.019, p<0.05). Results showed that positive expression rate of LRP16 was significantly higher in patients with advanced stage, poorly differentiated group, large tumor size group and lymphatic metastasis group than in clinically early stage, highly differentiated group, small tumor size group and no lymphatic metastasis group. Expression of Ki67 was correlated with clinical stage, histological grade, tumor size and lymphatic metastasis (χ^2=18.869, 10.407, 5.107, 7.185, p<0.05). Positive expression rate of Ki67 was significantly higher in clinically advanced stage, poorly differentiated group, large tumor size group and lymphatic metastasis group than in the clinically early stage, highly differentiated group, small tumor size group and no lymphatic metastasis group. Expression of EGFR was correlated with histological grade (χ^2=6.483, p<0.05) but not significantly correlated with clinical stage, tumor size and lymphatic metastasis (χ^2=2.326, 0.331, 0.293, p>0.05). Each index had no statistical difference in age, p>0.05; expression of LRP16 and Ki67 in the clinical factors was statistically significant, p<0.05.

Correlation Between Expression Levels of LRP16, Ki67 and EGFR in Breast Cancer and the Prognosis

Among 86 patients, local recurrence and metastasis occurred in 23 patients after surgery and 9 patients died. Overall survival rate was 89.5%; postoperative disease-free survival rate was 73.3%. Postoperative local reoccurrence and metastasis time showed no significant correlation with the expression of EGFR (t=1.117, p>0.05) but showed significant correlation with the expression of LRP16 and Ki67 (t=2.472, 2.158, p<0.05). Correlations of the expression levels of LRP16, Ki67 and EGFR with relapse and metastasis time were statistical significant (p<0.05).

Discussion

With increasing incidence rate of breast cancer, treatment and prognosis of breast cancer have become a research hot topic all over the world over years; evaluation of comprehensive clinical treatment of breast cancer and various chemotherapy regimens as well as survival and prognosis factors of breast cancer have attracted extensive attention in the field of tumor study. Presently, the commonly use treatments of breast cancer are still surgery and standardized chemotherapy, radiotherapy and endocrine therapy after surgery. Those comprehensive treatments bring long-term impacts on disease free survival of patients[11-13]. With the standardized treatment of breast cancer, the cure rate and long-term survival rate of breast cancer patients in China have been significantly improved during last 20 years. The proper comprehensive treatment mode, effective individualized therapy and increase in long-term survival rate are the main aims for clinical study. In terms

Table III. Correlation between expression of LRP16, Ki67, EGFR and relapse and metastasis time.

Indicator	Negative group (x±s)	Positive group (x±s)	*t*	*p*
LRP16	72.82±12.543	62.4±20.976	2.472	0.023
Ki67	72.97±11.176	65.8±18.819	2.158	0.035
EGFR	70.15±15.362	57.2±28.428	1.117	0.347

of prognostic prediction, the evaluation model of prognosis is just developed in recent ten years. A recognized prognosis evaluation model is to make a qualitative evaluation according to the relapse and metastasis risk evaluation criteria[14]. With the development of molecular biotechnology, more and more studies showed that prognosis of breast cancer was closely related to several molecular markers. Therefore, a better understanding of biological behavior of breast cancer at the molecular level is important for selecting clinical treatment evaluating prognosis and optimizing individual treatment[14]. The function of LRP16 has been well studied. Lu et al[15] found that LRP16 gene promoter sequence was a typical Type II RNA polymerase promoter with multiple steroid hormone receptors binding sites, and LRP16 might be involved in biological function of steroid hormone. To further study the role of LRP16 gene in development of breast tumor, Ma et al[16] and Han et al[17] studied the effect of LRP16 on MCF-7 cells proliferation. This study found that over expression of LRP16 in MCF-7 cells significantly promoted the proliferation of MCF-7 cells; in contrast, LRP16 expression inhibition in MCF-7 cells by siRNA interference significantly inhibited cell proliferation, indicating that LRP16 can promote the proliferation, invasion and metastasis of breast cancer cells[16,17]. Ki-67 is a proliferating cell nuclear antigen and an important proliferation indicator in guiding clinical chemotherapy of breast cancer[18]. EGFR is one of the four members of epidermal growth factor (EGF) gene family. Endogenous ligands of EGFR include epidermal growth factor, transforming growth factor, amphiregulin, heparin-binding epidermal growth factor, etc. Epidermal growth factor and transforming growth factor are the most important excitatory ligands. EGFR monomer is the inactive state of EGFR, while EGFR dimer can bind to ligand to enter into cells to serve as a receptor or a ligand complex. By inhibiting tyrosine kinase, catalytic activity of the receptor and downstream signal transduction can be blocked so that cell proliferation and differentiation will be controlled[19].

Conclusions

At present, more and more biological markers are applied in clinic. This paper explored the correlations between expressions of LRP16, Ki67 and EGFR in 86 breast cancer tissues and clinical pathological factors and prognosis, which provided guidance for clinical practices.

Conflict of interest

The authors declare no conflicts of interest.

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