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Correlation analysis of hormone receptors and the expressions of HER-2 and Ki-67 in breast cancer

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Summary

Objective: This study aims to investigate the correlation and clinical significance of hormone receptors and the expressions of HER-2 and Ki-67 in breast cancer primary lesions and lymph node metastatic tissues. *Methods:* 83 cases were studied, who were performed breast cancer surgeries and confirmed the ipsilateral axillary lymph node metastasis by the postoperative pathological diagnosis. Immunohistochemical method was used to simultaneously detect the expressions of ER, PR, HER-2 and Ki-67 in the primary lesions and lymph node metastases. *Results:* ER exhibited the expression concordance rate as 85.5% in primary lesions and metastases, with significant difference ($P = 0.039$); the expression concordance rates of PR and HER-2 in primary lesions and metastases were 90.4% and 89.2%, respectively, without significant difference ($P = 0.289$, 0.180); between the Ki-67-highly-expressed primary lesions and Ki-67-lowly-expressed metastases, the expressions of ER in primary lesions and metastases exhibited statistical significance, with P as 0.031. *Conclusions:* The primary lesions and lymph node metastases had higher consistency, while there was still about 10% patients showed differentiated expression. The simultaneous detection of breast cancer primary lesions and lymph node metastases was still very necessary.

Key words: breast cancer; ER; PR; HER-2; Ki-67; immunohistochemistry; correlation analysis.

Introduction

Breast cancer is the most common malignancy in female, and the molecular-level understanding of the biological behavior of breast cancer would contribute to the clinical diagnosis and treatment. The occurrence and development of tumor is a complex, multifactorial and multi-step process, with tumor heterogeneity as one of the most important factors that affect cancer treatment and prognosis. The expressions of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor (HER-2) in breast cancer have important guiding significance towards the molecular typing of breast tumors, the prognosis judgment and treatment options. The high expression of ER and PR in breast cancer indicate a higher degree of histological differentiation, and the endocrine therapy would have good results; HER-2 plays an essential part in regulating cell proliferation, adhesion, motility, and survival [1, 2], Trastuzumab, an anti-HER-2 monoclonal antibody, is one such therapy that has had a dramatic impact on the treatment of HER-2-positive breast cancer [3, 4]. The previous adjuvant therapy of clinical treatment of breast cancer were mainly judged on the basis of disease stage, expression status of primary lesions' hormone receptors and HER-2, while normally ignored the expressions of the above 3 factors in the metastases, and whether the changes would affect the patient's treatment and prognosis was also ignored. Many studies about the differentiated expressions of ER, PR and HER-2 in breast cancer primary lesions and metastases were not the same. Some scholars believed that the ER [4-8] and PR [6-9] of the primary lesion and metastases in the same breast cancer patient were inconsistent, while some other literature reported the inconsistency of HER-2 in primary lesion and metastases, and this result was not uniform [10-13, 14], proposing the necessity of biological characteristics re-evaluation towards the recurrent lesion through further biopsy of the breast cancer metastases. In recent years, some scholars also advocated that ER, PR and HER-2 immunohistochemistry should be performed not only to all breast cancer primary lesions, but also to the metastases, after that the treatment programs could be selected which would take both the original lesions and metastases into account [15].

Ki-67 (Nuclear-associated antigen) is the nuclear antigen associated with cell proliferation, playing an important role in tumor progression, and its expression changes with the changes of cell cycle. Many studies have shown that the development, metastasis and prognosis of tumor are related to the expression levels of Ki-67, and Ki-67 has been noted as a very important indicator towards the molecular subtypes, prognosis and treatment prediction of breast cancer clinically [16, 17]. How is the expression of Ki-67 in breast cancer metastases and its relationship with the expressions of ER and PR still need further study.

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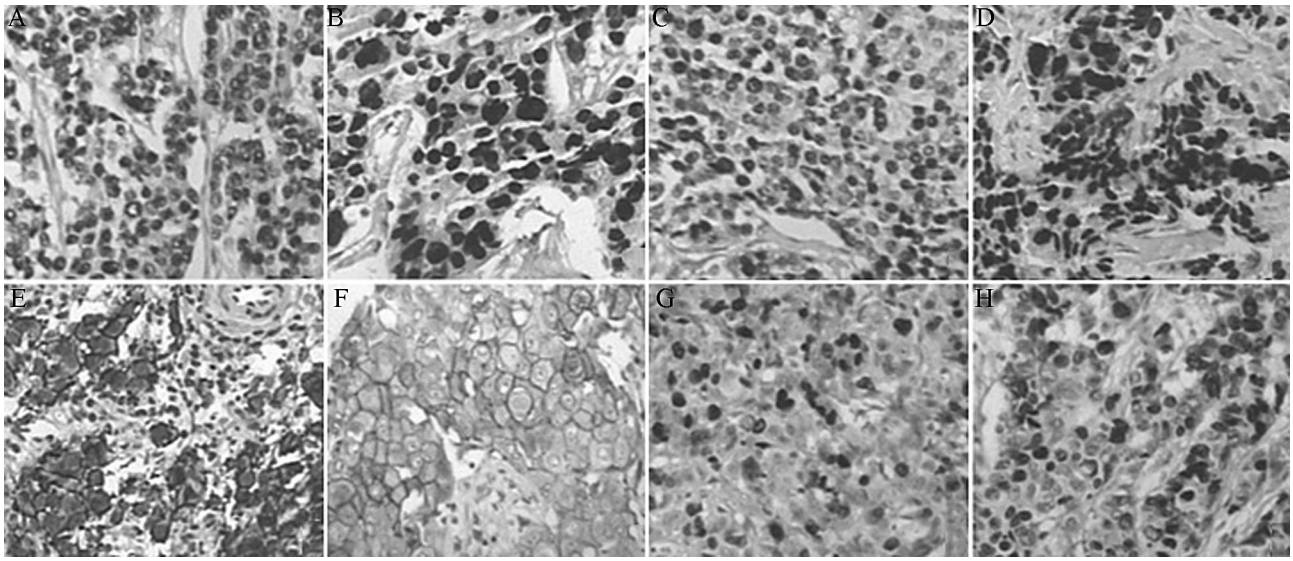

Figure 1. — Expression of ER, PR, HER-2 and Ki-67 (SP×200). A) primary lesions ER (3+); B) lymph node metastases ER (3+); C) primary lesions PR (3+); D) lymph node metastases PR (3+); E) primary lesions HER-2 (3+); F) lymph node metastases HER-2 (3+); G) primary lesions Ki-67 (+about 30%); H) lymph node metastases Ki-67 (+60~70%).

In this study, we detected the expressions of ER, PR, HER-2 and Ki-67 in 83 cases of primary lesions and metastatic tissues of breast cancer patients, to investigate their clinical significance in the treatment and prognosis of breast cancer.

Materials and Methods

Clinical data

236 cases of breast cancer were performed surgical treatment in the 2nd Clinical Section, Shandong Tumor Hospital, from Jan. 2012 to Sep. 2012, among who there were 83 cases were pathologically diagnosed as ipsilateral axillary lymph node metastasis, aging from 24 to 73 years, with a median age of 47 years old. According to the 2003 edition of TNM staging, 48 patients were in clinical stage II, 27 patients were in stage III and 8 patients were in stage IV. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Shandong Cancer Hospital. Written informed consent was obtained from all participants.

Experimental methods

The primary lesions of breast cancer and axillary lymph node specimens were cut postoperatively, fixed in 10% formalin, at the same time, the primary lesions and pathologically-confirmed axillary lymph node metastases were cut and performed 4μm serial sections. The above sections were dewaxed with xylene and dehydrated with graded ethanol on the same slide and under the same conditions and time. Then used 3% H_2O_2 to treat the sections and washed with PBS thoroughly, then performed the antigen hotfix with microwave oven, ER, PR and HER-2 were repaired with citrate buffer, and Ki-67 was repaired with EDTA buffer. After the hotfix, cooled the slides to the room temperature, added ER, PR, HER-2 and Ki-67 monoclonal antibodies (Fujian Maixin Biotechnology Development Co., Ltd., Fuzhou, China), 50μL to each slide and then placed them in the wet box, cultivated overnight in 4°C refrigerator. After restored to the room temperature, washed the slides with PBS and added biotinylated secondary antibody (Fujian Maixin Biotechnology Development Co., Ltd., Fuzhou, China), incubated at the room temperature and then rinsed with PBS thoroughly. The horseradish peroxidase-labeled streptavidin was then added for the incubation at the room temperature, then rinsed with PBS thoroughly, DAB staining (Fujian Maixin Biotechnology Development Co., Ltd., Fuzhou, China), hematoxylin restaining and neutral gum mounting. The known positive section was set as the positive control, the primary antibody was replaced by PBS for the negative control, then observed and photographed with a microscope.

Results judgment

5 high power fields were randomly selected in both primary lesions and metastases, counting more than 500 cells. The nuclear staining was judged as positive [18], and scored according to the number of positive cells: non-positive cell, 0 poiint; l% ~ 30% positive cells: l point; 3l% ~ 70% positive cells: 2 points; 7l% ~ l00% positive cells: 3 points; then according to positive staining intensity, light brown was recorded as l point, brown as 2 points, dark brown as 3 points. The total points of each slice was the accumulation of the above 2 scores, 0 point meant negative, l ~ 2 points was labeled as (+), 3 ~ 4 as (2+), 5 ~ 6 as (3 +), among which the above "(+)" results of ER, PR and Ki-67 were recorded as positive, while the "(3 +)" results of HER-2 were recorded as positive, and the suspicious HER-2-positive (2 +) patients should be performed FISH analysis, and the positive FISH results would result in HER-2 positive, the negative FISH result would result in HER-2 as negative (Figure 1). The expression of Ki-67 was based on the identified criteria in 2011 St.Gallen Breast International conference [19], Ki-67 <14% referred to the low expression, ≥ 14% referred to the high expression.

Statistical analysis

The experimental data were analyzed by SPSS17.0 statistical software, the counting data were expressed as the percentage, intergroup data were compared with X^2 test, and the test level was set as $\alpha = 0.05$.

Results

Expressions of ER, PR and HER-2

The positive and negative expression of ER in primary lesions were 55 cases and 28 cases, respectively, while 47 positive cases and 36 negative cases in metastases, the expression of ER was consistent in primary lesions and metastases, namely negative/negative or positive/positive were 26 cases and 45 cases, with a total of 71 cases, the concordance rate was 85.5%; the positive and negative expression of PR in primary lesions were 55 cases and 28 cases, respectively, while 49 positive cases and 26 negative cases in metastases, the expression of PR was consistent in primary lesions and metastases, namely negative/negative or positive/positive were 49 cases and 26 cases, with a total of 75 cases, the concordance rate was 90.4%; the positive and negative expression of HER-2 in primary lesions were 16 cases and 67 cases, respectively, while 11 positive cases and 72 negative cases in metastases, the expression of HER-2 was consistent in primary lesions and metastases, namely negative/negative or positive/positive were 65 cases and 9 cases, with a total of 74 cases, the concordance rate was 89.2%. Statistical analysis revealed that there was statistically significant difference in the ER expression between primary lesions and metastases, $P = 0.039$, while no significant differences in the PR and HER-2 expression between primary lesions and metastases, P values were 0.289 and 0.180, respectively (Table 1, 2).

Table 1. — *Expression status of ER, PR and HER-2 in breast primary lesions and lymph node metastatic tissues of the 83 cases*

Indicator		Primary lesion	Metastatic tissues	Concordance numbers
ER	Positive	55	47	45
	Negative	28	36	26
PR	Positive	55	51	49
	Negative	28	32	26
HER-2	Positive	16	11	9
	Negative	67	72	65

Correlation of Ki-67 expression and ER, PR and HER-2

Among the primary lesions, 40 cases showed low expression of Ki-67, while 43 cases showed high expression. In the low-expression cases, 2 cases were negative/positive ER expression, while the expressions of PR and HER-2 were exactly the same in primary lesions and metastases, namely all were negative/negative or positive/positive; 4 cases were negative/positive ER expression in primary lesions and metastases, 2 cases for PR and 4 cases for HER-2. In the primary-lesion-highly-expressed Ki-67 patients, the negative/positive PR and HER-2 expression patients were all 2 cases, and ER expression was exactly the same in primary lesions and metastases, all were negative/nega-

Table 2. — *Correlation analysis of ER, PR and HER-2 expression in primary lesions and lymph node metastatic tissues of the 83 breast cancer cases*

Indicator	Primary lesions	Metastatic tissues			*P*	R
		Positive	Negative	Summary		
ER	Positive	45	10	55	0.039	0.712
	Negative	2	26	28		
PR	Positive	49	6	55	0.289	0.796
	Negative	2	26	28		
Her-2	Positive	9	7	16	0.180	0.620
	Negative	2	65	67		

Table 3. — *Correlation analysis of Ki-67 expression in primary lesions and the changes of ER, PR and HER-2 between primary lesions and metastases*

Ki-67		Primary lesions	Metastases			*P*	R
			Positive	Negative	Summary		
ER	Low expression	Positive	28	4		0.687	0.577
		Negative	2	6	40		
	High expression	Positive	17	6		0.031	0.754
		Negative	0	20	43		
PR	Low expression	Positive	30	2		0.500	0.866
		Negative	0	8	40		
	High expression	Positive	19	4		0.687	0.725
		Negative	2	18	43		
HER-2	Low expression	Positive	2	2		0.500	0.688
		Negative	0	36	40		
	High expression	Positive	7	5		0.453	0.572
		Negative	2	29	43		

Table 4. — *Correlation analysis of Ki-67 expression in metastases and the changes of ER, PR and HER-2 between primary lesions and metastases*

Ki-67		Primary lesions	Metastases			*P*	R
			Positive	Negative	Summary		
ER	Low expression	Positive	27	6		0.031	0.715
		Negative	0	10	43		
	High expression	Positive	18	4		0.687	0.748
		Negative	2	16	40		
PR	Low expression	Positive	33	2		0.500	0.868
		Negative	0	8	43		
	High expression	Positive	15	4		1.000	0.649
		Negative	3	18	40		
HER-2	Low expression	Positive	5	1		1.000	0.731
		Negative	2	35	43		
	High expression	Positive	4	5		0.063	0.619
		Negative	0	31	40		

tive or positive/positive; 6 cases expressed ER as negative/positive or positive/negative in primary lesios and metastases, 6 cases showed PR, and 7 cases showed HER-2. The metastatic low expression of Ki-67 had 43 cases, while 40 cases with high Ki-67 expression. Among the low expression cases, 2 cases exhibited HER-2 expression as negative/positive, respectively, while ER and PR expression did not change. The positive/negative expression of ER, PR and HER-2 were 6, 2 and 1 case, respectively. In the metastatic high Ki-67expression cases, negative/positive expression of ER and PR were 2 and 3 cases, respectively, while HER-2 expression did not change. The positive/negative expression of ER, PR and HER-2 were 4, 4 and 5 cases, respectively. Statistical analysis revealed that there was statistically significant difference in the ER expression in primary lesions and metastases only in the high Ki-67 expression primary lesion cases and low Ki-67expression metastases cases, $P = 0.031$ (Table 3, 4).

Discussion

Breast cancer has the characteristic of heterogeneity, because of the abnormal expression of a series of breast cancer-related genes, the different combinations of gene expression could prompt the prognosis and predict therapeutic effects. The usage of immunohistochemical detection of ER, PR, HER-2 and Ki-67 could perform the molecular typing of breast cancer, which would facilitate the development of treatment strategies [20]. Endocrine therapy and targeted therapy play an important role in the combined treatment towards breast cancer. In normal circumstances, endocrine treatment would be effective towards the hormone receptor-positive patients, with low probability of recurrence and metastasis [21]. HER-2-positive tumors have highly malignant degree, and the disease normally would progress quickly. Trastuzumab-targeted therapy is considered as the standard therapy in HER-2 positive cases [22], patients who were performed the targeted therapy exhibited the significantly improved prognosis. Currently, lymph node metastasis is still an important indicator towards the prognosis prediction of breast cancer. The researches about the clinical significance of relevant immunohistochemical indicators in metastases, whether the changed results could change our conventional treatment plans and optimize treatment strategies, so that patients could get the best treatment, are the issues worthy of exploration in current breast cancer treatment.

Recent studies have found that the expressions of ER, PR and HER-2 had differences between breast primary lesions and lymph node metastases. In 2011, Deng [23] found that there was statistically significant difference in ER expression between primary lesions and recurrent metastases, the change rate was 66.67%; while there were no significant differences in PR and HER-2 expression between primary lesions and recurrent metastases, the change rate were 17.78% and 13.33%, respectively. Zhao [24] reported that there were no significant difference in ER, PR and HER-2 expression between primary lesions and lymph node metastases. This study found that there was significant difference in ER between primary lesions and metastases of breast cancer, while no significant difference in PR and HER-2. Monaco [25] found that the concordance rates of ER and PR expression in primary lesions and metastases were 81% and 65%, and in this study, the concordance rate were 85.5% for ER and 90.4% for PR, slightly higher than the above study. The differences in the results might involve many subjective and objective factors, such as patient selection, sample collection time, sampling sites and methods, laboratory reagents, methods and evaluation standards, *etc*. The specimens in this study were obtained from surgical resection, the immunohistochemistry of primary lesions and metastases were at the same time, on the same slide, under the same experimental conditions and judged by the same chief physician. Towards the patients with inconsistent expression between primary lesions and metastases, if the primary lesions exhibited negative ER and PR while positive in metastases, the endocrine therapy would be ad-

ministrated on the basis of conventional chemotherapy and radiotherapy, whether it could improve the efficacy of clinical practice still needed further verification.

The detection of HER-2 includes protein-level immunohistochemistry and gene-level FISH test, ASCO / CAP suggested that if the immunohistochemical results of HER-2 was (2 +), the FISH should be performed [26]. Lower [14] carried out the research on 382 recurrent patients of breast cancer, finding that 254 cases (66%) exhibited the concordance of HER-2 in primary lesions and metastatic sites, 90 cases had positive results in primary lesions and negative in metastases, while 37 cases were just the opposite. In 2011, Monaco [25] reported that the concordance rate of HER-2 in breast cancer primary lesions and metastases was 71%. In this study, it was found that the expression concordance rate of HER-2 in primary lesions and metastases was 89.2%, which was a high concordance rate, and the inconsistent cases were mostly positive in primary lesions and negative in metastases, which might indicate that some mechanism might inhibit the expression of HER-2 during the cancer *in vivo* metastasis to the lymph nodes. Thus, towards the patients with HER-2 expression as positive/negative, if they were administrated the targeted therapy based on the HER-2 expression in primary lesions, whether the clinical efficacy would be the same as positive/negative; while as for the patients with HER-2 expression as negative/positive, whether the clinical treatment was deficiencies, and whether the patients had missed opportunities of targeted therapy, the above still need further study.

Ki-67 antigen is a nuclear antigen present in proliferating cell nucleus, with short half-life, it begins the expression in G1 phase of the cell cycle, and reaches the highest peak in M phase, and would rapidly degrade after the completion of the cell cycle. As a cell proliferation marker, it's the reliable indicator of reflecting cell proliferation [27]. In the early stage of breast cancer, Ki-67 expression occurs a high proportion in lymph node metastasis, with poor prognosis. Tawfik [28] found that detection of Ki-67 expression status in lymph nodes was superior to evaluate the expression of Ki-67 in primary lesions, and would help to select the treatment options. In 2011, St.Gallen Breast International Conference formally proposed Ki-67 as an important reference index of molecular classification of breast cancer. In this study, the correlation of different expression levels of Ki-67 and ER, PR and HER-2 expression in primary lesions and metastases of breast cancer patients were explored, finding that in primary lesions with high Ki-67 expression and metastasis with low Ki-67 expression, the ER expression exhibited statistical significance between primary lesions and metastases, therefore, when use immunohistochemistry to detect the primary lesions of breast cancer patients, it should not neglect the Ki-67 expression status in lymph node metastases. In differences Ki-67 expression levels, PR and HER-2 expression also showed differences between primary lesions and metastases, although no statistically significant was found yet, the tumor with high Ki-67 expression had biological behavior of high proliferation, during the administration of hormone therapy or targeted therapy, the chemotherapy was also the measures which should not be ignored.

Conclusions

Hormone receptors and the expressions of HER-2 and Ki-67 of primary lesions and lymph node metastases in breast cancer had higher consistency, while there were still about 10% patients showed differentiated expression. The simultaneous detection of breast cancer primary lesions and lymph node metastases was still very necessary. Management of breast cancer still requires particular attention to metastatic site, long term follow-up after breast cancer therapy, more aggressive adjuvant therapy may be useful in different hormone receptors and the expressions of HER-2 and Ki-67 of metastatic lesions, and to access to the best effect.

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