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HER2 and VEGF expression in breast cancer and their correlations

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Abstract ***Objective:*** The aim of our study was to detect the correlation between the expressions of HER2 and VEGF in breast cancer, and their relations with some pathological factors. ***Methods:*** By immunohistochemistry technique, the expressions of HER2 and VEGF in the post-operation samples of 117 cases with breast cancer were assessed, and their relations with some pathological factors were analysed by statistical methods. Fifty samples of hyperplasia of mammary glands were observed as the control. ***Results:*** The positive expression rates of HER2 and VEGF in breast cancer were both significantly higher than those in hyperplasia of mammary gland ($P < 0.05$). The expressions of HER2 and VEGF were both correlated to lymph node metastasis ($P < 0.05$), but showed no relations with age, histological type, histological stage, tumor size ($P > 0.05$). The positive expression rate of HER2 had a positive correlation with those of VEGF ($P < 0.05$, $r = 0.373$). ***Conclusion:*** The expressions of HER2 and VEGF have no correlations with age, histological type, histological stage, tumor size, but are closely related with lymphatic metastasis. The positive expression rates of HER2 shows a positive correlation with those of VEGF.

Key words breast cancer; HER2; VEGF; immunohistochemistry

In this study, the expressions of HER2 and VEGF in 117 samples of breast cancer were observed by immunohistochemistry method. Our research also analyzed their correlations based on some pathological factors such as patient age, histological type, histological grade, tumor diameter, lymph node metastasis, etc..

Materials and methods

Specimens

A total of 117 operated samples with breast cancer from August 2006 to March 2009 were collected in this study. All the patients were confirmed by pathological examination and never did chemotherapy or radiotherapy before operation. In addition, 50 samples of benign lesions (hyperplasia of mammary glands) were collected as controls.

The operated samples were all from female. The age ranged from 30 to 89 years old and the mean age was 49.86 years old. < 40 years, 27 cases; 41–59 years, 67 cases; > 60 years, 23 cases.

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Histological type

According to WHO classification criteria of histological types of breast cancer: invasive ductal carcinoma, 86 cases; invasive lobular carcinoma, 8 cases; intraductal carcinoma, 7 cases; mucinous carcinoma, 6 cases; papillary carcinoma, 3 cases; medullary carcinoma, 3 cases; other types of breast cancer, 4 cases.

Histological grade

Grade I, 11 cases; Grade II, 59 cases; Grade III, 16 cases.

Tumor size

Less than 2 cm in tumor diameter, 49 cases; 2–5 cm in tumor diameter, 38 cases; more than 5 cm in tumor diameter, 30 cases.

Lymph node status

With lymph node metastasis, 40 cases; with no lymph node metastasis, 77 cases.

Methods

All the tissue samples by formalin-fixed and paraffin-embedded were cut in 3 μm thickness. HE and immunohistochemistry staining were done with each slice.

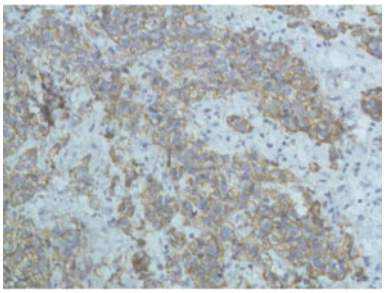

Fig. 1 HER2 expression in breast cancer tissues (+++) (SABC × 150)

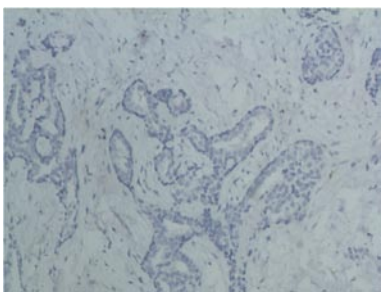

Fig. 2 HER2 expression in hyperplasia of mammary glands tissues (–) (SABC × 150)

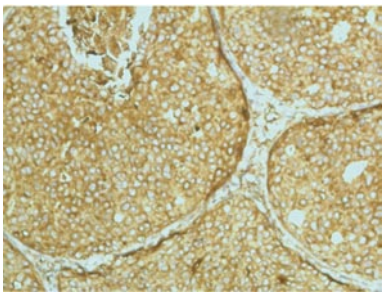

Fig. 3 VEGF expression in breast cancer tissues (+++) (SABC ×150)

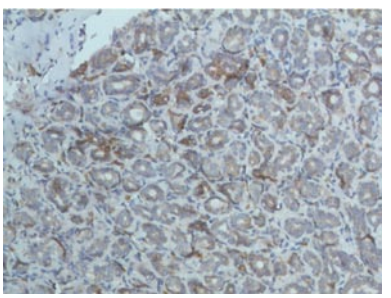

Fig. 4 VEGF expression in hyperplasia of mammary glands tissues (+) (SABC × 150)

The slices with HE staining were used for pathological diagnoses. Some samples were selected for immunohistochemistry staining. The expressions of HER2 and VEGF were observed with SABC immunohistochemistry methods. Mono-antibodies of HER2 (ZA-0023) and VEGF (ZA-0509) were applied. All the antibodies (HER2 and VEGF) and assay kits are produced by Zhongshan Golden Bridge Biotechnology Co., Ltd (China). PBS solution was used instead of first antibody as negative controls. Known breast cancer slices with positive expression were used as positive controls.

Assessment criteria

It was determined as positive expression when brown or tan color particles showed in cell membrane or cytoplasm. Each slice was observed in three HPF randomly and the results were defined according to the average proportion of positive cells.

The HER2-positive cells showed membrane staining. The slice with no staining was determined as negative (–); while staining cells > 10% was determined as positive; without successive and strong staining as weakly positive (+); middle part of discontinuous staining as positive (++); strong and successive staining as strong positive (+++). The VEGF-positive cells showed cytoplasm staining. The slice without staining was determined as negative (–); while staining cells < 25% was determined as weakly positive (+); staining cells 26%–50% as positive (++); positive staining cells > 50% as strong positive (+++).

Statistical analysis

All the data were processed with SPSS 10.0 statistical software and the result was analysed by Chi-square test and Pearson correlation coefficient. $P < 0.05$ was considered as statistical significant difference.

Results

HER2 and VEGF expression in the tissues of breast cancer and hyperplasia of mammary gland

Breast cancer tissues had positive expression of HER2 in membrane (Fig. 1), whereas benign breast disease (hyperplasia of mammary glands) tissues rarely expressed (Fig. 2). Breast cancer tissues had positive expression of VEGF in cytoplasm (Fig. 3), while benign breast disease (hyperplasia of mammary glands) tissues also had a certain degree of expression (Fig. 4).

Table 1 showed that the rate of HER2 expression was 35.0% in breast cancer tissues while 0 in hyperplasia of mammary glands tissue, which showed a significant difference ($P = 0$). The rate of VEGF expression was 76.9% in breast cancer tissues compared with 38.0% in hyperplasia of mammary glands tissues with a significant difference ($P = 0.0480$).

Correlation between HER2 or VEGF expression and pathological factors of breast cancer

Table 2 showed that in different age, histological type, histological grade, tumor diameters, both HER2 and

Table 1 HER2 and VEGF expression in the tissues of breast cancer and hyperplasia of mammary gland

Types of specimen (*n*)	HER2 expression		VEGF expression	
	+ (%)	–	+ (%)	–
Breast cance tissues (117)	41 (35.0)	76	75 (76.9)	27
Hyperplasia of mammary gland tissues (50)	0	50	31 (38.0)	19
P value	0		0.0480	

VEGF positive expression rates showed no significantly difference ($P > 0.05$). In the group with Lymph node metastasis, both HER2 and VEGF positive expression rates were significantly higher than the group without lymph node metastasis. *P* values were 0.0418 and 0.0039 respectively, which showed a significantly difference ($P < 0.05$).

Correlation between the HER2 and VEGF expression

Table 3 showed that in 90 cases with VEGF positive expression, 41 cases had positive expression of HER2. And VEGF was expressed in all 41 cases with HER2 positive expression. A total of 68 cases had consistent expressions of HER2 and VEGF (with HER2 and VEGF expression positive or negative in line), accounting for 58.1%. The HER2 and VEGF expression was positively correlated ($P < 0.05$, $r = 0.373$).

Discussion

HER2, also known as C-erbB2 or neu gene, is located in human chromosome No. 17, coding phosphorylated protein. It is a member of EGF receptor family and similar to epidermal growth factor receptor. So it is also called human epidermal growth factor-related genes. HER2 mainly expresses in the human body during the period of embryonic growth, and plays a role in a variety of tissue and organic developments. In adult normal tissues, HER2 often exists in single copy, with a lower or rare level of expression. In many tumor cells originated from epithelium, such as breast cancer, ovarian cancer and gastric cancer, HER2 often has an amplified or abnormal expression. It participates in regulating cell growth, proliferation and tumor cell differentiation, and its expression level has a closed correlation to tumor occurrence, development and prognosis [1]. Vitro studies had shown that transfected HER2 of breast cancer cell lines increased the DNA synthesis, accelerated cell growth and enhanced invasiveness. Also the tumorigenicity and metastatic potency in nude mice were strengthened. The results suggested that HER2 had positive expression in the membrane of breast cancer cells. But in benign breast disease (hyperplasia of mammary glands) tissues, it almost did not express. The positive expression rates of the two had a significant difference ($P < 0.05$), which was consistent with the results reported in

Table 2 The correlation between HER2 or VEGF expression and pathological factors of breast cancer

	Cases	HER2		*P* value	VEGF		*P* value
		+	–		+	–	
Age (years)				0.4226			0.0861
≤ 40	27	7	20		25	2	
41–59	67	24	43		48	19	
≥ 60	23	10	13		17	6	
Histological type				0.9609			0.0587
Invasive ductal carcinoma	86	31	55		70	16	
Invasive lobular carcinoma	8	3	5		3	5	
Intraductal carcinoma	7	2	5		4	3	
Mucinous carcinoma	6	1	5		4	2	
Medullary carcinoma	3	1	2		3	0	
Papillary carcinoma	3	1	2		2	1	
Other types of breast cancer	4	2	2		4	0	
Histological grade				0.4152			0.2556
I	11	2	9		7	4	
II	59	23	36		50	9	
III	16	6	10		13	3	
Tumor diameter (cm)				0.783			0.0636
< 2	49	16	33		33	16	
2–5	38	15	23		30	8	
> 5	30	10	20		27	3	
Lymph node metastasis				0.0418			0.0039
Yes	40	19	21		37	3	
No	77	22	55		53	24	

Table 3 The correlation between HER2 and VEGF expression

	VEGF expression		Total	P, *r*
	+	–		
HER2 expression				
+	41	0	41	*P* = 0, *r* = 0.373
–	49	27	76	
Total	90	27	117	

the literature studies. In addition, the pre-clinical study found that the high-level expression of HER2 could enhance the metastatic potency of breast cancer cells and present more invasive clinicopathological features. Studies on the application of ibuprofen (tyrosine kinase inhibitor) had shown that ibuprofen not only inhibited the HER2 receptor tyrosine kinase activity, but also lowered the metastatic capacity and growth rate of breast cancer cells [2, 3]. The results of this study showed that the positive expression rate of HER2 in the group with lymph node metastasis was significantly higher than that in the group without lymph node metastasis ($P < 0.05$). It suggested that HER2 expression in breast cancer is closely related to lymph node metastasis and HER2 play an important role in the course of breast cancer metastasis.

VEGF is one of the most important known factors of regulating angiogenesis and neovascularization in solid tumor [4]. VEGF includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor PIGF. They activate the tyrosine kinase receptor and guide the signal conversion of cell function. Normal adult body fluids and tissues also contain a minute amount of VEGF, but with low-level expression, which is maintenance of normal vascular density and permeability. In tumor tissues, VEGF can stimulate the division and proliferation of endothelial cell, thus promote tumor angiogenesis. It can induce proteolytic enzymes, interstitial collagenase and tissue factors to promote angiogenesis, but also to enhance vascular permeability, fibrinogen extravasation resulted in the promotion of tumor interstitial edema and the changes in extracellular matrix, which provided a suitable basis for the tumor infiltration and metastasis [5]. And Peng [6] reported that VEGF expression had correlations with histological type and grade of breast cancer. Early invasive ductal carcinoma, invasive ductal carcinoma, papillary adenocarcinoma showed a high positive expression, followed by carcinoma simplex, small tubular adenocarcinoma. The positive rate of VEGF enhanced with the increase of histological grade. Namely, the higher histological grade of malignant tumors was, the higher-level expression of VEGF would show. The results of this study showed that, in different histological type and grade groupings, the positive expression rates of VEGF were not significantly different ($P > 0.05$). It didn't agree with the conclusion mentioned above, which might be partly due to the small number of cases in different histological types and grades. And the specific mechanisms remain to be further studied.

Related studies have shown that HER2 may be the initiation factors of angiogenesis of breast cancer. It may have a positive regulation of VEGF expression through the HIF-la, and participate in angiogenesis of breast cancer [7]. VEGF is a downstream target of HER2 signaling pathway. Breast cancer cells with HER2 over-expression have an increase of VEGF expression. Laughner [8] confirmed that, in 3T3 and MCF-7 breast cancer cell lines, the overexpressed HER2 can increase the synthesis of HIF-la protein and VEGF mRNA expression through the signaling pathway, such as phosphatidylinositol-3-kinase (PI-3K)/protein kinase 13 (AKT)/FKPB-rapamycin-associated protein (FRAP), SRC or mitogen-activated protein kinase (MAPK), thereby promote tumor angiogenesis. Vitro studies found that, in breast cancer cells, through the method of transfection, HER2 with over-expression can increase the expression of VEGF from the levels of RNA and protein [9]. And using Herceptin to deal with the HER2 with over-expression can significantly reduce the VEGF level. In tumor animal models, it can also be observed that the relevant vessel diameter, the number of blood vessels, vascular permeability were significantly decreased. Yang [10], who used siRNA to silence HER2 in the tumor cells with high expression of HER2, also observed a drop in VEGF levels. In this study, in 90 cases with VEGF positive expression, 41 cases had positive expression of HER2. And VEGF was expressed in all the 41 cases with HER2 positive expression. A total of 68 cases have consistent expressions of HER2 and VEGF (with HER2 and VEGF expression positive or negative in line), accounted for 58.1%. The expression of HER2 and VEGF was positively correlated ($P < 0.05$, $r = 0.373$).

Invasion and metastasis are the main biological characteristics of malignant tumors, and the most important cause of death and failure of surgery, radiotherapy and chemotherapy. In the courses of tumor invasion and metastasis, HER2 and VEGF play an important role. And their expression levels in tumor tissues are closely linked to the progression and prognosis of breast cancer. At present, the HER2 and VEGF are considered respectively as the progression and prognostic factors of breast cancer, which has been accepted by most scholars. And the researches about HER2 or VEGF have become hot spots in the field of oncogene. But there are very few studies about the correlation between HER2 and VEGF. The results suggested that HER2 and VEGF expression weren't related to age, histological type, histological grade, tumor diameter and so on, but have a significant correlation with lymph node metastasis. Also, the expression of the two have a positive correlation.

The above results suggested that the combination of both HER2 and VEGF be expected to offer a better adju-

vant reference for the clinical pathological assessment of breast cancer patients. And it would help the analysis of recurrence and metastasis factors of breast cancer, which would be helpful for the prediction of the prognosis and the clinical individual treatment of breast cancer. But the specific mechanism remains to be further studied.

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