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Correlation between the expression of vascular endothelial growth factor c and C-erbB-2 in human breast cancer*

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Abstract *Objective:* We aimed to study the transcription level of VEGF-C in human breast cancer tissue, and explore the correlations with the expression of C-erbB-2. *Methods:* The expression of VEGF-C mRNA in 51 cases of human breast cancer was assessed by hybridization in situ. The expressions of C-erbB-2 was assessed by immunohistochemistry. *Results:* The positive rate of VEGF-C mRNA was 54.9% in 51 cases of breast cancer. The transcription level had correlation with tumor size and status of lymph nodes ($P < 0.05$). The expression of VEGF-C mRNA had a positive correlation with the expression of C-erbB-2 ($P < 0.05$). *Conclusion:* The up-expression of VEGF-C has a significant correlation with the malignancy level and clinical stage of breast cancer. The combined detection of VEGF-C, C-erbB-2 may help to estimate the prognosis of patients with breast cancer and study on thetherapeutic implications.

Key words breast cancer; vascular endothelial growth factor C; C-erbB-2

Breast cancer is a malignant disease with complicated syndromes. The leading reason for death is the dissemination and metastasis of tumor cells. Clinical pathology researches have shown that lymphangiogenesis of tumor is the early stage of distance dissemination, in which many cytokines have participated in the regulation process of lymphangiogenesis [1]. Recently, growth factors have attracted increasing attention by its potential contribution to the formation in lymphangiogenesis of tumor. Vascular endothelial growth factor C (VEGF-C), generated by hypoxia, has effect on lymphangiogenesis directly. VEGF-C was the mitogen of endotheliocyte, whose hyperplasia and movement would cause the metastasis of tumor cell [2]. The objective of this research was to observe the transcriptional expression of VEGF-C and its correlation with expression of C-erbB-2, further to discuss the clinical significance of expression of VEGF-C with C-erbB-2.

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Materials and methods

Patients and tumor specimens

Fifty-one tissues specimens of breast cancer used in this study were obtained from The General Hospital of Shenyang Military Region. All specimens were resected surgically between 2005 and 2008. The pathological diagnosis of these patients was all invasive breast cancer. Patients with median age of 55 years old (Range: 18–56 years old) were examined by B-ultrasonic, CT, tumor markers and PET-CT before operation to exclude the second type tumor. The tumors were classified into 4 stages according to the AJCC· TNM phased standard : I phase (13), II phase (23), III phase (15), IV phase (0). The situation of lymph nodes metastasis：lymph nodes metastasis > 3 in 15 cases, lymph nodes metastasis 1–3 in 13 cases, 23 patients have negative lymph nodes metastasis. 10 normal breast tissues were used as control. All specimens were cut into 4 μm serial sections.

Antibodies and kits used in the experiment

Instant-applied mouse anti-human C-erbB-2 monoclonal antibodies were purchased from Zhongshan Jinqiao Biotechnology Company (China). Instant-applied Ultra™ SP kit for immunohistochemistry and DAB-0031 kit

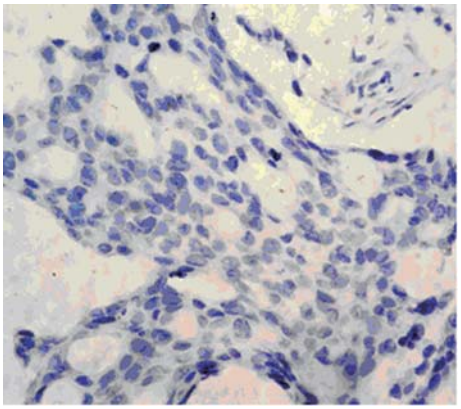

Fig. 1 Negative expression of VEGF-C mRNA in breast cancer tissue detected by hybridization in situ (SP × 400)

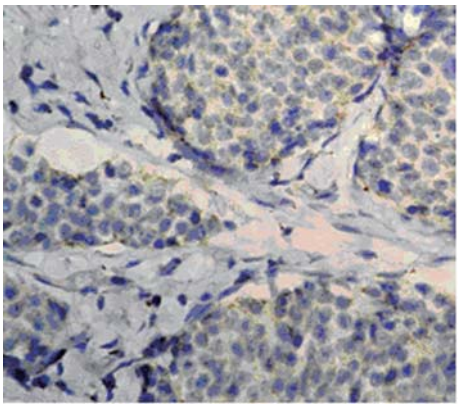

Fig. 2 Positive expression of VEGF-C mRNA in breast cancer tissue detected by hybridization in situ (SP × 400)

for DAB coloring were purchased from Maixin biological Ltd. in Fuzhou (China). In situ hybridization (ISH) kit for VEGF-C mRNA (including pepsin, 2 mL pre-hybridize liquid and 2 mL oligonucleotides probe liquid, 5 mL blocking liquid, 5 mL biotined mouse anti-digoxin, 5 mL SABC-POD, 5 mLL biotined peroxidase) was purchased from Boshide biological engineering Co. Ltd. in Wuhan (China).

ISH of VEGF-C mRNA

Expression of VEGF-C mRNA was measured in 51 breast cancer specimens and 10 normal breast tissues tumors by ISH. VEGF-C oligonucleotides probe was marked by digoxin. Hybridization signal was stained by DAB. Specific steps were done according to the kit manual. Positive control slides were provided by Department of Pathology, The First Affiliated Hospital of China Medical University. In the process of hybridization, omitting the step of probe was considered negative control.

Immunohistochemical (IHC) analysis of C-erbB-2

Streptomycin avidin-peroxidase was used to examine the expression of C-erbB-2 in 51 histological specimens of breast cancer patients and 10 normal breast tissues. Colorectal cancer specimens provided by Zhongshan Jinqiao Biotechnology Company (China) were used as positive control. PBS only was considered as Negative control.

Judgment of ISH and IHC results

VEGF-C mRNA was mainly stained as pale yellow or brown granules in cytoplasm of breast cancer cells. C-erbB-2 located in cell membrane or in partly cytoplasm. Staining intensity was confirmed by semi-quantitative analysis method as follows: choose the most intensive areas; observed the positive cells at low magnification (× 100); calculated the percentage of positive cells in tumor cells. Stained cells > 25% was considered positive (+).

Statistical analysis

Statistical analysis, including the Chi-square test and *t* test were carried out using the software package SPSS 10.0. The significance level was set at 5% for all analysis.

Results

Expression of VEGF-C mRNA in breast cancer

VEGF-C mRNA expressed in 28 invasive breast cancer specimens among 51 cases and the positive rate was 54.9% (28/51). VEGF-C mRNA mainly expressed as pale yellow or brown granules in cytoplasm of breast cancer cells and could not been found in cell membrane and nucleus (Fig. 1 and 2). In 10 normal breast tissue, positive expression rate of VEGF-CmRNA was 30% (3/10), which was much lower than that of breast cancer tissue ($P < 0.05$).

Relationship between VEGF-C mRNA expression with clinical and pathological features

Expression rate of VEGF-C mRNA had correlation with tumor size and metastasis of lymph nodes ($P < 0.05$, $\chi 2 = 0.785$; $P < 0.05$, $\chi^2 = 9.879$), but no correlation with age ($P > 0.05$, $\chi^2 = 0.005$). Along with the increase of tumor volume and metastasis of axillary lymph nodes, the expression rate of VEGF-C mRNA increased gradually (Table 1).

The positive rate of C-erbB-2 in 51 invasive breast cancer specimens was 56.8% (29/51) respectively. The expression level of VEGF-C mRNA correlate with that of C-erbB-2 ($P < 0.05$, $\chi^2 = 5.370$). Along with the increase of the expression level of VEGF-C mRNA, the positive rate of C-erbB-2 increased gradually (Table 2).

Table 1 Relationship between VEGF-C mRNA expression with clinical and pathological features

Characteristic	*n*	VEGF-C mRNA (–)		VEGF-C mRNA (+)		*P*
		n	%	*n*	%	
Age (years)						
≤ 45	18	8	44.4	10	55.6	0.954
> 45	33	15	45.5	18	54.5	
Tumor size (cm)						
< 2	14	11	78.6	3	21.4	
2–5	30	10	33.3	20	66.7	0.012
> 5	7	2	28.6	5	71.4	
Lymph node metastasis						
0	23	15	65.2	8	34.8	
1–3	13	6	46.2	7	53.8	0.007
> 3	15	2	13.3	13	86.7	

Table 2 Relationship between expression of VEGF-C mRNA and the expression of C-erbB-2 [*n* (%)]

Group	*n*	VEGF-C mRNA		*P* (χ^2)
		–	+	
C-erbB-2				
–	22	14 (63.6)	8 (36.4)	0.026
+	29	9 (31.0)	20 (69.0)	

Discussion

VEGFs is an important growth factor in the occurrence and progression of tumors [2–3]. VEGF-C, a member of VEGFs, plays an important role in tumor progression by both stimulating lymphangiogenesis and tumoral proliferation [3–4]. This will promote metastasis of breast cancer. Ciobanu [5] detected the expression of VEGF-C in 25 cases of breast cancer by immunohistochemistry. The positive rate in their study was similar to ours, which demonstrate that high expression of VEGF-C would have an important significance in the process of breast cancer.

We analyzed the relationship between the expression of VEGF-C mRNA and different clinicopathologic factors of breast cancer. Significant correlation was discovered between the expression of VEGF-C mRNA with the number of metastatic axillary lymph node and tumor size ($P < 0.05$). The results indicated that expression of VEGF-C in breast cancer tissue was relevant to clinical stage and malignancy degree. VEGF-C was very important in the growth, invasion and metastasis of breast cancer. Therefore, VEGF-C could be an important index for judging the prognosis of breast cancer, which was in accordance with the effect of VEGF-C in other tumors .

C-erbB-2, expressed in 30% human tumors, enhanced the proliferation and activity of tumor cells and promoted the formation of vessels by activating tyrosine kinase in cell signal transduction [6–7]. The over-expression of C-erbB-2 has resulted in the shortening of survival period, the increasing of recurrence and metastasis in human breast cancer. Our results showed that expression intensity of VEGF-C mRNA was related to that of C-erbB-2. This may suggest some relation between VEGF-C and C-erbB-2 in the occurrence and development of breast cancer. The combined detection of VEGF-C and C-erbB-2 would play an important role in forecasting the prognosis of breast cancer. Some experiments reported that gradually increase of VEGF-C expression partly mediated the biological invasive phenotype in C-erbB-2 high-expression breast cancer [8–9].

Chinese researches found that expression of VEGF-C and C-erbB-2 had obvious relation in C-erbB-2 high-expression phenotype ($P < 0.05$) [10]. However, the mechanism on interaction of VEGF-C and C-erbB-2 is unclear so far. Laughner's research on transgenic mice demonstrated that C-erbB-2 could control the transcriptional level of VEGF-C expression, which promoted the expression of VEGF-C mRNA and the lymphangiogenesis in breast cancer. Furthermost, the correlation between C-erbB-2 and VEGF-C may suggest an HER2 blocking therapy that could reduce not only tumor progression, but also lymphangiogenic metastasis [11].

Taken together,the combined detection of VEGF-C mRNA and C-erbB-2 has important significance in the prognosis of breast cancer. It will become an effective biological index for predicting prognosis. However, further prospective study should be done to confirm the clinical value of VEGF-C mRNA in judging the prognosis and directing the therapy of breast cancer.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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