Expression of Vascular Endothelial Growth Factor C and Its Relationship with Lymph nodes Metastasis in Breast Cancer

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Abstract **Objective** Study the expression of VEGF-C in breast cancer and its relationship with lymph nodes metastasis to clarify the role of VEGF-C in lymphatic metastasis and probe the underlying mechanism. **Methods** Paraffin-embedded specimens from 76 patients with breast cancer after radical mastectomy were studied by VEGF-C protein. **Results** There are expressions of VEGF-C protein in the cytoplasm of some breast cancer cells (28/67). Furthermore, the expression of VEGF-C in breast cancer cells of lymph nodes positive group was notably higher than lymph nodes negative group. **Conclusion** The expression of VEGF-C in breast cancer has a close correlation with lymph nodes metastasis of cancer cells.

Key words VEGF-C; breast cancer; neoplasm metastasis

Breast cancer is one of the most frequently-occurred tumors in female, second only to cervical cancer. In the development of breast cancer, metastasis of cancer cells is usual. Although a portion of the metastatic lymph nodes can be removed by surgery, there are still many patients died of lymph nodes metastasis. This significantly affects the prognosis of the disease. Therefore, investigation of the underlying mechanism to develop effective treatments will contribute greatly to the breast cancer therapy[1,2] Studies have suggested that the expression of EGFR, c-erbB2, c-met, VEGF and E-cadherin is closely related to lymph nodes metastasis of cancer cells. However, none of these factors has been demonstrated to play a role in the hyperplasia of lymphatics which is often observed in metastatic lymph nodes. As a specific lymphatic growth factor, vascular endothelial growth factor C (VEGF-C) is being paid more and more attention for its ability to promote lymphatic hyperplasia[3]. But little has been reported about its expression in tumors. In the present study, we examine the expression of VEGF-C in breast cancer using immunohistochemistry method and probe the mechanism of lymphatic metastasis.

MATERIALS AND METHODS

Specimens

76 specimens were obtained from patients who accepted radical mastectomy or improved radical mastectomy and was diagnosed of breast cancer by pathological histology. Axilliary lymph nodes metastasis was found in 46 cases. The specimens were embedded with paraffin and cut into slices with thickness of 4~5 μ m.

Reagents

Goat anti-human VEGF-C antibody, HIGH-SABC immunohistochemistry kit and DAB dye were purchased from Wuhan Boster Biotechnology Co. Ltd.

Methods

Adopted the immunohistochemistry method of HIGH-SABC, coloration with DAB after stained with hemoatoxylin. Briefly, the slides were dewaxed and immersed in methanol containing 2% H_2O_2 at room temperature for 30 minutes to deactivate H_2O_2 enzyme, then washed with distilled water and PBS. Compound digestive solution was added and incubated for 5 to 10 minutes at room temperature, followed by washing with distilled water. The slides were coated with normal rabbit serum for 10 to 20 minutes to prevent nonspecific reaction. Then VEGF-C(1 : 100) were added to the slides and incubated in wet kit at 4℃ overnight. Slides were thoroughly washed for 2 minutes with PBS three times. Added rabbit anti-goat secondary antibody labeled with biotin and incubated for 20 minutes at room temperature, followed by thorough wash for 2 minutes with PBS three times. After staining slightly by hematoxylin, dehydration, penetration and sealing with neutral gum, slides were colorated with DAB and washed with distilled water. PBS (pH=7.4) was used instead of first antibody as negative control. We

considered the slides with notable stain as VEGF-C positive, while the slides with weak or no stain as VEGF-C negative. The data were analyzed by chi-square test.

RESULTS

34 out of the 76 primary breast cancer tissue specimens showed positive stained granules, which were light brown and located in cytoplasm (Fig. 1, 2). After classified specimens by age, tumor size and metastasis of lymph nodes (table 1), we found that there was a notable correlation between VEGF-C protein and lymph nodes metastasis. VEGF-C expression rate in specimens with lymph nodes metastasis was significantly higher than that in specimens without lymph nodes metastasis ($P<0.01$). However, VEGF-C had no notable correlation with age and size of tumor ($P>0.05$).

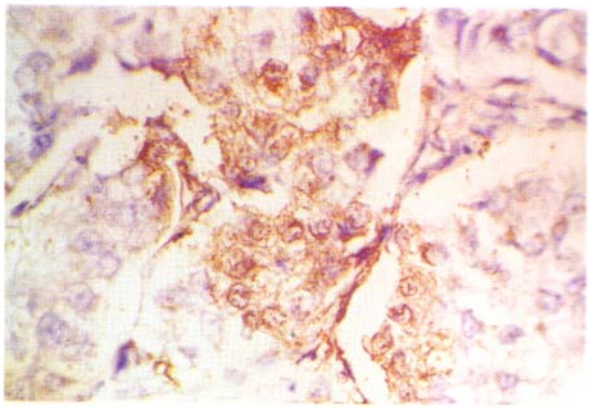

Fig.1 VEGF-C expressed as light brown granules located in cytoplasm, adopted immunohistochemistry method of HIGH-SABC, colorated by DAB. × 400

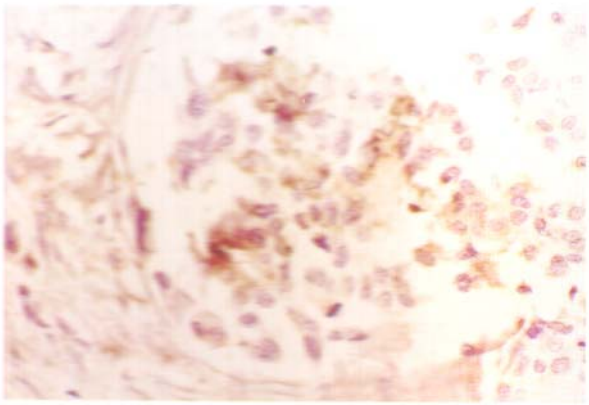

Fig.2 VEGF-C expressed as light brown granules located in cytoplasm, adopted immunohistochemistry method of HIGH-SABC, colorated by DAB. × 400

DISCUSSION

VEGF is a family of growth factors that act on vascular endothelial cells. Its members include VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and PIGF[3,4,5]. Among which VEGF-C is most important in lymphangiogenesis. VEGF-C promotes the hyperplasia of lymphatic endothelial cells and lymphatics by activating VEGFR-3 or VEGFR-2 in the membrane of endothelial cells. Experiment with transgenic mice have demonstrated that high expression of VEGF-C can accelerate hyperplasia of lymphatic vessels in skin[6]. In adults, VEGF-C functions are linked with VEGFR-3 more than VEGFR-2. VEGFR-3 is mainly expressed on lymphatic vessels endothelium and is a specific regulatory factor of lymphatic vessels[7]. Currently most people believe that

Table 1 Correlation between the expression of VEGF-C and age, Size of tumors, metastasis

	Specimens	expression of VEGF-C	
		Positive	negative
age			
<50	15	7	8
50-70	42	19	23
>70	19	10	9
size of tumor(cm)			
<3.5	48	19	29
>3.5	28	13	15
lymph nodes			
metastasis	46	26	20 *
without metastasis	30	8	22

* $P<0.01$

the activation of VEGFR-3 by VEGF-C leads to regeneration of lymphatic vessels.

Hyperplasia of lymphatic vessels endothelial cells and lymphatic vessels occur in breast cancer. Our researches demonstrated that VEGF-C is expressed in some breast cancer cells while there are no expression of VEGF-C in normal breast tissues. Furthermore, the expression rate of VEGF-C has a significant correlation with metastasis of axillary lymph nodes. The mechanism of VEGF-C production in breast cancer is still unclear. But since VEGFR-3 is present in lymphatic vascular endothelial cells, VEGF-C can activate VEGFR-3 to cause hyperplasia of lymphatic vessels in primary tumor and metastasis of cancer cells through lymphatic vessels. Blocking VEGFR-3 by antibody may reduce metastasis via lymphatic vessels in breast cancer. Besides, VEGF-C is a very potent vascular permeability factor. It can increase the permeability of micrangium and lymphatic vessels, which will also facilitate the metastasis of endothelial cells[8]. In conclusion, the production of VEGF-C in cancer cells can result in lymph nodes metastasis of cancer cells.

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