Study on the Difference in Expression of VEGF and bFGF and their Receptors in Young and Postmenopausal Women with Breast Cancer

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OBJECTIVE To study the difference in expression of VEGF and bFGF and their receptors in young and postmenopausal women with breast cancer.

METHODS Immunohistochemical methods (SABC) were used to study the expression of VEGF, FLK-1, bFGF and FLG on paraffin-embedded sections from 40 cases of young and 30 cases of postmenopausal women with breast cancer, The relationship between axillary lymph node metastasis and the expression of the growth factors and their receptors was studied.

RESULTS The mean expression of VEGF and bFGF and the positive rate of axillary lymph nodes in the young group were higher than that in the postmenopausal group ($P<0.01$ or $P<0.05$); the mean values of VEGF, bFGF, FLK-1 and FLG in cases of axillary lymph node metastasis were higher in patients without axillary lymph node metastasis in each group ($P<0.05$ or $P<0.01$); there was a significant difference between the mean expression of VEGF, bFGF, FLK-1 and FLG in cases of stage 0 ~ II comparaed to cases of stage III~IV ($P<0.05$ or $P<0.01$).

CONCLUSION The tumor vasculature is directly related to the high breast cancer aggressiveness in young women, a characteristic that might be due to the high expression of VEGF and bFGF.

KEYWORDS: breast cancer, vasculare endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), receptors for vasculare endothelial growth factor, receptors for basic fibroblast growth factor.

Breast cancer in young women is rare and they have a poorer prognosis compared to older women. [1] This phenomenon is mainly due to the fact that tumors in young patients more frequently exhibit aggressiveness and are prone to metastasize. Research on the biology of this tumor has reached a high level of interest. Several studies have found that the expression of some genes is significantly different in young women patients with breast cancer compared to older women, These genes have included CerebB-2, H-ras, P53 and BRCA1, etc., [2,3] but none of the reports have indicated whether there was a difference in angiogenesis. Therefore we attempted to detemine if there was a difference in the expression of vasculare endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and their receptors, FLK-1 and FLG in tissue of young and postmenopausal

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women with breast cancer.

MATERIALS AND METHODS

Clinical data

The young group

We collected 40 cases of young women(age ≤ 35years) with breast cancer who had received an operation and their disease verified by clinical pathology in our hospital from Januany 1997 through December 2001. The range of their ages were 28~35 years, average, 32.6 ± 2.5; Aggressive cancer accounted for 39 cases (97.5%), and unaggressive cancer account for one (2.5%). All cases were treated by radical mastectomy.

The postmenopausal group

We randomly selected 30 cases of postmenopausal patients with breast cancer who received radical mastectomy and whose tumors were verified by clinical pathology during the same period in our hosipital, Range of ages was 50~72 years, average, 61.4± 7.2. All cases were without preoperative anticancer therapy. Samples of the tumor tissues were routinely embedded in paraffin, serial sections of 5 μm made, and H&E and immunobiochemical staining applied.

Immunobiochemical staining

A SABC method was used and verified by testing sections of large intestinal cancer as a positive control and alteration of the first antibody by PBS as negative one. All reagents such as McAb for VEGF (1:200), McAb for bFGF (1:500), polycolonal antibody for FLK-1/KDR(1:400), polycolonal antibody for FLG (1: 400) and SABC were purchased from WuHan Boster Biolofical Technology LTD. The main immunobiochemical staining steps were followed as previously published.[4]

Evaluation of results

The results of VEGF and bFGF staining were evaluated based on intesity of staining and on the number of positive cells [4] as follows:staining intensity (a): 0=no, 1=weak, 2=medium, 3=strong; positive cell counts (b): 0=no, 1=<25%, 2=25%~50%, 3=>50%. The degree of expression was indicated by calculating a positive coefficient as follows: a+b score 0~1, has a positive coefficient of 0; a+b score 1, has a positive coefficient of 1; a+b score 3, has a positive coefficient of 2; a+b score 4~5, has a positive coefficient of 3; a+b score 6, has a positive coefficient of 4. The degree of expression of staining for FLK-1 and FLG was evaluated on the following bases: positive coefficient showing: 0=negaitive; 1=dubious positive; 2=weak positive; 3=medium positive; 4=strong positive. Positive VEGF is seen in Fig. 1. Positive bFGF is seen in Fig.2. Positive FLK-1 is seen in Fig. 3. And positive FLG is seen in Fig. 4.

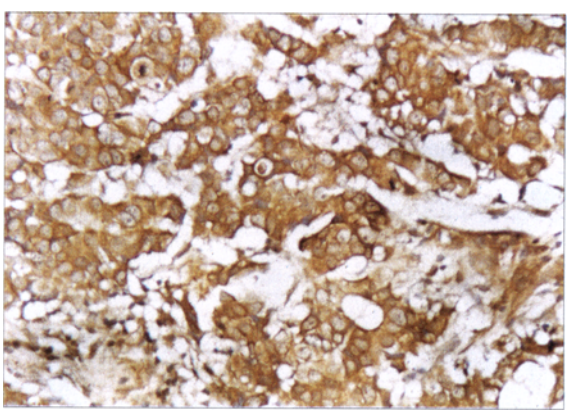

Fig.1. Breast cancer, the positive cytoplasma expression of VEGF, SABC × 200.

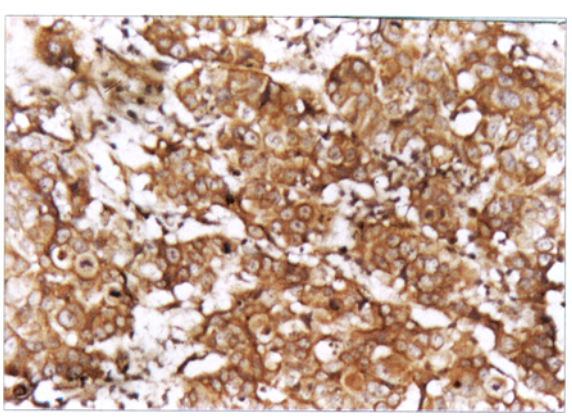

Fig.2. Breast cancer, the positive cytoplasma expression of bFGF, SABC × 200.

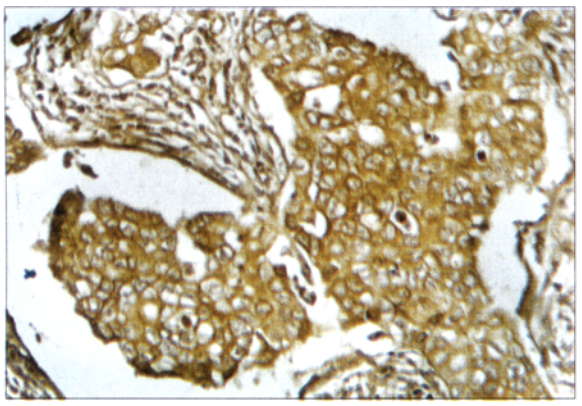

Fig.3. Breast cancer, the positive cytoplasma expression of FLK-1, SABC × 200.

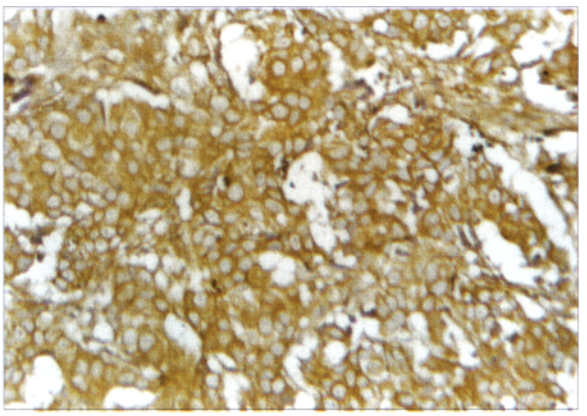

Fig.4. Breast cancer, the positive cytoplasma expression of FLG, SABC × 200.

Statistical methods

Statistical analysis was performed by SPSS 10.0 statistic software. Data are reported as $\bar{x} \pm s$. For simple factor analysis the t-test was used and for the rate analysis the χ^2 test was used.

RESULTS

The positive rate of estrogen receptor (ER) and progestin receptor (PR) expression, positive axillary lymph nodes (ALN) and the results of clinical staging

Table 1. Comparision of the positive rates of ER, PR and ALN and clinical staging by TMN in the two groups

						TMN clinical stages					
		ER positive		PR positive		0~II stage		III~IV stage		ALN positive	
Group	*n*	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Young	40	16	40.0	16	40.0	26	65.0	14	35.0	28	70.0*
Postmenopausal	30	17	56.7	15	50.0	19	63.3	11	36.7	12	40.0

Note:comparision with postmenopausal group * $P<0.05$.

Table 2. Compraision of the positive coefficients of VEGF, bFGF, FLK-1and FLG in the two groups

Group	*n*	VEGF	bFGF	FLK-1	FLG
Young	40	2.75±1.24	2.70±0.91	2.10±1.06	1.95±1.26
0~II stage	26	2.38±1.30	2.54±0.86	1.85±1.19	1.62±1.30
III~IV stage	14	3.43±0.76*	3.00±0.96	2.57±0.51**	2.57±0.94**
ALN positive	28	3.14±1.15*	3.00±0.86*	2.50±0.92*	2.57±0.92*
ALN negative	12	1.83±0.94	2.00±0.60	1.17±0.72	0.50±0.52
Postmenopausal	30	1.40±1.13	1.43±1.04	1.90±1.09	1.97±1.13
0~II stage	19	0.95±0.91	1.21±1.08	1.58±1.07	1.63±0.96
III~IV stage	11	2.18±1.08*	1.82±0.87	2.45±0.93**	2.55±1.21**
ALN positive	12	2.25±1.05*	2.00±0.85**	2.25±0.97*	2.58±1.16*
ALN negative	18	0.83±0.79	1.05±1.00	1.67±1.14	1.56±0.92

Note: *$P<0.01$;**$P<0.05$.

by TMN are showing in Table 1 in the young and postmenopausal groups. The positive rate of ALN in the young group was significantly higher than that in the postmenopausal group ($P<0.05$); and so was ER, but no significant difference. There was no significant difference between the positive rate of PR and the clinical TMN stage in the two groups. No significant difference was found between the positive coefficient of VEGF, bFGF, FLK-1 and FLG in the positive and negitive ER or PR groups ($P>0.05$).

The positive coefficients for VEGF, bFGF, FLK-1 and FLG in the two groups are shown in Table 2. There was a highly significant difference between the positive coefficients for VEGF and bFGF in the two groups ($P<0.01$), but no significant difference between that for FLK-1 and FLG ($P>0.05$). We found a significant or highly significant difference between the positive coefficient for VEGF and FLK-1 and FLG in 0~II and III~IV stages in each group ($P<0.05$ or $P<0.01$), but there was no significiant difference between that for bFGF ($P>0.05$); There was a significant or highly significant difference between the positive coefficient for VEGF, bFGF, FLK-1 and FLG in the ALN-positive and negative groups in the young and postmenopausal groups($P<0.05$ or $P<0.01$).

DISCUSSION

The growth and metastases of solid carcinomas greatly depends on continued, uncontrolled angiogenesis. Tumor cells produce many vascular growth factors to promote angiogenesis, and immature vessels not only offer a base for tumor cell nourishment, but also excrete some cell factors which promote tumor cell growth. The angiogenesis of tumors dependeds on the induction and regulation of various factors, among which, VEGF is known to be one of the most important angiogenesis factors and might serve as a metabolic and metastatic mark of a tumor. [5] During regulation and control of angiogenesis, bFGF may play a key role in enhancing tumor growth. It might promote regeneration of epidermal tissue, stimulate endothelial phagocytes and the division of vascular endothelial phagocytes, induce vascular endothelial phagocytes to separate from the stroma to initiate chemotaxis of endothelial phagocytes toward the tumor and form a tubular structure. Furthermore it might enhance activation factors for plasminogen in the tissue (Pas), namely induce endothelial phagocytes to produce other proteases, as a more direct inducer of angiogenesis.

VEGF not only promotes angiogenesis and development of tumors by a paracrine proeess, but also directly initiates proliferation and migration of tumor cells as a kind of autocrine regulator. [4,5] bFGF also promotes growth and metastasis of tumors by paracrine or autocrine mechanisms. Receptors for VEGF consist of VEGFR-1 (FLT-1), VEGFR-2 (FLK-1/KDR) and VEGFR-3 (FLT-4) all of which have tyrosine kinase activity. FLK-1 is a main modulator of angiogenesis and known to have chemical chemotactic and mitogenic activity.

In the two groups of young and postmenopausal women with breast cancer in our study, we found that the positive coefficient of VEGF, bFGF, FLK-1 and FLG in the ALN positive group was significantly higher than that in the ALN negative group. There was a significant difference between the positive coefficient of VEGF, FLK-1 and FLG in TNM 0~II stages verus that in the TNM~stages. This findings indicates that angiogenesis is corelerated with tumor proliferation and lymph node aggression of young and postmenopausal women with breast cancer. In addition, There was no significant difference between the positive coefficient for VEGF, bFGF, FLK-1 and FLG in either the positive or negative groups for ER or PR, which indicates that the ER and PR have no obvious effect on angiogenesis of those tumors. The results was basically similar to the reports at home and abroad.[6,7]

Young women with breast cancer have different clinical biological features compared to older patients, mainly by showing aggressive features, such as larger tumor size, higher histologic grade, positive lymph nodes, an absence of steroid receptors and a high S-phase fraction of the cell cycle. [1,8] Our study showed that there was a significant difference between the positive rate of ALN in young and postmenopausal women groups with breast cancer. Similar results have been reported, [1,2,8] but in our study there was no

significant difference between the positive rate of ER or PR and the TNM clinical stage in the two groups, a finding that is not consistent with other reports in the literature.[1,2,8] This difference might be related to our smaller sample size and higher TNM clinical stages.

VEGF gene is regulated by oncogenes,such as v-raf, v-ras, h-ras, v-src, K-ras, p53,[9] bcl-2 [10]etc., and bFGF also is regulated by genes, such as bcl-2 and p53.[6] A significant difference has been reported between the expression of CerebB-2, H-ras, P53 and BRCA1 in young versus old women with breast cancer. [2,3] Therefore there might also be a difference between angiogenesis in young and older patients. It was confirmed by our study, that there was a significant difference between the positive coefficient of VEGF and bFGF in young and postmenopausal groups, but for FLK-1 and FLG then was no difference, indicating that the main characteristics might be the higher expression of VEGF and bFGF, not their receptors.

Our study indicated that lymph node infilration in young breast cancer patients was higher than in postmenopausal patients. The higher expression of VEGF and bFGF and their receptors was related to the degree of tumor proliferation and lymph node involvement of young and postmenopausal women with breast cancer. The aggression of the breast cancers in young women is related to a higher expression of VEGF and bFGF, but not to their FLK-1 and FLG receptors.

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