Breast Cancer

Expression and Clinical Significance of Vascular Endothelial Growth Factor in Benign and Malignant Tissues of Breast

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ABSTRACT

Objective: To detect the expression of vascular endothelial growth factor (VEGF) and microvessel density (MVD) count in breast benign affection, breast atypical hyperplasia and breast carcinoma *in situ*, and to clarify the relationship between VEGF expression, MVD and the clinicopathological features of these diseases. **Methods:** The expression of VEGF and MVD count in 115 cases breast benign diseases (including 40 breast fibroid tumor，40 breast cystic hyperplasia and 35 intraductal papilloma, 19 breast atypical hyperplasias and 32 breast carcinomas *in situ* were examined by immunohistochemistry staining (SP-method). **Results:** The positive rate of VEGF in breast benign diseases, breast atypical hyperplasia and breast carcinoma in situ were 21.74%(25/115)、31.58.% (6/19)and 53.13%(17/32) respectively. It was the lowest in breast benign affection group, and was the highest breast carcinoma *in situ* group. The expression of VEGF increased gradually in the three groups (P<0.05). The MVD count of the three groups were 14.41±2.59, 18.89±4.47 and 21.13±4.12 respectively, It was the lowest in breast benign affection group, and was the highest breast carcinoma *in situ* group. The MVD count of the three groups increased gradually (P<0.05). In VEGF positive group, MVD count was 19.41±4.78; In VEGF negative group, MVD count was 14.91±3.15. The MVD count was higher in VEGF positive group than that in VEGF negative group (P<0.05). **Conclusion:** The results of this study suggested that VEGF could promote microvessel growth in breast tumors. The occurrence and progression of breast cancer might be related with the expression of VEGF.

Key Words: Breast benign disease; Breast atypical hyperplasia; Breast carcinoma in situ; Vascular endothelial growth factor(VEGF); Microvessel density (MVD)

Lots of researches had been done to discuss the expression and significance of VEGF in breast cancer, but it was rare to research the expression of VEGF and MVD count in breast benign disease, breast atypical hyperplasia and breast carcinoma *in situ*. So we used immunohistochemistry staining (SP-method) to detect the expression of VEGF and MVD count in breast benign disease, breast atypical hyperplasia and breast carcinoma *in situ* and to study the relationship between VEGF expression, MVD count and clinicopathologic features.

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MATERIALS AND METHODS

Patients

We collected 100 cases with breast benign disease (including 35 cases with breast fibroid tumor，35 cases with breast cystic hyperplasia and 30 cases with intraductal papilloma) 15 cases with breast atypical hyperplasia and 25 cases with breast carcinoma *in situ* in the Affiliated Kailuan Hospital from Jan, 1997 to Dec, 2007. The patients were all females and they did not receive radiotherapy or chemotherapy before operation

Immunohistochemistry Staining

The main reagents were products of Zhongshan Corporation(Beijing, China). Specimens were cut into 4 μm thick and dewaxed. Immunohistochemistry staining was undergone according to the directory of SP box. For negative control PBS solution displaced primary antibody. Microvessel density (MVD) was evaluated by immunostaining of factor VIII-RA, following Weidner's method[1]. The sections were first observed under low-power filed (×100) optic lens to determine neoformative vascular density in the tumor. High density showed an active neoformative vascular region. And then under the high-power filed (×200), random observation was performed in 3 fields of tumor microvessel on every section. Their average counts served as MVD of the tumor. VEGF staining showed brown granules in cell plasm of tumor cells. Three high power fields were selected, and a sample with less than or equal to 25% positive staining cells was defined as(-), then a sample with more than 25% positive staining cells was defined as(+)

Statistical Analysis

χ^2 test and rank sum test were performed by SPSS10.0 for windows, $P<0.05$ was considered significant.

RESULTS

Expression of VEGF in Breast Benign Disease

In breast fibroid tumor group, VEGF positive rate was 20.00% (8/40), in breast cystic hyperplasia group, VEGF positive rate was 22.50%(9/40), in intraductal papilloma group, VEGF positive rate was 23.86%(8/35). We did not find significant difference among the three groups about expression of VEGF($P>0.05$).

Expression of VEGF in Breast Benign Disease, Breast Atypical Hyperplasia, and Breast Carcinoma *in situ*

In breast benign disease group, VEGF positive rate was 21.74% (25/115), in breast atypical hyperplasia group, VEGF positive rate was 31.58% (6/19), in breast carcinoma in situ group, VEGF positive rate was 53.13%(17/32). The distribution of VEGF positive cells were not equal in breast carcinoma *in situ*, strong expression ones located mainly near basement membrane. Breast benign disease group was the lowest, breast carcinoma *in situ* group was the highest. The expression of VEGF increased gradually in the three groups ($P<0.05$).

MVD Count in Breast Benign Disease

In breast fibroid tumor group, MVD count was 15.13±2.71; In breast cystic hyperplasia group, MVD count was 13.45±2.34; In intraductal papilloma group, MVD count was 14.68±2.33. We did not find significant difference among the three groups about MVD count ($P>0.05$).

MVD Count in Breast Benign Disease, Breast Atypical Hyperplasia, and Breast Carcinoma *in situ*

In breast benign disease group, MVD count was 14.41±2.59, in breast atypical hyperplasia group, MVD count was 18.89±4.47, in breast carcinoma *in situ* group, MVD count was 21.13±4.12. Breast benign disease group was the lowest, breast carcinoma *in situ* group was the highest. The MVD count of the three groups increased gradually ($P<0.05$).

Relationship between MVD Count and VEGF

In VEGF positive group, MVD count was 19.41±4.78; In VEGF negative group MVD count was 14.91±3.15. MVD count was higher in VEGF positive group than that in VEGF negative group ($P<0.05$).

DISCUSSION

Angiogenesis is the formation of new blood vessels from the preexisting vascular network. Tumor can not exceed 1-2 mm in size without angiogenesis[2]. Folkman[3] first indicated that the growth of tumor relies on angiogenesis in 1971. Weidner[1] first reported the concept and measure method of MVD in 1991.

Ferrara[4] first found VEGF in 1989 and now we know there is a existing a VEGF family-VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E[5], PLGF[6] and many VEGF receptors including VEGFR-1 (Flt-1), VEGFR-2 (Kdr/Flk-1), VEGFR-3 (Flt-4)[7] and Neuropilins (NP-1, NP-2)[8].

Lots of scholars[9-14] had discussed the relationship between VEGF and MVD in tumors, and most of them believed that the MVD count increased significantly with the increase of the expression of VEGF. In our study, we also find MVD count was higher in VEGF positive group