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Relationship of vascular endothelial growth factor expression and microvessel density of clinicopathological features of triple negative-breast cancer

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Abstract: Objective To detect the expression of vascular endothelial growth factor (VEGF) and microvessel density in cancer tissues of breast cancer patients, and to analyze their relationship with clinicopathological characteristics of breast cancer. **Methods** Ninety-six breast cancer patients treated at the Fourth Affiliated Hospital of Nanjing Medical University from December 2021 to December 2022 were selected as the research subjects. According to whether the pathology was confirmed as triple-negative breast cancer (TNBC), they were divided into the TNBC group (24 cases) and the non-TNBC group (72 cases). Immunohistochemistry was used to detect the expression of VEGF protein in breast cancer tissues, and microvessel density was evaluated by immunohistochemical labeling of endothelial cells followed by counting the number of microvessels. The relationship between the two and the clinicopathological characteristics of breast cancer was analyzed. **Results** The positive rate of VEGF in TNBC patients (66.67%) was higher than that in non-TNBC patients (25.00%), with a significant difference (χ^2 =13.662, P<0.01); the microvessel density in TNBC patients (66.04±10.29) was higher than that in non-TNBC patients (61.07±10.36), with a significant difference (t=2.039, P=0.044). In the TNBC group, the positive expression rate of VEGF in patients with clinical stage I-II and no lymph node metastasis was significantly lower that in patients with clinical stage III and lymph node metastasis (P<0.05), and the microvascular density in patients with tumor diameter ≥2 cm and clinical stage I-II was significantly higher. clinical stage I-II was significantly higher than that in patients with tumor diameter <2 cm and clinical stage III (P<0.05). In non-TNBC group, there was no significant difference in VEGF positive expression rate and microvessel density between patients with different In non-TNBC group, there was no significant difference in VEGF positive expression rate and microvessel density between patients with different clinicopathological characteristics (P > 0.05). **Conclusion** In TNBC patients, the high expression of VEGF and the increase of microvessel density are related to the tumor diameter, clinical stage and lymph node metastasis of TNBC, which is helpful to predict the prognosis of patients and provide a new way to target VEGF pathway or microvessel therapy.

Keywords: Breast cancer, triple-negative; Immunohistochemical staining; Vascular endothelial growth factor; Microvessel density; Clincopathological feature

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The diagnosis of breast cancer usually relies on a variety of methods, such as clinical examination, imaging (mammography, ultrasound), etc. and histopathologic examination [1]. The incidence of triple-negative breast cancer (TNBC) is as high as 10.0%-20.8% among breast cancers, and due to its special biological and clinicopathological characteristics, higher requirements for its diagnosis and treatment should be put forward [2]. Efforts are being made to identify new drug targets against TNBC to improve patient survival and quality of life [3]. Vascular endothelial growth factor (VEGF) is a cytokine that plays an important role in tumor progression, which can promote angiogenesis, provide nutrient and oxygen supply to tumors, and increase their invasiveness and metastasis [4]. Microvessel density is the number of microvessels per unit area, and is an important means of evaluating tumor neovascularization in tumor research. The higher the microvessel density, the greater the ability of tumor growth, invasion and metastasis [5]. So far, there are relatively few in-depth studies on VEGF expression and microvessel density in the field of TNBC. Therefore, the present study is intended to take TNBC as the research object and take VEGF and microvessel density as the entry point to explore the correlation between VEGF and microvessel density in TNBC, so as to provide new ideas for the treatment of TNBC.

1 Materials and methods

1.1 General information

Patients with breast cancer admitted to the Fourth Affiliated Hospital of Nanjing Medical University from December 2021 to December 2022 were retrospectively collected, and 96 cases met the inclusion and exclusion criteria, of which 24 were TNBC and 72 were non-TNBC. The study was ethically reviewed by the Ethics Committee of the Fourth Affiliated Hospital of Nanjing Medical University (Ethics No. 20240814-K084).

1.2 Inclusion and exclusion criteria

Inclusion criteria: (1) meet the diagnostic criteria for breast cancer [6]; (2) ≥ 30 years; (3) complete clinicopathologic data; (4) VEGF expression and microvessel density can be tested with the completeness and reliability of the samples.

Exclusion criteria: (1) Combined with some other serious diseases or complications, which affected the results of the study; (2) Incomplete medical records or obvious errors; (3) Refused to participate in the study or withdrew from the study during the study period; (4) Family history of other tumors.

1.3 Methods

1.3.1 Examination and treatment

All patients with breast cancer were admitted to the hospital for registration of baseline data, imaging examination, blood sample collection, and clarification of past medical history.

1.3.2 Clinical data collection

Baseline data were collected by physicians through telephone, interview, access to the hospital's electronic case system, and questionnaires included (1) baseline characteristics: age, gender, and previous cancer history (2) Clinical and pathological features: tumor size, clinical stage, lymph node status, and depth of tumor infiltration.

1.4 Diagnosing criteria of TNBC

(1) Human epidermal growth factor receptor 2 (HER2) negative: first of all, the surgical excision specimen of breast cancer should be tested by immunohistochemistry (IHC), and IHC +++ should be judged as HER2 positive, IHC 0 and + are judged as HER2 negative, IHC ++ need to further apply fluorescence *in situ* hybridization (FISH) method to detect the amplification status of *HER2* gene to judge whether HER2 is positive or not. If the result is negative and there is no gene amplification, it can be judged as HER2 negative. (2) Negative is judged when the expression level of estrogen receptor (ER) is very low or not expressed at all by IHC assay. (3) If the expression level of progesterone receptor (PR) is very low or completely absent by IHC, it will be judged as negative. Breast cancer can only be judged as TNBC if all three of the above indicators are negative.

1.5 VEGF expression and microvessel density detection

The expression of VEGF protein in the tissues was detected by IHC; microvessel density detection was assessed by IHC labeling of endothelial cells and then counting the number of microvessels. Detection of VEGF protein expression: Samples were collected using ethylenediaminetetraacetic acid (EDTA) tube and sent to the medical laboratory department for molecular diagnostics. Microvessel density detection: tumor tissues were prepared into 4-μm- or 5-μm-thick tissue sections, then appropriate vascular endothelial cell (EC) markers [CD34 antibody and factor VIII antibody] were selected for labeling microvessels in the tissue sections. IHC was used to for detecting microvessels in the tissue sections. The entire tissue section was scanned under the low magnification field of view (×40, ×100), and the optical microscope field of view showed the densest number of microvessels, clear EC staining, and good contrasts. The number of all stained microvessels was counted at 200× field of view (0.72 mm^2) within the above selected field of view. The number of microvessels in each of the three fields of view from the three microvessel-dense areas was counted separately, and the mean number of microvessels was used to express the microvessel density.

1.6 Statistical methods

SPSS 26.0 was used for data processing. Count data were expressed as cases (%) using chi-square test, corrected chi-square test or Fisher exact test. Measurement data conforming to normal distribution were expressed as $\bar{x} \pm s$ using independent samples t-test. If P<0.05, then the differences are statistically significant.

2 Results

2.1 Description of general clinicopathological characteristics of patients in the two groups

No statistically significant differences were found between the two groups in terms of age, menstrual status, rate of lymph node metastasis and family history (P>0.05). The proportion of patients with tumour diameter ≥2 cm, clinical stage III and with lymph node metastasis was higher in the TNBC group than in the non-TNBC group. The differences were all statistically significant (P <0.05). **[Table 1]**

Tab.1 Baseline clinicopathological characteristics of patients in two groups [case (%)]

Item	TNBC (n=24)	Non-TNBC (n=72)	χ^2 value	P value
Age				
>45 years	11 (45.83)	25 (34.72)	0.948	0.330
≤45 years	13 (54.17)	47 (65.28)		
Tumor diameter				
<2 cm	6 (25.00)	35 (48.61)	4.101	0.043
≥2 cm	18 (75.00)	37 (51.39)		
Clinical staging				0.039
Phase I-II	19 (79.17)	40 (55.56)	4.236	
Phase III	5 (20.83)	32 (44.46)		
Menstrual status				
non-menopausal	11 (45.83)	23 (31.94)	1.518	0.218
menopausal	13 (54.17)	49 (68.06)		
Family history				
+	3 (12.50)	6 (8.33)	0.041	0.839
-	21 (87.50)	66 (91.67)		
Lymph node metastasis				
+	14 (58.33)	23 (31.94)	5.292	0.021
-	10 (41.67)	49 (68.06)		

2.2 Expression levels of VEGF and microvessel density detection in breast cancer

TNBC patients had a higher rate of positive VEGF expression (16/24, 66.67%) than non-TNBC patients (18/72, 25.00%), with a statistically significant difference (χ^2 =13.662, P=0.01); and the microvessel density value (66.04±10.29) was higher than that of non-TNBC patients (61.07±10.36), with a statistically significant difference (t=2.039, P=0.044).

2.3 Relationship between VEGF expression, microvessel density in TNBC and clinicopathologic features

In the TNBC group, the positive VEGF expression rate was significantly higher in patients with clinical stage I-II and no metastasis in lymph nodes than in patients with clinical stage III and metastasis in lymph nodes (P<0.05), and the difference of the VEGF expression rate in patients with other different clinicopathologic features was not statistically significant (P>0.05), see Table 2. The difference in microvessel density values between patients of different ages, menstrual status, lymph node metastasis, and family history was not statistically significant (P > 0.05); the microvessel density values of patients with tumor diameters of ≥2 cm and clinical stage I to II were greater than those of patients with tumor diameters of <2 cm and clinical stage III, and the difference was statistically significant (P < 0.05) . **[Table 3]**

2.4 Relationship between VEGF expression, microvessel density in non-TNBC and clinico-pathologic features

Among the non-TNBC patients, the differences in VEGF-positive expression rates among patients with different clinicopathologic features were not statistically significant (P>0.05). **[Table 4]** The differences in microvessel density values among patients with different pathologic features were not statistically significant (P>0.05). **[Table 5]**

Tab.2 Association between VEGF expressions and clinico-pathological characteristics in TNBC patients [case(%)]

Item	Case	VEGF-positive (n=16)	VEGF-negative (n=8)	P value
Age				
>45 years	11	7 (43.75)	4 (50.00)	1.000
≤45 years	13	9 (56.25)	4 (50.00)	
Tumor diameter				
<2 cm	6	3 (18.75)	3 (37.50)	0.362
≥2 cm	18	13 (81.25)	5 (62.50)	
Clinical staging				
Phase I-II	5	1 (8.33)	4 (50.00)	0.028
Phase III	19	15 (93.75)	4 (50.00)	
Menstrual status				
non-menopausal	11	8 (50.00)	3 (37.50)	0.679
menopausal	13	8 (50.00)	5 (62.50)	
Family history				
+	3	1 (6.25)	2 (25.00)	0.249
-	21	15 (93.75)	6 (75.00)	
Lymph node metastasis				
+	14	12 (75.00)	2 (25.00)	0.032
-	10	4 (25.00)	6 (75.00)	

Note: Fisher's exact test was used in this table.

Tab.3 Association between microvessel density values and clinicopathological characteristics in TNBC patients ($\bar{x} \pm s$)

Item	Case	Microvascular density	t value	P value
Age				
>45 years	11	67.73±9.11	0.731	0.472
≤45 years	13	64.62±11.35		
Tumor diameter				
<2 cm	6	57.17±8.95	2.773	0.011
≥2 cm	18	69.00±9.08		
Clinical staging				
Phase I-II	5	53.20±5.12	4.058	0.001
Phase III	19	69.42±8.46		
Menstrual state				
non-menopausal	11	62.82±10.27	1.445	0.162
menopausal	13	68.77±9.87		
Family history				
+	3	64.33±11.93	0.301	0.766
-	21	66.29±10.34		
Lymph node metastasis				
+	14	65.71±9.89	0.181	0.858
-	10	66.50±11.35		

Tab.4 Relationship between VEGF expressions and clinicopathological characteristics in non-TNBC patients[case(%)]

Item	Case	VEGF-positive (n=18)	VEGF-negative (n=54)	χ^2 value	P value
Age					
>45 years	25	7 (38.89)	18 (33.33)	0.184	0.668
≤45 years	47	11 (61.11)	36 (66.67)		
Tumor diameter					
<2 cm	35	12 (66.67)	23 (42.59)	3.132	0.077
≥2 cm	37	6 (33.33)	31 (57.41)		
Clinical staging					
Phase I-II	32	5 (27.78)	27 (50.00)	2.700	0.100
Phase III	40	13 (72.22)	27 (50.00)		
Menstrual state					
non-menopausal	23	9 (50.00)	14 (25.93)	3.599	0.058
menopausal	49	9 (50.00)	40 (74.07)		
Family history					
+	6	3 (16.67)	3 (5.56)	0.970	0.325
-	66	15 (83.33)	51 (94.44)		
Lymph node metastasis					
+	23	8 (44.44)	15 (27.78)	1.725	0.189
-	49	10 (55.56)	39 (72.22)		

Tab.5 Association between microvessel density values and clinico- pathological characteristics in non-TNBC patients ($\bar{x} \pm s$)

Item	Case	Microvascular density	*t* value	*P* value
Age				
>45 years	25	63.84±9.43	1.677	0.098
≤45 years	47	59.60±10.62		
Tumor diameter				
<2 cm	35	62.06±12.01	0.785	0.435
≥2 cm	37	60.14±8.58		
Clinical staging				
Phase I-II	32	62.94±9.57	1.378	0.173
Phase III	40	59.58±10.83		
Menstrual state				
non-menopausal	23	58.50±9.80	1.515	0.134
menopausal	49	62.43±10.47		
Family history				
+	6	56.50±14.34	1.131	0.262
-	66	61.48±9.96		
Lymphatic node metastasis				
+	23	63.48±10.73	1.360	0.178
-	49	59.94±10.09		

3 Discussion

Globally, there are approximately 1.68 million new cases of breast cancer each year, and the incidence is closely related to a variety of factors [7]. TNBC is a uniquely characterized type of breast cancer that is highly invasive, prone to metastasis, and has a poor prognosis [8]. Compared with other types of breast cancer, TNBC lacks sensitivity to endocrine therapy and targeted therapy, and treatment options are limited [9-10]. Malignant tumors are seriously damaging diseases characterized by the uncontrolled development of abnormal cells [11-12]. The research aims to investigate the relationship between VEGF expression, microvessel density and the clinicopathologic features of breast cancer, which is expected to provide more basic research data for clinical treatment.

Several studies have shown that VEGF expression is associated with microvessel density and TNBC clinicopathologic features. Feng et al. [13] showed that VEGF positive rate was significantly higher in tissues with lymph node metastasis than in tissues without lymph node metastasis in 102 specimens. Li *et al.* [14] found that VEGF expression was high in TNBC, and that the metastasis rate of lymph nodes, the degree of lymphatic infiltration, and the microvessel density of the tumor tissue were significantly higher than other types of breast cancer. The study of Tian et al.[15] also showed that VEGF positive rate correlated with the clinical pathology of patients' tumor size and clinical stage. Sun *et al.* [16] analyzed 89 samples with breast cancer and showed that microvessel density values were significantly higher in patients with breast cancer and axillary lymph node metastasis. Li and Jitariu *et al.*[17-18] also concluded that the microvessel density of patients with TNBC were correlated with tumor size, clinical stage, and lymph node metastasis.

In this study, VEGF expression and microvessel density were correlated with tumor size, clinical stage and lymph node metastasis in TNBC patients. The mechanism may be that bulky tumor cells often crave for more blood flow supply to obtain the required nutrients and oxygen in many cases. In this process, the level of VEGF expression in the tumor may rise, which stimulates and accelerates angiogenesis and angiectasis. In terms of clinical stage, as the disease progresses, the invasiveness of the tumor will change, requiring more neovascularization to support its growth and metastasis. Levels of VEGF expression and microvessel density may change accordingly. In tumor spreading and invasion, lymph node metastasis is a key step. Tumor cells need to rely on the neovascular network to provide nutrients and oxygen. VEGF plays a crucial role in this process, which not only promotes angiogenesis, but also regulates the density of the microvessels [19]. Based on the above results, it can be analyzed that the high expression of VEGF and higher microvessel density may suggest a poor prognosis. On the contrary, lower microvessel density and VEGF expression may reflect to a certain extent that the growth and spread of the tumor is somewhat limited, which may be associated with a better prognosis [20].

In summary, VEGF expression and microvessel density correlate with tumor size, clinical stage and lymph node metastasis in TNBC patients, which is helpful in predicting the prognosis of patients and may provide a new idea for targeting the VEGF pathway or microvessels.

Conflict of interest None

Reference

[1] Wu S, Jiang ZF.Adjuvant therapy strategies for breast cancer based on the efficacy of neoadjuvant therapy[J].Chin J Surg, 2024, 62(2):104-109. **[In Chinese]**
[2] Han JS, Xie XH, Wu MW, et al. Progress in immunotherapy of triple negative breast cancer[J]. Tumor, 2022, 42(9): 591-601. **[In Chinese]**
[3] Derakhshan F, Reis-Filho JS. Pathogenesis of triple-negative breast cancer[J]. Annu Rev Pathol, 2022, 17: 181-204.
[4] Zhao S, Ma D, Xiao Y, et al. Molecular subtyping of triple-negative breast cancers by immunohistochemistry: molecular basis and clinical relevance[. J]. 2020, 25(10): e1481-e1491.
[5] Ribatti D, Nico B, Ruggieri S, et al. Angiogenesis and antiangiogenesis in triple-negative breast cancer[J]. Transl Oncol, 2016, 9(5): 453-457.
[6] Yin L, Duan JJ, Bian XW, et al. Triple-negative breast cancer molecular subtyping and treatment progress[J]. Breast Cancer Res, 2020, 22(1): 61.
[7] Akram M, Iqbal M, Daniyal M, et al. Awareness and current knowledge of breast cancer [J]. Biol Res, 2017, 50(1): 33.
[8] Yang F, Xiao Y, Ding JH, et al. Ferroptosis heterogeneity in triple-negative breast cancer reveals an innovative immunotherapy combination strategy[J]. Cell Metab, 2023, 35(1): 84-100.e8.
[9] Zhang RN, Yang YJ, Dong WJ, et al. D-mannose facilitates immunotherapy and radiotherapy of triple-negative breast cancer via degradation of PD-L1[J J]. Proc Natl Acad Sci U S A, 2022, 119(8): e2114851119.
[10] Akingbesote ND, Owusu D, Liu R, et al. A review of the impact of energy balance on triple-negative breast cancer[J]. J Natl Cancer Inst Monogr, 2023, 2023(61): 104-124.
[11] Wei XX, Chen YH, Jiang XJ, et al. Mechanisms of vasculogenic mimicry in hypoxic tumor microenvironments[J]. Mol Cancer, 2021,

20(1): 7.
[12] Sadick M, Müller-Wille R, Wildgruber M, et al. Vascular anomalies (part I): classification and diagnostics of vascular anomalies[J]. Rofo, 2018, 190(9): 825-835.
[13] Feng CB. Expression and significance of EGFR and VEGF in triple-negative breast cancer[J]. China Mod Dr, 2017, 55(14): 32-34, 37.**[In Chinese]**
[14] Li ZY, Wang SM, Wang P, et al. The relationship between VEGF expression, microvessel density, lymphatic vessel density and clinicopathological The relationship between VEGF expression, microvessel density, lymphatic vessel density and clinicopathological characteristics of triple negative breast cancer and non triple negative breast cancer [C]//Proceedings of the 13th National Ultrasound Medical Academic Conference of the Chinese Medical Association Kunming, 2013: 449. **[In Chinese]**
[15] Tian QG, Wu YT, Liu Y, et al. Expressions and correlation analysis of HIF-1α, survivin and VEGF in patients with hepatocarcinoma[J]. Eur Rev Med Pharmacol Sci, 2018, 22(11): 3378-3385.
[16] Sun J. Relationship between VEGF expression and microvessel density and clinicopathological features of triple negative breast cancer and non-triple negative breast cancer[D]. Nanning: Guangxi Medical University, 2010. **[In Chinese]**
[17] Li C, Li R, Song HT, et al. Significance of AEG-1 expression in correlation with VEGF, microvessel density and clinicopathological characteristics in triple-negative breast cancer[J]. J Surg Oncol, 2011, 103(2): 184-192.
[18] Jitariu AA, Cîmpean AM, Ribatti D, et al. Triple negative breast cancer: the kiss of death[J]. Oncotarget, 2017, 8(28): 46652-46662.
[19] Bender RJ, Mac Gabhann F. Expression of VEGF and *semaphorin* genes define subgroups of triple negative breast cancer[J]. PLoS One, 2013, 8(5): e61788.
[20] Choi SY, Chang YW, Park HJ, et al. Correlation of the apparent diffusion coefficiency values on diffusion-weighted imaging with prognostic factors for breast cancer[J]. 2012, 85(1016): e474-e479.

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·论　著·

血管内皮生长因子表达及微血管密度与三阴性乳腺癌临床病理特征的关系

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摘要：目的　检测乳腺癌患者癌组织中血管内皮生长因子(VEGF)表达及微血管密度,分析其与乳腺癌临床病理特征的关系。**方法**　将 2021 年 12 月至 2022 年 12 月南京医科大学第四附属医院收治的 96 例乳腺癌患者作为研究对象,根据病理是否确诊为三阴性乳腺癌(TNBC),分为 TNBC 组 24 例,非 TNBC 组 72 例。采用免疫组织化学(IHC)法检测乳腺癌组织中 VEGF 蛋白的表达;通过 IHC 标记内皮细胞并计数微血管数量,即微血管密度。分析两者与乳腺癌临床病理特征的关系。**结果**　TNBC 患者 VEGF 阳性表达率(66.67%)高于非 TNBC 患者(25.00%),差异有统计学意义($\chi^2=13.662$, $P<0.01$);微血管密度值(66.04±10.29)高于非 TNBC 患者(61.07±10.36),差异有统计学意义($t=2.039$, $P=0.044$)。TNBC 组中,临床分期Ⅰ~Ⅱ期、淋巴结无转移患者的 VEGF 阳性表达率显著低于临床分期Ⅲ期、淋巴结有转移患者($P<0.05$);肿瘤直径≥2 cm、临床分期Ⅲ期患者的微血管密度值大于肿瘤直径<2 cm、临床分期Ⅰ~Ⅱ期患者,差异有统计学意义($P<0.05$)。非 TNBC 组中,不同临床病理特征患者的 VEGF 阳性表达率及微血管密度值差异均无统计学意义($P>0.05$)。**结论**　在 TNBC 患者中,VEGF 高表达、微血管密度增高,二者与 TNBC 的肿瘤直径、临床分期、淋巴结转移有一定相关性,对患者预后的预测有一定帮助,可为靶向 VEGF 通路或微血管的治疗提供新途径。

关键词： 乳腺癌，三阴性；免疫组织化学染色；血管内皮生长因子；微血管密度；临床病理特征

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Abstract: Objective　To detect the expression of vascular endothelial growth factor (VEGF) and microvessel density in cancer tissues of breast cancer patients, and to analyze their relationship with clinicopathological characteristics of breast cancer. **Methods**　Ninety-six breast cancer patients treated at the Fourth Affiliated Hospital of Nanjing Medical University from December 2021 to December 2022 were selected as the research subjects. According to whether the pathology was confirmed as triple-negative breast cancer (TNBC), they were divided into the TNBC group (24 cases) and the non-TNBC group (72 cases). Immunohistochemistry was used to detect the expression of VEGF protein in breast cancer tissues, and microvessel density was evaluated by immunohistochemical labeling of endothelial cells followed by counting the number of microvessels. The relationship between the two and the clinicopathological characteristics of breast cancer was analyzed. **Results**　The positive rate of VEGF in TNBC patients(66.67%) was higher than that in non-TNBC patients (25.00%), with a significant difference ($\chi^2=13.662$, $P<0.01$); the microvessel density in TNBC patients(66.04±10.29) was higher than that in non-TNBC patients (61.07±10.36), with a significant difference ($t=$

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2.039, $P=0.044$). In the TNBC group, the positive expression rate of VEGF in patients with clinical stage Ⅰ-Ⅱ and no lymph node metastasis was significantly lower than that in patients with clinical stage Ⅲ and lymph node metastasis ($P<0.05$), and the microvascular density in patients with tumor diameter≥2 cm and clinical stage Ⅲ was significantly higher than that in patients with tumor diameter<2 cm and clinical stage Ⅰ-Ⅱ ($P<0.05$). In non-TNBC group, there was no significant difference in VEGF positive expression rate and microvessel density between patients with different clinicopathological characteristics ($P>0.05$). **Conclusion** In TNBC patients, the high expression of VEGF and the increase of microvessel density are related to the tumor diameter, clinical stage and lymph node metastasis of TNBC, which is helpful to predict the prognosis of patients and provide a new way to target VEGF pathway or microvessel therapy.

Keywords: Breast cancer, triple-negative; Immunohistochemical staining; Vascular endothelial growth factor; Microvessel density; Clincopathological feature

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乳腺癌的诊断通常依赖多种方法,如临床检查、影像学检查(乳腺 X 线摄影、超声)等以及组织病理学检查[1]。三阴性乳腺癌(triple-negative breast cancer, TNBC)在乳腺癌中的发病率为 10.0%~20.8%,由于其特殊的生物学特性和临床病理特征,对其诊断和治疗提出了更高的要求[2]。目前学者正致力于寻找新的针对 TNBC 的药物靶标,以改善患者的生存质量[3]。血管内皮生长因子(vascular endothelial growth factor, VEGF)是一种在肿瘤进展过程中发挥重要作用的细胞因子,能够促进血管生成,为肿瘤提供营养和氧气供应,增加肿瘤的侵袭性和转移能力[4]。微血管密度是单位面积上的微血管数目,在肿瘤研究中,其是评价肿瘤新生血管的重要手段。微血管密度越高,肿瘤生长、侵袭及转移的能力越强[5]。到目前为止,在 TNBC 这一领域中,对于 VEGF 表达以及微血管密度的深入探究相对较少。因此,本课题拟以 TNBC 患者为研究对象,探讨 VEGF 和微血管密度与 TNBC 的相关性,为 TNBC 的治疗提供新的思路。

1　资料与方法

1.1　一般资料　回顾性收集 2021 年 12 月至 2022 年 12 月南京医科大学第四附属医院收治的乳腺癌患者,符合纳入排除标准 96 例,其中有 24 例 TNBC 和 72 例非 TNBC 的患者。本研究通过南京医科大学第四附属医院伦理委员会伦理审查(伦理编号 20240814-K084)。

1.2　纳入及排除标准　纳入标准:(1) 符合乳腺癌诊断标准[6];(2) 年龄≥30 岁;(3) 有完整的临床病理资料;(4) 能够进行 VEGF 表达和微血管密度的检测,确保样本的完整性和可靠性。排除标准:(1) 合并其他严重疾病或出现严重并发症,影响研究结果;(2) 病历资料不完整或存在明显错误;(3) 拒绝参与研究;(4) 有其他肿瘤家族史。

1.3　方法

1.3.1　检查与治疗　所有乳腺癌患者入院后,均进行一般资料登记、影像学检查和血样采集,明确既往病史。

1.3.2　临床资料收集　医生通过电话、访谈、查阅医院电子病历系统等方式收集患者的资料并负责填写调查问卷,包括(1) 患者基本资料:年龄、性别、既往肿瘤史等。(2) 临床和病理特征:肿瘤大小、临床分期、淋巴结状态、肿瘤浸润深度等。

1.4　TNBC 的判定标准　(1) 人表皮生长因子受体 2(human epidermal growth factor receptor 2, HER2)阴性:首先对乳腺癌手术切除标本做免疫组织化学(immunohistochemistry, IHC)检测,IHC +++ 判断为 HER2 阳性,IHC 0 和+则判断为 HER2 阴性。IHC ++者需进一步应用荧光原位杂交(Fluorescence in situ hybridization, FISH)的方法进行 *HER2* 基因扩增状态检测以确定 HER2 是否为阳性。若结果为阴性,且无基因扩增,则可判定为 HER2 阴性。(2) 利用 IHC 检测手段,发现雌激素受体(ER)的表达水平很低或根本没有表达,即可判定为阴性。(3) 通过 IHC 检测,若孕激素受体(PR)表达水平极低或完全缺失,则判定为阴性。只有当上述三个指标均为阴性时,乳腺癌才能被判定为 TNBC。

1.5　VEGF 表达及微血管密度检测方法　采用 IHC 法检测组织中 VEGF 蛋白的表达情况;通过 IHC 标记内皮细胞,然后计数微血管数量来评估微血管密度。VEGF 表达检测:使用粉盖促凝管(含分离胶)采集样本,采集后及时送至检验科分子诊断中心进行处理。微血管密度检测:将肿瘤组织制备成 4 μm 或

5 μm厚的组织切片;选择适当的血管内皮细胞(EC)标记物,抗原簇(CD)34 抗体和第八因子抗体,用于标记和检测组织切片中的微血管;采用 UltraSensitive SP 超敏浓缩试剂盒的 IHC 技术,对组织切片中的微血管进行标记和检测;在光学显微镜低倍视野下(×40,×100)扫视整个组织切片,找到微血管数量最密集、EC 染色清晰、背景对比良好的视野。在上述选定视野范围内,以 200 倍视野(0.72 mm^2)计数所有染色的微血管数。分别计数 3 个微血管密集区的 3 个视野的微血管数,以其均数表示观察组织标本的微血管计数,即微血管密度。

1.6　统计学方法　采用 SPSS 26.0 软件进行数据处理。计数资料用例(%)表示,采用 χ^2 检验、校正 χ^2 检验或 Fisher 确切概率检验;计量资料用 $\bar{x}\pm s$ 表示,采用独立样本 t 检验。$P<0.05$ 为差异有统计学意义。

2 结　果

2.1　两组患者一般临床病理特征描述　两组年龄、月经状况和家族史差异无统计学意义($P>0.05$)。TNBC 组肿瘤直径≥2 cm、临床分期Ⅲ期、有淋巴结转移患者占比高于非 TNBC 组,差异均有统计学意义($P<0.05$)。见表 1。

2.2　两组乳腺癌患者中 VEGF 表达、微血管密度检测结果　TNBC 组患者的 VEGF 阳性表达率(16/24, 66.67%)高于非 TNBC 组患者(18/72, 25.00%),差异有统计学意义($\chi^2=13.662$, $P<0.01$);TNBC 组患者的微血管密度值(66.04±10.29)高于非 TNBC 组患者(61.07±10.36),差异有统计学意义($t=2.039$, $P=0.044$)。

2.3　TNBC 患者中 VEGF 的表达及微血管密度与临床病理特征的关系　TNBC 患者中,临床分期Ⅲ期、淋巴结有转移患者的 VEGF 阳性表达率显著高于临床分期Ⅰ~Ⅱ期、淋巴结无转移患者($P<0.05$),VEGF 表达率在其他不同临床病理特征患者中差异均无统计学意义($P>0.05$),见表 2。不同年龄、月经状态、有无淋巴结转移、有无家族史患者的微血管密度值差异无统计学意义($P>0.05$);肿瘤直径≥2 cm、临床分期Ⅲ期患者的微血管密度值大于肿瘤直径<2 cm、临床分期Ⅰ~Ⅱ期患者,差异有统计学意义($P<0.05$)。见表 3。

2.4　非 TNBC 患者中 VEGF 的表达及微血管密度与临床病理特征的关系　非 TNBC 患者中,不同临床病理特征患者的 VEGF 阳性表达率比较差异均无统计学意义($P>0.05$),见表 4。不同病理特征患者的微血管密度值比较差异也均无统计学意义($P>0.05$),见表 5。

表 1　两组患者一般临床病理特征　[例(%)]

Tab. 1　Baseline clinicopathological characteristics of patients in two groups　[case(%)]

项目	TNBC($n=24$)	非 TNBC($n=72$)	χ^2 值	P 值
年龄				
>45 周岁	11(45.83)	25(34.72)	0.948	0.330
≤45 周岁	13(54.17)	47(65.28)		
肿瘤直径				
<2 cm	6(25.00)	35(48.61)	4.101	0.043
≥2 cm	18(75.00)	37(51.39)		
临床分期				
Ⅰ~Ⅱ期	5(20.83)	32(44.46)	4.236	0.039
Ⅲ期	19(79.17)	40(55.56)		
月经状态				
未绝经	11(45.83)	23(31.94)	1.518	0.218
已绝经	13(54.17)	49(68.06)		
家族史				
有	3(12.50)	6(8.33)	0.041	0.839
无	21(87.50)	66(91.67)		
淋巴结转移				
是	14(58.33)	23(31.94)	5.292	0.021
否	10(41.67)	49(68.06)		

表 2　TNBC 患者中 VEGF 的表达与临床病理特征的关系　[例(%)]

Tab. 2　Association between VEGF expressions and clinicopathological characteristics in patients with TNBC　[case(%)]

项目	例数	VEGF 阳性($n=16$)	VEGF 阴性($n=8$)	P 值
年龄				
>45 周岁	11	7(43.75)	4(50.00)	1.000
≤45 周岁	13	9(56.25)	4(50.00)	
肿瘤直径				
<2 cm	6	3(18.75)	3(37.50)	0.362
≥2 cm	18	13(81.25)	5(62.50)	
临床分期				
Ⅰ~Ⅱ期	5	1(8.33)	4(50.00)	0.028
Ⅲ期	19	15(93.75)	4(50.00)	
月经状态				
未绝经	11	8(50.00)	3(37.50)	0.679
已绝经	13	8(50.00)	5(62.50)	
家族史				
有	3	1(6.25)	2(25.00)	0.249
无	21	15(93.75)	6(75.00)	
淋巴结转移				
是	14	12(75.00)	2(25.00)	0.032
否	10	4(25.00)	6(75.00)	

注:本表均采用 Fisher 确切概率检验。

表 3　TNBC 患者中微血管密度值与临床病理特征的关系　($\bar{x}\pm s$)

Tab. 3　Association between microvessel density values and clinicopathological characteristics in TNBC patients　($\bar{x}\pm s$)

项目	例数	微血管密度	t 值	P 值
年龄				
>45 周岁	11	67.73±9.11	0.731	0.472
≤45 周岁	13	64.62±11.35		
肿瘤直径				
<2 cm	6	57.17±8.95	2.773	0.011
≥2 cm	18	69.00±9.08		
临床分期				
Ⅰ～Ⅱ期	5	53.20±5.12	4.058	0.001
Ⅲ期	19	69.42±8.46		
月经状态				
未绝经	11	62.82±10.27	1.445	0.162
已绝经	13	68.77±9.87		
家族史				
有	3	64.33±11.93	0.301	0.766
无	21	66.29±10.34		
淋巴结转移				
是	14	65.71±9.89	0.181	0.858
否	10	66.50±11.35		

表 4　非 TNBC 患者中 VEGF 的表达与临床病理特征的关系　[例(%)]

Tab. 4　Relationship between VEGF expressions and clinicopathological characteristics in non-TNBC patients　[case(%)]

项目	例数	VEGF 阳性表达 (n=18)	VEGF 阴性表达 (n=54)	χ^2 值	P 值
年龄					
>45 周岁	25	7(38.89)	18(33.33)	0.184	0.668
≤45 周岁	47	11(61.11)	36(66.67)		
肿瘤直径					
<2 cm	35	12(66.67)	23(42.59)	3.132	0.077
≥2 cm	37	6(33.33)	31(57.41)		
临床分期					
Ⅰ～Ⅱ期	32	5(27.78)	27(50.00)	2.700	0.100
Ⅲ期	40	13(72.22)	27(50.00)		
月经状态					
未绝经	23	9(50.00)	14(25.93)	3.599	0.058
已绝经	49	9(50.00)	40(74.07)		
家族史					
有	6	3(16.67)	3(5.56)	0.970	0.325
无	66	15(83.33)	51(94.44)		
淋巴结转移					
是	23	8(44.44)	15(27.78)	1.725	0.189
否	49	10(55.56)	39(72.22)		

3 讨　论

在全球,每年新发的乳腺癌病例数量大约有 168 万,其形成原因与多种因素密切相关[7]。TNBC 是一种具有独特特征的乳腺癌,其侵袭力强,易发生转移,且预后不佳[8]。与其他类型的乳腺癌相比,TNBC 对内分泌治疗和靶向治疗缺乏敏感性,治疗选择较为有限[9-10]。近年研究认为,血管生成拟态是侵袭性肿瘤新生血管形成的新模型,可以为肿瘤生长提供血液供

表 5　非 TNBC 患者中微血管密度值与临床病理特征的关系　($\bar{x}\pm s$)

Tab. 5　Association between microvessel density values and clinicopathological characteristics in non-TNBC patients　($\bar{x}\pm s$)

项目	例数	微血管密度	t 值	P 值
年龄				
>45 周岁	25	63.84±9.43	1.677	0.098
≤45 周岁	47	59.60±10.62		
肿瘤直径				
<2 cm	35	62.06±12.01	0.785	0.435
≥2 cm	37	60.14±8.58		
临床分期				
Ⅰ～Ⅱ期	32	62.94±9.57	1.378	0.173
Ⅲ期	40	59.58±10.83		
月经状态				
未绝经	23	58.50±9.80	1.515	0.134
已绝经	49	62.43±10.47		
家族史				
有	6	56.50±14.34	1.131	0.262
无	66	61.48±9.96		
淋巴结转移				
是	23	63.48±10.73	1.360	0.178
否	49	59.94±10.09		

应,因此,血管生成拟态抑制剂与常规抗血管生成治疗联合可能是一种提高靶向血管生成治疗效果的有前途的策略[11-12]。本研究从该视角出发,探讨 VEGF 表达及微血管密度与乳腺癌临床病理特征的关系,期望能为临床治疗提供参考。

多项研究显示,VEGF 表达与微血管密度和 TNBC 临床病理特征相关。冯传宝[13]通过对 102 例 TNBC 的研究表明,VEGF 阳性率在有淋巴结转移组织中显著高于无淋巴结转移组织;李紫瑶等[14]将 100 例乳腺癌患者分组进行分析后发现,在 TNBC 中,VEGF 呈现出高表达状态,并且淋巴结的转移率、淋巴管的浸润程度以及肿瘤组织的微血管密度都明显提高;同时 Tian 等[15]的研究也显示 VEGF 阳性率与肝癌患者的肿瘤大小和临床分期之间有关联;孙洁[16]对 89 例乳腺癌样本进行了分析,结果显示在有腋窝淋巴结转移的乳腺癌患者中,其微血管密度值显著偏高;Li 和 Jitariu 等[17-18]的研究也认为,微血管密度值在 TNBC 患者中与肿瘤大小、临床分期和淋巴结转移有关。本研究发现,VEGF 表达及微血管密度与 TNBC 患者的肿瘤大小、临床分期和淋巴结转移具有相关性。分析原因:在许多情况下,体积庞大的肿瘤细胞常常需要更多的血流供应,以便能够获得所需的养分与氧气,这一过程中,肿瘤内 VEGF 的水平可能会上升,从而刺激和加速血管的生成和扩展;在临床分期方面,随着病情进展,肿瘤的侵袭性会发生改变,需要更多的血管新生来支持其生长和转移,VEGF 表达和微血管密度可能会随之变化;在癌症的扩散和侵

袭中,淋巴结的转移是一个关键步骤,为了完成这一过程,肿瘤细胞需要依赖新生血管网络来提供营养和氧气,VEGF 则在此过程中扮演了至关重要的角色,它不仅促进了血管的生成,而且还调节着微血管的密度[19]。根据以上结果可分析出 VEGF 的高表达以及较高的微血管密度可能提示预后不良,相反,较低的微血管密度和 VEGF 的表达可能在一定程度上反映出肿瘤的生长和扩散受到一定限制,可能与较好的预后相关[20]。

综上所述,在 TNBC 患者中 VEGF 表达及微血管密度与肿瘤大小、临床分期及淋巴结转移相关,对患者预后的预测有一定帮助,可为靶向 VEGF 通路或微血管的治疗提供新思路。

利益冲突　无

参考文献

[1] 吴淞,江泽飞.基于新辅助治疗效果的乳腺癌辅助治疗策略[J].中华外科杂志, 2024, 62(2):104-109.
Wu S, Jiang ZF. Adjuvant therapy strategies for breast cancer based on the efficacy of neoadjuvant therapy[J]. Chin J Surg, 2024, 62(2): 104-109.

[2] 韩佳澍,谢霄鹤,吴孟闻,等.三阴性乳腺癌免疫治疗进展[J].肿瘤,2022,42(9):591-601.
Han JS, Xie XH, Wu MW, et al. Progress in immunotherapy of triple negative breast cancer[J]. Tumor, 2022, 42(9): 591-601.

[3] Derakhshan F, Reis-Filho JS. Pathogenesis of triple-negative breast cancer[J]. Annu Rev Pathol, 2022, 17: 181-204.

[4] Zhao S, Ma D, Xiao Y, et al. Molecular subtyping of triple-negative breast cancers by immunohistochemistry: molecular basis and clinical relevance[J]. 2020, 25(10): e1481-e1491.

[5] Ribatti D, Nico B, Ruggieri S, et al. Angiogenesis and antiangiogenesis in triple-negative breast cancer[J]. Transl Oncol, 2016, 9(5): 453-457.

[6] Yin L, Duan JJ, Bian XW, et al. Triple-negative breast cancer molecular subtyping and treatment progress[J]. Breast Cancer Res, 2020, 22(1): 61.

[7] Akram M, Iqbal M, Daniyal M, et al. Awareness and current knowledge of breast cancer[J]. Biol Res, 2017, 50(1): 33.

[8] Yang F, Xiao Y, Ding JH, et al. Ferroptosis heterogeneity in triple-negative breast cancer reveals an innovative immunotherapy combination strategy[J]. Cell Metab, 2023, 35(1): 84-100.e8.

[9] Zhang RN, Yang YJ, Dong WJ, et al. D-mannose facilitates immunotherapy and radiotherapy of triple-negative breast cancer *via* degradation of PD-L1[J]. Proc Natl Acad Sci U S A, 2022, 119(8): e2114851119.

[10] Akingbesote ND, Owusu D, Liu R, et al. A review of the impact of energy balance on triple-negative breast cancer[J]. J Natl Cancer Inst Monogr, 2023, 2023(61): 104-124.

[11] Wei XX, Chen YH, Jiang XJ, et al. Mechanisms of vasculogenic mimicry in hypoxic tumor microenvironments[J]. Mol Cancer, 2021, 20(1): 7.

[12] 张宇飏,王洪江.乳腺癌脉管标记物的临床应用与研究进展[J].中国临床研究,2024,37(3):329-333.
Zhang YY, Wang HH. Clinical application and research progress of lymphovascular markers for breast cancer[J]. Chin J Clin Res, 2024, 37(3): 329-333.

[13] 冯传宝.EGFR 与 VEGF 在三阴性乳腺癌中的表达及意义[J].中国现代医生,2017,55(14):32-34,37.
Feng CB. Expression and significance of EGFR and VEGF in triple-negative breast cancer[J]. China Mod Dr, 2017, 55(14): 32-34, 37.

[14] 李紫瑶,王淑梅,王平,等.VEGF 表达及微血管密度、淋巴管密度与三阴性乳腺癌及非三阴性乳腺癌临床病理特征的关系[C]//中华医学会第十三次全国超声医学学术会议论文集.昆明,2013:449.
Li ZY, Wang SM, Wang P, et al.The relationship between VEGF expression, microvessel density, lymphatic vessel density and clinicopathological characteristics of triple negative breast cancer and non triple negative breast cancer [C]//Proceedings of the 13th National Ultrasound Medical Academic Conference of the Chinese Medical Association Kunming, 2013: 449.

[15] Tian QG, Wu YT, Liu Y, et al. Expressions and correlation analysis of HIF-1α, survivin and VEGF in patients with hepatocarcinoma[J]. Eur Rev Med Pharmacol Sci, 2018, 22(11): 3378-3385.

[16] 孙洁.VEGF 表达及微血管密度与三阴性乳腺癌及非三阴性乳腺癌临床病理特征的关系[D].南宁:广西医科大学,2010.
Sun J. Relationship between VEGF expression and microvessel density and clinicopathological features of triple negative breast cancer and non-triple negative breast cancer[D]. Nanning: Guangxi Medical University, 2010.

[17] Li C, Li R, Song HT, et al. Significance of AEG-1 expression in correlation with VEGF, microvessel density and clinicopathological characteristics in triple-negative breast cancer[J]. J Surg Oncol, 2011, 103(2): 184-192.

[18] Jitariu AA, Cîmpean AM, Ribatti D, et al. Triple negative breast cancer: the kiss of death[J]. Oncotarget, 2017, 8(28): 46652-46662.

[19] Bender RJ, Mac Gabhann F. Expression of *VEGF* and *semaphorin* genes define subgroups of triple negative breast cancer[J]. PLoS One, 2013, 8(5): e61788.

[20] Choi SY, Chang YW, Park HJ, et al. Correlation of the apparent diffusion coefficiency values on diffusion-weighted imaging with prognostic factors for breast cancer[J]. Br J Radiol, 2012, 85(1016): e474-e479.

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