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Original Article

Differential expression of EGFR and VEGF between Han and Uygur patients with triple-negative breast cancer

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Abstract: This study is to analyze the expression of epidermal growth factor receptor (EGFR) and vascular endothelial growth factor (VEGF) in Han and Uygur patients with triple-negative breast cancer (TNBC). Totally 187 female TNBC patients, 134 Han and 53 Uygur, were included. Expression of EGFR and VEGF was detected with immunohistochemistry. Association between EGFR/VEGF expression, lymph node metastasis, and TNM staging were assessed. Immunohistochemistry showed that, the positive expression rates of EGFR and VEGF in Han TNBC patients were significantly lower than Uygur TNBC patients. Association analysis showed that, the EGFR/VEGF expression was positively associated with lymph node metastasis in the Han and Uygur TNBC patients. Highest expression levels of EGFR and VEGF were noted in the sub-groups with ≥ 4 metastatic lymph nodes. Moreover, the expression levels of EGFR and VEGF were positively associated with the TNM staging in the Han and Uygur TNBC patients. In the follow-up period, our results indicated no differences in the 5-year disease-free survival rate between the Han and Uygur TNBC patients. Recurrence and metastasis were noted in 24.6% (33/134) of the Han TNBC patients and in 32.1% (17/53) of the Uygur TNBC patients, without statistically significant differences. Expression levels of EGFR and VEGF in the Han TNBC patients were significantly lower than the Uygur TNBC patients, without significantly different 5-year disease-free survival rates. EGFR and VEGF could be considered as independent prognostic factors for TNBC, which might contribute to the disease diagnosis and treatment in clinic concerning different ethnics.

Keywords: Triple-negative breast cancer (TNBC), endothelial growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), Han and Uygur

Introduction

Triple-negative breast cancer (TNBC) refers to breast cancer lacking the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2). TNBC accounts for about 15% of all the breast cancer cases, especially with high incidences in young African American females [1, 2]. It has been found that, TNBC shares the same genotypes with the majority of basal cell breast cancers [1]. Compared with non-TNBC, TNBC is associated with increased invasion ability, higher risk of recurrence and metastasis, decreased survival rate, and poorer prognosis. Moreover, young and elderly TNBC patients have comparable survival rates in clinic [2, 3].

Epidermal growth factor receptor (EGFR) is the expression product of oncogene C-erbB1, which is involved in a series of cellular signal transduction pathways. EGFR is expressed in epithelial, mesenchymal, and neural tissues, and EGFR signaling pathway plays important roles in various physiological processes, including cell growth, proliferation, and differentiation [4]. On the other hand, vascular endothelial growth factor (VEGF) is an important angiogenesis regulatory factor during mitosis in endothelial cells. VEGF overexpression could induce the division, proliferation, and migration of vascular endothelial cells. Moreover, VEGR has also been shown to be closely related to tumor growth process, which would promote neovascularization and tumor angiogenesis [5]. EGFR and VEGF are both important gene regulators during the pathogenesis of malignant tumors (including TNBC), interacting with signaling pathways involved in tumor angiogenesis [6].

China is a vast multi-ethnic country, and Chinese people share substantially similar genetic background, accompanied with individual local

characteristics. In recent years, the differences in the TNBC incidence and prognosis, as well as expression of related genes, between different ethnics, have attracted more and more attention. In this study, the expression levels of EGFR and VEGR in Han and Uygur female patients with TNBC in Xinjiang, China, were analyzed and compared. Moreover, the relationship between the EGFR/VEGR expression, lymph node metastasis, and TNM staging was also investigated and discussed.

Materials and methods

Study subjects

Totally 187 TNBC patients (134 Han and 53 Uygur) were included in this study, who were admitted to our hospital from January 2008 to January 2010. Inclusion criteria were as follows: (1) Han or Uygur female breast cancer patients who received the first treatment in our hospital; (2) patients with complete clinical and pathological data; (3) patients negative for ER, PR and HER-2 expression (confirmation of TNBC). Prior written and informed consent were obtained from every patient and the study was approved by the ethics review board of Xinjiang Medical University.

Immunohistochemistry

Expression levels of EGFR and VEGF were detected with the PV-9000 immunohistochemical method (ZSGB-BIO, Beijing, China), according to the manufacturer's instructions. After washing with PBS, the tissue section was treated with 3% H_2O_2 at room temperature for 5-10 min. Then the section was incubated with mouse anti-human EGFR antibody (1:100 dilution; ZSGB-BIO), or mouse anti-human VEGF antibody (1:100 dilution; ZSGB-BIO), at 37°C for 1.5 h. After washing with PBS, the section was treated with poly pelper reagent 1 at room temperature for 20 min, followed by the incubation with polyer peroxidase-anti-mouse IgG at room temperature for 20 min. After coloration with DAB reagent for 5 min, the section was washed, dehydrated, sealed, and then observed. Known cancer tissues and tissues samples treated with PBS were used as positive and negative controls, respectively.

The result interpretation for EGFR was as follows: 0, negative expression (no staining or non-specific staining); 1, low positiveness (> 10% of the vision field exhibiting brown or tan staining, with no continuous membrane staining); 2, moderate positiveness (> 10% of the vision field exhibiting brown or tan staining, with continuous membrane staining, without complete morphology); and 3, strong positiveness (> 10% of the vision field exhibiting brown or tan staining, with continuous membrane staining and complete morphology). On the other hand, for VEGF: 0, negative experssion (brown staining in cytoplasm accounting for < 25%); 1, low positiveness (brown staining in cytoplasm accounting for 25%-50%); 2, moderate positiveness (brown staining in cytoplasm accounting for 50%-75%); and 3, strong positiveness (brown staining in cytoplasm accounting for > 75%). Considering the scores for EGFR and VEGF staining, the total score ≤ 2 was regarded as negative expression (-), and total score > 3 was regarded as positive expression (+).

Follow-up observation

Follow-up investigation included admission review and telephone follow-up interviews, which started immediately after the first chemotherapy following surgery. Recurrence and metastasis were considered as the termination events. The follow-up period lasted for 5 y, which ended on January 30, 2015. The prognosis indicators included the recurrence proportion, 5-year disease-free survival rate, and expression of EGFR and VEGF, as well as its relationship with lymph node metastasis and TNM staging.

Statistical analysis

SPSS 18.0 software was used for statistical analysis. The χ^2 test was performed for group comparison. Spearman rank correlation analysis was used to analyze the association between the EGFR/VEGF expression, lymph node metastasis, and TNM staging. $P < 0.05$ was considered as statistically significant.

Results

Expression of EGFR and VEGF in Han and Uygur TNBC patients

To investigate the expression of EGFR and VEGF in Han and Uygur TNBC patients, PV9000 immunohistochemistry was performed. Our results showed that, positive staining of EGFR and VEGF was mainly observed in cell cyto-

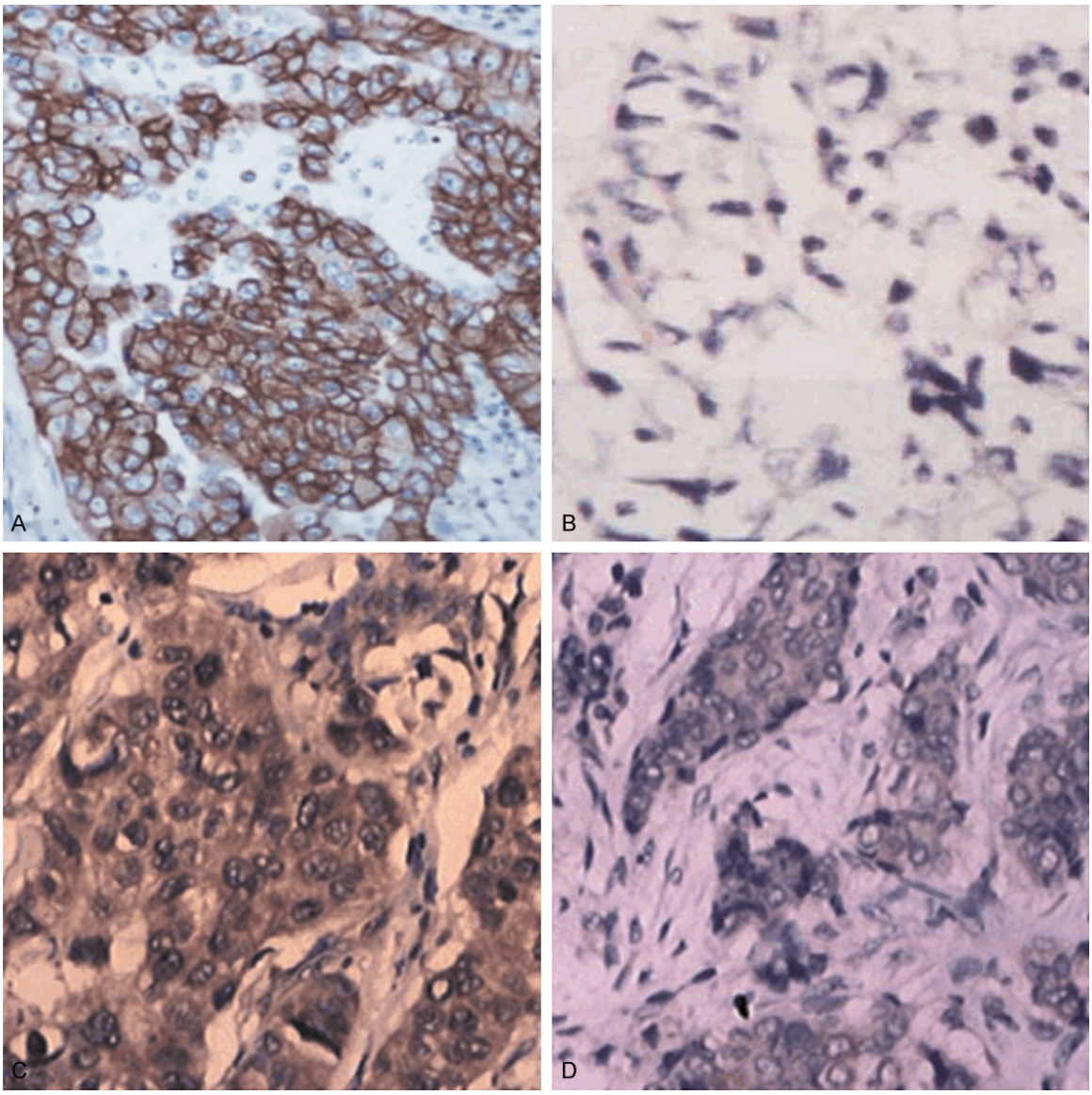

Figure 1. Expression of EGFR and VEGF in Han and Uygur TNBC patients. The expression of EGFR (A) and VEGF (C) in Han and Uygur TNBC patients were detected with PV9000 immunohistochemistry (×200). Corresponding negative controls were shown in (B) and (D), respectively.

plasm and on cellular membrane (**Figure 1**). As shown in **Table 1**, for EGFR, the positive expression rate in Han TNBC patients was 40.3% (54/134), which was significantly lower than the positive expression rate 56.6% (30/53) in Uygur TNBC patients ($P < 0.05$). On the other hand, similar results were observed for the expression of VEGF. The positive expression rate of VEGF in Han TNBC patients was 48.8% (65/134), which was significantly lower than the positive expression rate of 66.0% (35/53) in Uygur TNBC patients ($P < 0.05$). These results suggest that, the positive expression rates of VEGF and EGFR in the Han TNBC patients are significantly lower than the Uygur patients.

Association between EGFR/VEGF expression and lymph node metastasis in Han and Uygur TNBC patients

Association between the EGFR/VEGF expression and lymph node metastasis in these Han and Uygur TNBC patients was next analyzed. According to the lymph node metastasis conditions, both the Han and Uygur TNBC groups were divided into three sub-groups (with 0, 1-3, and ≥ 4 metastatic lymph nodes, respectively). Our results showed that, in both Han and Uygur groups, the expression levels of EGFR and VEGF were increased along with increasing metastatic lymph nodes (**Table 2**). Highest

Table 1. Expression of EGFR and VEGF in Han and Uygur TNBC patients

	EGFR		VEGF	
	Positive (+)	Negative (-)	Positive (+)	Negative (-)
Han, % (n)	40.3% (54/134)	59.7% (80/134)	48.5% (65/134)	51.5% (69/134)
Uygur, % (n)	56.6% (30/53)	43.4% (23/53)	66.0% (35/53)	34.0% (18/53)

Table 2. Association between EGFR/VEGF expression and lymph node metastasis in Han and Uygur TNBC patients

	Metastatic lymph notes	EGFR positive rate, % (n)	χ^2	*r*	VEGF positive rate, % (n)	χ^2	*r*
Han	0	20.0% (10/50)	23.01	0.404	32.0% (16/50)	17.80	0.345
	1-3	39.2% (20/51)			45.1% (23/51)		
	≥ 4	72.7% (24/33)			78.8% (26/33)		
Uygur	0	36.8% (7/19)	6.99	0.368	47.4% (9/19)	6.24	0.343
	1-3	55.6% (10/18)			66.7% (12/18)		
	≥ 4	81.3% (13/16)			87.5% (14/16)		

Table 3. Association between EGFR/VEGF expression and TNM staging in Han and Uygur TNBC patients

	TNM staging	EGFR positive rate, % (n)	χ^2	*r*	VEGF positive rate, % (n)	χ^2	*r*
Han	I	18.6% (8/43)	24.01	0.411	23.3% (11/43)	23.33	0.417
	II	36.4% (20/55)			49.1% (27/55)		
	III	72.2% (26/36)			77.8% (28/36)		
Uygur	I	25.0% (3/12)	8.779	0.404	14.5% (4/12)	8.566	0.384
	II	54.5% (12/22)			68.2% (15/22)		
	III	78.9% (15/19)			84.2% (16/19)		

expression rates of EGFR and VEGF were noted in the sub-groups with ≥ 4 metastatic lymph nodes. The EGFR expression rate in the Han TNBC patients with ≥ 4 metastatic lymph nodes was 72.7%, while the EGFR positive rate was 81.3% in corresponding Uygur patients. On the other hand, the EGFR expression rates in the Han and Uygur TNBC patients with ≥ 4 metastatic lymph nodes were 78.8% and 87.5%, respectively. According to the association analysis, the expression of EGFR and VEGF was positively associated with the metastatic lymph node numbers in both the Han and Uygur TNBC groups ($0 < r < 1$; $P < 0.05$).

Association between EGFR/VEGF expression and TNM staging in Han and Uygur TNBC patients

Association between the EGFR/VEGF expression and TNM staging in the Han and Uygur TNBC patients was then analyzed. According to the TNM staging, these TNBC patients were divided into Stages I, II, and III. Our results showed that, differences in the expression of EGFR and VEGF were observed between different TNM stages in both Han and Uygur TNBC patients ($P < 0.05$) (**Table 3**). For both Han and Uygur groups, the expression rates of EGFR and VEGF for patients at Stage I were significantly lower than the expression rates for patients at Stages II and III. Moreover, the association analysis showed that, the expression of EGFR and VEGF was positively associated with the TNM staging in both the Han and Uygur TNBC patients ($0 < r < 1$; $P < 0.05$).

Analysis of recurrence and metastasis in Han and Uygur TNBC patients

Recurrence and metastasis conditions in Han and Uygur TNBC patients were investigated during the 5-year follow-up period. Our results showed that, recurrence and metastasis were noted in 24.6% (33/134) of the Han TNBC patients, while 32.1% (17/53) of the Uygur TNBC patients were associated with recurrence and metastasis ($P > 0.05$) (**Table 4**). No signifi-

Table 4. Recurrence and metastasis in Han and Uygur TNBC patients

	Han, % (n)	Uygur, % (n)	OR	CI, 95%	*P*
Local recurrence	10.44% (14)	15.09% (8)	0.692	0.308-1.553	0.450
Distant metastasis	20.89% (28)	24.52% (13)	0.852	0.479-1.500	0.695
Local distant metastasis					
Bone	10.44% (14)	16.98% (9)	0.615	0.284-1.335	0.225
Lung	14.92% (20)	16.98% (9)	0.879	0.428-1.805	0.823
Other sites	8.21% (11)	5.66% (3)	0.544	0.232-1.277	0.182
Total	24.6% (33)	32.1 (17)	0.768	0.470-1.255	0.360

cant differences were observed in the local recurrence, distant metastasis, and metastatic sites between the Han and Uygur TNBC patients ($P > 0.05$). Moreover, no significant differences were observed in the 5-year disease-free survival rate between Han and Uygur TNBC patients. Taken together, these results suggest that, there are no significant differences in the recurrence and metastasis rate between Han and Uygur TNBC patients.

Discussion

Pathogenesis and development of breast cancers are related to various genetic, physiological, and environmental factors. Since Perou *et al.* [7] proposed the concept of TNBC, the disease has become the research hot spot due to its rapid development, poor prognosis, and complex molecular characteristics. In China, current clinical reports of TNBC mainly focus on the Han people, without comparing the pathogenic processes among different ethnics. In the present study, the expression of EGFR and VEGF in TNBC was investigated and compared between the Han and Uygur patients.

EGFR is one member of the epidermal growth factor receptor family, which plays important roles in the cell growth, proliferation, and differentiation. EGFR is a transmembrane protein encoded by the oncogene C-erbB1, and is mainly expressed in normal epithelial cells. It has been shown that, EGFR is often abnormally expressed and activated during tumor pathogenesis, which might be associated with the tumor angiogenesis, invasion, and metastasis [8]. The binding of EGFR and its ligand would lead to tyrosine phosphorylation and the subsequent activation of signal transduction pathways. For example, EGFR could activate the MAPK signaling pathway and stimulate the oncogene c-fos to enhance the malignant proliferation of cells, and induce the hormone-independent growth in TNBC cells [4]. There have already been molecular therapeutic drugs targeting on EGFR, one representative of which is gefitinib. Gefitinib mainly act on the tyrosine kinase in the intracellular domain of EGFR, competing with ATP and inhibiting its function. It has been found that, the expression levels of EGFR and p53 are significantly up-regulated, while PTEN expression level is down-regulated, in TNBC, which would synergistically induce the disease pathogenesis and development [9]. In this study, our results showed that, the EGFR expression rate was 45% (84/187) in total, in line with a previous report from Nielsen *et al.* [10], who have found that the expression rate of EGFR in TNBC is between 45%-70%. Moreover, our results showed that, the EGFR expression rate in the Han TNBC patients (40.3%) was significantly lower than the Uygur TNBC patients (56.6%). Further in-depth studies are still needed to find out the causes of this phenomenon.

VEGF is an angiogenesis regulatory factor, which specifically binds to vascular endothelial cells, stimulating endothelial cell proliferation and tumor angiogenesis [11]. Studies have shown that, the expression levels of VEGF were dramatically elevated in TNBC patients compared with non-TNBC patients, and the VEGF over-expression is associated with poor prognosis [12, 13]. VEGF has been gradually becoming a novel target for the treatment of TNBC [14, 15]. At present, there are mainly two kinds of therapeutic drugs targeting on VEGF: one kind is the anti-VEGF antibody (bevacizumab Avastin), and the other kind is endostatin (Endostar). Compared with paclitaxel, bevacizumab could dramatically improve the disease-free survival for TNBC. It has been shown that, combination of epirubicin, cyclophosphamide, docetaxel, and bevacizumab could increase the pathologic complete response rate in TNBC

patients, from 27.9% to 39.3% [16]. Our results showed that, the total expression rate of VEGF in Han and Uygur TNBC patients was 53.4% (100/187), which was in line with a previous finding from Yang *et al.* (45.0%) [17]. However, the VEGF expression rate in the Han TNBC patients (48.5%) was significantly lower than that in the Uygur TNBC patients (66.0%). Similar results were found about the EGFR expression levels. This difference might be caused by the relatively late staging of TNBC for the Uygur patients at admission.

The follow-up results indicated that, the recurrence rate within 5 y for the Han TNBC patients was 24.6%, while the recurrence rate for the Uygur patients was 32.1%. No differences were observed in the recurrence rate between the Han and Uygur TNBC patients. Then the association of EGFR/VEGF expression, lymph node metastasis, and TNM staging was investigated. Our results showed that EGFR and VEGF were over-expressed in TNBC, which were associated with the lymph node metastasis and TNM staging. Patients over-expressing EGFR/VEGF are prone to suffer from early recurrence and metastasis, and are generally related to poor prognosis [18]. In contrast, Jobim *et al.* [19] have found that, the lymph node metastasis, tumor size, and histological staging are not related to the expression of VEGF. A previous study has shown that, the protein expression levels would be dramatically changed during different stages of TNBC [20]. Our results showed that, the expression rates of EGFR and VEGF are significantly associated with the metastatic lymph node numbers and TNM staging in both Han and Uygur TNBC patients, suggesting that EGFR and VEGF might contribute to the pathogenesis and development of TNBC. Lymph node metastasis and TNM staging are important indicators for the clinical prognosis, and they are also important reference for the individual comprehensive treatment. Based on our results, the differential expression of EGFR/VEGF should be taken into consideration for the diagnosis and treatment of TNBC concerning different ethnics. The individual targeting therapeutic strategy might improve the cure rate and elevate the survival rate for the clinical treatment of TNBC.

In conclusion, our result showed that, the expression levels of EGFR and VEGF in the Han TNBC patients were significantly lower than those in the Uygur TNBC patients. However, no significant differences in the 5-year disease-free survival rate were observed between Han and Uygur TNBC patients. In addition, the EGFR/VEGF expression was positively associated with lymph node metastasis and TNM staging in TNBC. These results suggest that, EGFR and VEGF could be considered as independent prognostic factors for TNBC, whose over-expression might predict poorer disease prognosis. Moreover, different prognostic factors would exist for Han and Uygur TNBC patients. These findings might contribute to the analysis of TNBC concerning different ethnics and provide evidence for the understanding of prognostic factors for TNBC in clinic.

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Disclosure of conflict of interest

None.

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References

[1] Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, Lickley LA, Rawlinson E, Sun P and Narod SA. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007; 13: 4429-34.

[2] Millikan RC, Newman B, Tse CK, Moorman PG, Conway K, Dressler LG, Smith LV, Labbok MH, Geradts J, Bensen JT, Jackson S, Nyante S, Livasy C, Carey L, Earp HS and Perou CM. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat 2008; 109: 123-39.

[3] Bulut N and Altundag K. Excellent clinical outcome of triple-negative breast cancer in younger and older women. J BUON 2015; 20: 1276-81.

[4] Ciardiello F and Tortora G. EGFR antagonistsin cancer treatment. N Engl J Med 2008; 358: 1160-74.

[5] Kim MS, Park TI, Lee YM, Jo YM and Kim S. Expression of Id-1 and VEGF in non-small cell lung cancer. Int J Clin Exp Pathol 2013; 6: 2102-11.

[6] Normanno N, Campiglio M, Maiello MR, De Luca A, Mancino M, Gallo M, D'Alessio A and

Menard S. Breast cancer cells with acquired resistance to the EGFR tyrosine kinase inhibitor gefitinib show persistent activation of MAPK signaling. Breast Cancer Res Treat 2008; 112: 25-33.

[7] Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO and Botstein D. Molecular portraits of human breast tumours. Nature 2000; 406: 747-52.

[8] Sliwkowski MX and Mellman I. Antibody therapeutics in cancer. Science 2013; 341: 1192-8.

[9] Li X, Wang Q, Fu L, Liu M and Yu X. Expression of PTEN, p53 and EGFR in the molecular subtypes of breast carcinoma and the correlation among them. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2015; 40: 973-8.

[10] Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, Hernandez-Boussard T, Livasy C, Cowan D, Dressler L, Akslen LA, Ragaz J, Gown AM, Gilks CB, van de Rijn M and Perou CM. Immunohistochemical and clinica characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004; 10: 5367-74.

[11] Kim MS, Park TI, Lee YM, Jo YM and Kim S. Expression of Id-1 and VEGF in non-small cell lung cancer. Int J Clin Exp Pathol 2013; 6: 2102-11.

[12] Greenberg S and Rugo HS. Triple-negative breast cancer: role of antiangiogenic agents. Cancer J 2010; 16: 33-8.

[13] Linderholm BK, Hellborg H, Johansson U, Elmberger G, Skoog L, Lehtiö J and Lewensohn R. Significantly higher levels of vascula-r endothelial growth factor (VEGF) and shorter survival times for patients with primary operable triple-negative breast cancer. Ann Oncol 2009; 20: 1639-46.

[14] Bahhnassy A, Mohanad M, Shaarawy S, Ismail MF, El-Bastawisy A, Ashmawy AM and Zekri AR. Transforming growth factor-β, insulin-like growth factor I/insulin-like growth factor I receptor and vascular endothelial growth factor-A: prognostic and predictive markers in triple-negative and non-triple-negative breast cancer. Mol Med Rep 2015; 12: 851-64.

[15] Rydén L, Jirström K, Haglund M, Stål O and Fernö M. Epidermal growth factor receptor and vascular endothelial growth factor receptor-2 are specific biomarkers in triple-negative breast cancer. Results from a controlled randomized trial with long-term follow-up. Breast Cancer Res Treat 2010; 120: 491-8.

[16] von Minckwitz G, Eidtmann H, Rezai M, Fasching PA, Tesch H, Eggemann H, Schrader I, Kittel K, Hanusch C, Kreienberg R, Solbach C, Gerber B, Jackisch C, Kunz G, Blohmer JU, Huober J, Hauschild M, Fehm T, Müller BM, Denkert C, Loibl S, Nekljudova V, Untch M; German Breast Group and Arbeitsgemeinschaft Gynäkologische Onkologie-Breast Study Groups. Neoadjuvant chemotherapy and bevacizumab for HER2-negative breast cancer. N Engl J Med 2012; 366: 299-309.

[17] Yang YF, Liu J, Jiang ZS and Gu L. Expression of VEGF in TNBC and its clinical significance. Chinese J Clinical Oncology 2012; 39: 439-41.

[18] Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF and Ellis IO. prognostic markers in triple-negative breast cancer. Cancer 2007; 109: 25-32.

[19] Jobim FC, Schwartsmann G, Xavier NL, Uchoa Dde M, Saciloto M and Chemello N. Expression of MMP-9 and VEGF in breast cancer :correlation with other prognostic indicators. Rev Bras Ginecol Obstet 2008; 30: 287-93.

[20] Tsai CH, Chiu JH, Yang CW, Wang JY, Tsai YF, Tseng LM, Chen WS and Shyr YM. Molecular characteristics of recurrent triple-negative breast cancer. Mol Med Rep 2015; 12: 7326-34.