Observational Study

Medicine®

OPEN

Expression analysis of E-cad and vascular endothelial growth factor in triple-negative breast cancer patients of different ethnic groups in western China

Weihua Jiang, MD, Yongtao Li, MD, Jianghua Ou, PhD, Xiaowen Wang, MD, Chenguang Zhang, MD, Lina Yi, MD, Laiti Xue, MD, Mingshuai Zhang, MD*

Abstract

The aim of this article is to investigate the expression of E-cadherin (E-cad) and vascular endothelial growth factor (VEGF) in triple-negative breast cancer (TNBC) of Han and Uygur women patients in western China, and their relationship with clinical features of TNBC.

Totally, 172 cases of Han TNBC patients and 79 cases of Uighur TNBC patients were enrolled. The expressions of E-cad and VEGF were detected with immunohistochemistry. The correlation of E-cad and VEGF expression with lymph node metastasis, TNM stage, and histological grade were analyzed. The 5-year disease-free survival rate of the 2 groups was also evaluated.

There was no significant difference in the 5-year disease-free survival rate ($P > .05$) and the expression of E-cad between the 2 groups. The positive rate of VEGF in Han was significantly lower than that in Uygur ($P < .05$). The expression of E-cad was negatively correlated with lymph node metastasis, TNM stage, and histological grade ($-1 \leq r < 1$, $P < .05$). However, the expression of VEGF was positively correlated with lymph node metastasis and TNM staging ($0 < r < 1$, $P < 0.05$), but not with histological grading.

The expression of E-cad and VEGF and their relationship with clinical features of TNBC suggest that Uygur TNBC patients might have different prognostic factors as compared with Han patients.

Abbreviations: E-cad = E-cadherin, ER = estrogen receptor, HER-2 = human epidermal growth factor receptor 2, N-TNBC = non-triple-negative breast cancer, PR = progesterone receptor, TNBC = triple-negative breast cancer, VEGF = vascular endothelial growth factor.

Keywords: E-cadherin, Han, triple-negative breast cancer, Uighur, vascular endothelial growth factor

1. Introduction

Triple-negative breast cancer (TNBC) is currently the focus of breast cancer research. Researches[1,2] have demonstrated that the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2) are absent in patients with TNBC. Compared with non-triple-negative breast cancer (N-TNBC), patients with TNBC have short survival and poor clinical prognosis.[2] In addition, elderly and young patients have similar survival rates.[3]

E-cadherin (E-cad), the most important member of the cell adhesion molecule family, is synthesized by the cadherin gene located on chromosome 16q22.1.[4] E-cad is a cell adhesion molecule that mediates cell molecular recognition and binding, tumor infiltration, and metastasis.[4] It is composed of 3 domains, a cytoplasmic domain, a transmembrane domain, and an extracellular domain, which is bound to Ca^{2+}.[5] Highly conserved cytoplasmic domain of E-cad forms a complex with the actin fibers to maintain the stability of cell adhesion and polarity, mediate cell-cell adhesion, and involve in cell signaling.[6] Abnormalities of the E-cad expression can result in the metastasis of epithelial tumors from the primary tumor and across the basement membrane.[4,7]

Vascular endothelial growth factor (VEGF) is an important angiogenesis regulator identified in the process of mitosis of endothelial cells.[8] Overexpression of VEGF leads to the proliferation and migration of vascular endothelial cells.[9] VEGF promotes neovascularization and tumor angiogenesis, and therefore is closely associated with tumor growth.[10]

Studies[11,12] have shown that molecular characteristics of TNBC are different among ethnic groups in China. Compared with the Han, tumors in Uygur TNBC patients are large and staged late.[13,14] In this study, we detected the expression levels of E-cad and VEGF in Han and Uygur female TNBC

Editor: Alfons Lawen.

This work was supported by the Natural Science Foundation of the Xingjian Uyghur Autonomous Region (grant No. 2016D01C350).

Authors' contributions: Conception and design—WJ, MZ; acquiring and analysis of data—WJ, YL, JO, XW, CZ, LY, LX; drafting the manuscript—WJ, TL.

All authors read and approved the final manuscript.

The authors disclose no conflicts of interest.

Department of Breast Surgery, Affiliated Tumor Hospital, Xinjiang Medical University, Urumqi, P.R. China.

* Correspondence: Mingshuai Zhang, Department of Breast Surgery, Affiliated Tumor Hospital, Xinjiang Medical University, No.789, Suzhou East Road, Urumqi, 830011 Xinjiang, China (e-mail: 28394970@qq.com).

Medicine (2017) 96:42(e8155)

Received: 20 April 2017 / Received in final form: 11 August 2017 / Accepted: 1 September 2017

http://dx.doi.org/10.1097/MD.0000000000008155

patients in Xinjiang, China. Their relationship with prognosis was analyzed.

2. Materials and methods

2.1. Patients

A total of 251 cases of TNBC patients from the Affiliated Tumor Hospital between January 2011 and December 2011 were enrolled in this study. TNBC was confirmed by immunohistochemistry. There were 172 cases of Han patients and 79 cases of Uygur patients. The clinical data of patients were shown in Table 1. The inclusion criteria were as follow: subjects were Han or Uygur women breast cancer patient treated for the first time; the clinical and pathological data of subjects were complete; subjects had negative expressions of ER, PR, and HER-2. The exclusion criteria were as follow: the clinical and pathological data of subjects were incomplete; subjects were not TNBC patients. Breast cancer tissues were collected during surgery. Previous written and informed consent were obtained from every patient and the study was approved by the ethics review board of Xinjiang Medical University.

Table 1

The clinical data of patients.

Clinical data	Patients	
	Han	Uygur
Total	172	79
Age		
<40	46	24
≥40	126	55
Lymph node metastasis		
Positive	82	47
Negative	90	32
TNM staging		
I	56	20
II	74	31
III	42	28
histological grading		
1–2	120	49
3	52	30

2.2. Immunohistochemistry staining

The expression of E-cad and VEGF was measured by immunohistochemical staining using a PV-9000 kit (Zhong Shan-Golden Bridge Biological Technology CO., Ltd., Beijing, China). Briefly, tissues were fixed, embedded in paraffin, and cut into tissue sections. Then tissue sections were dewaxed in xylene and rehydrated in graded alcohols. Then sections were incubated with 0.3% hydrogen peroxide to inactivate endogenous peroxidase activity. Antigen retrieval was achieved by incubating with sodium citrate (pH 6.0). After blocking, sections were incubated with primary antibodies against E-cad and VEGF (ZSGB-bio, Beijing) at 37°C for 90 minutes. After washing with PBS, secondary antibodies of anti-mouse IgG were added and incubated for 20 minutes. Then sections were developed with DAB chromogenic reagent. Positive control was set up. For negative control, PBS instead of the primary antibody was used.

2.3. Evaluation of staining results

Cells with yellow or brown staining were positive cells. Based on the percentage of positive staining, immunohistochemistry staining results were scored as follows: score 0, positive staining percentage <25%; score 1, 25% to 50% of positive staining percentage; score 2, 50% to 75% of positive staining percentage and score 3, positive staining percentage >75%. According to the staining intensity, immunohistochemistry staining results were evaluated as follows: score 0, no staining; score 1, light yellow; score 2, brownish yellow and score 3, tan. The degree of staining was calculated by adding the scores of positive staining percentage and the intensity of staining. And the overall degree of staining was defined as follows: total score of ≤2 points was defined as negative staining and total score >3 points was defined as positive staining.

2.4. Follow-up

Follow-up was performed in 2 ways, hospital regular review and telephone. The start time of the operation was defined as the follow-up start time and the metastasis was the termination time. The follow-up time was in units of months and for a total of 5 year. The deadline was December 1, 2016.

2.5. Statistical analyses

The data were analyzed using SPSS 18.0 software. The χ^2 test was used to compare the differences between 2 ethnic groups. The relationships of E-cad, VEGF and the number of lymph node metastasis, TNM staging, and histological grading were compared by Spearman rank correlation analysis. Kaplan-Meier method was used for calculating 5-year disease-free survival and the log-rank test was used to compare the statistical difference. A P value <0.05 was considered statistically significant.

3. Results

3.1. Expression of E-cad and VEGF in TNBC patients of different ethnic groups

To investigate the expression of E-cad and VEGF in TNBC patients, immunohistochemical staining method was used. The results showed that the expression of E-cad and VEGF was located in the membrane and cytoplasm of the tumor cells, which were stained brown and tan (Fig. 1). As shown in Table 2, E-cad positive expression was found in 78 of 172 Han patients (45.3%) and 30 of 79 Uygur patients (38.0%). The positive expression of VEGF in Han and Uygur patients was 81 of 172 (47.1%) and 48 of 79 (60.8%). Statistically, the expression of VEGF in Han women was significantly lower than that in Uighur women ($P < .05$). However, the expression of E-cad showed no significant difference between the 2 groups. These results suggest that there are significant differences in expression of VEGF in TNBC patients of different ethnic groups.

3.2. Correlation of E-cad and VEGF with lymph node metastasis

The correlation between expression of E-cad and VEGF and lymph node metastasis was analyzed. As shown in Table 3, the positive expression of E-cad decreased with the increase of the number of lymph node metastasis. In the cases without lymph node metastasis, the expression of E-cad was 55.6% in Han and 56.3% in Uygur. In the case with lymph node metastasis, especially the number of metastases ≥4, the positive expression of

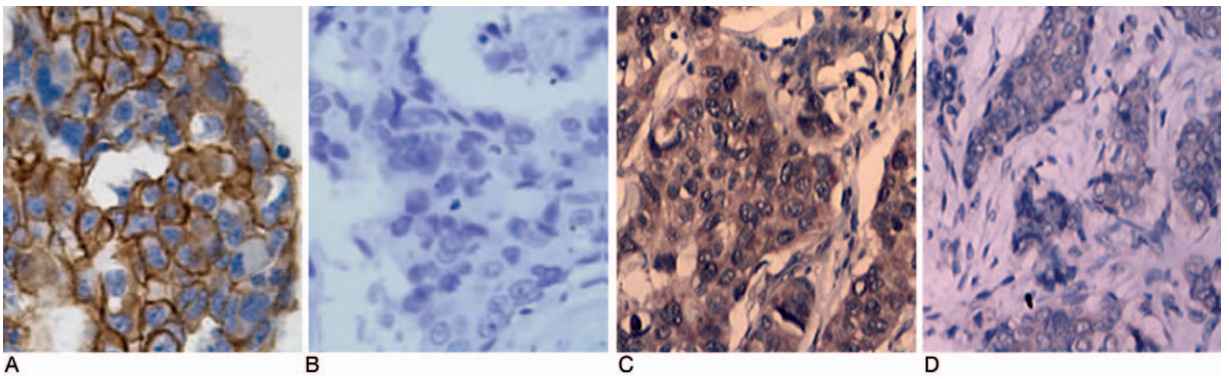

Figure 1. The expression of E-cad and vascular endothelial growth factors (VEGFs) detected by immunohistochemical straining. The expression of E-cad and VEGF was detected using PV9000 kit. The images were magnified at 200×. (A) The positive expression of E-cad. (B) The negative expression of E-cad. (C) The positive expression of VEGF. (D) The negative expression of VEGF.

Table 2

Comparison of the expression of E-cad and VEGF in the Han and Uygur TNBC patients.

Patients	E-cad		VEGF	
	Positive expression	Positive rate (%)	Positive expression	Positive rate (%)
Han (n = 172)	78	45.3	81	47.1
Uygur (n = 79)	30	38.0	48	60.8
χ^2	1.201		4.048	
P	0.273		0.044	

E-cad = E-cadherin, TNBC = triple-negative breast cancer, VEGF = vascular endothelial growth factor.

E-cad was only 32.1% in Han and 21.3% in Uygur. There was a negative correlation between the E-cad and lymph node metastasis ($-1 \leq r < 1$, $P < .05$). With the number of lymph node metastasis increased, positive expression of VEGF gradually increased. When the number of lymph node metastases was ≥4, the positive expression of VEGF was 71.4% in Han and 73.7% in Uygur, respectively. There was a positive correlation between the VEGF and lymph node grouping ($-1 \leq r < 1$, $P < .05$). These results indicate that lymph node metastasis is related to the loss of E-cad and the overexpression of VEGF in TNBC patients.

3.3. Correlation of E-cad and VEGF with TNM staging of different ethnic groups

The expression and correlation of E-cad and VEGF in different TNM stages were statistically analyzed in Han and Uygur women TNBC patients. The results showed that the expression of E-cad and VEGF in the TNM staging of TNBC were significantly different ($P < .05$) (Table 4). In both groups, the positive expression of E-cad in stage I was higher than that in stage II and III, whereas the positive rate of VEGF in stage III was higher than that in stage I and II. Correlation analysis showed that the expression of E-cad decreased gradually with the TNM clinical stage increased ($P < .05$), and the difference was statistically significant ($-1 \leq r < 1$, $P < .05$). Statistically, the positive rate of VEGF was gradually increased with the increase of TNM clinical stage ($0 < r < 1$, $P < .05$). These results demonstrate that TNM staging correlates with the loss of E-cad and the overexpression of VEGF in TNBC patients.

3.4. Correlation of E-cad and VEGF with histological grading of TNBC in different ethnic groups

The expression and correlation of E-cad and VEGF in different histological grades were statistically analyzed in Han and Uygur women TNBC patients. The results are shown in Table 5. The

Table 3

The expression of E-cad and VEGF in Han and Uygur TNBC patients with or without lymph node metastasis.

Patients	Lymph node metastasis	E-cad		Positive rate	Correlation		VEGF		Positive rate	Correlation	
		+	−	(%)	r	P	+	−	(%)	r	P
Han	0	50	40	55.6	−0.211	<.01	33	57	36.7	0.247	<.01
	1–3	19	35	35.2			28	26	51.9		
	≥4	9	19	32.1			20	8	71.4		
Uygur	0	18	14	56.3	−0.307	<.01	15	17	46.9	0.232	.040
	1–3	8	20	28.6			19	9	67.9		
	≥4	4	15	21.3			14	5	73.7		

+ = positive expression, − = negative expression, E-cad = E-cadherin, TNBC = triple-negative breast cancer, VEGF = vascular endothelial growth factor.

Table 4

The expression of E-cad and VEGF in TNM staging of Han and Uygur TNBC patients.

		E-cad		Positive rate	Correlation		VEGF		Positive rate	Correlation	
Patients	**Stage**	**+**	**−**	**(%)**	***R***	***P***	**+**	**−**	**(%)**	***r***	***P***
Han	Ⅰ	34	22	60.7	−0.259	<.01	15	41	26.8	0.335	<.01
	Ⅱ	33	41	44.6			36	38	48.6		
	Ⅲ	11	31	26.2			30	12	71.4		
Uygur	Ⅰ	13	7	65.0	−0.335	.003	6	14	30.5	0.333	.003
	Ⅱ	11	20	35.5			21	10	67.7		
	Ⅲ	6	22	21.4			21	7	75.0		

+ = positive expression, − = negative expression, E-cad = E-cadherin, TNBC = triple-negative breast cancer, VEGF = vascular endothelial growth factor.

Table 5

The expression of E-cad and VEGF in the histological grading of Han and Uygur TNBC patients.

		E-cad		Positive rate	Correlation		VEGF		Positive rate	Correlation	
Patients	**grade**	**+**	**−**	**(%)**	***r***	***P***	**+**	**−**	**(%)**	***r***	***P***
Han	1–2	61	59	50.8	−0.167	0.028	54	66	45.0	0.064	.406
	3	17	35	32.7			27	25	51.9		
Uygur	1–2	23	26	46.9	−0.236	0.036	27	22	55.1	0.148	.193
	3	7	23	23.3			21	9	70.0		

+ = positive expression, − = negative expression, E-cad = E-cadherin, TNBC = triple-negative breast cancer, VEGF = vascular endothelial growth factor.

results showed that the expression of E-cad in the Han and Uygur women were negatively correlated with histological grading ($-1 \leq r < 1, P < .05$). With the increase of histological grade, loss of E-cad expression was more obvious. There was no significant correlation between the expression of VEGF and the histological grading ($P > .05$). These results show that histological grading is associated with the loss of E-cad, but not with the overexpression of VEGF in TNBC patients.

3.5. Disease-free survival time

All patients were followed up regularly. Until December 2016, there were 44 (44/172, 25.6%) and 26 (26/79, 32.9%) case of TNBC patients with recurrence and metastasis in Han and Uighur, respectively. There was no significant difference between the 2 groups in the 5-year disease-free survival (Log rank = 1.566, $P = .211$) (Fig. 2).

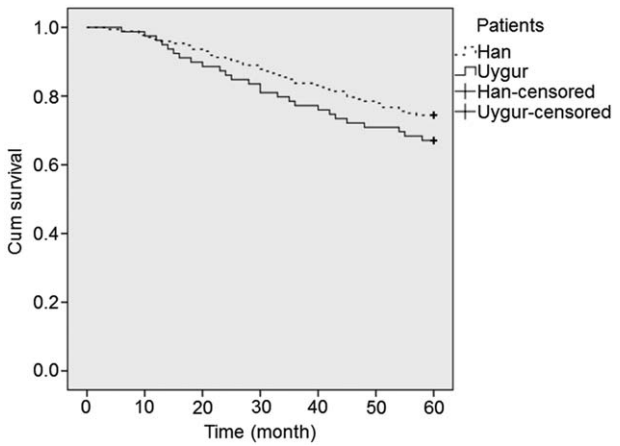

Figure 2. The 5-year disease-free survival of non-triple-negative breast cancer (TNBC) in Han and Uygur patients. The 5-year disease-free survival rate of TNBC was 74.4% in Han patients and 67.1% in Uygur patients and there was no statistically significant difference between the 2 groups.

4. Discussion

Occurrence and development of breast cancer is related to genetic and environmental factors.[15] Compared with N-TNBC, TNBC has complex molecular characteristics with different clinical and pathological features and prognosis.[1,2] There are significant differences in molecular characteristics of TNBC among different ethnic groups.[11,12] However, the clinical reports[16] on TNBC are limited to the Han patients and there is a lack of control research among different ethnic groups. In this article, the expression levels of E-cad and VEGF, and their relationship with clinical characteristics of TNBC patients were investigated.

E-cad, a cell adhesion molecule, has a transmembrane glycoprotein structure distributed in 3 different regions of extracellular, transmembrane, and intracellular domain, which plays a role in signal transduction.[17] E-cad plays a key role in cell movement and the process of epithelial-mesenchymal transition and migration during tumor invasion.[7] The lack of E-cad expression is critical for epithelial tumor invasion and metastasis.[18] Studies have shown that low expression of E-cad is a sign of poor tumor prognosis, and is significantly related to disease-free survival.[19,20] In this study, the results showed that 78 cases of patients had positive expression of E-cad in 172 cases of Han patients with the positive rate of 45.3%, and 30 cases of patients had positive expression of E-cad in 79 cases of Uygur patients with the positive rate of 38.0%. There was no significant difference between the 2 groups. These results suggest that there is no significant difference in expression of E-cad in TNBC patients of different ethnic groups.

VEGF can stimulate vascular endothelial cell proliferation and promote neovascularization of tumor cells.[9] It is closely related to tumor growth.[10] Studies have shown that the high expression of VEGF and 5-year disease-free survival rate is significantly related in TNBC patients and overexpression of VEGF corresponds to poor prognosis.[21,22] VEGF may become a new target for TNBC therapy.[23] The combination of bevacizumab (anti-VEGF), epirubicin, cyclophosphamide, and docetaxel have increased the rate of pathologic remission in TNBC patients from 27.9% to 39.3%.[24] Our results showed that the

positive rate of VEGF expression in Han patients was significantly lower than that in Uygur patients. The reason for this difference may be because Uighur TNBC patients usually go to treatment at a late stage. This requires further study to confirm.

Through the follow-up, we found that the number of recurrence and metastasis was 44 cases (26.6%) and 26 cases (32.9%) in Han and Uygur TNBC patients, respectively, without significant difference. There was no significant difference in 5-year disease-free survival between 2 groups.

Study has shown that the invasion of tumor cells and expression levels of E-cad are negatively correlated.[25] By overexpression of E-cad, the invasion of tumor cells can be effectively inhibited.[26] E-cad plays an important role in the development and progression of TNBC and can be used as a prognostic indicator of TNBC.[19,27] In this study, we found that the expression of E-cad was negatively correlated with the lymph node status, TNM staging, and histological grading in both the Han and Uygur groups. With the increased number of lymph node metastasis, the later clinical stage, the higher histological grade, lack of E-cad expression is more obvious, which affects the prognosis of patients with TNBC. The 5-year overall survival and disease-free survival rates of E-cad-negative patients with 61% and 27% were significantly lower than those of E-cad-positive patients with 89% and 65%, respectively.[28]

Overexpression of VEGF in TNBC has correlation with lymph node metastasis and tumor stage, and is prone to cause early recurrence and metastasis of TNBC and poor prognosis.[29] A study of TNBC recurrence has shown that the expression of VEGF is significantly different in different stages of TNBC.[30] In 2011, Song et al showed HIF1a, VEGF related to Wnt, and β-catenin and in patients with AML, but the mechanism has not been investigated.[31] Our study showed that the positive expression rate of VEGF was positively correlated with the number of lymph node metastasis and TNM staging in Han and Uygur TNBC patients, but not the histological grading, which was consistent with previous report.[32] With the increased number of lymph node metastasis and TNM stage, the positive expression of VEGF gradually increased, indicating that VEGF plays a certain role in the progress of TNBC.

Lymph node metastasis, TNM staging, and histological grading are important basis for clinical judgment of prognosis and reference clues for development of individualized comprehensive treatment program.[33] The correlation of E-cad and VEGF with lymph node metastasis, TNM stage, and histological grade further suggests that we should pay great attention to the differences of E-cad and VEGF expression in different ethnic TNBC patients. E-cad and VEGF may have clinical significance as therapy targets. Individual treatment strategies for TNBC patients of different ethnic groups should be taken to obtain a higher cure rate and survival rate.

In conclusion, we reported that the 5-year disease-free survival rate of TNBC patients in Han and Uygur patients had no significant difference, but the difference of VEGF expression rate was statistically significant, indicating that VEGF could be used as an independent factor in the prognosis of TNBC. With the increase of VEGF expression, the prognosis is worse.[34] Meanwhile, this study also showed that the positive expression rate of VEGF in Uygur TNBC patients was higher than that in Han patients, but the 5-year disease-free survival rate and expression of E-cad were not significantly different. In the prognosis of N-TNBC, it has been demonstrated that the 6-year disease-free survival rate of Uygur Lumina A breast cancer patients is lower than that of Han patients.[13] Therefore, these results suggest that the prognosis factors of Uygur TNBC patients may be different from those of Han TNBC patients.

References

[1] Adamo B, Anders CK. Stratifying triple-negative breast cancer which definition(s) to use. Breast Cancer Res 2011;13:105.

[2] Dent R, Trudeau M, Pritchard KI, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007;13: 4429–34.

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

[4] Martin-Belmonte F, Perez-Moreno M. Epithelial cell polarity, stem cells and cancer. Nat Rev Cancer 2012;12:23–38.

[5] Hirohashi S. Molecular aspects of adhesion-epigenetic mechanisms for inactivation of the E-Cadherin-mediated cell adhesion system in cancers. Verh Dtsch Ges Pathol 2000;84:28–32.

[6] Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002;2:442–54.

[7] Shibata K, Suzuki A, Watanabe T, et al. ZEB-1 and E-cadherin expression may predict recurrence-free survival in patients with invasive ductal breast carcinoma. Yamagata Med J 2015;33:61–9.

[8] Sauter ER, Nesbit M, Watson JC, et al. Vascular endothelial growth factor is a marker of tumor invasion and metastasis in squamous cell carcinomas of the head and neck. Clin Cancer Res 1999;5:775–82.

[9] Adams J, Carder PJ, Downey S, et al. Vascular endothelial growth factor (VEGF) in breast cancer: comparison of plasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen. Cancer Res 2000;60: 2898–905.

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

[11] Jiang W, Li Y, Wang X. Differential expression of EGFR and VEGF between Han and Uygur patients with triple-negative breast cancer. Int J Clin Exp Med 2016;9:11824–30.

[12] Jiang W, Li Y, Wang X. Han and Uygur triple-negative breast cancer epidemiological characteristics of difference. Acta Univ Med Anhui 2016;51:724–7.

[13] Qiu X, Cheng F, Liu Y. Clinicopathological characteristics and prognosis of 1 006 Uygur and Han patients with diff erent molecular subtypes of breast cancer. Tumor 2015;35:292–300.

[14] Jiang W, Ou J, Zhang G, et al. Triple-negative breast cancer of Han and Uygur: clinical features and prognosis. Chin J Clin Oncol 2011;38:1579–83.

[15] Palli D, Rizzolo P, Zanna I, et al. SULT1A1 gene deletion in BRCA2-associated male breast cancer: a link between genes and environmental exposures? Cell Mol Med 2013;17:605–7.

[16] Yuan ZY, Wang SS, Gao Y, et al. Clinical characteris tics and prognos is of triple -negative breast cancer: a report of 305 cases. Chinese J Cancer 2008;27:561.

[17] Abu Taha A, Schnittler HJ. Dynamics between actin and the VE-Cadherin/catenin complex: novel aspects of the ARP2/3 complex in regulation of endothelial junctions. Cell Adh Migr 2014;8:125–35.

[18] Shen T, Zhang K, Siegal GP, et al. Prognostic Value of E-Cadherin and β-catenin in Triple-Negative Breast Cancer. Am J Clin Pathol 2016.

[19] Tang D, Xu S, Zhang Q, et al. The expression and clinical significance of the androgen receptor and E-cadherin in triple-negative breast cancer. Med Oncol 2012;29:526–33.

[20] Kashiwagi S, Yashiro M, Takashima T, et al. Advantages of adjuvant chemotherapy for patients with triple-negative breast cancer at Stage II: usefulness of prognostic markers E-cadherin and Ki67. Breast Cancer 2011;13:R122.

[21] Greenber GS, Ruho HS. Triple-negative breast cancer: role of antiangiogenic agents. Cancer Journal (Sdbury Mass) 2010;16:33–8.

[22] Linderholm BK, Hellbor GH, Johansso NU, et al. Signifi cantly higher levels of vascula -r endothelial growth factor(VEGF) and shorter survival times for patients with primary operable triple-negative breast cancer. Ann Onc 2009;20:1639–46.

[23] Bahhnassy A, Mohanad M, Shaarawy S, et al. 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.

[24] Minkwitz G, Eidtmann H, Rezai M, et al. Neoadjuvant chemotherapy and bevacizumab for HER2 - negative breast cancer. Eng J Med 2012; 366:299–309.

[25] Canel M, Serrels A, Frame MC, et al. E-cadherin-integrin crosstalk in cancer invasion and metastasis. J Cell Sci 2013;126:393–401.

[26] Techasen A, Loilome W, Namwat N, et al. Loss of E-cadherin promotes migration and invasion of cholangiocarcinoma cells and serves as a potential marker of metastasis. Tumour Biol 2014;35:8645–52.
[27] Pang H, Lu H, Song H, et al. Prognostic values of osteopontin-c, E-cadherin and β-catenin in breast cancer. Cancer Epidemiol 2013;37: 985–92.
[28] Sun T, Yao Y, Gong J, et al. Expression of E-cadherin in the triple negative breast cancer. J Basic Clin Oncol 2014;27:8–10.
[29] Rakha EA, EI-sayed ME, Green AR, et al. Prognostic markers in triple-negative breast cancer. Cancer 2007;109:25–32.
[30] Tsai CH, Chiu JH, Yang CW, et al. Molecular characteristics of recurrent triple-negative breast cancer. Mol Med Rep 2015;12:7326–34.
[31] Song S, Christova T, Perusini S, et al. Wnt inhibitor screen reveals iron dependence of β-catenin signaling in cancers. Cancer Res 2011;71:7628–39.
[32] Tan L, Qin H, Piao Y, et al. Expression and clinical significance of MTDH and VEGF in triple-negative breast cancer. Zhonghua Zhong Liu Za Zhi 2015;37:827–32.
[33] Giltnane JM, Rapp J, Moeder C, et al. Construction of a five-marker protein-based model for stage-independent assessment of prognosis in breast cancer. Clin Oncol 2009;27:11013.
[34] Sheridan W, Scott T, Caroline S, et al. Breast cancer in young women: have the prognostic implications of breast cancer subtypes changed over time? Breast Cancer Res Treat 2014;147:617–29.