Biomedical Research 2017; Special Issue: S537-S541 *ISSN 0970-938X*
www.biomedres.info

The study of correlation between neoadjuvant chemotherapy and the expression of VEGF, HIF-1α and MVD in breast cancer.

Tian Ruihua[1], Li Yongqiang[2], Zheng Zongzhu[2*]

[1]The First Oncology Department, Shandong Tai'an City Center Hospital, Tai'an, Shandong, PR China

[2]The Otolaryngological Department, Shandong Tai'an City Center Hospital, Tai'an, Shandong, PR China

Abstract

Objective: To explore the influence of neoadjuvant chemotherapy on expression of VEGF, HIF-1α and MVD in breast cancer tissue by detection before and after neoadjuvant chemotherapy and to analyse the correlation between the changes of these biological factors and the curative effect of chemotherapy.
Methods: Fifty female patients diagnosed as IIB-IIIC stage breast infiltrating ductal carcinoma in our hospital from September 2013 to September 2014 were treated with CET chemotherapy for 4-6 cycles, and also in surgical therapy to evaluate the efficacy of neoadjuvant chemotherapy. The expression of VEGF, HIF-1α, and MVD in core-needle biopsy specimens and postoperative specimens were detected by immunohistochemical method before and after neoadjuvant chemotherapy. SPSS13.0 software was used for statistical analysis.
Results: The MVD value of breast cancer tissue before chemotherapy was 32.17 ± 0.51, while 25.43 ± 0.68 after chemotherapy with the statistically significant difference (P<0.05). The effective rate of neoadjuvant chemotherapy was 95.23% in VEGF-negative group before chemotherapy, which was significantly higher than that in positive expression group (62.07%, P<0.05). The effective rate was 80.00% in HIF-1α-negative group, which was higher than that in the positive group (74.29%), there was no significant difference (P>0.05). Before chemotherapy, MVD value of the effective group was 31.62 ± 1.10, while chemotherapy ineffective group was 33.19 ± 0.88 without statistically significant difference (P>0.05).
Conclusions: 1. Neoadjuvant chemotherapy can significantly reduce the expression of VEGF and HIF-1α, and also the MVD value in breast cancer tissue. 2. The effective rate of neoadjuvant chemotherapy in VEGF-negative group was significantly higher than the positive group, suggesting that VEGF can be used as a predictor of neoadjuvant chemotherapy. 3. HIF-1α and MVD cannot be used as predictors of neoadjuvant chemotherapy.

Keywords: Breast cancer, Neoadjuvant chemotherapy, Vascular endothelial growth factor, Hypoxia inducible factor-1, Microvessel density.

Accepted on March 30, 2017

Introduction

Breast cancer is one of the most common malignancies in women, and its incidence is increasing year by year [1-4]. In the early 1970s, Neoadjuvant Chemotherapy (NAC) was first reported to be used in the treatment of Locally Advanced Breast Cancer (LABC) [5] and initially exhibited a satisfactory curative effect. There is a variety of advantages in neoadjuvant chemotherapy, including tumor degradation [6]. In current, neoadjuvant chemotherapy is being more used for larger-size tumor and resectable breast cancer, and has become a standard treatment for local advanced breast cancer and one of the standard treatment options of resectable breast cancer [7]. Although there are many advantages of NAC, but reported in the clinical neoadjuvant chemotherapy after the clinical efficacy is generally 60%-90% [8]. Breast cancer is an angiogenesis-dependent malignant tumor, while Vascular Endothelial Growth Factor (VEGF) is the most powerful pro-angiogenic factor directly acting on vascular endothelial cells, leading to neovascularization [9-11]. Hypoxia-Inducible Factor-1 (HIF-1) regulates the expression of Vascular Endothelial Growth Factor (VEGF) and promotes tumor angiogenesis, which is of great significance in evaluation of clinical therapeutic effect. Microvessel Density (MVD) can be used to measure the degree of tumor angiogenesis activity [12,13], which at present was measured by CD34 [14].

In this study, the expression of VEGF, HIF-1α and MVD in paraffin-embedded specimens of breast cancer before and after chemotherapy were detected by immunohistochemistry. The correlation between these biological factors and the curative effect of chemotherapy was explored based on the changes of

expression to infer whether they can be used as predictors of curative effect of neoadjuvant chemotherapy.

Material and Methods

Patient selection

50 female patients diagnosed as IIB-IIIC stage breast infiltrating ductal carcinoma in our hospital from September 2013 to September 2014 were recruited. The general information was shown in Table 1.

Table 1. *Clinicopathological features of patients.*

Clinical features		Cases	Proportion (%)
Age	≥ 50	23	46
	<50	27	54
Menopausal status	Premenopause	24	48
	Postmenopause	26	52
Primary tumor location	Right	24	48
	left	26	52
Primary tumor size	≤ 5 cm	41	82
	>5 cm	9	18
Regional lymph nodes	N0	8	16
	N1	10	20
	N2	20	40
	N3	12	24
Clinical stages	IIB stage	10	20
	IIIA stage	15	30
	IIIB stage	9	18
	IIIC stage	16	32
ER	Positive	31	62
	Negative	19	38
PR	Positive	26	52
	Negative	24	48
HER-2	Positive	12	24
	Negative	38	76
Molecular subtyping	Triple-negative	8	16
	Luminal A	13	26
	Luminal B	20	40
	Over-expression of HER-2	9	18
Pathological grading	I grade	9	18
	II grade	25	50
	III grade	16	32

Evaluation before chemotherapy

Primary breast cancer of all the cases was diagnosed by needle aspiration biopsy to clear histopathological diagnosis and immunohistochemistry status. Patients have no history of chemotherapy, radiotherapy, endocrine therapy or immunotherapy before chemotherapy, and without other tumors simultaneously. The research was discussed and approved by the hospital ethics committee. Full communication with the patient's family was conducted to accept the experiment and sign informed consent.

Main reagents and instruments

1) VEGF monoclonal antibody (Wuhan Boster Co., Ltd.); (2) HIF-1α monoclonal antibody (Wuhan Boster Co., Ltd); (3) CD34 monoclonal antibody working solution (Hangzhou Yanke Biotechnology Co., Ltd.); (4) hollow-core ejection gun (USA BARD Co., Ltd.); (5) shells hollow needle (14G or 16G) (Germany, Barton Co., Ltd.); (6) SSA-240A ultrasound system (Japan, Toshiba), the frequency of the probe is 5-12 MHz.

Treatment method

Patients with primary breast cancer were treated with core needle biopsy under the guidance of the ultrasound positioning system, obtain 5-6 cylindrical specimens were obtained from each lesion. Once the pathological examination of II B-III C stage invasive ductal carcinoma, the case would be recruited into the group. CET was used as preoperative neoadjuvant chemotherapy: intravenous bolus of 500 mg/m^2 cyclophosphamide d1+intravenous infusion of 100 mg/m^2 epirubicin d1+intravenous infusion of 75 mg/m^2 docetaxel d1, every 21 days as a cycle for 4-6 cycles. Every time before chemotherapy and preoperative, they will be in physical examination, and ultrasonic testing. Once the disease is in progress, the neoadjuvant chemotherapy and surgical treatment will be immediately terminated. The first assessment of efficacy was initiated on the last day of the second cycle of chemotherapy, i.e. the beginning of the third cycle of the program. After 2 cycles of chemotherapy, the efficacy of CR discontinued neoadjuvant chemotherapy and received surgical treatment; while PR and SD continue to neoadjuvant chemotherapy. After 4 cycles of chemotherapy, the patients have not yet reached the evaluation of PR, will terminate neoadjuvant chemotherapy for surgical treatment; sustained PR will continue to neoadjuvant chemotherapy up to 6 cycles. The Ultrasonic measurement method is length × width × thickness as an indicator. 10-14 days after the end of the last cycle of chemotherapy, surgery will be performed.

Immunohistochemical method

According to the registered pathology number, archives puncture and pathological wax block of pathology of Central Hospital, Tai'an City, Shandong Province will be accessed. Hematoxylin Eosin (HE staining), and immunohistochemical staining of VEGF, HIF-1α and CD34 will be processed respectively.

Criteria for the evaluation of neoadjuvant chemotherapy

Based on the RECIST criteria, it is divided into complete remission, partial remission, stable disease and progression disease. Complete Remission (CR): all target lesions disappeared; Partial Remission (PR): compared with the sum of the longest diameter of the target lesion and the baseline state, at least 30% decrease; Progression Disease (PD): compared with the sum of the longest diameter of the target lesion and the baseline state with a 20% increase, or the emergence of one or more new lesions. Stable Disease (SD)-between partial remission and progression disease. Disease control rate=(total number of cases-progress cases)/total number of cases × 100%; effective rate of treatment=(total number of cases-the number of stable cases-the number of progress cases)/total number of cases × 100%. Pathological Complete Remission (pCR) has the following indicators: the disappearance of breast lumps and axillary lymph nodes, necrotic tissue, calcification, fibrosis in primary tumor area of surgical specimens without cancer cell infiltration. pCR is the definition of surgical resection of the primary tumor and axillary lymph nodes at the same time without any invasive cancer residue.

Staining result determination

The determination of VEGF-positive: The tumor cells with claybank or dark brown particles in cytoplasm or membrane under optical microscope were counted as positive cells, referring to the criteria by Kinoshita et al. [15]. The determination of HIF-1α positive was based on the method by Zhong [16]. The counting method established by Weidner [14] was used for MVD assay.

Statistical analysis

All the data were analysed by SPSS13.0. Single factor variance analysis count data analysis was used for χ^2 test. The test level was P=0.05. The quantitative data was presented as mean ± standard deviation. Comparison between the two groups was conducted with t test, P<0.05 means the difference with statistical significance.

Results

The efficacy of neoadjuvant chemotherapy

Of 50 patients, 18 cases received 4 cycles of chemotherapy, 2 cases of 5 cycles, 30 cases of 6 cycles. 16 cases achieved clinical complete remission (CR, 32%) after chemotherapy, 6 cases of pathologic complete remission (pCR, 12%), 23 cases of partial remission (PR, 46%), 10 of stable disease (SD, 20%) and 1 case of progressive disease (PD, 2%) for the enlargement of breast and axillary lymph node. The overall effective rate of neoadjuvant chemotherapy (CR+PR) was 78% (39/50) (Table 2).

Table 2. *The efficacy of neoadjuvant chemotherapy.*

Efficacy	Cases	Percent
Pathologic Complete Remission (pCR)	6	12%
Complete Remission (CR)	16	32%
Partial Remission (PR)	23	46%
Stable disease (SD)	10	20%
progressive Disease (PD)	1	2%
Clinical Effective (CR+PR)	39	78%
Clinical Invalid (SD+PD)	11	22%

The relationship between the expression of VEGF and chemotherapy before neoadjuvant chemotherapy

All the biopsy specimens before chemotherapy were analysed: 29 cases were VEGF-positive with the effective chemotherapy rate of 62.07%. The effective rate of chemotherapy for the 21 VEGF-negative patients was 95.23%, indicating the chemotherapy was more effective for the VEGF-negative than the positive with significant difference (P<0.05, Table 3).

Table 3. *The relationship between the expression of VEGF and chemotherapy before neoadjuvant chemotherapy.*

Groups	VEGF		P value
	Positive	Negative	
Effective response	18	20	<0.05
Invalid response	11	1	

The effect of neoadjuvant chemotherapy on VEGF expression

The positive expression rate of VEGF was 58.00% in breast cancer before neoadjuvant chemotherapy while 34.00% in breast cancer after chemotherapy. The positive expression rate of VEGF in breast cancer tissue after chemotherapy was lower than that before chemotherapy with a significant difference (P<0.05, Table 4).

The relationship between HIF-1α expression before neoadjuvant chemotherapy and chemotherapeutic efficacy

The puncture specimens before the chemotherapy were analysed statistically: The positive rate of chemotherapy was 74.29% in HIF-1α-positive group, 15 cases were in the HIF-1α-negative group with the positive chemotherapy rate of 80.00%. The HIF-1α-negative cases had higher efficacy than

the positive ones without statistical difference (P>0.05, Table 5).

Table 4. The effect of neoadjuvant chemotherapy on VEGF expression.

Groups	VEGF		P value
	Positive	Negative	
Before chemotherapy	29	21	<0.05
After chemotherapy	17	33	

Table 5. The relationship between HIF-1α expression before neoadjuvant chemotherapy and chemotherapeutic efficacy.

Groups	HIF-1α		P value
	Positive	Negative	
Effective response	26	12	>0.05
Invalid response	9	3	

The effect of neoadjuvant chemotherapy on the HIF-1α expression

The positive expression rate of HIF-1α was 70.00% in breast cancer before neoadjuvant chemotherapy while 48.00% in breast cancer after chemotherapy. The positive expression rate of HIF-1α in breast cancer tissue after chemotherapy was lower than that before chemotherapy with a significant difference (P<0.05, Table 6).

Table 6. The change of positive expression rate of HIF-1α before and after neoadjuvant chemotherapy.

Groups	HIF-1α		P value
	Positive	Negative	
Before chemotherapy	35	15	<0.05
After chemotherapy	24	26	

The relationship between MVD value before neoadjuvant chemotherapy and chemotherapeutic efficacy

The puncture specimens before the chemotherapy were analysed statistically: The MVD value of chemotherapy-effective group was 31.62 ± 1.10 and 33.19 ± 0.88 of the invalid group respectively. There was no significant difference between the two groups (P>0.05).

The effect of neoadjuvant chemotherapy on MVD

MVD value in breast cancer before chemotherapy was 32.17 ± 0.51, while 25.43 ± 0.68 after chemotherapy. The MVD in breast cancer tissue after chemotherapy was lower than that before chemotherapy with a significant difference (P<0.05).

Discussion

Our study suggests VEGF as a predictor of neoadjuvant chemotherapy was possibly used to facilitate screening of neoadjuvant chemotherapy-sensitive patients. So that sensitive patients can maximize the efficacy of neoadjuvant chemotherapy, insensitive patients may avoid unnecessary economic loss, refrain from the pain of chemotherapy, and even delay of the disease, indicating further guide clinical practice and decision-making. In addition, whether these biological factors can guide the second-line treatment of patients with recurrence and postoperative individual therapy and whether it can provide reference information for the new anti-angiogenic target therapy remains to be further explored and in practice. Although anti-VEGF therapy is still in the experimental stage, with the further study of VEGF, vascular growth inhibitors are expected to become a novel and comprehensive treatment of breast cancer. In conclusion, neoadjuvant chemotherapy can significantly reduce the expression of VEGF, HIF-1α, and the MVD value in breast cancer; the response rate of VEGF-negative group after neoadjuvant chemotherapy was significantly higher than that of the positive group, suggesting that VEGF can considered as a predictor of neoadjuvant chemotherapy, while HIF-1α and MVD cannot be used as predictors.

References

1. Hua-Hsi W, Wen-chang L, Kuo-Wang T. Advances in studies on prognostic biomarkers of gastric. J Clin Oncol 2010; 15: 177.
2. Bang N, Nielsen MB, Vejborg I, Mogensen AM. Clinical report: contrast enhancement of tumor perfusion as a guidance for biopsy. Eur J Ultrasound 2000; 12: 159.
3. Brown JM, Chaloupka J, Taylor KJ, Quedens Case C, Alderman J, Greener Y. Contrast-enhanced ultrasound for guidance of local tumor ablation. Ultrasound Med Biol 1999; 25: 1213.
4. Fisher B, Saffer EA, Fisher ER. Studies concerning the regional lymph node in cancer. Thymidine uptake by cells from nodes of breast cancer patients relative to axillary location and histopathologic discriminants. Cancer 1974; 33: 271.
5. De lena M, Zucali R, Viganotti G, Valagussa P, Bonadonna G. Combined chemotherapy-radiotherapy approach in locally advanced (T3b-T4) breast cancer. Canc Chemother Pharmacol 1978; 1: 53.
6. Garces CA, Cance WG. Neoadjuvant chemotherapy of breast cancer. Am Surg 2004; 70: 565-569.
7. Kaufmann M, Hortobagyi GN, Goldhirsch A, Scholl S, Makris A, Valagussa P, Blohmer JU, Eiermann W, Jackesz R, Jonat W. Recommendations from an international expert panel on the use of neoadjuvant (Primary) systemic treatment of operable breast cancer: an update. J Clin Oncol 2006; 24: 1940.

8. Shannon C, Smith I. Is there still a role for neoadjuvant therapy in breast cancer? Crit Rev Oncol Hematol 2003; 45: 77-90.
9. Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med 285: 1182-1186.
10. Kaio E, Tanaka S, Kitadai Y, Sumii M, Yoshihara M, Haruma K, Chayama K. Clinical significance of angiogenic factor expression at the deepest invasive site of advanced colorectal carcinoma. Oncol 2003; 64: 61.
11. Folkman J. Angiogenesis and growth and metastasis of tumor. J Int Oncol 2001; 28: 118.
12. Weidner N. Current pathologic methods for measuring intratumoral microvessel density within breast carcinoma and other solid tumors. Breast Cancer Res Treat 1995; 36: 169.
13. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med 1991; 324: 1.
14. Fina L, Molgaard HV, Robertson D, Bradley NJ, Monaghan P, Delia D, Sutherland DR, Baker MA, Greaves MF. Expression of the CD34 gene in vascular endothelial cells. Blood 1990; 15: 2417-2426.
15. Kinoshita J, Kitamura K, Kabashima A, Saeki H, Tanaka S, Sugimachi K. Clinical significance of vascular endothelial growth factor-c (VEGF-C) in breast cancer. Breast Cancer Res Treat 2001; 66: 159.
16. Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW. Overexpression of hypoxia-inducible factor 1a in common human cancers and their metastases. Cancer Res 1999; 59: 5830.

*Correspondence to

Zheng Zongzhu

The Otolaryngological Department

Shandong Tai'an City Center Hospital

PR China