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Effect of *EGFR* gene mutation in patients with TNBC treated with bevacizumab combined with gemcitabine.

Shihui Ma*, Feihai Ling, Shifeng Chen, Anping Gui

Department of Mammary Gland Surgery, Zhongshan Hospital, Sun Yat-sen University, Guangzhou, PR China

Abstract

Objective: To investigate the effect of Epidermal Growth Factor Receptor (*EGFR*) gene mutation in patients with Triple Negative Breast Cancer (TNBC) treated with bevacizumab combined with gemcitabine.

Methods: 180 patients with triple-negative breast cancer admitted to our hospital from September 2011 to September 2013 were enrolled in this study. According to their DNA sequencing results, the patients were divided into *EGFR* mutation group and the non-mutation group, and according to the patient's genetic mutation type, patients in mutation group were divided into *exon 19* deletion group, *exon 21* mutation group. All patients were treated with bevacizumab and gemcitabine, and the prognosis of each group was compared.

Results: *EGFR* mutations were found in 51 cases (28.3%). The sex ratio and smoking history of *EGFR* mutant group were significantly different (P<0.01) compared with non-mutated group. For *EGFR* mutation group, there were 30 cases (58.8%) of *exon 19* deletion, and 21 cases of *exon 21* mutation (41.2%). The progression-free survival time and 1-year survival rate were significantly higher in mutant group than in non-mutation group (P<0.05). The total effective rate in EGFR exon 19 deletion group, exon 21 mutation group was 80.0% and 76.2%, higher than 40.3% in the non-mutation group, the disease control rate was also higher than the mutation group, the difference was statistically significant (P<0.05). There was no significant difference in total effective rate and disease control rate between exon 19 deletion mutation group and exon 21 mutation group (P>0.05). Adverse reactions were concentrated in the rash, diarrhea, with mild degree and tolerable. For the incidence of adverse reactions in three groups, the difference was not statistically significant (P>0.05).

Conclusion: *EGFR* mutations are more sensitive in patients with triple-negative breast cancer treated with bevacizumab and gemcitabine, with better clinical efficacy, and the progression-free survival of tumors is prolonged. *EGFR* mutation has no significant effect on the safety of treatment.

Keywords: Epidermal growth factor receptor, Gene mutation, Bevacizumab, Gemcitabine, Triple negative breast cancer.

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Introduction

Three negative breast cancer is a common subtype of breast cancer, with aggressive, high recurrence rate, distant metastases and other characteristics. The prognosis of patients is often in poor quality [1]. The combination of bevacizumab and gemcitabine in the treatment of triple-negative breast cancer after chemotherapy failed in the second and third line treatment has been widely concerned about, the safety and tolerance of which are better for patients with advanced triple-negative breast cancer. It also has certain significance in improving the quality of life [2]. In recent years, some scholars pointed out that Epidermal Growth Factor Receptor (EGFR) and tumor angiogenesis, invasion and metastasis are closely related, and *EGFR* gene mutations have some impact in patients with chemotherapy [3]. To determine the *EGFR* gene mutation in triple negative breast cancer in the treatment of bevacizumab combined with gemcitabine, 180 cases of triple-negative breast cancer patients in our hospital from September 2011 to September 2013 were prospectively studied, intending to evaluate the differences between efficacy and adverse reactions, the research process and conclusions are reported as follows.

General information

Case information: One hundred and eighty patients with triple-negative breast cancer admitted to our hospital from September 2011 to September 2013 were included in the study after informed consent obtained and approved by the Medical Ethics Transfer Committee. The mean age of patients was 32~75 years old (54.20 ± 19.17) years old. The ECOG functional status score was 0~1, with mean of (0.71 ± 0.20). Menstrual state: 74 cases of menopause, 96 cases of no

menopause. Metastatic sites: 57 cases of liver metastasis, lung metastasis in 26 cases, 17 cases of other parts of the metastasis.

Inclusion criteria and exclusion criteria: Inclusion criteria: (1) the first diagnosis of triple negative breast cancer using pathological histology [4,5]. (2) Histological stage of IIIa~IV, with no radical surgery indications. (3) Measurable lesions ≥ 1. (4) The expected survival ≥ 3 months. (5) No previous history of chemotherapy or chemotherapy failure. Exclusion criteria: (1) Refusal to participate in the study, or drug compliance is poor. (2) The complicated with heart, liver, kidney and other vital organs lesions or tumors.

Research Methods

Drug regimen

180 patients were treated with bevacizumab and gemcitabine. Gemcitabine (trade name: Yu Jie, Guoyaozhunzi H20063675, Harbin Yu Heng Pharmaceutical Co., Ltd., specifications of 1.0 g) was administered by intravenous infusion, each time for 1.0 g/m^2 at day 1 and day 8. Bevacizumab (Trade name: Avastin, Registration No. S20100023, Roche Pharmaceutical Company, Switzerland, specification 100 mg: 4 mL) was given at days 2-4, each time for 60~70 mg/m^2. 21 day for a treatment cycle, continuous treatment of 6 months, or with the end of treatment for tumor progression or unable to tolerate toxic and side effects [6].

Grouping method

The EGFR gene was detected by digestion-DNA extraction and purification-PCR amplification-sequencing [7]. The sequencing equipment was ABI 3730XL automated DNA sequencer (US Applied Biosystems). According to their DNA sequencing results, patients were divided into *EGFR* mutation group and the non-mutation group, and according to the patient's genetic mutation type, they were divided into *exon 19* deletion group and *exon 21* mutation group.

Observation indicators

The patients were followed up for 18 months by telephone and outpatient follow-up. Their progression-free survival time, adverse reaction status and tumor progression-free survival time were recorded at the end of follow-up in March 2015. Tumor progression-free survival: treatment initiation to tumor progression or death time. Survival: time of treatment start to death.

Clinical efficacy was evaluated at the time of last follow-up with reference to the Response Evaluation Criteria in Solid Tumors (RECIST) [8]. Complete remission (CR): the lesion completely disappeared; 2. Partial remission (PR): the sum of the longest diameter of lesions decreased ≥ 30% than before the treatment; 3. Stable Disease (SD): the sum of the longest diameter of lesions decreased<30% than before the treatment; 4. Progressive Disease (PD): the sum of the longest diameter of lesions increased ≥ 20% than before the treatment, or new lesions appeared. Total effective rate=(CR+PR)/total number of cases × 100%, disease control rate=(CR+PR+SD)/total number of cases × 100%.

Statistical analysis

All data for this clinical study were analysed using SPSS 18.0, count data expressed in (n/%), and with the use of χ^2 test. Measurement data were represented as ($\bar{x} \pm s$), and tested with t test. Test level was set to α=0.05. P<0.05 was considered statistically significant, with P<0.01 was statistically extremely significant.

Results

EGFR gene mutations in patients

EGFR mutations were found in 51 patients (28.3%). The sex ratio and menstrual status of *EGFR* mutant group were significantly different (P<0.01) compared with non-mutation group (Table 1). In *EGFR* mutation patients, there were 30 cases of *exon 19* deletion (58.8%), and 21 cases of *exon 21* mutation (41.2%).

Table 1. *Clinical data comparison between EGFR gene mutations and non-mutations (n/%).*

Indexes		Mutation group (n=51)	Non-mutation group (n=129)	t/χ^2	P
Age (years)		53.95 ± 18.42	55.10 ± 18.85	0.139	>0.05
Functional status score (points)		0.70 ± 0.13	0.73 ± 0.15	0.047	>0.05
Menopausal status	Menopause	35 (68.6)	39 (30.2)	19.365	<0.01
	No menopause	16 (31.4)	80 (62.0)		
Metastatic sites	Local lymph nodes	38 (74.5)	107 (82.9)	0.747	>0.05
	Liver	16 (31.4)	41 (31.8)	0.085	>0.05
	Lung	9 (17.6)	17 (13.2)	0.224	>0.05

Other parts	5 (9.80)	12 (9.30)	0.139	>0.05

EGFR mutation in survival of patients

The tumor progression-free survival time and 1-year survival rate were significantly higher in mutant group than in non-mutation group (P<0.05, Table 2).

Table 2. *Survival time comparison between EGFR gene mutations and non-mutations ($\bar{x} \pm s$).*

Indexes	Mutation group (n=51)	Non-mutation group (n=129)	t/χ2	P
Tumor progression-free survival time (Months)	11.96 ± 2.84	7.21 ± 3.90	4.663	<0.05
1 year survival rate (n/%)	40 (78.4)	73 (56.6)	9.721	<0.05

EGFR mutation in the clinical efficacy of patients

The total effective rate in EGFR *exon 19* deletion group, *exon 21* mutation group was 80.0% and 76.2%, higher than 40.3% in the non-mutation group, the disease control rate was also higher than the mutation group, the difference was statistically significant (P<0.05). There was no significant difference in total effective rate and disease control rate between *exon 19* deletion mutation group and *exon 21* mutation group (P>0.05, Table 3).

Table 3. *Comparison of clinical efficacy among the groups (n/%).*

Clinical efficacy	Mutation group (n=51)		Non-mutation group (n=129)
	EGFR exon 19 deletion group (n=30)	EGFR exon 21 mutation group (n=21)	
CR	3 (10.0)*	2 (9.5)*	16 (12.4)
PR	21 (70.0)*	14 (66.7)*	36 (27.9)
SD	5 (16.7)	4 (19.0)	25 (19.4)
PD	1 (3.3)*	1 (4.8)	52 (40.3)
Total effective	24 (80.0)*	16 (76.2)*	52 (40.3)
Disease control	29 (96.7)*	20 (95.2)*	77 (59.7)

Note: compared to non-mutated group, *P<0.05.

EGFR mutations in patients with adverse effects

Adverse reactions were concentrated in the rash, diarrhea, with mild degree and tolerable. For the incidence of adverse reactions in three groups, the difference was not statistically significant (P>0.05, Table 4).

Table 4. *Comparison of adverse reactions among the groups (n/%).*

Adverse reactions	EGFR exon 19 deletion group (n=30)	EGFR exon 21 mutation group (n=21)	Non-mutation group (n=129)
Rash	17 (56.7)	11 (52.4)	68 (52.7)
Diarrhea	7 (23.3)	5 (23.8)	31 (24.0)
Xerosis cutis	4 (13.3)	3 (14.3)	18 (14.0)
Anorexia	4 (13.3)	2 (9.5)	13 (10.1)
Others	8 (26.7)	6 (28.6)	37 (28.7)

Discussion

Breast cancer is the first and second killer in female malignant tumors by incidence and mortality, of which three negative breast cancer is a subtype of breast cancer to negative estrogen receptor, progesterone receptor and human epidermal growth factor 2 as the main feature, of such patients with poorly differentiated cancer cells and high invasion [9-11]. At the same time, because of hormone receptor expression were negative, sensitivity of triple negative breast cancer to endocrine therapy is poor, with the lack of effective treatment. In recent years, molecular targeted technology has been a focus of attention in triple-negative breast cancer treatment. These drugs target at the tumor cell line of specifically, and with less damage to normal cells. They bring a new hope for treatment of patients with triple-negative breast cancer of poor general situation and low chemotherapy tolerance.

Bevacizumab is a humanized monoclonal antibody directed against Vascular Endothelial Growth Factor (VEGF), which is composed of 7% murine structure and 93% human Immunoglobulin G (IgG). It can be combined with VEGF receptor by competitive binding, so as to inhibit VEGF biological activity, thus blocking VEGF playing the role in endothelial cell mitosis, neovascularization, finally inhibiting the growth of tumor cells to achieve the purpose. At the same time, bevacizumab has effect on reducing vascular permeability and interstitial pressure. It not only can promote the normalization of tumor blood vessels, but also enhance the

concentration of other chemotherapy drugs [12-14]. Bevacizumab has been widely used in the first-line treatment of metastatic colon cancer and breast cancer since the beginning of the 21st century.

Gemcitabine is a nucleoside antimetabolite whose metabolites have unique antitumor activity by interfering with DNA strand synthesis and depletion of deoxynucleotides, inhibiting DNA synthesis, leading to gemcitabine-induced apoptosis. A large number of studies have shown that the mechanism of gemcitabine had no cross-resistance with anthracycline or taxane, so it can still achieve high efficacy after the treatment failure of anthracycline or taxane in second or third line chemotherapy [15,16].

In recent years, a number of clinical studies found *EGFR* gene mutation in triple-negative breast cancer patients, and that this mutation had an impact on the treatment effect of molecular targeted drug [17,18]. On this basis, we selected 180 cases of triple-negative breast cancer patients for clinical study and found that *EGFR* mutation rate was 28.3%, and exon 19 deletion, exon 21 mutation based, consistent with the conclusions of the study of Lim et al. [19]. The patients with *EGFR* mutations had a significantly better progression-free survival time and clinical efficacy than those of the non-mutation group after treatment with bevacizumab plus gemcitabine. The safety and tolerability were excellent, suggesting that bevacizumab combined with gemcitabine have a better effect on treatment of *EGFR* mutations in triple-negative breast cancer patients, significantly higher than the study of Prat et al. [20] who used gemcitabine only. The reason may be that *EGFR* mutation causes *EGFR* overexpression, activates Ras/Raf/MEK/ERK-MAPK pathway, enhances the sensitivity of micrometastasis, remnant tumor and sensibility of tumor cells to chemotherapeutic drugs, and increases the benefit of chemotherapy.

In summary, there is triple-negative breast cancer patients with *EGFR* mutations, who can achieve a more significant benefit after receiving bevacizumab combined with gemcitabine treatment. The tumor progression-free survival time and clinical efficacy were better than that in non-mutated patients. Patients with the program had a good tolerance. In future clinical treatment, patients with *EGFR* gene mutation should be detected first, the implementation of reasonable treatment programs should be implemented to improve the quality of their prognosis and prolong their survival. The gemcitabine combined bevacizumab chemotherapy on prolonged survival rate of patients should also be studied.

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***Correspondence to**

Shihui Ma

Department of Mammary Gland Surgery

Zhongshan Hospital

Sun Yat-sen University

PR China