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Effect of CTCs and INHBA level on the effect and prognosis of different treatment methods for patients with early breast cancer

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Abstract. – OBJECTIVE: We aimed at investigating the changes in the number of circulating tumor cells (CTCs) in patients with early breast cancer (BCa) before and after different treatments, and the influence of INHBA expression on patients' therapy efficacy and prognosis prediction was also evaluated.

PATIENTS AND METHODS: Treatment plans were formulated based on patient's condition and willingness. 160 patients with early BCa were divided into trastuzumab adjuvant group (80 cases) and surgery group (80 cases). The correlation between the number of CTCs and Inhibin, beta A (INHBA) level was assessed by Spearman correlation analysis. The cumulative survival curve was depicted using Kaplan-Meier method to evaluate the prognosis of patients in different groups.

RESULTS: The number of detected CTCs and INHBA expression in the two groups were both reduced as compared with those before chemotherapy. After 3 months of treatment, CTCs and INHBA level in the surgical group were detected lower than those in the trastuzumab group. The number of CTCs was positively correlated with INHBA level. The overall survival rate of patients in the surgery group was remarkably higher than those in the adjuvant trastuzumab group.

CONCLUSIONS: The CTCs in the peripheral blood of patients with early BCa showed a great relevance to INHBA expression, which may thus serve as predictors of treatment effect and prognosis of BCa patients in early stage. Meanwhile, we demonstrate that surgery should be the first choice for early BCa patients after chemotherapy.

Key Words:
Breast cancer, Circulating tumor cells, INHBA, Surgery prognosis.

Introduction

Breast cancer (BCa) as one of the most common malignant tumors threatens the health of women worldwide. With the continuous improvement of diagnostic techniques and treatment methods, BCa has become a controllable cancer with a 5-year survival rate of 90% in early diagnosed cases. However, there are still more than half a million people who die of BCa each year due to high incidence of BCa metastasis, which is the leading cause of BCa deaths[1,2].

Paget[3] proposed in 1889 that the metastasis and spread of tumor cells are restricted by the interaction between tumor cells and host organs, and it is currently believed that epithelial-mesenchymal transition (EMT) is the main mechanism promoting the spread of cancer cells[4]. Collective migration and invasion through tumor buds and CTCs may be the main mode of metastasis, and a single CTC can also participate in metastasis[5,6]. The number of CTCs is remarkably relevant to the metastasis and recurrence of early BCa[7]. Therefore, accurately detecting the distribution of CTCs in peripheral blood of BCa patients may have important clinical significance.

Inhibin, beta A (INHBA), as a member of the TGF-β superfamily, is composed of α and β subunit, which is involved in hypothalamic and pituitary hormone secretion, gonadal hormone secretion, germ cell development and maturation, red blood cell differentiation, insulin secretion, nerve cell survival, embryonic axial development or bone growth[8]. Increased expression of INHBA is closely associated with the occurrence of gastric cancer[9], esophageal cancer[10], and colorectal cancer[11]; meanwhile, overexpression of INHBA in mesenchymal cells enhances the colony forming potential of epithelial cells and accelerates the progression of BCa[12].

Some researches[13,14] have shown that patients with high expression of human epidermal growth factor receptor-2 (HER2) gene in CTCs can ob-

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tain considerable efficacy after trastuzumab targeted therapy. In this study, we analyzed the link between CTCs and INHBA in peripheral blood of patients with early BCa and the prognosis of different therapies, so as to provide a reference for clinical diagnosis and treatment of early BCa.

Patients and Methods

Patients

160 early BCa patients treated in the hospital from July 2016 to May 2019 were selected as the research objects. Inclusion criteria are based on the Eighth Edition of the AJCC Cancer Staging Manual: Breast Cancer: 1) BCa was diagnosed by preoperative puncture pathological examination; 2) tumor node metastasis (TNM) stage was T2-3 N0-2 M0 stage with surgical indication; 3) no history of chemotherapy. Exclusion criteria: 1) pregnant or lactating women; 2) inflammatory breast cancer; 3) patients with poor adherence; 4) patients' withdrawal from the study. Patients and family members were informed of the study and voluntarily signed informed consent. This investigation was approved by the Ethics Committee of Qishan County Hospital of Shaanxi Province. Signed written informed consents were obtained from all participants before the study.

Treatment Method

Patients were treated with chemotherapy before trastuzumab or before surgery. Specific chemotherapy regimen is docetaxel 75 mg/m^2 and epirubicin 90 mg/m^2, intravenous drip, day 1. 21 days were taken as a chemotherapy cycle, and a total of 6 consecutive cycles of chemotherapy were applied. Patients in the trastuzumab group received trastuzumab 1 month after the chemotherapy, with a dose of 110 mg per week and intravenous injection. One week was a treatment cycle for 52 consecutive weeks. Patients in the surgery group received early breast-conserving surgery for BCa 1 month after the end of chemotherapy.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from tissue specimens or cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed using PrimeScript™ RT kit (TaKaRa, Otsu, Shiga, Japan). qRT-PCR was carried out with the SYBR Green kit according to the product instructions. Primer sequences used in this study is as follows: INHBA upstream primer 5'-ACACAACAACTTTTGCTGCC-3', downstream primer 5'-TCGTGTCACCACTGTCTTCTC-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) upstream primer 5'-ACCCATCACCATCTTCCAGGAG-3', downstream primer 5'-GAAGGGGCGGAGATGATGAC-3 '. The relative expression of the genes were calculated using the 2-DDCt method.

CTCs Detection

7.5 mL of peripheral blood was collected from patients before chemotherapy, 1 month after chemotherapy, 3 months after surgery or trastuzumab treatment. Cell Search kits (purchased from Wuhan Merck Biotech, Wuhan, China) were used for detection. The prepared samples were processed using the Cell Tracks Auto prep system, tumor cells were identified and counted by Cell Tracks Analyzer II, and CTCs were detected by immunofluorescence staining.

Postoperative Follow-Up

5-year follow-up was performed in the form of telephone and outpatient follow-up the first day after surgery to the death or follow-up deadline of the patients.

Statistical Analysis

Statistical analysis was carried out using Statistical Product and Service Solutions (SPSS) 19.0 (IBM Corp., Armonk, NY, USA). Data were represented as mean ± SD (Standard Deviation). Differences between two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). p less than 0.05 is statistically significant.

Results

Comparison of Clinical Data Between the Two Groups of Study Subjects

Table I shows that the patients in the trastuzumab group were 37 males and 43 females aged 30 to 55 years, with an average age of (45.3 ± 9.6) years. In terms of TNM stage, 36 cases were in stage I and 44 cases in stage II. The patients in the surgery group were 46 males and 34 females aged 32 to 57 years (46.0 ± 10.6 years), with 42 cases in stage I and 38 cases in stage II. Statistics

Table I. Comparison of clinical data between two groups of study subjects.

Variable	N	Trastuzumab (n = 80)	Surgery (n = 80)	χ^2	*p*
Sex					
Male	83	37	46	2.028	0.205
Female	77	43	34		
Age					
< 45	71	39	32	1.241	0.34
≥ 45	89	41	48		
TNM staging					
I	78	36	42	0.901	0.429
II	82	44	38		

indicate that gender, age, and TNM staging of the two groups of patients in this study were comparable ($p>0.05$).

Comparison of CTCs Detection Rates

Before chemotherapy, CTCs were detected in peripheral blood of both groups of patients. One month after chemotherapy, no significant difference was found in the detection rate of CTCs between the two groups ($p>0.05$). After 3 months of treatment, the detection rates of CTCs in the two groups were both remarkably reduced in comparison to 1 month after chemotherapy, especially the surgery group (Table II). The above results indicated that both trastuzumab adjuvant therapy and surgical treatment reduced CTCs detection rate in early BCa patients, among which, the efficacy of the latter is more significant than that of the former.

Comparison of the Number of CTCs

Table III shows the number of CTCs detected at different time points between two groups of patients. Before chemotherapy, the number of CTCs showed no marked difference between the two groups ($p>0.05$). After 1 and 3 months of treatment, the number of CTCs detected in both groups was remarkably reduced, especially the surgery group ($p<0.05$). The above results indicate that both trastuzumab adjuvant therapy and surgical treatment lead to a reduction in CTCs number, among which, surgery shows a better efficacy.

Comparison of INHBA Levels

RT-PCR showed no significant difference in INHBA expression between the two groups of patients before chemotherapy ($p<0.05$). However, after 1 and 3 months of treatment, INHBA expression in the surgical group was reduced as compared to trastuzumab group ($p<0.05$). In addition, Table IV indicates a statistically significant difference between the two groups of patients with different treatments in terms of INHBA expression ($p<0.05$); a comparison of INHBA expression levels at different time points of the same treatment was also significant ($p<0.01$). The above results suggest that both treatments reduce INHBA level in the peripheral blood of early BCa patients in a time-dependent manner, among which, surgery results in a greater decrease.

Table II. Comparison of CTC detection rates at different time points between the two groups of patients [n (%)].

Groups	Trastuzumab (n = 80)	Surgery (n = 80)	χ^2	*p*
Before chemotherapy	80 (100)	80 (100)	–	–
1 month after the end of chemotherapy	72 (90)	70 (87.5)	0.25	0.803
3 months after the end of chemotherapy	39 (48.75)[a]	11 (13.75)ab	22.807	< 0.001
χ^2	32.035	87.039		
p	< 0.001	< 0.001		

Note: A: compared with the group 1 month after the end of chemotherapy, $p<0.01$; B: compared with trastuzumab at the same time point, $p<0.01$.

Table III. Number of CTCs detected at different time points between two groups of patients ($\bar{x} \pm s$).

Groups	Trastuzumab (n = 80)	Surgery (n = 80)	χ^2	*p*
Before chemotherapy	81.29 ± 23.94	79.64 ± 21.06	0.463	0.644
1 month after the end of chemotherapy	69.64± 18.61[a,b]	58.33 ± 15.92[a,b,c]	4.131	< 0.001
3 months after the end of chemotherapy	37.91 ± 14.88[a,b]	18.76 ± 9.73[a,b,c]	9.634	< 0.001
F	18.93	26.13		
p	< 0.001	< 0.001		

Note: A: compared with the group 1 month after the end of chemotherapy, $p<0.01$; B: compared with trastuzumab at the same time point, $p<0.01$.

Table IV. INHBA expression levels at different time points of the two groups of patients ($\bar{x} \pm s$).

Groups	Trastuzumab (n = 80)	Surgery (n = 80)	χ^2	*p*
Before chemotherapy	5.35 ± 2.87	5.41 ± 2.96	0.13	0.897
1 month after the end of chemotherapy	4.02 ± 2.11[a,b]	3.37 ± 1.84[a,b,c]	2.077	0.039
3 months after the end of chemotherapy	3.22 ± 1.73[a,b]	1.48 ± 1.07[a,b,c]	7.651	< 0.001
F	6.31	10.78		
p	< 0.001	< 0.001		

Note: A: compared with the group 1 month after the end of chemotherapy, $p<0.01$; B: compared with trastuzumab at the same time point, $p<0.01$.

Correlation Between the Number of CTCs and INHBA and Analysis of Patient Prognosis

To further clarify whether there is a correlation between the number of CTCs and INHBA expression, we conducted a Spearman rank correlation analysis. It showed that the Spearman coefficient was 0.778, $p<0.05$, in patients of the surgery group and 0.752, $p<0.05$ in patients of the adjuvant trastuzumab group, indicating that the number of CTCs is positively linked to INHBA expression in BCa patients. After 5 years of follow-up, the Kaplan-Meier survival analysis curve revealed that the overall survival rate of the surgeon was remarkably higher than that of trastuzumab adjuvant therapy (HR=8.676, $p=0.0032$) (Figure 1).

Discussion

Currently, breast cancer, a systemic disease of mammary epithelium origin, has become the main cause leading to death of women[15]. What's worse, the number of clinical BCa patients is on the rise, making the research on BCa treatment become an important project in the clinical field[16].

CTCs refer to all kinds of tumor cells in peripheral blood, which are derived from primary tumor or metastatic tumor and have the ability to escape from basement membrane and enter blood vessels through tissue matrix[16]. At present, in terms of prediction of the prognosis of BCa, traditional imaging diagnosis and serum tumor marker examination have the disadvantage of low early detection rate of micro-metastases, while CTCs can detect micro-metastases early[17,18]. Moss et al[19] have found that CTCs can serve as monitors for the progression and prognosis of BCa.

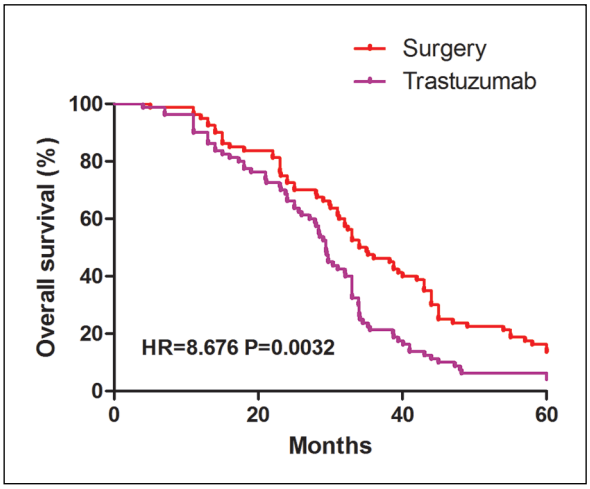

Figure 1. Survival analysis of two groups of patients. The 5-year overall survival rate of the surgery group was remarkably higher than that of the trastuzumab adjuvant group (HR=8.676, $p=0.0032$).

In this study, the number of CTCs was detected in the peripheral blood of BCa patients in early stage. We demonstrate that the detection rate and number of CTCs in both trastuzumab and surgery group decreased, and the treatment effect of patients in the operation group was better.

The INHBA gene encodes the βA subunit, which is involved in regulating the reproductive and developmental processes of the body through the formation of statins and activators[20]. INHBA is overexpressed in a variety of malignant tumors, and thus is engaged in the occurrence and development of tumors[21]. In esophageal adenocarcinoma, the mRNA level of INHBA is remarkably higher than that in Barrett's esophagus and esophageal dysplasia[22]. In gastric cancer, INHBA mRNA level in tumor tissues was also remarkably higher than that in adjacent normal tissues[23]. Besides, INHBA plays a crucial role in the development of BCa and can be used as a potential prognostic indicator[24]. These conclusions are similar to the results of this study that the INHBA level in BCa patients of two groups (trastuzumab and surgery) was both reduced after treatment, among which, the treatment effect of patients in the surgery group was better.

Some reports have demonstrated a correlation between EGFR mutation status in peripheral blood and tumor tissues by detecting peripheral blood free DNA[25,26]. In recent years, there has been much research on the detection of EGFR mutations by peripheral blood CTCs, most of which have confirmed the consistency of EGFR mutations with tumor tissues[27,28]. In this research, we found that the number of CTCs was positively correlated with INHBA expression in the peripheral blood of BCa patients, but the specific mechanism remained to be further explored. In addition, surgery was found to remarkably improve survival in patients with early BCa after 5 years of follow-up.

In this study, we found that there was a positive correlation between the number of CTCs in peripheral blood and the expression of INHBA in patients with early-stage breast cancer. This helps to better understand the molecular biological heterogeneity and complexity of breast cancer. Our research further confirms that surgery should be the first treatment option for patients with early breast cancer after chemotherapy. We also revealed that the number of CTCs in peripheral blood and the expression of INHBA can be used as a potential biomarker for monitoring the prognosis of breast cancer patients, which can provide a more accurate judgment for the prognosis of patients.

Conclusions

In summary, the number of CTCs detected and INHBA expression in early BCa patients remarkably changed before and after treatment. In addition, surgery can be the first choice of treatment for BCa patients after chemotherapy, but only for patients with early-stage BCa. However, whether it is suitable for BCa patients with different tumor stages still needs to be further explored.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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