RESEARCH COMMUNICATION

Expression of β-tubulin III and Survivin in Advance Stage Breast Cancer Correlates with Chemotheraputic Effects of Docetaxel

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Abstract

Aims: To investigate the relationship between the expression of β-tubulinIII and survivin in advanced breast cancers and chemotherapeutic effects of docetaxel. Methods: Clinical pathological data of 74 patients with advanced breast cancer were retrospectively analyzed after docetaxel chemotherapy. Expression of β-tubulinIII and survivin was assessed by immunohistochemistry and analyzed with reference to therapeutical and adverse effects of docetaxel. Results: The positive expression rate of β-tubulinIII was 38.1% (32/84), while that of survivin was 76.2% (64/84). The effective rate (complete response + partial response) was 52.4%. That for patients with the positive expression of β-tubulinIII or/and survivin was significantly lower than for those with negative expression (P<0.05). There were significant differences in the non-progression of median diseases, 1-year and 2-year survival rates of between the patients with positive and negative expression (P<0.05). The main side effects were myelosuppression, alimentary canal response and alopecie, no differences being observed between groups. Conclusions: The combined detection of β-tubulinIII and survivin is a predictive index for chemotherapy effects of docetaxel in metastatic breast cancer.

Keywords: Breast cancer - docetaxel - β-tubulinIII - survivin - chemotherapy

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Introduction

Breast cancer is one of the most common malignant tumors which threaten women health. In recent years, the incidence and mortality of breast cancer have been rapidly increased. For the treatment of palindromic and metastatc patients with late breast cancer, chemotherapy occupies an extreme important position. With the applications of the third generation chemotherapy drugs such as docetaxel (TXT) and the improvement of chemotherapy program, the effects of chemotherapy have been furtherly improved (Biganzoli et al., 2004; Jones et al., 2005). However, the choice of the patients, especially who failed to adjuvant chemotherapy with the application of taxane and anthracycline, on current clinical chemotherapy was still blind. So far, no standard chemotherapy regimen has been recommended, and when re-treatment, the patients may lead to ineffective chemotherapy. The clinicians found that the different patients with breast cancer even if at the same stage, the same pathological type, and adopted the same chemotherapy containing docetaxel, the efficacy and survival rate of the patients were quite different. Therefore, how to effectively predict the sensitivity of chemotherapeutic drugs is a problem with clinical significance.

Previous studies showed (Banerjee et al., 2002; Grisko et al., 2006) that the response rate on docetaxel of the patients with low expression of β-tubulin III in tumor was higher than that of the patients with high expression of β-tubulin III, while the level of survivin expression was correlated to the drug resistance of chemotherapy drug docetaxel targeted on microtubule. In this study, the levels of β-tubulin III and survivin protein from 84 patients with late breast cancer patients, combined with chemotherapy efficacy of docetaxel combined with doxorubicin and cyclophosphamide, were retrospectively analyzed and biological indicators of drug sensitivity were preliminarily determined when choosing this program.

Materials and Methods

The objects of investigation

84 patients with late breast cancer from June 2003 to December 2008 patients were enrolled in our hospital. The age of the patients was 35-78 years with a median age of 55 years. All the patients were performed with

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surgery, and confirmed as invasive late breast cancer by postoperative pathology. Among them, the patients includes 40 cases of invasive lobular carcinoma and 44 cases of infiltrating ductal carcinoma. According to degree of tissue differentiation, the patients includes 20 cases of well differentiated breast cancer, 29 cases of moderate differentiation breast cancer and 35 cases of poorly differentiated breast cancer. 60 patients with estrogen receptor (ER)-positive, progestogen receptor (PR) positive 55 cases, human epidermal growth factor receptor 2 (Her-2) positive of 24 cases. Pathological type of cancer was according to WHO histologic classification (1998) standard and the phases of cancer were according to the standard established by the International Union Against Cancer (UICC) by 1997. All the patients were enrolled in the following conditions including the patients with breast cancer confirmed by pathological diagnosis, the patients previously received the chemotherapy of taxane or no preoperative chemotherapy and radiotherapy, and endocrine therapy during chemotherapy, the Karnofaky scores of the patients exceed 70 and the predictable survival dates exceed 3 months, the patients with objective observable indexes to evaluate the therapeutic effect, and the patients with the normal routine examination of blood, the function of liver and kidney and ECG and no detected contraindication of chemotherapy.

Chemotherapy scheme

The patients were intravenously injected with docetaxel at 75 mg/m^2 for one hour, d1; and with doxorubicin at 50mg/m^2, d1 and cyclophosphamide at 500 mg/m^2, d1. 21 days was a cycle of chemotherapy. The patients were treated with at least 2 cycles of chemotherapy. For the prevention of fluid retention and allergic reaction after the use of docetaxel, the oral use of dexamethasone was started from the day before the administration of Docetaxel for 3 days at 8mg/bid. In addition, the patients were intramuscular injected with 25mg Flanagan 30 min before administration, and intravenously injected with 50 mg ranitidine. The patients were routinely administrated with 5HT3 receptor antagonists (8mg ondansetron) to prevent nausea and vomiting.

Immunohistochemistry

After the slice of the biopsy specimen, the slice will be treated by pro-streptavidin biotin-peroxidase method (SP). The sections were treated with xylene and graded ethanol and dewaxed to water, then incubated with 3% H_2O_2 for 15 minutes to block the activity of endogenous peroxidase. The sections were treated with 10 mmol/L citrate buffer by microwave heating to repair antigen and dropped with goat serum blocking solution after cooling and incubated at room temperature for 5 minutes to block non- specific antibodies. The sections were dropped with monoantibody after excess serum on the section was removed and incubated on 4 °C overnight. The sections were dropped with biotinylated goat anti-mouse IgG and horseradish peroxidase Streptomyces avidin working solution, and incubated at room temperature for 15 minutes. Then the sections were DAB colored and counterstained with hematoxylin. The positive control and negative control were set up for each antibody. positive control was known tissue sections with positive expression and a negative control, where the primary antibody was replaced with either mouse or rat IgG at the same dilution, was always included.

The evaluation of immunohistochemistrial resutls

β-tubulin III protein and survivin was localized in cellular skeleton and cellular cytoplasm, respectively. The brown particles in sections were considered as positive expression. The standard of evaluating positive expression: less than 25% positive cells in sections were considered as negative, 25 - 50% positive cells in sections were considered as weakly positive (+), 50 ~ 75% as moderate positive(+ +), greater than 75% as strongly positive (+ + +).

The evaluation of therapeutic effect

After two cycles of chemotherapy, the therapeutic effect of docetaxel was evaluated. According to the treatment of RECIST solid tumor, the evaluation criteria included complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD). The objective effective rate was calculated by CR + PR. In addition, the progression time, 1-year and 2-year survival rate of median diseases were also analyzed.

The evaluation of toxicity criteria

The evaluation criteria of toxicity was mainly according to the toxicity criteria formulated by U.S. NCI (CTC 3rd version).

Statistical analysis

All the data were processed with SPSS 13.0 statistical package. The relationship between protein expression, the effective rate of chemotherapy and clinico- pathological parameters were analyzed with χ^2 test. The comparison between β- tubulin III and survivin was analyzed with Spearman rank correlation. $P < 0.05$ was considered as significant statistically. The estimated survival analysis of patients was used with Kaplan-Meier curve and Log-rank test.

Results

Results of immunohistochemical staining

In the 84 specimens of the patients with advanced breast cancer tissues, the positive rate of β-tubulin III was 38.1% (32/84). Among them, the positive rate of β-tubulin III from invasive lobular carcinoma tissues and invasive ductal carcinoma was 37.5% and 38.6%, respectively. The positive rate of β-tubulin III from the patients aged ≥50 years and 50 years old was 35.4% and 40.1%, respectively. The positive rate of β-tubulin III from the patients with and without lymph node metastasis was 39.3% and 35.7%, respectively. The positive rate of β-tubulin III from the patients with and without positive expression of ER was 38.3% and 37.5%, respectively. The positive rate of β-tubulin III from the patients with and without positive expression of PR 38.2% and 37.9%, respectively. The positive rate of β-tubulin III from the patients with

Table 1. Expression of β-tubulinIII and Survivin in Breast Cancer Tissues

Clinical Characteristics	n	β-tubulinIII expression +	β-tubulinIII expression –	Survivin expression +	Survivin expression –
age					
<50	31	11(35.4)	20(64.6)	23(74.2)	8(25.8)
≥50	53	21(40.1)	32(59.9)	41(77.4)	12(22.6)
pathotypes					
ILC	40	15(37.5)	25(62.5)	29(72.5)	11(27.5)
IDC	44	17(38.6)	27(61.4)	35(79.5)	9(20.5)
tumor size					
≤5cm	39	15(38.5)	24(61.5)	30(76.9)	9(23.1)
>5cm	45	17(37.8)	28(62.2)	34(75.6)	11(24.4)
LN metastasis					
+	56	22(39.3)	34(60.7)	46(73.2)	10(26.8)
–	28	10(35.7)	18(64.3)	18(64.3)	10(35.7)
ER expression					
+	60	23(38.3)	37(61.7)	44(73.3)	16(26.7)
–	24	9(37.5)	13(62.5)	20(83.3)	4(16.7)
PR expression					
+	55	21(38.2)	34(61.8)	42(76.4)	13(23.6)
–	29	11(37.9)	18(62.1)	22(75.9)	7(24.1)
Her-2 expression					
+	24	9(37.5)	13(62.5)	17(70.9)	7(29.1)
–	60	23(38.3)	37 (61.7)	47(78.3)	13(21.7)

+,represents positive expression;–,represents negative expression; ILC, infiltrating lobular carcinoma; IDC, nfitrating ductal carcinoma; LN, lymph node; ER, estrogen receptor; PR, progestogen receptor; human epidermal growth factor receptor 2, Her-2

Table 2. The Relationship Between the Expressions of β-tubulinIII and Survivin and Therapeutic Effects

	PD	SD	PR	CR	RR	χ^2	P
β-tubulinIII							
+	5	15	11	1	37.5	9.936	0.002
_	0	20	27	5	61.54		
Survivin							
+	4	29	31	0	48.4	6.771	0.009
_	1	6	7	6	65		

+, represents positive expression; –, represents negative expression; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease

and without positive expression of Her-2 was 37.5% and 38.3%, respectively. The above data were analyzed by χ^2 test and there were no statistical significance between the two groups (P> 0.05).

In the 84 specimens of the patients with advanced breast cancer tissues, the positive rate of survivin was 76.2% (64/84). Among them, the positive rate of survivin from invasive lobular carcinoma tissues and invasive ductal carcinoma was 72.5% and 79.5%, respectively. The positive rate of survivin from the patients aged ≥50 years and 50 years old was 77.4% and 74.2%, respectively. The positive rate of survivin from the patients with and without lymph node metastasis was 73.2 % and 64.3%, respectively. The positive rate of survivin from the patients with and without positive expression of ER was 73.3% and 83.3%, respectively. The positive rate of survivin from the patients with and without positive expression of PR 76.4% and 75.9%, respectively. The positive rate of survivin from the patients with and without positive

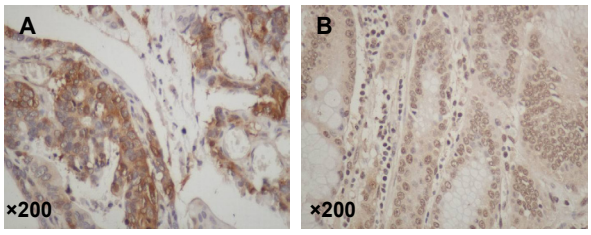

Figure 1. Expression of A) β-tubulin III and B) Survivin in Breast Cancer Tissues

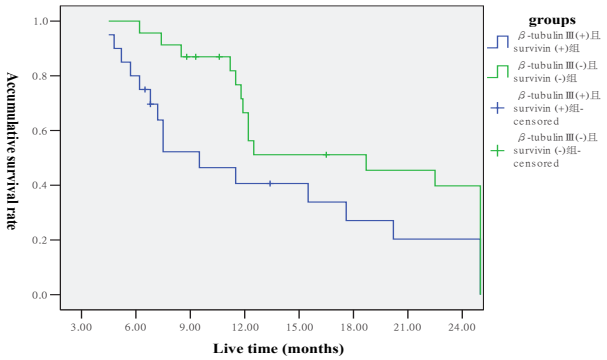

Figure 2. Cumulative Survival of the Patients with Advanced Breast Cancer

expression of Her-2 was 70.9% and 78.3%, respectively. The above data were analyzed by χ^2 test and there were no statistical significance between the two groups (P> 0.05). The results of Spearman rank correlation test showed that the expression of β-tubulin III and was positively correlated to that of Survivin (r = 0.525, P = 0.000) (Figure 1, Table l).

The curative effect of chemotherapy

After two cycles of chemotherapy, the therapeutic effect of docetaxel was evaluated. Among the 84 patients with advanced breast cancer, the patients of CR was 6 cases, the patients of PR was 38 cases, SD was 35 cases and PD was 5 cases. The total effective rate was 52.38% (44/84). There were significant difference between the effective rate of the patients with the positive expression of β-tubulin III (37.50%) and that of the patients with the negative expression of β-tubulin III (61.54%) (P <0.01). There were significant difference between the effective rate of the patients with the positive expression of survivin (48.40%) and that of the patients with the negative expression of survivin (65.00%) (P<0.05). The analysis of combined detection showed that there were significant difference between the effective rate of the patients with the positive expressions of β-tubulin III and surviving (25.0%) and that of the patients with the negative expressions of β-tubulin III and surviving (73.91%) (P <0.01) (Table 2).

Survival condition

As shown in Figure 2, all the patients have been followed up. The follow up rate was 100%, and the time of follow up was 2 years. The time was terminated in December 2010, 2 years after the last patient were enrolled. The non-progression period of median disease of the patients with positive expression of β-tubulin III and surviving was 3.9 months. In addition, 1-year and

Table 3. The Relationship Between the Expressions of β-tubulinIII and Survivin and Toxicant and Side Effects of Chemotherapy

toxicant and side effects	T(+) S(+)	T(+) S(-)	T(-) S(+)	T(-) S(-)
nausea and vomiting				
I-II°	7	8	6	7
III-IV°	0	1	1	0
bone marrow suppression				
I-II°	8	7	8	6
III-IV°	1	1	1	0

T, represents β-tubulinIII; S, represents Survivin; +, represents positive expression ; -, represents negative expression

2-year survival rate was 34.5% and 15.1%, respectively. The non-progression period of median disease of the patients with negative expression of β-tubulin III and surviving was 7.7 months. In addition, 1-year and 2-year survival rate was 61.6% and 31.4%, respectively. There were significant difference between the two groups (P <0.05). The overall survival period of the patients were not investigated because the treatments of the patients after evaluating curative effect were different.

Toxicity and side effects

As shown in Table 3, allergic reaction was not happened in any enrolled patients. The main adverse reactions were gastrointestinal symptoms, bone marrow suppression and alopecia, et al. The patients with nausea and vomiting were 41 cases (48.8%), including 2 cases of patients with III - IV°. The patients with leucopenia were 62 cases (73.8%), including 3 cases of patients with III -IV° bone marrow suppression. The patients with slight alopecie were 50 cases (59.5%). There were no significant difference between the groups (P> 0.05). The patients have tolerated and finished the treatment after symptomatic and supportive treatment.

Discussion

Breast cancer is one of the most common malignancy for women, whose incidence accounted for the first incidence of women malignant tumors. About 50% of breast cancer patients have happened recurrence or metastasis. The survival time of median disease was more than two years. Chemotherapy is a major rescue treatment for palindromic metastatic breast cancer. Because many patients after adjuvant therapy and palindromic metastasis were treated with anthracycline, the incidence of anthracycline-resistant breast cancer was significantly increased. It is very difficult to choose further treatment. Systemic chemotherapy can improve the survival time and life quality of patients. A number of studies showed that either alone or combined treatment of docetaxel has shown obvious advantages on the treatment of advanced breast cancer and the tolerance of patients treated with docetaxel was well (Khan et al., 2003; Biganzoli et al., 2004). However, it made the clinicians so confused that the different patients with breast cancer even if at the same stage, the same pathological type, and adopted the same chemotherapy containing docetaxel, the efficacy and survival rate of the patients were quite different. It also suggested that the sensitivity to docetaxel of every patient was different. Docetaxel is a new semi-synthetic anticancer drug extracted from yew needles. Its mechanism is to promote the polymerization of tubulin and prevent the depolymerization of microtubule for the purpose of inhibiting the mitosis and proliferation of cancer cell. For palindromic metastatic breast cancer, the effective rate of the single treatment of docetaxel was 34% to 58% (Campone et al., 2009).

Survivin is a novel member of the family inhibitor of apoptosis (IAps), which can block the apoptosis process by inhibiting the activity of caspase-3, etc (Kappler et al., 2007). It is one of the key factors that affect cell cycle, mitosis and apoptosis. Research showed that the high expression of survivin can inhibit apoptosis induced by a variety of factors, such as anticancer drugs, and can increase the survival rate of tumor cells in the process of tumor progression and chemotherapy. Survivin is expressed in cell cycle-dependent manner in the G2 / M phase, and exerted effect afer the combination with spindle and microtubules. It is closely related to microtubules and spindle, so the onset of resistance is likely to lie in the interaction with microtubules (Altieri, 2003; Wang et al, 2006). β-tubulin III is one of 7 subtypes of β tubulin and expressed in a variety of tumor tissues. β-tubulin III plays an critical role in cell cycle, cell proliferation, differentiation and tumorigenesis due to its unique depolymerization activity of microtubule. In addition, β-tubulin III also can control the cell cycle, and thus alter cell proliferation, differentiation, and other biologic behaviors. In recent years many studies showed that β-tubulin subunits of β III were associated with taxane resistance. In vitro studies, many experimental results showed that chemotherapy sensitivity to taxane drugs was significantly decreased on the tumor cell lines with the high expression of β-tubulin III protein or gene (Ranganathan et al, 2008).

With the progress of the molecular biological technology, the further apprehension of mechanism of tumor drug resistance and the breakthrough of gene targeting treatment, more and more sensitive molecular markers were detected to predict curative effect and drug selection was based on sensitivity of anti-cancer drugs so as to improve the efficiency of drugs and predict the prognosis of diseases. Current experiments have proved that the expression of β-tubulin III and survivin was related to the resistance of taxane drugs, but the combined detections of two genes to predict the sensitivity to docetaxel of advanced breast cancer patients has not been reported. Therefore, this study was designed to detect by the expressions of β-tubulin III and survivin of 84 cases of breast cancer patients by immunohistochemistry. The results showed that the positive rate of β-tubulin III and survivin was 38.1% and 76.2%, respectively. The positive staining area located in the cytoskeleton and kytoplasm. It also showed that the gene expression of β-tubulin III and survivin was not related to age, pathological type, tumor size, lymph node metastasis and hormone receptor expression (p> 0.05). Further retrospective analysis of 84 cases of breast cancer programs containing docetaxel

chemotherapy, (TAT + ADM + CTX) showed that the total effective rate was 52.38% (44/84).Among them, The effective rate of the patients with the positive expression of β-tubulinIII was significantly lower than that of the patients with negative expression of β-tubulinIII (37.50% vs 61.54 %, P<0.05). The effective rate of the patients with the positive expression of survivin was significantly lower than that of the patients with negative expression of survivin (48.40% vs 65.50%, P<0.05). In addition, the effective rate of the patients with positive expressions of β-tubulinIIIand survivin was significantly lower than that of the patients with negative expressions of β-tubulinIIIand survivin (25.0% vs 73.91%, P<0.05). The main adverse reactions were gastrointestinal symptoms, bone marrow suppression and alopecia. The patients with nausea and vomiting were 41 cases (48.8%), including 2 cases of patients with III - IV°. The patients with leucopenia were 62 cases (73.8%), including 3 cases of patients with III - IV ° bone marrow suppression. The patients with slight alopecie were 50 cases (59.5%). There were no significant difference between the groups (P> 0.05). The patients have tolerated and finished the treatment after symptomatic and supportive treatment.

In summary, the high expression of β-tubulin III and survivin may be responsible for the drug resistance to docetaxel in patients with advanced breast cancer. The detection of β-tubulin III and survivin on specimens can guide clinical therapy improve the curative effect of chemotherapy before chemotherapy, and its clinical significance needs further investigation. we will further increase the cases of patients and prospectively forecast the curative effect of chemotherapy for the patients with advanced breast cancer according to the detection of two protein levels.

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