Effect of Neoadjuvant CAF Regimen on the Expression of BCSG1 in Breast Cancer*

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* Supported by a grant from the Health Department of Hebei Province (No. 02276103D-12).

Received: 22 September 2005 / Revised: 15 November 2005 / Accepted: 3 May 2006

Abstract Objective: To evaluate the efficacy of neoadjuvant chemotherapy and explore a sensitive and objective way in the evaluation of neoadjuvant chemotherapy, the pathological changes and BCSG1 expression were studied by pathological and immunohistochemical method in breast cancer patients with CAF neoadjuvant chemotherapy (Cyclophosphamide, Adriamycin and Fluorouracil, CAF) and those without at the same period. Methods: Specimens were obtained from 34 breast cancer patients receiving neoadjuvant CAF regimen chemotherapy (CAF group) and 110 breast cancer patients not receiving neoadjuvant chemotherapy (control group). The BCSG1 expression was detected by SP immunohistochemistry. Correlation between BCSG1 expression and pathological response to CAF neoadjuvant chemotherapy was analyzed. Results: Overall response rate to neoadjuvant chemotherapy was 79.4%. The strong cytoplasm expression of BCSG1 was significantly lower in CAF group than in control group (29.4% vs. 64.5%, P<0.01). In CAF group, the positive cytoplasm expression in partial response (PR) (grade II) cases was significantly lower than that in no response (NR) (grade III) cases (P=0.002). Conclusion: Neoadjuvant chemotherapy of CAF regimen could decrease the nuclear expression of BSCG1 in breast cancer.

Key words: breast cancer; neoadjuvant chemotherapy; immunohistochemistry; BSCG1

The significance of neoadjuvant chemotherapy (preoperative chemotherapy) in breast cancer is to reduce or prevent recurrence and metastasis, increase tumor resectability by reducing the size of primary tumor, and improve local control rate, survival rate and quality of life (QOL). It also opens an important area, in which a perfect drug sensitivity model in vivo can be offered[1]. In the past, the evaluation of the effects of neoadjuvant chemotherapy in breast cancer was limited to morphologic changes under light microscopy after chemotherapy[2]. As the fast increase in the number and identification of different tumor markers in recent years, the possible usage of tumor markers in the evaluation of the effects of chemotherapy has caused the concern of oncolgists. BCSG1, a breast cancer specific gene, was found in 1997. Ji et al[3] reported that BSCG1 expression played an important role in development of human breast cancer. Up to now, studies on the putative significance of changes in the BSCG1 expression after chemotherapy have not been seen.

To evaluate the efficacy of neoadjuvant chemotherapy and explore a sensitive and objective way in the evaluation of neoadjuvant chemotherapy, the pathological changes and BSCG1 expression were studied with pathological and immunohistochemical methods in breast cancer patients with CAF neoadjuvant chemotherapy (Cyclophosphamide, Adriamycin and Fluorouracil, CAF) and those without at the same period.

Materials and methods

Clinical Data
Specimens were obtained from 144 hospitalized patients with breast cancer in the Fourth Affiliated Hospital of Hebei Medical University from June 2000 to October 2003. The diagnosis was further confirmed by two experienced pathologists according to the standard described by Chinese Anti-Cancer Association[2]. All patients were women with a median age of 47 (ranging from 31 to 74). Of the 144 patients, 34 received preoperative neoadjuvant chemotherapy (2 cycles of CAF regimen, CAF group), and other 110 cases were treated with surgical method alone without chemotherapy or radiotherapy (control group). According to the TNM staging system of the UICC (1997), there were 7 cases in stage IIA, 15 cases in stage IIB, 9 cases in stage IIIA and 3 cases in stage IIIB.

Neoadjuvant chemotherapy treatment
For the 34 cases in CAF group, two cycles of CAF regimen were used before operation as neoadjuvant chemotherapy. CAF regimen consisted of Adriamycin (ADM) 60
mg/m² in d1; Cyclophosphamide (CTX) 600 mg/m² in d1 and d8; Fluorouracil (5-Fu) 500 mg/m² in d1 and d8; q3w.

**Immunohistochemical analysis of BCSG1 expression**

Caprine anti-human monoclonal antibody against BCSG1 (sc-10699) and SP Immunohistochemical staining kit were purchased from Beijing Zhongshan Biotechnology (China).

The monoclonal antibody against BCSG1 was diluted at 1:100. Normal breast tissue was used as positive control, and normal breast tissue sections treated with PBS (0.01 mol/L, pH 7.4) instead of the primary antibodies were used as negative controls. Counterstaining was performed with haematoxylin.

**Evaluation of pathological response and immunohistochemical staining**

Treatment efficacy was evaluated pathologically according to the standard described by Yi et al.[4]. The pathological change after chemotherapy was graded into three. Grade I was complete response (CR), including grade IA (tumor disappeared completely and was replaced by fibrous tissue), and IB (tumor could not been seen by the unaidsed eye, but a few cancer cells were observed under microscope). Grade II was partial response (PR), including IIA (the percentage of degeneration and necrosis of tumor cells was over 60%) and IIB (the percentage of degeneration and necrosis of tumor cells was from 20% to 60%). Grade III was NR (No response), in which the percentage of degeneration and necrosis of tumor cells was <20% or no changes could be seen.

BCSG1 expression was evaluated according to the method described by Mohsin et al.[5]. Clearly brown staining restricted to cytoplasm was considered as positive reaction for BCSG1, while no staining restricted to nucleus. The mean percentage of positive tumor cells was assigned one of the following 4 categories: 0, <25%; 1, 25%–50%; 2, 51%–75%; 3, >75%. Cases with <3 were defined as weak expression, and cases with >3 as strong expression.

**Statistical analysis**

Statistical evaluation was performed by Chi-square test. A value of $P<0.05$ was considered significant.

**Table 1** BCSG1 expression in cytoplasm in two groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>BCSG1 expression [n, (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low expression</td>
</tr>
<tr>
<td>Control</td>
<td>110</td>
<td>39 (35.5)</td>
</tr>
<tr>
<td>Neoadjuvant chemotherapy</td>
<td>34</td>
<td>24 (70.6)</td>
</tr>
</tbody>
</table>

$\chi^2=13.027$, $P<0.01$

**Table 2** Relationship between BCSG1 expression and pathological changes after neoadjuvant chemotherapy

<table>
<thead>
<tr>
<th>Pathohistological changes</th>
<th>n</th>
<th>BCSG1 expression [n, (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low expression</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>27</td>
<td>23</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

Fisher’ exact test, $P=0.002$

**Results**

**BCSG1 expression in breast cancer cells**

The strong positive expression rate of BCSG1 in cytoplasm in neoadjuvant chemotherapy group was significantly lower than that in control group (29.4% vs. 64.5%, $P<0.01$; Table 1, Fig. 1, 2).

**Pathological evaluation of neoadjuvant chemotherapy**

Pathologically, no CR (grade I) cases were found among the 34 cases. PR (grade II) was achieved in 27 cases and NR (grade III) was seen in the other 7 cases (Fig. 3). The total effective rate (CR+PR) was 79.4% (27/34).

**Correlation of BCSG1 expression to pathological changes in CAF group**

Significant correlation could be found between grades of pathological changes and the positive expression of BCSG1 after neoadjuvant chemotherapy. The positive BCSG1 expression rate was lower in grade II cases than in grade III cases (Table 2).