Downregulation of BCSG1 may correlate with better outcome of neoadjuvant chemotherapy for triple-negative breast cancer

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Abstract. The aim of this study was to investigate the correlation between breast cancer-specific gene 1 (BCSG1) and the effect of neoadjuvant chemotherapy (NAC) in patients with triple-negative breast cancer (TNBC). Real-time RT-PCR and immunohistochemistry were used to determine the expression of BCSG1 mRNA and protein levels of 32 TNBC patients before and after NAC. Tumor size was reduced significantly after NAC in all 32 TNBC patients. The expression of BCSG1 was also decreased after NAC at both mRNA and protein levels. There was a negative correlation between BCSG1 levels after NAC and the effect of NAC. BCSG1 may be a potential target for NAC in the treatment of TNBC.

Introduction

Triple-negative breast cancer (TNBC) is a type of high-risk breast cancer in which the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2/ErbB2) are all negative (1). TNBC accounts for 15-20% of all breast cancer cases. Since the effective targeted endocrine therapy cannot be used, patients with TNBC usually have a poor prognosis. In addition to surgical therapy, the main treatment for TNBC is chemotherapy. Neoadjuvant chemotherapy (NAC) has been widely accepted in the treatment of breast cancer. Despite the relative chemosensitivity, less than 25% of all patients with TNBC treated with standard NAC achieve complete pathological response (pCR). However, the evaluation of the effect of NAC is limited to the clinical and pathological changes of the tumors and metastatic lymph nodes. Therefore, a method to simply and accurately evaluate the effect of NAC in the treatment of TNBC would be extremely valuable.

A breast cancer-specific gene, BCSG1, was identified by Ji *et al* (2) in 1997. BCSG1 is highly expressed in human-infiltrating breast carcinomas but not expressed in normal or benign breast tissues and the expression of BCSG1 is also stage-specific for breast cancer (3). Overexpression of BCSG1 in breast cancer cells increases the motility and invasiveness *in vitro* and stimulates metastasis *in vivo* (4). In a clinical trial, patients with BCSG1-positive breast tumors generally had shorter disease-free survival and overall survival and higher probability of mortality compared with the patients with BCSG1-negative tumors (5). Therefore, BCSG1 may be used as a marker for breast cancer progression and prognosis (5,6). Although a number of studies have established the significance of BCSG1 in breast cancer, few results concerning the correlation between BCSG1 and TNBC have been reported (7). Therefore, we analyzed the correlation between BCSG1 expression and the effect of NAC in the treatment of TNBC in the present study to determine the role of BCSG1 in the treatment of TNBC with NAC.

Patients and methods

Patients and treatment. All 32 patients (female, 27-45 years old; median age, 40) were treated at the Center of Breast Diseases at the Second People's Hospital of Shenzhen between September 2009 and August 2011. All patients were diagnosed with triple-negative invasive non-specific cancer by pathological evaluation and hormone receptors test. Patients underwent breasts, double axillary and liver type-B ultrasonic scan, chest X-ray and whole body bone scan prior to chemotherapy. The TNM stages were: IIA, 11; IIB, 14; IIIA, 5; IIIB, 2 (UICC/AJCC, 2003). A total of 18 patients were found to have ipsilateral axillary lymph node metastasis. No other treatment was administered prior to definite diagnosis. No serious heart, liver or kidney damage was detected (Karnofsky score=100).

All the 32 patients were administered a combination of 600 mg/m^2 cyclophosphamide (CTX), 80 mg/m^2 epirubicin

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(EPI) and 500 mg/m^2 fluorouracil (5-FU) on day 1 and then every 21 days. After 2 cycles of chemotherapy, patients underwent modified radical mastectomy or breast-conserving surgery. The changes of lesions were evaluated according to the criteria of the World Health Organization (WHO) for anticancer drugs prior to and following NAC-based clinical response and B ultrasound (3). Complete response (CR) and partial response (PR) were calculated as clinical overall response. Breast cancer tissues were obtained through core-needle biopsy prior to NAC or surgery following NAC. Written informed consent was obtained from the patients. The study was approved by the ethics committee of the First Affiliated Hospital of Shenzhen University, Shenzhen, Guangdong, China.

Immunohistochemistry. Immunohistochemistry was performed according to the manufacturer's instructions with BCSG1 polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA; 1:100 dilution). Brain tissue was used as a positive control and PBS as a negative control. BCSG1-positive cells were defined according to the standard reported by Mohsin *et al* (8). The BCSG1 expression score was calculated from the proportion of positive cells and the color intensity of the cells: i) number of positive cells, grade 0, <25%; grade 1, 26-50%; grade 2, 51-75%; grade 3, ≥75%; ii) color intensity, grade 1, weak; grade 2, moderate; grade 3, strong. The score for each slice is the summation of the two parts. A total score <3 was considered to indicate low expression and ≥3 to indicate high expression.

Real-time RT-PCR. Total RNA was isolated from breast cancer tissue with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration and purity of total RNA were determined by a spectrophotometer (Eppendorf, Hamburg, Germany). Reverse transcription was performed using Random primer RT mixtures with M-MLV (20 U/μl; Promega, Madison, WI, USA) reverse transcriptase 20 U/20 μl, total RNA 1 μg/20 μl. cDNA was stored at -20˚C. Quantitative real-time PCR was performed with an ABI stepone plus Real-time PCR system. The sequences of the primers were as follows: BCSG1, forward 5'-AGGAGGGGGTCATGTATGTG-3', reverse 5'-TTCTCTTTGGATGCCTCACC-3'; GAPDH forward 5'-GGAAGGTGAAGGTCGGAGT-3', reverse 5'-CCTGGAAGATGGTGAGGG-3'. PCR mixtures contained 1 μl cDNA, 12.5 μl SYBR® Premix 2X (Toyobo, Osaka, Japan) and 0.16 μmol/l forward and reverse primers in a total volume of 25 μl. Reactions were started with a polymerase activation step at 94˚C for 5 min followed by 35 cycles of 94˚C for 30 sec, 57˚C for 45 sec and 72˚C for 30 sec. Fluorescence data were acquired after each cycle. The amount of specific mRNA in samples was calculated using the ΔΔCT method.

Statistical analysis. Measurement data are expressed as mean ± SD. Data were analyzed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA). The paired Student's t-test was used for comparing the BCSG1 expression difference before and after NAC. The χ^2 test was used for comparing the clinical overall response rate in patients with high or low BCSG1 expression. The correlation between the effect of NAC and BCSG1 expression was measured by Spearman rank correlation analysis. P<0.05 was considered to indicate a statistically significant result.

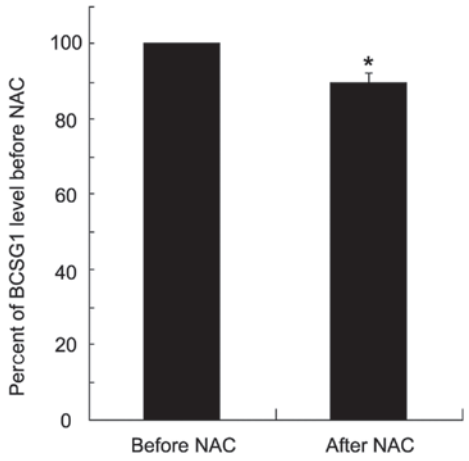

Figure 1. Expression of BCSG1 mRNA in the breast cancer tissues. Before and after NAC, breast cancer tissues were obtained through core-needle biopsy or surgery, respectively. The BCSG1 mRNA levels were determined by real-time RT-PCR. The amounts of specific mRNA in samples were calculated using the ΔΔCT method. Data are expressed as mean ± SD (n=32). *P<0.05, compared with before NAC. BCSG1, breast cancer-specific gene 1; NAC, neoadjuvant chemotherapy.

Results

Response to neoadjuvant chemotherapy. The tumor softened in 62.5% of patients (20/32) 10 days after NAC and reduced in size in 71.9% of patients (23/32) after one cycle of NAC. Tumor size shrank markedly in 84.4% (27/32) patients after 2 cycles of NAC. There were 3 CR, 24 PR and 5 stable disease (SD) with an overall response rate of 84.4% (27/32). In 28 cases of postoperative cancer tissue specimens, a clear boundary between the cancer tissue and the breast tissue and white cut surface and little necrosis on tumor tissue were observable with the naked eye. Various degrees of cell degeneration and necrosis of the tumor cells, nuclear contraction, rupture and cytoplasmic coagulation necrosis of the cells surrounding the tumor tissue and vascular endothelial hyperplasia and blood vessel narrowing or occlusion were observed with a light microscope. In the remaining four cases, various degrees of cell degeneration were observed, but necrosis was not clear. Various degrees of nuclear contraction and fragmentation, necrosis, calcification and fibrosis were also observed in the cancer cells in the axillary lymph nodes.

BCSG1 mRNA expression. Real time RT-PCR was performed to examine the BCSG1 mRNA level in the cancer tissues of each patient prior to and following NAC (Fig. 1). The BCSG1 mRNA level in breast cancer tissues after NAC was decreased significantly compared with that before NAC (a decrease of ~10%).

Correlation of the BCSG1 level and the effect of NAC. The expression of BCSG1 protein was determined with immuno-

Table I. Correlation of BCSG1 expression and curative effect of NAC (n=32).

Curative effect of NAC	BCSG1 expression			
	High expression	Low expression	P-value	r
CR + PR	5	22	<0.01	-0.584
SD	4	1		

BCSG1, breast cancer-specific gene 1; NAC, neoadjuvant chemotherapy; CR, complete response; PR, partial response; SD, stable disease.

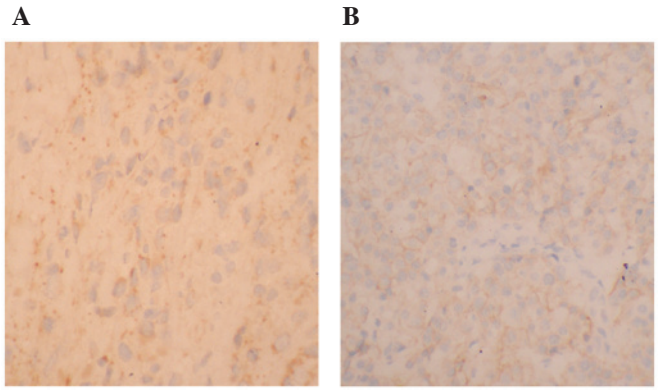

Figure 2. Immunohistochemistry for BCSG1 expression. (A) BCSG1 was markedly expressed in TNBC tissues prior to NAC (x100). (B) BCSG1 expression was lowered in TNBC tissues following NAC (x100). BCSG1, breast cancer-specific gene 1; TNBC, triple-negative breast cancer; NAC, neoadjuvant chemotherapy.

histochemistry (Fig. 2). BCSG1 protein was highly expressed in 22 patients (68.8%) before NAC, but only in nine patients after NAC (28.1%). The difference was significant. There was a negative correlation between the BCSG1 level and the effect of NAC (r=-0.584, P<0.01; Table I).

Discussion

In the present study, we found that the expression of BCSG1 was decreased following NAC in TNBC patients. There was a negative correlation between the BCSG1 level and the effect of NAC in TNBC. Our results indicate that there is a correlation between the BCSG1 levels and the effect of NAC in patients with TNBC.

BCSG1, also known as SNCG (γ-synuclein gene), was identified in 1997 by direct sequencing of cDNA gene in breast cancer (2). BCSG1 is not expressed in normal breast tissue but is highly expressed in most invasive and metastatic breast cancers. It has been shown that overexpression of BCSG1 promoted the invasion and metastasis of breast cancer cells (4). Overexpression of BCSG1 is also an event in advanced breast cancer and predicts poor clinical outcome in breast cancer (5,6) These results suggest that BCSG1 is a predicator for the tumor invasion and metastasis and a target for gene therapy. Therefore, detecting BCSG1 may aid the evaluation of the invasive and metastatic ability and the prognosis of breast cancer. In our study, we found that BCSG1 was also highly expressed in TNBC patients before NAC, suggesting a potential therapy target for the TNBC. Our results showed that TNBC patients who gained more benefit from NAC had lower BCSG1 expression, indicating that BCSG1 is involved in the NAC treatment. TNBC is a subtype of breast cancer. Although TNBC is an initially chemosensitive disease, less than 25% of patients with TNBC who received standard NAC achieved pCR and the remaining patients usually have a poor prognosis (9). Several approaches have been reported to improve the NAC efficacy in previous studies, including different anthracycline-based regimens, anthracycline-taxane combinations, sequential regimens and dose-dense schedules (9). Furthermore, certain researchers have revealed other characteristics of TNBC, including overexpression of EGFR and c-KIT, increased proliferative rate through MAP kinase and Akt pathways (10) and providing some basis for the targeted therapy in those patients. Poly-ADP-ribose polymerase (PARP), a DNA-repair nuclear enzyme, has gained attention as a therapeutic target for cancer. Inhibition of PARP may improve the efficacy of certain DNA-damaging chemicals, including platinum compounds and topoisomerase inhibitors (11,12). However, lack of consistency and the complexity of the analysis and interpretation of molecular classification data hindered the application of these targets in the treatment of TNBC (9). Since TNBC patients cannot benefit from target endocrine therapy and anti-HER-2 treatment, systemic chemotherapy treatment is the only one option in combination with local surgery and radiotherapy. In the present study, we showed that patients with lower BCSG1 levels after NAC gained more benefit from NAC than patients with high BCSG1 levels. Since the expression level of BCSG1 has been indicated to correlate with the effect of chemotherapy, BCSG1 would be used as a new target in NAC and a screening compound for TNBC.

The current study has certain limitations. First, the number of patients enrolled in the study was small and further research is required to support the conclusion. Second, the correlation between the BCSG1 levels and the long-term prognosis of the TNBC patients also requires further investigation in the future.

In summary, the results of the present study revealed a correlation between the BCSG1 level after NAC and the effect of NAC, indicating that BCSG1 may act as a target for chemotherapy and be used in the screening of new agents for TNBC treatment.

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