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Glutathione *S*-transferase polymorphisms influence chemotherapy response and treatment outcome in breast cancer

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ABSTRACT. The aim of this study was to evaluate the role of *GSTM1* null/present, *GSTT1* null/present, and GSTP1 IIe105Val polymorphisms in the clinical response to chemotherapy and treatment outcome of patients with breast cancer. A total of 262 subjects were randomly selected from among patients with a histologically confirmed breast cancer. The genotypes of *GSTM1*, *GSTT1*, and GSTP1 IIe105Val polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism analysis. Our study found that the null genotype of *GSTM1* was associated with a better response to chemotherapy, and the odds ratio [95% confidence interval (CI)] was 1.78

(1.03-3.08). In the Cox proportional hazard model, the hazard ratio (95%CI) for overall survival (OS) in patients carrying the null genotype of *GSTM1* was 0.57 (0.32-0.98) using the non-null genotype as the reference variable. However, we observed no significant association between the *GSTT1* and GSTP1 polymorphisms and response to chemotherapy and OS in patients with breast cancer. In conclusion, our study found that the *GSTM1* polymorphism plays an important role in influencing the chemotherapy response and OS in patients with breast cancer.

Key words: Glutathione *S*-transferases; Polymorphism; Chemotherapy; Clinical outcome

INTRODUCTION

Breast cancer is the leading cause of cancer mortality in women worldwide and the incidence is still increasing in China (Jemal et al., 2010; Fan et al., 2014). It is well known that chemotherapy for breast cancer is used as an adjuvant systemic therapy following primary surgery or as neoadjuvant therapy before surgery in patients with locally advanced breast cancers (van der Hage et al., 2001). Although many clinicopathologic characteristics have been identified as being imprecise in predicting the efficacy of chemotherapy, increasing evidence has suggested that drug-metabolizing enzymes play an important role in determining interindividual variations in therapeutic response (Arun et al., 2010; Franco et al., 2012).

The human glutathione *S*-transferases (GSTs) are a superfamily of dimeric phase-II metabolic enzymes that play a key role in the cellular defense system (Strange and Fryer, 1999). GST enzymes detoxify chemotherapeutic drugs or their metabolites by catalyzing the reduction of these compounds through their conjugation with glutathione. It has been suggested that genetic polymorphisms in *GST* genes could reduce the effectiveness of the detoxification of cytotoxins generated by chemotherapeutic agents in the treatment of breast carcinoma (Townsend and Tew, 2003).

Many previous studies have reported the association between GSTs and overall survival (OS) of patients with breast cancer, but they have yielded inconsistent results (Bai et al., 2012; Franco et al., 2012; Duggan et al., 2013; Oliveira et al., 2014). Therefore, the aim of this study was to evaluate the role of *GSTM1* null/present, *GSTT1* null/present, and GSTP1 IIe105Val polymorphisms in the clinical response to chemotherapy and the treatment outcome of patients with breast cancer.

MATERIAL AND METHODS

Subjects

A total of 262 subjects were randomly selected from among patients with a histologically confirmed breast cancer at Hongqi Hospital of Mudanjiang Medical University. The eligibility criterion for this study was diagnosis with singular, unilateral or primary neoplasia of the breast with a UICC stage II or III invasive ductal histology.

Patients received 35 mg/m^2 intravenous docetaxel on days 1 and 8, and 60-65 mg/m^2 intravenous thiotepa on day1; cycles were repeated every 21 days until disease progression

or unacceptable toxicity occurred, with a maximum of 6 cycles. Premedication consisted of 7.5 mg oral dexamethasone twice daily beginning on the day before docetaxel infusion and continuing for a total of 3 days.

Tumor responses were evaluated by contrasted computed tomography scan and/or magnetic resonance imaging every two cycles to document complete response (CR), partial response (PR), stable disease, or progressive disease according to the Response Evaluation Criteria in Solid Tumors (RECIST criteria) version 1.1. The response was confirmed over 4 weeks later.

OS was calculated from the date of chemotherapy to the date of death or last clinical follow-up. The study was approved by the Hongqi Hospital of Mudanjiang Medical University. All participants provided written informed consent for genetic polymorphism analysis before enrollment, and the study was carried out in accordance with the Declaration of Helsinki.

Genotyping

Genomic DNA was isolated from peripheral blood lymphocytes using a Qiagen blood mini kit (Qiagen, Germany) according to the manufacturer protocol. The genotypes of *GSTM1*, *GSTT1*, and GSTP1 IIe105Val polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Design primers of *GSTM1*, *GSTT1*, and GSTP1 IIe105Val for PCR were performed by the Sequenom Assay Design 3.1 software (Sequenom®) to amplification and single base extension assays. The PCR conditions were as follows: initial denaturation at 95°C for 5 min; 35 cycles of amplification with denaturation at 95°C for 30 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s; and a final extension step of 7 min at 72°C.

Statistical analysis

Continuous variables are reported as means ± standard deviation, and categorical variables were shown by number of subjects (%). Survival curves were analyzed by the Kaplan-Meier method. Meanwhile, baseline characteristics were adjusted in order to avoid potential confounding effects, such as age, gender, smoking history, histological types, and TNM stage at entry. The associations between response to chemotherapy and *GSTM1*, *GSTT1*, and GSTP1 IIe105Val polymorphisms were described as odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression analysis. The prognostic value of *GSTM1*, *GSTT1*, and GSTP1 IIe105Val polymorphisms for the OS was estimated by multivariate analysis using the Cox proportional hazard models, describing as the hazard ratio (HR) and 95%CI. P values < 0.05 with two-sided were considered to be statistical differences. Data analysis was conducted by the statistical software SPSS Statistics (version 11.0, SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 262 patients with breast cancer were included in our study. The median age of included subjects was 56.2 years (Table 1). Of 262 patients, 138 (52.67%) were premenopausal, 49 (18.70%) had tumor size >4.0 cm, 75 (28.63%) were at the III-IV clinical stage, 154 (58.78%) had positive estrogen receptor status, and 143 (54.58%) had positive progesterone receptor status.

Table 1. Clinical characteristics of 262 patients with breast cancer.

Characteristics	Number of patients with breast cancer	%
Median age (range, years)	56.2 ± 6.2	
<50	114	43.51
≥50	148	56.49
Menopausal status		
Premenopausal	138	52.67
Postmenopausal	124	47.33
Tumor size		
<2.0 cm	76	29.01
2.1-4.0 cm	137	52.29
>4.0 cm	49	18.70
Clinical stage		
I-II	187	71.37
III-IV	75	28.63
Estrogen receptor status		
Negative	108	41.22
Positive	154	58.78
Progesterone receptor status		
Negative	119	45.42
Positive	143	54.58

The associations between *GSTM1*, *GSTT1*, and GSTP1 IIe105Val polymorphisms and response to chemotherapy are shown in Table 2. Of 262 patients, 151 showed responsive to CR and PR chemotherapy, with a response rate of 57.63%. Our study found that the null genotype of *GSTM1* was associated with a better response to chemotherapy, and the OR (95%CI) was 1.78 (1.03-3.08). However, we did not find significant association between *GSTT1* or GSTP1 IIe105Val polymorphisms and response to chemotherapy.

Table 2. Relationship between response to chemotherapy and *GST* polymorphisms.

SNP	CR + PR	%	SD + PD	%	OR (95%CI)	P value
GSTM1						
Present	83	54.97	76	68.47	1.0 (Ref.)	-
Null	68	45.03	35	31.53	1.78 (1.03-3.08)	0.03
GSTT1						
Present	79	52.32	62	55.86	1.0 (Ref.)	-
Null	72	47.68	49	44.14	1.15 (0.68-1.95)	0.57
GSTP1						
IIe/IIe	68	45.03	53	47.75	1.0 (Ref.)	-
IIe/Val	37	24.50	25	22.52	1.15 (0.59-2.26)	0.65
Val/Val	46	30.46	33	29.73	1.09 (0.59-2.01)	0.78

GST = glutathione *S* transferase; SNP = single nucleotide polymorphism; OR = odds ratio; CI = confidence interval.

In the Cox proportional hazard model, after adjusting for potential confounding factors, the HR (95%CI) for OS in patients carrying the null genotype of *GSTM1* was 0.57 (0.32-0.98) using the non-null genotype as the reference variable (Table 3). However, we observed no significant association between the *GSTT1* and GSTP1 IIe105Val polymorphisms and OS in patients with breast cancer.

Table 3. Overall survival for patients with *GST* polymorphisms.

SNP	Deaths (N = 108)	%	Surviving (N = 154)	%	Median survival time (months)	HR (95%CI)	P value
GSTM1							
Present	77	71.30	90	58.44	32.25	1.0 (Ref.)	-
Null	31	28.70	64	41.56	43.54	0.57 (0.32-0.98)	0.03
GSTT1							
Present	62	57.41	79	51.30	36.50	1.0 (Ref.)	-
Null	46	42.59	75	48.70	38.35	0.78 (0.46-1.32)	0.33
GSTP1							
Ile/Ile	49	45.37	72	46.75	36.10	1.0 (Ref.)	-
Ile/Val	25	23.15	37	24.03	37.50	0.99 (0.51-1.94)	0.98
Val/Val	34	31.48	45	29.22	36.20	1.11 (0.60-2.05)	0.72

GST = glutathione *S* transferase; SNP = single nucleotide polymorphism; HR = hazards ratio; CI = confidence interval.

DISCUSSION

It is well known that the GST super-family belongs to the phase II group of enzymes, which are involved in the metabolism of a wide range of xenobiotics and drugs including a variety of cytotoxic cancer chemotherapeutic agents (Ge et al., 2013). Since the first report that GSTs might play an important role in chemotherapy efficacy (Anderer et al., 2000), the results of various subsequent studies have demonstrated the inconsistent nature of this relationship. Our study examined the association of *GSTM1*, *GSTT1*, and GSTP1 polymorphisms and the treatment response and outcomes of chemotherapy in patients with breast cancer. Our results suggested that *GSTM1* polymorphism plays an important role in influencing the chemotherapy response in patients with breast cancer, and that this gene polymorphism can influence their OS as well. This association was independent of traditional predictors of prognosis.

An increasing number of studies have investigated the role of *GSTM1*, *GSTT1*, and GSTP1 polymorphisms in the susceptibility to chemotherapeutic agent toxicity and have shown that individuals carrying *GST* variant genotypes are less able to detoxify the metabolites of drugs and carcinogens used for treating colorectal cancer, bladder cancer, osteosarcoma, and breast cancer, among others (Zhang et al., 2012; Djukic et al., 2013; Duggan et al., 2013; Vreuls et al., 2013; Kap et al., 2014). Kap et al. (2014) conducted a study involving 755 patients with colorectal cancer and found that *GSTM1* might be a predictive marker for the success of oxaliplatin therapy. Djukic et al. (2013) conducted a study with 105 patients with muscle invasive bladder cancer and reported that the *GSTT1* active genotypes might have a prognostic role for treatments. Zhang et al. (2012) reported the predictive value of *GST* gene polymorphisms for the prognosis of osteosarcoma patients receiving chemotherapy; the results showed that the *GSTP1* gene polymorphism might have an important role in this cohort.

Many previous studies have reported that GST polymorphisms play an important role in the prognosis of breast cancer (Oliveira et al., 2010; Mishra et al., 2011; Bai et al., 2012; Duggan et al., 2013; Tulsyan et al., 2013). Tulsyan et al. (2013) reported that *GSTM1* and *GSTP1* polymorphisms influence the chemotherapy response and toxicity in patients with breast cancer. Duggan et al. (2013) assessed the association between *GST* genes and mortality in survivors of breast cancer, and the results showed that *GSTP1* variants increased the risk for all causes of mortality in breast-cancer survivors. Oliveira et al. (2010) found that carriers of a *GSTP1* gene polymorphism had a significantly higher response rate to chemotherapy among patients with breast cancer. However, some studies did not find significant association between

GST gene polymorphisms and chemotherapy response in patients with breast cancer, such as that by Mishra et al. (2011). These inconsistent results might be due to differences between the studies in ethnicities, sources of patients, disease stages, sample sizes, or to chance.

In conclusion, our study found that a *GSTM1* polymorphism plays an important role in influencing the chemotherapy response in patients with breast cancer, and that this gene polymorphism can influence the OS of patients with breast cancer. The *GSTM1* polymorphism might substantially contribute to the future design of individualized cancer treatment for patients with breast cancer. Therefore, further multicenter studies involving various populations are required to confirm our results.

Conflicts of interest

The authors declare no conflict of interest.

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