Predictive potential role of glutathione S-transferases polymorphisms in response to chemotherapy and breast cancer prognosis

P. Yuan[1], L. Yuan[2], B.L. Xu[3], C.Z. Wang[1], H.Z. Yang[1] and Y. Li[4]

[1]Breast Cancer Center,
The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, China
[2]Department of Surgery,
The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, China
[3]Central Laboratory,
The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, China
[4]Department of Thoracic Surgery,
The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, China

Corresponding author: Y. Li
E-mail: liyin_yin5@163.com

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ABSTRACT. The aim of this study was to evaluate the role of *GSTM1* null/present, *GSTT1* null/present, and *GSTP1* polymorphisms in the clinical response to chemotherapy and treatment outcome of breast cancer. The *GSTM1*, *GSTT1*, and *GSTP1* Ile105Val polymorphism genotypes were analyzed using polymerase chain reaction coupled with restriction fragment length polymorphism. Conditional logistic regression analysis revealed that breast cancer patients carrying the GG genotype of *GSTP1* Ile105Val showed a significantly better response to chemotherapy compared to those expressing the AA genotype [odds ratio = 2.66, 95% confidence interval (CI) = 1.24-5.91, P = 0.007]. The Cox proportional hazards model indicated

that the GG genotype of *GSTP1* Ile105Val in breast cancer patients was correlated with a lower risk of death from all causes than those with AA genotype. The adjusted hazard ratio (95%CI) for the GG genotype of *GSTP1* Ile105Val was 0.44 (0.18-0.99; P = 0.03). In conclusion, the results of our study indicated that the GG genotype of *GSTP1* Ile105Val was significantly associated with better response to chemotherapy and longer overall survival, compared to the wide-type genotype.

Key words: Glutathione S-transferases; Polymorphism; Chemotherapy; Breast cancer

INTRODUCTION

Breast cancer is, by far, the most frequent type of cancer affecting women, and the leading cause of malignancy-related deaths in many countries (Jemal et al., 2009). An estimated 1.67 million new cases of cancer have been diagnosed in 2012, accounting for 25% of all cancers (IARC, 2012). Chemotherapy is an adjuvant systemic therapy administered after primary surgery, or a neo-adjuvant chemotherapy applied before surgery in patients with locally advanced breast cancers (van der Hage et al., 2001). Although many clinicopathological characteristics have been unable to precisely predict the efficacy of chemotherapy, increasing evidence has suggested that drug-metabolizing enzymes play an important role in determining inter-individual variations in therapeutic response (Arun et al., 2010).

GSTs detoxify chemotherapeutic drugs or their metabolites by catalyzing the reduction of these compounds with glutathione. GSTM1, GSTT1 and GSTP1 are three common enzymes belonging to the GST superfamily. Allelic deletions in the *GSTM1* and *GSTT1* genotypes are correlated with reduced enzyme production (Strange et al., 2001). Polymorphisms in *GSTP1* are associated with lower substrate-specific catalytic activity. Previous studies have suggested that genetic polymorphisms in the GST genes could influence the effectiveness of detoxification of the cytotoxins generated by the chemotherapeutic agents used in breast cancer treatment (Bai et al., 2012; Tulsyan et al., 2013; Vivenza et al., 2013; Oliveira et al., 2014); however, these studies have yielded inconsistent results. Therefore, the aim of this study was to evaluate the role of GSTM1 null/present, GSTT1 null/present, and GSTP1 polymorphisms in the clinical response to chemotherapy and the outcome of breast cancer treatment.

MATERIAL AND METHODS

Patients

Two hundred and ninety-two subjects were recruited between April 2009 and April 2012 from the Affiliated Cancer Hospital of Zhengzhou University for this study. This cohort of breast cancer patients had been newly diagnosed, were histopathologically confirmed, and were untreated. Among these, 273 breast cancer patients agreed to participate in this study (participation rate, 93.49%), and signed informed consent forms to this effect.

The clinical characteristics of the enrolled patients, including tumor size, clinical stages,

lymph mode metastasis, and estrogen receptor (ER) and progesterone receptor (PR) status, were collected from the patient medical records. A standardized questionnaire was used to collect the demographic data, including mean age and menopausal status.

All 273 patients were treated with chemotherapy; these breast cancer patients received chemotherapy treatment evaluation based on the RECIST criteria (Duffaud and Therasse, 2000). Response to chemotherapy was stratified into the response (complete response or partial response) and non-response (stable disease or progressive disease) types. All patients were followed up until April 2014. the overall survival (OS) of breast cancer patients was defined as the time from the beginning of study (April 2009) to death. Breast cancer patients who were alive at the time of analysis were excluded from the study on the day of the final follow-up.

DNA extraction and genotyping

DNA was extracted from peripheral blood samples (collected from patients) using TIANamp Blood DNA kit (Tiangen Biotech Co., Ltd., Beijing, China). The *GSTM1*, *GSTT1*, and *GSTP1* Ile105Val genotypes were analyzed by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). The PCR fragments were subsequently digested with their specific restriction enzymes. The digestion products were separated by electrophoresis on an ethidium bromide stained agarose gel, and visualized under UV light.

Statistical methods

Statistical analyses were performed on a STATA v.9.0 software platform (StataCorp LP, College Station, TX, USA); statistical significance was determined by two-sided tests. Survival curves were analyzed by the Kaplan-Meier method to evaluate the impact of the *GSTM1*, *GSTT1*, and *GSTP1* Ile105Val polymorphisms on OS. The association between the *GSTM1*, *GSTT1*, and *GSTP1* Ile105Val polymorphisms and response to chemotherapy was analyzed by a conditional logistic regression method; this association was presented as the odds ratio (OR) and 95% confidence interval (CI). The association between the *GSTM1*, *GSTT1*, and *GSTP1* Ile105Val polymorphisms and OS was analyzed by the Cox proportional hazards model; this association was expressed as the hazard ratio (HR) with a 95%CI. Two-sided P values < 0.05 were considered to be statistically significant.

RESULTS

The clinical and demographic characteristics of 273 breast cancer patients are summarized in Table 1. The mean age of the inducted breast cancer patients was 55.7±11.4 years. One hundred and forty six of the included patients (53.48%) were premenopausal, and 127 (46.52%) were postmenopausal. The tumor size of 96 patients (35.16%) was < 2.0 cm and that of 177 (64.84%) patients was >2.0 cm. One hundred and eighty two patients (66.67%) were at clinical stages I-II, and 91 (33.33%) were at stages III-IV. Furthermore, 153 patients (56.04%) exhibited positive lymph node metastasis, and 120 (43.96%) showed negative lymph mode metastasis. One hundred and sixty five patients (60.44%) showed positive ER status, while 143 (52.38%) showed positive PR status.

Table 1. Characteristics of 273 breast cancer patients.

Characteristics	Number	%
Mean age, years	55.7 ± 11.4	
Age, years		
≤50	122	44.69
>50	151	55.31
Menopausal status		
Premenopausal	146	53.48
Postmenopausal	127	46.52
Tumor size, cm		
≤2.0	96	35.16
>2.0	177	64.84
Clinical stages		
I-II	182	66.67
III-IV	91	33.33
Lymph node metastasis		
Negative	120	43.96
Positive	153	56.04
ER status		
Negative	108	39.56
Positive	165	60.44
PR status		
Negative	128	46.89
Positive	143	52.38

At the end of the follow-up period, 169 breast cancer patients (61.90%) exhibited CR + PR to chemotherapy, while 104 (38.10%) showed a SD + PD status (Table 2). Conditional logistic regression analysis revealed that patients carrying the GG genotype of the *GSTP1* IIe105Val polymorphism showed a significantly better response to chemotherapy, compared to the AA genotype (OR = 2.66, 95%CI = 1.24-5.91, P = 0.007). However, we observed no significant association between the *GSTM1* and *GSTT1* polymorphisms and response to chemotherapy in breast cancer patients (P > 0.05).

Table 2. Association between the *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val polymorphisms and response to chemotherapy.

Genotype	Patients	%	Response to chemotherapy				OR (95%CI)[1]	P value
			Responder (CR+PR)	%	Non-responder (SD+PD)	%		
GSTM1								
Present	156	57.14	93	55.03	63	60.58	1.0 (Ref.)	-
Null	117	42.86	76	44.97	41	39.42	1.26 (0.74-2.13)	0.37
GSTT1								
Present	121	44.32	73	43.20	48	46.15	1.0 (Ref.)	-
Null	152	55.68	96	56.80	56	53.85	1.12 (0.67-1.90)	0.63
GSTP1 IIe105Val								
AA	105	38.46	55	32.54	50	48.08	1.0 (Ref.)	-
AG	113	41.39	73	43.20	40	38.46	1.66 (0.93-2.96)	0.07
GG	55	20.15	41	24.26	14	13.46	2.66 (1.24-5.91)	0.007

[1]Adjusted for age, menopausal status, tumor size, clinical stage, lymph node metastasis, ER status and PR status. OR = odds ratio; CI = confidence interval.

Sixty-seven patients died during the follow-up period by the end of April 2014; the five year survival rate of breast cancer patients was calculated to be 24.54%. The GG genotype of the *GSTP1* IIe105Val polymorphism in breast cancer patients was correlated with a lower risk of death from all causes by the Cox proportional hazards model, compared to the AA genotype (Table 3).

The adjusted HR (95%CI) for the GG genotype of the *GSTP1* IIe105Val polymorphism was 0.44 (0.18-0.99) (P = 0.03). However, the *GSTM1* and *GSTT1* gene polymorphisms did not influence the overall survival of breast cancer patients (P > 0.05).

Table 3. Association between the *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val polymorphisms and overall survival of breast cancer patients.

Genotype	Patients	%	Event	%	Alive	%	HR (95%CI)[1]	P value
GSTM1								
Present	156	57.14	39	58.21	114	55.34	1.0 (Ref.)	-
Null	117	42.86	28	41.79	92	44.66	0.89 (0.49-1.61)	0.68
GSTT1								
Present	121	44.32	30	44.78	89	43.20	1.0 (Ref.)	-
Null	152	55.68	37	55.22	117	56.80	0.94 (0.52-1.70)	0.82
GSTP1 IIe105Val								
AA	105	38.46	28	41.79	59	28.64	1.0 (Ref.)	-
AG	113	41.39	27	40.30	89	43.20	0.64 (0.33-1.25)	0.16
GG	55	20.15	12	17.91	58	28.16	0.44 (0.18-0.99)	0.03

[1]Adjusted for age, menopausal status, tumor size, clinical stage, lymph node metastasis, ER status and PR status. HR = hazard ratio; CI = confidence interval.

DISCUSSION

In this study, we evaluated the association between the *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val polymorphisms and the response to chemotherapy in, and overall survival of, breast cancer patients in a Chinese population. The results of our study indicated that the GG genotype of *GSTP1* IIe105Val was associated with good response to chemotherapy, and was correlated with high overall survival of breast cancer.

The GST super-family of enzymes is a part of the phase II group of enzymes; GST enzymes play an important role in the metabolism of many xenobiotics and drugs, including cytotoxic cancer chemotherapeutic agents (Tew, 1994). Many previous studies have reported that glutathione S-transferases may play an important role in determining the efficacy of chemotherapy on cancer (Ruwali et al., 2011; Suneetha et al., 2011; Bai et al., 2012; Goričar et al., 2015); however, the results of subsequent studies have shown the inconsistent nature of this relationship.

Recent studies have investigated the association of genetic polymorphisms in the *GSTM1*, *GSTT1*, and *GSTP1* IIe105Val region with breast cancer prognosis. People with variant GST genotypes display a reduced ability to detoxify drug metabolites, thereby ensuring a longer overall breast cancer survival. Several previous studies have reported that GSTs play a role in the prognosis of breast cancer patients subjected to chemotherapy (Syamala et al., 2008; Gor et al., 2010; Zhang et al., 2010; Bai et al., 2012; Romero et al., 2012; Zhou et al., 2015). Zhang et al. (2010), in a study conducted in a Chinese population, reported an association between polymorphisms in the *GSTP1* gene and good response and light toxicity in breast cancer patients. Bai et al. (2012), who also conducted a study in a Chinese population, reported that null *GSTM1* and *GSTP1* Val/Val genotypes showed significantly better response rates to chemotherapy, compared to wide-type genotypes. On the other hand, Romero et al. (2012) discovered an association between the *GSTP1* polymorphism and a lower risk of chemo-resistance, when treated with doxorubicin. However, some inconsistent results have also been reported. Gor et al. (2010) did not find a significant association between GST gene polymorphisms and disease-free survival and overall survival in breast cancer patients. Syamala et al. (2008), in a study conducted in an Indian population, reported that polymorphisms

in GST genes did not affect the overall survival of sporadic breast cancer patients, while Zhou et al. (2015), in a study with 420 breast cancer patients, reported that the *GSTP1* Ile105Val polymorphism was associated with an increased risk of death from breast cancer and poor tumor response to chemotherapy. These inconsistency results may be attributed to the differences in ethnicities and source of patients, as well as the disease stages and sample size.

There were several limitations to our study: the breast cancer patients were selected from one hospital only, which may cause selection bias. The sample size used for the analysis of association between GST polymorphisms and breast cancer survival was relatively small; therefore, some of the findings may be undervalued because of the limited number of studies available for analyses. Therefore, studies with larger sample sizes must be performed to confirm the results obtained in this study.

In conclusion, the results of our study indicated that the GG genotype of the *GSTP1* Ile105Val polymorphism was significantly associated with better response to chemotherapy and longer overall survival, compared to the wide-type genotype. Further prospective studies with larger sample sizes are required to validate this association.

Conflicts of interest

The authors declare no conflict of interest.

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