RESEARCH ARTICLE

Predictive Role of GSTs on the Prognosis of Breast Cancer Patients with Neoadjuvant Chemotherapy

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Abstract

Objective: To evaluate the predictive value of GST gene polymorphisms with regard to prognosis of breast cancer patients receiving neoadjuvant chemotherapy. Methods: A total of 159 patients were included in our study between January 2005 and January 2007. All the patients were followed up until January 2012. Genotyping was based upon the duplex polymerase-chain-reaction with the PCR-CTPP method. Results: Patients with null GSTM1 and GSTP1 Val/Val genotypes had significantly had better response rates to chemotherapy when compared with non-null GSTM1 and GSTP1 Ile/ Ile genotypes (OR=1.96 and OR=2.14, respectively). Patients with the GSTM1 null genotype had a longer average survival time and significantly lower risk of death than did those with non-null genotypes (HR=0.66). Similarly, those carrying the GSTP1 Val/Val genotype had 0.54-fold the risk of death of those with GSTP1 Ile/ Ile (HR=0.54). Conclusion: A significant association was found between GSTM1 and GSTP1 gene polymorphisms and clinical outcomes in breast cancer cases.

Keywords: GSTM1 - GSTT1 - GSTP1 - breast cancer - chemotherapy

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Introduction

Breast cancer is by far the most frequent cancer among women with an estimated 1.38 million new cancer cases diagnosed in 2008 (23% of all cancers), and ranks second overall (10.9% of all cancers). In China, the incidence is 14.2/105, and it is one of the most leading causes of death in Chinese women (IARC, 2008). Neoadjuvant chemotherapy (NAC) for primary breast cancer patients is known to enhance to operability of patients with advanced tumors previously considered inoperable, as well as making breast-conserving surgery more feasible for patients for whom such surgery was previously not feasible due to large tumor size. Anthracycline-based chemotherapy regimens are preferred for downstaging breast cancer tumors (Bafaloukos et al., 2005).

Response to chemotherapy cannot be predicted for patients, but the polymorphism in genes encoding for metabolizing enzymes and drug transporters can affect drug efficacy and toxicity (Bosh et al., 2006). Glutathione S-transferases (GSTs) are a family of cytosolic enzymes involved in the detoxification of various exogenous as well as endogenous reactive species.1, 2 GSTs function as dimers by catalyzing the conjugation of mutagenic electrophilic substrates to glutathione. In humans, 4 major subfamilies of GSTs can be distinguished and are designated as GSTa, GSTμ, GSTθ and GSTπ. Each of these subfamilies is composed of several members, some of which display genetic polymorphism. Homozygozity of GSTM1 and GSTT1, members of GSTμ and GSTθ, leads to absence of phenotypic enzyme activity. The polymorphism of GSTP1 at codon 105 (IIe105Val), a member of GSTπ subfamily, shows different catalytic activities (Mishra et al., 2001).

The polymorphisms of GSTs in tumor cells present association with resistance to chemotherapy. Several in vitro studies using various human cells have indicated that GSTM1, GSTT1 and GSTP1 expression is associated with resistance to chemotherapy (Satta et al., 1992; Whelan et al., 1992; Lourenço et al., 2010). However, the results of GSTs polymorphisms and response to chemotherapy in breast cancer are conflicting (Wang et al., 2009; Arun et al., 2010; Franco et al., 2012). Moreover, there is no study conducted in Chinese population on the association of GSTs expression with response to chemotherapy in breast cancer patients. We conducted a prospective study to investigate the association of GSTs expression with survival of breast cancer patients with chemotherapy.

Materials and Methods

Study population

Our study included 159 newly diagnosed breast cancer cases in the First Affiliated Hospital of Xinxiang Medical College. The cases were histological confirmed between March 2007 and March 2008. All the patients

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were treated with neoadjuvant chemotherapy (NAC). The chemotherapy included anthracycline-based (epirubicin [E] or doxorubicin [A]) chemotherapy. Anthracycline-based chemotherapy consists of cyclophosphamide (C), the anthracycline agent (E or A), and/or 5-fluorouracil (F), (CEF and CAF regimens) combined with radiotherapy. Patients were subsequently grouped as responders (complete+partial response) or non-responders (stable+progressive disease). Cases with secondary or recurrent tumors were excluded. All patients were followed up till March 2011.

Genotyping

The DNA samples were obtained from stored blood samples using the Qiagen Blood Kit (Qiagen, Chastworth, CA). Genotyping for GSTM1, GSTT1 and GSTP1 polymorphisms by PCR-RFLP assay was done following a modified method of Ateş et al (Ateş et al., 2005). The primer sequences of GSTM1, GSTT1 and GSTP1 polymorphisms were 5'-GAACTCCCTGAAAAGCTAAGC-3' (forward) and 5'-GTTGGGGCTCAAATATACGGTGG-3' (reverse), 5'-TTCCTTACTGGTCCTCACATCTC-3' (forward) and 5'-TCACCGGATCAGGCCAGCA-3' (reverse), and 5'-ACCCCAGGGCTCTATGGGAA-3' (forward) and 5'-TGAGGGCACAAGAAGCCCCT-3' (reverse), respectively. Polymerase chain reaction conditions were used as follows: an initial melting step of 5 min at 94°C; 35 cycles of denaturation for 30 s at 94°C; annealing for 30 s at 55°C; extension for 45 s at 72°C, followed by a 5 min final extension at 72°C. We also performed the genotyping of internal positive control samples, use of no template controls, and use of replicates for 10% samples for quality control. These results of the quality control analysis confirmed 100% concordance.

Table 1. Clinical Characteristics of Responders and Non-responders to Neoadjuvant Chemotherapy for Breast Cancer

Characteristic	Responders (n=83)	%	Nonresponders (n=76)	%	P value
Age at diagnosis, years					
≤45	48	57.83	35	46.05	
>45	35	42.17	41	53.95	0.137
Menopausal status					
Premenopausal	28	33.73	19	25	
Postmenopausal	55	66.27	57	75	0.228
First-degree family history of breast cancer					
No	76	91.57	63	82.89	
Yes	7	8.43	13	17.11	0.1
Tumor grade					
I	7	8.43	7	9.21	
II	45	54.22	53	69.74	
III	31	37.35	16	21.05	0.07
Tumor size, mm					
0-20	26	31.33	32	42.11	
21-50	46	55.42	37	48.68	
>50	11	13.25	7	9.21	0.34
Estrogen receptor (ER)					
Positive	31	37.35	24	31.58	
Negative	52	62.65	52	68.42	0.45
Progesterone receptor (PR)					
Positive	24	28.92	11	14.47	
Negative	59	71.08	65	85.53	<0.05
Therapeutic regimen					
Antracycline-based chemotherapy	44	53.01	41	53.95	
Other chemotherapies or treatment	39	46.99	35	46.05	0.91

Statistical analysis

Statistical analysis was performed by using SPSS version 16.0 statistical software (SPSS, Chicago, IL, USA). The descriptive data for the major characteristics of study groups are expressed as mean and percent. Pearson's 2×2 χ^2-test (gender) and independent sample t-test(mean age) were used for analysis the differences of several qualitative and quantitative data. The association of polymorphisms of GSTM, GSTT1 and GSTP1 with response to chemotherapy in breast cancer patients was calculated by odds ratios (OR). The odds ratio was expressed with a corresponding 95% confidence interval (CI). The relative risk [hazard ratio (HR)] and 95% CI were calculated with the Cox regression model for all significant predictors from cancer diagnosis to the endpoint of the study (event). A primary death from breast cancer was defined as a failure event, and the survival time was defined as the time between diagnosis and death. The cause of death was defined by specialists based on clinical documents and reports by patients' family members. If a patient died from a cause other than ovarian cancer, her data was censored at the date of death. Statistical significance was set at $P<0.05$ and all tests were two-sides.

Results

Subject characteristics

The clinical features of 159 breast cancer patients are summarized in Table 1. The median age at diagnosis is 14.7±9.6 years (range7 to 39 years). Among 159 patients, 83 patients were responders and 76 were non-responders to chemotherapy. Among the responders, 33 showed a complete response and 50 showed a partial response. Patients with positive PR had higher response rate to chemotherapy ($P<0.05$).

Among 83 responders, about 51% of them showed the null GSTM1 genotype, 49.4% showed GSTT1 genotype,

Table 2. Distribution of GSTM1, GSTT1 and GSTP1 in Responders and Non-responders to Neoadjuvant Chemotherapy for Breast Cancer

Genotypes	Responders (n=83)	%	Nonresponders (n=76)	%	Odds ratio (95% CI)[1]
GSTM1					
Present	49	59.04	53	69.74	-
Null	34	40.96	23	30.26	1.96(1.04 -4.26)
GSTT1					
Present	42	50.6	43	56.58	-
Null	41	49.4	33	43.42	1.43(0.78-2.57)
GSTP1					
Ile/ Ile	40	48.19	41	53.95	-
Ile/Val	17	20.48	18	23.68	1.05(0.49-2.44)
Val/Val	26	31.33	17	22.37	2.14(1.07-4.77)

[1]Adjusted for age, menopausal status, tumor grade, tumor size, ER; PR and therapeutic regimen

Table 3. Hazard Ratios for Overall Survival in Breast Cancer Patients with Chemotherapy

Genotypes	N	%	Median Survival time (months)	HR (95% CI)[1]
GSTM1				
Present	102	64.15	31.6	-
Null	57	35.85	37.4	0.66(0.31-0.93)
GSTT1				
Present	85	53.46	34.4	-
Null	74	46.54	34.7	0.88(0.67-2.01)
GSTP1				
Ile/ Ile	81	50.94	32.8	-
Ile/Val	35	22.01	32.7	0.98(0.58 -1.89)
Val/Val	43	27.04	37.1	0.54(0.29-0.90)

[1]Adjusted for age, menopausal status, tumor grade, tumor size, ER; PR and therapeutic regimen

and 31.3% were GSTP1 val/val genotype. Of GSTM1 polymorphisms, null GSTM1 genotype had significantly higher rates of response to chemotherapy when compared to the non-null GSTM1 genotype [OR (95% CI) = 1.96(1.04-4.26)]. In the case of GSTP1, the GSTP1 Val/Val genotype had significantly higher rates of response to chemotherapy [OR (95% CI) = 2.14 (1.07-4.77)]. However, we did not find significant odds of response in favor of patients with null GSTT1 genotype.

Among all patients, the median median survival time was 31.6 month. Patients with GSTM1 null genotype had a longer average survival time and significantly lower risk of death than did those with non-null genotypes [HR (95% CI) = 0.66(0.31-0.93)]. Similarly, those carrying GSTP1 Val/Val genotype had 0.54-fold the risk of death of those with GSTP1 Ile/ Ile [HR (95% CI) = 0.54(0.29-0.90)]. There was no significant association between GSTT1 gene polymorphisms and risk of death.

Discussion

The present study investigates the association between GST polymorphisms, GSTM1, GSTT1 and GSTP1 genotypes and survival of breast cancer with chemotherapy. Our results showed null GSTM1 and wide-type GSTP1 Val/Val genotype had better response to chemotherapy among breast cancer patients, moreover, the two genotypes could improve the survival of gastric cancer. However, no association was found between GSTT1 gene polymorphism and breast cancer prognosis.

Since this is the first study on the association between GSTs polymorphisms and the response to chemotherapy. Previous evidences showed the GSTM1, GSTT1 and GSTP1 are involved in response to chemotherapy in various cancers, such as gastric cancer, myeloid leukemia, colorectal cancer and ovarian cancer (Mossallam et al., 2006; Nagle et al., 2007; Ott et al., 2008; Funke et al., 2010). However, there are few studies in Chinese breast cancer patients. Only several studies conducted in western countries investigation the association of GSTs with chemotherapy response and survival of breast cancer, but the results are conflicting (Satta et al., 1992; Whelan et al., 1992; Ott et al., 2008; Lourenço et al., 2010; Oliveira et al., 2010; Mishra et al., 2011). A study conducted in Brasil showed combination of null GSTT1 and GSTP1 105Val have poor response than combination of non-null GSTT1 and GSTP1 105IIe (Oliveira et al., 2010). However, another study in Indian, it did not find a significant association between glutatinione S-transferases and responses to chemotherapy (Mishra et al., 2011), and another study conducted in Germany did not found a significant responses to chemotherapy among individuals with GSTT1, GSTM1 and GSTP1 (Ott et al., 2008). Our study finds a significant association of null GSTM1 and GSTP1 Val/Val genotypes with breast cancer survival and response to chemotherapy. These inconsistency results might be due to differences in ethnicities, source of patients, disease stages, sample size and by chance. Further multicenter studies are warranted to establish the impact of GST genotypes on chemotherapy.

In our study, the GSTM1 and GSTP1 gene polymorphisms influenced clinical outcome in breast cancer patients. Previous study did not report a significant association of null GSTM1 and GSTP1 Val/Val genotypes and response to chemotherapy in breast cancer patients (Ott et al., 2008; Mishra et al., 2011). However, the GSTs polymorphisms were associated with resistance to chemotherapy in other vivo and in vitro studies (Wang et al., 2003; Zheng et al., 2011). In our study, we found the null GSTM1 and GSTP1 Val/Val genotypes were association with prongsis of breast cancer. The explanation might be that genetic polymorphisms in GSTs gene polymorphisms influence the efficacy of detoxifying cytotoxins generated by chemotherapeutics. Due to the impairment of the GSTM1 and GSTP1 capacity, patients with inactive variant allele may be less capable of detoxifying chemotherapeutic regimens when compared with patients carrying active genotype. Therefore, the null GSTM1 and GSTP1 Val/Val genotypes might decrease the risk of death from breast cancer.

In conclusion, we found significant association between GSTM1 and GSTP1 gene polymorphisms and clinical outcomes, but no association was found between GSTT1 polymorphism and risk of death from breast cancer. Further prospective studies incorporating larger numbers of patients are needed to validate these associations.

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