Glutathione S-transferase pi polymorphism contributes to the treatment outcomes of advanced non-small cell lung cancer patients in a Chinese population

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ABSTRACT. We analyzed the association between polymorphisms in three glutathione S-transferase genes (*GSTP1*, *GSTM1*, and *GSTT1*) and the treatment outcome for advanced non-small cell lung cancer (NSCLC). We recruited 284 NSCLC patients at advanced stage from Department of Radiotherapy in Peace Hospital Attached to Changzhi Medical College between May 2009 and May 2011, who had received cisplatin-based chemotherapy. The *GSTP1*, *GSTM1*, and *GSTT1* genotyping for was determined using DNA pyrosequencing on an ABI Prism 3100 DNA analyzer. In the Cox proportional hazards model, the IIe/Val and Val/Val genotypes of *GSTP1* were associated with lower risk of disease progression compared with the IIe/IIe genotype, and the HRs (95%CIs) were 0.37 (0.18-0.74) and 0.15 (0.06-0.35), respectively. The IIe/Val and Val/Val genotypes

significantly decreased risk of death from all causes in patients with NSCLC, and the HRs (95%CIs) were 0.52 (0.29-0.92) and 0.37 (0.17-0.79), respectively No significant association was observed between *GSTM1* and *GSTT1* polymorphisms and progression-free survival and overall survival in the NSCLC patients. In summary, we suggest that *GSTP1* polymorphisms might influence the treatment outcome of advanced NSCLC patients, and our results could help improve individualized therapy.

Key words: *GSTP1*; *GSTM1*; *GSTT1*; Polymorphism; NSCLC

INTRODUCTION

Lung cancer was the most prevalent and lethal form of cancer in both men and women in 2012 worldwide (IARC, 2012). It is estimated that over a million people are affected by lung cancer globally each year (IARC, 2012). In 2012, lung cancer was the first cause of death among people with malignant tumors in China (Ferlay et al., 2010). Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. Although both individual and combination treatments, such as surgery, radiotherapy, and chemotherapy, have improved the survival of patients with NSCLC in recent years, long-term survival could still be improved. The TNM classification system is the basis for the prognostic management of NSCLC; however, it does not provide sufficient information about biological tumor progression (Chansky et al., 2009). There is still demand for biomarkers that can predict patient survival.

GSTP1, *GSTM1*, and *GSTT1* are three members of the human glutathione S-transferases (GSTs) super family of genes, and they are polymorphic in humans. Previous studies have reported that GST polymorphisms can influence the effectiveness of the detoxification of cytotoxins generated by cisplatin-based chemotherapy in several kinds of cancer, including osteosarcoma, cervical cancer, and gastric cancer (Daukantienė et al., 2014; Yu et al., 2014; Goričar et al., 2015). The authors of several studies have investigated the relationship between GST polymorphisms and the prognosis of NSCLC, but the results are inconsistent. Therefore, we conducted a study to examine the association between *GSTP1*, *GSTM1*, and *GSTT1* polymorphisms and treatment outcomes for advanced NSCLC in a Chinese population.

MATERIAL AND METHODS

Patients

We recruited 284 NSCLC patients at advanced stage from department of radiotherapy in Peace Hospital Attached to Changzhi Medical College between May 2009 and May 2011, who had received cisplatin-based chemotherapy. All the patients were pathologically confirmed as being at stage IIIA, IIIB, or IV. The exclusion criteria for patients with advanced NSCLC were those that had not previously underwent any systemic anticancer therapies prior to enrollment, and had severe complications, acute and chronic infection diseases, liver and renal dysfunctions, and/or organ failure and brain metastasis, were excluded from this study.

Assessment of treatment outcome

After enrollment into this study, advanced NSCLC patients underwent cisplatin-based combination chemotherapy. The advanced NSCLC patients received physical examination and computed tomography scan before cisplatin-based combination chemotherapy. The patients received cisplatin-based combination chemotherapy for at least four cycles unless there was unacceptable toxicity or disease progression to continue treatment. Chemotherapy response was evaluated based on the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) using computed tomography scan examination. Progression-free survival (PFS) and overall survival (OS) were used as the endpoints. PFS was calculated from the date of chemotherapy to the data of confirmed progression, death from any cause or the end of follow-up. OS was calculated from the date of chemotherapy to the data of death from any cause or the end of follow-up. Patients were followed-up by return visits and telephone conversations, and patients were followed-up at the end of May 2014. Up to May 2014, patients were followed-up for 2-60 months, with a median follow-up time of 24.35 months. A total of 271 cases were followed-up, while 13 cases were lost to follow-up.

DNA extraction and genotyping

Each subject was asked to provide a peripheral blood sample (2 mL) and the samples were stored at -20°C until required. Genomic DNA was extracted from peripheral blood lymphocytes using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer instructions. The *GSTP1*, *GSTM1*, and *GSTT1* genotyping for was determined using DNA pyrosequencing on an ABI Prism 3100 DNA analyzer (Applied Biosystems, USA). The forward and reverse primer sequences for *GSTP1* were 5'-GAA GAG CCA AGG ACA GGT CC-3' and 5'-CAA CTT CAT CCA CGT TCA AC-3', respectively; those for *GSTM1* were 5'-CTG GGG TAC TTG ATT GAT CCC-3' and 5'-CTG GAT TGT AGC AGA TCA TGC-3'; and those for *GSTT1* were 5'-TTC CTT ACT GGT CCT CAC ATC CC-3' and 5'-TCA GGC GAT CAT GGC CAG AC-3'; those for β-globin were 5'-GAA GAG CCA AGG ACA GGT CC-3' and 5'-CAA CTT CAT CCA CGT TCA AC-3'. The length of digested fragment for *GSTP1*, *GSTM1*, *GSTT1* and β-globin were 215, 480, 176 and 286 bp, respectively.

Statistical analysis

The demographic and clinical characteristics of patients with advanced NSCLC were expressed by frequencies and percentages. The associations between *GSTP1*, *GSTM1*, and *GSTT1* polymorphisms and PFS and OS were testing the hazard ratios (HRs) and 95% confidence intervals (95%CIs) from multivariate Cox proportional hazards regression analysis, and the common genotypes of *GSTP1*, *GSTM1*, and *GSTT1* were used as reference group. Data were subjected to statistical SPSS Statistics software (version 19.0, SPSS Inc., Chicago, IL, USA), and two-sided P values < 0.05 was considered the threshold for statistically significance.

RESULTS

The demographic and clinical information of the advanced NSCLC patients are described in Table 1. The advanced NSCLC is composed of 97 females and 187 males, and

their mean age was 63.60 ± 11.65 years. In the NSCLC patients, 171 patients smoked tobacco, 39 had a family history of cancer, 134 had squamous carcinoma, 150 had adenocarcinoma, 216 were at TNM stages IIIA or IIIB, 68 were at TNM stage IV, 127 had NSCLC located in the left lung, and 146 had NSCLC located in the right lung.

Table 1. Demographic and clinical information of the non-small cell lung cancer (NSCLC) patients.

Parameter	Patients	%
Age, years		
<60	162	57.04
≥60	122	42.96
Gender		
Female	97	34.15
Male	187	65.85
Tobacco consumption		
Never	113	39.79
Sometimes	171	60.21
Family history of cancer		
No	245	86.27
Yes	39	13.73
Histological types		
Squamous carcinoma	134	47.18
Adenocarcinoma	150	52.82
TNM stage		
IIIA and IIIB	216	76.06
IV	68	23.94
Location		
Left	127	44.72
Right	146	51.41
Other	11	3.87

Up to the end of follow-up, 206 patients showed progression, and 78 showed non-progression. After adjustment for clinical variables, the IIe/Val and Val/Val genotypes of *GSTP1* had a shorter PFS time compared with the IIe/IIe genotype (for IIe/Val *vs* IIe/IIe, 21.65 *vs* 16.60 months; for Val/Val *vs* IIe/IIe, 22.52 *vs* 16.60 months). In the Cox proportional hazards model, the IIe/Val and Val/Val genotypes of *GSTP1* were associated with lower risk of disease progression compared with the IIe/IIe genotype, and the HRs (95%CIs) were 0.37 (0.18-0.74) and 0.15 (0.06-0.35), respectively (Table 2). However, no significant association was found between *GSTM1* and *GSTT1* polymorphisms and PFS in NSCLC.

Table 2. Association between *GSTP1*, *GSTM1*, and *GSTT1* polymorphisms and progression-free survival (PFS) innon-small cell lung cancer (NSCLC).

Genotypes	Patients	%	Progression	%	Non-progression	%	HR (95%CI)[1]	P value
GSTM1								
Present	162	57.2	121	58.74	41	62.86	1.0 (Ref.)	-
Null	122	42.8	85	41.26	37	37.14	0.78 (0.45-1.36)	0.35
GSTT1								
Present	155	54.5	118	57.28	37	53.33	1.0 (Ref.)	-
Null	129	45.5	88	42.72	41	46.67	0.67 (0.39-1.17)	0.14
GSTP1								
IIe/IIe	115	40.6	99	48.06	16	33.33	1.0 (Ref.)	-
IIe/Val	121	42.6	84	40.78	37	35.24	0.37 (0.18-0.74)	0.002
Val/Val	48	16.8	23	11.17	25	32.38	0.15 (0.06-0.35)	<0.001

[1]Adjusted for age, gender, and TNM stage.

Up to May 2014, a total of 173 patients with NSCLC died from all causes, and the 5-year overall survival rate was 39.08%. The IIe/Val and Val/Val genotypes of *GSTP1* were

associated with longer overall survival of NSCLC compared with the IIe/IIe genotype (for IIe/Val *vs* IIe/IIe, 25.32 *vs* 21.85 months; for Val/Val *vs* IIe/IIe, 27.40 *vs* 21.85 months). In the Cox proportional hazards model, the IIe/Val and Val/Val genotypes significantly decreased risk of death from all causes in patients with NSCLC, and the HRs (95%CIs) were 0.52 (0.29-0.92) and 0.37 (0.17-0.79), respectively (Table 3). No significant association was observed between *GSTM1* and *GSTT1* polymorphisms and OS in NSCLC.

Table 3. Association between *GSTP1*, *GSTM1*, and *GSTT1* polymorphisms and overall survival (OS)innon-small cell lung cancer (NSCLC).

Genotypes	Patients	%	Death	%	Alive	%	HR (95%CI)[1]	P value
GSTM1								
Present	162	57.2	102	58.96	60	54.05	1.0 (Ref.)	-
Null	122	42.8	71	41.04	51	45.95	0.82 (0.49-1.36)	0.42
GSTT1								
Present	155	54.5	98	56.65	57	51.35	1.0 (Ref.)	-
Null	129	45.5	75	43.35	54	48.65	0.81 (0.49-1.34)	0.38
GSTP1								
IIe/IIe	115	40.6	82	47.40	33	29.73	1.0 (Ref.)	-
IIe/Val	121	42.6	68	39.31	53	47.75	0.52 (0.29-0.92)	0.02
Val/Val	48	16.8	23	13.29	25	22.52	0.37 (0.17-0.79)	0.005

Adjusted for age, gender, and TNM stage.

DISCUSSION

Increasing evidence has indicated that the genetic variations of drug-metabolizing enzymes, drug transporters, and drug targets involve in the inter-individual differences in the treatment efficacy of chemotherapy for cancers. In this study, we evaluated whether the *GSTP1*, *GSTM1*, and *GSTT1* polymorphisms could have an impact on the treatment outcomes for NSCLC in a Chinese population. We revealed that the IIe/Val and Val/Val genotypes were associated with a lower risk of disease progression and death from all causes compared to the IIe/IIe genotype. No significant association was found between *GSTM1* and *GSTT1* polymorphisms and progression and survival in NSCLC.

GST enzymes were reported to have a crucial role in metabolizing many xenobiotics and cytotoxic cancer chemotherapeutic agents (Tew et al., 1996). Previous study reported that *GSTP1* is the most abundant isoenzyme among GSTs and is over-expressed in cancer or precancerous tissues (Tiseo et al., 2013). Genetic variation of *GSTP1* could result in amino acid substitution changes of *GSTP1* and cause to hydroliphobicity of amino acids, which could contribute to the enzymatic stability and catalytic capability.

Currently, many studies have investigated the relationship between *GSTP1* genetic polymorphism and treatment outcome of chemotherapy in several kinds of cancer, such as bone tumors, colorectal cancer, breast cancer and gastric cancer (Jun et al., 2009; Li et al., 2010; Romero et al., 2012; Yang et al., 2012; Eralp et al., 2013; Ye et al., 2013). Jun et al. (2009) conducted a study with 122 advanced colorectal cancer patients, and reported that the Val/Val genotype of *GSTP1* is correlated with a higher clinical response to chemotherapy and with better survival of patients. Li et al. (2010) conducted a study in a Chinese population and reported that Val allele had better prognosis and response to oxaliplatin regimen for patients with advanced gastric cancer. Romero et al. (2012) carried out a study in Spanish population and reported that varied expression of *GSTP1* contributes to the clinical outcome of breast cancer. Yang et al. (2012) conducted a study with 187 patients with osteosarcoma, and

indicated that GSTP1 genetic polymorphism might play a role in the response to chemotherapy and prognosis of bone tumors. Eralp et al. conducted a study in a Turkish population, and indicated that downregulation of GSTP1 could improve progression-free survival in advanced breast cancer patients (Eralp et al., 2013). The results of these studies reported that *GSTP1* polymorphisms affect chemotherapy sensitivity and the prognosis for several kinds of cancers.

Many studies have reported the relationship between *GSTP1* polymorphisms and prognosis of advanced NSCLC, but the results are inconclusive (Booton et al., 2006; Lu et al., 2006; Vlachogeorgos et al., 2008; Kalikaki et al., 2009; Sun et al., 2010; Lv et al., 2014; Han et al., 2015). Sun et al. (2010), Han et al. (2015) and Lv et al., (2014) conducted studies in Chinese populations and reported that *GSTP1* genetic variations might be the predictive markers for the treatment outcome of advanced NSCLC. Booton et al. (2006), Vlachogeorgos et al. (2008) and Lu et al. (2006) carried out studies in Caucasians and they reported that *GSTP1* polymorphisms may predict response to treatment and survival in patients with advanced NSCLC. However, Kalikaki et al. (2009) did not revealed a significant correlation between *GSTP1* genetic polymorphisms and treatment outcomes in advanced NSCLC. The inconsistency in these studies may be attributed to the discrepancies in ethnicity, selection of patients, and sample size.

In summary, we suggest that *GSTP1* polymorphisms might influence the treatment outcome of advanced NSCLC patients, and our results could help improve individualized therapy.

Conflicts of interest

The authors declare no conflict of interest.

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